

IMPACTS OF EARLY- AND LATE-SERIAL MYCORRHIZAE DURING RESTORATION IN SEASONAL TROPICAL FOREST, MEXICO

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Abstract. Disturbance of vegetation and soil may change the species composition of arbuscular mycorrhizal fungi (AMF), which may in turn affect plant species responses to AMF. Seasonal tropical forest in Mexico is undergoing rapid conversion to early-successional forest because of increased wildfire and may require restoration. The responses of six early- and late-successional tree species were tested using early- and late-successional AMF inoculum. The plants were germinated in the shadehouse and received three inoculum treatments: (1) soil from a two-year-old burned site, (2) soil from a mature forest site, or (3) uninoculated controls. They were transplanted as seedlings to a site prepared by burning, and their growth was measured from September 1997 to November 2000. All six species had the greatest growth response to early-seral inoculum, but the response to late-seral inoculum varied. Two tree species, *Ceiba pentandra* and *Guazuma ulmifolia*, were smallest with late-seral inoculum, even smaller than the uninoculated plants, and the other species, *Brosimum alicastrum*, *Havardia albicans*, *Acacia pennatula*, and *Leucaena leucocephala*, had intermediate growth with late-seral inoculum. Of these, *Brosimum*, *Havardia*, and *Ceiba* occur in late-successional forest, and the others are early seral. Of the several growth measurements (height, cover, biomass, stem diameter), stem-diameter responses to inoculum were still significantly different into the third year for four of the species. The uninoculated plants became infected by residual inoculum in the burned experimental site within three months of transplanting, yet mycorrhizal responses persisted. The treatment size differences may be due to different species composition of the inocula. The early-seral inoculum was dominated by small-spored *Glomus* spp., while the late-seral inoculum had a higher density of large-spored Gigasporaceae. The latter are known from greenhouse experiments to promote a smaller plant-growth response than *Glomus*. Mature forest trees may withstand the carbon drain from Gigasporaceae better than establishing seedlings, so the growth patterns we observed with inoculum source are consistent with a rapidly growing successional forest, followed by slower-growing mature forest. The results suggest that early-seral AMF should be used when seedlings are inoculated for restoration, even for late-seral tree species.

Key words: *Acacia pennatula*; agriculture, slash and burn; arbuscular mycorrhizal fungi (AMF); *Brosimum alicastrum*; *Ceiba pentandra*; *Guazuma ulmifolia*; *Havardia albicans*; *Leucaena leucocephala*; Quintana Roo, Mexico; seasonal tropical forest; tropical forest restoration.

INTRODUCTION

Seasonal tropical forests are undergoing rapid conversion to agriculture faster than any other tropical ecosystem. Currently <10% of the original Mesoamerican seasonal tropical forest remains (Murphy and Lugo 1986, 1995), so restoration will be essential for preserving diversity and ecosystem functioning in this region. Various methods of restoration have been tested, including tree planting and the promotion of natural succession processes through vegetation management (e.g., Janzen 1988, Lugo 1988, Holl et al. 2000). My-

corrhizae are also likely to be an important component for restoring forests to former pastures and croplands, as most trees in tropical forests are mycorrhizal (Janos 1980a, Mikola 1980, Högberg 1982, Trappe 1987). Because soil disturbance can alter the functioning of mycorrhizae and, in turn, tree survival and growth, inoculation may be key in the restoration process. In this study, we examined the effects of inoculation with arbuscular mycorrhizal fungi (AMF) from early- and late-seral forests on early- and late-seral tree species in a seasonal tropical forest in Mexico.

Tropical trees have a wide range of responses to inoculation with AMF—from no significant increase in growth to obligately mycorrhizal (Janos 1980b, 1987, Siquiera and Saggin-Junior 2001). In some studies the obligately mycorrhizal species were mature forest trees, while early-successional trees were facultative or even had no growth response to inoculation (Janos

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1980b, Huante et al. 1993). Alternatively, other studies showed that the early-successional species were more responsive to AMF inoculum (Siquiera et al. 1998, Kiers et al. 2000, Zangaro et al. 2000). In those three studies the large-seeded late-successional trees were more dependent on their seed reserves than small-seeded early-successional trees in greenhouse trials. Although the long-term responses of trees in the field are more difficult to assess, they are important in understanding the role of mycorrhizae in succession and community dynamics.

Mycorrhizal inoculum is ubiquitous in natural soils, and is typically not limiting for naturally regenerating or restored trees unless the soil has been disturbed, causing reduced inoculum density or possibly changed fungal species composition. Janos (1980a) noted that nonmycorrhizal sedges colonized abandoned agricultural lands; these soils initially had reduced inoculum that increased over successional time. He hypothesized that mycorrhizal-dependent trees could not colonize until the inoculum attained sufficient density. Different sites and kinds of disturbances have different impacts on AMF inoculum. For instance, natural tree-fall gaps did not change inoculum density or AMF spore species in wet tropical forest (Guadarrama and Alvarez-Sanchez 1999) or in seasonal tropical forest (Allen et al. 1998) in Mexico, so natural gaps should not limit plant growth due to reduced inoculum. However, spore species were depleted in density and diversity in planted pastures compared to adjacent seasonal tropical forest (Allen et al. 1998). By contrast, pasture and forest inoculum were comparable in AMF spore species composition in two Costa Rican moist forests (Johnson and Wedin 1997, Picone 2000), but were higher in density in pasture than in another moist forest in Costa Rica (Fischer et al. 1994). Agricultural and grazing lands managed by burning (Gómez-Pompa 1980) may also have altered mycorrhizal inoculum, although field bioassay plants showed no reduction in percentage of root mycorrhizal infection under standard tropical agricultural practices (Maldonado et al. 2000, Rosemeyer et al. 2000).

Plant growth is affected by both the density and species composition of AMF inoculum. Various species assemblages of mycorrhizal fungi will promote different growth responses by the host plant species (van der Heijden et al. 1998), suggesting that any practices that change soil inoculum would potentially affect growth of inoculated or naturally colonizing plants. Reciprocal inoculation of host plant species with their associated and neighboring inoculum showed host-specific and inoculum-specific growth responses in tropical tree seedlings (Kiers et al. 2000) as well as temperate old-field plants (Bever et al. 2001). A Costa Rican tree species grew equally fast in forest and pasture soil inoculum, and both were better inocula than inoculum collected from the rhizosphere of a native fern (Asbjornsen and Montagnini 1994), but in another study forest inoculum

was not as effective as pasture inoculum in promoting plant growth (Fischer et al. 1994). These studies and others suggest that mycorrhizal fungi may control not only the biomass of individual species, but also the diversity of the vegetation community because of varying responses of individual species to inocula (Allen et al. 1995). The studies used various methods for assessing AMF inoculum (spore density and species composition, infected root length in the field, and infection or growth of bioassay plants), and they had contrasting conclusions about spore species diversity, density, and effectiveness as inoculum. In addition, they were done using whole soil or roots, and included associated microorganisms in the inoculum. Thus, they leave many open questions about changes and functions of inoculum from disturbed soils on forest dynamics during succession.

Few studies have followed the fate of AMF-inoculated tropical trees beyond the seedling stage, and even fewer have been done in the field. The latter include a study on *Inga edulis* in Costa Rica that demonstrated nearly a 10-fold height increase in inoculated compared to uninoculated plants eight months after transplanting to the field (Janos 1988); the legume *Centropogon tomentosum* in Brazil inoculated with rhizobium and with one effective and one ineffective AMF and transplanted to the field (Marques et al. 2001); and studies on citrus, which had improved growth after inoculated seedlings were transplanted to the field (Menge et al. 1981). Field inoculation studies have been done more frequently for herbaceous vegetation in tropical (Cuenca et al. 1998) and temperate sites for restoration purposes. Field restoration experiments present a unique opportunity to understand mycorrhizal responses, especially in situations where the inoculum has been reduced by disturbance. For instance, grasses seeded into inoculated soil in degraded land in Venezuela had increased biomass (Cuenca et al. 1998), and increased growth of seeded grasses and colonizing weeds, and successional patterns were changed with field inoculation on a Wyoming (USA) surface mine (Allen and Allen 1986, 1988). A field mycorrhizal transplant study of *Artemisia tridentata* into benomyl-treated soil showed survival of transplanted fungi up to four years (Weinbaum et al. 1996) and variable growth responses of the host plant depending upon the species of mycorrhizal fungus used (Allen et al. 1992).

