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The establishment of microorganisms in vesicular-arbuscular mycorrhizal and control treatments

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Summary. We examined methods for the introduction and establishment of microorganisms in soils treated with inocula commonly used in research with vesicular-arbuscular mycorrhizal (VAM) fungi. Our purpose was to test the assumption that similar microbial groups become established in VAM fungal and control treatments. Microbial activity (CO_2 evolution) and/or the populations of protein-, chitin-, and cellulose-decomposing organisms, fluorescent *Pseudomonas* spp., and total fungi, bacteria, and protozoa were quantified. In closed, nonplanted systems, the establishment of microbial groups in soil, and their populations, was influenced by the type of inoculum used. In open, planted pots, the type of inoculum used affected microbial populations, shoot dry weight, and shoot and root P content, but did not affect root dry weight or shoot and root N content. We conclude that similar functional groups of microorganisms can establish in VAM and control treatments if a VAM-fungal inoculum wash is applied to both treatments. Inoculum prepared from a non-VAM plant does not provide an adequate control treatment.

Key words: Mycorrhiza – Inoculation – Microbial populations

Vesicular-arbuscular mycorrhizal (VAM) fungi cannot grow abundantly in the absence of a host plant. As a consequence, most types of VAM-fungal inocula are obtained from open pot cultures and are contaminated with a diverse assemblage of soil biota. Saprophytic organisms, including those associated with open pot cultures of VAM fungi, can be important in mineralizing nutrients which then become available for plant uptake (Coleman et al. 1983). Murdoch et al. (1967) compared the growth of nontreated controls with plants inoculated with washings of mycorrhizal roots. They found an increase in plant dry mass in the wash-treated plants over controls, and attributed this to the microorganisms associated with the VAM-fungal inoculum. However, in a later study by Manjunath and Bagyaraj (1981), washings of a VAM-fungal pot culture inoculum did not result in growth differences between the wash-treated and control plants. Since microflora associated with VAM-fungal inoculum have the potential to affect plant growth, it is necessary to introduce these organisms into non-VAM treatments to obtain meaningful controls. Only then can growth differences between mycorrhizal and non-mycorrhizal plants be attributed to the VAM fungus.

Most experiments with VAM fungi include a control treatment into which some type of "control inoculum" has been introduced. Methods for establishing VAM plants and appropriate controls have been reviewed by Gerdemann (1964, 1968), Menge and Timmer (1982), Linderman and Hendrix (1982), and Abbot and Robson (1984). In all cases, it has been assumed that VAM and non-VAM inocula would contain equivalent numbers and/or species of microorganisms (except the VAM fungus), and that these organisms would colonize the soil and influence plant growth equally in all treatments. We were not aware of any studies which tested the validity of these assumptions. Therefore, we conducted experiments to address the following questions: (1) Do the same functional groups of organisms become established in soil after inoculation with various types of VAM-fungal and

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control inocula? (2) Does the type of inoculum affect microbial populations? (3) Do the various types of inocula affect plant growth?

Materials and methods

The three experiments described below can be distinguished on the basis of inoculum type, system sterility, and presence or absence of a host plant. Experiment 1 utilized spore inocula of *Glomus macrocarpum* Tul. & Tul. while pot culture material (spores, soil, hyphae, and mycorrhizal root fragments) of the same VAM fungus was used in experiment 2. Both of these experiments examined the introduction and establishment of microorganisms in closed, sterilized, nonplanted soil systems. Experiment 3 used inocula similar to that of experiments 1 and 2; however, an open-pot, planted soil system was used.

