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Mycorrhizal dependency, inoculum potential and habitat preference of native woody species in South Brazil

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ABSTRACT. Seedlings from 43 native woody species belonging to different successional groups from the Tibagi River Basin, Paraná State, South Brazil were studied to obtain information on the importance of colonization by native arbuscular mycorrhizal (AM) fungi. The experiment was carried out in a greenhouse for 15 to 45 wk, with soil-mix treatments and four successional groups. The mycorrhizal dependency was 90, 48, 12 and 14% of the pioneer, early secondary, late secondary and climax species, respectively. The content of P, Ca and K was 20, 17 and 23 times greater, respectively, in the leaves of the pioneer species than in the other successional groups. The colonization by AM fungi in field was studied in seedlings of 36 native woody species collected in the interior of the forest of the Mata dos Godoy State Park, and in open area at the beginning of arboreal succession. The mycorrhizal colonization in the field was 55.5, 26.9, 6.1 and 2.2% for the pioneer, early secondary, late secondary and climax species, respectively. To assess the mycorrhizal inoculum potential, rhizosphere soil was collected in the interior of the forest and a gap in the same forest and in a cleared area abandoned for natural regeneration. The inoculum potentials and the spore number in the area at the beginning of succession were 5.6 and 53.4 times greater than in the interior of the forest. The results show that the initial growth of the woody species which take part in the initial phases of succession may be more dependent on the AM fungi, in soils poor in minerals, while those that make up the final succession phases may be less dependent. The potential of the AM fungi inoculum decreases throughout the successional process and there is a relation between the inoculum potential found in the field and the occurrence for the different habitats of the species of adult plants belonging to different successional groups.

KEY WORDS: arbuscular mycorrhizal fungi, inoculation, inoculum potential, mycorrhizal colonization, root symbiosis, successional groups

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INTRODUCTION

In tropical regions, low soil fertility and limited P availability favour the formation of the mycorrhizal association which is essential for a large variety of plant species (Janos 1983, Newsham *et al.* 1995). The arbuscular mycorrhizal (AM) fungi may increase the P supply for plants which is reflected in an increase in the plant biomass (Cooperband *et al.* 1994, Merryweather & Fitter 1996).

Janos (1980a) inoculated AM fungi in 28 native woody species from a tropical forest in Costa Rica, and found that the mycorrhizal association increased the growth of 23 species, the survival of six and cotyledon retention in another five. Janos (1980a,b; 1983, 1995) has suggested that the tree species belonging to the mature forest tend to be obligately mycotrophic. These tropical species have large seeds, which may favour the persistence of the seedlings in the field and allow the colonization of roots by AM fungi, because the nutritional availability of the seed reserve guarantees the meeting of roots with the inoculum and maintains adequate photosynthesis capacity to support initial colonization. Janos (1980a, 1983) suggested that woody species from the understorey maintain the mycorrhizal association because of its importance for seedling growth, when gaps are opened in the canopy. Many pioneer and early successional species are facultatively mycotrophic or non-mycorrhizal, with small seeds with few nutritional reserves, and are very efficient in uptake of nutrients and may rapidly establish themselves without having to wait for AM fungi colonization. On the other hand, Allsopp & Stock (1992) report that in an ecosystem in South Africa, the obligately mycotrophic species have small seeds with low P content and the response of the plants to mycorrhizal colonization decreased with the increase in seed size and its P content. Similarly, Siqueira *et al.* (1998), working with native woody species from Southeast Brazil, observed that pioneer species have light seeds, high susceptibility to infection and colonization rate and are very responsive to AM fungi. The late successional species have large seeds and are more dependent upon their seed reserves than upon AM fungi for initial growth.

The formation of a mycorrhizal association depends on environmental factors, host physiology, soil microorganisms and the quantity and composition of arbuscular mycorrhizal AM fungi. Alterations in some of these factors may influence the number of mycorrhizal associations formed (Tommerup 1992), called inoculum potential. The low inoculum potential found in secondary forest in Costa Rica was explained by the disturbance of the root-hypha web, the small quantity of spores in the soil, by the host species used in the bioassays of soil dilution (Fischer *et al.* 1994) and by Janos' hypothesis (1980b) which proposes that the dependence of obligately mycotrophic plant species might decrease after maturity (Asbjornsen & Montagnini 1994). Janos (1980b, 1983, 1995) reported that in the tropical rain forest of Costa Rica few AM fungi spores are found in mature forest because infection spreads as hyphae from

Table 1. Relative contribution in percentage of AM fungi species used as inoculum sampled from rhizosphere soil from different pioneer woody species of south Brazil.

Species	%
Glomus macrocarpum Tulasne and Tulasne	25
G. occultum Walker	25
Entrophospora colombiana Spain and Schenck	15
Glomus etunicatum Becker and Gerdemann	10
Acaulospora mellea Spain and Schenck	5
A. morrowiae Spain and Schenck	5
Scutellospora pellucida (Nicol. and Schenck) Walker and Sanders	5
S. weresubiae Koske and Walker	5
Acaulospora foveata Trappe and Janos	1
A. tuberculata Janos and Trappe	1
A. scrobiculata Trappe	1
A. longula Spain and Schenck	1
Non-identified	1

root to root among neighbouring mycotrophic plants which are very plentiful, and low spore production is compensated.

This study reports the existence of differences in the responses to inoculation with indigenous AM fungi in native woody species belonging to different successional groups and the influence of mycotrophism in the availability of AM fungi inoculum in areas of tropical forest fragments in the south of Brazil.

METHODS

AM fungi spores for inoculation were obtained from rhizosphere soil from different native tree species in an area with natural vegetation dominated by pioneer species. The spores were separated by sieving and decanting, centrifuged in 60% sucrose and de-infested with sodium hypochlorite 1% (Sieverding 1991). Table 1 shows the relative contribution in percentage of AM fungi species used as inoculum.

Seeds from the native woody species were de-infested with 1% sodium hypochlorite, washed in distilled water and placed on sterilized sand to germinate. Table 2 shows the native woody species studied and their probable position in the different successional groups, according to studies by Ferretti *et al.* (1995), Gandolfi *et al.* (1995), Kageyama (1992) and Leitão-Filho (1993).

Subsoil (85%) and sand (15%) were mixed and placed in black plastic bags with 4.0 kg capacity and fumigated with methyl bromide. The mixture had 5.1 pH, C (1.9 g 100 g^{-1}), P (1.8 mg kg^{-1}), Al (0.08 meq 100 ml^{-1}), Ca (5.47 meq 100 ml^{-1}), Mg (2.46 meq 100 ml^{-1}) and K (0.61 meq 100 ml^{-1}). One hundred ml of soil filtrate without AM fungi were added to each bag (Abbott & Robson 1984). For the encounter of root seedlings with inoculum, about 350 indigenous AM fungi spores were applied in the planting hole to each cultivation bag, totalling 10 bags per species. A further 10 bags did not receive spores, making the control group. The seedlings were transplanted between 6 and 15 d after emergence of the shoot. The pioneer, early secondary, late secondary and

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Table 2. Native woody species listed according to their successional status, from south Brazil. Seedlings were collected in the interior of the forest (I), area at the beginning of succession (B) or not collected in the field (C).

