BIOTROPICA 26(4): 369–377 1994

Mycorrhiza Inoculum Potentials in Tropical Secondary Succession[1]

Christine R. Fischer[2]

Department of Forest Science, Oregon State University, Corvallis, Oregon 97331, U.S.A.

David P. Janos

Department of Biology, University of Miami, P.O. Box 249118, Coral Gables, Florida 33124, U.S.A.

David A. Perry

Department of Forest Science, Oregon State University, Corvallis, Oregon 97331, U.S.A.

Robert G. Linderman

USDA-ARS Horticultural Research Laboratory, 3420 Orchard Street, Corvallis, Oregon 97331, U.S.A.

and

Philip Sollins

Department of Forest Science, Oregon State University, Corvallis, Oregon 97331, U.S.A.

ABSTRACT

Vesicular-arbuscular mycorrhizae can be indispensable for establishment and growth of tree seedlings in infertile lowland wet tropical soils. Persistence of mycorrhizal fungi after disturbance, however, is problematic to assess. We used a greenhouse bioassay employing *Psidium guajava* L. and *Allium cepa* L. to estimate the most probable number of mycorrhizal fungus propagules in two Costa Rican soils (Oxic Dystropepts) with different vegetation histories. We collected soils at the La Selva Biological Station from sites in secondary forest, abandoned pasture, and plots kept bare of vegetation for four and six years.

Both bioassay hosts yielded positively correlated estimates of mycorrhiza propagule numbers. Median propagule estimates per 100 gm dry soil for the pasture site are 57 and 63; these estimates significantly exceed those for the other sites which range from 0.2 to 10 for bare plots and were estimated to be 0.6 and 10 for secondary forest. These bioassay estimates are positively correlated (Pearson $r = 0.66$ and 0.72) with counts of whole spores in these soils, but not with counts of sporocarps, spore clusters, or the most numerous, spores apparently empty of cytoplasmic contents or parasitized. Growth of both bioassay hosts in pasture soil significantly exceeded growth in soils from the other three sites in accord with the bioassays and whole spore counts.

RESUMEN

Las micorrizas vesículo-arbusculares pueden ser indispensables para el crecimiento y establecimiento de plantones de árboles en suelos tropicales infértiles de tierras bajas húmedas. La persistencia de hongos micorrícicos en suelos después de una perturbación no es fácil de estimar. Se realizó un bioensayo en invernadero utilizando *Psidium guajava* L. y *Allium cepa* L. como plantas huésped para estimar el número más probable de propágulos de hongos micorrícos en 2 tipos de suelos de Costa Rica (Oxic Dystropepts) de 4 sitios con differentes usos. Los suelos se colectaron en la Estación Biológica La Selva en sitios con bosque secundario, pastizales abandonados y en 2 áreas que habian sido mantenidas desprovistas de vegetación por durante 4 y 6 años.

Ambos huéspedes del bioensayo mostraron una respuesta similar en el numero de propágulos micorrícicos en todos los suelos muestreados. La mediana del número de propágulos por 100 g de suelo seco del pastizal se estimó en 57 y 63 en los dos huespedes, valores que excedieron significamente los de los otros sitios. En las areas desprovistas de vegetación, la mediana varió entre 0.2 y 10, y en el bosque secundario se estimó en 0.6 y 10. Las estimaciones del bioensayo están positivamente correlacionadas (Pearson $r = 0.66$ y 0.72) con el número de esporas enteras en el suelo pero no con el número de esporocarpos, esporas agrupadas o esporas parasitadas o aparentemente vacias de contenido citoplásmico, que eran las más numerosas. El crecimiento de ambos huéspedes fue significativamente mayor en el suelo del pastizal que en suelos provenientes de los otros tres sitios, de acuerdo con los resultados del bioensayo y con el número de esporas enteras.

Key words: Glomales; lowland humid tropics; mycorrhiza inoculum potential; pasture; Psidium guajava; *reforestation; secondary succession; vesicular-arbuscular mycorrhizae.*

[1] Received 9 August 1993; accepted 11 January 1994.

[2] Present address: Pacific Northwest Research Station, Forestry Sciences Laboratory, 3200 SW Jefferson Way, Corvallis, Oregon 97331, U.S.A.

MYCORRHIZAE, PLANT ABSORBTIVE ORGANS formed by mutualistic association of certain fungi with plant roots, can be indispensable for survival and growth of tropical tree seedlings, especially on typical, phosphorus-poor, lowland humid tropical soils (Janos 1980a, 1987; see also Sieverding 1991). Mycorrhizae benefit host plants by aiding acquisition of water and mineral nutrients, by increasing rates of photosynthesis, and by improving root resistance to pathogens and root longevity (Linderman 1988). In the tropics, tree seedlings may fail to establish because of slow or sparse mycorrhiza formation, especially when the seedlings are in competition with plant species that have little requirement of mycorrhizae (Bowen 1980; Janos 1980b, 1992). Pioneer and colonizing species often comprise the latter, able to grow independently of mycorrhizae; some, such as sedges (Cyperaceae), typically do not form mycorrhizae (Tester *et al.* 1987). Because vesicular-arbuscular mycorrhizal (VAM) fungi, those forming the most common type of mycorrhizae, are unable to proliferate if not associated with host plant roots, pioneer plant communities that sustain few mycorrhizae may retard succession (Janos 1980b; see also Allen 1991). In the face of human disturbances to ecosystems that disrupt nutrient cycling (Whitmore 1989), mycorrhiza formation may be necessary for recovery and stabilization of plant communities (Perry *et al.* 1989, Pankow *et al.* 1991).