We tested the hypothesis that AMF inoculum from early- and late-seral forest will promote different growth responses by early- and late-successional tree species in Quintana Roo, Mexico. The field site was chosen because it is in need of restoration. The area has vast expanses of forest burned within the last 25 years, with <1% mature forest remaining (Carranza Sanchez et al. 1996). We first characterized the occurrence of AMF genera in inoculum from recently burned and mature seasonal tropical forest, and followed changes in inoculum composition during the course of

the experiment. A field transplant experiment was performed to understand the long-term effects of the inoculum on tree growth, both from a practical standpoint for restoration purposes and to understand how early-seral and late-seral AMF might affect successional processes.

METHODS

Experimental site

The study was carried out at El Edén Ecological Reserve, a 1500-ha seasonal tropical forest reserve (description available online).⁵ The reserve is in the northeast Yucatán Peninsula (21°12.61' N, 87°10.93' W) ~30 km west of Cancún, in the state of Quintana Roo. The region has 1500–2000 mm annual precipitation, most falling during June–December (Isphording 1975), with semi-deciduous trees in the dry season. The soils are thin and rocky with highly organic pockets that lie over karstic limestone bedrock (Weidie 1985, Estrada Medina 2000). Line transects at the research site following experimental burning (see *Field-site preparation...*, below) revealed that 23% of the surface was exposed bedrock. Soil analyses are described below (see *Study species: Inoculation and plant propagation*).

The remnant local stands of mature forest are about 15 m tall with a predominance of trees in the family Leguminosae (Gómez-Pompa 1998, Schultz 2001). The major natural disturbance is hurricanes that increase woody debris and promote fire in the following dry season (Whigham et al. 1991, Whigham and Olmstead 2003). Most of the Reserve is secondary forest in a region with an increasing frequency of anthropogenic fires (Carranza Sanchez et al. 1996). The reserve has evidence of inhabitation by ancient Maya more than 2000 years ago (Fedick et al. 2000). Post-European settlement and recent uses include slash-and-burn agriculture, selective tree harvesting, and grazing (Allen et al. 2003). The rocky soils preclude pasture establishment; instead, the rhizomatous *Pteridium caudatum* (L.) Maxon colonizes following forest fires (Allen et al. 2003). The successional forest used as a study site was maintained in an early-seral state for cattle grazing until 1990. It burned during an extensive wildfire in 1995, two years prior to the experiment. The mature forest at the reserve, by contrast, has likely not burned in many decades or centuries (no fire scars are visible), although there is evidence of selective tree harvest from the late 19th century (Allen et al. 2003). Thus this forest has been occupied from early Maya times to the recent ranching era, but since the mature forest was not clearcut or recently burned it would have an intact soil microflora that suits the purposes of this study.

Study species

Even though successional stages are difficult to identify in seasonal tropical forests (Gomez-Pompa 1998),

the study of Schultz (2001) allowed us to select tree species based on their abundance during succession. Three of the species are early successional and may establish after fire, *Leucaena leucocephala* Benth., *Acacia pennatula* (Cham. & Schltdl.) Benth. (both Fabaceae), and *Guazuma ulmifolia* Lam. (Sterculiaceae); these three do not persist into late-successional stages. The other three species occur in late-seral forest, but have different requirements for stage of establishment. *Havardia albicans* (Kunth) Britton & Rose (Fabaceae) also colonizes after fire or other disturbance but persists into late succession, while *Brosimum alicastrum* Swartz (Moraceae) and *Ceiba pentandra* (L.) Gaertn. (Bombacaceae) colonize under an established canopy. Thus *Havardia* can be found at any stage of succession while the other species are early to mid-successional (*Leucaena*, *Acacia*, *Guazuma*) or mid- to late successional ones (*Brosimum*, *Ceiba*) (genus names will be used henceforth for the study trees).

Seeds of the six species were collected in March–June 1997 in or near the Reserve. Seed mass varied greatly. The seeds of *Acacia* averaged 49.2 mg, *Ceiba* seeds weighed 116 mg, *Guazuma* were 4.9 mg, *Leucaena* were 31.6 mg, *Havardia* were 68 mg, and *Brosimum* were the heaviest at 2.5 g. *Brosimum* seed germinated readily upon collection with no treatment. The others have thick seed coats that were nicked with a razor blade, resulting in nearly 100% germination for all the species.

Inoculation and plant propagation.—Two kinds of mycorrhizal inoculum were collected, late successional using soil from the mature forest, and early successional from a two-year-old burned forest. The mature forest was about 2 km distant from the early-successional forest. The successional-forest soil was collected adjacent to the planting site, which was dominated in some areas by *Pteridium caudatum*. The inoculum soil was collected from the top 10 cm of soil from each forest type from 5–6 locations within 100 m of each other. Inoculum consisted of AMF (arbuscular mycorrhizal fungi) spores, infected root fragments, and hyphae. The mycorrhizal spores were extracted by sucrose flotation (Allen et al. 1979) and counted and identified to genus (Schenck and Pérez 1988).

Additional soil was collected for nutrient analyses in both forest types. Soil samples of ~100 g ($n = 5$ samples in each forest type) were collected to 10-cm depth using a trowel, as coring is impossible in these rocky soils. Analyses included percentage organic matter by combustion (450°C), pH (50% water solution), total Kjeldahl N (Bremner and Mulvaney 1982), total P (Sah and Miller 1992, Meyer and Keliher 1992), and bicarbonate-extractable P (Olsen and Sommers 1982). Nitrogen and phosphorus analyses were done at the University of California, Davis, Analytical Laboratory (Davis, California, USA).

Seeds were planted 2–5 mm deep on 28 June 1997 in pots (height 15 × 6 cm diameter) holding approx-

⁵ URL: (http://maya.ucr.edu/pril/el_eden/Home.html)

imately 500-g dry-mass sterilized soil. The soil was collected near the planting site and steam sterilized for 2 h at 90°C. Three inoculum treatments were used—25 g of fresh late-seral forest soil, 25 g of fresh early-seral forest soil, and the uninoculated controls. The inocula were each homogenized to include root segments and soil, and placed ~3 cm beneath the surface of the steamed soil. Forty-two individuals of each of the six species were planted in each of the three inoculum treatments for the field restoration experiment. The total was 42 replicates \times 6 tree species \times 3 inoculum treatments = 756 tree seedlings. Extras of each species in each treatment were grown to account for mortality and to harvest for biomass in the shadehouse. Twelve of the 42 individuals were also harvested later in the field, leaving 30 replicates for each species in each treatment for long-term observation. The pots were placed in a shadehouse that reduced sunlight by 25%, and allowed rainfall to enter as a fine mist. The plants were grown for about 12 weeks, at which time they were from 2–15 cm tall, the smallest being *Guazuma*, the largest *Brosimum*.

Field-site preparation and experimental design

The field site was located within the 1995 wildfire, and it was prepared on 2 July 1997 by burning the woody resprouts. Vegetation was slashed in an area of 60 \times 90 m and burned. Soil samples to 10 cm deep were taken from each plot one week prior to the burn and again after the burn on 17 July. These were each subject to soil chemical analysis as above, mycorrhizal spore counts, and root infection (described below) before and after the burn.

The experimental design was seven replicate blocks each with the three inoculum treatments: (1) seedlings with late-seral forest inoculum, (2) seedlings with early-seral forest inoculum, and (3) uninoculated seedlings. The site was divided into a 3 treatment \times 7 block grid of 21 plots total, each plot 10 \times 10 m with a 4-m aisle between plots. Each inoculum treatment was placed in a separate plot rather than having inocula randomized within a plot, to reduce contamination among the inocula. Within the 10 \times 10 plot, the seedlings were planted in rows and columns of six species each, for 36 plants per plot in a Latin-square design. There was a 2-m spacing between seedlings.

The seedlings were transplanted to the field 13–16 September 1997 by placing them in holes dug large enough to accommodate the 15 cm \times 6 cm diameter root ball. They were each watered with about 1 L initially, but were not watered again as precipitation was abundant. The annual precipitation at El Edén for 1998–2000 was 2239, 3323, and 2545 mm, respectively—three years with high hurricane and tropical-storm activity. Each seedling was surrounded with a plastic mesh plant protector to avoid deer (*Mazama americana*) browsing. The protectors were 40 cm tall by 10-cm diameter with a 1-cm mesh that did not pre-

vent insect herbivory. After the seedlings reached the height of the protective mesh, the mesh was removed (beginning in December 1997 for the largest species, April 1998 for the smallest). No deer browsing was observed until the second growing season, and limited insect herbivory was noted on occasional seedlings. Seedlings that died within a month of planting were replaced, but after that no seedlings were replaced. However, initial mortality was low; only seven of the smallest *Guazuma* (with late-successional inoculum) and individuals of three other species were replaced. The resprouting vegetation around the planted seedlings was weeded at approximately 2–3 week intervals during the rainy season with a machete. Lianas were especially abundant, and 1–2 lianas were removed near each transplant at each weeding.