Species	Site
Pioneer	
Cecropia pachystachya Trec. (Cecropiaceae)	B
Cestrum intermedium Semdth. (Solanaceae)	B
Croton floribundus Spreng. (Euphorbiaceae)	B
C. urucurana Baill. (Euphorbiaceae)	C
Peschiera australis (M. Arg.) Miers (Apocynaceae)	B
Schinus terebinthifolius Raddi (Anacardiaceae)	B
Senna macranthera (Collad.) Irwin et Barn. (Caesalpiniaceae)	B
Trema micrantha (L.) Blum. (Ulmaceae)	B
Early secondary	
Acacia polyphylla DC. (Mimosaceae)	C
Anadenanthera macrocarpa (Benth.) Brenan (Mimosaceae)	C
Bauhinia forficata Link. (Caesalpiniaceae)	C
Colubrina glandulosa Perk. (Rhamnaceae)	B
Ficus guaranitica Schodat (Moraceae)	I
Guazuma ulmifolia Lam. (Sterculiaceae)	B
Heliocarpus americanus L. (Tiliaceae)	B
Inga striata Benth. (Mimosaceae)	I
Jacaranda mimosaefolia D. Don (Bignoniaceae)	C
J. puberula Cham. (Bignoniaceae)	I
Lonchocarpus campestris Mart. & Benth. (Fabaceae)	C
L. muehlbergianus Hassl. (Fabaceae)	I
Luehea candicans Mart. Zucc. (Tiliaceae)	B
Parapiptadenia rigida (Benth.) Brenan (Mimosaceae)	B
Prunus sellowii Hoehne (Rosaceae)	I
Sebastiania commersoniana (Baill.) Smith & Downs (Euphorbiaceae)	B
Vitex montevidensis Cham. (Verbenaceae)	B
Late secondary	
Astronium graveolens Jacq. (Anacardiaceae)	I
Bougainvillea spectabilis Willd. (Nyctaginaceae)	I
Cedrela fissilis Vell. (Meliaceae)	I
Centrolobium tomentosum Guill. Ex Benth. (Fabaceae)	I
Chorisia speciosa St. Hil. (Bombacaceae)	I
Enterolobium contorfisiliquum (Vell.) Morong (Mimosaceae)	C
Lithraea molleoides (Vell.) Engl. (Anacardiaceae)	I
Ocotea puberula (Reich.) Ness (Lauraceae)	I
Peltophorum dubium (Spreng.) Taub. (Caesalpiniaceae)	I
Ruprechtia laxiflora Meisn. (Polygonaceae)	I
Strichinus brasiliensis (Spreng.) Mart. (Styracaceae)	I
Syagrus romanzoffiana (Cham.) Glassm. (Arecaceae)	I
Climax	
**Actinostemon concolor* (Spreng.) M. Arg. (Euphorbiaceae)	I
Aspidosperma polyneuron M. Arg. (Apocynaceae)	I
Copaifera langsdorffii Desf. (Caesalpiniaceae)	C
Euterpe edulis Mart. (Arecaceae)	I
**Guarea kunthiana* A. Juss. (Meliaceae)	I
Ormosia arborea (Vell.) Harms (Mimosaceae)	C
Trichilia claussenii C.DC. (Meliaceae)	I
T. elegans C.DC. (Meliaceae)	I

* Seedlings collected in the interior of the forest but not used in greenhouse experiments.

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climax species grew on average for 17, 18, 20 and 23 wk, respectively. A further eight bags were prepared for each non-inoculated plant species because of the small growth of some species, and to obtain a sufficient quantity of dry leaves to quantify the minerals. Due to the long persistence of the cotyledon and the slow growth of *S. romanzoffiana*, *S. brasiliensis*, *A. polyneuron*, *T. elegans*, *T. claussenii* and *E. edulis*, five inoculated bags and their controls were kept for 45 wk to assess the influence of the cotyledons on growth and mycorrhizal colonization and response of these species. The bags were distributed randomly in the greenhouse.

To estimate the mycorrhizal colonization 1 g of roots from each plant were clarified in 10% KOH and 5% H_2O_2, acidified with HCL 1%, washed in running water and stained with 0.05% trypan blue in lactoglycerol solution (Kormanik & McGraw 1982). Segments of fine roots, *c.* 1 cm long were used, and total colonization of roots was calculated as total intersections observed (Giovanetti & Mosse 1980). Roots and shoots were placed in a drying chamber at 65 °C until they reached a constant weight to obtain the dry biomass. Using shoot dry matter data, degree of response to mycorrhiza (dependency) was calculated as being the difference between the biomass of the shoot of inoculated and non-inoculated plants and was expressed as a percentage of the dry biomass of inoculated plants (Plenchette *et al.* 1983). The relative growth rate (RGR) was determined by the equation: $RGR = (\ln W_{t2} - \ln W_{t1}) / (t2 - t1)$, where W is the total mean of the dry plant biomass, expressed in mg and t is the time in d (see Huante *et al.* 1993).

The P, Ca and K concentrations in the leaves of the different species were determined in the Radiochemical and Chemical Analytical Section of the Center of Nuclear Energy in Agriculture (CENA), Piracicaba, SP. The nutrient content in the leaf was calculated based on the concentration in the leaf and leaf biomass. The nutrient content ratio was calculated by quantity of nutrient content in the leaves of the inoculated plants divided by the non-inoculated plants.

To assess the inoculum potential of AM fungi, soil from the rhizospheres of different woody species seedlings were collected under canopy in mature forest in the Mata dos Godoy State Park (23°27′S and 51°15′W), in one gap created by tree-fall, with *c.* 15 m diameter in the same forest, and in a cleared area used as pasture for 40 y, abandoned for natural recuperation 12 y ago, dominated by pioneer woody species, adjacent to the forest in Londrina county, Paraná State, Brazil. Rhizosphere soil and fine roots were obtained from each woody species seedling, and were mixed thoroughly by cutting roots into small pieces and mixing by hand. Thirty samples from each site were collected. The soils were diluted (0–100% in 10% steps) using a subsoil (80%) and sand (20%) mixture and fumigated with methyl bromide. The diluted soils were placed in 1.0 kg capacity black plastic bags with three replicates. *Cecropia pachystachya* Trec. (Cecropiaceae) seedlings were used as host plant. The bioassay was set up the

day after harvesting the different soils in the field. After 90 d growth in a greenhouse, the seedlings were harvested and their roots washed in running water. Segments of fine roots *c.* 1 cm long were examined for each soil dilution and total colonization of roots was calculated.

