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# Effects of Mycorrhizae and Nontarget Organisms on Restoration of a Seasonal Tropical Forest in Quintana Roo, Mexico: Factors Limiting Tree Establishment

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## Abstract

We initiated a study of the effects of mycorrhizal fungal community composition on the restoration of tropical dry seasonal forest trees. Tree seedlings were planted in a severely burned experimental site (1995 fire) during the growing season of 1998 at the El Edén Ecological Reserve, in north Quintana Roo, Mexico. Seedlings of *Leucaena leucocephala*, *Guazuma ulmifolia*, *Caesalpinia violacea*, *Piscidia piscipula*, *Gliricidia sepium*, and *Cochlospermum vitifolium* were germinated in steam-sterilized soil and either remained uninoculated (nonmycorrhizal at transplanting) or were inoculated with mycorrhizal fungi in soils from early-seral (recently burned) or late-seral (mature forest) inoculum. Inoculum from the early-seral soil was largely *Glomus* spp., whereas a diverse community of arbuscular mycorrhizal fungi were reintroduced from the mature forest including species of *Scutellospora*, *Gigaspora*, *Glomus*, *Sclerocystis*, and *Acaulospora*. Plants grew

better when associated with the mature forest inoculum, unlike a previous experiment in which plants grew taller with the early-seral inoculum. Reasons for the different responses include a less-intense burn resulting in more residual organic matter. In addition to mycorrhizal responses, plants were severely affected by deer browsing. One tree species, *C. vitifolium* found in the region but not in the reserve, was eliminated by a resident fungal facultative pathogen. Several practical conclusions for restoration can be made. The common nursery practice of soil sterilization may be detrimental because it eliminates beneficial mycorrhizal fungi; species not native to the site may not survive because they may not be adapted to the local pathogens; and herbivory can be severe depending on the landscape context of the restoration.

**Key words:** *Fusarium*, herbivory, mycorrhizae, plant disease, tropical seasonal forest.

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## Introduction

Seasonal dry tropical forests are a widespread vegetation type, but these forests are being converted to agriculture and rangelands, with less than 10% of the original mature forest remaining (Murphy & Lugo 1995). Due to rapidly increasing population, the Yucatan Peninsula is subject to increasing fire frequency that threatens to convert mature forests protected as conservation reserves to secondary forests, with a forest structure and composition less suitable for many species of animals and plants (e.g., Whigham et al. 1998). Although large-scale restoration may generally be impractical, patches may be restored (Aide 2000; Aide et al. 2000) that link forest fragments or that can serve as source populations for plants, mutualistic microbes, and animals that may be crucial to an overall conservation strategy.

Tropical ecosystem structure and composition may be especially responsive to mycorrhizae (Went & Stark 1968; Mikola 1980; Janos 1980a, 1980b; Allen & Allen 1990; Huante et al. 1993; Asbjornsen & Montagnini 1994; Cuenca et al. 1998; Siqueira & Saggin-Junior 2001). Just as important may be the composition of the arbuscular mycorrhizal (AM) fungal community (Allen 1991). In temperate ecosystems, species composition and richness of mycorrhizal fungi affected both plant production and interactions (van der Heijden et al. 1998; Bever et al. 2001). In restoring forests for conservation purposes, one goal is reintroducing a diverse, native inoculum that will facilitate forest recovery and resilience (Mikola 1980; Allen et al. 2003a).

Large-scale disturbance alters the composition and activity of mycorrhizal fungi as well as the host plants. In seasonal tropical forests in western Mexico (Chamela, Jalisco), disturbance associated with large-scale conversion to grassland caused a shift from a diverse mycorrhizal inoculum to predominantly small *Glomus* spp. (Allen et al. 1998). In northeastern Yucatan Peninsula, *Glomus* spp. predominated following fire compared with a diverse suite of fungi in the mature forest (Allen et al. 2002, 2003a). But this response is not ubiquitous. In pastures in Costa Rica, the conversion to grass did not change

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inoculum composition (e.g., Johnson & Wedin 1997; Picone 2000). In part, this may relate to the scale and intensity of disturbance and replacement vegetation. In the Yucatan, the soil is high in organic matter (>30%) and hot fires often burn the organic layer to the limestone bedrock. In a preserve in western Mexico (Chamela), small patches of disturbance (blowdowns) did not result in any AM fungal species change (Allen et al. 1998), and in Costa Rica, soils tend to be deeper, providing inoculum that is not heated to the same extent. In these areas the diverse inoculum may, however, be lost following severe erosion (Carpenter et al. 2001). A few previous studies have introduced mycorrhizal inoculum to enhance restoration, but a major issue is the source of the inoculum (Mikola 1980).

