Revista Brasileira de Ciência do Solo

Division - Soil Processes and Properties | Commission – Soil Biology

Dependency and Response of *Apuleia leiocarpa* to Inoculation with Different Species of Arbuscular Mycorrhizal Fungi

Joel Quintino de Oliveira Júnior[(1)], Ederson da Conceição Jesus[(2)], Marcos Gervasio Pereira[(3)*], Rodrigo Camara[(1)], Ariovaldo Machado Fonseca Júnior[(3)] and Ana Carolina Oliveira Sousa[(1)]

[(1)] Universidade Federal Rural do Rio de Janeiro, Instituto de Florestas, Programa de Pós Graduação em Ciências Ambientais e Florestais, Seropédica, Rio de Janeiro, Brasil.
[(2)] Empresa Brasileira de Pesquisa Agropecuária, Embrapa Agrobiologia, Seropédica, Rio de Janeiro, Brasil.
[(3)] Universidade Federal Rural do Rio de Janeiro, Departamento de Solos, Seropédica, Rio de Janeiro, Brasil.

*** Corresponding author:**
E-mail: mgervasiopereira01@gmail.com

Received: April 9, 2016

Approved: September 23, 2016

How to cite: Oliveira Júnior JQ, Jesus EC, Pereira MG, Camara R, Fonseca Júnior AM, Sousa ACO. Dependency and response of *Apuleia leiocarpa* to inoculation with different species of arbuscular mycorrhizal fungi. Rev Bras Cienc Solo. 2017;41:e0160174.
https://doi.org/10.1590/18069657rbcs20160174

ABSTRACT: Inoculation with arbuscular mycorrhizal fungi (AMF) is a strategy to improve the efficiency of forest plantations, reducing costs and increasing the survival of plant species. The objective of this study was to assess the response and mycorrhizal dependency of seedlings of the forest species *Apuleia leiocarpa* (Vogel) J.F. Macbr to inoculation with AMF. The experiment was conducted in a completely randomized design using a 5 × 5 factorial arrangement with six replications. The treatments consisted of combinations of five P rates (0, 24, 71, 213, and 650 mg kg^{-1}) with five types of inoculations with AMF (inoculation with the fungi *Rhizophagus clarus, Gigaspora margarita*, *Dentiscutata heterogama,* inoculation with an AMF mix of these three species, and a treatment without inoculation). The *A. leiocarpa* showed the highest biomass accumulations in inoculation with *D. hetorogama* combined with the P rates of 213 and 650 mg kg^{-1}, and in the AMF mix combined with the P rates of 71, 213, and 650 kg^{-1}. Biomass accumulation showed a linear, positive response to inoculation with *D. heterogama* combined with the different P rates, and a positive square root fit to inoculation with the AMF mix. The plants inoculated with *G. margarita* had no significant biomass accumulation. The plant species had a positive response to inoculation with *R. clarus* combined with the lowest P rates; however, it had a negative response to combination with the highest P rate (650 mg kg^{-1}). The relative benefit of inoculation with these fungi was more than 100 % in most treatments, showing the high mycorrhizal dependency of *A. leiocarpa* and the nutritional benefit of AMF inoculation for this species. However, this response is dependent on the fungus species that colonize the plant roots. The best combination between fungus and P rate was inoculation with the AMF mix combined with the P rate of 71 mg kg^{-1}.

Keywords: endomycorrhiza, phosphorus, nutritional characteristics.

INTRODUCTION

The inoculation of seedlings with arbuscular mycorrhizal fungi (AMF), which are the symbionts most commonly associated with higher plants (Smith and Read, 1997), contributes to successful revegetation of degraded areas and reduces costs by accelerating plant development (Franco and Faria, 1997; Chaer et al., 2011). These fungi occur in most terrestrial ecosystems and contribute to the productivity and competitiveness of their host plants (Sieverding et al., 1991), thus favoring plant diversification (van der Heijden et al., 1998). Arbuscular mycorrhizal fungi can exploit a more extensive volume of soil than root systems due to the length of their hyphae, contributing to increased nutrient and water uptake by plants. Therefore, mycorrhizal growth on plants is considered one of the most important associations between plants and microorganisms (Wang and Qiu, 2006).