Experiment 1. The soil for this experiment was a Balcom series (fine-loamy, mixed calcareous, thermic, Typic Xerorthents) which was screened (1.0 mm) and mixed (1:2, v:v) with a fine washed sand. This mix contained 0.7% organic matter, 2 µg NH_4^+-N g^{-1}, 1 µg NO_3^--N g^{-1}, 4 µg P (NH_4HCO_3-DTPA extracted) g^{-1} and had a pH of 8.2. Air-dry soil equivalent to 20 g (72 h – 60 °C oven dry weight) was placed in each 50 ml Erlenmeyer flask and moistened with 2.0 ml sterile 0.1% Difco Bacto-Peptone. The sterile 0.1% Bacto-Peptone (SBP) solution was used because it is less damaging to microbial membrane integrity than distilled water. Flasks were closed with a foam plug, incubated at 25 °C for 24 h, autoclaved for 30 min, incubated for an additional 24 h, and then inoculated.

For the VAM fungal inocula, 300 spores of *G. macrocarpum* were wet sieved from field-collected Balcom soil and placed in SBP. Spores were swirled in SBP for several minutes to obtain a spore wash. One hundred spores were surface sterilized by placing them in filter-sterilized solutions of 5% chloramine T for 15 min, transferring them to a streptomycin sulfate (250 µg ml^{-1})-gentamicin sulfate (100 µg ml^{-1}) solution for 15 min, then washing them in SBP. The remaining 200 nonsterile spores were used directly (100) or squashed (100) prior to inoculation. For the squashed spore treatment, ten spores for each replication were squashed with sterile forceps. The squashed spores and the 1.0-ml solution they were squashed in were used for inoculation. The inoculum for each flask consisted of, or was suspended in, 1.0 ml SBP and applied to the soil surface. There were seven replications for each of the following treatments: (1) SBP, (2) ten surface-sterilized spores, (3) ten nonsterile spores, (4) ten squashed nonsterile spores, and (5) spore wash. The moisture content of the soil in each flask was brought to 16% with SBP. After each flask was inoculated, it was sealed in a 470-ml airtight jar along with a 20-ml vial containing 2.0 ml 1.0 *N* NaOH for trapping CO_2. The time of each jar closure was recorded and jars were incubated at 25°–28 °C.

Carbon dioxide evolution is a good indicator of microbial activity (Waksman and Starkey 1924), and was determined for each flask on approximately a 24-h basis for 13 days. At each sampling, the vial containing NaOH was removed and closed with an airtight seal. A fresh vial of NaOH was placed in the jar which was then resealed. Carbon dioxide trapped in the NaOH was determined by titration (Coleman et al. 1978).

Flasks were sampled for microorganisms beginning 13 days after inoculation. This date was chosen based on stabilizing rates of CO_2 evolution. One flask from all treatments was sampled each day for 6 days because of the time involved for microbial population determinations. The soil from each flask was mixed and 1.0-g subsamples were removed for dry weight and microbial population determinations. Dilution plates were prepared for each microbial group. Protein decomposers were counted after 48 h on azocoll-Difco Nutrient Agar (4 ml) layered onto water agar plates (Caplan and Fahey 1982). Chitin-decomposing organisms were counted after 7 days on plates with chitin agar (Lingappa and Lockwood 1962) layered onto water agar. Cellulose decomposers were enumerated after 5 days. The medium used for these organisms was modified from that of Eggins and Pugh (1962) by using washed cellulose-azure (type 2) at 4.0 g 100 ml^{-1}. Four milliliters of this medium was layered on water agar plates. Chitin-, cellulose-, and protein-decomposing organisms were detected by the presence of a cleared zone around each colony. Total counts of fungi, bacteria, and fluorescent *Pseudomonas* spp. were made on Difco Potato Dextrose Agar (PDA), Difco Nutrient Agar, and Kings Medium B (King et al. 1954), respectively. Soil protozoan populations were estimated by the most probable number method using the techniques of Darbyshire et al. (1974).

Colony count data were transformed to $\log_{10}$ colony-forming units (CFU) g^{-1} dry weight of soil and analyzed as a randomized complete block design with days as the blocks. When a significant ($\alpha = 0.05$) treatment effect was found, a Student's *t*-test (LSD) was used to compare treatment means at the 0.05 probability level.