We initiated research on the introduction of local mycorrhizal inoculum into restoration of seasonal tropical forest in Quintana Roo, Mexico. We postulated that inoculating seedlings with a diverse, mature forest inoculum would best enhance recovery of trees (Allen et al. 2003a). Surprisingly, we found that during the first 2 years, the early-seral inoculum, consisting of a few *Glomus* spp., stimulated plant growth more than the diverse, mature forest inoculum. In some cases, the diverse inoculum inhibited growth compared with uninoculated controls (Allen et al. 2003a). However, conditions for two or more restoration efforts are never identical. In the year following that project, we initiated a second restoration experiment immediately adjacent to the first to determine how similar the results would be to the outcome of the first experiment.

There are both practical and theoretical reasons to conduct this type of repetition. Is there really an "optimum" inoculum that can be applied to enhance restoration? Two variants on this question emerge. Is there a single inoculum source or fungus that can be tailored to the known site conditions, or should a strategy of a diverse inoculum source(s) be further studied? Many horticultural applications are dependent on finding the best inoculum using field or pot trials, which can then be sold commercially (Corkidi et al. 2004). Alternatively, according to the work of van der Heijden et al. (1998), richness is the cornerstone of mycorrhizal benefits. This is especially important given that there are local variations in both species composition and environment, both spatially and temporally. An additional final question was, do the initial plant responses to inoculum persist over time?

## Materials and Methods

The site was located in the El Edén Ecological Reserve, a 1,500-ha, seasonally dry tropical forest ([http://maya.ucr.edu/pril/el\\_eden/Reserve.html](http://maya.ucr.edu/pril/el_eden/Reserve.html)), in the northeast Yucatan Peninsula (lat 21°12.61'N, long 87°10.93'W) in the state of Quintana Roo (Gómez-Pompa et al. 2003). The region averages 1,500–2,000 mm annual precipitation, most falling during June–December (Giddings & Soto 2003). The soils are extremely thin (often only a few centi-

meters) and rocky with highly organic pockets that lie over karstic limestone bedrock (Weidie 1985; Estrada Medina 2000). The remnant local stands of mature forest (selva) are about 15 m tall, with a preponderance of trees in the family Fabaceae (Gómez-Pompa 1998; Schultz 2003). The mature forest (selva) probably has not burned in several decades, but selective tree harvest occurred in the late nineteenth century (Allen et al. 2003b). Hurricanes are the major natural disturbance, which increase woody debris and may be followed by fire in the dry season (Whigham et al. 1991, 2003). Most of the reserve is secondary forest (acahual) in a region with an increasing frequency of anthropogenic fires (Carranza Sanchez et al. 1996). Ancient Maya occupied the reserve more than 1,500 years ago (Fedick 2003). Post-European settlement and recent uses include slash-and-burn agriculture, selective tree harvesting, and grazing (Allen et al. 2003b). Following fire, the rocky soils facilitate the establishment of the rhizomatous bracken fern, *Pteridium caudatum* (L.) Maxon (Allen et al. 2003b).

The acahual was maintained in an early-seral stage and used for cattle grazing until 1990. The site is located on limestone bedrock consisting of thin (0–20 cm), highly organic soil within fissures and sinkholes in the bedrock. The soil is relatively nutrient rich because it is comprised almost exclusively of decomposing plant material (approximately 30% organic matter). It burned during an extensive wildfire in 1995. The burn eliminated all vegetation and much organic matter above the limestone bedrock.

For this experiment we chose five fast-growing secondary species. Two of the species overlapped with the prior restoration study (Allen et al. 2003a), *Leucaena leucocephala* (Lam.) De Wit (Fabaceae) and *Guazuma ulmifolia* Lam. (Sterculiaceae). *Leucaena* is an important forage tree, and both native animals and livestock use its foliage. *Guazuma* is a common midseral plant at the site. In addition, we chose three additional species from the site that tend to be mid- to late-seral species but can colonize rapidly after fire. They are also valuable to the local people, as well as for their conservation value. These included *Caesalpinia violacea* (Miller) Standl. and *Piscidia piscipula* (L.) Sarg., used for wood and fodder, and *Gliricidia sepium* (Jacq.) Steudel, an important forage and shade tree. Finally, we selected an early-seral species, *Cochlospermum vitifolium* (Willd.) Sprengel, found in the area but not on site. It is used locally as an ornamental for its flowers and is important for some local pollinators. We speculated that this species had been present but extirpated due to the small remaining area of the remnant forest and might be suitable for reintroduction. All seeds were collected on site, except for *Cochlospermum*, which was collected near Cancun, about 30 km distant.