Ten additional plants of each species in each mycorrhizal treatment in the shadehouse were measured and shoots and roots were harvested in September 1997. They were dried to constant mass at 65°C and weighed. Leaves were separated from stems, and were analyzed for tissue Kjeldahl N (Isaac and Johnson 1976, Carlson et al. 1990) and P (Meyer and Keliher 1992, Sah and Miller 1992). Roots were harvested from five extra individuals of each species in each treatment, and they were examined for mycorrhizal infection. The roots were stained with trypan blue stain (Kormanik et al. 1980) and microscopically assessed for the presence of mycorrhizal hyphae, vesicles, and arbuscules (Allen 1983). Presence of nonmycorrhizal fungi was also assessed, including potential pathogens. Other indicators of plant health, including root browning, leaf color, lesions, or wilting, were noted.

Measurements

The height of the seedlings was measured immediately after they were planted on 17–18 September, then monthly during the first season when growth rates were rapid, and less frequently later on. In addition, crown width and length (perpendicular) was measured after the plant protectors were removed and seedlings were >10 cm in width. Crown cover was calculated using the formula for an ellipse. Stem diameter was measured with calipers at 10-cm height when the stems were larger than 1-cm diameter.

A subset of seedlings ($n = 6$ individuals) for each species in each treatment was destructively harvested in the field on 18 December 1997 and again on 12 April 1998 to assess shoot biomass and root infection. These were from blocks 1 and 7, respectively. The blocks were first analyzed statistically to assure there were no block-by-treatment interactions. This left 30 replicates of each species in each treatment for continued field measurements. The roots were excavated with a trowel to 10 cm deep from the harvested plants, and only those attached to the plants were used (to assure proper identification). Roots were stained and assessed for mycorrhizal infection as above. Roots were also collected

nondestructively on later dates using a trowel at the base of each transplant, and taking care to collect roots that were connected to the seedling. One plant per treatment of each species was collected, for five replicates in the five remaining unharvested blocks. Roots and soil ($n = 5$ individuals) of *Brosimum* and *Havardia* were collected from plants in the mature forest during the same times as the experimental plants. Rhizosphere soil to 10 cm depth was collected with the roots for analysis of mycorrhizal spores. Spores were extracted from 5 g of soil by sucrose flotation as described above, and identified to genus and counted (individual spores were counted for sporocarpic forms, mainly *Sclerocystis*).

Statistical analyses

Data were checked for normality and arcsine transformed as needed, and analyzed using repeated-measures ANOVA to show trends in height, crown cover, and stem diameter over time. Repeated-measures analyses were performed separately for each of the years, as growth patterns were different in different years. Potential block-by-treatment interactions were statistically tested, but the block-interaction P value in the repeated-measures ANOVA was not significant for any of the tree species ($P > 0.05$). ANOVA was used to show statistical differences in percentage mycorrhizal infection and spore density on each measurement date (the soil and root samples were from different individual trees each time, so this was not a repeated measure). The biomass harvest data were used to calculate relative growth rate (RGR; Hunt et al. 2002). RGR was also calculated using the height data. Both sets of RGR data were subject to ANOVA to test effects of inoculum treatment on growth rate of each species. Significant differences for the ANOVAs are shown using the least significant difference at $P = 0.05$ ($LSD_{0.05}$) (Zar 1974).

RESULTS

Plant growth response to mycorrhizae

Height growth, crown cover, stem diameter, and relative growth rate of all species responded significantly to inoculum during one or all three years (Figs. 1, 2, and 3). The early-successional inoculum provided the greatest growth stimulus for tree-seedling height growth, but the second-best inoculum varied by host plant species (Fig. 1). Four of the species, *Leucaena*, *Acacia*, *Havardia*, and *Brosimum*, had a pattern of greatest height with early-seral inoculum, second best with late-seral inoculum, and smallest when uninoculated. The other two species, *Guazuma* and *Ceiba*, also had greatest height with early-seral inoculum, but were smallest with late-seral inoculum. The height differences were most pronounced and statistically significant for all species during the first growing season (September 1997 to February 1998). During the second and third years (April to November 1998, April 1999

to October 2000) three species continued to have early-seral inoculated trees as the tallest, namely *Guazuma*, *Ceiba*, and *Havardia*. *Brosimum* decreased in mean size due to deer herbivory in 1999–2000 and suffered high mortality, with 4–5 survivors of $n = 30$ individuals per inoculum treatment. Terminal buds were removed from most trees, and some trees were largely defoliated. Mortality of *Guazuma* was also fairly high by October 2000, with 22 survivors in late-seral inoculum, 27 in early-seral, and 25 in the uninoculated treatment. The other four species had nearly 100% survival.

Crown cover was measured beginning June of the second growing season when all plant protectors were removed and the tree seedlings were large enough. *Brosimum* had a small crown (<10 cm across) and was not measured. *Guazuma* and *Ceiba* had significantly larger canopies for the early-seral than for the late-seral inoculum treatments in both the second and third years using repeated-measures ANOVA (Fig. 2). The other three species' canopies were not significantly different by treatment in either the second or third years.

Stem diameter at 10 cm height was the most consistent measurement to assess species response to mycorrhizal treatment. Four of the five measured species had significantly larger stems with early-seral inoculum by the final measurement in October 2000 based on repeated-measures $P < 0.05$ (Fig. 3). Data are shown for 1999–2000, as stems were too thin to measure in all individuals earlier, and *Brosimum* still had stems <10 mm by the third year. The patterns of stem diameter were similar to those of height and cover, with the largest stems in the early-seral inoculum for all five measured species. Uninoculated *Leucaena* had the thinnest stems among the three inoculum treatments, while the other three species (*Guazuma*, *Ceiba*, *Havardia*) by October 2000 had the thinnest stems when treated with late-seral inoculum.

The relative growth rates (RGR) of shoots were calculated from seed mass at shadehouse planting time (June 1997) through the first harvest from shadehouse pots in September 1997 (field transplanting time), the second harvest in the field in December 1997, and the third harvest in April 1998 (Table 1). Only two of the six species (*Ceiba*, *Guazuma*) showed significant effects of mycorrhizal inoculum on RGR during the three months in the shadehouse, June–September 1997. For *Ceiba* the late-seral inoculated plants had smaller RGR than the other two treatments; for *Guazuma* the early-seral inoculated plants had greater RGR than the other two treatments. All six species except *Acacia* had significant treatment differences in RGR during the first three months in the field, September–December. In each case the uninoculated plants had the lowest RGR, the early-seral inoculated plants had greatest RGR, and the late-seral inoculated plants were either intermediate, equal, or lowest in the case of *Guazuma*, reflecting the height growth curves (Fig. 1). During the dry season,

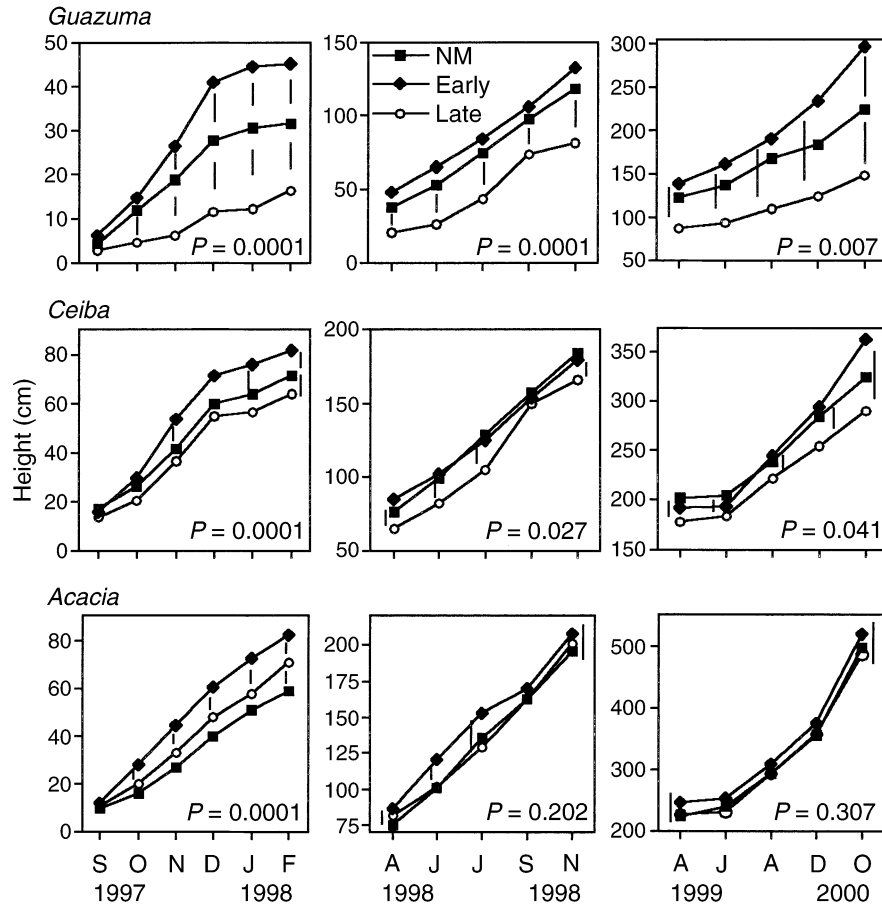


FIG. 1. Heights of six tree species over time, inoculated as seedlings with soil from early-seral or late-seral forest, or grown in sterile soil and initially nonmycorrhizal (NM). Seedlings were inoculated in the shadehouse and transplanted to the field in September 1997. Vertical bars show least significant difference at $P = 0.05$ ($LSD_{0.05}$) for each date. P values indicate treatment differences with a repeated-measures ANOVA. The x -axes of the individual rows of panels are drawn to show each of the first three growing seasons separately, with one point (October) from the fourth (2000) growing season added to the 1999 panel. Note different values of y -axes, which show different heights during different growing seasons.