Conversion of tropical forest to pasture has produced serious obstacles to reforestation in some instances (Nepstad *et al.* 1990). Janos (1988) has shown that a lack of VAM inoculum in an overgrazed, sedge-filled pasture in Costa Rica significantly retarded the growth of seedlings of a leguminous tree species, *Pithecellobium longifolium* (H. and B.) Standl. Nevertheless, tropical pasture grasses may sustain and benefit from VAM fungi (Salinas *et al.* 1985) and tropical pasture soils may contain VAM fungus spores (Toro & Sieverding 1986). Where sedge dominance or soil erosion do not contribute to the loss of VAM fungi, the capacity of pasture soils to produce mycorrhizae needs to be assessed as a factor potentially limiting reforestation.

VAM inocula comprise spores, hyphae associated with living roots, and hyphae associated with dying roots or root fragments (Janos 1992). Of these, spores easily can be extracted from soil and counted (Gerdemann & Nicolson 1963), but spore counts may not be correlated with mycorrhiza formation (An *et al.* 1990, Johnson *et al.* 1991). Quantification of VAM fungus hypha presence in soil is laborious, and hypha viability and infectiveness are difficult to determine (see Sieverding 1991). Because VAM fungi cannot be grown in axenic cultures apart from host roots, only by using host plants can soils be bioassayed for the presence of infective propagules of these fungi (Sieverding 1991).

In this study, we used two VAM plant species to bioassay tropical soils for their mycorrhiza inoculum potential (MIP), an integrative measure of the capacity of VAM fungus propagules within the soils to produce mycorrhizae. We compared the MIP under abandoned grass pasture to those of a secondary forest and plots bare of vegetation. We expected that bare plots would be devoid of VAM inoculum, or nearly so, that secondary forest dominated by mycorrhizal plants would have a high MIP, and that pasture would be intermediate between these two extremes. We examined the correlation of MIP with spore presence.

METHODS

SITES AND SOIL COLLECTION.—In early July 1990, we collected soil samples from the La Guaria Annex of the La Selva Biological Research Station (10°26'N, 83°59'W) in northeastern Costa Rica. La Selva is located in a region of lowland tropical wet forest with average annual precipitation of 4000 mm, and a mean annual temperature of 24°C (see Sollins & Radulovich 1988). All soils that we sampled are highly weathered, infertile acid soils derived from andesitic to basaltic parent materials. The soils are well-aggregated, with bulk densities near 0.7 Mg/m^3 at 0–10 cm depth (Radulovich *et al.* 1989).

We collected soil from four sites, all of which had been cleared of primary forest during the 1950s and 1960s (Pierce 1992). The first collection was from a secondary forest located on Matabuey soil developed in volcanic alluvium lying atop a weathered lava flow (Sollins *et al.* 1993). This site (Tables 1 and 2: "Forest") was a 10-yr-old, secondary mixed forest, approximately 400 m^2 in area, dominated by *Pentaclethra macroloba* (Willd.) Kuntze, *Rollinia microsepala* Standley, and *Vismia* sp.

The other three sites are all located on Helechal soil within a two hectare area on an upper Sarapiqui river terrace. They were cleared of forest by 1960, grazed intermittently until about 1981, and then abandoned (Sollins & Radulovich 1988). Small plots within two of these sites were completely cleared of vegetation and kept bare by periodic hand weeding as part of a study of nutrient availability (see Sollins *et al.* 1988). One pair, approximately 12 × 12 m each, had been bare of vegetation since 1984, or six years at the time of this collection (Tables 1 and 2: "6-yr bare"), and a second set of plots, approx-

TABLE 1. *Soil attributes, median most probable number (MPN) of vesicular-arbuscular mycorrhizal fungus propagules estimated with* Psidium guajava *and* Allium cepa *bioassays, and growth of both bioassay species after 14 weeks in undiluted soils from four sites. Median MPNs are shown with 95% confidence limits in parentheses. Within a row, parameters followed by the same letter are not significantly different at* P = 0.05 *by ANOVA; for growth parameters* N = 18.

