

Effect of vesicular-arbuscular mycorrhizae on seedling growth of four tree species from the tropical deciduous forest in Mexico

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Abstract. The influence of vesicular-arbuscular mycorrhizae on the growth of seedlings of *Caesalpinia eriostachys*, *Cordia alliodora*, *Ipomoea wolcottiana* and *Pithecellobium mangense* was investigated in a greenhouse experiment conducted at the Biological Station of Chamela on the Pacific coast of Mexico. Dry biomass production, relative growth rate, root/shoot ratio and mycorrhizal dependency were quantified for 75-day-old seedlings. With the exception of the pioneer species *I. wolcottiana*, mycorrhizal infection resulted in increases in biomass production, relative growth rate and leaf area. The root/shoot ratios attained for the species, however, did not show a consistent trend with infection. Nevertheless, all species had root/shoot ratios below 1 with infection and only one, *Cordia alliodora*, had a ratio greater than 1 without infection. The two late successional species from the mature part of the forest, *Caesalpinia eriostachys* and *P. mangense*, showed a larger mycorrhizal dependency than the two associated with disturbed environments.

Key words: Tropical deciduous forest – Tree seedlings – Growth analysis – Vesicular-arbuscular mycorrhizae

Introduction

Mycorrhizal fungi are important for the growth of a number of species of tropical forest trees, but theory and some evidence suggest that early seral species have a smaller response to infection than late seral species (Janos 1980a, b, 1987; Mikola 1980; Sieverding 1991). Janos (1980b) divided tropical tree species into obligately or facultatively mycorrhizal species based on their response to infection by vesicular-arbuscular (VA) mycorrhizal fungi, where the obligately mycorrhizal species tended to be from mature forest and the facultative species were from disturbed forest. Our objectives were to examine the responses to VA mycorrhizae of two early

and two late successional species in tropical seasonal forest, an environment quite different from tropical wet forest, but one which may offer further insights into the influence of mycorrhizae on succession.

Most advances in our understanding of mycorrhizal response concern temperate ecosystems (Allen 1991; Harley and Smith 1983; Koide 1991), although there have been some studies in wet tropical forest (Janos 1980a, 1980b, 1987; Mikola 1980). The lack of basic information about mycorrhizae in the tropics is the main obstacle to addressing the more encompassing question about the role of mycorrhizae in highly diverse and more complex communities. Very little attention has been paid to the seasonal tropical deciduous forest in North America. However, research on this system is important because 40% of all tropical forest in the world is tropical deciduous forest (Murphy and Lugo 1986) and it has been subject to severe exploitation and destruction.

The research reported here was designed to determine the effect of VA mycorrhizae on the growth of seedlings of four coexisting tree species from the tropical deciduous forest on the Pacific coast of Mexico. This forest is characterized by its highly seasonal climate and high plant diversity (Lott 1985). Research on species establishment and regeneration in tropical deciduous forest is very scarce, and mycorrhizal studies are unknown. We chose two early successional species typical of canopy gaps, *Cordia alliodora* DC. (Boraginaceae) and *Ipomoea wolcottiana* Rose (Convolvulaceae), and two that are dominants in the mature forest, *Caesalpinia eriostachys* Benth. (Leguminosae) and *Pithecellobium mangense* (Jacq.) MacBride (Leguminosae), and examined their response to mycorrhizal infection.

Materials and methods

The study was carried out at the Biological Station of Chamela (19° 30' N, 105° 03' W) on the Pacific coast of Mexico, which is dominated by tropical deciduous forest. The species richness is higher than in other neotropical deciduous forests and has been estimated at 758 species in the ca. 2000-ha reserve (93 plant species per 1000 m²) (Lott et al. 1987). The climate is remarkably seasonal

with an average annual temperature of 24.9°C and an average annual (1977–1984) precipitation of 748 mm, but 80% of the rain occurs between July and October (Bullock 1986). In this forest, the rainy season provides the most favorable growth conditions for most of the species (Bullock and Solís Magallanes 1990). Additional information on productivity and species composition can be found in Martínez-Yrizar et al. (1992).

