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# Shifts in Soil Microflora Induced by Velvetbean *(Mucuna deeringiana)* in Cropping Systems to Control Root-Knot Nematodes

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**This project is part of work underway in our laboratories to test the hypothesis that the induction of soil suppressiveness to plant parasitic nematodes that occurs following planting of velvetbean *(Mucuna deeringiana* (Bort) Merr.) is associated with the development of an antagonistic microflora in soils and rhizospheres. The specific objective of this investigation was to examine long-term microbial shifts associated with the use of velvetbean in rotations to control nematodes. A crop rotation study was conducted in microplots, consisting of three crop cycles. Cycle 1 involved planting of either velvetbean or cowpea *(Vigna unguiculata* L.) in the first spring. Cycle 2 during the next fall and winter was fallow or cover-cropped with wheat *(Triticum aestivum* L.) or crimson clover *(Trifolium incarnatum* L.). Cycle 3 the next spring was soybean *(Glycine max* (L.) Merr.). Populations and species diversity of bacteria and fungi in soils or rhizospheres were investigated at the end of each cropping cycle. Rhizosphere fungal populations were significantly smaller on velvetbean than on cowpea at the end of cycle 1. The use of velvetbean in cycle 1 significantly decreased rhizosphere bacterial populations on crops in cycle 2, compared to treatments which had cowpea in cycle 1. Velvetbean also influenced bacterial diversity, generally increasing frequency of bacilli, *Arthrobacter* spp. and *Burkholderia cepacia,* while reducing fluorescent pseudomonads. Some of these effects persisted through cycle 3. Fungal diversity was influenced in cycle 1 by velvetbean; however, effects generally did not persist through cycles 2 and 3. The results indicate that the use of velvetbean in a cropping system alters the microbial communities of the rhizosphere and soil, and they are consistent with the hypothesis that the resulting control of nematodes results from induction Of soil suppressiveness.** © 2000 Academic Press

It has long been recognized that soilborne diseases do not occur at high levels whenever the pathogen is present due to a phenomenon termed "suppressive soils" (Cook, 1982; Hornby, 1983). The reduction in disease, compared to theoretically expected levels, in suppressive soils is generally considered to be due to activity of natural antagonists (Cook, 1982), and depending on the nature of these antagonists, soil suppressiveness has been classified as "general" or "specific" suppression (Cook and Baker, 1983). General suppression relates to total microbial activity in soil without preferential involvement of one specific group of microorganisms, while specific suppression relates to antagonism of an individual or select group of microorganisms (Cook and Baker, 1983). Several reviews on this subject have suggested that suppressiveness could be induced and used as a practical alternative to pesticides for managing diseases (Hornby, 1983; Cook, 1982; Baker and Chet, 1982).

One limitation to using the concept of induced suppressiveness in practical agriculture is that this is classically done through multiple years of crop monoculture (Hornby, 1983; Cook and Baker, 1983). Stirling (1991) stated that with efforts to induce nematode-suppressive soils, the long cropping cycle allowed for considerable nematode damage before suppressiveness developed. In addition, Stirling (1991) indicated that "all documented examples of effective natural suppression of plant parasitic nematodes appear to be due mainly to the action of one or two specific biological control agents," and that these are highly host specific.

Despite the emphasis in the literature on using monoculture to develop specific suppression by increasing a limited number of antagonists, monoculture often does not lead to induction of suppressiveness, especially in highly eroded soils with low organic matter, low cation exchange capacity, and low fertility, indicative of many tropical and subtropical agricultural areas, including the southeastern United States (Bunch, 1994; Rodríguez-Kábana and Canullo, 1992). In Alabama, for example, monoculture of soybean *(Glycine*



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*max* (L.) Merr.) for 8 years or peanut for 12 years failed to induce suppressiveness against plant parasitic nematodes (reviewed in Rodríguez-Kábana and Canullo, 1992). In both cases, monoculture led to continually declining yields and continually increasing population densities of the parasitic nematodes. However, there is substantial evidence that suppressiveness can still be induced in these soils but through the use of specific crop rotation systems that include rotation with tropical legumes. One such legume is velvetbean *(Mucuna deeringiana* (Bort) Merr.), which has shown nematode control potential as either a rotational crop or an inter-planted crop in diverse geographical areas, including the southeastern United States (McSorley and Gallaher, 1992; Rodríguez-Kábana *et al.,* 1992a; Weaver *et al.,* 1993), Puerto Rico (Acosta *et al.,* 1991), Mexico (Garcia-Espinoza, 1994; Marciel and Garcia, 1986), and Spain (Rodríguez-Kábana *et al.,* 199213).

Velvetbean produces nematicidal compounds, which were initially considered to account for all of the observed protection against nematodes (Vincente and Acosta, 1987). However, with a 3-year field-rotation study, Rodríguez-Kábana *et al.* (1992a) reported that end-of-season populations of *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 juveniles in peanut, which followed 2 years of cropping with velvetbean, were significantly lower than continual peanut, demonstrating that reductions in nematode populations which occurred due to velvetbean were maintained during the winter and throughout the following season on a susceptible host. In contrast, the reductions in plant parasitic nematode population densities which occur with nematicides in the year of treatment typically do not continue to show reduced end-of-season populations in the following year without reapplication (Rodríguez-Kábana, personal communication), and sometimes the end-of-season populations are not different between nematicide treatment and nontreated controls in the same season that the nematicide was used, which was the case in the second year of the 3-year study cited above (Rodríguez-Kábana *et al.,* 1992a). This prolonged effect of velvetbean in suppressing populations of nematodes suggests that the mechanism is not due exclusively to toxic compounds produced by the velvetbean and would be consistent with induced suppressiveness due to microbial antagonists. Supporting this idea are the results of Chavarría-Carvajal and Rodríguez-Kábana (1998) in which amendment of soil with velvetbean resulted in increased antagonism of *Meloidogyne incognita* eggs.

To test the hypothesis that velvetbean can induce suppressiveness, we previously compared the microflora from rhizospheres of velvetbean and soybean (as a susceptible host) (Kloepper *et al.,* 1991, 1992). Population densities of both total aerobic bacteria and fungi were significantly lower in velvetbean than in soybean rhizospheres, while physiological characterization of isolated bacteria indicated that the frequency of several phenotypes associated with biological control increased with velvetbean compared to soybean (Kloepper *et al.,* 1991). Bacterial identification revealed that 80% of the rhizosphere bacteria isolated from mature soybean were *Bacillus* spp., while from mature velvetbean, only 6% were bacilli and 48% were *Burkholderia (Pseudomonas) cepacia* or the closely related *Burkholderia (Pseudomonas) gladioli,* a group not encountered from soybean rhizospheres. These results clearly demonstrate that velvetbean selected a taxonomically and physiologically distinct microflora from soil in comparison to soybean, which would tend to support our hypothesis that velvetbean can induce suppressiveness through microbial shifts. If this hypothesis is correct, the microbial shifts caused by velvetbean should extend beyond the season in which velvetbean is grown, since, as discussed above, the control of nematodes extends beyond this time. The main objective of this study was to examine the long-term microbial shifts associated with use of velvetbean in rotations to control nematodes.

## MATERIALS AND METHODS

### *Rotation Regime*

The experiment described here for assessing microflora shifts was part of a larger rotational study to determine effects of velvetbean on nematodes over five plantings (Vargas-Ayala, 1995). For microbial shifts, the rotation regime consisted of three cycles as follows. Cycle 1 was velvetbean or cowpea *(Vigna unguiculata* L.) cultivar "pink eye purple hull" planted in summer, 1992. Each of the treatments from cycle 1 was followed in cycle 2 by three treatments in winter, 1993: fallow or cover-cropped with wheat *(Triticum aestivum* L.) cultivar "Coker 9766" or crimson clover *(Trifolium incarnaturn* L.). Cycle 3, which was planted in spring, 1993, consisted of soybean planted to all treatments. Cowpea was used in cycle 1 as a control to velvetbean with the rationale that both crops are N-fixing legumes but cowpea is susceptible to *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949, which accounts for why in the early 1900s cowpea as a winter legume was replaced by velvetbean in rotations with cotton (Buckes *et al.,* 1998). The different winter treatments were used to test if persistence of microflora shifts expected from planting velvetbean in cycle 1 was affected by choice of fallow or cover-crop. Soybean was planted in cycle 3 as the susceptible host for the indigenous nematodes.