	Site			
	6-yr Bare	4-yr Bare	Pasture	Forest
Soil consociation	Helechal	Helechal	Helechal	Matabuey
Soil pH	4.2	4.1	4.5	4.4
Extractable P (ppm)	0.4	1.7	1.1	0.6
MPN (propagules/100 g soil)				
Psidium	7.0[a]	10.4[a]	62.8[b]	10.2[a]
	(4.1–11.9)	(6.1–17.7)	(36.8–107.2)	(5.9–17.3)
Allium	0.2[a]	0.8[a]	56.7[b]	0.6[a]
	(0.1–0.9)	(0.3–2.4)	(21.3–151.3)	(0.2–1.7)
Psidium guajava				
Height (cm)	3.8[a]	4.8[a]	6.0[b]	4.3[a]
Shoot dry mass (g)	0.014[a]	0.044[a]	0.113[b]	0.031[a]
Root dry mass (g)	0.017[a]	0.034[a]	0.058[b]	0.026[a]
Allium cepa				
Shoot dry mass (g)	0.041[a]	0.033[a]	0.07[b]	0.025[a]

imately 27 × 15 m each, had been kept bare since 1986, or four years at the time of this collection (Tables 1 and 2: "4-yr bare"). Secondary succession had been allowed at the third site (Tables 1 and 2: "Pasture") which was dominated by the grass *Olyra latifolia* L. and by ferns (Sollins & Radulovich 1988) at the time we collected soil. *Conostegia subcrustulata* (Beurl.) Tr., *Plukenetia volubilis* L., *Panicum maximum* Jacq., and *Passiflora vitifolia* HBK. also were present.

We collected six 1600 cm³ soil samples from each of the four sites. We removed litter and surface roots before collecting soil and included fine roots to a depth of 5 cm. Within one week of collection, we transported 1200 cm³ of refrigerated soil from each sample to the USDA-ARS Horticultural Crops Research Laboratory in Corvallis, Oregon. At La Selva, we examined the remaining 400 cm³ from each sample and four additional 400 cm³ collections from each site for the presence of spores. Soil pH in water and extractable phosphorus (by the dilute acid fluoride method) are shown in Table 1.

MYCORRHIZA INOCULUM POTENTIAL ASSESSMENT.—We used the "most probable number" (MPN) method (Porter 1979, An *et al.* 1990) to compare the mycorrhiza inoculum potentials of sites. This method, also known as the "dilution end-point" method, was developed by microbiologists to estimate population sizes of microorganisms from observations of the greatest dilutions at which microorganisms remain detectable (Alexander 1982). We used the presence of VAM in the root systems of two bioassay plant species, *Psidium guajava* L. (guava) and *Allium cepa* L. (onion) to calculate most probable numbers of VAM fungal propagules in the four sites. Guava is strongly dependent on VAM for growth in La Selva old alluvial soil (Janos 1980a), and onion is similarly responsive to VAM (Thomas *et al.* 1986).

We made three replicates of a twofold dilution series from each field sample by successively mixing 25 cm³ of soil with 25 cm³ sterile sand seven times, so that the greatest dilution was 2^{-7} (1/128) of the original soil. Including the undiluted soils, this gave 576 soil mixtures (four sites × six samples × eight concentrations × three replicates) for each bioassay host species which we placed in 60 cm³ pine tubes (Leach Container Nursery, Aurora, Oregon).

We planted onion seeds (variety Hardy White Bunching obtained from Nichols Garden Nursery of Albany, Oregon) directly into the tubes on 15 August 1990 and thinned to one plant per pot after germination. We transplanted guava seedlings to tubes on 25 October 1990, six weeks after germination in a sterile sand and peat mixture. We had collected ripe guava fruits at La Selva in July 1990, removed, washed and air-dried their seeds, and stored them at room temperature for four weeks

TABLE 2. *Mean numbers of propagules of glomalean fungi per 100 g dry soil by category and size class for four sites. Within a row, totals followed by the same letter are not significantly different at* P ≤ *0.05 by ANOVA and 95% FPLSD;* N = *24.*

	Site			
	6-yr Bare	4-yr Bare	Pasture	Forest
Sporocarps				
0.425–1.0 mm	0.21	0.00	0.04	0.00
0.250–0.425 mm	4.86	4.64	0.04	0.04
0.150–0.250 mm	0.14	0.04	0.00	0.00
0.063–0.150 mm	0.00	0.00	0.00	0.00
Total	5.21a	4.68a	0.08b	0.04b
Spore clusters				
0.425–1.0 mm	3.25	0.82	0.21	0.00
0.250–0.425 mm	6.11	0.21	0.00	1.21
0.150–0.250 mm	3.36	0.29	0.04	2.21
0.063–0.150 mm	0.00	0.00	0.00	0.00
Total	12.72a	1.32b	0.25b	3.42b
Whole spores				
0.425–1.0 mm	0.39	0.25	0.21	0.04
0.250–0.425 mm	2.04	3.21	2.29	3.89
0.150–0.250 mm	7.75	0.32	1.07	10.71
0.063–0.150 mm	0.00	0.00	34.79	0.00
Total	10.18a	3.78a	38.36b	14.64a
Empty spores				
0.425–1.0 mm	0.00	0.00	0.00	0.00
0.250–0.425 mm	0.68	0.07	0.04	0.11
0.150–0.250 mm	0.39	6.21	20.25	0.57
0.063–0.150 mm	2082.13	2196.41	1182.13	960.71
Total	2083.20a	2202.69a	1202.42a	961.39a

before transport to Oregon. In Oregon, we surface sterilized these seeds with hydrogen peroxide, immersed them in water, and refrigerated them for two weeks before sowing at 2 mm depth in a greenhouse flat to produce transplants.