December 1997–April 1998, the late-seral inoculated plants had lowest RGR for three species, *Leucaena*, *Guazuma*, and *Brosimum*. There were no significant differences during this growth period for the other three species. The net effect of higher RGR of early-seral inoculated plants in the wet season and lower RGR during the dry season, was that the treatments for *Leucaena* and *Brosimum* tended to become more similar, with no statistical differences in height or cover the second season for *Leucaena*. For *Guazuma*, the RGR differences that occurred during shadehouse growth continued through the first year, and into the second and subsequent years (Figs. 1 and 2). Overall, *Brosimum* had the lowest RGR values, but had the tallest seedlings in the shadehouse (15 cm). This was related to the large seed size, as it also had the heaviest seeds (2.5 g/seed) by several orders of magnitude. The RGR of *Guazuma* was highest in the shadehouse, even

though this species had the shortest seedlings (2–4 cm) but these began from the smallest seeds (4.9 mg).

The RGR analysis for height (Table 2) shows that the initial height growth promoted by early-seral inoculum was maintained only during the first season (September 1997–February 1998). Four of the species—*Guazuma*, *Ceiba*, *Acacia*, and *Havardia*—had significantly greatest height growth during the first season. By the second or third season the height growth of the late-seral or uninoculated plants of these species became relatively more rapid, explaining why these treatments became more similar in height to the early-seral inoculated plants over time. This may be related to the natural infection process of soil-borne inoculum in the burned field. In the case of *Acacia* there were no longer differences in height by the second season (Fig. 1). In the case of *Leucaena* only biomass RGR reflected the treatment effect of the inocula in the September–December 1997

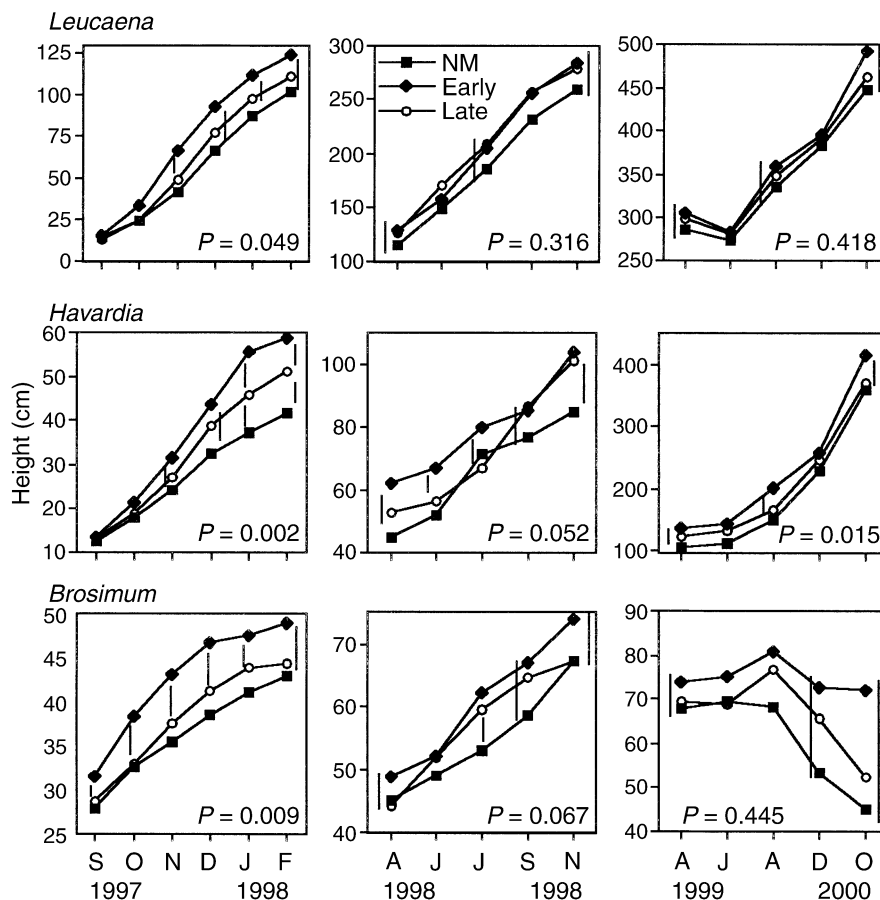


FIG. 1. Continued.

portion of the first season (Table 1), as there were no significant differences in height growth RGR.

Plant and soil nutrients

The leaf N and P analyses showed that mycorrhizal inoculation did not significantly increase the concentration of nutrients in the December 1997 harvest (Fig. 4). For three of the six species the early-seral inoculum treatment had significantly lower P than the uninoculated plants, with late-seral plants having intermediate levels. This suggests a dilution effect of higher biomass on P concentration, and that mycorrhizal infection did not cause increased P uptake. Overall, *Ceiba* had the highest P concentrations, while *Havadia* had the lowest ($P = 0.0001$ for differences in phosphorus concentrations among species). The N concentrations were not significantly different by treatment within any of the species, but some species had higher concentrations of N than others. *Leucaena* was especially high at 4.1% N, while *Brosimum* was the lowest at 2.5% ($P = 0.0001$). Since the uninoculated plants had already become contaminated by residual mycorrhizae at this harvest date (Fig. 5), we expected no additional effects of mycorrhizae on P uptake. The inoculum treatments

were in effect becoming more similar by contamination, and no further nutrient analyses were done.

Soil N and P were analyzed from the mature forest and in the experimental plot before the burn, after the burn, and two years later. Both mature and successional forest had moderate to high bicarbonate-extractable P, surprising for a calcareous soil, and they had high total P (Table 3). They also had high total N and very high organic matter. Values of organic matter, total N, total P, and extractable P were not significantly different between the forest and the experimental plot prior to the burn (June 1997), even though the vegetation of the preburn plot was recovering from a 1995 wildfire. The organic matter decreased from 34 to 15.9% in the research plot following the experimental burn in July 1997, but neither extractable P nor total N declined after burning. The percentage organic matter recovered two years following the experimental burn, to a level slightly higher than before the burn. This may be an artifact of the experiment, as resprouting vegetation was weeded around the transplants, adding an elevated amount of organic matter. The pH was slightly basic (Table 3).