Seedlings of 36 woody species (Table 2) were collected from 1993 to 1997 in the interior undercanopy of the tropical forest of the Mata dos Godoy State Park, and the same area at the beginning of succession abandoned for natural regeneration. All the seedlings were collected without cotyledons and between 30 and 60 cm in height. Three to five seedlings of each species were collected with their root system, which were washed in running water to remove soil and separate the roots of other plants. The fine roots were stored in FAA, later cleared, stained and total colonization of roots was calculated.

At each sampling area, ten mineral soil samples were collected from the surface horizon, to a depth of *c.* 20 cm. Soils were air-dried and passed through a 2-mm sieve prior to analysis. The mineral content of the soils was determined in the soil laboratory of the Paraná Agronomic Institute (IAPAR), and analysed for pH, C, Al, Ca, Mg, K and P available. Soils were extracted with 1.25 M ammonium acetate at pH 4.8 and analysed for Al, Ca, Mg and K. Total carbon content were determined by dry combustion. The extract was analysed for available P colorimetrically with ammonium molybdate. The concentration of soil nutrients from the mature forest had 4.9 pH, C (4.2 g 100 g^{-1}), P (9.96 mg kg^{-1}), Al (0.04 meq 100 ml^{-1}), Ca (11.7 meq 100 ml^{-1}), Mg (3.61 meq 100 ml^{-1}) and K (0.73 meq 100 ml^{-1}). The concentration of soil nutrients from the area at the beginning of succession had 5.2 pH, C (1.8 g 100 g^{-1}), P (1.8 mg kg^{-1}), Al (0.09 meq 100 ml^{-1}), Ca (6.89 meq 100 ml^{-1}), Mg (2.77 meq 100 ml^{-1}) and K (0.52 meq 100 ml^{-1}). SAS (Statistical Analysis System) was used for the statistical analyses. Analyses of variance (ANOVA), the Tukey and Tukey–Kramer tests were performed at a level of 5% significance.

RESULTS

The responses of the native woody species showed that the inoculum used contained effective species for the growth of the majority of the host plants. The controls did not have mycorrhizal colonization. The cotyledon fall of the non-inoculated plants occurred at 3.8, 6.5, 11.1 and 12.7 wk for the pioneer, early secondary, late secondary and climax species, respectively. The average seed weight was 21 ± 5.9 (n = 8), 141 ± 20.3 (n = 17), 395 ± 77.1 (n = 12) and 487 ± 98.4 (n = 6) mg for the pioneer, early secondary, late secondary and climax species, respectively. The cotyledon fall of the non-inoculated plants was positively related to the seed weight ($r^2 = 0.44$, $P < 0.0001$, n = 43; Figure 1a) and to root / shoot rate ($r^2 = 0.21$, $P < 0.01$) and inversely related to the mycorrhizal response ($r^2 = 0.47$, $P < 0.0001$; Figure 1b) and colonization ($r^2 = 0.44$, $P < 0.0001$). These relationships disappeared in the inoculated treatment and the cotyledons remained active and attached to the plant for a much longer

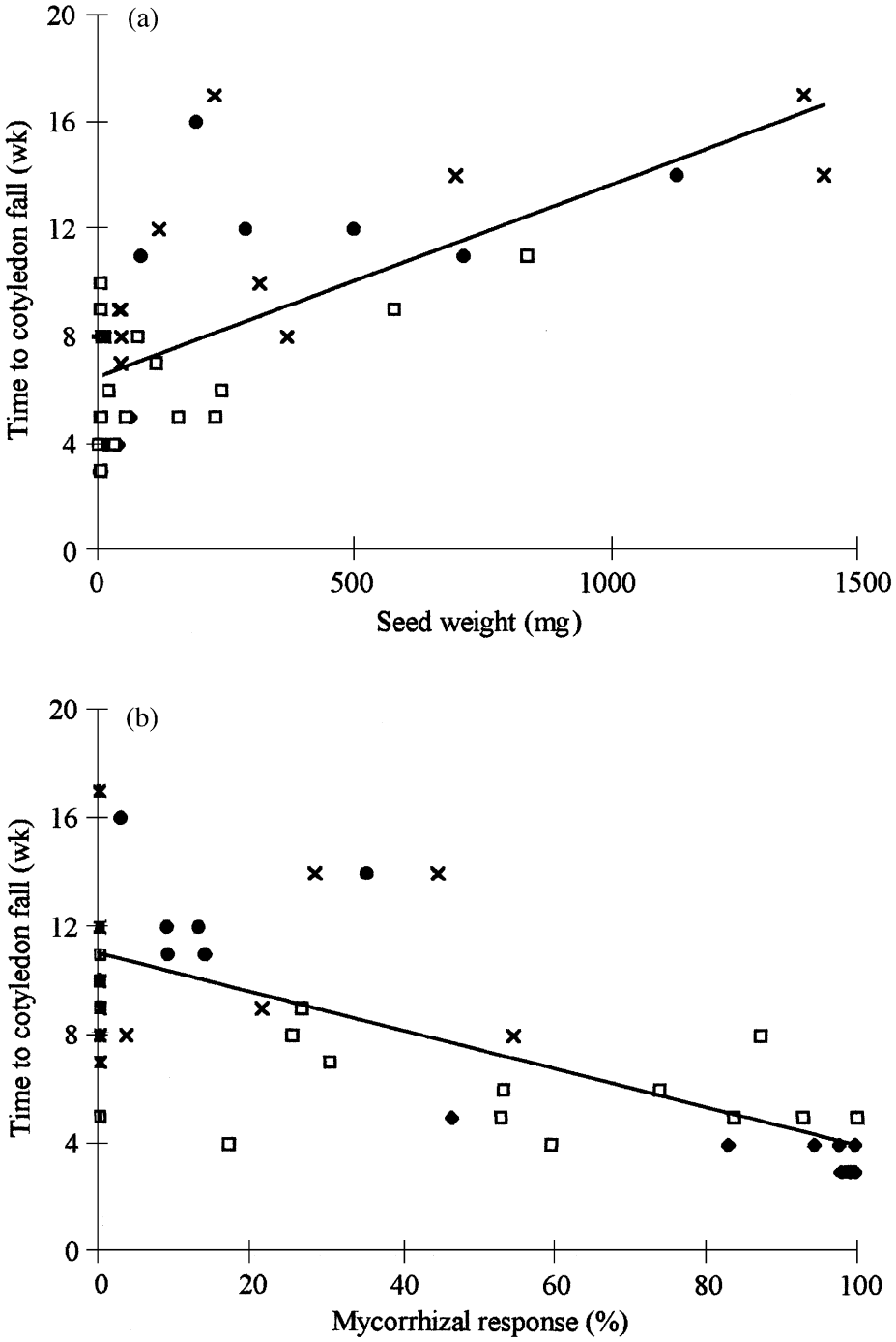

Figure 1. Relationships between time to cotyledons fall of non-inoculated plants and (a) seed weight ($y = 0.007x + 6.5$), and (b) mycorrhizal response ($y = -0.070x + 11.01$), for native woody species of south Brazil belonging to the different successional stages: pioneer (◆), early secondary (□), late secondary (×), and climax (●). Statistics are shown in the text.