In order to reduce potential contamination by spores that might be splashed from one soil to another on greenhouse benches, we arranged tubes in trays of the same site and dilution. We changed the positions of trays twice weekly to eliminate bench effects. In addition, we spread a thin layer of autoclaved sand on the surface of each tube. As a check for contamination, we filled an additional 24 tubes with autoclaved soil, planted them with onion seeds, and interspersed these tubes among the others. No contamination of the roots of these plants occurred.

We fertilized both bioassay species weekly with Long Ashton's nutrient solution (Hewitt 1966) with ¼ strength phosphorus (11 ppm). We provided supplemental greenhouse lighting at an intensity of 240 $\mu mols \cdot s^{-1} m^{-2}$ for 12 hours per day. Daytime temperature was 26°C, and night temperature was 22°C.

After 14 weeks we harvested the bioassay plants. We oven-dried shoots at 70°C for one week and determined their dry masses. For guava seedlings, we measured shoot height and fresh root mass as well as the fresh and dry masses of roots of additional (nonbioassay) guava seedlings of similar sizes which we used to determine a dry mass to fresh mass ratio. By using this ratio we estimated dry masses for bioassay guava roots. We cleared fresh roots of all bioassay plants with KOH and stained them with trypan blue in lactoglycerol (Kormanik & McGraw 1982).

We examined each root system microscopically and scored it for the presence or absence of VAM colonization. We considered VAM to be present if we found at least two of the following three diagnostic features of VAM: aseptate hyphae penetrating root cells; arbuscules, or coiled hyphae characteristic of VAM fungi; and typical VAM fungus vesicles (see Brown & King 1982).

TABLE 3. *Pearson product-moment correlation coefficients (r) between counts of propagules of glomalean fungi and log transformed most probable number (MPN) estimates of vesicular-arbuscular mycorrhizal fungus propagules based upon* Psidium guajava *and* Allium cepa *bioassays for four sites. Asterisks indicate significant correlations at* P = *0.01 of Bonferroni probabilities;* N = *24 for all correlations.*

	Sporocarps	Spore clusters	Whole spores	Empty spores	MPN (*Psidium*)
Spore clusters	0.358				
Whole spores	−0.342	−0.231			
Empty spores	0.314	−0.075	−0.258		
MPN (*Psidium*)	−0.498	−0.408	0.659*	−0.296	
MPN (*Allium*)	−0.305	−0.259	0.718*	−0.320	0.750*

Spore counts.—Fungi capable of forming vesicular-arbuscular mycorrhizae belong to the order Glomales (class: Zygomycetes), and single spores are large enough to be discernable with a dissecting microscope, typically ranging in size from 0.050 mm to 0.250 mm (Morton 1988). We counted glomalean fungus propagules in ten 400 cm^3 samples from each site by using a modified wet-sieving and decanting method (Gerdemann & Nicolson 1963). We recognized four categories of propagules (*i.e.*, sporocarps; clusters of spores attached to hyphae and root fragments; cytoplasm-filled, viable-appearing individual spores; and floating spore forms that appeared empty or parasitized) in four size-fractions: 0.063–0.150 mm, 0.150–0.250 mm, 0.250–0.425 mm, and 0.425–1.0 mm. We did not attempt to enumerate species.

Statistical analysis.—We constructed a table of MPN values for 2-fold dilutions with 3 replicates per dilution based on the general equation of Halvorson and Zigler (Alexander 1982) that we used to estimate the most probable number of infective propagules per gram of dry soil. We compared MPN values among soil treatments with analysis of variance (ANOVA). To correct for unequal variances, we transformed guava MPN values as log(MPN) and onion MPN values as log(MPN + 0.001). We also compared heights, and shoot and root dry weights of plants grown in nondiluted soils, and counts of the different categories of propagules by ANOVA without transformations. We used Fisher's 95 percent Protected Least Significant Differences (95% FPLSD) to separate means.

We sought correlations between propagule counts and log-transformed MPN values by computing Pearson Product-moment Correlation coefficients among them and assessing the significance of these correlations with Bonferroni-probabilities that adjusted for multiple, *a posteriori* comparisons. We based these correlations only on the six bulked soil samples from each site that we subsampled for both propagule counts and MPN bioassays. Before transformation, we scaled MPN values to the same volume of soil used for propagule counts. In addition, we tabulated the size-class distribution of whole spores in "pasture" and "forest" soils and tested for association with a Likelihood Ratio Chi-square statistic (the "G-statistic" of Sokal and Rohlf (1981)).

RESULTS

Analysis of variance of MPN values from both bioassay host species indicates that infective propagules of VAM fungi are more numerous in the "pasture" soil ($P < 0.001$) than in soils from the other three sites (Table 1). Both bioassay host species gave similar median estimates of 56.7 and 62.8 propagules per 100 g dry soil for the "pasture" site versus a range from 0.2 to 10.4 propagules for the other sites. There are no significant differences (95% FPLSD) in MPNs of propagules among the latter sites.