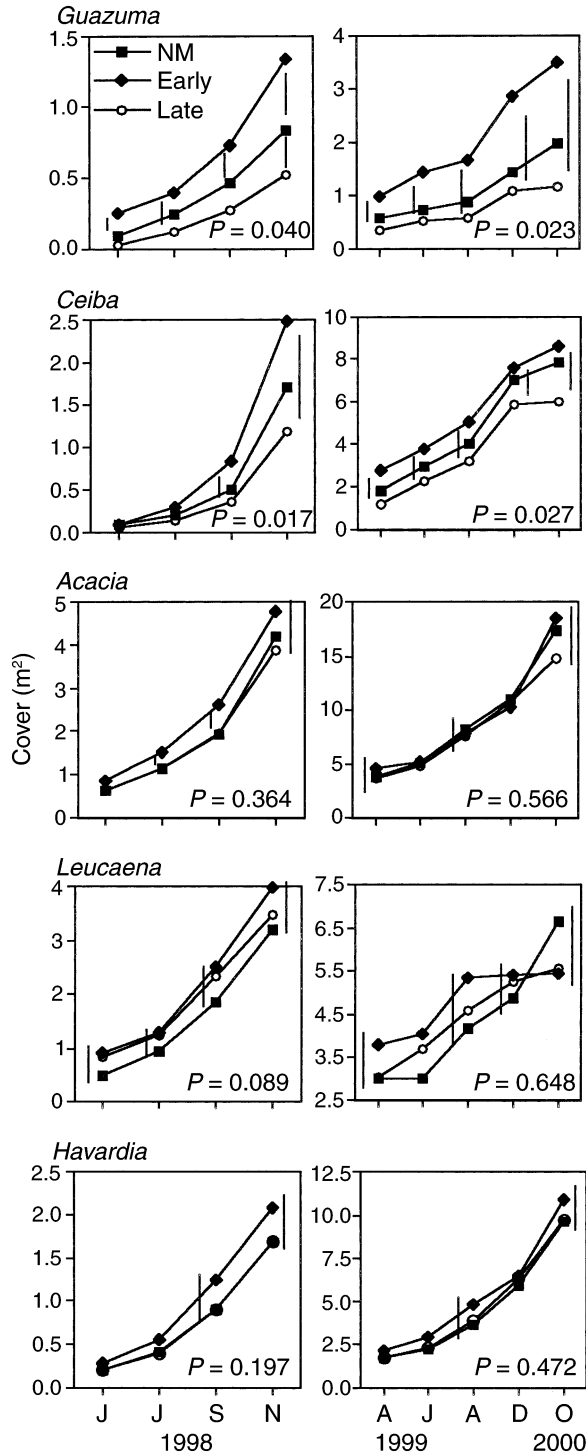


FIG. 2. Crown cover (maximum width by perpendicular length) of five tree species over time (*Brosimum* was not measured because of small stature), inoculated as seedlings with soil from early-seral or late-seral forest, or grown in sterile soil and initially nonmycorrhizal (NM). Seedlings were inoculated in the shadehouse and transplanted to the field in September 1997. Measurements were begun after trees were 10 cm in width. Note different values of y-axes. Vertical bars are $LSD_{0.05}$ for each date. *P* values indicate treatment differences with a repeated-measures ANOVA.

Mycorrhizal infection and spores

The mycorrhizal spore density of the preburn plot (June 1997) was 4.0 ± 0.6 spores/g dry soil (mean \pm SE; $n = 21$ replicate soil samples). The spore density after the burn was 0.8 ± 0.2 spores/g the following September at planting time. Only spores of *Glomus* were found immediately following the burn. In spite of the low postburn spore density, there was still enough inoculum to cause root infection of $32.5 \pm 8.6\%$ ($n = 10$ replicate soil samples of a random sample of roots from resprouting vegetation in September 1997). Some vesicles (characteristic of *Glomus*) were found in these roots, but no auxilliary cells or thick hyphae (characteristic of Gigasporaceae, Hart et al. 2001). This did not represent the inoculum density across the plot, only of those areas where resprouts occurred, with a low cover of only 1% vegetation at this time. Thus we fully expected that the transplanted seedlings would rapidly become contaminated by the background soil inoculum.

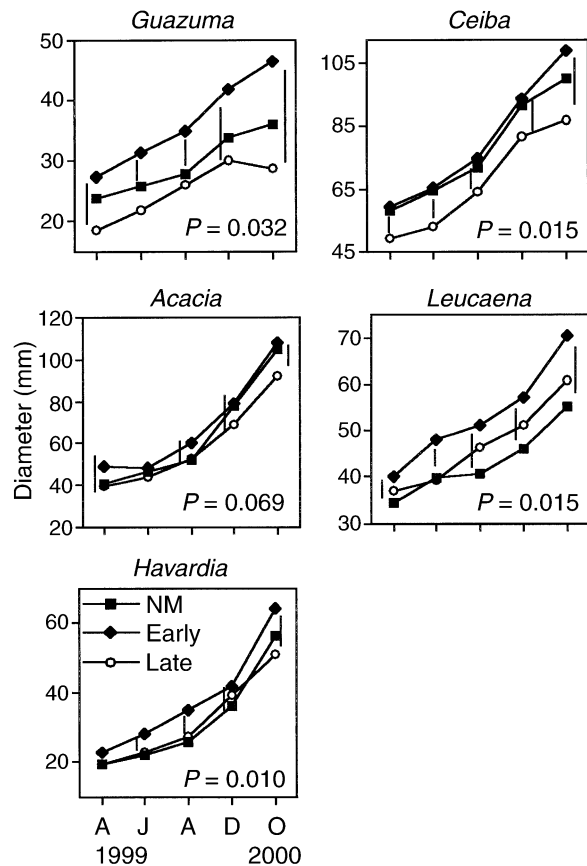


FIG. 3. Stem diameter at 10-cm height of five tree species, inoculated as seedlings with soil from early-seral or late-seral forest, or sterile soil and initially nonmycorrhizal (NM). Measurements were begun when all individuals of a species had stem diameter >10 mm. The sixth species, *Brosimum*, had most individuals with a diameter <10 mm.

TABLE 1. Relative growth rate based on biomass showing differences among mycorrhizal inoculum treatments (early- and late-seral inoculum and uninoculated [NM = nonmycorrhizal]) within each of three time intervals for six tree species.

Time interval	Relative growth rate, RGR [†] (mg·g ⁻¹ ·d ⁻¹)			P	LSD _{0.05}
	NM	Early	Late		
<i>Guazuma</i>					
Jun–Sep 1997	45.3 ^a	49.1^b	41.8 ^c	0.0001	2.4
Sep–Dec 1997	18.0 ^a	26.4^b	5.7 ^c	0.0001	7.4
Dec 1997–Apr 1998	6.1 ^a	3.2 ^b	10.3^c	0.0001	2.5
<i>Ceiba</i>					
Jun–Sept 1997	22.2^a	21.4^a	18.1 ^b	0.001	2.2
Sep–Dec 1997	27.0 ^a	33.9^b	26.3 ^a	0.005	4.9
Dec 1997–Apr 1998	14.3	12.5	14.0	0.222	NS
<i>Acacia</i>					
Jun–Sep 1997	8.0	13.7	12.3	0.140	NS
Sep–Dec 1997	45.3	51.0	46.6	0.107	NS
Dec 1997–Apr 1998	19.5	17.2	17.0	0.154	NS
<i>Leucaena</i>					
Jun–Sep 1997	44.3	44.3	43.9	0.125	NS
Sep–Dec 1997	19.6 ^a	27.9^b	23.7 ^{ab}	0.003	4.6
Dec 1997–Apr 1998	14.4^a	10.2 ^b	13.3^a	0.002	2.4
<i>Havardia</i>					
Jun–Sep 1997	17.3	18.2	17.4	0.760	NS
Sep–Dec 1997	26.4 ^a	30.6^b	29.0 ^{ab}	0.008	2.7
Dec 1997–Apr 1998	8.3	8.9	7.9	0.641	NS
<i>Brosimum</i>					
Jun–Sep 1997	5.3	5.4	5.5	0.613	NS
Sep–Dec 1997	2.8 ^a	3.9^b	3.3 ^{ab}	0.043	0.9
Dec 1997–Apr 1998	3.0^a	2.1 ^b	2.4 ^b	0.006	0.6

[†] RGR is based on biomass from seed mass (planted June 1997), shadehouse harvest corresponding to field planting date (September 1997), and two field harvests (December 1997 and April 1998). Values in boldface are the highest within each time interval. Data with the same lowercase letter superscripts are not significantly different among treatments within a time interval based on least significant difference at $P = 0.05$ (LSD_{0.05}). NS = nonsignificant.

The mycorrhizal infection levels varied among the six tree species, but showed some similarity of patterns (Fig. 5). The uninoculated treatments had infection of 0–1% following 12 weeks in the shadehouse on September 1997, the date of transplanting to the field. The shadehouse values for individual inoculated species ranged from 1% for *Havardia* to 55% for *Leucaena*. The plants with either early- or late-seral inoculum generally continued to have higher infection than the uninoculated plants through the first 2–3 sample dates until April or September 1998. After that there were few or no significant differences among inoculum treatments. In December 1998 all of the plants experienced a reduction in infection nearly to 0%, which mimicked the low values found in individuals of *Havardia* and *Brosimum* collected in the mature forest. These low values occurred even though the soil was moist and fine, and white roots were abundant. The values of the two species from mature forest somewhat mimicked the general trends in infection of the planted trees, except for a low value in April 1998. These two species were averaged together to show infection patterns in the mature forest, as their levels of infection were not significantly different from each other. Observations of

vesicles and arbuscules were <10% of the root length for any species on any date of observation, and usually only 0–2%. No arbuscules were observed in the September 1997 shadehouse seedlings and only a few vesicles. Vesicles and arbuscules were virtually absent during dry-season observations, especially April and December, in planted seedlings and forest trees.