The rotation study was conducted in microplots on the Auburn University campus using four replications per treatment arranged in a randomized complete

block design, in which each replication consisted of one terra cotta container 36 cm X 36 cm and 1.0 m deep. Field soil from a soybean field near Elberta, Alabama was collected, classified as a Norfolk sandy loam (fine loamy, siliceous thermic, Typic Paleudults), and mixed 1:1 (volume:volume) with sand prior to placing into microplots. This sand:soil mixture contained several indigenous plant parasitic nematodes, including *M. incognita, M. arenaria, Heterodera glycines* Ichinohe, 1952, and low populations of *Helicotylenchus dihystera* (Cobb, 1893) Sher, 1961, and *Tylenchorhynchus* spp.

### *Sampling and Enumeration of Microflora*

Soil or rhizosphere soil samples were taken at the end of each cropping cycle by removing three cores per microplot from the center using a 2.5-cm-diameter soil probe at a depth of 25 cm. At this time, when a crop was present, the sampled soil contained plant roots and, therefore, represented rhizosphere soil. The cores from each microplot were mixed, and a 10-g sample from each was mixed with 90 ml 0.02 M sterile potassium phosphate buffer (pH 6.8) and shaken on an orbital shaker at 150 rpm for 30 min. Three serial 10-fold dilutions were prepared, and the $10^{-2}$ and $10^{-3}$ dilutions were plated using a spiral plater (Spiral System Instruments, Bethesda, MD) onto 5% tryptic soy agar (5% TSA) (Difco, Detroit, MI) for enumeration of total aerobic bacteria. The $10^{-1}$ and $10^{-2}$ dilutions were spiral-plated onto Ohio agar (Johnson and Curl, 1972) for enumeration of total fungi. Plates were inverted and incubated at 28°C for 48-72 h. Bacterial colonies were enumerated using a laser colony counter with Bacterial Enumeration Software (Spiral System Instrument), while fungal colonies were enumerated manually For each replication, treatment means were calculated by averaging the log of colony-forming units/g of dry soil (cfu/g), and data were analyzed using the general linear models in PC SAS (SAS, Release 6.04, SAS Institute, Cary, NC). Treatment means were compared using LSD at $P = 0.05$.

### *Bacterial and Fungal Diversity*

Bacterial and fungal diversity were assessed using the same plates on which populations were enumerated. With bacteria, 40 bacterial colonies from each replication were selected by sequentially removing each individually spaced colony on the outer most edge of the spiral and transferring these to 1.2-ml cryovials containing 0.75 ml sterile tryptic soy broth (TSB; Difco, Detroit, MI). Vials were placed in a Nalgene cryobox and allowed to grow on an orbital shaker at 150 rpm for 24 h. After inoculation, 0.25 ml of sterile glycerin was added to the vials which were shaken for an additional hour. The vials were stored in an ultra-cold freezer at −80 ± 1°C. Bacteria were identified by fatty acid analysis using fatty acid methyl-esters (FAMEs) (Sasser, 1990). Frozen bacterial isolates were streaked on TSA and incubated for 24 h at 28°C. Bacterial growth was checked visually for purity A 4-mm-diameter loopful of each isolate (approx 40 mg) was placed at the bottom of a 10-mm glass tube. Saponification, methylation, and FAME extraction were performed as described previously (Sasser, 1990). Bacteria were identified using the Aerobic Bacterial Library of MIDI (Microbial Identification, Newark, DE). Strains generating no match to those in the FAME library were classified as "unknown" and isolates exhibiting no growth in TSB were classified as "oligotrophic." Diversity among bacterial communities was characterized by calculating the total number of species (richness) and their abundance (evenness) according to Ludwig and Reynolds (1988). Significant differences in richness of predominant genera or species among rotation systems were determined using single-degree-of-freedom contrast analysis in the general linear models of PC SAS.

Predominant fungal colony types were selected from the plates used to quantify fungal population densities at each sampling time, and these were identified to species level by microscopic and stereoscopic analysis, using taxonomic keys. Uncertain fungal species were identified to genus level only, while nonsporulating fungi were classified as "unknown." When required, fungal isolates were transferred to fresh plates containing Ohio agar or potato dextrose agar (PDA; Difco, Detroit, MI). Species of *Penicillium* were isolated on Czapek agar (Difco, Detroit, MI) for their identification. Diversity of fungal communities was measured by calculating richness and evenness as described above for bacteria. Significant differences in fungal richness among rotation systems were analyzed using single-degree-of-freedom contrast analysis in the general linear models of PC SAS.

## RESULTS

### *Enumeration of Microflora*

Analysis of total aerobic bacterial population densities (Table 1) revealed no significant differences in mean cfu/g between cowpea and velvetbean rhizospheres at the end of the first cycle. As expected, populations at the end of cycle 2 were significantly lower with fallow compared to cropping with either cowpea or velvetbean. In plots planted in cycle 2 with cowpea or velvetbean, i.e., nonfallow treatments, populations were significantly less in plots cropped in cycle 1 with velvetbean than in plots cropped previously with cowpea. After cycle 3, all soybean treatments which were cropped in cycle 1 with cowpea had statistically

equivalent populations, while two of the three treatments originally cropped with velvetbean were significantly less.

The effects of cropping systems on total fungal population densities (Table 2) were different from the effects on bacterial populations. Fungal populations were significantly lower on velvetbean than on cowpea at the end of the first cycle. In contrast to the results with bacterial populations, there generally was no effect of using velvetbean in cycle 1 on subsequent fungal populations in cycles 2 or 3.

*Bacterial Diversity*

The diversity of several bacterial taxa based on single-degree-of-freedom contrast analyses was significantly different in rhizospheres of velvetbean and those of cowpea at the end of cycle 1 (Table 3). Compared to cowpea, velvetbean hosted higher frequencies of total bacilli, total *Arthrobacter* spp., and *B. cepacia,* which was isolated 3 times more frequently from velvetbean than from cowpea. Frequencies of the taxa were significantly less in rhizospheres of velvetbean than those of cowpea, including *Pseudomonas chlororaphis, Xylophila ampelinus,* and *Cytophaga johnsonae,* which was 15 times less frequent on velvetbean than on cowpea.

At the end of cycle 2, some effects of using velvetbean in cycle 1 were still evident (Table 4). The frequency of isolation of total *Arthrobacter* spp. in cycle 2 was approximately 2 and 2.5 times greater for wheat and clover, respectively, following velvetbean than from the same crops following cowpea (in cycle 1). Total *Bacillus* spp. were approximately 3 times less frequent in rhizospheres of wheat and clover which were previously cropped to velvetbean than from the same crops previously cropped to cowpea.

**TABLE 1**

Population Densities of Soil and Rhizosphere Bacteria in a Cropping Sequence with Velvetbean

| Cropping sequence[a] | Mean population (log cfu/g sample) of bacteria on 5% TSA[b] | | |
|---|---|---|---|
| | First cycle | Second cycle[c] | Third cycle[c] |
| C-F-S | 4.91 | 5.07 D | 5.43 AB |
| C-W-W | | 5.67 A | 5.28 BC |
| C-Cl-S | | 5.61 AB | 5.19 BC |
| V-F-S | 4.92 | 5.04 D | 4.9 2D |
| V-W-S | | 5.5 BC | 5.66 A |
| V-CI-s | | 5.4 C | 5.13 CD |
| $LSD_{0.05}$ | NS | 0.16 | 0.24 |

[a] Letters refer to cropping sequence in cycles 1,2, and 3. C, cowpea; F, fallow; S, soybean; W, wheat; Cl, crimson clover; and V, velvetbean.
[b] Mean of 12 replications with three samples/replication for the first cycle and 4 replications with three samples/replication for the second and third cycles. TSA, tryptic soy agar.
[c] Values followed by the same letter do not differ significantly at $P = 0.01$ according to the general linear models ANOVA.

**TABLE 2**

Population Densities of Soil and Rhizosphere Fungi in a Cropping Sequence with Velvetbean

| Cropping sequence[a] | Mean population (log cfu/g sample) of fungi on OA[b] | | |
|---|---|---|---|
| | First cycle[c] | Second cycle[c] | Third cycle[c] |
| C-F-S | 3.88 A | 3.09 | 3.95 A |
| C-W-S | | 3.22 | 3.97 A |
| C-CI-S | | 3.21 | 4.11 A |
| V-F-S | 3.69 B | 3.02 | 3.64 B |
| V-W-S | | 3.21 | 4.11 A |
| V-CI-S | | 3.24 | 3.97 A |
| $LSD_{0.01}$ | 0.11 | NS | 0.28 |

[a] Letters refer to cropping sequence in cycles 1,2, and 3. C, cowpea; F, fallow; S, soybean; W, wheat; Cl, crimson clover; and V, velvetbean.
[b] Mean of 12 replications with three samples/replication for the first cycle and 4 replications with three samples/replication for the second and third cycles. OA, Ohio agar.
[c] Values followed by the same letter do not differ significantly ($P = 0.01$) according to the general linear models ANOVA.