Height, shoot and root dry weights of guava are significantly greater (ANOVA and 95% FPLSD, $N = 18$, $P < 0.001$) when grown in nondiluted "pasture" soil than when grown in soil from the other sites (Table 1), as are shoot dry weights for onion (ANOVA, and 95% FPLSD, $N = 18$, $P < 0.05$). As in the MPN study, there are no significant differences (95% FPLSD) among any other measured growth parameters for guava or onion seedlings grown in the other three nondiluted soils.

Among the glomalean fungus propagules that we recovered, we identified *Sclerocystis coremioides* Berk. and Broome emend. Almeida and Schenck, *Glomus clavisporum* (Trappe) Almeida and Schenck (= *Sclerocystis clavispora* Trappe), *Glomus fasciculatum* (Thaxter) Gerdemann and Trappe emend. Walker and Koske, and *Acaulospora foveata* Trappe and Janos. Counts of "sporocarps" (Table 2) in-

cluded the former two species, while " spore clusters" include only *Glomus* species.

We partitioned propagule counts from each site by size class (Table 2). Clusters of spores (3 to 40 spores per cluster) sometimes attached to root fragments are most abundant (12.7 clusters/100 g soil) in the site kept bare of vegetation for 6 yr, and are least abundant in the pasture site (0.25 clusters/100 g). Sporocarps are significantly more common in the bare than in the pasture and forest sites (95% FPLSD), averaging 4.7 and 5.2 sporocarps/100 g in the 4-yr bare and 6-yr bare sites, respectively, and fewer than 0.1/100 g in the pasture and forest sites. We found empty spores in great abundance in the smallest size-fraction (0.063–0.150 mm) of all sites, but detected no significant differences among sites in total number of empty spores.

We found significantly more total whole spores in the pasture than in other sites (95% FPLSD; Table 2). Whole spores predominate in the 0.063–0.150 mm size fraction of the pasture site (34.8 spores/100 g). In a comparison of only pasture and forest sites, the size-class distributions of whole spores differ significantly ($N = 20$, Likelihood Ratio Chi-square = 1315, $P < 0.001$).

Transformed MPN estimates from both bioassay host species are significantly correlated (Table 3; Pearson $r = 0.750$, Bonferroni $P < 0.001$). Among propagule categories, however, only counts of whole spores are significantly correlated with MPN estimates from both hosts (Table 3; guava: Pearson $r = 0.659$, Bonferroni $P = 0.007$; onion: Pearson $r = 0.718$, Bonferroni $P = 0.001$).

DISCUSSION

Our results indicate that, notwithstanding some having been pasture for approximately two decades, the sites we examined contain VAM fungus inocula. As measured by guava and onion bioassays, mycorrhiza inoculum potential is relatively low in sites kept bare of vegetation for several years and in secondary forest, but is high in abandoned grass pasture (Table 1). These results are similar to those of Cuenca and Lovera (1992) who found that grasses and legumes planted on severely disturbed sites enhanced MIP. The pasture site we studied, however, is neither seriously eroded, nor severely overgrazed as was that studied by Janos (1988). Presence of whole spores is strongly, positively correlated with MIPs estimated from both guava and onion bioassays, which agree well with one another (Table 3), such that viable spores are likely to have been the principal form of VAM inoculum in our bioassays. Bioassay host growth responded significantly to the high MIP of the pasture site, notwithstanding that this is not the most fertile of the four sites (Table 1).

We expected that MIP would be low in bare sites, but the low MIP of the forest site is surprising. It may reflect a bias inherent in extractive bioassays. Soil collection inevitably disturbs root-and-hyphal networks in the soil. Because lowland wet tropical forest soils typically contain few spores (see Janos 1992), consistent with our findings (Table 2), hyphal networks likely are the primary form of inocula under forest. Jasper *et al.* (1989a, b) showed that soil disturbance reduced survival and infectivity of hyphae external to roots. Moreover, removal of the litter layer prior to sample collection, may have eliminated some VAM roots from our samples. Rose and Paranka (1987a) found that in a tropical wet forest in northeastern Brazil roots in the litter and humus layers supported a significantly higher proportion of VAM than roots in the mineral soil.

Neither of the aforementioned explanations is likely to account for the low MIP of sites kept bare of vegetation (Table 1) which are devoid of roots. Because of the lack of hosts on these sites we did not expect to find apparently viable propagules of VAM fungi (Tables 1 and 2). Presence of VAM fungus spores has been shown to be positively correlated with plant cover in a temperate-zone prairie (Anderson *et al.* 1983). Because of their large size, glomalean fungus spores are poorly adapted for wind dispersal in humid conditions (Gerdemann & Trappe 1974), so the presence of viable spores in our bare plots may be a consequence of activity by animal spore vectors such as rodents (Janos 1992) and ants (Janos 1993). If so, however, our small bare plots likely over-represent the spore input which might occur in large disturbed areas. Nevertheless, the preponderance in the bare sites of sporocarps and spore clusters which, in contrast to single spores, are commonly associated with ants and rodents is consistent with animal activity.