Mature seeds of all species were collected from at least 10 different individuals. The seed dry mass of the four species ($n = 50$, randomly selected from different trees) was as follows: *Caesalpinia eriostachys*, 219.5 (21.13) mg; *Cordia alliodora*, 14.7 (2.5) mg, *I. wolcottiana*, 60.2 (5.4) mg, *P. mangense*, 39.0 (4.0) mg, and the seeds were germinated on sterilized pure silica sand inside a greenhouse located at the station. When necessary the seeds were scarified prior to planting to ensure rapid and synchronous germination. Five days after germination, the seedlings were transplanted to black plastic bags (20 × 30 cm) filled with a mix of soil and pure silica sand (3:1). Soils at Chamela are young, weakly developed entisols on substrates of rhyolite and basalt; the organic matter content is between 3 and 5%. The soil used in this investigation was characterized as sandy loam (64% sand, 13% loam, 23% clay), with a pH of 6.5–7.1. The bicarbonate-extractable soil P was 37.3 ($SE = 11.5$) $\mu\text{g/g}$ and total Kjeldahl N was 0.32 (0.07)% (E. Solís, personal communication). The soil was sterilized in a microwave oven and allowed to rest for 2 weeks before the beginning of the experiment as a precaution against possible phytotoxic effects due to the sterilization process (Rovira and Bowen 1966).

Spores for inoculation were isolated from 1-year-old soil pot cultures, from the tropical deciduous forest, following the procedure of Ianson and Allen (1986). Spore identification is still in progress; however, *Acaulospora* and *Glomus* were the main genera of spores observed in the pot cultures. The 5-day-old seedlings were placed randomly in pots in a field greenhouse at the Biological Station and watered every second day. The experiment comprised nine replicates per species inoculated with 1000 spores and nine replicates without any spore addition, with an initial and a final harvest. Spore washings derived from the pot cultures, following the procedure of Koide and Li (1989), were added to both treatments in order to reincorporate the nonmycorrhizal soil microorganisms to the sterilized soil. The seedlings were harvested after 75 days, only slightly less than the 4-month normal wet season. Three replicates for each treatment were randomly selected and the roots carefully separated from the soil and stained, following the method of Phillips and Hayman (1970), in order to determine presence of infection. No attempt was made to establish percentage of root infection. The remaining six replicates were harvested and shoot and root were carefully separated. Total leaf area was determined with a LI-COR 1300 leaf-area instrument. Roots and shoots were dried at 80°C for 48 h to obtain the dry weight. The average dry biomass for root and shoot were obtained from these data. The mean relative growth rate (RGR, dry weight increment per unit total plant weight per unit time, mg/mg/day) of each species was determined according to Hunt (1982): $RGR = (\ln W_{t_2} - \ln W_{t_1}) / (t_2 - t_1)$, where W is the mean total plant dry biomass in mg and t is time in days. The relation between root and shoot dry matter (R/S) (Evans 1972; Hunt 1982) was also calculated. Mycorrhizal dependency (MC) (Gerdemann 1974) was calculated as the difference between the average total dry biomass of the mycorrhiza-inoculated and uninoculated plants and expressed as a percentage of total dry biomass of inoculated plants (Plenchette et al. 1983).

Significant differences between the treatments for each measured parameter were tested by analysis of variance, using log-transformed data where necessary to meet assumptions of normality (Zar 1974).

Results

Microscopic observations of the stained roots showed that all inoculated treatments were mycorrhizal and all

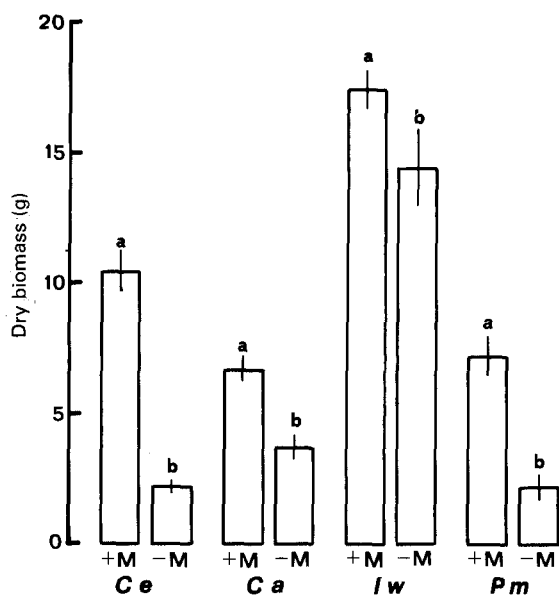


Fig. 1. Total average dry weight of seedlings of *Caesalpinia eriostachys* (*Ce*), *Cordia alliodora* (*Ca*), *Ipomoea wolcottiana* (*Iw*) and *Pithecellobium mangense* (*Pm*) in the presence and absence of vesicular arbuscular (VA) mycorrhizae (*M*). Vertical bars show standard deviation. Different letters indicate a significant difference between treatments at the $P < 0.05$ level