We also made note of other species of fungi that occurred in the roots of the transplants and the trees from mature forest. These were mostly unidentified, but *Pythium* spp. and *Phytophthora* spp. were present in mature forest and transplanted seedlings that were inoculated or uninoculated. Fewer than 1 out of 30 of the samples (5 replicates × 6 species) observed on any date had any potential pathogen present, and where present the potential pathogens constituted only 1–2% of any root sample observed. In addition, none of the seedlings exhibited symptoms associated with these root pathogens, such as wilting, browning of roots or leaf tips, or leaf drop.

The spore composition by genus at the time of seeding in the shadehouse, June 1997, was assessed from the field inoculum for each treatment (Fig. 6). The late-seral inoculum had significantly higher densities of the

TABLE 2. Relative growth rate (RGR) based on height data of Fig. 1 showing differences among mycorrhizal inoculum treatments (early- and late-seral inoculum, and uninoculated [NM = no mycorrhizal inoculum]) within each of three time intervals between September 1997 and December 1999 for six tree species.

Time interval	RGR (mm·cm ⁻¹ ·d ⁻¹)			P	LSD _{0.05}
	NM	Early	Late		
<i>Guazuma</i>					
Sep 1997–Feb 1998	0.109 ^{ab}	0.122^b	0.098 ^a	0.039	0.019
Apr 1998–Nov 1998	0.045 ^a	0.040 ^a	0.063^b	0.0001	0.009
Apr 1999–Dec 1999	0.014	0.019	0.008	0.116	NS
<i>Ceiba</i>					
Sep 1997–Feb 1998	0.086 ^a	0.099^b	0.088 ^{ab}	0.054	0.012
Apr 1998–Nov 1998	0.035 ^a	0.033 ^a	0.043^b	0.025	0.007
Apr 1999–Dec 1999	0.016	0.019	0.016	0.349	NS
<i>Acacia</i>					
Sep 1997–Feb 1998	0.105 ^a	0.118^b	0.111 ^{ab}	0.037	0.009
Apr 1998–Nov 1998	0.041^a	0.032 ^b	0.041^a	0.0001	0.004
Apr 1999–Dec 1999	0.021	0.021	0.022	0.680	NS
<i>Leucaena</i>					
Sep 1997–Feb 1998	0.113	0.126	0.123	0.115	NS
Apr 1998–Nov 1998	0.034	0.032	0.031	0.562	NS
Apr 1999–Dec 1999	0.013	0.013	0.011	0.473	NS
<i>Havardia</i>					
Sep 1997–Feb 1998	0.069 ^a	0.086^b	0.080 ^{ab}	0.171	0.011
Apr 1998–Nov 1998	0.025	0.021	0.026	0.427	NS
Apr 1999–Dec 1999	0.039^a	0.032 ^b	0.033 ^b	0.031	0.006
<i>Brosimum</i>					
Sep 1997–Feb 1998	0.028	0.030	0.026	0.420	NS
Apr 1998–Nov 1998	0.019	0.018	0.018	0.813	NS
Apr 1999–Dec 1999

Notes: Values in boldface are the highest within each time interval. Data with the same lowercase letter superscripts are not significantly different among treatments within a time interval based on LSD_{0.05}. Mortality of *Brosimum* was high during 1999 from deer browsing, so RGR was not calculated.

large-spored Gigasporaceae (*Gigaspora* plus *Scutellospora*, 100–250 μm) and *Acaulospora* (110–200 μm), while the early-seral inoculum had few of these species. Both inoculum sources had approximately equal densities of *Glomus* (50–100 μm), ~ 2 spores/g soil. Over time the densities of spores increased from none initially in the rhizospheres of uninoculated plants. For *Sclerocystis* (80–100 μm) and *Glomus* there were no significant differences among the treatments, and *Acaulospora* was significantly higher in mature-forest inoculum at two observation dates. The Gigasporaceae continued to be higher in the rhizospheres of plants inoculated with late-seral soil through July 1999. At the final assessment in November 2000 there was no longer a significant difference among the treatments in counts of Gigasporaceae. Counts of *Glomus* in November 2000 were about one order of magnitude higher than previous dates (~ 50 spores/g), although the other genera were within the same order of magnitude during all the observations. Because of the large differences in size, we also calculated the biovolume of the different species. The Gigasporaceae spore volume averaged $18 \times 10^{-4} \text{ mm}^3$ per spore, *Sclerocystis* was $3.8 \times 10^{-4} \text{ mm}^3$, *Acaulospora* was $25 \times 10^{-4} \text{ mm}^3$, and *Glomus* was $2.8 \times 10^{-4} \text{ mm}^3$. Although the Gigaspor-

aceae were not very abundant, they contributed almost as much total volume as the small-spored *Glomus* combined, except in November 2000 when *Glomus* sporulated profusely (Fig. 6).

Spore genus data were collected separately for each host plant, and we noted that all fungal genera were associated with all host plants. Since there was no differential association of host plants with spore genera, we show only the mean values of spores in the rhizospheres of the combined six tree species, rather than by individual tree species (Fig. 6). A further analysis of spore composition by species is underway from prepared slides that may show specialization of certain spore species for certain tree species. We have tentatively distinguished some 43 arbuscular mycorrhizal taxa to date.

DISCUSSION

Successional models of arbuscular mycorrhizal fungi (AMF) suggest that as late-seral plant species colonize, inoculum density increases and later seral species of fungi colonize as well (Janos 1980a, Allen 1991, Hart et al. 2001). Thus our initial working hypothesis was that late-seral fungi would be beneficial for plant establishment, especially of the late-seral tree species,

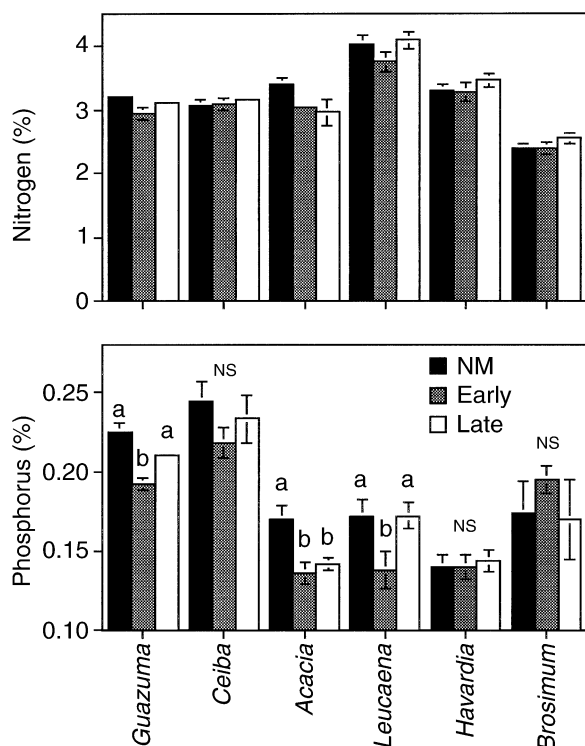


FIG. 4. Percentage of N and P in leaves of plants harvested from the field in December 1997, three months after planting. Treatments are inoculated as seedlings with soil from early-seral or late-seral forest, or grown in sterile soil and initially nonmycorrhizal (NM). Error bars are ± 1 SE. Different lowercase letters show significantly different values for P within a species; NS = significant.

but clearly this was not the case here. Early-seral inoculum was more beneficial in all cases. The poor response of *Ceiba* and *Guazuma* to late-seral inoculum was also surprising, as these plants were even smaller than uninoculated plants. *Ceiba* is a mid- to late-seral tree, while *Guazuma* is early to mid-seral, so there is no specific beneficial relationship between late-seral AM fungi and late-seral trees based on this set of tree species. In addition, both early- and late-seral trees had similarly high positive responses to early-seral inoculum, unlike other studies where either the early-seral trees (Siqueira et al. 1998, Kiers et al. 2000, Zangaro et al. 2000) or the late-seral trees (Janos 1980b, Huante et al. 1993) derived greater growth benefits from inoculation.

Most prior studies that compared early- and late-seral tree response used only early-seral or pot-cultured inoculum (Janos 1980a, Huante et al. 1993, Siqueira et al. 1998, Zangaro et al. 2000). Studies using late-seral inoculum have had mixed results; forest inoculum was better than pasture inoculum for an early-seral tree (Fischer et al. 1994), but the two sources of inocula produced similar growth in a late-seral tree (Asbjornson and Montagnini 1994). The study of Kiers et al. (2000) had species-specific interactions, with late-seral

inoculum best for a pioneer tree in one case, and early-seral inoculum best for a mature-forest tree in another. Our study, by contrast, showed a consistent growth benefit of the early-seral inoculum.