In all sites, spores empty of cytoplasmic contents or apparently parasitized are common (Table 2), but neither their presence nor that of sporocarps and spore clusters is significantly correlated with MIP (Table 3). Empty spores probably do not represent viable propagules, but are only recalcitrant, chitinous spore walls. In a lowland wet tropical environment, rapid loss of VAM fungal spores to predation by bacteria, saprophytic fungi, and soil fauna can be expected (Janos 1980b, see also Fitter 1985). Most of the spores in sporocarps and spore clusters also appeared to lack contents when ex-

amined microscopically (400×). Lack of contents, or a need for spores to be freed from sporocarps before germination may explain the lack of correlation of sporocarp and spore cluster presence with MIPs.

Another possible explanation for the low MIP of the forest site may be that the greenhouse environment or host species employed is unfavorable for spore germination or mycorrhiza formation by species of VAM fungi present in the forest soil. Although we did not quantitatively contrast glomalean fungus species composition among sites, because whole spores predominated in different size classes in forest versus pasture soil (Table 2), glomalean fungus species composition likely differs at least partially between these sites. Johnson *et al.* (1991) similarly reported that glomalean fungus species composition changed during temperate-zone secondary succession from field to forest, and Schenck *et al.* (1989) found a change in the VAM species composition with change from native cerrado vegetation in Brazil to an agroecosystem. Rose and Paranka (1987b) found *Glomus* spores under sugar cane in Brazil, although they recovered *Gigaspora* spores from adjacent forest. VAM fungus species display nearly no restriction of host ranges (Janos 1980a, 1987; Gianinazzi-Pearson & Diem 1982), but can differ markedly in tolerance of soil conditions (see Janos 1987) and, in consequence, in response to different host species (Bevege & Bowen 1975). Guava and onion in the greenhouse may present conditions more suitable for VAM fungus species that occur in the pasture site than for those that occupy the forest site.

All existing techniques for estimating mycorrhiza inoculum potential have inherent limitations (Sieverding 1991). Direct counts of mycorrhiza propagules such as spores, colonized roots, and external hyphae do not indicate how rapidly they are activated or how rapidly infective hyphae grow through soil. Neither can greenhouse, extractive bioassays assess conditions as they prevail in the field. MPN techniques homogenize the distribution of inocula through soil, and lose information on small-scale spatial heterogeneity (Janos 1992). Moreover, host species which differ in capacity for root spread before mycorrhiza formation may experience the same inoculum differently (Janos 1992). Notwithstanding these limitations, the agreement between our bioassays conducted with two different host species, and correlation of MPNs of propagules with counts of whole spores (Table 3) suggest that our study accurately represents the relative MIPs of bare and grass pasture sites. Low MIP of the forest site, however, may be an artifact.

Anthropogenic, large-scale disturbances that result in nonregenerating, highly degraded pastures as described by Uhl *et al.* (1988) need to be assessed for MIP because the presence and persistence of VAM fungus inocula in soil may be a key factor limiting tropical reforestation (Janos 1988, 1992). In a degraded Amazonian pasture, Nepstad *et al.* (1990) identified drought tolerance and ability to compete for limited soil resources as critical to the survival and growth of tree seedlings. Effective VAM colonization can improve drought tolerance (see Janos 1987, Nelson & Safir 1982), and may provide competitive advantages to tree seedlings (Janos 1985). Nepstad *et al.* (1990) suggested that strategies for accelerating forest regrowth should minimize inputs of capital and human labor and maximize the contribution of natural processes. We submit that creative and intelligent lowland wet tropical forest regeneration programs must consider and should maximize appropriate mycorrhiza inocula.

ACKNOWLEDGMENTS

We thank the Organization for Tropical Studies for the opportunity to conduct research in Costa Rica and the staff at La Selva Biological Research Station for field support; technical help in the lab was provided by D. Ianson, N. Mosier, J. Trappe and E. Cázares. We also wish to thank T. Bell, C. Colinas-González and K. Martin for statistical assistance.

LITERATURE CITED

ALEXANDER, M. 1982. Most probable number method for microbial populations. *In* Methods of soil analysis, Part 2. Chemical and microbiological properties: Agronomy Monograph no. 9 (2nd Edition). ASA-SSSA, 677 S. Segoe Road, Madison, Wisconsin.

ALLEN, M. F. 1991. The ecology of mycorrhizae. Cambridge University Press, Cambridge, England.

AN, Z.-Q., J. W. HENDRIX, D. E. HERSHMAN, AND G. T. HENSON. 1990. Evaluation of the "Most Probable Number" (MPN) and wet-sieving methods for determining soilborne populations of endogonaceous mycorrhizal fungi. Mycologia 82: 576–581.

ANDERSON, R. C., A. E. LIBERTA, L. A. DICKMAN, AND A. J. KATZ. 1983. Spatial variation in vesicular-arbuscular mycorrhiza spore density. Bull. Torrey Bot. Club 110: 519–525.