Results from identifications of rhizosphere bacteria in cycle 3 (Table 5) indicated that the choice of winter cropping system (cycle 2) impacted the magnitude of differences in the bacterial communities of soybean among treatments originally cropped to velvetbean or cowpea (in cycle 1). With *B. cepacia,* there was no substantial difference between the use of cowpea or velvetbean (in cycle 1) in the frequency of isolation from soybean rhizospheres (cycle 3) for the treatments which had clover in cycle 2. However, when wheat was used in cycle 2, *B. cepacia* was twice as frequent from soybean in cycle 3 which had velvetbean in cycle 1 than from soybean which had cowpea in cycle 1. Frequencies of total pseudomonads were lower in soybean rhizospheres for all three treatments which had velvetbean in cycle 1 than in the corresponding three treatments which had cowpea in cycle 1. Frequencies of isolation of total *Bacillus* spp. and total *Arthrobacter* spp. were significantly greater in soybean which had fallow in cycle 2 and velvetbean in cycle 1 than in soybean with fallow in cycle 2 and cowpea in cycle 1. However, when clover or wheat was cropped in cycle 2, the frequencies of total *Bacillus* spp. and total *Arthrobacter* spp. in soybean rhizospheres were not substantially different between treatments receiving velvetbean or cowpea in cycle 1.

*Fungal Diversity*

Some differences in the frequencies of isolation of fungal taxa were noted between velvetbean and cowpea

## TABLE 3

Bacterial Diversity in Rhizospheres of Cowpea and Velvetbean After Cycle 1

| | | Isolation frequency | | | |
|---|---|---|---|---|---|
| | | Cowpea | | Velvetbean | |
| Bacterial group | Taxon | No.[a] | % | No.[a] | % |
| Gram-negative | *Acidovorax* spp.[b] | 4 | 0.8 | 2 | 0.4 |
| | *Agrobacterium* spp.[c] | 2 | 0.4 | 1 | 0.2 |
| | *Alcaligenes eutrophus* | 9 | 1.9 | 10 | 2.1 |
| | *Burkholderia cepacia** | 12 | 2.5 | 34 | 7.1 |
| | *B. gladioli* | 0 | 0 | 1 | 0.2 |
| | *B. pickettii* | 148 | 30.8 | 161 | 33.5 |
| | *Burkholderia* (total) | 160 | 33.3 | 196 | 40.8 |
| | *Chromobacterium violaceum* | 0 | 0 | 2 | 0.4 |
| | *Chryseobacterium* spp.[d] | 4 | 0.8 | 0 | 0 |
| | *Comamonas acidovorans* | 4 | 0.8 | 0 | 0 |
| | *Cytophaga johnsonae** | 15 | 3.1 | 1 | 0.2 |
| | *Enterobacter intermedius* | 1 | 0.2 | 0 | 0 |
| | *Erwinia chrysanthemi* | 0 | 0 | 8 | 1.7 |
| | *Hydrogenophaga pseudoflava* | 4 | 0.8 | 2 | 0.4 |
| | *Methylobacterium* spp. | 1 | 0.2 | 2 | 0.4 |
| | *Phyllobacterium myrsinacearum* | 4 | 0.8 | 7 | 1.5 |
| | *P. rubiacearum* | 3 | 0.6 | 6 | 1.2 |
| | *Pseudomonas* spp.[e] | 6 | 1.2 | 7 | 1.5 |
| | *Ps. chlororaphis** | 13 | 2.7 | 4 | 0.8 |
| | *Ps. putida* | 0 | 0 | 3 | 0.6 |
| | *Ps. stutzeri* | 4 | 0.8 | 0 | 0 |
| | *Ps. syringae* | 3 | 0.6 | 7 | 1.5 |
| | *Pseudomonas* (total) | 26 | 5.3 | 21 | 4.4 |
| | *Ralstonia solanacearum* | 0 | 0 | 11 | 2.3 |
| | *Serratia marcescens* | 0 | 0 | 1 | 0.2 |
| | *Sphingomonas capsulata* | 0 | 0 | 1 | 0.2 |
| | *Sphingobacterium* spp.[f] | 2 | 0.4 | 6 | 1.2 |
| | *Variovorax paradoxus* | 5 | 1 | 4 | 0.8 |
| | *Xanthomonas axonopodis* | 0 | 0 | 1 | 0.2 |
| | *Xylophila ampelinus** | 34 | 7.1 | 25 | 5.2 |
| | Subtotal | 278 | 57.9 | 307 | 64 |
| Gram-positive | *Arthrobacter ramosus* | 5 | 1 | 9 | 1.9 |
| | *A. aurescens* | 3 | 0.6 | 0 | 0 |
| | *A. globiformis* | 3 | 0.6 | 9 | 1.9 |
| | *A. ureafaciens* | 0 | 0 | 3 | 0.6 |
| | Total *Arthrobacter* spp.[g] | 15 | 3 | 23 | 4.8 |
| | *Aureobacterium saperdae* | 0 | 0 | 1 | 0.2 |
| | *Bacillus megaterium* | 2 | 0.4 | 5 | 1 |
| | *Paenibacillus polymyxa* | 1 | 0.2 | 3 | 0.6 |
| | *B. sphaericus* | 2 | 0.4 | 0 | 0 |
| | Total bacilli[h] | 7 | 1.4 | 11 | 2.2 |
| | *Brevibacterium helvolum* | 2 | 0.4 | 0 | 0 |
| | *Clavibacter michiganensis* | 2 | 0.4 | 3 | 0.6 |
| | *Corynebacterium aquaticum* | 0 | 0 | 2 | 0.4 |
| | *Curtobacterium flaccumfaciens* | 0 | 0 | 2 | 0.4 |
| | *Micrococcus* spp.[i] | 5 | 1 | 5 | 1 |
| | *Staphylococcus* spp.[j] | 11 | 2.3 | 5 | 1 |
| | Subtotal | 42 | 8.8 | 52 | 10.8 |
| Unknown[k] | | 77 | 16 | 56 | 11.6 |
| Oligotrophic[l] | | 83 | 17.3 | 65 | 13.5 |
| Grand total | | 480 | | 480 | |

[a] Values represent the number of isolates of 480 tested which were identified to each taxon using fatty acid methyl-ester (FAME) analysis.

[b] *Acidovorax* spp. include *A. delafieldi* and *A. facilis.*

[c] *Agrobacterium* spp. include *A. rubi* and A. *radiobacter.*

[d] *Chryseobacterium* spp. include *C. balustinum* and *C. indologenes.*

[e] *Pseudomonas* spp. include *P. aeruginosa, Acidovorax* (formerly *Pseudomonas) avenae, Burkholderia caryophylli* (formerly *Pseudomonas), P. cichorii, P. fluorescens, Herbaspirillum* (formerly *Pseudomonas) rubrisubalbicans,* and *P. saccharophila.*

[f] *Sphingobacterium* spp. include *S. multivorum* and *S. spiritivorum.*

[g] Total *Arthrobacter* spp. include *A. atrocyaneus, A. nicotianae,* and *A. uratoxydans.*

[h] Total bacilli include *B. laterosporus, B. maroccanus, B. megaterium, B. pumilis, B. sphaericus, B. thuringiensis,* and *Paenibacillus polymyxa.*

[i] *Micrococcus* spp. include *M. kristinae, M. luteus, M. lylae,* and *M. varians.*

[j] *Staphylococcus* spp. include *S. aureus, S. epidermidis, S. hominis,* and *S. warneri.*

[k] Unknown strains are those with no match to taxa in the FAME library

[l] Oligotrophic strains are those with no growth on TSA media.

* Indicates significant difference ($P = 0.05$) in isolation frequency between velvetbean and cowpea according to single-degree-of-freedom contrast analyses.