A number of studies have recognized changes in AM species composition during succession (Nicolson and Johnston 1979, Allen et al. 1987, 1993b), but detailed analyses of the functions of AMF from different stages are more recent (Hart et al. 2001). In this study we used whole-soil inoculum, so we added not only mycorrhizal fungi, but all of the soil organisms. Reduced growth responses from soil inoculum may occur if the mycorrhizal fungus acts as a carbon drain, but in whole soil any growth reductions may also be attributed to soil pathogens (Allen et al. 1993a). Two alternative hypotheses on plant response to the inocula are (1) the early- and late-seral fungi have different effects on plant growth, and (2) there are other species of microorganisms or edaphic factors in the early and late-stage soils that also affect plant growth.

The hypothesis that early- and late-successional fungi had different effects on plant growth is supported by several lines of evidence. First, *Scutellospora* and *Gigaspora* were more abundant in the late-successional than the recently burned forest, and in the restoration plot they maintained their relatively higher abundance until July 1999, almost two years after planting. Disturbance is known to change the species composition of mycorrhizal fungi, including loss of the large-spored Gigasporaceae and an increase in the generally smaller *Glomus* spp. This occurred, for instance, in soils impacted by anthropogenic nitrogen deposition in southern California, where large-spored species declined with high soil N (Egerton-Warburton and Allen 2000, Egerton-Warburton et al. 2001). *Glomus* spp. also dominated in recently mined, revegetated lands in Australia (Brundrett et al. 1999) and Montana (Allen et al. 1987), whereas nearby undisturbed sites had a diversity of large-spored fungi. Pastures planted with exotic grasses on the west coast of Mexico had mainly *Glomus* species compared to the higher diversity in seasonal tropical forest (Allen et al. 1998). This contrasts with results for Costa Rican grasslands and nearby wet tropical forest, which were similar in AMF spore species composition (Johnson and Wedin 1997, Picone 2000).

While not all disturbances in tropical forest or elsewhere have caused a shift in AMF species composition, we documented a difference in soil inoculum in recently burned soils compared to mature forest caused by a reduced density of large-spored Gigasporaceae. The Gigasporaceae had <1 spore/g soil, but their large size ensured that they had a similar volume as the many small *Glomus* spores did. Biovolume is a better approximation of microbial biomass than spore density (Van Veen and Paul 1979, Allen and MacMahon 1985), and larger spores give rise to more germ tubes that can initiate infections in roots (Gerdemann 1968). Gigasporaceae produced four times more extraradical hyphae

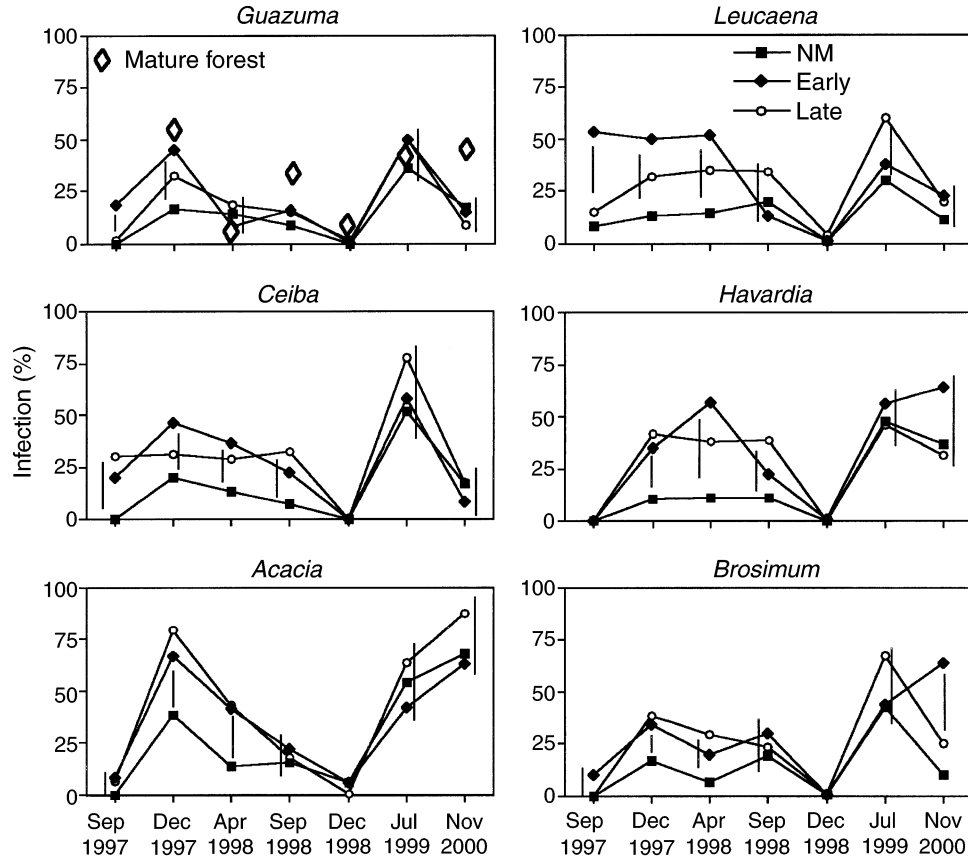


FIG. 5. Percentage of mycorrhizal infection over time of each of six tree species that were inoculated as seedlings with soil from early-seral or late-seral forest, or grown in sterile soil and initially nonmycorrhizal (NM). Each data point is based on $n = 5$ individual plants except for *Brosimum* on the last date, when only four plants survived per treatment. Bars are $LSD_{0.05}$ to compare treatment means for each individual sample date.

TABLE 3. Soil measurements in the research plot pre- and postburn and two years following planting, and in the inoculum soil collected from the mature forest.

Soil characteristic	Mature forest, Inoculum soil, 15 June 1997	Restoration plot		
		Preburn, 14 June 1997	Postburn, 4 July 1997	At two years, 12 July 1999
pH	7.2	7.7	7.6	7.5
Total P ($\mu\text{g/g}$)				
Mean	710.6	564.6	591.1	no data
1 SE	88.0	55.2	49.8	
Extractable P ($\mu\text{g/g}$)				
Mean	20.2 ^a	29.1 ^b	29.4 ^b	14.5 ^c
1 SE	6.4	3.0	2.5	1.6
Organic matter (%)				
Mean	26.8 ^a	34.0 ^a	15.9 ^b	43.2 ^c
1 SE	6.8	1.2	0.7	1.1
Total N (%)				
Mean	2.04 ^a	1.39 ^a	1.52 ^a	0.87 ^b
1 SE	0.65	0.08	0.09	0.05

Note: Data with the same lowercase superscript letters are not significantly different among treatments based on least significant difference at $P = 0.05$.

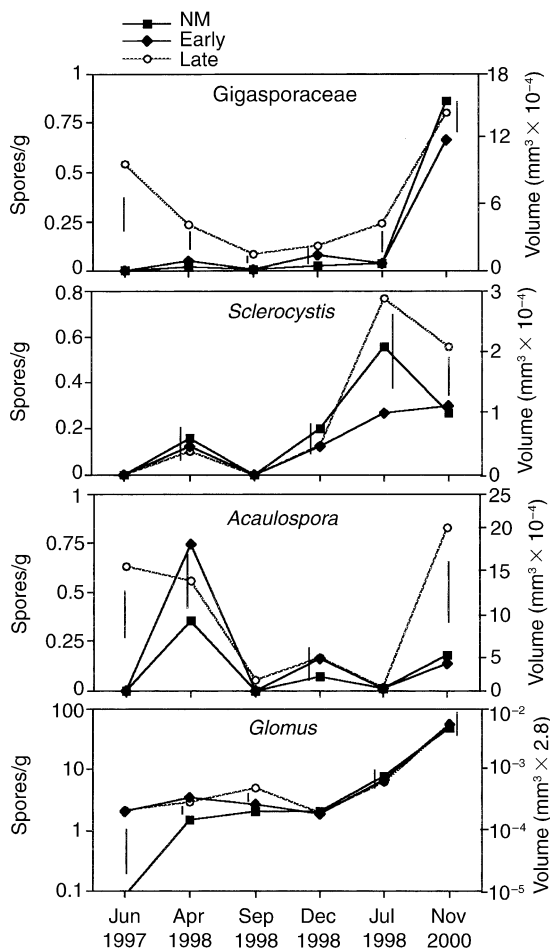


FIG. 6. Density of mycorrhizal spore genera (number of spores per gram of dry soil) in soil samples from the rhizospheres of six tree species over time, inoculated as seedlings with soil from early-seral or late-seral forest, or grown in sterile soil and initially nonmycorrhizal (NM). The vertical bars are LSD at $P = 0.05$.

than Glomaceae and Acaulosporaceae (Hart and Reader 2002).