Experiment 2. The soil-flask system was identical to that of experiment 1, except that different types of inocula were placed between two 10-g layers of soil rather than applied to the soil surface. Inocula were prepared from a strawberry (*Fragaria vesca* L. Duchesne cv. UC5) pot culture of *G. macrocarpum,* or from a non-VAM strawberry pot culture. These planted pots were maintained for 4 months under the same conditions except that one was inoculated with surface-sterilized VAM fungal spores. A VAM pot culture inoculum wash was prepared by mixing 25 g pot inoculum in 100 ml SBP and passing this twice through a sterile 45-µm-mesh screen. There were five replications for each of the following treatments: (1) 3.5 g (fresh weight) VAM pot culture inoculum, (2) 3.5 g VAM pot inoculum plus 1.0 ml pot inoculum wash, (3) 3.5 g autoclaved VAM pot inoculum, (4) 3.5 g autoclaved pot inoculum plus 1.0 ml pot inoculum wash, (5) 3.5 g non-VAM pot inoculum, (6) 1.0 ml VAM pot inoculum wash, and (7) 1.0 ml SBP. Soil moisture content, incubation conditions, microbial activity (CO_2 evolution), microbial data collection, and statistical analyses were the same as for experiment 1.

Experiment 3. The Balcom soil for experiment 3 was screened through a 2-mm mesh and mixed with sand (1 soil:2 sand, v:v) before autoclaving. The soil was stored in a closed can for 1 week prior to use. This soil mix contained 0.2% organic matter, 1 µg NH_4-N g^{-1}, 1 µg NO_3-N g^{-1}, 4 µg P (NH_4HCO_3-DTPA extracted) g^{-1}, and had a pH of 8.2. Forty-nine plastic pots (10 cm × 10 cm × 9.3 cm) each received 1.05 kg (72 h – 60 °C dry weight basis) of soil. A hole 2 cm wide by 5 cm deep was made in the soil in the center of each pot to accomodate the inoculum and plant seedling.

Inoculum from the VAM strawberry pot culture was wet sieved to collect spores of *G. macrocarpum.* One thousand spores which appeared to be viable and unparasitized were placed in 100 ml SBP and mixed, and the spore wash was passed through a 45-µm mesh twice and retained. Five hundred spores were stored in SBP while the remaining 500 were surface sterilized as described previously. A VAM-fungal pot inoculum wash was prepared by placing 100 g of this material in a sterile flask containing 500 ml SBP, and mixed. A microbial suspension, minus infective propagules of the VAM fungus, was obtained by passing the pot inoculum wash twice through a 45-µm-mesh screen. There were seven treatments, each with five replications as follows: (1) 50 surface-sterilized spores, (2) 50 nonsterile spores, (3) 10 ml spore wash, (4) 10 g VAM pot culture inoculum, (5) 50 ml VAM pot inoculum wash, (6) 10 g non-VAM pot culture inoculum, and (7) noninoculated control. All pots received a total of 50 ml SBP which took into account the amount applied with the various inocula.