**TABLE 4**

Bacterial Diversity in Soil and Rhizospheres after Cycle 2

| Bacterial group | Taxon | Isolation frequency | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C-F[a] | | C-W[a] | | C-CL[a] | | V-F[a] | | V-W[a] | | V-Cl[a] | |
| | | No.[b] | % | No.[b] | % | No.[b] | % | No.[b] | % | No.[b] | % | No.[b] | % |
| Gram-negative | *Acidovorax* spp.[c] | 0 | 0 | 1 | 0.6 | 2 | 1.2 | 1 | 0.6 | 0 | 0 | | 0.6 |
| | *Agrobacterium* spp.[d] | 2 | 1.2 | 2 | 1.2 | 2 | 1.2 | 0 | 0 | 2 | 1.2 | 0 | 0 |
| | *Alcaligenes* spp.[e] | 3 | 1.9 | 2 | 1.2 | 1 | 0.6 | 2 | 1.2 | 2 | 1.2 | 0 | 0 |
| | *Burkholderia cepacia* | 7 | 4.4 | 8 | 5 | 3 | 1.9 | 16 | 10 | 6 | 3.7 | 4 | 2.5 |
| | *B. gladioli* | 1 | 0.6 | 0 | 0 | 1 | 0.6 | 3 | 1.9 | 3 | 1.9 | 2 | 1.2 |
| | *B. pickettii* | 4 | 2.5 | 4 | 2.5 | 1 | 0.6 | 5 | 3.1 | 5 | 3.1 | 2 | 1.2 |
| | *Burkholderia* (total) | 12 | 7.5 | 12 | 7.5 | 5 | 3.1 | 24 | 15 | 14 | 8.8 | 8 | 5.0 |
| | *Chryseobacterium* spp.[f] | 0 | 0 | 5 | 3.1 | 6 | 3.7 | 0 | 0 | 4 | 2.5 | 4 | 2.5 |
| | *Comamonas testosteroni* | 1 | 0.6 | 3 | 1.9 | 0 | 0 | 0 | 0 | 1 | 0.6 | 0 | 0 |
| | *Cytophaga spp.*[g] | 04 | 2.5 | 51 | 0.6 | 3 | 1.9 | 0 | 0 | 4 | 2.5 | 0 | 0 |
| | *Hydrogenophaga pseudoflava* | 1 | 0.6 | 0 | 0 | 4 | 2.5 | 1 | 0.6 | 2 | 1.2 | 0 | 0 |
| | *Janthinobacterium lividum* | 0 | 0 | 1 | 0.6 | 0 | 0 | 2 | 1.2 | 0 | 0 | 1 | 0.6 |
| | *Methylobacterium extorquens* | 0 | 0 | 1 | 0.6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *Ochrobactrum anthropi* | 0 | 0 | 1 | 0.6 | 1 | 0.6 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *Pantoea* spp.[h] | 1 | 0 | 3 | 1.9 | 5 | 3.1 | 0 | 0 | | 0.6 | | 0.6 |
| | *Phyllobacterium* spp.[i] | 3 | 1.9 | 3 | 1.9 | 2 | 1.2 | 1 | 0.6 | 5 | 3.1 | 4 | 2.5 |
| | *Pseudomonas* spp.[j] | 12 | 7.5 | 16 | 10 | 8 | 5 | 7 | 4.4 | 11 | 6.9 | 7 | 4.4 |
| | *Ralstonia solanacearum* | 0 | 0 | 1 | 0.6 | 2 | 1.2 | 0 | 0 | 1 | 0.6 | 1 | 0.6 |
| | *Sphingobacterium* spp.[k] | 1 | 0.6 | 5 | 3.1 | 4 | 2.5 | 0 | 0 | 8 | 5 | 4 | 2.5 |
| | *Variovorax paradoxus* | 4 | 2.5 | 1 | 0.6 | 1 | 0.6 | 1 | 0.6 | 5 | 3.1 | 3 | 1.9 |
| | *Vibrio metschnikovii* | 0 | 0 | 1 | 0.6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *Weeksella zoohelcum* | 0 | 0. | 1 | 0.6 | 0 | 0 | 0 | 0 | 1 | 0.6 | 0 | 0 |
| | *Xanthomonas* spp.[l] | 2 | 1.2 | 2 | 1.2 | 0 | 0 | 3 | *1.9* | *4* | *2.5* | *0* | *0* |
| | *Yersinia frederiksenii* | 0 | 0 | 0 | 0 | | 0.6 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Subtotal | 45 | 28.1 | 59 | 36.9 | 47 | 29.4 | 42 | 26.2 | *64* | *40* | 34 | 21.2 |

rhizospheres in cycle 1 (Table 6). *Paecilomyces* spp. constituted 19% of isolates from cowpea and 28% of isolates from velvetbean. *Gliocladium roseum* was two times more frequent, and *Zygorrhynchus molleri* was four times more frequent on velvetbean than on cowpea. *Penicillium* spp. were dominant on both crops, accounting for 80% of cowpea isolates and 67% of velvetbean isolates; however, there were differential frequencies of some species. *Pencillium herquei* was twice more frequent, and *P. janthinellum* was twice less frequent on velvetbean than on cowpea.

In cycle 2, there were generally fewer differences in fungal frequencies than in bacterial frequencies, which were related to the use of velvetbean or cowpea in cycle 1 (Table 7). *G. roseum,* which had increased with velvetbean in cycle 1, was isolated at the same frequency on all three cycle 2 treatments which received velvetbean or cowpea in cycle 1. *Paecilomyces* spp., which were slightly more frequent in cycle 1 on velvetbean than on cowpea, were generally not isolated from the three treatments in cycle 2 which received velvetbean in cycle 1. Z. *molleri,* was three times more frequent in fallow after velvetbean than in fallow after cowpea; it was four times more frequent in cycle 2 on wheat which had velvetbean in cycle 1 than on wheat which had cowpea in cycle 1; however, it was not more frequent on clover after velvetbean than on clover after cowpea.

On soybean in cycle 3, the dominant fungal taxon isolated from all six treatments was *Paecilomyces lilacinus* (Table 8). There were almost no substantial differences in fungal diversity in cycle 3 which related to the crop used in cycle 1. *Metarrhizium anisopliae,* which was not detected in cycle 2, was much more frequent on soybean which had clover in cycle 2 (and either velvetbean or cowpea in cycle 1) than on soybean which had wheat or fallow in cycle 2.

## DISCUSSION

Use of velvetbean in a cropping system alters the microbial communities of the rhizosphere and soil. Some of these alterations which began on the velvetbean plant roots persisted through a 12-week fallow period and were seen at the end of the next season in the rhizosphere of soybean. The trend toward reduced total bacterial and fungal populations following velvetbean, observed in this microplot rotation study, agrees