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time than on the non-inoculated ones in the pioneer species and majority of the early secondary species.

The relative growth rate was positively related with mycorrhizal response (r^2> = 0.53, $P < 0.0001$; Figure 2a), and colonization ($r^2 = 0.34$, $P < 0.001$; Figure 2b) and inversely related with root / shoot rate ($r^2 = 0.26$, $P < 0.001$). The mycorrhizal response was positively related to colonization ($r^2 = 0.72$, $P < 0.0001$; Figure 3a) and weakly inversely related to seed weight ($r^2 = 0.10$, $P < 0.04$; Figure 3b). The mycorrhizal colonization in greenhouse ($r^2 = 0.15$, $P < 0.01$, $n = 43$) and in field ($r^2 = 0.20$, $P < 0.01$, $n = 36$) conditions was also inversely related to seed weight.

The mycorrhizal response decreased for the species of later successional groups (Figure 4a), where the pioneer species differed significantly ($P < 0.05$) from the early secondary species and these from the late secondary and climax species, which were alike. The mycorrhizal colonization in the greenhouse and field decreased the advance of the successional groups (Figure 4b), where pioneer and early secondary species had a high degree of colonization, while the late secondary and climax species had low colonization.

Long after cotyledon fall, the mineral supply from cotyledons to seedlings did not significantly change the biomass, relative growth rate, degree of mycorrhizal colonization and response in the species which grew for 45 wk, when compared to those which grew 21–24 wk (Table 3).

The concentration and content of the macronutrients in the leaves of the pioneer species increased with inoculation (Table 4). When non-inoculated, the pioneers had lower concentrations than the species belonging to other successional groups, except for Ca in the early secondary species. With inoculation, the P, Ca and K concentrations almost doubled in the pioneer species but did not increase in the other groups, which caused the disappearance of the differences among all the successional groups for P and Ca, and a significant increase in the K concentration in the pioneer species when compared to the secondary and climax species. Inoculation significantly increased the content of minerals in the pioneer and early secondary species, but there was no difference in the late secondary and climax species. The pioneer, early secondary and late secondary species accumulated similar amounts of P, which were significantly greater than those of the climax species. Regarding Ca and K, the pioneers accumulated greater quantities than the early and late secondary species and even more than the climax species. The nutrient content for all the successional groups, expressed as the ratio between the inoculated treatment/non-inoculated treatment showed that the accumulation potential for the three elements was, on average, 28.6 times for the pioneer species, 2.1 times for the early secondary species and 1.1 times for the late secondary and climax species.

The soil inoculum potential of AM fungi from the area at the beginning of succession had 62% of initial mycorrhizal colonization which decreased only with 50% of inoculum dilution, with a 22% fall in colonization at 50% dilution

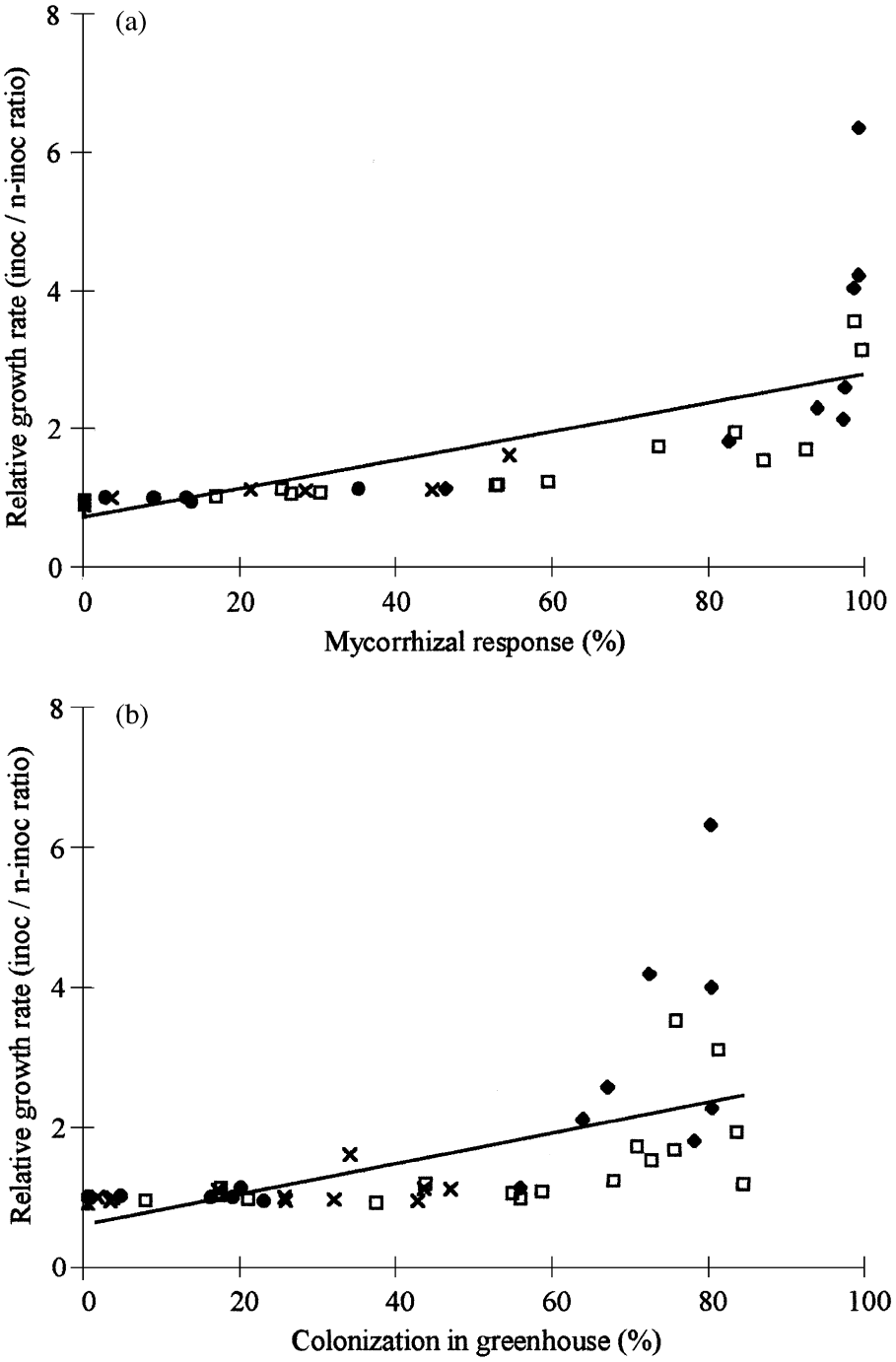

Figure 2. Relationships between relative growth rate and (a) mycorrhizal response (y = 0.020x + 0.74), and (b) colonization in greenhouse (y = 0.022x + 0.61), for native woody species of south Brazil belonging to the different successional stages: symbols as Figure 1. Statistics are shown in the text.