## Inoculation and Plant Propagation

Two kinds of mycorrhizal inoculum were collected, late-seral soil from the mature forest and early-seral soil from

a 2-year-old burned forest. The mature forest was about 2 km distant from the early successional forest. The early-seral soil was collected adjacent to the planting site and was dominated in some areas by *P. caudatum*. The inoculum soil was collected from the top 10-cm soil from each forest type from five to six locations within 100 m of each other. Inoculum consisted of AM fungal spores, infected root fragments, and hyphae. The mycorrhizal spores were extracted by sucrose flotation (Allen et al. 1979), counted, and identified (Schenck & Perez 1986).

Seeds of the six tree species were collected in the region during the dry season of 1997–1998. The seeds have thick coats that were nicked with a razor blade, resulting in nearly 100% germination for all species. Seeds were planted 2–5 mm deep in early July 1998 in pots (15 × 6-cm diameter) holding approximately 500 g dry mass sterilized soil. The soil was collected near the planting site and steamed at 90°C for 2 hours to kill mycorrhizal fungi, then allowed to slowly cool over a 24-hour period. Three inoculum treatments were used: 25 g per pot of fresh late-seral forest soil, 25 g of fresh early-seral forest soil, and the uninoculated (nonmycorrhizal) controls. The inocula were each homogenized to include root segments and soil, and placed approximately 3 cm beneath the surface of the steamed soil. Thirty individuals of each of the six species were planted in each of the three inoculum treatments for the field plantings: the total was 30 replicates × 6 tree species × 3 inoculum treatments = 540 seedlings. Extras of each species in each treatment were grown to replace a few individuals that died within the first 3 weeks and to assess infection prior to outplanting. 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- to 15-cm tall.

#### Field Site Preparation and Experimental Design

The field site was located within the 1995 wildfire, immediately adjacent to the previous study (Allen et al. 2003a), but it was prepared in June of 1998. Woody resprouting vegetation was slashed in an area of 60 × 70 m and burned (Fig. 1). However, the summer rains started slightly earlier than during the previous study. Consequently, the vegetation was partially green with some new growth. The resulting fire was patchy, with herbaceous cover and the soil organic matter remaining only partially burned. Soils had moderate levels of total soil N (14 g/kg), extractable P (29 mg/kg), and a high amount of organic matter (34%). Organic matter was analyzed by combustion (450°), N was Kjeldahl N (Bremner & Mulvaney 1982), and P was bicarbonate extractable (Olsen & Sommers 1982). Nitrogen and phosphorus analyses were done at the University of California, Davis, Analytical Laboratory.

The experimental design consisted of five replicate blocks (each 18 × 60 m), each with the three inoculum treatments and a control (no planting), creating a five-block



Figure 1. Site preparation for restoration experiment, mimicking slash-and-burn agriculture, a process that, under less-controlled conditions, can lead to escaped fires.

grid of 15 planted and 5 control plots total. An individual plot was 10 × 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 × 10 plot, the seedlings were planted in rows and columns of six species each, for 36 plants per plot randomized by species within the plot with a 2-m spacing between individual seedlings.

The seedlings were transplanted to the field in mid-September 1998, into holes large enough to accommodate the 15 × 6-cm-diameter root ball. Each was initially watered with 1 L but was not watered again because the wet season was underway. These procedures mimic those used by local farmers for planting orchards and crops. The annual precipitation at El Edén for 1998, 1999, and 2000 was 2,239, 3,323, and 2,545 mm, respectively. These were 3 years with high hurricane and tropical storm activity. Each seedling was surrounded with a plastic mesh plant protector to deter deer (*Mazama americana*) browsing. The protectors were 40-cm tall by 10-cm diameter with a 1-cm mesh that did not prevent insect herbivory. The protectors were left in place throughout the experiment. The resprouting vegetation around the seedlings was cut at approximately 2- to 3-week intervals during the rainy season with a machete.

Ten additional plants of each species in each mycorrhizal treatment in the shadehouse were measured, and shoots and roots were harvested in September 1998 at the time of transplanting.

Seedling heights were measured periodically during the wet season throughout the study period. Mycorrhizae were assessed early in the growing season (generally July) and toward the end of the growing season (November). Roots and soil were collected using a trowel adjacent to the base of each transplant, and taking care to collect roots that were connected to the seedling. One plant was randomly collected from each plot per treatment of each species. The roots were stained with trypan blue (Kormanik et al. 1980) and microscopically assessed for the presence of mycorrhizal hyphae, vesicles, and arbuscules. Presence of nonmycorrhizal fungi and potential pathogens was also assessed using direct observations. Other indicators of plant health, including root browning, leaf color, lesions, and wilting, were noted.