**TABLE 4—*Continued***

| Bacterial group | Taxon | Isolation frequency C-F[a] No.[b] | % | C-W[c] No.[b] | % | C-CL[a] No.[b] | % | V-F[a] No.[b] | % | V-W[a] No.[b] | | V-Cl[a] No.[b] | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gram-positive | *Arthrobacter ramosus* | 3 | 1.9 | 6 | 3.7 | 6 | 3.7 | 4 | 2.5 | 4 | 2.5 | 6 | 3.7 |
| | *A. atrocyaneus* | 0 | 0 | 2 | 1.2 | 2 | 1.2 | 7 | 4.4 | 4 | 2.5 | 0 | 0 |
| | *A. globiformis* | 7 | 4.4 | 5 | 3.1 | 8 | 5 | 9 | 5.6 | 16 | 10 | 30 | 18.7 |
| | *A. nicotianae* | 6 | 3.7 | 2 | 1.2 | 2 | 1.2 | 7 | 4.4 | 4 | 2.5 | 9 | 5.6 |
| | *A. ureafaciens* | 1 | 0.6 | 0 | 0 | 1 | 0.6 | 1 | 0.6 | 2 | 1.2 | 2 | 1.2 |
| | Total *Arthrobacter* spp.[m] | 24 | 15 | 18 | 11.1 | 20 | 12.3 | 31 | 19.4 | 34 | 21.2 | 53 | 32.9 |
| | *Aureobacterium* spp. | 2 | 1.2 | 1 | 0.6 | 0 | 0 | 0 | 0 | 5 | 3.1 | 0 | 0 |
| | *Bacillus megaterium* | 7 | 4.4 | 2 | 1.2 | 1 | 0.6 | 13 | 8.1 | 1 | 0.6 | 4 | 2.5 |
| | *Paenibacillus polymyxa* | 8 | 5 | 1 | 0.6 | 6 | 3.7 | 7 | 4.4 | 0 | 0 | 0 | 0 |
| | Total bacilli[n] | 27 | 16.9 | 12 | 7.4 | 32 | 19.9 | 30 | 18.7 | 4 | 2.5 | 12 | 7.5 |
| | *Brevibacterium helvolum* | 1 | 0.6 | | 0.6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *Brochothrix* spp.[o] | 0 | 0 | 1 | 0.6 | 2 | 1.2 | 4 | 2.5 | 1 | 0.6 | 1 | 0.6 |
| | *Cellulomonas cellulans* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.6 | 0 | 0 | 0 | 0 |
| | *Clavibacter michiganensis* | 2 | 1.2 | 0 | 0 | | 0.6 | 1 | 0.6 | 0 | 0 | 2 | 1.2 |
| | *Corynebacterium* spp.[p] | 0 | 0 | 2 | 1.2 | | 0.6 | 1 | 0.6 | 5 | 3.1 | 3 | 1.9 |
| | *Curtobacterium* spp.[q] | 4 | 2.5 | 2 | 1.2 | 3 | 1.9 | 2 | 1.2 | 8 | 5 | 6 | 3.7 |
| | *Microbacterium* spp.[r] | 2 | 1.2 | 1 | 0.6 | 0 | 0 | 2 | 1.2 | 0 | 0 | 1 | 0.6 |
| | *Micrococcus* spp.[s] | 5 | 3.1 | 3 | 1.9 | 1 | 0.6 | 2 | 1.2 | 0 | 0 | 4 | 2.5 |
| | *M. kristinae* | 0 | 0 | 4 | 2.5 | 5 | 3.1 | 2 | 1.2 | 2 | 1.2 | 2 | 1.2 |
| | *Rhodococcus erythropolis* | 1 | 0.6 | 0 | 0 | 0 | 0 | 2 | 1.2 | 2 | 1.2 | 4 | 2.5 |
| | *Staphylococcus* spp.[t] | 6 | 3.7 | 3 | 1.9 | 1 | 0.6 | 2 | 1.2 | | 0.6 | 4 | 2.5 |
| | Subtotal | *75* | *46.9* | *51* | *31.9* | *66* | *41.2* | *80* | *50* | *63* | *39.4* | *92* | *57.5* |
| Unknown[u] | | 20 | 12.5 | 20 | 12.5 | 19 | 11.9 | 16 | 10 | 16 | 10 | 13 | 8.1 |
| Oligotrophic[v] | | 20 | 12.5 | 30 | 18.7 | 28 | 17.5 | 22 | 13.7 | 17 | 10.6 | 21 | 13.1 |
| Grand total | | 160 | | 160 | | 160 | | 160 | | 160 | | 160 | |

[a] C, cowpea; F, fallow; W, wheat; Cl, crimson clover; and V, velvetbean.
[b] Values represent the number of isolates of 160 tested which were identified to each taxon using fatty acid methyl-ester (FAME) analysis.
[c] *Acidovorax* spp. include *A. delafieldii* and *A. facilis.*
[d] *Agrobacterium* spp. include *A. rubi* and *A. radiobacter*
[e] *Alcaligenes* spp. include *A. eutrophus* and *A. xylosoxydans.*
[f] *Chryseobacterium* spp. include *Flavobacterium aquatile, C. balustinum, Aureobacterium esteraromaticum, C. indologenes, C. meningosepticum,* and *F. thalpophilum.*
[g] *Cytophaga* spp. include *C. aquatilis, C. heparina,* and *C. johnsonae.*
[h] *Pantoea* spp. include *P. agglomerans* and the closely related taxa of *Enterobacter mundtii* and *Erwinia chrysanthemi.*
[i] *Phyllobacterium* spp. include *P. myrsinacearum* and *P. rubiacearum.*
[j] *Pseudomonas* spp. include *P. aeruginosa, Acidovorax* (formerly *Pseudomonas) avenae, P. chlororaphis, P. coronafaciens, P. diminuta, P. fluorescens, P. putida, P. saccharophila,* and *P. syringae.*
[k] *Sphingobacterium* spp. include *S. multivorum* and *S. spiritivorum.*
[l] *Xanthomonas* spp. include *X. axonopodis* and *Stenotrophomonas maltophilia.*
[m] Total *Arthrobacter* spp. include *A. aurescens, A. citreus, A. mysorens, A. oxydans, A. pascens, A. uratoxydans,* and *A. viscosus.*
[n] Total bacilli include *Paenibacillus alvei, B. amyloliquefaciens, B. cereus, B. circulans, B. coagulans, B. freudenreichii, B. lentus, B. laterosporus, B. longisporus, P macerans, P. marinus, P. pabuli, B. pumilis, B. sphaericus, B. subtilis,* and *B. thuringiensis.*
[o] *Brochothrix* spp. include *B. campestris* and *B. thermosphacta.*
[p] *Corynebacterium* spp. include *C. aquaticum* and *C. mediolanum.*
[q] *Curtobacterium* spp. include *C. citreum, C. flaccumfaciens,* and *C. pusillum.*
[r] *Microbacterium* spp. include *M. lacticum, M. imperiale,* and *M. laevaniformans.*
[s] *Micrococcus* spp. include *M. agilis, M. luteus, M. lylae, M. roseus,* and *M. varians.*
[t] *Staphylococcus* spp. include *S. aureus, S. capitis, S. epidermidis, S. haemolyticus, S. hominis,* and S. *warneri.*
[u] Unknown strains are those with no match to taxa in the FAME library.
[v] Oligotrophic strains are those with no growth on TSA media.

with our previous greenhouse study in which bacterial and fungal populations were lower in the rhizosphere of velvetbean than in soybean (Kloepper *et al.*, 1991).

In addition to causing changes in population densities of culturable microorganisms, velvetbean was associated with shifts in the microbial community diversity of the rhizosphere. At the end of cycle 1, compared to cowpea, velvetbean had higher frequencies of total bacilli, total *Arthrobacter* spp., and *Burkholderia cepacia* (Table 3), while it had lower frequencies of three