BEVEGE, D. I., AND G. D. BOWEN. 1975. Endogone strain and host plant differences in development of vesicular-arbuscular mycorrhizas. *In* F. E. Sanders, B. Mosse, and P. B. Tinker (Eds.). Endomycorrhizas, pp. 77–104. Academic Press, London, England.

BOWEN, G. D. 1980. Mycorrhizal roles in tropical plants and ecosystems. *In* P. Mikola (Ed.). Tropical mycorrhiza research, pp. 165–190. Clarendon Press, Oxford, England.

BROWN, M. F., AND E. J. KING. 1982. Morphology and histology of vesicular-arbuscular mycorrhizae. *In* N. C. Schenck (Ed.). Methods and principles of mycorrhizal research, pp. 15–21. The American Phytopathology Society, St. Paul, Minnesota.

CUENCA, G., AND M. LOVERA. 1992. Vesicular-arbuscular mycorrhizae in disturbed and revegetated sites from La Gran Sabana, Venezuela. Can. J. Bot. 70: 73–79.

FITTER, A. H. 1985. Functions of vesicular-arbuscular mycorrhizas under field conditions. New Phytol. 99: 257–265.

GERDEMANN, J. W., AND T. H. NICOLSON. 1963. Spores of mycorrhizal endogone species extracted from soil by wet-sieving and decanting. Trans. Brit. Mycol. Soc. 46: 235–244.

———, AND J. M. TRAPPE. 1974. The endogonaceae in the Pacific Northwest. Memoir No. 5. The New York Botanical Garden, New York, New York.

GIANINAZZI-PEARSON, V., AND H. G. DIEM. 1982. Endomycorrhizae in the tropics. *In* Y. R. Dommergues and H. G. Diem (Eds.). Microbiology of tropical soils, pp. 209–251. Martinus Nijhoff, The Hague, The Netherlands.

HEWITT, F. J. 1966. Sand and water culture methods used in the study of plant nutrition. *In* Technical communication, Revised Edition 2, No. 22. Commonwealth Agricultural Bureaux, London, England.

JANOS, D. P. 1980a. Vesicular-arbuscular mycorrhizae affect lowland tropical rain forest plant growth. Ecology 61: 151–162.

———. 1980b. Mycorrhizae influence tropical succession. Biotropica 12(suppl.): 56–64.

———. 1985. Mycorrhizal fungi: agents or symptoms of tropical community composition? *In* R. Molina (Ed.). Proceedings of the Sixth North American Conference on Mycorrhizae, pp. 98–103. Forest Research Laboratory, Corvallis, Oregon.

———. 1987. VA mycorrhizas in humid tropical ecosystems. *In* G. R. Safir (Ed.). Ecophysiology of VA mycorrhizal plants, pp. 107–134. CRC Press, Boca Raton, Florida.

———. 1988. Mycorrhiza applications in tropical forestry: are temperate-zone approaches appropriate? *In* F. S. P. Ng (Ed.). Trees and mycorrhiza, pp. 133–188. Forest Research Institute, Malaysia, Kuala Lumpur, Malaysia.

———. 1992. Heterogeneity and scale in tropical vesicular-arbuscular mycorrhiza formation. *In* D. J. Read, D. H. Lewis, A. H. Fitter, and I. J. Alexander (Eds.). Mycorrhizas in ecosystems, pp. 276–282. CAB International, Oxon, England.

———. 1993. Vesicular-arbuscular mycorrhizae of epiphytes. Mycorrhiza 4:1–4.

JASPER, D. A., L. K. ABBOTT, AND A. D. ROBSON. 1989a. Soil disturbance reduces the infectivity of external hyphae of vesicular-arbuscular mycorrhizal fungi. New Phytol. 112: 93–100.

———, ———, AND ———. 1989b. Hyphae of a vesicular-arbuscular mycorrhizal fungus maintain infectivity in dry soil, except when the soil is disturbed. New Phytol. 112: 101–108.

JOHNSON, N. C., D. R. ZAK, D. TILMAN, AND F. L. PFLEGER. 1991. Dynamics of vesicular-arbuscular mycorrhizae during old field succession. Oecologia 86: 349–358.

KORMANIK, P. P., AND A.-C. MCGRAW. 1982. Quantification of vesicular-arbuscular mycorrhizae in plant roots. *In* N. C. Schenck (Ed.). Methods and principles of mycorrhizal research, pp. 37–45. The American Phytopathology Society, St. Paul, Minnesota.

LINDERMAN, R. G. 1988. VA (vesicular-arbuscular) mycorrhizal symbiosis. ISI Atlas Sci. Anim. Plant Sci. 1: 183–188.

MORTON, J. B. 1988. Taxonomy of VA mycorrhizal fungi: classification, nomenclature, and identification. Mycotaxon 32: 267–324.

NELSON, C. E., AND G. R. SAFIR. 1982. Increased drought tolerance of mycorrhizal onion plants caused by improved phosphorus nutrition. Planta 154: 407–413.