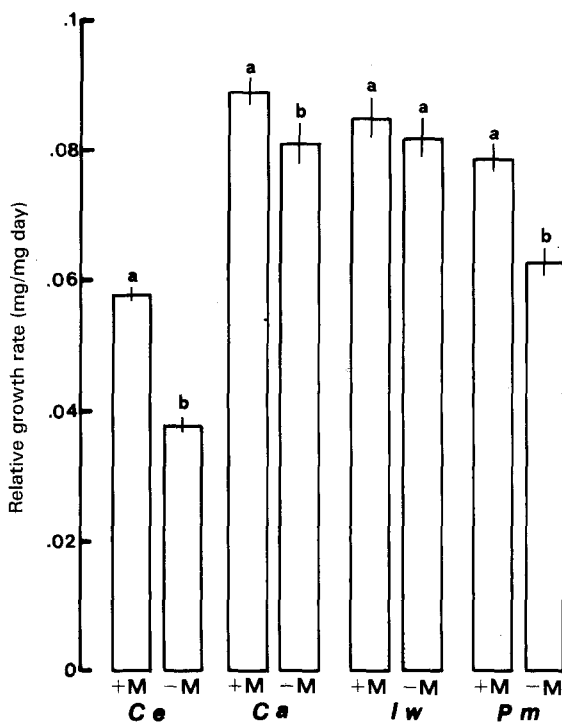


Fig. 2. Relative growth rate of *Caesalpinia eriostachys* (*Ce*), *Cordia alliodora* (*Ca*), *I. wolcottiana* (*Iw*) and *P. mangense* (*Pm*) in the presence and absence of VA mycorrhizae (*M*). Vertical bars show standard deviations. Different letters indicate a significant difference between treatments at the $P < 0.05$ level

uninoculated treatments had no mycorrhizal formation.

The influence of the VA mycorrhizae on dry biomass production for each the species studied is shown in Fig.

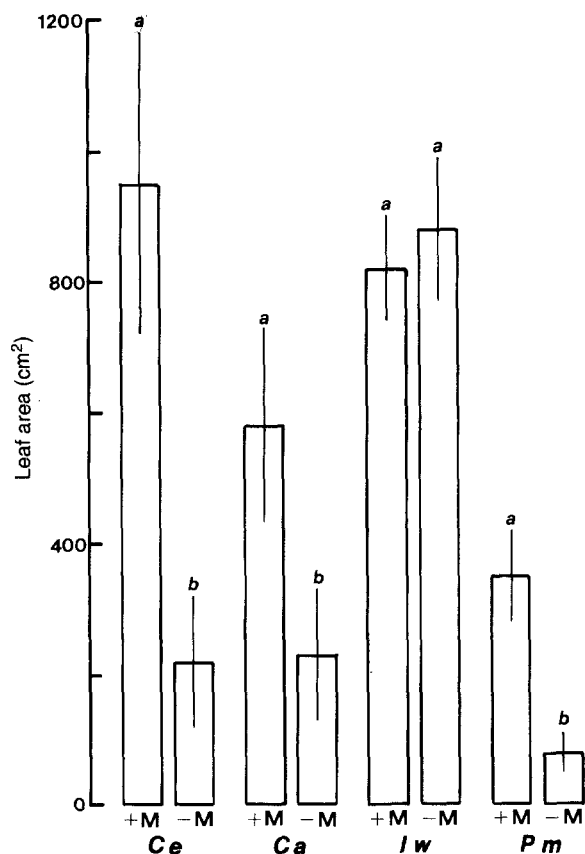


Fig. 3. Average total leaf area of *Caesalpinia eriostachys* (*Ce*), *Cordia alliodora* (*Ca*), *I. wolcottiana* (*Iw*) and *P. mangense* (*Pm*) after 75 days growth inside a greenhouse in presence and absence of VA mycorrhizae (*M*). Vertical bars represent standard deviations. Different letters indicate a significant difference between treatments at the $P < 0.05$ level