### Statistical Analyses

Data were checked for normality and arcsine transformed as needed, and analyzed using repeated measures analysis of variance (ANOVA) to show trends in height over time. Repeated measures analyses were performed separately for each of the years because 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 ( $p < 0.05$ ). Therefore, ANOVA was used to show statistical differences in percent mycorrhizal infection and spore density on each measurement date (the soil and root samples were from different individual trees each time, so not a repeated measure). Significant differences for the ANOVAs for plant responses were shown using the least significant difference (LSD) at  $p = 0.05$  ( $LSD_{0.05}$ ) (Zar 1974). For the mycorrhizal activity, both infection and spore counts were highly variable, and we used an alpha level of 0.05 to indicate significant differences and 0.15 to indicate important trends (Klironomos et al. 1999).

### Results

Uninoculated seedlings were nonmycorrhizal at the time of outplanting. Seedlings inoculated with early- or late-seral inoculum did not differ in infection frequency at outplanting ( $p = 0.60$ ) nor were there significant differences among plant species ( $p = 0.25$ ). Infection of individual seedling roots ranged from 1 to 13% of the root length and was localized just behind the root tips. However, almost every new root was mycorrhizal, indicating a high degree of activity (Allen 2001).

Mycorrhizal inoculation consistently increased the growth rates of the trees. Initially, the most effective inoculum was the early-seral fungi (Fig. 2). In *Cochlospermum*, *Gliricidia*, and *Leucaena*, the early-seral inoculum continued to increase plant growth over other treatments until the end of the first growing season. In *Caesalpinia*, the

early- and late-seral inocula promoted growth similarly through both growing seasons. By the end of the first growing season, or into the second growing season, plants inoculated with the late-seral inoculum had higher growth than early-seral inoculum. In the case of *Piscidia*, early-seral inoculum actually inhibited growth compared with the other treatments.

Most of the trees continued growing during the study period. However, *Guazuma* and *Leucaena* grew to the height of the plant protectors by the end of the second growing season. After this time, both species were heavily browsed by deer and never extended above the plant protectors.

By the beginning of the second rainy season, July 1999, there was no difference in percent mycorrhizal root infection among plant species ( $p = 0.99$ ), but there was a difference between treatments ( $p = 0.046$ ) (Fig. 3A). The plants inoculated with the late-seral inoculum (large-spored fungi) had higher percentage of mycorrhizal root length than those of the nonmycorrhizal treatment ( $p = 0.02$ ) but was not different from the early-seral inoculum (*Glomus* spp.) ( $p = 0.51$ ). Plants that were nonmycorrhizal or had early-seral inoculum were marginally different ( $p = 0.08$ ).

There were no differences among plant species ( $p = 0.65$ ) or treatments ( $p = 0.28$ ) in *Glomus* spp. sporulation. Spore densities of large-spored ( $>150 \mu\text{m}$ ) fungal genera from the late-seral inoculum (*Acaulospora*, *Gigaspora*, *Scutellospora*) also did not differ between host species ( $p = 0.58$ ), but there were important differences between treatments (Fig. 3B). In July 1999, spore densities of large-spored species were uniformly low. By November 2000, densities of these fungi were high and not different between the late-seral inoculum treatments and the nonmycorrhizal (uninoculated) treatments ( $p = 0.73$ ). But they differed between the late-seral and early-seral inoculum ( $p = 0.05$ ) and showed a trend toward significant differences between the nonmycorrhizal and early-seral inoculum ( $p = 0.11$ ).

By the end of the 2000 growing season (November), both infection and spore counts were not different among treatments (Fig. 3). Percentage of infection was low during this part of the growing season, but sporulation was high. Spore counts among treatments were not different for either *Glomus* spp. ( $p = 0.71$ ) or the late-seral taxa ( $p = 0.31$ ). By November of 2000, we found no differences in species composition among treatments. *Scutellospora calospora* (Nicol. & Gerd.) Walker & Sanders, *Sc. persica* Koske & Walker, *Gigaspora gigantea* (Nicol. & Gerd.) Gerdemann & Trappe, *A. denticulate* Sieverding & Toro, and *A. spinosa* Walker & Trappe were commonly found among the large-spored taxa, along with *Sclerocystis rubiformis* (Gerdemann & Trappe) that commonly formed sporocarps. *Glomus* was dominated by numerous morphological varying species, but of interest, *Paraglomus brasilianum* (Spain & Miranda) Morton et Redecker, *Gl. macrocarpum* Tul. & Tul., *Gl. constrictum* Trappe, and *Gl. mossea* (Nicol. & Gerd.) Gerdemann & Trappe were found.