## TABLE 5

Bacterial Diversity in Soybean Rhizospheres after Cycle 3

| Bacterial group | Taxon | Isolation frequency | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C-F-S[a] | | C-W-S[a] | | C-Cl-S[a] | | V-F-S[a] | | V-W-S[a] | | V-Cl-S[a] | |
| | | No.[b] | % | No.[b] | % | No.[b] | % | No.[b] | % | No.[b] | % | No.[b] | % |
| Gram-negative | Acidovorax avenue | 4 | 2.5 | 4 | 2.5 | 0 | 0 | 3 | 1.9 | 7 | 4.4 | 3 | 1.9 |
| | *Acinetobacter baumannii* | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 1.9 | 0 | 0 | 0 | 0 |
| | *Actinobacillus lignieresii* | 2 | 1.2 | 0 | 0 | 1 | 0.6 | 0 | 0 | 1 | 0.6 | 0 | 0 |
| | *Aeromonas masoucida* | 0 | 0 | 1 | 0.6 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.6 |
| | *Agrobacterium radiobacter* | 5 | 3.1 | 2 | 1.2 | 1 | 0.6 | 1 | 0.6 | 2 | 1.2 | 2 | 1.2 |
| | *Aquaspirillum autotrophicum* | 0 | 0 | 1 | 0.6 | 0 | 0 | 0 | 0 | 1 | 0.6 | 0 | 0 |
| | *Alcaligenes spp.*[c] | 6 | 3.7 | 3 | 1.9 | 7 | 4.4 | 6 | 3.7 | 0 | 0 | 6 | 3.7 |
| | *Burkholderia cepacia* | 20 | 12.5 | 9 | 5.6 | 18 | 11.2 | 12 | 7.5 | 20 | 12.5 | 14 | 8.7 |
| | *B. gladioli* | 1 | 0.6 | 3 | 1.9 | 4 | 2.5 | 0 | 0 | 2 | 1.2 | 1 | 0.6 |
| | *B. pickettii* | 16 | 10 | 8 | 5 | 15 | 9.4 | 28 | 17.5 | 5 | 3.1 | 17 | 10.6 |
| | *Burkholderia (total)* | 37 | 23.1 | 20 | 12.5 | 37 | 23.1 | 40 | 25 | 27 | 16.9 | 32 | 20 |
| | *Chryseobacterium spp.*[d] | 6 | 3.7 | 1 | 0.6 | 5 | 3.1 | 5 | 3.1 | 5 | 3.1 | 3 | 1.9 |
| | *Comamonas acidovorans* | 1 | 0.6 | 6 | 3.7 | 3 | 1.9 | 1 | 0.6 | 0 | 0 | 4 | 2.5 |
| | *Cytophaga johnsonae* | 7 | 4.4 | 12 | 7.5 | 5 | 3.1 | 2 | 1.2 | 6 | 3.7 | 4 | 2.5 |
| | *Enterobacter* spp.[e] | 2 | 1.2 | 1 | 0.6 | 2 | 1.2 | 0 | 0 | 3 | 1.9 | 1 | 0.6 |
| | *Erwinia chrysanthemi* | 2 | 1.2 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.6 | 0 | 0 |
| | *Flavimonas oryzihabitans* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.6 | 1 | 0.6 |
| | *Hydogenophaga pseudoflava* | 4 | 2.5 | 2 | 1.2 | 1 | 0.6 | 1 | 0.6 | 2 | 1.2 | 2 | 1.2 |
| | *Klebsiella terrigena* | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1.2 | 0 | 0 | 2 | 1.2 |
| | *Methylobacterium mesophilicum* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.6 | 0 | 0 | 1 | 0.6 |
| | *Ochrobactrum anthropi* | 1 | 0.6 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.6 | 0 | 0 |
| | *Phyllobacterium* spp.[f] | 1 | 0.6 | 10 | 6.2 | 3 | 1.9 | 7 | 4.4 | 7 | 4.4 | 3 | 1.9 |
| | *Ps. chlororaphis* | 1 | 0.6 | 2 | 1.2 | 1 | 0.6 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *Ps. syringae* | 4 | 2.5 | 14 | 8.7 | 7 | 4.4 | 5 | 3.1 | 3 | 1.9 | 7 | 4.4 |
| | Total pseudomonads[g] | 18 | 11.2 | 30 | 18.6 | 22 | 13.7 | 8 | 5 | 21 | 13.1 | 14 | 8. |
| | *Ralstonia solanacearum* | 3 | 1.9 | 2 | 1.2 | 1 | 0.6 | 1 | 0.6 | 3 | 1.9 | 3 | 1.9 |
| | *Salmonella typhimurium* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1.2 |
| | *Sphingobacterium* spp.[h] | 8 | 5 | 7 | 4.4 | 2 | 1.2 | 2 | 1.2 | 6 | 3.7 | 3 | 1.9 |
| | *Variovoraxparadoxus* | 1 | 0.6 | 1 | 0.6 | 0 | 0 | 0 | 0 | 1 | 0.6 | 0 | 0 |
| | *Vibrio vulnificus* | 1 | 0.6 | 0 | 0 | 0 | 0 | 1 | 0.6 | 0 | 0 | 0 | 0 |
| | *Weeksella zoohelcum* | 0 | 0 | 0 | 0 | 3 | 1.9 | 0 | 0 | 7 | 4.4 | 0 | 0 |
| | *Xanthomonas* spp.[i] | 3 | 1.9 | 3 | 1.9 | 4 | 2.5 | 2 | 1.2 | 4 | 2.5 | 3 | 1.9 |
| | *Yersinia enterocolitica* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.6 | 0 | 0 |
| | Subtotal | 112 | 70 | 106 | 66.2 | 97 | 60.6 | 86 | 53.8 | 107 | 66.9 | 90 | 56.2 |
| Gram-positive | *Arthrobacterglobiformis* | 1 | 0.6 | 1 | 0.6 | 2 | 1.2 | 3 | 1.9 | 0 | 0 | 5 | 3.1 |
| | *A. ilicis* | 1 | 0.6 | 1 | 0.6 | 3 | 1.9 | 4 | 2.5 | 1 | 0.6 | 4 | 2.5 |
| | *Total Arthrobacter* spp.[j] | 4 | 2.5 | 4 | 2.5 | 8 | 5 | 10 | 6.3 | 2 | 1.2 | 15 | 9. |
| | *Aureobacterium liquefaciens* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.6 | 0 | 0 | 0 | 0 |
| | *Bacillus laterosporus* | 1 | 0.6 | 1 | 0.6 | 3 | 1.9 | 4 | 2.5 | 0 | 0 | 5 | 3.1 |
| | *B. megaterium* | 5 | 3.1 | 7 | 4.4 | 5 | 3.1 | 10 | 6.2 | 5 | 3.1 | 6 | 3.7 |
| | *Paenibacillus polymyxa* | 1 | 0.6 | 1 | 0.6 | 2 | 1.2 | 3 | 1.9 | 0 | 0 | 3 | 1.9 |
| | Total bacilli[k] | 1 | 6.8 | 20 | 12.5 | 16 | 9.9 | 26 | 16.2 | 7 | 4.3 | 22 | 9.2 |
| | *Cellulomonas fimi* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.6 |
| | *Clavibacter michiganensis* | 1 | 0.6 | 0 | 0 | 1 | 0.6 | 4 | 2.5 | 0 | 0 | 0 | 0 |
| | *Corynebacterium* spp.1 | 0 | 0 | 0 | 0 | 1 | 0.6 | 0 | 0 | 1 | 0.6 | 0 | 0 |
| | *Micrococcus* spp.[m] | 0 | 0 | 1 | 0.6 | 0 | 0 | 4 | 2.5 | 1 | 0.6 | 3 | 1.9 |
| | *Rathayibacter tritici* | 1 | 0.6 | 2 | 1.2 | 3 | 1.9 | 1 | 0.6 | 1 | 0.6 | 1 | 0.6 |
| | *Rhodococcus erythropolis* | 0 | 0 | 0 | 0 | 1 | 0.6 | 0 | 0 | 1 | 0.6 | 0 | 0 |
| | Subtotal | 17 | 10.6 | 77 | 16.9 | 30 | 18.8 | 46 | 28.8 | 13 | 8.12 | 42 | 26.2 |
| Unknown[n] | | 14 | 8.7 | 10 | 6.2 | 17 | 10.6 | 17 | 10.6 | 22 | 13.7 | 11 | 6.9 |
| Oligotophic[o] | | 17 | 10.6 | 17 | 10.6 | 16 | 10 | 11 | 6.9 | 18 | 11.2 | 17 | 10.6 |
| Grand total | | 160 | | 160 | | 160 | | 160 | | 160 | | 160 | |

[a] C, cowpea; F, fallow; S, soybean; W, wheat; Cl, crimson clover; and V, velvetbean.

[b] Values represent the number of isolates of 160 tested which were identified to each taxon using fatty acid methyl-ester (FAME) analysis.

[c] *Alcaligenes* spp. include *A. eutrophus, A. piechaudii,* and A. *xylosoxydans.*

[d] *Chryseobacterium* spp. include *C. balustinum, Aureobacterium esteraromaticum, C. indologenes,* and *C. meningosepticum.*

[e] *Enterobacter* spp. include *Pantoea agglomerans* and *E. cancerogenus.*

[f] *Phyllobacterium* spp. include *P. myrsinacearum* and *P. rubiacearum.*

[g] Total pseudomonads include *P. aeruginosa, Brevundimonas diminuta, P. fluorescens P. mendocina, P. putida, Herbaspirillum rubrisubalbicans, P. sacc harophila,* and *B. vesicul*

[h] *Sphingobacterium* spp. include *S. multivorum* and *S. spiritivorum.*

[i] *Xanthomonas* spp. include *X. axonopodis* and *Stenotrophomonas maltophilia.*

[j] Total *Arthrobacter* spp. include *A. mysorens, A. nicotianae, A. oxidans, A. pascens, A. ramosus, A. uratoxydans,* and *A. viscosus.*

[k] Total bacilli include *Paenibacillus alvei, B. cereus, B. circulans, B. coagulans, P. macerans, P. pabuli, B. pumilis, B. sphaericus,* and *B. thuringiensis.*

[l] *Corynebacterium* spp. include *C. aquaticum* and *C. bovis.*

[m] *Micrococcus* spp. include *M. kristinae* and *M. luteus.*

[n] Unknown strains are those with no match to taxa in the FAME library.

[o] Oligotrophic strains are those with no growth on TSA media.