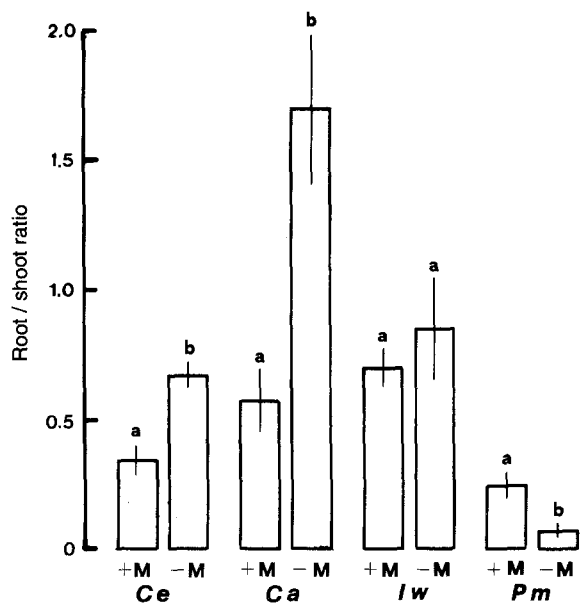


Fig. 4. Average root/shoot ratio attained by mycorrhizal and non-mycorrhizal 75-day-old seedlings of *Caesalpinia eriostachys* (*Ce*), *Cordia alliodora* (*Ca*), *I. wolcottiana* (*Iw*) and *P. mangense* (*Pm*) in the presence and absence of VA mycorrhizae (*M*). Vertical bars show standard deviations. Different letters indicate a significant difference between treatments at the $P < 0.05$ level

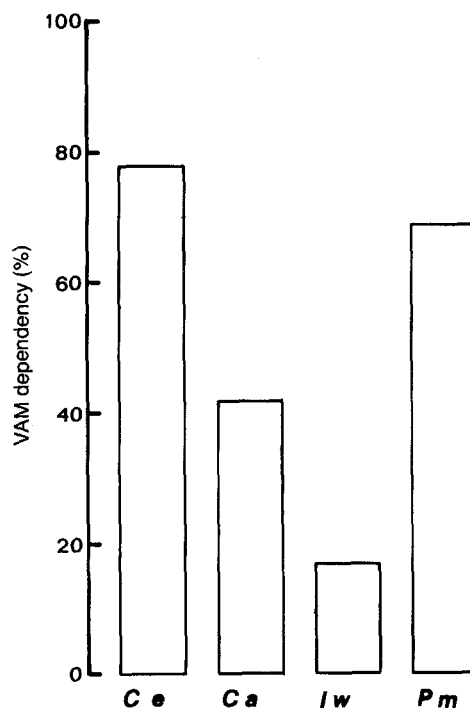


Fig. 5. VA mycorrhizal dependency of tree seedlings from the tropical deciduous forest. *Caesalpinia eriostachys* (*Ce*), *Cordia alliodora* (*Ca*), *I. wolcottiana* (*Iw*) and *P. mangense* (*Pm*)

1. The total average dry biomass was significantly higher for all species in the presence of mycorrhizae. Although *I. wolcottiana* had the highest weight, it had the lowest mycorrhizal response.

With respect to RGR, all species except *I. wolcottiana* achieved significantly higher rates in the presence of mycorrhizae (Fig. 2). *Caesalpinia eriostachys* had the lowest RGR. Leaf area followed a similar trend (Fig. 3), since *I. wolcottiana* was the only species that was unresponsive to mycorrhizal infection. *Caesalpinia eriostachys* had the greatest leaf area of the mycorrhizal plants, a surprising result as it had the lowest RGR of all four species in the mycorrhizal treatment.

In terms of the biomass allocation, R/S did not follow a consistent pattern (Fig. 4). *Caesalpinia eriostachys* and *Cordia alliodora* showed similar responses; the R/S was significantly lower in the presence of mycorrhizae. The R/S achieved by *I. wolcottiana* did not differ significantly between the two treatments, while *P. mangense* allocated more biomass to roots in the presence of the VA mycorrhizae.