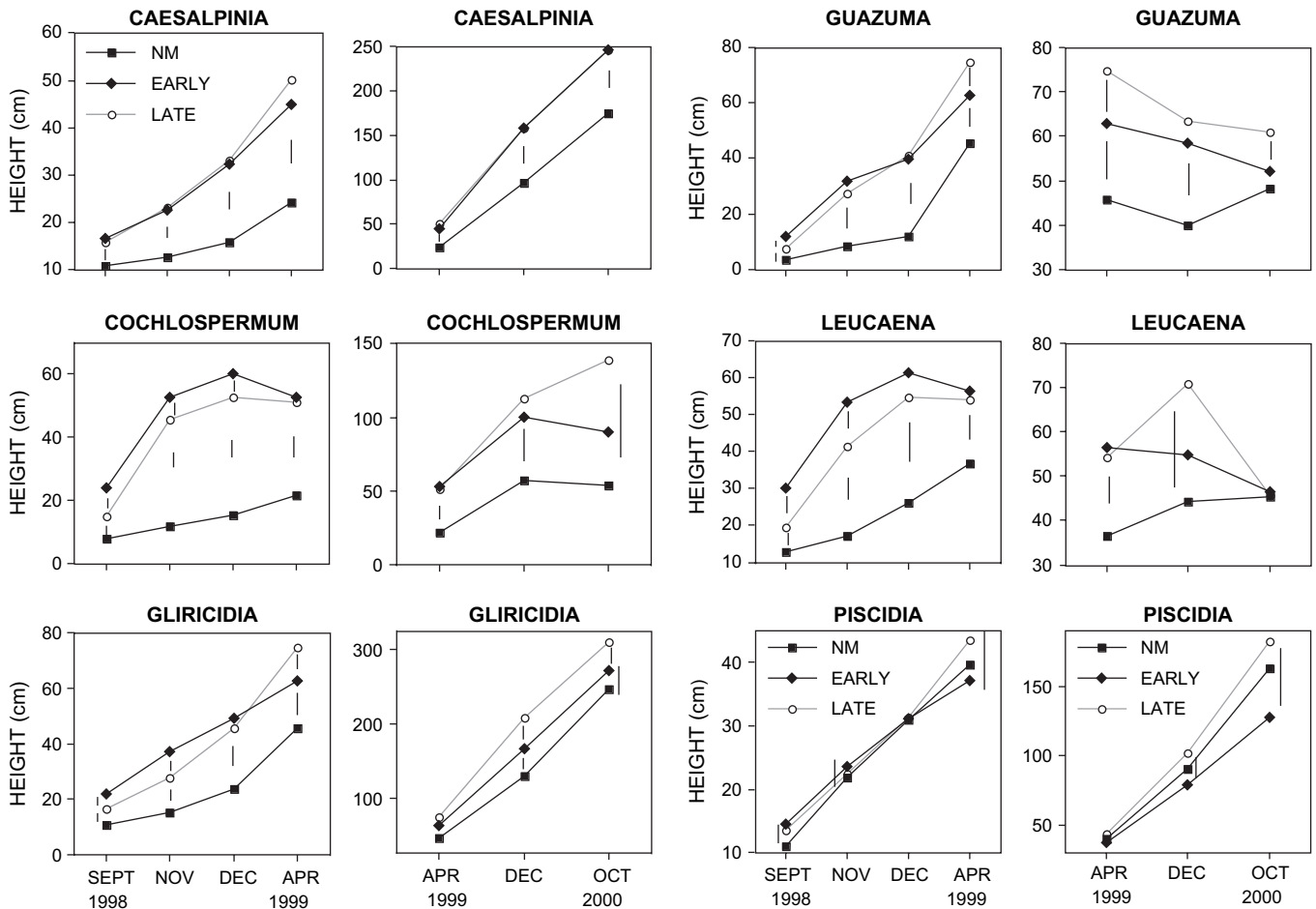


Figure 2. Tree height (cm) over the first two growing seasons. Shown are the means and  $LSD_{0.05}$  at each date for inoculum treatments, through the 1998–1999 and 1999–2000 growing seasons. The inoculum treatments are nonmycorrhizal (NM), early-seral inoculum, or late-seral inoculum. A repeated measures ANOVA showed significant ( $p < 0.001$ ) effects of inoculum treatment for all species during both 1998–1999 and 1999–2000 growing seasons. The only exception was *Piscidia*, which did not respond significantly to inoculum ( $p = 0.761$ ) in 1998–1999 but was significant ( $p = 0.007$ ) in 1999–2000.

