AN ABSTRACT OF THE THESIS OF

Christine R. Fischer for the degree of Master of Science in Forest Science presented on February 3, 1992.

Title: Inoculum Potential of Vesicular-Arbuscular Mycorrhizal Fungi in Two Costa Rican Soils with Different Vegetation Covers

Abstract approved: Signature redacted for privacy.

David A Perry

Mycorrhizae are important for plant growth, particularly in nutritionally poor soils. Vesicular-arbuscular mycorrhizae (VAM) are the primary form of mycorrhizae found in tropical plants, and their persistence in the soil as colonizing fungal propagules following deforestation cannot be directly measured. Utilizing the "most probable number" (MPN) method for estimating infective propagules, values of mycorrhizal inoculum potential (MIP) were obtained for two Costa Rican soils with different vegetation histories following primary forest removal in 1950's-60's. The soils (Oxic Dystropepts) were collected at La Selva Biological Research Station from sites covered by secondary forest, pasture, and land bare of vegetation for four and for six years.

We hypothesized that MIP would be greater in the pasture and forest soils than in the soils bare of vegetation. Two VA-mycotrophic plants, *Psidium guajava* L. and *Allium cepa* L. were used as bioassays in greenhouse studies to obtain MPN values. Both bioassay studies gave estimates of 0.6 propagules/gr. dry soil from the pasture soil. For the other three soils both bioassays gave significantly lower estimates ($p<0.00001$) in

the range of 0.002-0.104 propagules/gr. dry soil. Growth responses for plants grown in the pasture soil were similarly greater than those plants grown in the other three soils.

The MPN values were correlated with spore counts made from the same soils using the wet-sieving and decanting technique. Correlations between spore counts and MPN values were not significant.

Inoculum Potential of Vesicular-Arbuscular Mycorrhizal Fungi in Two Costa Rican Soils with Different Vegetation Covers

by

Christine R. Fischer

A THESIS

submitted to

Oregon State University

in partial fulfillment of

the requirements for the

degree of

Master of Science

Completed February 3, 1992

Commencement June 1992

APPROVED:

Redacted for privacy

Professor of Forest Science in charge of major

Redacted for privacy

Head of the Department of Forest Science

Redacted for privacy

Dean of Graduate School

Date thesis is presented February 3, 1992

Typed by Researcher for Christine R. Fischer

ACKNOWLEDGEMENTS

I would like to acknowledge my committee members David Perry, Phillip Sollins, and Robert Linderman for the individual expertise and scientific perspective that each brought to this project and to my education. I would also like to acknowledge David Janos from the University of Miami for his assistance in Costa Rica and his continued interest and support for my work.

I am grateful for the educational encouragement from Logan Norris, and for the guidance and patience at the microscope from David Ianson, Nola Mosier and Efrén Cázares. And I am grateful for the opportunity and permission from the Organization for Tropical Studies to work at La Selva Biologic Research Station .

This work would have been impossible without the good humor, constructive criticism, assistance and camaraderie of many friends and graduate students. A special thanks to Carlos Colinas for his dedication to my education and to my father, Michael J. Fischer, for his inspiration and love of forest and fungi.

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INTRODUCTION

The discovery of mycorrhizal fungi (Frank 1985) has been followed by a century of work which has included identification and description of three major types--ectomycorrhiza, ericoid mycorrhiza, and vesicular-arbuscular mycorrhiza (VAM). This work has encompassed taxonomy, fungal and plant morphology and physiology, and the nature of fungal-host relationships and their ecological significance. Studies of the relationship between symbiotic mycorrhizal fungi and vascular land plants have demonstrated that the primary benefit to the host plant is the acquisition of water and mineral nutrients, particularly the relatively immobile element phosphorus (Cox et al. 1975; Nelson and Safir 1982). Other benefits to the host include increased root longevity, increased photosynthetic rate, and protection against root pathogens (Linderman 1988). Present concerns have focused on the significance of mycorrhizae in the stabilization of plant and forest communities and ecosystems in the face of increasing human disturbances (Pankow et al. 1991).

An important example of human disturbance is tropical deforestation. The Food and Agricultural Organization of the United Nations estimates the annual rate of tropical deforestation at 75,000 km^2 (WRI 1986). This is a result of commercial logging, ranching, shifting agriculture and fuel needs from rapidly expanding populations (Lanley 1985). Conversion of forest to pasture has resulted in serious reforestation obstacles in some tropical soils (Nepstad et al. 1991). Efforts at reforestation have shown that inoculation of forest trees with mycorrhizal fungi can stimulate tree growth in nutritionally poor soils (Bowen 1980). Much of this work has involved inoculation with

ectomycorrhizal fungi (Marx 1980), and the application of VAM inoculum is still in developmental stages (Wood 1982, Janos 1988).

The majority of tropical trees are either facultatively or obligately mycorrhizal, and of these the majority form VA mycorrhizae (Janos 1980). Facultative host plants benefit from the absorptive capacity of their fungal symbiont in nutritionally poor soils, but may thrive without the fungus in fertile soils. Obligately mycotrophic species are those which cannot survive to reproductive maturity if they are nonmycorrhizal at the soil fertility levels found in their natural habitats (Janos 1980). Deforestation in the tropics has resulted in the loss of mature forests to early-successional, nonmycorrhizal weed species (Janos 1980). This may reduce or alter mycorrhizal fungus populations due to the loss of suitable hosts. Loss of mycorrhizal fungal inoculum will subsequently influence plant succession, particularly where soil fertility is low enough to limit to establishment, growth and reproduction.

In tropical Oxisols and Ultisols available phosphorus is present in very low concentrations due to phosphorus fixation, or the absorbtion of phosphates by the aluminum and iron oxides present in highly weathered soils (Sanchez 1976; Sollins et al. 1988). Primary production in tropical agroecosystems as well as in some mature forests is controlled largely by phosphorus availability (Fox 1980 in Sollins et al 1988, Vitousek 1984). VAM fungal hyphae enhance phosphorus uptake through extensive soil exploration (Alexander 1989, review), and VAM-forming root mats serve as nutrient traps in the upper soil-litter layer of tropical forests (Medina and Cuevas 1989). Disturbances to tropical forest communities which damage the forest floor and VAM-forming root mats interfere with nutrient cycling and forest recovery (Whitmore 1989).

Beyond the important role of nutrient sequestering is the role of mycorrhizal fungi in below-ground processes which ultimately influences resiliency in above-ground plant communities. VAM colonization alters carbon allocation of root exudates to the rhizosphere through changes in root membrane permeability (Linderman 1988). This process, in turn, supports selective groups of beneficial bacteria including nitrifiers (Meyer and Linderman 1986; Ingham and Molina 1991, review). Fungal hyphae promote soil aggregation (Thomas et al. 1986) and provide an essential food source for many soil organisms including arthropods which in turn provide aeration and promote soil aggregation (Rabatin 1990). The complex mycorrhizosphere community which results from this altered environment provides positive feedback to the host plant (Perry et al. 1989). The implications of deforestation in destabilization of the rhizosphere and loss of this positive feedback loop are unknown. Because VAM play a pivotal role in this feedback loop, studies are needed to examine their persistence following forest disturbance.

OBJECTIVES

The purpose of this study was to compare the inoculum potential of VA mycorrhizal fungi from four deforested sites in two moist, lowland Costa Rican soils. For this study mycorrhizal inoculum potential (MIP) describes an integrative measure of the capacity of VAM fungi within the soil to colonize a host plant, given the soil fertility, seedling physiology, rhizosphere organisms and other soil organisms present.

Two approaches were used to evaluate and compare MIP of these soils. The first was the use of two VA-mycotrophic plants as bioassays in a dilution end-point study. The second was a correlation between spore counts and the estimates of VAM propagules from the dilution end-point study.

The following hypotheses were tested:

1.) VAM inoculum potential of the forest and pasture soils will be greater than those found in the soils bare of vegetation.

2.) Spore numbers present in the soils will be a poor indicator of inoculum potential, as observed in previous studies (Johnson et al., 1991; An et al., 1990).

MATERIALS AND METHODS

Soils

Soils were collected from a research site established in 1984 in the La Guaria Annex of the La Selva Biological Research Station (10° 26'N, 83° 59'W) (Figure 1) in northeastern Costa Rica (Sollins and Radulovich 1988). Developed in alluvially deposited volcanic materials, the soils are classified as Oxic Dystropepts, and are part of the Helechal and Matabuey consociations (Sollins et al.,in press)(Figure 2). Annual precipitation at the site averages 4000 mm, and mean annual temperature is 24° C (La Selva Meteorlogic Station, 1957-83 as cited in Sollins and Radulovich 1988.). The soils are well aggregated, with bulk densities near 0.7 Mg /m^3 at 0-10 cm depth (Radulovich et al. 1989).