NEPSTAD, D., C. UHL, AND E. A. SERRAO. 1990. Surmounting barriers to forest regeneration in abandoned, highly degraded pastures: a case study from Paragominas, Para, Brazil. *In* A. B. Anderson (Ed.). Alternatives to deforestation: steps toward sustainable use of the Amazon rain forest, pp. 215–229. Columbia University Press, New York, New York.

PANKOW, W., T. BOLLER, AND A. WIEMKEN. 1991. The significance of mycorrhizas for protective ecosystems. Experiencia (Basel) 47: 391–394.

PERRY, D. A., M. P. AMARANTHUS, J. G. BORCHERS, S. L. BORCHERS, AND R. E. BRAINERD. 1989. Bootstrapping in ecosystems. Bioscience 39: 230–237.

PIERCE, S. M. 1992. La Selva biological station history: colonization/land use/deforestation of Sarapiqui, Costa Rica. M.S. Thesis, Colorado State Univ., Fort Collins, Colorado.

PORTER, W. M. 1979. The "most probable number" method for enumerating infective propagules of vesicular-arbuscular mycorrhizal fungi in soil. Aust. J. Soil. Res. 17: 515–519.

RADULOVICH, R., E. SOLORZANO, AND P. SOLLINS. 1989. Soil macropore size distribution from water breakthrough curves. Soil Sci. Soc. Am. J. 53: 556–559.

ROSE, S. L., AND J. E. PARANKA. 1987a. The location of roots and mycorrhizae in tropical forest litter. *In* D. M. Sylvia, L. L. Hung, and J. H. Graham (Eds.). Mycorrhizae in the next decade: practical applications and research priorities, p. 165. 7th North American Conference on Mycorrhizae. Institute of Food and Agricultural Science, University of Florida, Gainesville, Florida.

———, AND ———. 1987b. Root and VAM distribution in tropical agricultural and forest soils. *In* D. M. Sylvia, L. L. Hung, and J. H. Graham (Eds.). Mycorrhizae in the next decade: practical applications and research priorities, p. 56. 7th North American Conference on Mycorrhizae. Institute of Food and Agricultural Science, University of Florida, Gainesville, Florida.

SALINAS, J. G., J. I. SANZ, AND E. SIEVERDING. 1985. Importance of VA mycorrhizae for phosphorus supply to pasture plants in tropical oxisols. Plant Soil 84: 347–360.

SCHENCK, N. C., J. O. SIQUIRA, AND E. OLIVEIRA. 1989. Changes in the incidence of VA mycorrhizal fungi with changes in ecosystems. *In* Vancura, V. and F. Kunc (Eds.). Interrelationships between microorganisms and plants in soil. Proceedings of an International Symposium. Elsevier, Science Publishing Co., Inc., New York.

SIEVERDING, E. 1991. Vesicular-arbuscular mycorrhiza management in tropical agrosystems. Deutsche gesellschaft für technische zusammenarbeit, Eschborn, Germany.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry. W. H. Freeman & Co., New York, New York.

SOLLINS, P., AND R. RADULOVICH. 1988. Effects of soil physical structure in solute transport in a weathered tropical soil. Soil Sci. Soc. Am. J. 52: 1168–1173.

———, G. P. ROBERTSON, AND G. UEHARA. 1988. Nutrient mobility in variable- and permanent-charge soils. Biogeochemistry (Dordr.) 6: 181–199.

———, F. SANCHO, R. MATA, AND R. SANFORD, JR. 1993. Soils of La Selva. *In* L. McDade (Ed.). La Selva: Ecology and natural history of a Neotropical rainforest, pp. 34–53. University of Chicago Press, Chicago, Illinois.

TESTER, M., S. E. SMITH, AND F. A. SMITH. 1987. The phenomenon of "nonmycorrhizal" plants. Can. J. Bot. 65: 419–431.

THOMAS, R. S., S. DAKESSIAN, R. N. AMES, M. S. BROWN, AND G. J. BETHLENFALVAY. 1986. Aggregation of a silty clay loam soil by mycorrhizal onion roots. Soil Sci. Soc. Am. J. 50: 1494–1499.

TORO, T. S., AND E. SIEVERDING. 1986. Evaluación cuantitativa y cualitativa de hongos formadores de micorriza vesiculo arbuscular en la región de Mondomo, Colombia. Suelos Ecuatoriales 16: 122–129.

UHL, C., D. NEPSTAD, R. BUSCHBACHER, K. CLARK, B. KAUFFMAN, AND S. SUBLER. 1990. Studies of ecosystem response to natural and anthropogenic disturbances provide guidelines for designing sustainable land-use systems in Amazonia. *In* A. B. Anderson (Ed.). Alternatives to deforestation: steps toward sustainable use of the Amazon rain forest, pp. 24–42. Columbia University Press, New York, New York.

WHITMORE, T. C. 1989. Tropical forest nutrients, where do we stand? A tour de horizon. *In* J. Proctor (Ed.). Mineral nutrients in tropical forests, pp. 1–13. Blackwell Scientific Publications, Oxford, England.