Until June 1999, most seedlings survived, with the exception of nonmycorrhizal *Cochlospermum* and *Guazuma* (Fig. 4). In October 2000, we observed a high mortality across the experimental area in *Cochlospermum* in all treatments. At this time, even though it was peak growing season and soils were moist, leaves of the *Cochlospermum* showed extensive wilting and yellowing. There was no spatial pattern in plant disease because trees from all treatments and blocks were simultaneously affected. Fungal pathogens were always observed (a trace to 1%) on the roots of all plant species. These fungi included species of *Fusarium*, *Rhizoctonia*, *Alternaria*, *Phytophthora*, and *Pythium*. Previously, in this study, we rarely observed pathogens in the roots of any plant. In this plant species in October 2000, over 50% of the roots were infected with hyphae of a *Fusarium*. We did not have permits to bring a live culture in to the United States or have facilities to culture the fungus at the station. However, based on the size and shape of the macroconidia and the chains of

intercalary chlamydospores, the fungus was tentatively identified as *F. semitectum* Berk. & Rav. (Fig. 5). Root necrosis was observed at many of these infections.

Measurements were discontinued after the 2000 growing season. *Guazuma* and *Leucaena* never grew above the plant protectors and were eventually completely browsed out. The *Cochlospermum* plants that survived the infection of 2000 persisted until 2002 but were gone by 2003. Only *Caesalpinia*, *Gliricidia*, and *Piscidia* persisted and were still alive in the growing season of 2003.

## Discussion

van der Heijden et al. (1998) predicted that increasing the diversity of mycorrhizal fungi improves the production and diversity of host plants. Based on this outcome, we postulated that inoculating seedlings with a diverse, mature forest inoculum would best enhance recovery of trees.

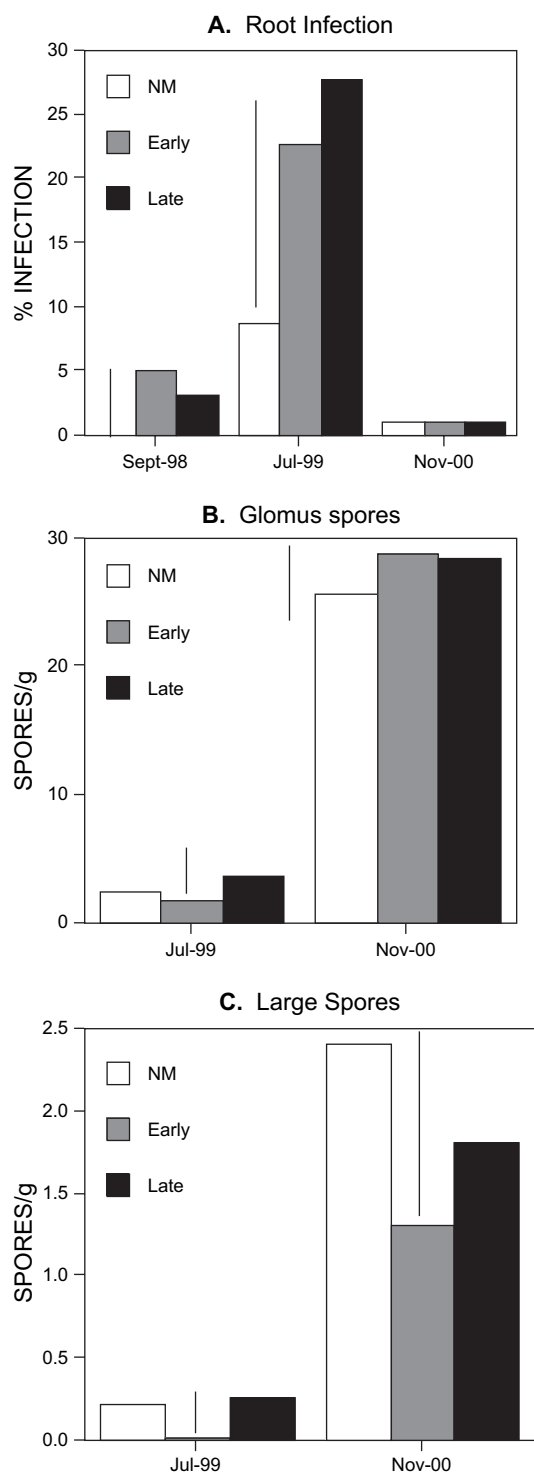


Figure 3. Mycorrhizal infection (A), spore counts of *Glomus* spp. (B), and spore counts of the large-spored species (C) in three treatments. Shown are the nonmycorrhizal (NM), early-seral inoculum, or late-seral inoculum treatments. Percent infection was measured in greenhouse plants at the time of transplanting to the field (September 1998), and spores and infection were measured from the field on two later dates. Large spores include species of *Acaulospora*, Gigasporaceae, and sporocarpic *Sclerocystis*. Bars are LSD<sub>0.05</sub> values indicating significant differences among treatments within a date.