Soils from four treatment sites were collected in early July 1990. All four sites (Figure 2) were part of the upper Sarapiqui river terrace, cleared of primary forest during the 1950's and 1960's (Pierce 1992; USAF aerial photo). Three sites were mostly cleared of forest by 1960, grazed intermittently until about 1981, and then left as abandoned pasture (Sollins and Radulovich 1988). Two of these sites had been cleared of vegetation, then hand-weeded and kept bare as part of a larger study of nutrient availability (Sollins et al. 1988). The first of these two plots had been kept bare of vegetation since 1984, or six years at the time of this collection and will be referred to here as treatment BA6. The second plot had been kept bare of vegetation since 1986, or four years at the time of this collection and will be referred to here as treatment BA4. The third plot had remained unaltered and allowed to proceed through secondary succession and will be referred to as treatment PAS. It was dominated by the grass *Olyria latifolia* L. and ferns *Pteridium* spp. (Sollins and Radulovich 1988). Also present on

this site at the time of soil collection were *Conostegia subcrustulata* (Beurl.) Tr., *Plukenetia volubilis* L., *Pannicum maximum* Jacq., and *Passiflora vitifolia* HBK. The litter and surface root mat were removed before soil was collected from the PAS site.

The fourth collection site was a 10-yr. old, secondary mixed forest, also cleared of primary forest in the 1960's, acquired by La Selva in 1981 (Pierce1992). At the time of this soil collection it was dominated by *Rollinia microsepala* Standley, *Pentaclethra macroloba* (Willd.) Kuntze, and *Vismia* sp. This soil will be referred to as treatment FOR. The litter layer was removed before collection was made from this site.

Samples of 1600 cm^3 were collected from six randomly chosen spots (A,B,C,D,E and F) on each of the four treatment sites. Samples were taken from the 0-5 cm layer, and 1200 cm^3 were refrigerated and transported within one week of collection to the USDA-ARS Horticultural Crops Research Laboratory in Corvallis, OR. The remaining 400 cm^3 from each of the six replicate samples and four additional 400 cm^3 from each site were sieved at the research site for VAM fungal spore counts. Soil pH(water) and extractable phosphorus (dilute acid flouride method) of the samples are shown in Table 1.

Figure 1. La Selva Biological Research Station operated by the Organization for Tropical Studies, in Northeastern Costa Rica.

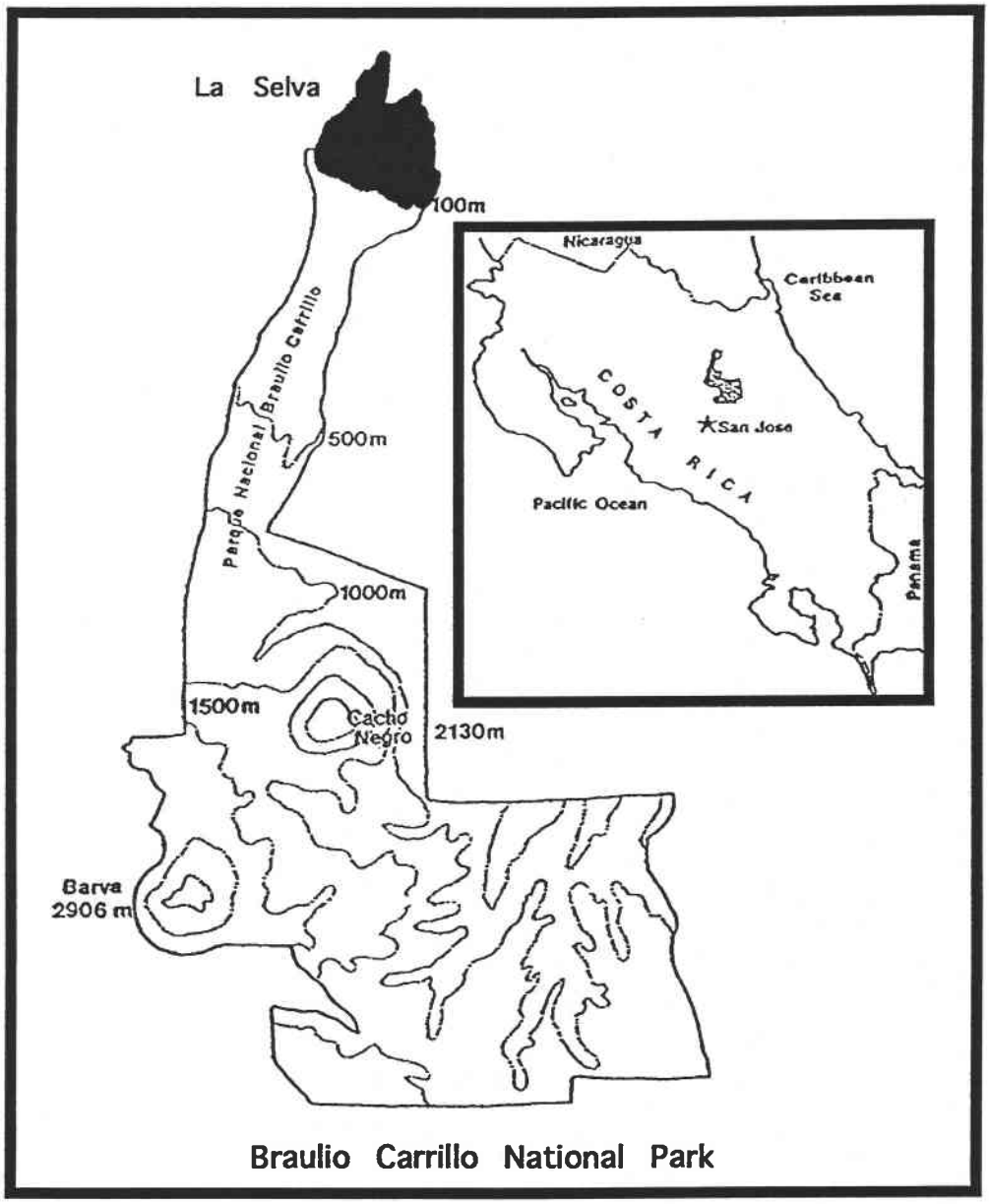


Figure 2. La Guaria Annex of La Selva Biological Research Station: Soil consociations and sampling sites.

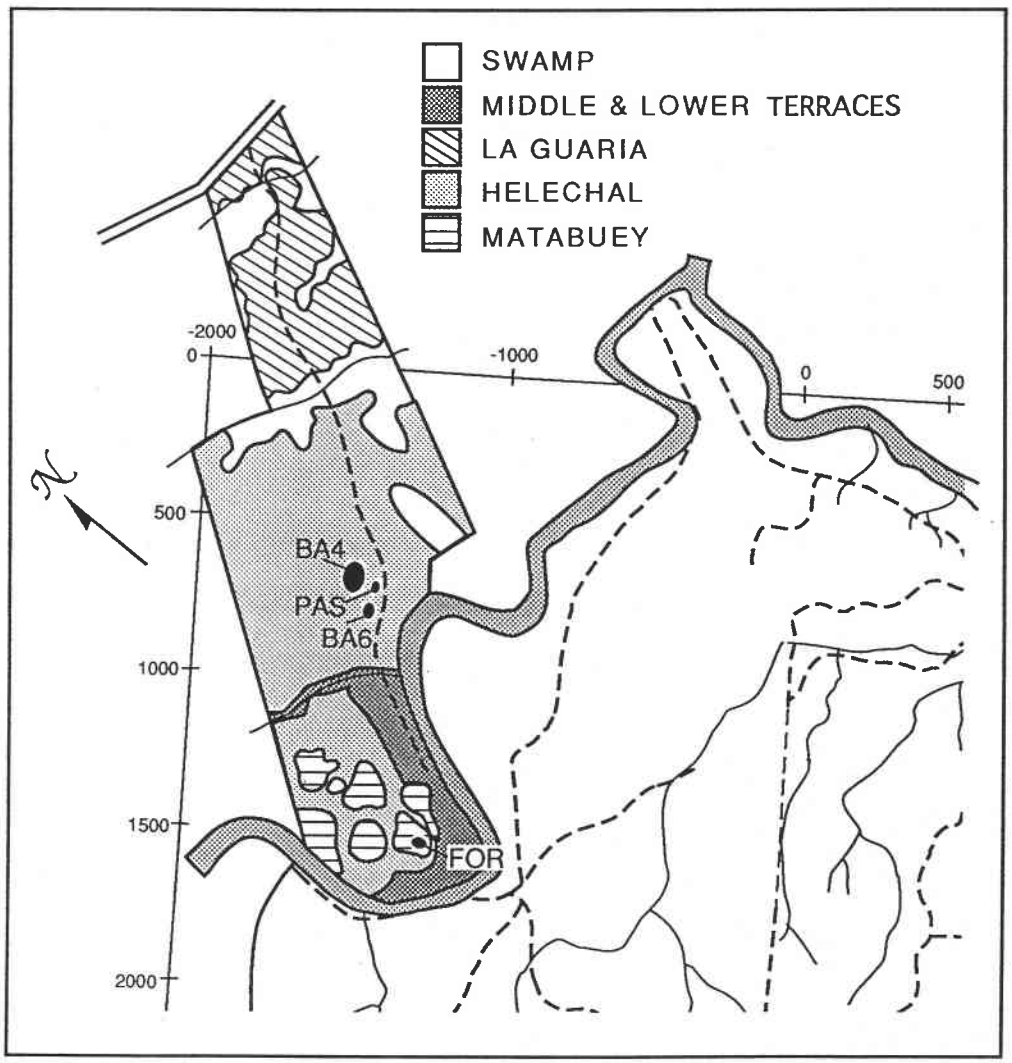


Table 1. Soil pH and extractable phosphorus of soil from the four treatment sites.