The nutritional benefits and the response of plants to AMF colonization, as shown by their increased growth and productivity (Rocha et al., 2006), may vary with the host plant, the AMF species, and the environmental conditions (Rogers et. al., 1994; Saggin-Júnior et al., 1994; Sanders et al., 1996). There is evidence for functional specificity between these symbionts (Bever, 2002), which may depend on the balance between the benefits and costs of the symbiosis to the host plant (Koide, 1991) and vary due to differences in colonization rates or in nutrient transport efficiency between the myco-symbiont and the phyto-symbiont (Abbott and Robson, 1982).

Testing the inoculation of different AMF in the same plant species is needed in order to select the most efficient strains and to maximize the benefits of the symbiosis to the host plant (Saggin Júnior and Siqueira, 1995). Moreover, it is important to evaluate the plant's mycotrophic potential, which is defined as the plant's degree of mycorrhizal dependency. The degree of mycorrhizal dependency is related to the concentration of phosphorus in the soil solution. The main contribution of AMF to plants is through P uptake due to the low mobility of this nutrient in the soil and due to the high amounts needed for plant growth and metabolism (Siqueira and Saggin-Júnior, 2001). The higher the P rate at which AMF inoculation benefits the plant, the higher the mycorrhizal dependency of the plant (Janos, 1988).

Despite the findings reported in the literature, the benefits of mycorrhizal symbiosis and the factors responsible for differential mycorrhizal dependency are not well understood. This is noticeable especially in tree species of the Brazilian Atlantic Forest, for which there is only incipient information regarding their association with mycorrhizal fungi. One of these species is *Apuleia leiocarpa* (Vogel) J.F. Macbr (Fabaceae), a pioneer species popularly known as "garapá" or "grápia". *A. leiocarpa* is ecologically important due to its usefulness in recovery of degraded soils and economic contribution from the value of its timber (Carvalho, 2003). Despite being a legume, *A. leiocarpa* does not fix N, but it is able to associate with mycorrhizal fungi.

The hypothesis was that *Apuleia leiocarpa* has distinct responses to inoculation with different species of AMF. The objective of this study was to assess mycorrhizal dependency and the response of *Apuleia leiocarpa* (Vogel) J.F. Macbr to inoculation with arbuscular mycorrhizal fungi (AMF).

MATERIALS AND METHODS

The experiment was conducted in a greenhouse using a completely randomized design in a 5 × 5 factorial arrangement with six replications. The treatments consisted of combinations of five P rates (0, 24, 71, 213, and 650 mg kg^{-1}) applied to the soil using potassium monophosphate (KH_2PO_4), with five types of inoculations with arbuscular mycorrhizal fungi (AMF), namely, inoculations with the fungi *Gigaspora margarita* Becker and Hall (GM), *Rhizophagus clarus* Becker and Gerdemann (RC), and *Dentiscutata* *heterogama* (Nicol and Gerd) Walker and Sanders (DH), inoculation with an AMF mix of these three species (GRD), and a treatment without inoculation. An absolute control was used, which was formed from combination of the treatment without inoculation with AMF and a 0 rate of P.

The P rates (0, 24, 71, 213, and 650 mg kg^{-1}) applied to the soil were established based on remaining P (Alvarez V et al., 2000). The K concentration of the different treatments was balanced with application of KCl. The available P in the soil was determined by the Mehlich-1 extractant, which showed that the applied P was highly correlated with the available P (Figure 1).

The containers used for planting consisted of a plastic tube (280 mL) and a disposable plastic pot (700 mL) filled with about 1 kg of soil from the B horizon (*Cambissolo Háplico Tb Distrófico típico* - SiBCS - Santos et al., 2013), which had pH in water (1:2.5) of 4.9, 1.89 mg dm^{-3} of P, and 64 mg dm^{-3} of K (Mehlich-1); and Al^{3+} 1.21 $cmol_c$ dm^{-3}, Ca^{2+} 0.46 $cmol_c$ dm^{-3}, and Mg^{2+} of 0.21 $cmol_c$ dm^{-3} (1 mol L^{-1} KCl). Liming was carried out with Minercal (TNP 91 %, CaO 39 %, and MgO 13 %) at 943 g Mg^{-1} of soil, based on soil chemical analysis. The substrate was incubated for 60 days.