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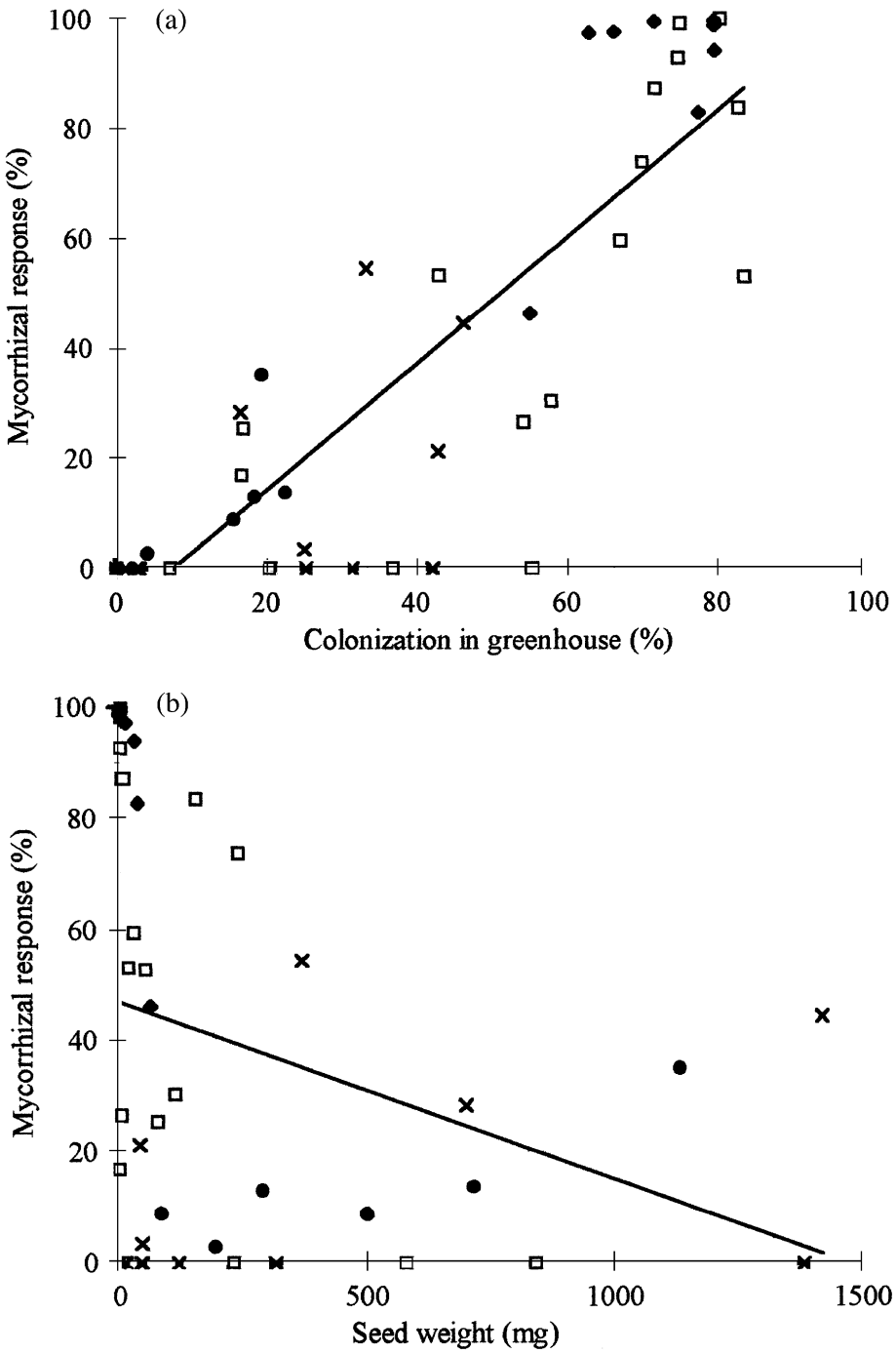

Figure 3. Relationships between mycorrhizal response and (a) colonization in the greenhouse (y = 1.142x – 8.72), and (b) seed weight (y = –0.032x + 47.1), for native woody species of south Brazil belonging to the different successional stages: symbols as Figure 1. Statistics are shown in the text.

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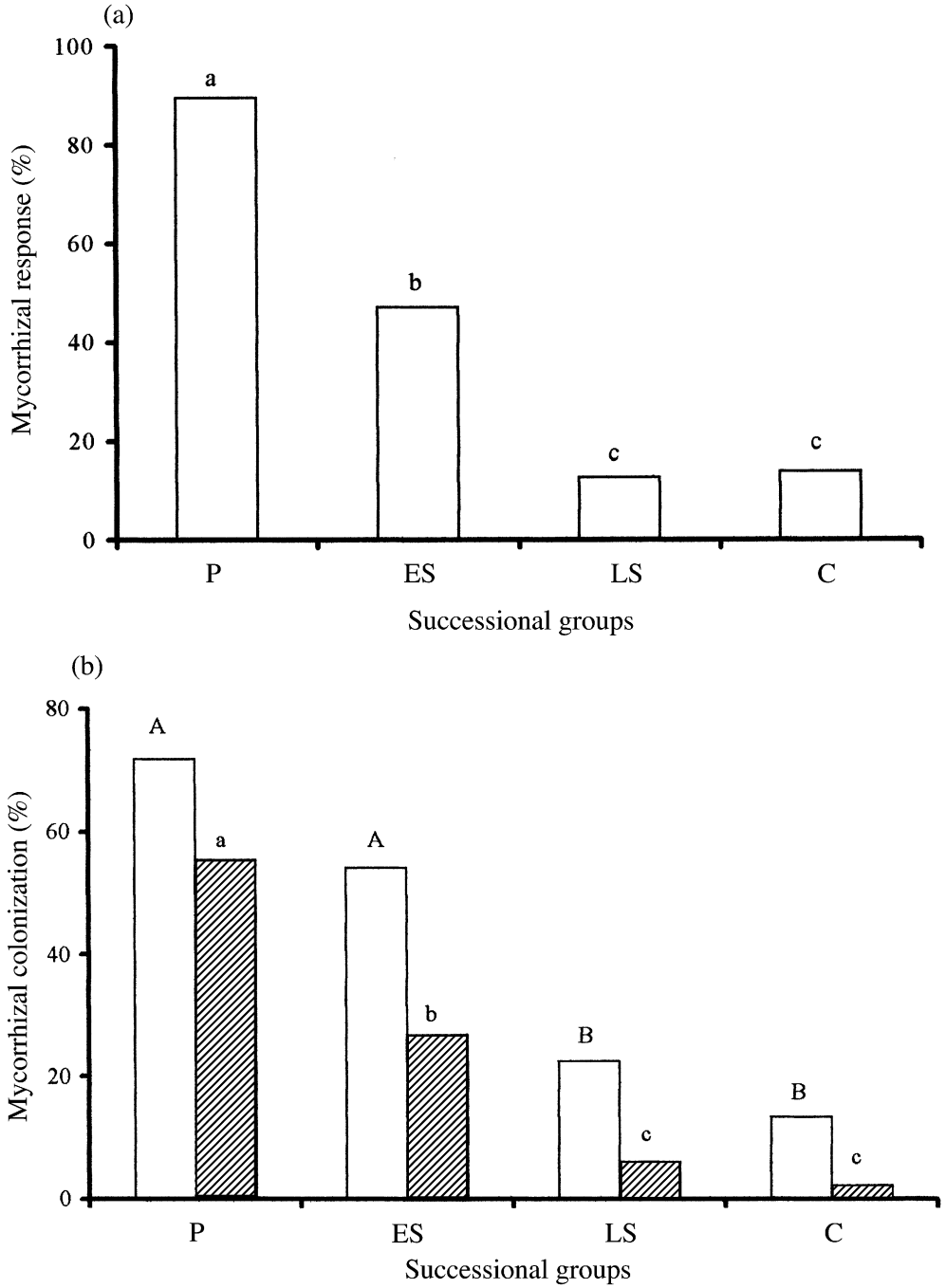