	Soil pH_w	Extractable $P_{(dilute\ acid)}$(ppm in soil soln.)
BA6	4.2	0.4
BA4	4.1	1.7
PAS	4.5	1.1
FOR	4.4	0.6

Experimental Procedures

The Most Probable Number method (Porter 1979; An et al. 1990) was used to compare the mycorrhizal inoculum potential (MIP) of these soils. The most probable number (MPN) method, also known as the dilution end-point method, has been developed by microbiologists to estimate population sizes of microorganisms by observing the highest dilution at which detection or growth of a microorganism occurs (Alexander 1982). In using this technique, it is essential that presence of the organism can be detected. Because there is no method for growing VAM fungi in pure culture, a bioassay method is required. The presence or absence of VAM in the root systems of two bioassay plants was used to calculate a most probable number of VAM fungal propagules in the four soil treatments. *Psidium guajava* L. (guava) and *Allium cepa* L. (onion) seedlings were used as bioassay plants.

Guava was found to be obligately mycotrophic in studies done at La Selva (Janos 1975). In July 1990, ripe guava fruits were collected from beneath trees growing in the understory openings in mixed secondary forests along the trail known as Lindero Occidental within La Selva Biological Station. The seeds were removed from the fruits, washed and air-dried. Seeds were kept at room temperature for four weeks, then surface sterilized with H_2O_2, immersed in water, and refrigerated for two weeks. The seeds were planted 2 mm deep in a sterile sand and peat mixture in a greenhouse flat. Six weeks later the seedlings were transplanted into the test soils.

Onion has been widely used as a VAM-responsive plant (Mosse 1973; Snellgrove et al. 1982). Seeds of variety Hardy White Bunching were obtained from Nichols Garden Nursery of Albany, OR, and planted directly into the test soils.

Each soil treatment consisted of six subsamples: A,B,C,D,E and F, corresponding to the field site collections. A two-fold dilution series with 3 replications was used. Fifty cm^3 of soil were serially diluted with 25 cm^3 sterile sand for seven dilutions, so that the greatest dilution was 2^{-7} (1/128) of the original soil. There were three replicates of each of eight dilutions, (undiluted soil included), six subsamples of each soil treatment and four soil treatments, for a total of 576 individual containers planted with each of the two bioassay species, and grown under greenhouse conditions.

Soil dilutions were put into 60 cm^3 pine tubes (Leach Container Nursery, Aurora, OR), and onion seeds were planted August 15th, 1990 and thinned after germination to one plant per pot. To reduce contamination by spores splashed from one soil type to another, the plants were arranged on the bench in trays with plants of the same treatment and similar strength soils. Also a thin layer of autoclaved sand was spread on the surface of each pot. The trays were rotated twice weekly to eliminate any bench effect. As a check for contamination, 24 pine tubes were filled with autoclaved soils, planted with onion seeds and interspersed among the treatments. No VAM were found in the roots of these plants.

Guava seedlings were transplanted to the soil dilutions in the pine tubes in a similar manner as the onions on October 25th, 1990. Plants of both species were fertilized weekly with Long Ashton's nutrient solution (Hewitt 1966) which was prepared with 1/4 strength phosphorus (11 ppm). Supplemental greenhouse lighting at an intensity of 240 $\mu mols\text{-}^s/m^2$ was provided for 12 hours per day. Temperatures were 26° C for daytime and 22° C at night. The bioassay experiment was carried out on a quarantined bench, and all water, soil and plant material was collected and autoclaved to assure containment of foreign organisms.

Plants were harvested after 14 weeks. Shoots were oven-dried (70° C) for one week and dry weights determined. For the guava seedlings, height and root fresh weights were also measured. Fresh and dry weights from nontest guava seedlings of similar size were used to establish a ratio of dry to fresh weight in order to calculate dry weights from fresh weight measurements for the guava roots. Fresh roots of all plants were cleared with KOH and stained with trypan blue in lactoglycerol (Phillips and Hayman 1970; Kormanik et al. 1980).

Each root system was examined microscopically and scored for the presence or absence of VAM colonization. For a positive score, at least two of the following three diagnostic features of VAM infection were required: 1) presence of nonseptate coarse to fine hyphae penetrating the stained root; 2) presence of characteristic VAM arbuscules, or coiled hyphae; and 3) presence of VAM vesicles (Brown 1982) (Figures 3 and 4).

For the onion roots, which are more easily cleared and stained than guava, presence or absence scores were also made for two other types of root endophytes: 1) fine, dark-staining hyphae, superficially attached to the root, extending from a central core in a stellar configuration, frequently associated with deteriorating root tissue (Figure 5); and 2) an irregularly septated fine- to medium- thick hyphae running parallel to the root cortex with Rhizoctonia-like(Peyronel 1924), or "dark-septate" features (Trappe, personal communication) (Figure 6).

Figure 3. Psidium guajava root segment (400x magnification) with blue-staining, coarse VAM hyphae and vesicles.

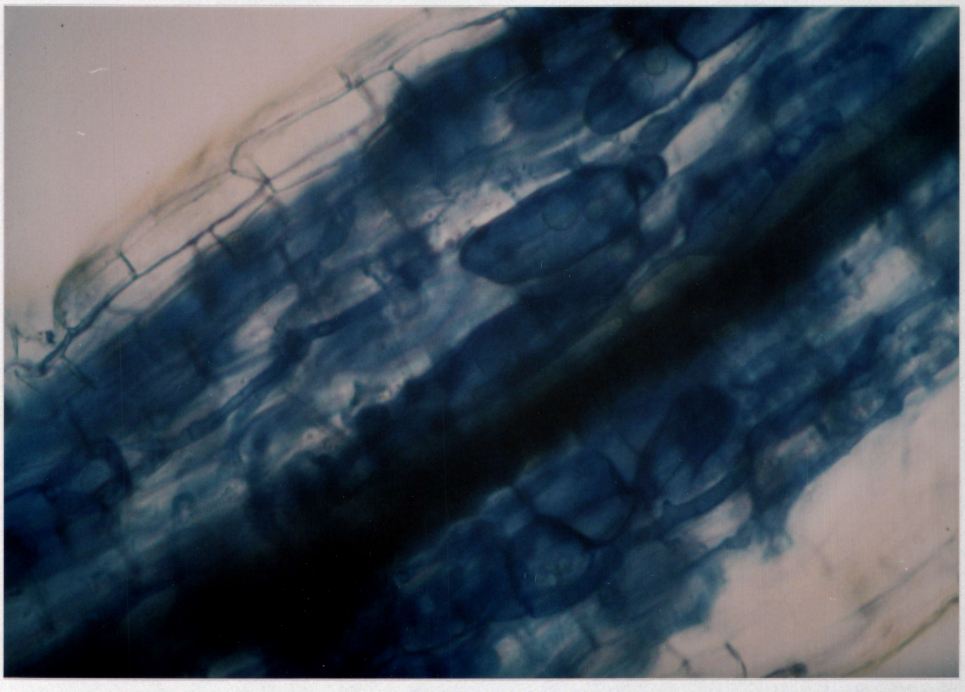


Figure 4. Psidium guajava root segment (400x magnification) with blue-staining, VAM arbuscules.

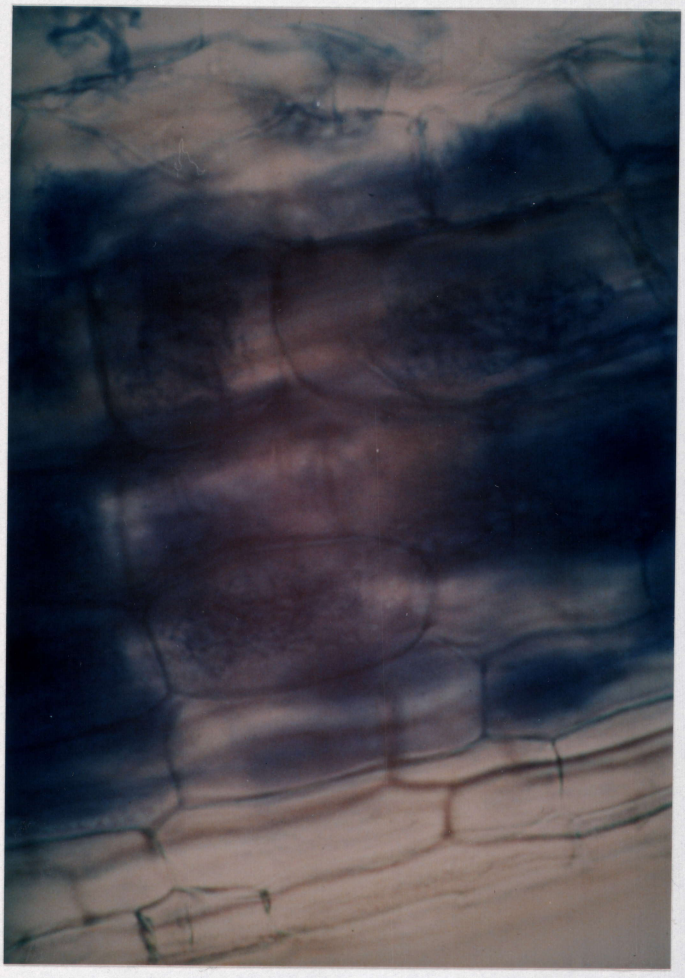


Figure 5. Allium cepa root segment (400x magnification) colonized by dark-staining nonmycorrhizal fungal endophyte with stellar configuration.