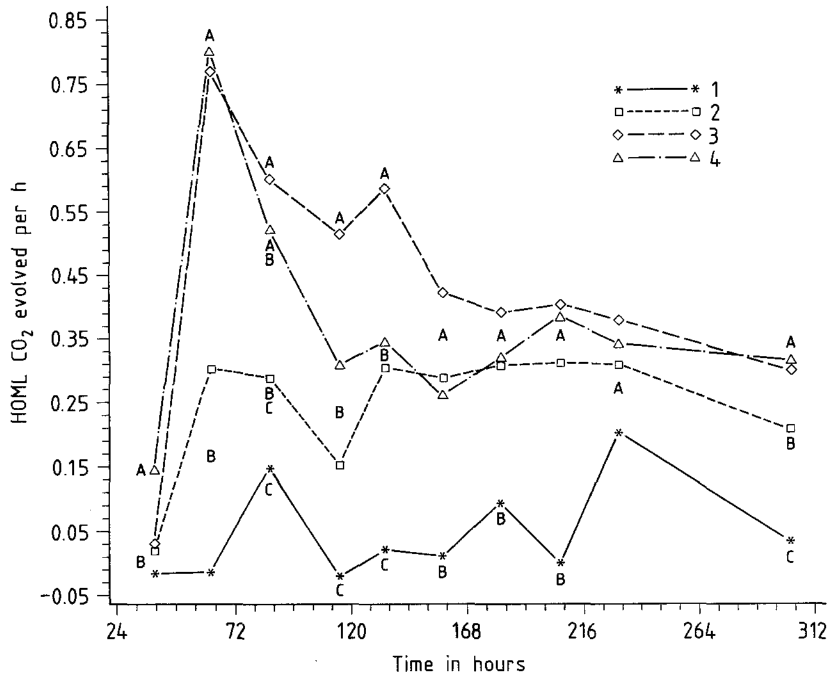

Fig. 1. Rates of CO_2 evolution (μmol CO_2 h^{-1} $flask^{-1}$) from soils inoculated with surface-sterilized spores of *Glomus macrocarpum* (*1*), nonsterile spores (*2*), squashed spores (*3*), or a wash from the nonsterile spores (*4*). Treatments not sharing the same letter (closest location within sample date) are significantly different ($P \leq 0.05$)

Table 1. Colony-forming units (CFU) of microorganisms cultured from soil 13 days after inoculation with *G. macrocarpum* spores or spore washings

Treatment	Substrate utilization			Fluorescent *Pseudomonas* spp.	Total		
	Protein	Cellulose	Chitin		Fungi	Bacteria	Protozoa
Noninoculated control	–	–	–	–	0	0	0
Surface-sterilized spores	–	–	–	–	0	0	0
Nonsterile spores	60 (4)[a]	0.004 (2)	6 (5)	140 (2)	0	TNTC (6)	0
Squashed spores	36 (6)	0	45 (4)	145 (2)	0	TNTC (6)	0
Spore wash	164 (6)	0	34 (5)	130 (6)	0	TNTC (6)	0

[a] All values are CFU $\times 10^6$ g^{-1} soil. Numbers in parentheses indicate number of replications out of six which contained that particular microbial group. Analysis of variance was invalid because of large variation and missing (lack of growth) values for some of the microbial groups. TNTC indicates colonies were too numerous to count at dilution factors used for this experiment

One-week-old barley (*Hordeum vulgare* L.) seedlings were transplanted (one per pot) into the center of each pot, and placed in a growth chamber in a completely randomized design. Growth conditions were set for a 14-h photoperiod (700 μmol $m^{-2} s^{-1}$, 27 °C) day, and a 10-h (18 °C) night. The relative humidity varied from 50% to 80%. Each morning for 60 days, the soil water content in each pot was brought to 11% based on weight determinations. One-half strength Ruakura nutrient solution (Smith et al. 1983) was modified to provide 4 μg P ml^{-1} and Fe as Na-EDDHA instead of ferric citrate. One hundred mililiters of nutrient solution was applied to all treatments weekly for the 1st month and twice weekly thereafter. After 60 days, one replication from each treatment was harvested each day for 5 days. Four soil-root cores (2×8 cm) were removed, one from each quadrant of the pot, with a cork bore. The central 2 cm was cut from each 8-cm-long core with an alcohol-flamed knife, and all four subsamples were placed in a sterile jar containing 200 ml SBP. The sealed jars were shaken 200 times by hand before removing samples for dilution plating. Roots were removed from each jar, washed, and the percentage arbuscular colonization was estimated microscopically at X60 by autofluorescence (Ames et al. 1982) based on 1000 intersections in a gridded (0.635-cm^2 squares) petri plate. Shoots and roots, including roots from cores, were dried (60 °C, 72 h), and their dry weight was determined and ground for N and P analysis. Plant N was determined by dry combustion on an ERBA model 1400 analyzer and P after the method of Allen (1940).