MD is shown in Fig. 5 and, consistent with the other results, *I. wolcottiana* was the least dependent species.

Discussion

The growth responses of the four tropical deciduous forest tree species support the hypothesis that early seral species are less dependent upon mycorrhizae than late seral species (Janos 1980a). The two species from mature parts of the forest had higher dry biomass produc-

tion, leaf area and RGR with infection than the early seral species. Both early seral species had a low dependence on mycorrhizae and, in fact, one of the early seral species, *I. wolcottiana*, had a significant response to infection in dry biomass but not in any of the other variables measured. A gradient of dependency (from high to low) in relation to habitat preference (from closed to open sites) could be established as follows: *Caesalpinia eriostachys*, *P. mangense*, *Cordia alliodora*, *I. wolcottiana*. From studies conducted in both temperate and tropical species, it has been suggested that the species from late seral stages tend to show the highest requirement for a mycorrhizal association (Allen 1991; Allen and Allen 1986, 1990; Janos 1980a, 1980b, 1987). In addition, the relationship between the RGR and MD appears to indicate that the seedlings with a low growth rate, the two late seral species, were also more dependent on the mycorrhizae. This relationship, however, needs to be tested further.

In order to consider further the mycorrhizal benefit of these species it is necessary to bear in mind that the ecological interpretation of responses of tropical deciduous seedlings to mycorrhizae may vary with other developmental characteristics of the species. In particular, seedling growth and establishment are also affected by dispersal patterns, seed size, germination requirements, water and nutrient use efficiency, light demands, effect of herbivores and tolerance to pathogens. We must also consider that different species of fungi may affect plant growth in different ways or present contrasting efficiency in soil resource capture and utilization (Allen 1991; Koide 1991). In addition, the cost-benefit ratio of the association must be evaluated comparatively in both disturbed and undisturbed tropical habitats. However, in a greenhouse experiment where conditions were maintained as uniformly as possible, the responses of the four species were as predicted by their seral status.

Plant ecological theory (Chapin 1980, 1988; Grime 1979) predicts that plants from habitats poor in soil resources have a greater dry weight allocation to roots. On the contrary, plants from nutrient-rich soils allocate more biomass to be above-ground structures. Based on this assumption, the tropical seedlings studied in this investigation appear to be adapted to a non-nutrient-limited environment, if we consider that with mycorrhizae all the species showed an R/S around 0.5. In fact, the soils from this forest have high N and P concentrations, as reported above. The effects of mycorrhizae on dry matter allocation patterns in environments with contrasting mineral nutrient availability are variable (Allen 1991). In this study, the mycorrhizae had a mixed effect on biomass allocation to root and shoot. However, the only species that did not show a significant difference between the mycorrhizal treatments was *I. wolcottiana*, a species found in disturbed parts of the forest.

Most tropical studies on mycorrhizae have been conducted in tropical rain forest, but there is no documentation in tropical environments on the relevance of mycorrhizae to water and nutrient uptake where seasonal water restriction controls temporal growth differentiation (phenological patterns) (Bullock and Solis-Magal-

lanes 1990). There are distinct environmental differences between wet and seasonal tropical forest that drive growth and establishment of species. For instance, gap dynamics are important for seedling establishment in wet forest (Denslow 1980), but the pronounced seasonality of deciduous forest acts as a perturbation that probably allows seasonal establishment at the beginning of the rainy season. In spite of this probable difference in the dynamics of seedling establishment, early versus late seral tree seedlings appear to show similar mycorrhizal responses in both forest types.

The study presented here includes only four tree species of a highly diverse forest. Further comparative growth experiments are needed to assess whether MD is related to species occupying undisturbed, mature parts of the forest. It also remains to be tested if MD varies as the phosphorus availability of the soil changes (Habte and Manjunath 1991). In addition, we must consider whether MD changes with other plant growth responses to resource supply, such as water or light availability. However, the results suggest that a pattern in MD exists in early versus late seral species, as has been previously shown in temperate shrub steppe (Allen and Allen 1991) and wet tropical forest (Janos 1980b).

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