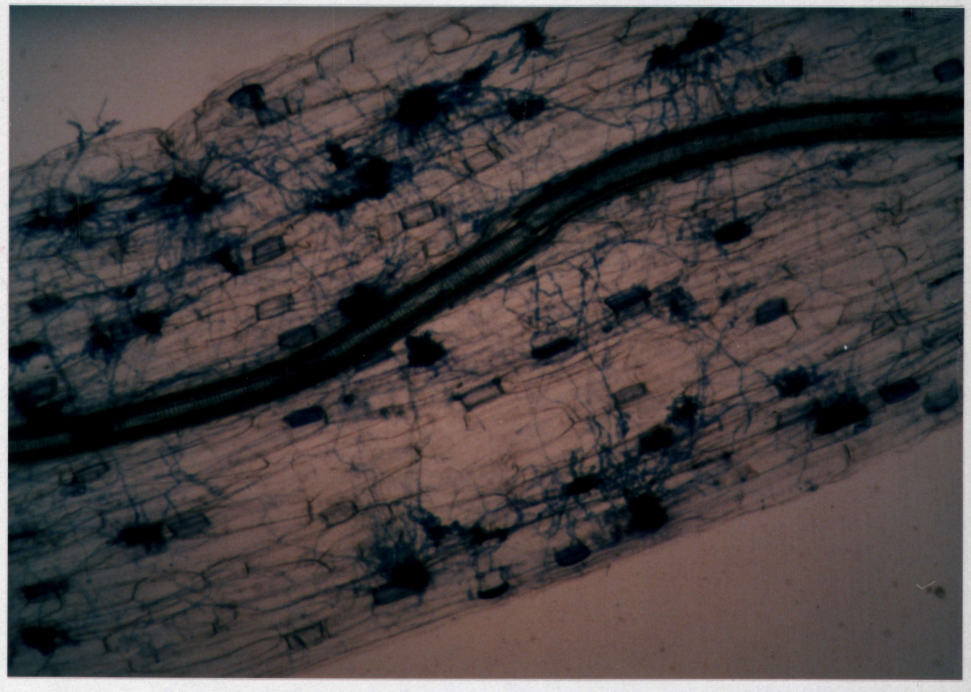
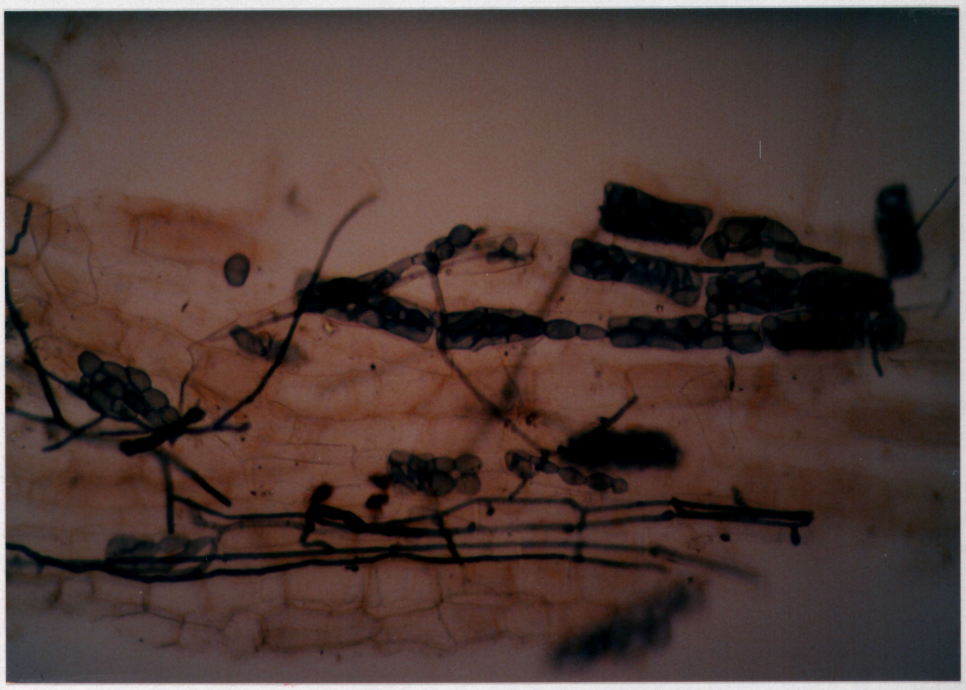


Figure 6. Allium cepa root segment (400x magnification) colonized by dark-staining nonmycorrhizal fungal fine endophyte with Rhizoctonial or "dark-septate" features.



Spore Counts

VAM fungal spore counts from ten 400 cm^3 samples of each soil were made using a modified wet-sieving and decanting method (Gerdemann and Nicholson 1963). Counts were made from the following four size-fractions: 0.063-0.150mm, 0.150-0.250mm, 0.250-0.425mm, and 0.425-1.0 mm. Totals from each size-fraction were divided into four categories: 1) spore clusters attached to hyphal and root fragments; 2) sporocarps; 3) viable-appearing, individuals with spore contents; and 4) free-floating spore forms that appeared empty or parasitized. Complete species identification was beyond the scope of this study but identification of a few prominent species will be discussed.

Statistical Analysis

A table of MPN for use with 2-fold dilutions and 3 tubes per dilution was created based on the general equation of Halvorson and Zigler (Alexander 1982). The most probable number of infective propagules per gram of dry soil for each treatment was calculated from this table. The MPN values for each soil treatment from both bioassays were compared using an analysis of variance (ANOVA). To correct for unequal variance, guava MPN values were transformed as log(MPN) and onion MPN values were transformed as log(MPN +0.001). Comparisons of height, shoot and root dry weights of plants from the non-diluted soils were also subjected to an analysis of variance without transformations. Fisher's 95% Protected LSD's were calculated for separation of means.

Mean spore counts were tested against MPN values in a correlation matrix. These counts were made on 400 cm^3 of the same soil from which the MPN values of guava and onion were obtained. The MPN of VAM

propagules from the two bioassay studies were converted to values per 400 cm^3 fresh soil. A correlation between spore counts, by category, and the MPN of guava and onion was determined using the Pearson-Product-Moment Correlation. Further comparison of filled individual spores from PAS and FOR was made using a CHI squared analysis.

RESULTS

Analysis of variance for MPN values from both bioassay species indicated that the MPN of infective propagules of VAM was greater in the PAS soil ($p<0.00001$) than in any of the other three soils (Table 2). The number of infective propagules in the pasture soil was estimated at 0.6 per gram of dry soil for both bioassay plants. The range in the other soil treatments was 0.002-0.104, all of which were not different using a 95% FPLSD comparison.

Height, shoot and root dry weights for guava were greater in the PAS soil ($p<0.00001$) (Figures 7 and 8). Shoot dry weights for onion were also greater in the PAS soil than in the other three treatments ($p=0.0052$) (Figure 9). As in the MPN study, none of the height or weight measurements for guava seedlings or onion plants from the other three soils were different based on a 95% FPLSD. Comparisons of weights and heights were made using plants from the non-diluted soils.

Analysis of variance for the MPN values obtained for the two unknown root endophytes found in the onion roots showed no significant differences among treatments. These fungi appeared to be present in nearly all onion roots from all soils and at most dilutions.

Mean spore counts from each soil treatment were divided into categories and soil size fractions to illustrate the variation in form and size observed in these soils (Table 3, Figures 10, 11, 12, and 13). Small clusters of spores (ranging from 3-40/cluster) attached to root and hyphal fragments were most abundant (35/400 cm^3 fresh soil) in the BA6 soil. Sporocarps averaged 13-14/400 cm^3 in the BA6 and BA4 soils, and less than 1/400 cm^3 in the PAS and FOR soils. Empty spore forms were found in great

abundance in all soil samples at the smallest size-fraction sieved (0.063-0.150mm). Viable-appearing individual spores were observed in all size classes and in all soil treatments, but the relative proportion by size class differs statistically (CHI squared value=1143.6, $p<0.001$). There were relatively more (107/400 cm^3) in the PAS soil, 0.063-0.150 size fraction, than in the FOR soil (Figure 10).