Microbial population data and statistical analyses were as described previously except that micorbial data were expressed as CFU per four cores.

Results

Experiment 1

Based on CO_2 evolution rates, microbial activity in the squashed-spore and spore-wash control inoculum treatments was greater at the peak and final sampling periods than that of the live spore treatments (Fig. 1).

No microorganisms were detected from soils which were noninoculated or inoculated with surface-sterilized spores (Table 1). Protozoa and fungi were not detected from any treatment. Not all of the remaining

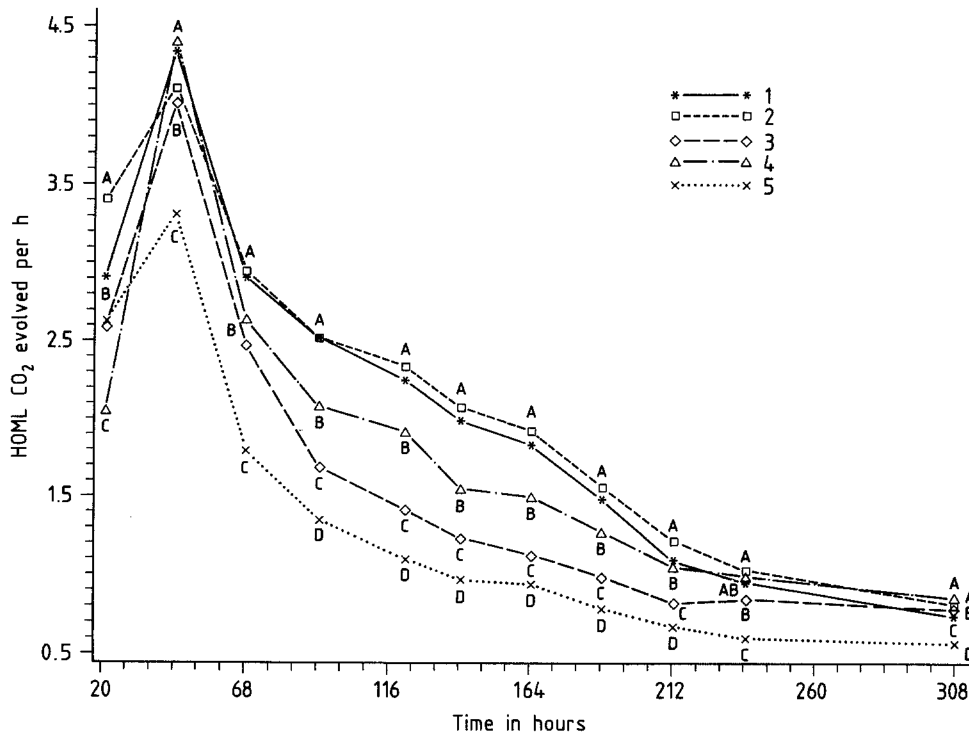

Fig. 2. Rates of CO_2 evolution (μmol CO_2 h^{-1} $flask^{-1}$) from soils inoculated with *Glomus macrocarpum* (VAM) pot culture inoculum (*1*), VAM pot inoculum plus VAM pot inoculum wash (*2*), autoclaved VAM pot inoculum plus VAM pot inoculum wash (*3*), pot inoculum from a non-VAM plant (*4*), or VAM pot inoculum wash (*5*). Treatments not sharing the same letter (closest location within sample date) are significantly different ($P \leq 0.05$)

Table 2. Colony-forming units (CFU) of microorganisms cultured from soil 13 days after inoculation with *G. macrocarpum* pot inoculum or control innocula