**TABLE 6**

Fungal Diversity in Rhizospheres of Cowpea and Velvetbean after Cycle 1

| | Isolation frequency | | | |
|---|---|---|---|---|
| | Cowpea | | Velvetbean | |
| Taxon | No.[a] | % | No.[a] | % |
| *Aspergillus* spp.[b] | 9 | 25 | 6 | 17 |
| *Cladosporium* spp.[c] | 5 | 14 | 6 | 17 |
| *Fusarium* spp.[d] | 1 | 3 | 2 | 5 |
| *Geotrichum candidum* | 1 | 3 | 1 | 3 |
| *Gliocladium catenulatum* | 1 | 3 | 1 | 3 |
| *Gliocladium roseum* | 2 | 5 | 4 | 11 |
| Total *Gliocladium* spp. | 3 | 8 | 5 | 14 |
| *Humicola fuscoatra* | 1 | 3 | 1 | 3 |
| *Metarrhizium anisopliae* | 3 | 8 | 1 | 3 |
| *Neocosmospora vasinfecta* | 2 | 5 | 0 | 0 |
| *Paecilomyces lilacinus* | 6 | 17 | 8 | 22 |
| *Paecilomyces nivea* | 1 | 3 | 2 | 5 |
| Total *Paecilomyces* spp. | 7 | 19 | 10 | 28 |
| *Penicillium* spp.[e] | 8 | 22 | 10 | 28 |
| *Penicillium herquei* | 2 | 5 | 4 | 11 |
| *Penicillium janthinellum* | 11 | 30 | 5 | 14 |
| *Penicillium lanosum* | 8 | 22 | 5 | 14 |
| Total *Penicillium* spp. | 29 | 80 | 24 | 67 |
| *Phoma* spp.[f] | 4 | 11 | 5 | 14 |
| *Pithomyces graminicola* | 2 | 5 | 0 | 0 |
| *Pseudospiropes ramichloridium* | 6 | 17 | 6 | 17 |
| *Trichoderma harzianum* | 6 | 17 | 6 | 17 |
| *Verticillium chlamydosporum* | 1 | 3 | 1 | 3 |
| *Zygorrhynchus molleri* | 2 | 5 | 8 | 22 |
| Others[g] | 6 | 17 | 3 | 8 |

[a] Values represent the number of plates of 36 sampled which contained the indicated fungus.

[b] *Aspergillus* spp. includes *A. fumigatus* and *A. terreus.*

[c] *Cladosporium* spp. includes *C. macrocarpum* and *C. sphaerospermum.*

[d] *Fusarium* spp. includes *F. oxysporum, F. redolens,* and *F. solani.*

[e] *Penicillium* spp. includes *P. brevi-compactum, P. chrysogenum, P. fellutanum, P. frequentans, P. griseofulvum, P. implicatum, P. islandicum, P. oxalicum, P. restrictum, P. steckii, P. variable,* and *P. velutinum.*

[f] *Phoma* spp. includes *P. eupyrena* and *P. herbarum.*

[g] Others includes *Acremonium strictum, Chloridium virescens, Emericellopsis terricola, Macrophomina* sp., *Mortierella* sp., *Myrothecium verrucaria, Scolecobasidium constrictum, Thielavia terricola,* and *Volutella minima.*

Gram-negative species. Some of these microbial alterations, relating to the use of velvetbean, persisted through the second cycle and were noted in the rhizosphere of winter cover crops, although the choice of cover crop vs fallow and the specific cover crop used affected the microbial shifts observed (Table 4).

The induction by velvetbean of microbial alterations distinct from cowpea and the persistence of these shifts through cropping cycles parallel the reductions in phytoparasitic nematodes reported with the use of velvetbean in the same cropping system used here (Vargas-Ayala, 1995), the 2-year protection reported in a field study with peanut and velvetbean (Rodríguez-Kábana *et al.,* 1992a), and the increase in antagonism of *M. incoginata* eggs associated with amendment of soil with velvetbean (Chavarría-Carvajal and Rodríguez-Kábana, 1998). Although it is not possible to prove a causal relationship between specific microbial shifts and induction of suppressiveness, it could be hypothesized that the microbial shifts induced by velvetbean contribute to the observed antagonism and that velvetbean induced suppressiveness to nematodes. Supporting this hypothesis is the work of Chavarría-Carvajal and Rodríguez-Kábana (1998) in which addition of velvetbean to soil resulted in enhanced parasitism of *M. incognita* eggs by diverse bacteria and fungi. It is generally considered that natural soil suppressiveness to pathogens results from "general suppressiveness" which involves total microbial activity, while "induced suppressiveness" results from activity of a very few specific microorganisms (Cook and Baker, 1983). Classic approaches to inducing suppressiveness to fungal (Hornby, 1983; Cook and Baker, 1983) and nematode (Stirling, 1991) pathogens require multiple years of crop monoculture to develop high populations of one or two specific antagonistic microorganisms, which are considered to be the active biocontrol agents. In contrast, velvetbean used as a rotation crop can induce suppressiveness to nematodes in 1 or 2 years (Vargas-Ayala, 1995; Rodríguez-Kábana *et al.,* 1992a). The results presented here suggest that the induced suppressiveness resulting from velvetbean is not associated with only one or two specific antagonistic microorganisms as defined by Stirling (1991). However, it is also most likely not general suppressiveness as defined by Cook and Baker (1983), since total microbial populations did not increase. In fact, total fungal populations in cycle 1 (Table 2) and total bacterial populations in cycle 2 (Table 1) decreased with velvetbean. Hence, we propose that inclusion of velvetbean into a crop rotation sequence for nematode control may represent a new case of induced suppressiveness in which the antagonism is due to neither total microbial activity nor activity of one or two specific biocontrol agents but is due to selected shifts in microbial communities. It may become possible in the future to test this idea by gaining more direct evidence of a causal role for microbial shifts in antagonism but, currently, methods to do this are limited.

Research aimed at determining if shifts in microbial communities are associated with biological control are complicated by at least two main factors. First, there is no single correct way to measure microbial alterations. The approach used here is based on shifts in frequency of known culturable aerobic-heterotroph microorganisms, which obviously does not account for noncultur-

**TABLE 7**

Fungal Diversity in Soil and Rhizospheres after Cycle 2

| Taxon | Isolation frequency | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C-F[a] | | C-W[a] | | C-Cl[a] | | V-F[a] | | V-W[a] | | V-Cl[a] | |
| | No.[b] | % | No.[b] | % | No.[b] | % | No.[b] | % | No.[b] | % | No.[b] | % |
| *Acremonium strictum* | 2 | 17 | 0 | — | 2 | 17 | 0 | — | 0 | — | 0 | — |
| *Alternaria* spp.[c] | 2 | 17 | 4 | 33 | 0 | — | 1 | 8 | 3 | 25 | 1 | 8 |
| *Aspergillus* spp.[d] | 4 | 33 | 3 | 25 | 2 | 17 | 3 | 25 | 1 | 8 | 1 | 8 |
| *Chrysosporium merdarium* | 0 | — | 1 | 8 | 0 | — | 0 | — | 1 | 8 | 0 | — |
| *Cladosporium sphaero-spermum* | 3 | 25 | 4 | 33 | 4 | 33 | 2 | 17 | 4 | 33 | 2 | 17 |
| *Cunninghamella elegans* | 3 | 25 | 1 | 8 | 0 | — | 2 | 17 | 1 | 8 | 1 | 8 |
| *Epicoccum purpurascens* | 0 | — | 0 | — | 2 | 17 | 0 | — | 3 | 25 | 3 | 25 |
| *Fusarium oxysporum* | 2 | 17 | 3 | 25 | 1 | 8 | 1 | 8 | 1 | 8 | 3 | 25 |
| *Fusarium redolens* | 2 | 17 | 1 | 8 | 0 | — | 0 | — | 3 | 25 | 3 | 25 |
| *Fusarium solani* | 2 | 17 | 1 | 8 | 1 | 8 | 2 | 17 | 0 | — | 1 | 8 |
| Total *Fusarium* spp. | 6 | 50 | 5 | 42 | 2 | 17 | 3 | 25 | 4 | 33 | 7 | 58 |
| *Gliocladium roseum* | 3 | 25 | 3 | 25 | 3 | 25 | 3 | 25 | 3 | 25 | 3 | 25 |
| *Humicola fuscoatra* | 3 | 25 | 1 | 8 | 3 | 25 | 1 | 8 | 2 | 17 | 4 | 33 |
| *Microsphaeropsis ofivacea* | 0 | — | 0 | — | 0 | — | 0 | — | 0 | — | 3 | 25 |
| *Mortierella gamsh* | 1 | 8 | 0 | — | 1 | 8 | 1 | 8 | 1 | 8 | 0 | — |
| *Neocosmospora vasinfecta* | 0 | — | 1 | 8 | 2 | 17 | 0 | — | 0 | — | 1 | 8 |
| *Paecilomyces filacinus* | 2 | 17 | 0 | — | 0 | — | 0 | — | 0 | — | 1 | 8 |
| *Penicillium* spp.[e] | 8 | 67 | 5 | 42 | 5 | 42 | 8 | 67 | 5 | 42 | 6 | 50 |
| *Pestalotiopsis* sp. | 0 | — | 0 | — | 0 | — | 1 | 8 | 0 | — | 1 | 8 |
| *Phoma* spp.[f] | 1 | 8 | 0 | — | 2 | 17 | 0 | — | 0 | — | 2 | 17 |
| *Phoma eupyrena* | 1 | 8 | 3 | 25 | 3 | 25 | 0 | — | 3 | 25 | 3 | 25 |
| *Phoma herbarum* | 1 | 8 | 0 | — | 3 | 25 | 1 | 8 | 0 | — | 3 | 25 |
| *Phoma macrostoma* | 2 | 17 | 2 | 17 | 1 | 8 | 0 | — | 4 | 33 | 1 | 8 |
| Total *Phoma* spp. | 5 | 42 | 5 | 42 | 9 | 75 | 1 | 8 | 7 | 58 | 9 | 75 |
| *Rhizopus oryzae* | 0 | — | 0 | — | 0 | — | 1 | 8 | 0 | — | 1 | 8 |
| *Trichoderma harzianum* | 4 | 33 | 1 | 8 | 2 | 17 | 2 | 17 | 1 | 8 | 1 | 8 |
| *Zygorrhynchus molleri* | 1 | 8 | 1 | 8 | 0 | — | 3 | 25 | 4 | 33 | 0 | — |
| Others[g] | 2 | 17 | 2 | 17 | 1 | 8 | 3 | 25 | 0 | — | 0 | — |