Correlations between spore counts and MPN values from both bioassays were not significant (Table 4). No category of spores counted was a reliable indicator for VAM infectivity as observed in the greenhouse bioassay studies.

Table 2. Medians for Most Probable Number of VAM propagules per gram dry soil by bioassays, with Psidium guajava and Allium cepa seedlings.

	BA6	BA4	PAS	FOR
Psidium guajava	0.07[a] (0.041-.119)	0.104[a] (0.061-0.177)	0.628[b] (0.368-1.072)	0.102[a] (0.059-0.173)
Allium cepa	0.002[a] (0.001-0.009)	0.008[a] (0.003-0.024)	0.567[b] (0.213-1.513)	0.006[a] (0.002-0.017)

For each species, medians followed by same lower case letter are not statistically different at $p=0.05$. The numbers in parentheses are 95% confidence intervals. Medians are from backtransformed logarithmic data.

Figure 7. Mean height (mm) for Psidium guajava seedlings grown in four treatment soils from Costa Rica.

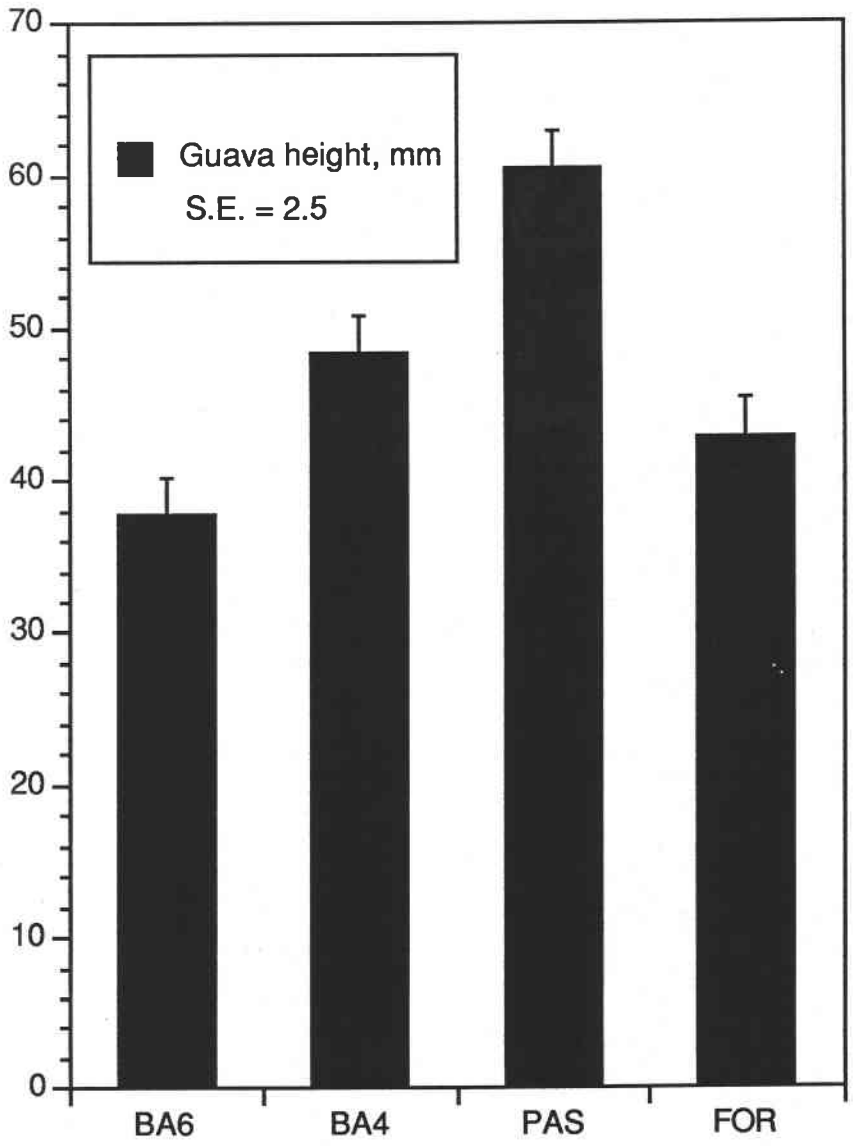


Figure 8. Mean shoot and root dry weights (grams) for Psidium guajava seedlings grown in four treatment soils from Costa Rica.

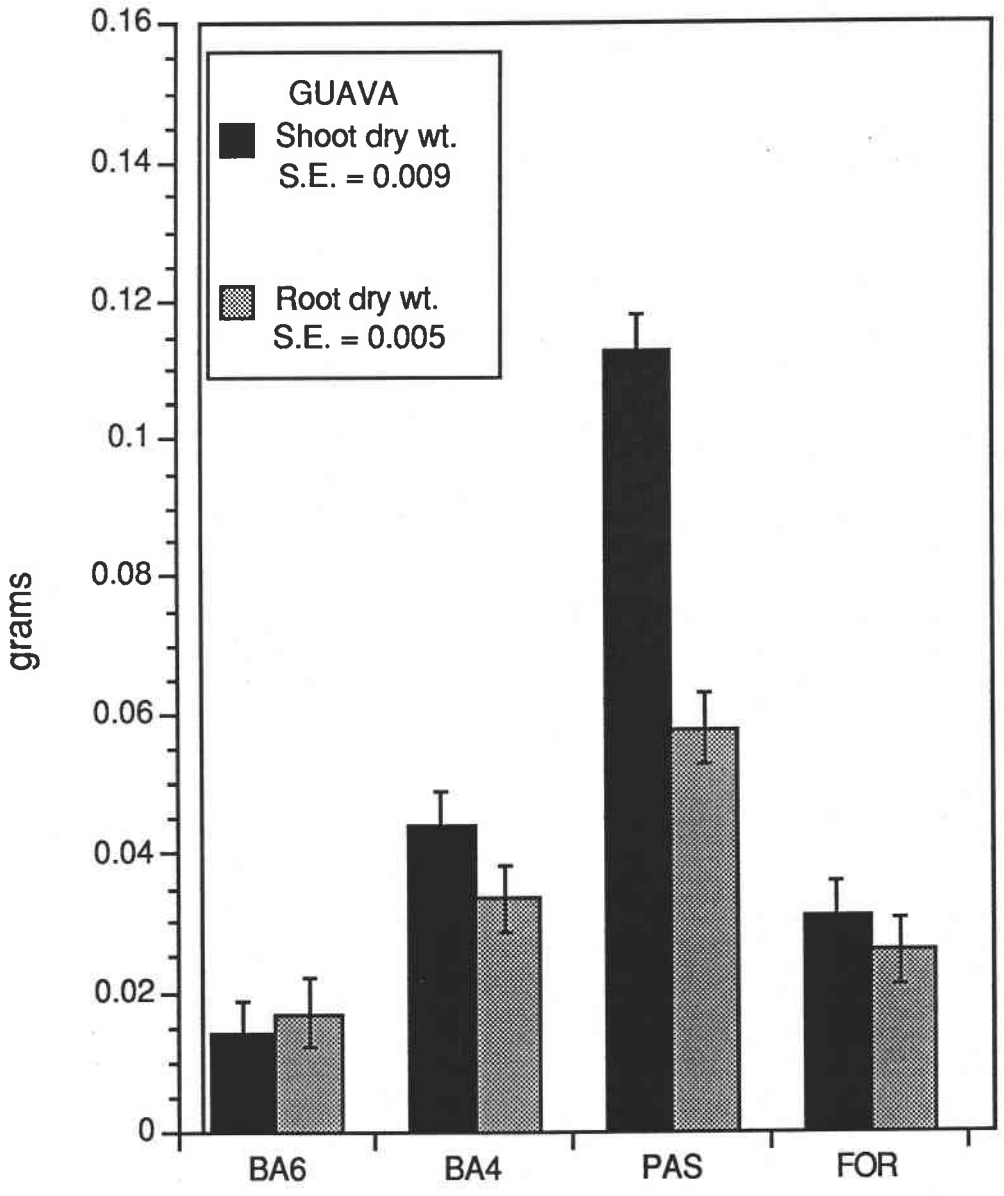


Figure 9. Mean shoot dry weights (grams) for Allium cepa plants grown in four treatment soils from Costa Rica.