In our first experiment, the 1997 burn was relatively hot and burned almost everything above the limestone bedrock. In this case, the AM fungi that best enhanced seedling growth was the early-seral inoculum consisting of a suite of small-spored *Glomus* species collected from a patch that had burned 2 years previously (1995) and supported largely *Pteridium caudatum* (L.) Maxon along with some early-seral vegetation (Allen et al. 2003a). We predicted that the responses of the 1998 planting should resemble those of the 1997 (Allen et al. 2003a) planting. However, plant growth and responses to inoculation differed in interesting ways from the previous study even though the plantings were adjacent to each other and only planted 1 year apart.

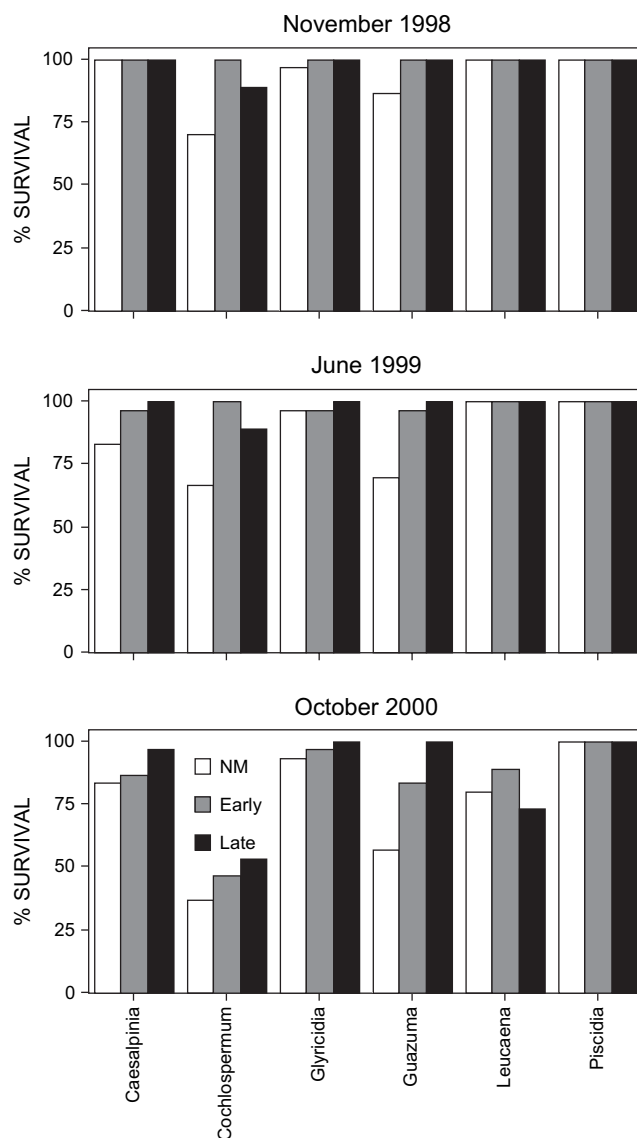


Figure 4. Percent survival for all tree seedlings in the restoration plot. Seeds were germinated in the shadehouse in June 1998 and planted in the field in September 1998.

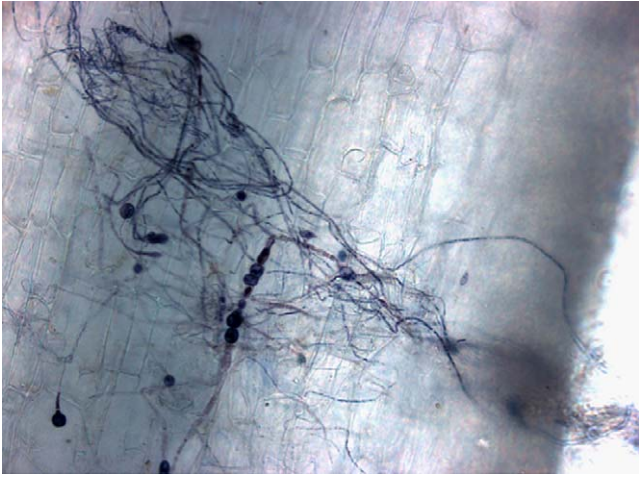


Figure 5. *Fusarium semitectum* infecting a root of *Cochlospermum*, showing intercalary chlamydospores and microconidia.