Figure 4. Mycorrhizal response (a), and mycorrhizal colonization (b), in the greenhouse (□) and field (▨) of native woody species of south Brazil belonging to the different successional stages: P, pioneer; ES, early secondary; LS, late secondary; C, climax. Means followed by same letter are not different by Tukey–Kramer test at the P = 0.05 level.

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Table 3. Comparison between different times of growth by late secondary and climax woody seedling species inoculated and non-inoculated with arbuscular mycorrhizal fungi.

Species and successional groups	Time after treatment (wk)	Time to loss of cotyledons (wk)	Ratio (inoc./non-inoc.) Dry mass			Colonization	MD
			Root	Shoot	RGR	(%)	(%)
Late secondary species							
Syagrus romanzoffiana							
	22	17	0.7 ns	1.2 ns	1.01 ns	2.5	0
	45	17	1.1 ns	1.0 ns	1.00 ns	3.2	0
Strichinus brasiliensis							
	21	14	1.2 ns	1.5*	1.15 ns	12.3	26.0
	45	14	1.2 ns	1.4*	1.11 ns	16.7	28.4
Climax species							
Aspidosperma polyneuron							
	24	11	1.1 ns	1.1 ns	1.05 ns	0	0
	45	11	0.8 ns	1.1 ns	1.01 ns	0	0
Trichilia elegans							
	23	12	1.0 ns	1.0 ns	1.01 ns	22.1	3.5
	45	12	1.2 ns	1.1 ns	1.01 ns	18.6	13.1
Trichilia claussenii							
	24	16	1.0 ns	1.2 ns	1.12 ns	5.2	8.5
	45	16	1.1 ns	1.0 ns	1.02 ns	4.3	2.8
Euterpe edulis							
	23	14	1.2 ns	1.2 ns	1.12 ns	14.3	11.4
	45	14	1.3 ns	1.4 ns	1.04 ns	19.6	15.7

Significance levels by Tukey test: * $P \leq 0.05$.
RGR, relative growth rate; MD, mycorrhizal dependency.

Table 4. Leaf nutrient concentrations and contents of pioneer (P), early secondary (ES), late secondary (LS) and climax (C) of woody seedling species from south Brazil inoculated (inoc.) and non-inoculated (non-inoc.) with arbuscular mycorrhizal fungi.

Successional groups	Nutrient concentration			Nutrient content		
	Non-inoc.	Inoc.	NCR[1]	Non-inoc.	Inoc.	NCR[2]
	P (mg 100 mg^{-1})			P (mg per leaf)		
P	0.07 b B	0.14 a A	2.00	0.17 b C	5.10 a A	30.0
ES	0.11 a A	0.14 a A	1.08	1.57 b A	3.52 a A	2.25
LS	0.14 a A	0.15 a A	1.07	3.22 a A	3.51 a A	1.09
C	0.15 a A	0.15 a A	1.00	0.93 a B	1.03 a B	1.11
	Ca (mg 100 mg^{-1})			Ca (mg per leaf)		
P	0.35 b B	0.57 a A	1.63	0.87 b C	20.7 a A	23.8
ES	0.47 a AB	0.55 a A	0.94	6.72 b A	13.8 a B	2.05
LS	0.57 a A	0.58 a A	1.02	13.1 a A	13.6 a B	1.04
C	0.66 a A	0.63 a A	0.95	4.09 a B	4.35 a C	1.06
	K (mg 100 mg^{-1})			K (mg per leaf)		
P	0.69 b B	1.51 a A	2.19	1.72 b C	54.9 a A	31.9
ES	1.03 a A	1.18 a B	1.12	14.8 b A	29.6 a B	2.01
LS	1.06 a A	1.09 a B	1.03	24.4 a A	25.5 a B	1.05
C	1.15 a A	1.14 a B	0.99	7.13 a B	7.87 a C	1.10

Means followed by the same letter (small in row and capital in column) are not different by Tukey–Kramer HSD test at the 0.05 level.
NCR[1], nutrient concentrations ratio between inoc./non-inoc.
NCR[2], nutrient contents ratio between inoc./non-inoc.

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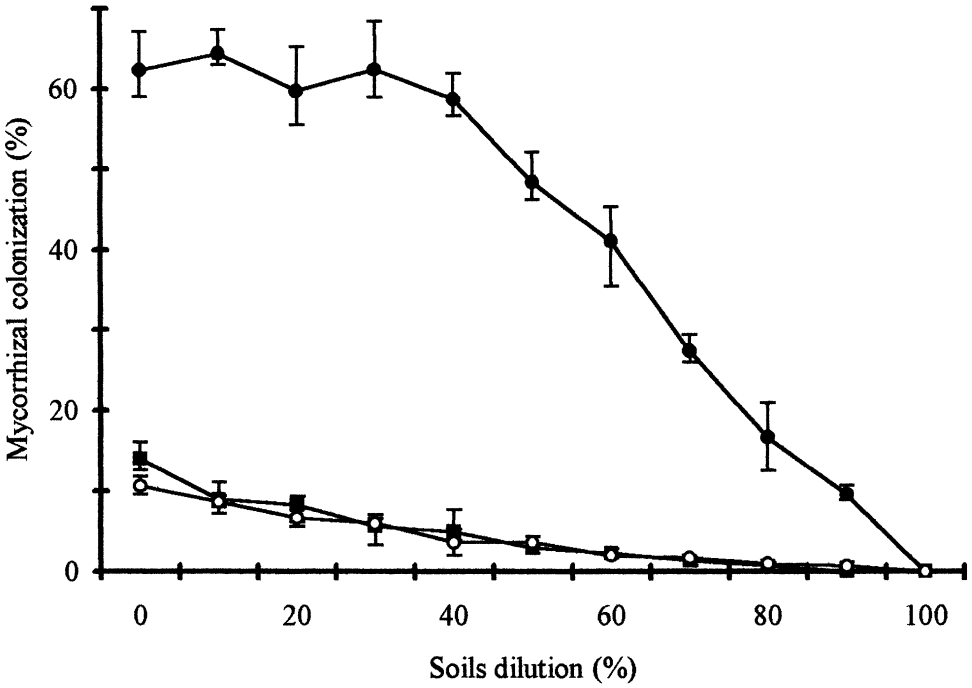

Figure 5. Soil dilution effect from the areas of early succession (●), gap (■) and climax forest (○) on mycorrhizal colonization of *Cecropia pachystachya*. Vertical bars show the ranges.