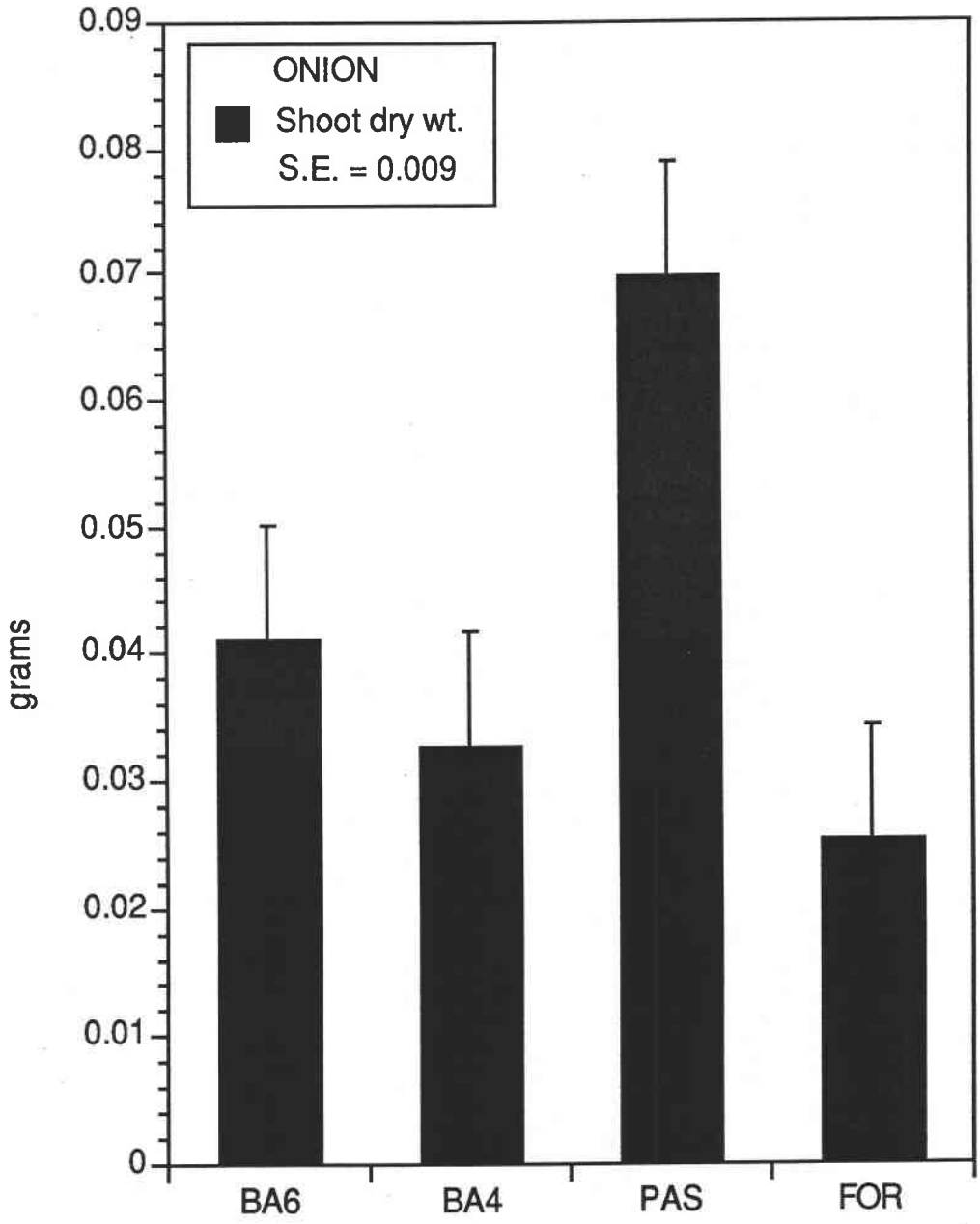


Table 3 Mean Spore Counts, by category and size class, per 400 cm^3 fresh soil collected on four treatment sites in Costa Rica.

	BA6	BA4	PAS	FOR
CLUSTERS				
0.425-1.0mm	9.1	2.3	0.6	0
0.250-0.425mm	17.1	0.6	0	3.4
0.150-0.250mm	9.4	0.8	0.1	6.2
0.063-0.150mm	0	0	0	0
TOTAL	**35.6**	**3.7**	**0.7**	**9.6**
SPOROCARPS				
0.425-1.0mm	0.6	0	0.1	0
0.250-0.425mm	13.6	13	0.1	0.1
0.150-0.250mm	0.4	0.1	0	0
0.063-0.150mm	0	0	0	0
TOTAL	**14.6**	**13.1**	**0.2**	**0.1**
EMPTY FORMS				
0.425-1.0mm	0	0	0	0
0.250-0.425mm	1.9	0.2	0.1	0.3
0.150-0.250mm	1.1	17.4	56.7	1.6
0.063-0.150mm	5830	6150	3310	2690
TOTAL	**5833**	**6167**	**3367**	**2692**
FILLED INDIVIDUALS				
0.425-1.0mm	1.1	0.7	0.6	0.1
0.250-0.425mm	5.7	9	6.4	10.9
0.150-0.250mm	21.7	0.9	3	30
0.063-0.150mm	0	0	97.4	0
TOTAL	**28.5**	**10.6**	**107.4**	**41**

Figure 10. Means for spore clusters, by size class, from four treatment soils of Costa Rica.

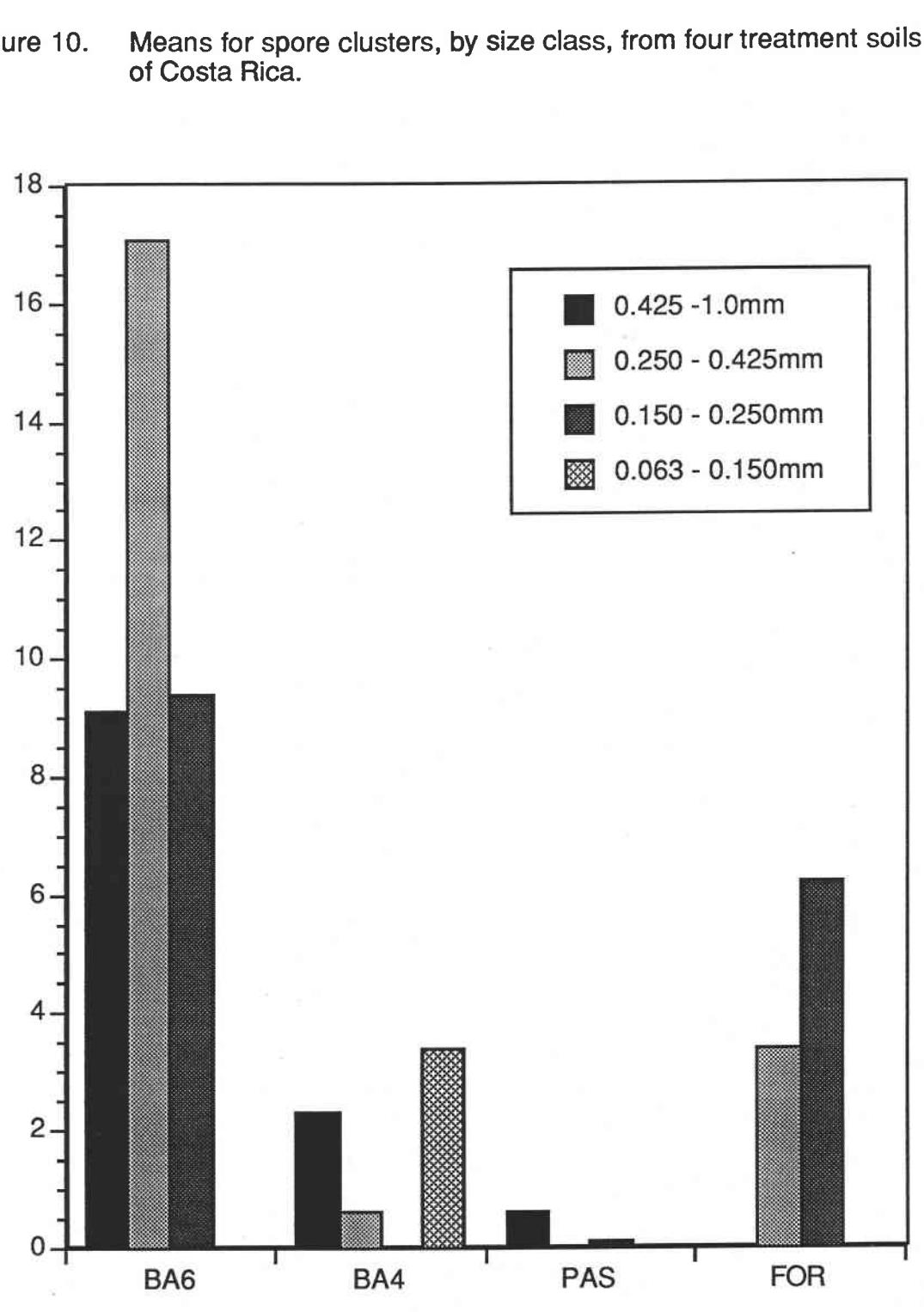


Figure 11. Means for sporocarps, by size class, from four treatment soils of Costa Rica.