The substrate of each container was inoculated with 1 g of soil and roots colonized with propagules of AMF according to each treatment. The fungi species were inoculated with different amounts: *Dentiscutata heterogama* (DHET A2 - CNPAB002) with 17 spores g^{-1}, *Gigaspora margarita* (GMAR A1 - CNPAB001) with 35 spores g^{-1}, and *Rhizophagus clarus* (RCLA A5 – CNPAB005) with 26 spores g^{-1}. Ten ml of a filtrate solution from the three AMF inocula, free of propagules, was added to all containers to standardize the treatments regarding the other components of the inoculum microbiota.

The *A. leiocarpa* seeds were sanitized with an H_2O_2 (30 %) solution for 2 min and immersed in an H_2SO_4 (98 %) solution for 15 min to overcome dormancy. Subsequently, seeds were germinated in petri dishes with filter paper and cotton inside a germination chamber (Biological Oxygen Demand) under constant light and temperature of 28 °C for 5 days.

Three pre-germinated seeds were placed in each container and the seedlings were thinned after 15 days, leaving one seedling per container.

Soil fertilization was performed monthly with application of 100 mL of a nutrient solution [$CaCl_2(H_2O)_2$ (2 mmol L^{-1}), $MgSO_4(H_2O)_7$ (1 mmol L^{-1}), KCl (3 mmol L^{-1}), $ZnSO_4(H_2O)_7$ (0.9 μmol L^{-1}), H_3BO_3 (4 μmol L^{-1}), $CuSO_4(H_2O)_5$ (1 μmol L^{-1}), $MnSO_4H_2O$ (6 μmol L^{-1}), $NaMoO_4(H_2O)_2$ (0.1 μmol L^{-1}), and Fe EDTA (1,66 %) (Bertrand et al., 2000), and 120 mg of N (NH_4NO_3)] to each seedling. The plants were irrigated daily by an automatic sprinkler system, maintaining soil moisture at 70 % of field capacity.

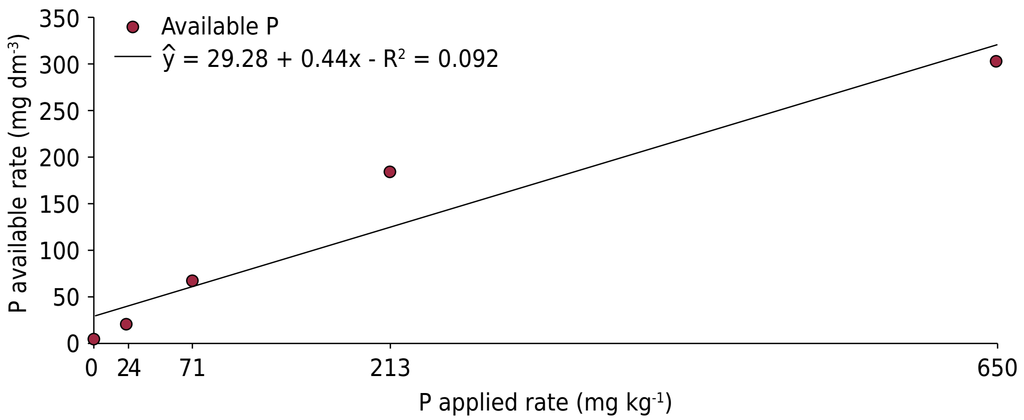

Figure 1. Relationship between applied P and available P (significant at 1%, by the F test) in the soil, evaluated through the Mehlich-1 extractant.

Seedling heights were measured from the root crown to the shoot apical meristem with a ruler (mm), and stem diameter was measured with a digital caliper (mm). Measurements were carried out every 21 days over 98 days, beginning after the first month after planting, for a total of four measurements for each variable.

The seedlings were removed from the containers and separated into shoots and roots. Colonization of the root system by the AMF was evaluated, with three replications. Fine roots (0.5 g) were taken from plants of each treatment and maintained in an ethanol solution (50 %) until the whitening and staining process (Koske and Gemma, 1989; Grace and Stribley, 1991). The percentage rate of root colonization by the AMF was evaluated through the gridline intersect method (Giovanetti and Mosse, 1980), adapted from the root length method (Newman, 1966).