(Figure 5). The rate of colonization reduction throughout the dilution was lower than the rate of soil dilution. Before the dilution of the gap and mature forest soils the mycorrhizal colonization was *c.* 12%. Colonization was reduced with dilution, decreasing by *c.* 72% with 50% dilution. The initial colonization rate of the soil from the area at the beginning of succession was about five times greater than the other areas.

The spore content in the soil was 5.7, 9.5 and 304.6 spores per 100 g dry soil from the mature forest, gap and the area at the beginning of succession, respectively. The mycorrhizal colonization of these species in the greenhouse was strongly correlated with mycorrhizal colonization in the field ($r^2 = 0.84$, $P < 0.0001$; Figure 6).

The occurrence of native woody species used in this study was compared with the floristic composition and the phytosociology of the arboreal strata, carried out by Soares-Silva & Barroso (1992) in 1 ha in the interior of the Mata dos Godoy State Park forest. Only two pioneer and one early secondary species which had high degrees of mycorrhizal response and colonization in the greenhouse and mycorrhizal colonization in the field occurred in the interior of the forest. The arboreal species which had little or no degree of mycorrhizal response and colonization in the greenhouse and field are the species which dominate in the interior of the forest, represented by late secondary and climax species.

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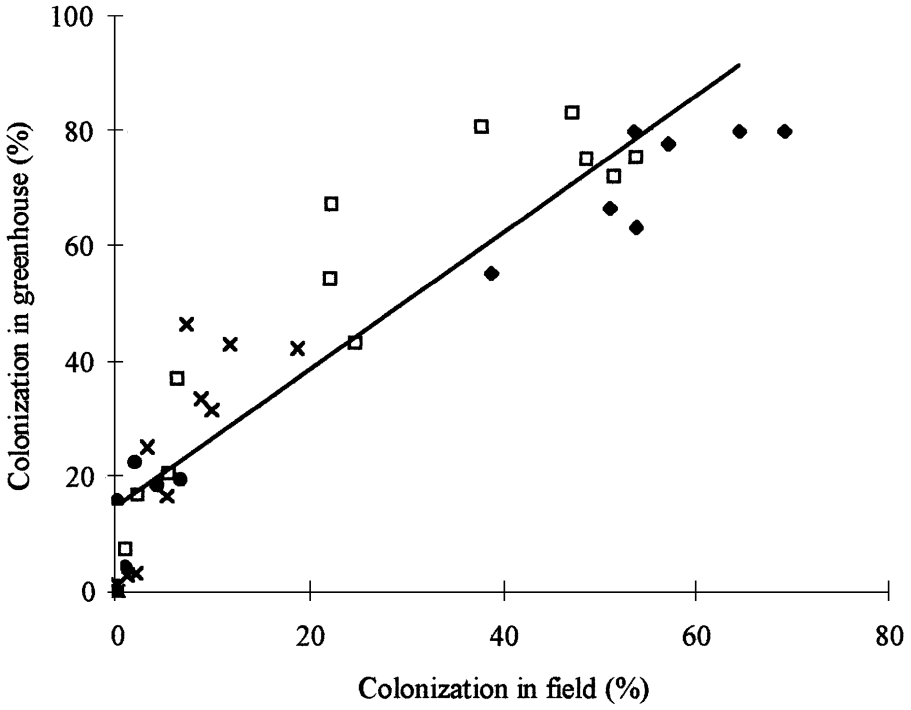

Figure 6. Relationships between mycorrhizal colonization in greenhouse with mycorrhizal colonization in the field (y = 1.18x + 15.1), for native woody species of south Brazil belonging to the different successional stages: symbols as Figure 1. Statistics are shown in the text.

DISCUSSION

The results obtained do not sustain the hypothesis that early successional species are less dependent on mycorrhizae than the late successional species, proposed by Janos (1980b, 1983) for tropical woody species in Costa Rica. Pioneer species and early secondary species were highly influenced by the AM fungi when compared with late secondary and climax species. In soils with little available P, the mycorrhizal colonization rate is generally correlated with the growth responses of the plant (Abbott & Robson 1984) and mycorrhizal dependency may be a phenotypic response of the fungus when faced with the great availability of C supplied by the host (Graham & Eissenstat 1994). The decrease in mycorrhizal colonization and response throughout the successional process indicates that the species which take part in the initial succession phases are markedly more colonized and responsive to AM fungi, and those belonging to the final phases are strongly less colonized and responsive to AM fungi, in low fertility soils.

The concentration and content of P, Ca and K in the leaves of the pioneer and early secondary species was increased in the inoculated plants, indicating that in low fertility soils AM fungi improve the uptake and increase the content of minerals in the woody species which begin the succession and may be less

important for those at the end of the succession. The plant species may differ in their mineral requirement, especially for P, due to the differences in their growth rates (Janos 1983), P uptake capacity and use (Koide 1991). The non-inoculated pioneer species have a greater limitation in the mineral acquisition capacity, and the greatest responses to inoculation were obtained by species with smaller seeds. Their small nutritional cotyledon reserve may increase the importance for the initial AM infection and colonization of the roots. Species which begin the succession are not only dependent on the AM fungi for growth, but they may also be very efficient in uptake of nutrients in soils where few nutrients are available, when their roots have mycorrhizal colonization. They are able to establish themselves very quickly after the event of infection and colonization, justifying the great aggressiveness of these species during the initial colonization of open and disturbed areas. This efficiency may be due to the colonization by mycorrhizal fungi, and thus the rapid initial growth would occur in spite of the small seed reserve.