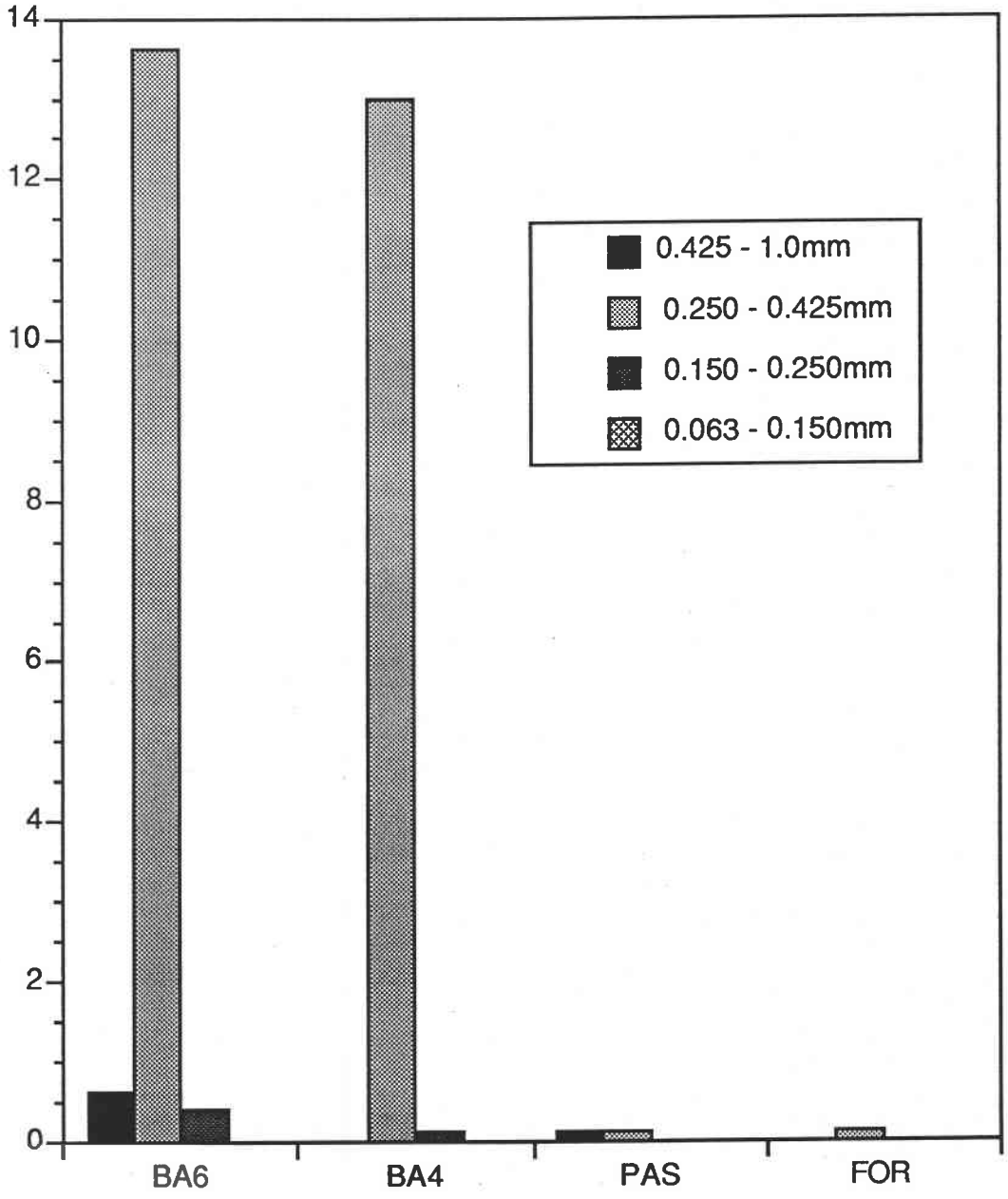


Figure 12. Means for viable-appearing, filled individual spores, by size class, from four treatment soils of Costa Rica.

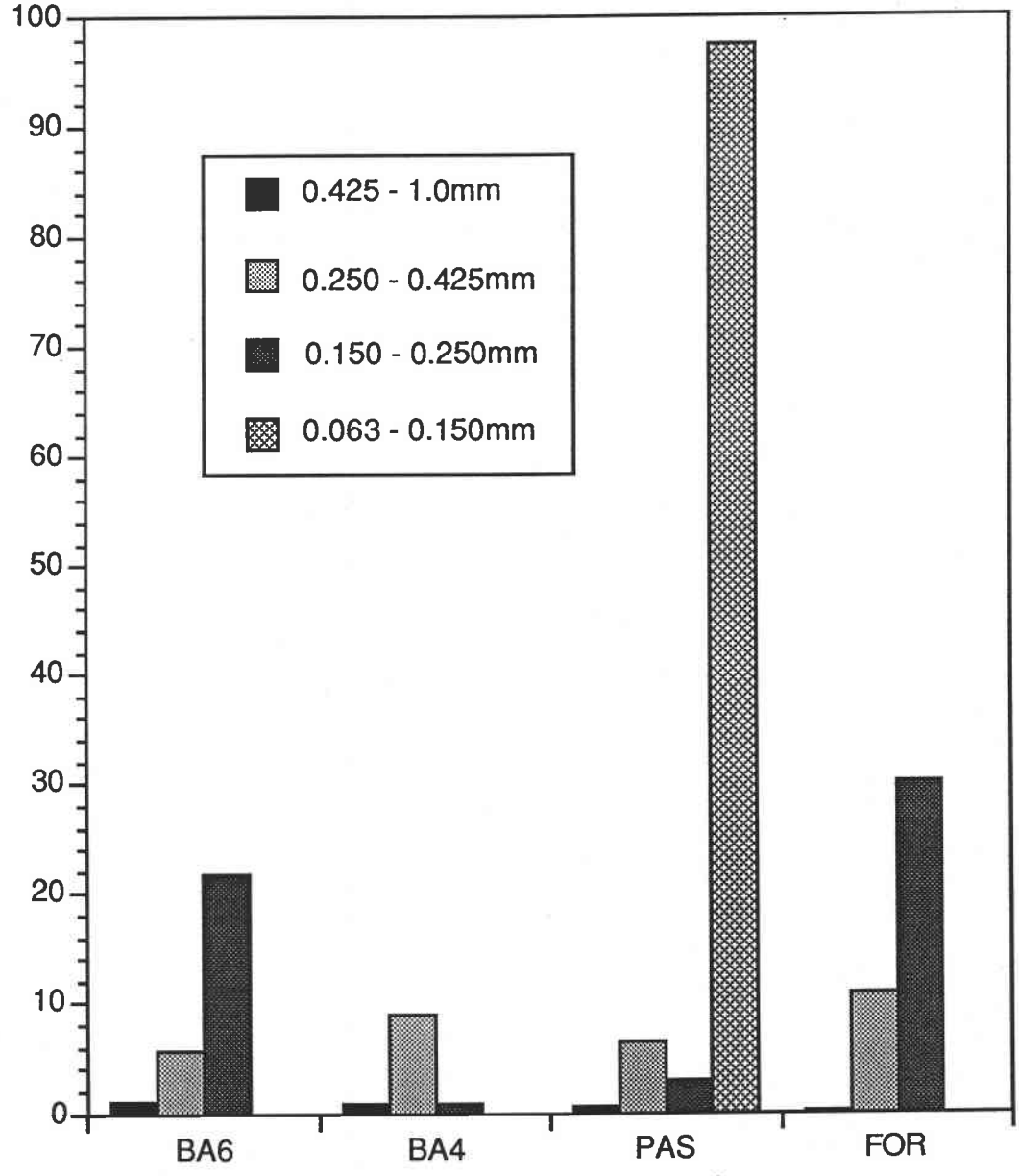


Figure 13. Means for empty spore forms, by size class, from four treatment soils of Costa Rica.