The root dry weight (RDW) was also evaluated with three replications, whereas the shoot dry weight (SDW) was evaluated with six replications. Plants of each treatment were dried in a forced-air circulation oven (65 °C, 72 h) and weighed (g) on a 3-decimal-place analytical balance to evaluate the RDW and SDW. The RDW to SDW ratio (RSr) was calculated from these two variables. Both materials were ground in an electric mill and subjected to nitric-perchloric digestion (2:1) for extraction of P and K, and sulfuric acid digestion for extraction of N (Silva, 1999). The P contents were determined by colorimetry, K by flame photometry (Silva, 1999), and N by the modified Kjeldahl method.

The P benefit, mycorrhizal benefit (increase in dry weight from the fungal species), and symbiotic efficiency were assessed using response curves to the P applied, estimated by regression analysis, as described by Saggin Júnior and Siqueira (1995).

The relative increase of biomass (RI) in the forest species due to the treatments was defined as the increase in dry weight by the inoculation and fertilization compared to the treatment without inoculation with AMF and with a P rate of 0 (absolute control). This calculation was performed for all P rates. This relation was converted into percentage of relative increase of root dry weight (RI-RDW) and relative increase of shoot dry weight (RI-SDW), which was calculated by the formula

$$RI = [(DWt - DWac)/DWac] \times 100$$

in which DWt is the dry weight (RDW or SDW) of the treatment, and DWac is the dry weight (RDW or SDW) of the absolute control (without inoculation with AMF and with P rate of 0).

All data were transformed (Box-Cox) to achieve normalization and subjected to analysis of variance. The Scott Knott test at 5 % significance was applied to compare the averages. Regression analysis was applied to the quantitative factors. The Sisvar 5.0 software (Ferreira, 2003) was used for statistical analyses.

RESULTS

Apuleia leiocarpa response to inoculation with arbuscular mycorrhizal fungi (AMF)

The species *A. leiocarpa* showed distinct responses to the different arbuscular mycorrhizal fungi (AMF) and P rates, as indicated by variations in plant biomass (root, shoot, and total dry weight) (Figure 2).

The total biomass of *A. leiocarpa* inoculated with *R. clarus* decreased linearly (Figure 2a). Inoculated seedlings accumulated more biomass than uninoculated seedlings with the four initial P rates (0, 24, 71, 213 mg kg^{-1}); however, this was reversed at the rate of 650 mg kg^{-1}, in which uninoculated seedlings accumulated more biomass. Based on the estimated model, the combination of P with AMF is negative as of the P rate of 444 mg kg^{-1} (value T') (Figure 2a), which represents 225 mg dm^{-3} of available P (Figure 1).

Contrary to what was observed for *R. clarus*, the total biomass of *A. leiocarpa* inoculated with the fungi *D. heterogama* and *G. margarita* increased linearly with the P rates (Figures 2b and 2c), denoting a synergistic effect of these species with P availability. The shoot biomass of plants inoculated with the fungus *D. heterogama* increased at all P rates compared to the plants without inoculation (Figure 2b). In contrast, plants inoculated with *G. margarita* had a weaker initial development up to the P rate of 139 mg kg^{-1} (98 mg dm^{-3} of available P), as estimated by the statistical model, with a positive response to inoculation beyond this rate (Figure 2c).

The accumulation of total biomass in response to inoculation with the AMF mix followed a square root model (Figure 2d). The dry weight of seedlings in this treatment increased from the P rate of 71 mg kg^{-1}, tending to stabilize as of the P rate of 213 mg kg^{-1}, with no significant variation up to the rate of 650 mg kg^{-1}.

The mycorrhizal benefit varied depending on the fungus species (Table 1). Higher mycorrhizal benefits promoted by the fungus *D. heterogama* and the AMF mix denote a symbiotic efficiency higher than 100 % in both treatments (Table 1).