[a] C, cowpea; F, fallow; S, soybean; W, wheat; Cl, crimson clover; and V, velvetbean.
[b] Values represent the number of plates with the fungus of 12 plates tested.
[c] *Alternaria* spp. includes *A. alternata* and *A. tenuissima.*
[d] *Aspergillus* spp. includes *A. jumigatus, A. terreus,* and *A. versicolor.*
[e] *Penicillium* spp. includes *P. janthinellum* and *P. lanosum.*
[f] *Phoma* spp. includes *P. americana, P. pomorum,* and *P. sorghina.*
[g] Others includes *Botrytis cinerea, Chaetomium* sp., *Coniothyrium cerealis, Geotrichum candidum, Pseudospiropes ramichloridium, Ramichloridium* sp., *Scolecobasidium constricturn,* and *Staphylotrichum coccusporum.*

able microorganisms and is widely accepted as underrepresenting actual populations of microorganisms in the environment (Wilson and Lindow, 2000). Second, changes in frequency of taxa may not reflect the physiological functions, due to the ecological concept of guild (Simberloff and Dayan, 1991), which was originally defined as a group of species that exploit the same class of environmental resources in a similar way (Root, 1967). In relation to microbial ecology, a guild may be all microorganisms in a specific habitat which share a physiological function, e.g., all rhizosphere bacteria which antagonize nematode eggs. If the frequency of one taxon within a guild diminishes, populations of different taxa in the same guild may increase, so that the physiological activity of interest may or may not be affected by microbial shifts as measured by diversity of taxa. Each approach to measuring populations and community structure has inherent limits (Mahaffee and Kloepper, 1997), and each can be used to examine community structure from a different perspective.

Ultimately, one would like to have direct evidence that shifts in microbial communities account for or do not account for biological control. However, this may be experimentally impossible, given the complex interactions which occur in mixed microbial communities and given that the physiological states of microorganisms in the environment are very different from those cultured in the laboratory where physiological traits are

**TABLE 8**

Fungal Diversity in Soybean Rhizospheres after Cycle 3

| | Isolation frequency | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C-F-S[a] | | C-W-S[a] | | C-Cl-S[a] | | V-F-S[a] | | V-W-S[a] | | V-Cl-S[a] | |
| Taxon | No.[b] | % | No.[b] | % | No.[b] | % | No.[b] | % | No.[b] | % | No.[b] | % |
| *Acremonium strictum* | 0 | — | 9 | 12 | 1 | 0.9 | 1 | 2.6 | 2 | 1.8 | 1 | 1.1 |
| *Aspergillus* spp.[c] | 0 | — | 1 | 1.3 | 2 | 1.8 | 0 | — | 2 | 1.8 | 2 | 2.3 |
| *Chaetomium* sp. | 2 | 2.6 | 1 | 1.3 | 2 | 1.8 | 0 | — | 0 | — | 1 | 1.1 |
| *Cladosporium sphaerospermum* | 1 | 1.3 | 2 | 2.7 | 2 | 1.8 | 1 | 2.6 | 8 | 7.4 | 3 | 3.4 |
| *Fusarium* spp.[d] | 1 | 1.3 | 1 | 1.3 | 0 | — | 0 | — | 1 | 0.9 | 0 | — |
| *Gliocladium roseum* | 0 | — | 1 | 1.3 | 3 | 2.7 | 2 | 5.3 | 2 | 1.8 | 2 | 2.3 |
| *Metarrhizium anisopliae* | 1 | 1.3 | 3 | 4 | 14 | 12.8 | 0 | — | 0 | — | 9 | 10.2 |
| *Microsphaeropsis olivacea* | 0 | — | 0 | — | 0 | — | 1 | 2.6 | 3 | 2.8 | 1 | 1.1 |
| *Myrothecium roridum* | 4 | 5.2 | 0 | — | 0 | — | 0 | — | 1 | 0.9 | 1 | 1.1 |
| *Neocosmospora vasinfecta* | 4 | 5.2 | 4 | 5.3 | 0 | — | 2 | 5.3 | 4 | 3.7 | 0 | — |
| *Paecilomyces lilacinus* | 44 | 57.1 | 39 | 52 | 66 | 60.5 | 22 | 57.9 | 71 | 65.7 | 49 | 55.7 |
| *Penicillium* spp.[e] | 3 | 3.9 | 1 | 1.3 | 0 | — | 1 | 2.6 | 0 | — | 0 | — |
| *P. janthinellum* | 1 | 1.3 | 1 | 1.3 | 6 | 5.5 | 4 | 10.5 | 2 | 1.8 | 2 | 2.3 |
| *P. lanosum* | 2 | 2.6 | 1 | 1.3 | 0 | — | 0 | — | 1 | 0.9 | 0 | — |
| Total *Penicillium* spp. | 6 | 7.8 | 3 | 4 | 6 | 5.5 | 4 | 10.5 | 3 | 2.8 | 2 | 2.3 |
| *Phoma* spp.[f] | 1 | 1.3 | 4 | 5.3 | 1 | 0.9 | 0 | — | 0 | — | 2 | 2.3 |
| *Scolecobasidium constrictum* | 0 | — | 1 | 1.3 | 0 | — | 1 | 2.6 | 0 | — | 2 | 2.3 |
| *Thielavia terricola* | 1 | 1.3 | 0 | — | 0 | — | 0 | — | 0 | — | 0 | — |
| *Trichoderma harzianum* | 0 | — | 2 | 2.7 | 0 | — | 0 | — | 1 | 0.9 | 0 | — |
| *Verticillium chlamydosporum* | 2 | 2.6 | 3 | 4 | 0 | — | 0 | — | 1 | 0.9 | 3 | 3.4 |
| *Zygorrhynchus molleri* | 1 | 1.3 | 0 | — | 0 | — | 0 | — | 0 | — | 0 | — |
| Unknown[g] | 9 | 11.7 | 1 | 1.3 | 12 | 11 | 3 | 7.9 | 9 | 8.3 | 10 | 11.4 |

[a] C, cowpea; F, fallow; S, soybean; W, wheat; Cl, crimson clover; and V, velvetbean.
[b] Values represent the number of isolates in 12 plates tested.
[c] *Aspergillus* spp. includes *A. jumigatus, A. niger* and *A. versicolor.*
[d] *Fusarium* spp. includes *F. oxysporum, F. redolens,* and *F. sporotrichioides.*
[e] *Penicillium* spp. includes *P. citrinum, P. fellutanum,* and *P. herquei.*
[f] *Phoma* spp. includes *P. americana, P. eupyrena,* and *P. herbarum.*
[g] Unknown are nonsporulating fungi *in vitro.*

studied. Nevertheless, further studies on possible microbial shifts which occur in relation to specific biological control approaches should aid efforts to understand and apply principles of soil suppressiveness. With the velvetbean system studied here, future work should be done to determine when the microbial shifts first occur and if they are specifically due to velvetbean root exudates.

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