The importance of the different genera of AMF in promoting different growth responses lies in their functional differences. Van der Heijden et al. (1998) showed that the effects on individual plant growth from adding additional AMF species to a spore mix becomes diminished after 6–8 species of fungi are present. This is the number of species that may typically be associated with an individual plant species, although there are more species across an entire stand (Allen et al. 1995, 2001). Thus, on the scale of an individual host plant the addition of large-spored Gigasporaceae may affect plant growth, and in this case the response was to reduce growth.

Several studies have shown *Glomus* to be a better mutualist in promoting plant growth than either *Scutellospora* or *Gigaspora*. Plants inoculated with *Scutellospora calospora* were smaller than those inocu-

lated with isolates of *Glomus* spp., most likely because *S. calospora* produced more external hyphae and utilized a higher proportion of plant carbon (Thomson et al. 1990, Pearson et al. 1993, Hart and Reader 2002). Nevertheless, *Glomus* initiated infections more rapidly than *Gigaspora* or *Acaulospora* (Hart and Reader 2002). *Scutellospora calospora* provided less P to plants than did a *Glomus* isolate (Pearson and Jakobsen 1993). When *S. calospora* and the *Glomus* isolate were inoculated together, *Glomus* had reduced infection, while *S. calospora* was unaffected by *Glomus* (Pearson 1994). In another study, *Gigaspora rosea* accumulated polyphosphates in hyphae, possibly making them less available to plants, while *Glomus manihotis* did not (Boddington and Dodd 1999). These studies may explain why the occurrence of *Gigaspora* plus *Scutellospora* in mature-forest soils, with biovolumes equivalent to *Glomus*, may have promoted smaller growth responses by the host trees.

We also considered the alternative hypothesis, that other species of soil microorganisms caused the reductions in growth of the trees with late-seral inoculum. Fungal pathogens (possibly *Pythium* and *Phytophthora*) occurred in <1% of the observations, and occurred in all treatments, so pathogens alone do not explain reduced plant growth in this study. Typically, pathogens that are known to affect plant health have a higher percentage of root infection. Root pathogens occur in many species of tropical-forest trees (da Silva and Minter 1995) and mycorrhizae in some cases have a beneficial effect in reducing pathogen impact (Declercq et al. 2002). In a separate experiment in a nearby field at El Edén, we observed one instance of *Fusarium* spp. that covered >50% of the root length of inoculated, planted *Cochlospermum vitifolium*, and caused >90% tree mortality (M. F. Allen, E. B. Allen, and A. Gómez-Pompa, *personal observations*). However, we did not see *Fusarium* sporulating on any of the plants in the current experiment, nor was there any evidence of mortality, leaf drop, or yellowing caused by root fungi. Finally, if pathogens had a role, they did not affect all of the seedlings equally. In the shadehouse *Acacia* had reduced relative growth rate (RGR) only of uninoculated seedlings, which is inconsistent with a pathogen-driven hypothesis. Both *Ceiba* and *Guazuma* had lowest RGR with late-seral inoculum in the shadehouse, but we observed insufficient evidence of fungi other than AMF to invoke a pathogen effect. The other three species had equal RGR in the three inoculum treatments from seed planting to field transplanting, so their growth was unaffected by any components of the soil inoculum until they were planted in the field.

The overall slow growth of *Brosimum* has been noted in other studies (Ramos and del Amo 1992) and is related to its life history as a late-seral tree. This is a shade-tolerant species that regenerates naturally in forest gaps, and had the highest growth rate in intermediate shade conditions (Ramos and del Amo 1992). It

had the lowest growth rate compared to early- and intermediate-successional species (Ramos and del Amo 1992, Ramos and Grace 1990). In our site *Brosimum* was planted in full sun where it would not have optimum growth. Although the three legume tree species were 4–5 m tall and started to shade *Brosimum* by the final measurement, deer herbivory increased, and both *Brosimum* and *Guazuma* suffered mortality, with *Brosimum* greatly decreased in height.

The changes in seasonality of spore density and mycorrhizal root infection both showed poor relationships with plant growth and were inconsistent from year to year. For instance, plants with early- and late-successional inoculum generally did not have different levels of infection after planting in the field, but plants with early-successional inoculum were larger for all species. The mean percentage infection of inoculated plants was 41–45% in December 1997, but it was only 1–2% in December 1998. Both years had relatively high rainfall during the summer–fall rainy season, and moist soils with many fine roots during the December sample period. The relationship between percentage infection and plant response is complicated by the fact that roots and fungi grow independently at different rates, and fungal growth rate may not be as rapid as fast-growing roots (Allen 2001). Roots in this soil penetrate into fissures in the rocky substrate, so total root length is impossible to obtain from the field, and would need to be obtained from greenhouse experiments. Percentage infection is an indication of presence of the fungi and may not necessarily be related to plant growth.

The spore density of *Glomus* increased by one order of magnitude between July 1999 and November 2000, when values averaged 50 spores/g dry soil (mean of the three inoculum treatments). We also noted *Glomus* densities of ~30 spores/g during October 1996 before this experiment started (L. Corkidi and E. B. Allen, *personal observations*), but other genera still had ~1 spore/g soil at this time. *Glomus* spore densities also fluctuated by an order of magnitude at another seasonal tropical forest site on the west coast of Mexico (Allen et al. 1998).

The mycorrhizal fungi did not increase plant P concentrations, as has been shown in numerous other studies (Smith and Read 1997). In this case mycorrhizal inoculum increased the biomass without increasing P concentration, resulting in significantly reduced P concentration in three of the species. The N:P ratios of the three non-legumes ranged from 13 to 14, within the range observed in other seasonal tropical forests (Jaramillo and Sanford 1995). The three legume species were higher in N:P, as expected, ranging from 21 to 25. We observed nodules on the roots of all three legume species at the first harvest in December 1997. Plants at our site had higher tissue concentrations of both N and P than most other seasonal tropical forest sites, and these soils are higher in N and P than most other sites (Jaramillo and Sanford 1995). The relatively

high values of extractable and total soil P and of leaf P suggest that P is less limiting in these soils than other seasonal tropical forests.

Another line of evidence that indicates that P is not limiting in these forests is a P-fertilization study at a forest some 40 km distant from our site on the same calcareous substrate that did not produce a plant growth response (Whigham et al. 1998). While we did not do a P-fertilization study here, such studies have been done to test mycorrhizal response of one of our study plants, *Leucaena leucocephala*. This species shows large growth responses to AMF inoculation under low P and is considered mycorrhizal dependent (Huang et al. 1985, Habte and Manjunatha 1987, Brandon et al. 1997). Mycorrhizal *Leucaena* in our study did not have elevated tissue P as these other studies showed. It was not responsive to inoculation during the first 12 weeks in the shadehouse as shown by RGR, but it was responsive to mycorrhizae in diameter growth after three years in the field. Given the relatively high levels of P in our soils, the mycorrhizal response of *Leucaena* and the other species we tested may also be caused by other physiological mechanisms that promote plant growth, such as water relations during the dry season or uptake of other soil nutrients (Huang et al. 1985, Allen 1991).

During secondary succession we can expect an increase in the abundance of Gigasporaceae, but considering their poor performance as mutualists in some studies (Pearson et al. 1993, Pearson 1994) their benefits can only be speculated. For instance, it is unclear how the accumulation of polyphosphates by Gigasporaceae might benefit plant growth in the long term (Boddington and Dodd 1999), and responses to other nutrients are poorly understood for Gigasporaceae. Their well-developed external hyphae and large spores may constitute a carbon drain for the host plant (Thomson et al. 1990, Pearson et al. 1993, Hart and Reader 2002). It is likely that mature or large trees can withstand this C drain better than establishing seedlings, so the successional patterns we observed in spore species are consistent with a rapidly growing successional forest, followed by slower-growing mature forest. Fire impacted the inoculum by reducing the relative abundance of Gigasporaceae, followed by rapid recovery of the genus *Glomus*. A previous study on fire in grassland also showed a loss of diversity in AM fungi, but no reduction in mycorrhizal infection following fire (Eom et al. 1999). Gigasporaceae may have a negative effect on the C balance of establishing seedlings, but may be more beneficial once the mycelial network has become established. Our results show the need for site-specific understanding of effects of disturbance on inoculum and on host-plant response. From a practical standpoint, the results suggest that, in these seasonal tropical forests, early-seral inoculum is the best for restoration of trees, and that the inoculum that remained following fire is beneficial to plant growth. All six species responded significantly to mycorrhizal inoculum with in-

creased height in the first year, a time when there is also rapid growth of resprouts following fire. In our study we cleared the resprouts, but during actual restoration or natural recolonization seedlings will likely suffer from competition, and taller or faster-growing inoculated seedlings may have a greater probability of survival. When inoculum is used in restoration, our study shows there is no benefit to the trees to use lateral inoculum for seedling establishment.

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