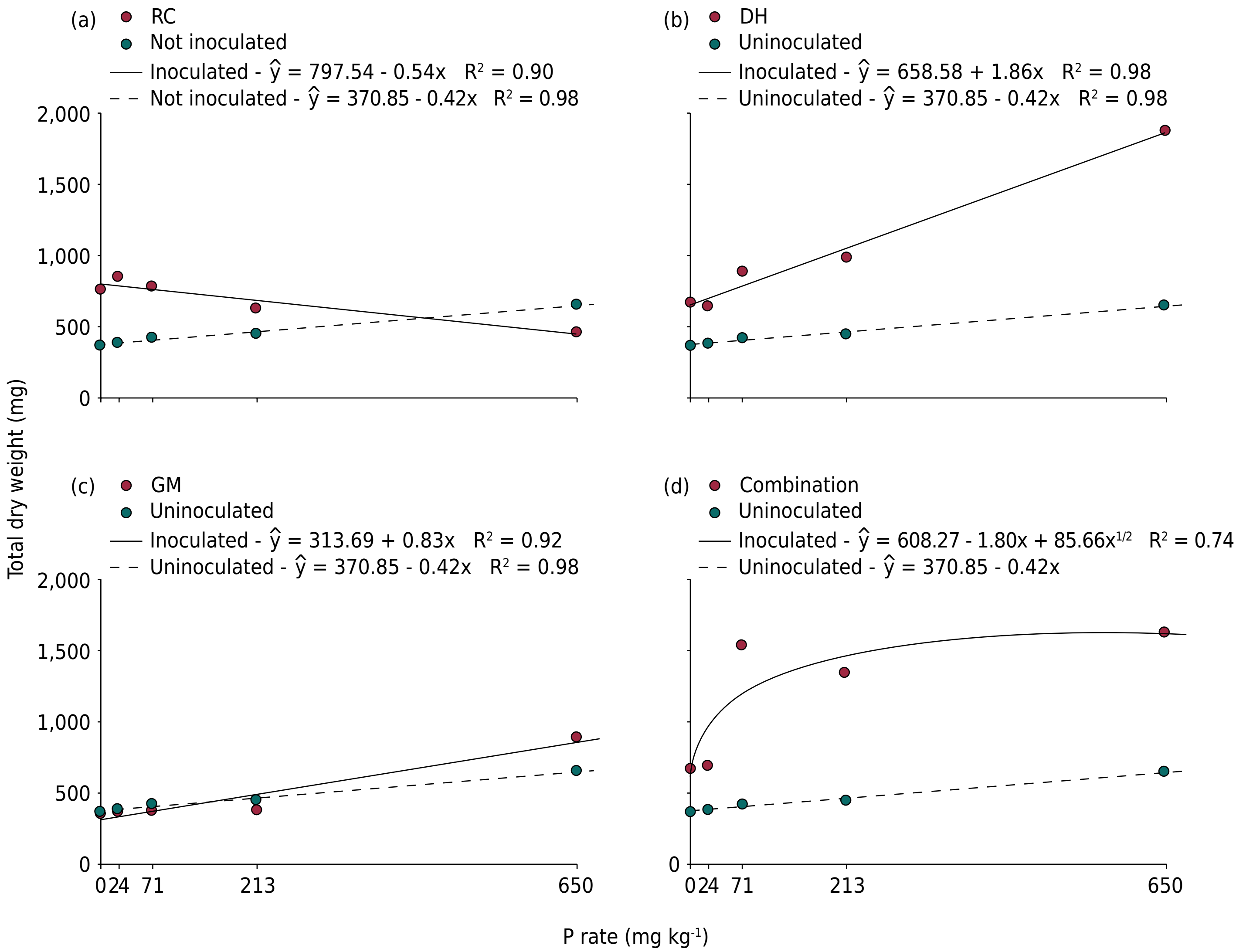

Figure 2. Response curves for phosphorus rates of *Apuleia leiocarpa* seedlings inoculated with (a) *Dentiscutata heterogama* (DH) and without inoculation, (b) *Rhizophagus clarus* (RC), (c) *Gigaspora margarita* (GM), and (d) with a mix of *Dentiscutata heterogama*, *Gigaspora margarita*, *and Rhizophagus clarus*.

Relative increase

Inoculation with *D. heterogama* combined with the highest P rate (650 mg kg^{-1}) promoted the highest increase in shoot dry weight (SDW) of *A. leiocarpa*, resulting in an increase of approximately 550 % compared with the absolute control (without inoculation and P rate of 0). Inoculation with the AMF mix combined with the highest P rate increased shoot development by 492 % compared to the absolute control, with the highest root growth increase at the P rate of 71 mg kg^{-1} (Figure 3).

Plant responses to inoculation with *R. clarus* were different since the highest relative increase of shoot biomass was observed at the lowest P rates, decreasing with increasing P rates. A relative increase in roots was expressive only in the combination with the P rate of 0 (Figure 3).