Janos (1980a, 1983) suggested that, for native Costa Rican species, the understorey seedlings allow the formation of the infection because of the nutritional reserve from the large seeds, maintaining the mycorrhizal association due to its importance for growth when gaps appear. In the present study the responses of the native woody plants to the mycorrhizal colonization and response decreased with the increase in seed weight. Species with large seeds, with large reserves, especially the late secondary and climax species, which may maintain initial growth in the seedlings may prevent the initial root colonization and avoid the carbon drain by the AM fungi. The initial growth of these species in the final succession phases may depend on the duration of the cotyledon reserves, mainly when they are in low fertility soils. These species do not have significant alteration in biomass production, mineral uptake, mycorrhizal colonization and response between 9 and 31 wk after cotyledon fall. The relative growth rate decreased after the cotyledon fall and the majority of the species had leaf yellowing and fall, indicating that after the cotyledon fall, these inoculated or non-inoculated species have little mineral uptake capacity from the soil. The use and consequent decrease in the quantity of minerals in the interior of the plants could provide greater stimulus in the mycorrhizal colonization, and late successional species may become mycorrhizal-dependent in later growth stages (Siqueira *et al.* 1998). The lack of significant change in biomass, relative growth rate, mycorrhizal colonization and response of late successional species which grew for 45 wk, when compared to those which grew for 21–24 wk, suggests that the majority of the species that take part in the final succession phases do not use the AM fungi to help in mineral uptake, and do not need to absorb great quantities because of their slow growth and limitation due to light.

The high AM fungi inoculum potential and the spore content from the area at the beginning of succession, dominated by pioneer woody species, indicate

that those species are very efficient in multiplying the AM fungi, producing a large quantity of propagules in the soil that may be favourable to recruitment of their seedlings. In spite of the light differences in forest floor between the gap and the interior of the forest canopy, the mycorrhizal colonization obtained in the soil dilution and number of spores were similar. This may indicate that the late species in the succession have low AM fungi requirements, being weak multipliers, which is reflected in the small quantity of propagules in the forest soil.

The low inoculum potential and the spore content of AM fungi in the forest interior and in the gap were similar to those obtained by Asbjornsen & Montagnini (1994), Fischer *et al.* (1994) and Janos (1992), in soils in the interior of the tropical forest in Costa Rica. Besides the difference in the composition of plant species and soils, another fundamental difference is clear: the mature forest of Costa Rica is dominated by obligately mycotrophic woody species (Janos 1980a,b; 1983, 1992, 1995) while the Mata dos Godoy State Park forest is dominated by species with low susceptibility to mycorrhizal infection, colonization and response to mycotrophic growth. Therefore, the small inoculum potential found in the interior of the forest may be due to the absence of adequate host plants.

The strong correlation between mycorrhizal colonization in the field with mycorrhizal colonization of the same species in the greenhouse suggest that the colonization percentage in the greenhouse may be a reliable index for predicting of mycorrhizal colonization in the field for native woody species in this tropical region. The quantification of the root colonization may be an indicator of the availability of the inoculum in the field (Gange *et al.* 1993).

Mycorrhizal fungi may play an important role in succession, either by altering the result of plant competition or by affecting species diversity (Gange *et al.* 1990, 1993). Non-mycotrophic plants could establish themselves where the quantity of inoculum was low, dominating the community, and mycotrophic plants may be recruited and dominate where inoculum is high (Allen 1996, Allen & Allen 1990). The results obtained in this study suggest that there is a relationship between the inoculum potential found in the field and the occurrence in the different habitats of adult plant species belonging to different successional groups. Mycorrhizal colonization and response gradient can be established for habitat preference. The pioneer and early secondary species have high susceptibility to infection, high colonization and high degree of response to mycorrhiza, and frequently dominate open areas with high inoculum potential, while late secondary and climax species have reduced degree of infection, colonization and response to mycorrhiza and dominate the interior of the forest where the inoculum potential is low. Therefore, the presence of AM fungi in the soil may be one of the determinants that leads to the distribution of different plant species in the field, especially in the beginning of successional process.

Forest ecosystems have great variation because of the availability of nutrients in the soils, which range from oligotrophic to eutrophic (Vitousek 1984). In the oligotrophic ecosystems, which generally have intensely leached soils poor in nutrients, the plants have mineral conservation mechanisms in their biomass and the translocation of these before leaf fall contributes to the low mineral nutrient levels in the soil (Jordan & Herrera 1981, Vitousek & Sanford 1986). Bearing in mind that oligotrophic soils have a low mineral reserve and therefore little nutrient availability for vegetation, Janos (1980a,b) working with woody species in the Costa Rican forests which are made up of poor soils (Asbjornsen & Montagnini 1994, Johnson & Wedin 1997) suggested that the AM fungi play a fundamental role in the uptake and transference mechanism of the mineral nutrients for plants which make up the mature forest.

Structured soils with a good nutritional chemical potential exercise less pressure on the species and may maintain all the vegetation that does not need to conserve its nutrients, returning it to the environment for recycling (Pagano 1989, Vitousek & Sanford 1986). The high rates of litter decomposition and lack of nutrient conservation mechanisms before the fall of the plant constitutents make the litter rich in nutrients, leading to high mineral content in the soil, which may be in forms available to the plants (Pagano 1989, Schlittler *et al.* 1993). The most important regulatory processes of mineral cycling take place in the soil-litter subsystem, where the great decomposing biological activity and the leaching action of rain water move the nutrients towards the root network localized below or in the mentioned subsystem. The more intense the rain, the greater the quantity of minerals made available to the root systems (Moraes & Domingos 1997, Santos 1989). According to EMBRAPA (1984) the soil in the Mata dos Godoy State Park is TRe3 type, a structured red soil, clay in texture, of eutrophic variety, and derived from basalt rocks from the Trapp spill/lava sheet. The chemical composition of the soil in the forest interior has a higher mineral content than the degraded soil from an area at the beginning of succession, mainly in the quantity of P which is about six times greater. High P and Ca content in soils is also a good indicator of the efficient supply of P for plants (Santos 1989), which seems to happen in the present case. It is further believed that the low Al content found at 0–20 cm depth in the forest soil, where most of the fine roots are found, prevents one of the main forms of P immobilization from occurring in the soil, by the formation of few soluble phosphates of Fe and Al (Cooperband *et al.* 1994, Jordan & Herrera 1981).

The differences among oligotrophic and eutrophic environments show that nutrient cycling and its use are different. The nutrient content available in the soil may determine the importance of the presence of mycorrhizal fungi inoculum in these different ecosystems. The high content of nutrients in the soil forest, low mineral requirement, slow growth and shading that contribute to low carbohydrate production, may influence the adaptation of plants to survive in the interior of the forest with no or low quantities of AM fungi. On the

other hand, pioneer seedlings, which only survive in well-lit environments and grow rapidly and must produce large quantities of carbohydrates, may shelter a sufficient quantity of AM fungi in their roots which may have helped the pioneer species to establish decisively in more hostile environments with low mineral nutrient availability.

The observations obtained in this study may be an important contribution to the understanding of AM fungi ecology. The high degree of mycotrophy of the woody species belonging to the early phases of succession, together with the capacity to uptake nutrients at high rates, rapid growth, rapid turnover in the vegetative structures, short life span and the later transference of biomass to the system, may place the AM fungi as one of the main biotic factors for the installation and progress of plant succession in these tropical regions, and may be used as an important tool in inoculation programmes in forest nurseries, when the objective is rehabilitation of degraded land with native woody species.

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