Treatment	CFU g^{-1} soil						
	Substrate utilization			Fluorescent *Pseudomonas* spp.	Total		
	Protein $\times 10^5$	Cellulose $\times 10^2$	Chitin $\times 10^4$	$\times 10^5$	Fungi $\times 10^2$	Bacteria $\times 10^6$	Protozoa $\times 10^2$
Noninoculated control	–	–	–	–	0	0	0
Sterile VAM pot inoculum	–	–	–	–	0	0	0
Sterile VAM pot inoculum plus inoculum wash	478 a[a]	77 ab	317 a	686 a	202 a	494 a	90 ab
VAM pot inoculum plus inoculum wash	262 a	128 a	313 a	628 a	219 a	358 a	78 bc
VAM pot inoculum	459 a	122 a	340 a	671 a	158 ab	697 a	102 a
VAM pot inoculum wash	445 a	11 c	344 a	877 a	111 b	672 a	103 a
Non-VAM pot inoculum	259 a	48 b	230 a	251 b	152 ab	366 a	67 c

[a] All values are a mean of five replications. Within columns, means followed by the same letter are not significantly different ($P > 0.05$)

functional groups were represented in every flask. In some treatments, only two of six replications were positive for a particular microbial group. A majority of the chitin-decomposing organisms in the spore-inoculated treatments were actinomycetes while bacterial chitin-decomposers were more common in the spore-wash treated soils. The various microbial groups were more likely to be introduced into the soil with the spore wash than with the other types of inocula. This is shown in Table 1 by the greater number of replications containing each microbial group.

Experiment 2

Carbon dioxide evolution rates were significantly higher from the live VAM pot inoculum treatments than from any of the control inoculum treatments at 7 of the 11 sampling periods (Fig. 2). The controls also differed significantly from each other at six sample dates in the following order: non-VAM pot inoculum > autoclaved VAM pot inoculum plus wash > inoculum wash. The addition of the pot inoculum wash to the live VAM pot inoculum did not signifi-

Table 3. Barley tissue dry weight and N and P content (mean of five replications) 60 days after inoculation with various types of VAM-fungal (*Glomus macrocarpum*) or control inocula

Treatment	Tissue dry weight (g)			Nutrient content (mg)			
	Roots from cores	Total		Total N		Total P	
		Root	Shoot	Root	Shoot	Root	Shoot
Noninoculated control	0.145 a[a]	8.18 a	5.77 b	54.88 a	62.16 a	5.56 a	3.57 c
Surface-sterilized spores	0.146 a	7.80 a	6.31 ab	50.74 a	62.40 a	4.84 b	3.90 abc
Nonsterile spores	0.155 a	7.98 a	6.91 a	50.51 a	66.92 a	4.79 bc	4.27 a
Spore wash	0.159 a	7.97 a	7.23 a	48.13 a	69.97 a	4.44 bc	4.17 ab
VAM pot inoculum	0.147 a	7.69 a	6.48 ab	48.48 a	62.31 a	4.45 bc	3.89 abc
VAM pot inoculum wash	0.140 a	7.74 a	5.90 b	49.81 a	61.31 a	4.31 c	3.65 bc
Non-VAM pot inoculum	0.141 a	7.50 a	6.38 ab	49.38 a	61.20 a	4.33 bc	3.42 c

[a] Within columns, means with the same letter are not significantly different ($P>0.05$)

Table 4. Colony-forming units (CFU) of microorganisms and percentage mycorrhizal colonization of roots (mean of five replications) detected from soil cores taken from potted barley plants 60 days after inoculation with various types of VAM-fungal (*Glomus macrocarpum*) or control inocula