In the current study, the early-seral *Glomus* inoculum promoted plant growth during the first year, but those inoculated with the mature forest inoculum improved growth, in many cases, as much as the early-seral inoculum and, by the second year, increased the plant growth beyond that of the early-seral inoculum. One factor is that the burn was not as effective in the present experiment as in the 1997 experiment. In 1998, the rainy season began earlier and much of the organic matter (already wet) persisted through the fire. The higher organic matter may have provided a better habitat for the mature forest fungi.

The second factor that was different between years was the degree of herbivory. Following the large regional fires of 1995, few deer were observed in the experimental area. There was little herbivory on the 1997 plantings. But by 1998, deer herbivory in all our research plots increased dramatically. *Brosimum alicastrum* Sw., *Guazuma*, and *Leucaena* from the 1997 experiment suffered from herbivory, but the plants were already large enough to withstand it. In the current experiment, deer browsed most of the leafy material whenever it emerged from the plant protectors for both *Leucaena* and *Guazuma*. Both species were unable to get enough of a start to successfully grow and died after three growing seasons.

A third factor that distinguished this experiment was the outbreak of a fungal root pathogen. *Fusarium semitectum* is a generalist fungal pathogen found in most tropical forests (Booth 1971; Domsch et al. 1980). We had observed this as well as other facultative and obligate root pathogens infrequently in roots from mature forest trees and from the 1997 plantings (Allen et al. 2003a). However, it had never infected more than 1 or 2% of the roots in all plants observed. Interestingly, this fungus is probably more of a facultative parasite, generally causing disease largely in introduced species (Domsch et al. 1980). It is known to cause severe wilting disease in a leguminous tree *Anadenanthera macrocarpa*, planted in Brazil (Dhingra et al.

2002). This was an interesting case in that the tree is naturally found in Brazilian primary or secondary forests in dry sandy or stony soil in a seasonal deciduous wet forest. These would be soils with lower water-holding capacity. At El Edén, *F. semitectum* appears to be common but only mildly infective. But during years of high hurricane activity and higher than normal precipitation, such as 1998–2000, disease could be a problem for species adapted to slightly drier conditions.

Together, these data demonstrate a high degree of complexity in replacing the structure and functioning of a local ecosystem. But there is no single fungus or inoculum combination that fits all restoration projects, even for a single site. The composition of mycorrhizal fungi and the effectiveness of different inocula sources depend on the extent of the perturbation, which may be a function of many factors such as the prior vegetation and the weather at the time of perturbation. Even the idea that a diverse inoculum is the best strategy is suspect because the outcome depends on the specific initial condition.

Both the AM fungi and the pathogens observed have broad host ranges (Domsch et al. 1980; Allen et al. 1995). The weather can also regulate the plant species composition by creating conditions in which indigenous pathogens can become epidemic. *Cochlospermum* is present nearby but occurs in somewhat drier forests with less than 1,500 mm of precipitation. But it is not present at the site. It may not be able to survive long term at El Edén because it was sensitive to the indigenous *Fusarium*. We are not aware of another study showing that a plant species distribution might be limited by a local pathogen. Most of what is known about pathogen control of native plants comes from invasives such as chestnut blight (*Cryphonectria parasitica*) (Roane et al. 1986) or jarrah die-back (*Phytophthora cinnamomi*) (Shea 1977). The impacts of native pathogens can only be determined from a restoration or other introduction experiment.

Finally, the role of herbivory remains interesting. Herbivory can devastate particularly desirable plants, but the herbivory can change markedly between years as a function of both the climate and the surrounding environment. When the burn occurred at the regional scale, herbivory was less of an issue because the entire regional population was likely lowered, and there were emerging seedlings across the landscape. However, when just a patch was created within conditions of a dense, surrounding secondary forest, our planted seedlings known to be useful deer forage (*Brosimum*, *Leucaena*, *Guazuma*) were devastated.

We can also make some recommendations for restoration in areas, like Quintana Roo, where funding for restoration is limited. The benefits gained from mycorrhizae, whether early seral when mineral soil predominates or late seral with more organic soils, clearly outweigh the expense and any perceived gains from the normal nursery practice of soil sterilization. At least under the conditions of our experiments, soil sterilization to eliminate pathogens was more detrimental than using natural soil inoculum. A mix

of sterilized soil plus a plug of known local inoculum to initiate mycorrhizal infection before transplanting provides an early growth stimulation that carries for many years. Careful consideration of tree species not found on site needs to be undertaken. There may be reasons such as pathogens or microclimate interactions that regulate local distributions. Finally, herbivory should be considered in restoration planning. Tree protectors certainly helped in the initial phases of establishment. But the longer-range establishment, surrounding landscape context, herbivore populations, and animal densities require consideration.

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