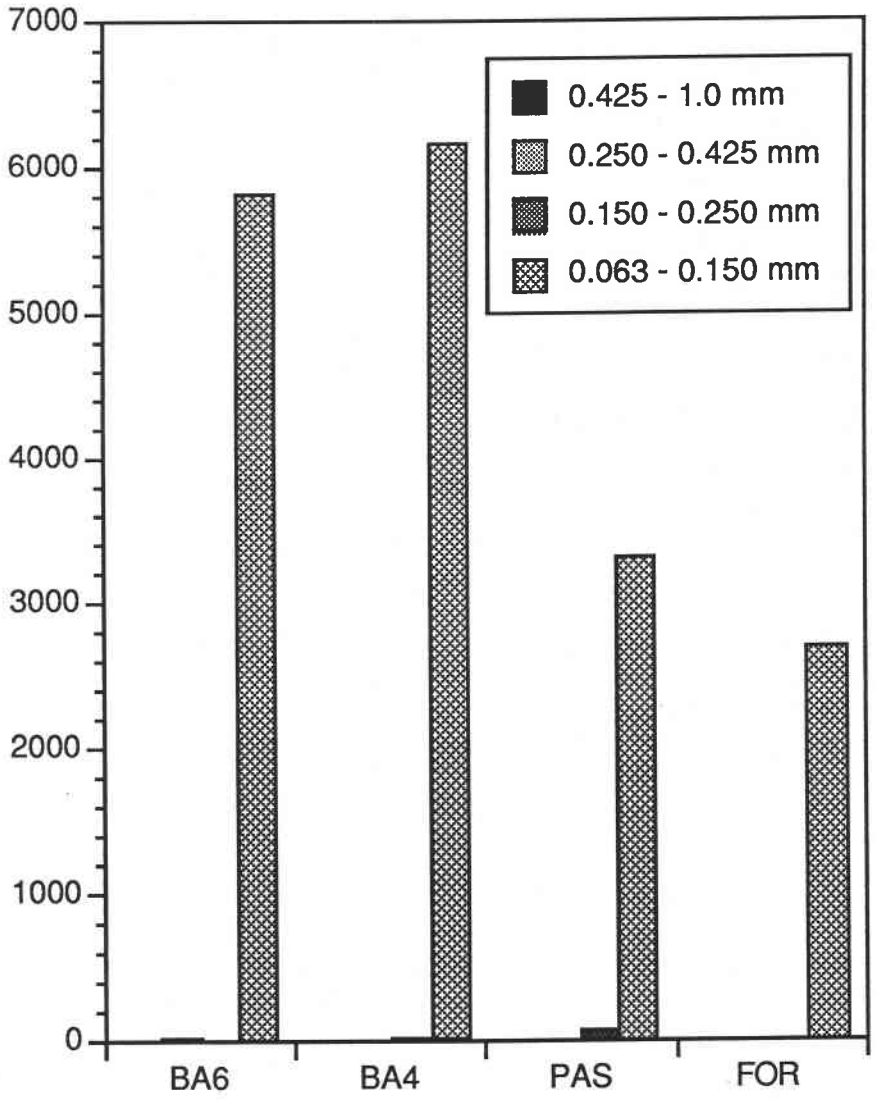


Table 4. Pearson Product-Moment Correlation Cefficient (r) for the estimated number of infective VAM propagules per 400 cm^3 fresh soil and mean spore counts, by category, from the same soil samples. The r values were calculated for the MPN values from Psidium guajava and from Allium cepa.

	Psidium guajava MPN/400 cm^3	Allium cepa MPN/400 cm^3
MPN/400 cm^3	1.00	1.00
Clusters	-0.036	0.128
Sporocarps	-0.5	-0.311
Empty forms	-0.248	-0.444
Filled Individuals	-0.147	-0.238

DISCUSSION

The presence or persistence of VAM fungal inoculum in soil may be a key factor in tropical reforestation. Among the barriers to forest regeneration in a degraded Amazon pasture, Nepstad et al. (1991) identified drought tolerance and the ability to compete for limited soil resources as critical to the survival and growth of transplanted seedlings from the mature forest. Effective VAM colonization may provide a competitive advantage for tree seedlings.

We found that VAM inoculum was lower in the bare sites than in the pasture site. We had hypothesized that VAM inoculum would also be greater in the forest patch than in the bare sites. Surprisingly, we found similarly low levels of inoculum in the soil with forest cover, relative to those from the pasture. These low MIP levels from the forest may reflect the form of inoculum measured in this study. VAM inoculum can be present in soil as extramatrical hyphae, VAM-colonized root fragments, and as spores (Friese and Allen 1991). Colonization is influenced by host-fungal compatibility, by the presence of viable hyphae or spores in the rooting zone, and by soil conditions, host physiology and the presence of other rhizosphere organisms (Perry et al.1987).

How long do vegetative hyphae remain viable in the soil? Warner and Mosse (1980) believed that external hyphae cannot survive after root death. In some species of VAM fungi, new hyphae have emerged from dead root fragments in dried soil (Tommerup 1981). Jasper et al. (1989) found the survival and infectivity of external hyphae was reduced with soil disturbance (chopped and mixed), presumably due to disruption of the hyphal network.

By removing soil from the study sites, disturbance to fungal hyphal networks was inevitable, and the inoculum potential we measured must reflect this bias. In the forest patch where light, temperature and moisture conditions are fairly constant, stimulation to sporulate may be minimal, and the primary source of inoculum in the forest patch would then be VAM root-hyphal networks. If so, physical disturbances may have significantly reduced VAM inoculum in our forest soil samples. The pasture samples had more viable-appearing individual spores in the fine soil fraction, and this difference in spore counts may have been reflected in the greater MIP in the pasture soil.

A second explanation for the low inoculum potentials observed in the forest soils may be a host-fungal compatibility dynamic. Relative to ectomycorrhizae, the VAM display minimal host specificity (Gianinazzi-Pearson and Diem 1982). However, there are differences (Ianson 1991) in VAM effectiveness --the degree of nutritional or other advantage resulting from the symbiotic association between a particular auto- or heterotroph (Mosse 1975). Bevege and Bowen (1975) reported marked variation between different host endophyte pairs with regard to germination time for spores, development of vesicles and arbuscules, spread of infection and growth of hyphae outside the root. Environmental conditions, shade or temperature fluctuations, may influence effectivity of VAM colonization. Johnson (1976) observed VAM infection levels to be sensitive to shading for tropical plants *Coprosma leptospermum* and *Microlaena* sp., but not for *Griselinia* sp. and *Parsonsia*.sp. Certain VA fungi may prefer a particular plant species(Mosse 1975). *P. guajava* and *A. cepa* may be more suitable hosts for the VAM fungi present in the open pasture soil than those present in a shaded forest soil.

Both of these host species are well-adapted to high light conditions. *P. guajava* can be observed in the open fields at La Selva, as well as in the dense forest . Another species of the same genus, *P. cattlemania* has been documented as an invasive tree in Hawaii with broad environmental tolerances (Huenneke and Vitousek 1990). Further greenhouse experiments in our laboratory with *P. guajava* seedlings have indicated that height and leaf area vary with VAM fungal species (Ianson, personal communication). Our MPN results may, in part, reflect MIP of species or strains of VAM fungi as selected by these bioassay plants.

A third explanation for these low MIP levels from the forest soil is heterogeneity of VAM fungi distribution compounded with a sampling anomaly and extractive studies. Our samples were taken from a relatively young (10 yr. old) and small forested area (approximately 400^2 meters). The litter layer was removed prior to collection. Rose and Paranka (1987) found that roots in the litter and humus layers supported significantly higher % VAM colonization than roots from mineral soil in a tropical wet forest in northeastern Brazil. Given these possibilities and sampling limitations, we cannot extrapolate these findings to a field study of soils in a mature tropical forest.

Spores were abundant in the plots bare of vegetation which had low levels of VAM fungal inoculum potential, and large root fragments were conspicuously missing from these soils. Spores of VAM are produced below-ground. They are relatively large (0.03-0.45 mm) and poorly adapted for wind dispersal in humid conditions (Gerdemann and Trappe 1974). Spatial distribution patterns of VAM spores have been found to correlate positively with plant cover and negatively with soil moisture (Anderson et al. 1983). In a tropical environment with high temperatures and heavy rainfall, we would

expect rapid loss of VAM fungal spores to predation by bacteria, saprophytic fungi, and soil fauna (Janos 1983; Fitter 1985). Viable spores present on our bare plots may be the result of activity by animal spore vectors such as ants and small rodents. Janos and Sahley (personal communication) found VAM spores and sporocarps in 69% of fecal samples collected from six species of rodents in an Amazonian Peruvian rainforest.

Identifiable spores in our samples included *Glomus fasciculatum* (Thaxter) Gerdemann and Trappe emend. Walker and Koske, *Acaulospora* sp. , and *Sclerocystis clavispora* (Berkeley and Broome). When examined microscopically (400x), most of the intact sporocarps and the root-hyphal clusters, found almost exclusively in the bare vegetation plots, appeared to be empty of cell contents. These may reflect VAM inoculum that has degraded in the abscence of a host, and leave only the recalcitrant cell walls remaining.

The pasture soil had significantly more (mean=98) of the filled, viable-appearing fine (0.063-0.150 mm) spores, whereas the forest had none. On the other hand, the forest soil had more (mean =30) filled individual spores in the 0.150-0.250 mm size compared to a mean of 3 in the pasture soil. These differences in size classes of VAM fungi found in the two soils may represent a species shift from pasture to forest. Johnson et al. (1991) reported that the species shift of VAM during secondary succession of field to forest in temperate conditions was related to plant community composition and to soil pH, soil C, N, and P, and root biomass. They also found that total number of spores was not significantly correlated with inoculum potential.

Our results indicate that VAM fungal inoculum in this pasture soil can support at least one early successional forest tree. This site was never seriously eroded, and therefore cannot be compared with tropical sites that

have sustained burning and heavy grazing. Future studies are needed to include such sites as described by Uhl et al. (1988) as anthropogenic, large-scale disturbances that result in nonregenerating, highly degraded pastures.

Techniques for estimating inoculum potential are labor intensive and have limitations. Spore counts can vary dramatically with seasonal changes. Greenhouse bioassays can never accurately measure field or forest conditions. The utilization of a mature forest species as a bioassay under field conditions may give further insight into VAM-host effectiveness. Variations in shading would help to compare the effects of solar radiation, moisture and temperature on VAM colonization. We agree with Nepstad et al. (1991). that strategies for accelerating forest regrowth should minimize inputs of capital and human labor and maximize the contribution of natural processes. Creative and intelligent forest regeneration must address and maximize VAM inoculum in tropical reforestation.

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