Treatment	Total CFU from four cores per pot							VAM (%)
	Substrate utilization			Fluorescent *Pseudomonas* spp.	Total			
	Protein $\times 10^7$	Cellulose $\times 10^3$	Chitin $\times 10^6$	$\times 10^6$	Fungi $\times 10^4$	Bacteria $\times 10^8$	Protozoa $\times 10^4$	
Noninoculated control	13.16 a[a]	0.69 b	7.39 c	51.49 a	8.55 a	16.55 c	6.10 bc	0
Surface-sterilized spores	14.79 a	1.45 b	1.84 d	39.21 ab	2.08 a	31.64 a	5.25 c	0
Nonsterile spores	14.70 a	3.20 b	5.85 cd	45.44 a	7.17 a	25.21 abc	6.88 bc	0.7
Spore wash	17.24 a	1.64 b	13.61 bc	35.96 ab	8.95 a	28.54 ab	10.41 abc	0
VAM pot inoculum	1.53 c	69.47 a	17.06 bc	12.52 c	6.72 a	28.73 ab	10.13 abc	9.3
VAM pot inoculum wash	6.24 b	81.43 a	39.67 ab	38.86 ab	7.93 a	18.99 bc	13.07 ab	0
Non-VAM pot inoculum	1.94 c	92.30 a	80.02 a	19.24 bc	11.37 a	6.81 d	18.65 a	0

[a] Within columns, means with the same letter are not significantly different ($P>0.05$)

cantly alter microbial activity from that of the live VAM pot inoculum alone.

Microorganisms were not detected in soil samples from the noninoculated or autoclaved inoculum treatments (Table 2). The population of cellulose decomposers was significantly less in the wash-inoculated treatment than treatments which received pot culture inoculum. Otherwise, the microbial populations were very similar for the VAM pot inoculum and VAM pot inoculum wash treatments. The use of pot inoculum from a non-VAM plant produced significantly lower populations in three of seven microbial groups compared with the VAM pot inoculum treatment.

Experiment 3

There were no significant differences between treatments in total root dry weight or the dry weight of roots extracted from soil cores (Table 3). There were no significant effects of treatment on the total N of shoots and roots: however, shoot dry weight and the total P of shoots and roots was affected.

No mycorrhiza formation was detected by autofluorescence in the surface-sterilized spore treatment and low levels were found in roots inoculated with nonsterile spores or VAM pot inoculum (Table 4). Mycorrhizae were not detected from the other treatments.

Microbial populations in soils inoculated with nonsterile spores did not differ from treatments which received surface-sterilized spores, a spore wash, or which were noninoculated (Table 4). The spore wash treatment was significantly higher in chitin decomposers than the surface-sterilized spore treatment. Chitin-decomposing organisms were more numerous and the total bacterial population was significantly lower in the noninoculated controls when compared with the surface-sterilized spore treatment.

Soils inoculated with VAM-fungal pot culture material were significantly lower in protein decomposers

and fluorescent *Pseudomonas* spp. than those treated with the VAM pot inoculum wash. The non-VAM pot inoculum treatment had a lower total bacterial population but higher chitin-decomposing population than the VAM pot inoculum treatment. Inoculum derived from pot cultures (VAM or non-VAM) caused a reduction in protein decomposers and fluorescent *Pseudomonas* spp. and an increase in cellulose- and chitin-decomposing organisms when compared with most of the other treatments.

Discussion

There are three assumptions behind the selection of appropriate controls for VAM plants: (1) The same species of microorganisms (except the VAM fungus) will be introduced into all treatments. (2) Microorganisms will have a equal chance to establish in all inoculated soils. (3) The VAM fungus and not the method of inoculation will affect plant growth and soil microbial populations. These assumptions should be valid for any plant-soil-VAM fungus combination in any given experiment. Below we discuss why we selected certain microbial groups for study and how the results of our experiments relate to the assumptions stated above.

We quantified protozoa, and protein, chitin, and cellulose decomposers because of their functioning in the mineralization of nutrients such as nitrogen (N) and phosphorus (P) from organic substrates. These elements are usually chemically immobilized in the soil matrix or tied up in microbial biomass. Low levels of N and P remain free in the soil solution and available to the plant. Therefore, the activities of the soil biota are important in cycling these elements to the soil solution and to the plant. VAM fungi can obtain P and N mineralized from inorganic or organic substrates, and transport them to the host plant (Azcón et al. 1976; Ames et al. 1983). In addition to nutrient cycling, soil microorganisms are involved in hormone production, plant pathogenesis, detoxification, or other functions which influence plant growth. For these reasons, it is essential to minimize or eliminate differences in microbial species which are introduced into VAM and control treatments.

In experiment 1 the significant differences in microbial activity demonstrate that the inocula were not equivalent in their nutritional or microbial composition. This is further supported by the fact that some functional groups of organsims were not introduced equally into all flasks. This experiment shows that when inoculating with low numbers of spores (ten in this experiment), there is a high probability that the same species of microorganisms will not be introduced or become established equally in all replications, thus not supporting assumptions one and two as stated above. Since the spore wash was much richer in its microbial composition than the squashed or nonsterile spore inocula, we recommend that a spore wash be applied to both VAM spore-inoculated and control treatments. We realize that frequently more than ten spores are used to obtain VAM plants but since spores are not all colonized by the same microflora it is necessary to apply a microbial suspension to all treatments.

VAM-fungal washings, from spores or pot culture inocula, can be prepared in a variety of ways. Although Linderman and Hendrix (1982) recommended passing washings through a 20-μm sieve, we feel that a larger mesh opening is more desirable. The advantage of using the largest possible mesh is to increase the diversity of microbes which can pass through and be introduced into controls. A 20-μm or smaller size opening can eliminate many nonplant parasitic nematodes and protozoa which occur in the VAM-fungal inoculum. These organisms can be very important in making nutrients available for plant growth or VAM-fungal uptake (Coleman et al. 1983). When inoculating with small-spored VAM species, one must weigh the risk of controls becoming mycorrhizal against the sieve size used for the inoculum wash. We have regularly used a 45-μm-mesh screen for the wash without obtaining mycorrhizal control plants.

As expected, pot culture inoculum or a pot culture inoculum wash contained representatives from all the microbial groups we examined. Thus in experiment 2 our first assumption that the same species of organisms would be introduced into all treatments is supported. We must caution here that we evaluated the populations of organisms for their ability to decompose a given substrate. This does not necessarily mean that all the chitin decomposers for example are of the same genus and species. In fact, the microbial activity (CO_2 evolution rates) in experiment 2 indicated that differences existed in the microorganisms present or the nutrient content of the inocula. A large difference was noted for the cellulose-decomposer population of the wash treatment, which was probably influenced by a low organic matter content.

Based on our microbial count data from experiment 2, we recommend against using pot culture inoculum from a non-VAM plant to treat control plants. Further support for this comes from studies by Barea et al. (1975), Azcón et al. (1976), Bagyaraj and Menge (1978), Hayman (1978), Krishna et al. (1982), Ames et al. (1984), and Meyer and Linderman (1986), all stating that rhizosphere microbial populations from VAM and non-VAM plants differ. Therefore if one uses inoculum from a non-VAM plant for the control

treatment, assumptions one and two will not be met for establishing and appropriate control.

The results from experiment 3 demonstrated that the method of inoculation can influence some microbial populations, shoot dry weight, and the P content of roots and shoots. The purpose of all our experiments was to test assumptions behind the establishment of non-VAM control treatments; therefore, not having a VAM-fungal growth response in experiment 3 does not detract from the results. The percentage VAM colonization of the roots was low by clearing and staining standards, but one must realize that the autofluorescence method assesses only the active arbuscules at the time of harvest. The total infected root length was undoubtedly larger.

With increasing interest in the role of VAM fungi in plant ecology, previously accepted theories on how these fungi function are being tested in greater detail. Unfortunately, the large number of plant-soil-VAM fungus combinations make comparisons between studies very difficult. The establishment of an appropriate, non-VAM control for each study is becoming more critical; yet the description of control treatments in publications is often inadequate and impossible to dublicate. Our studies do not demonstrate that all the assumptions behind establishing a appropriate non-VAM control can be met. However, we do present data which favor the use of a microbial suspension for minimizing non-VAM fungal induced differences between VAM and control treatments.

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