Inoculation with the fungus *G. margarita* resulted in relative increases similar to those of the treatments without inoculation, at all P rates. The most expressive response was for root growth at the highest P rate, which resulted in an increase of approximately 300 % (Figure 3).

The response of *A. leiocarpa* to increasing P rates in treatments without inoculation was 20 % (24 mg kg^{-1}), 13 % (71 mg kg^{-1}), 38 % (213 mg kg^{-1}), and 5 % (650 mg kg^{-1}), indicating that the roots of this species may be inefficient in taking up P, due to genetic characteristics. These responses were low compared with those observed in the treatments of inoculations with *D. heterogama* and the AMF mix. Seedlings in the treatments without

Table 1. Response of *Apuleia leiocarpa* to inoculation with different arbuscular mycorrhizal fungi (AMF) regarding mycorrhizal benefit, P benefit, and symbiotic efficiency, considering total plant dry weight

Treatment	Mycorrhizal benefit[2]	P benefit[3]	Symbiotic efficiency[4]
	Area		%
D. heterogama	150.73	128.99	117
R. clarus	32.56	128.99	25
G. margarita	0	128.99	0
AMF mix (GRD)[1]	138.81	128.99	108

[1] GRD: AMF mix of the three arbuscular mycorrhizal fungi. [2] Estimated by subtracting the area under the curve of the treatment without inoculation from the area under the curve of the treatments with inoculation. [3] Estimated by the definite integral of the curve of the treatments without inoculation. [4] Estimated by the relation between the mycorrhizal benefit and P benefit.

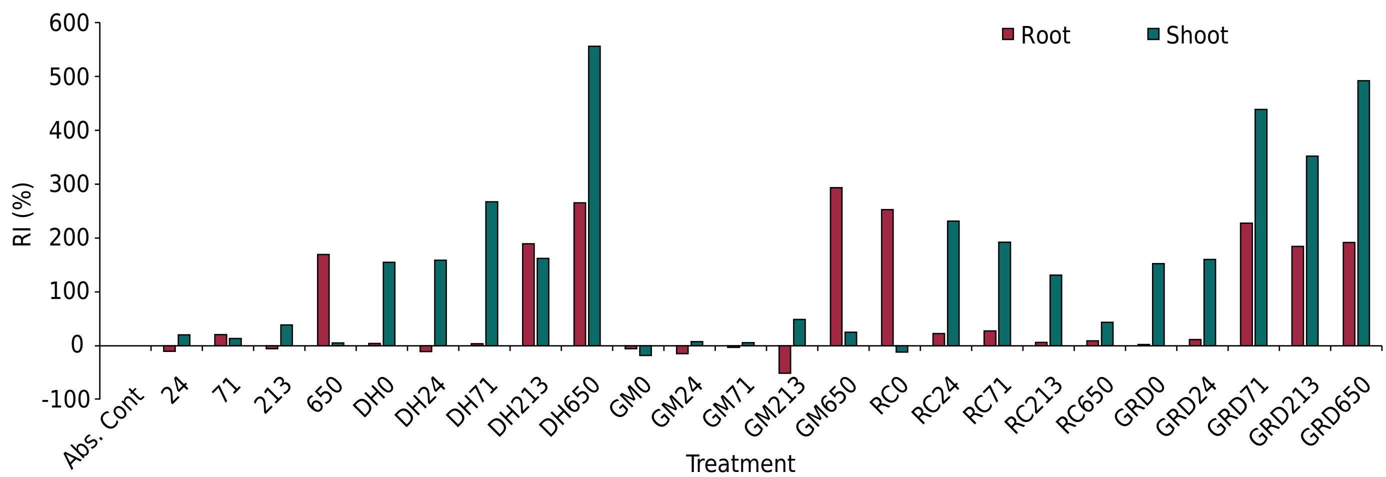

Figure 3. Relative increase (RI) of *Apuleia leiocarpa* seedlings due to inoculation with *Dentiscutata heterogama* (DH), *Gigaspora margarita* (GM), *Rhizophagus clarus* (RC), and GRD (DH+GM+RC) at different phosphorus rates (0, 24, 71, 213, and 650 mg kg^{-1}), according to the variables SDW (shoot dry weight) and RDW (root dry weight).

inoculation had the highest shoot growth at the highest P rate, reaching a growth approximately 200 % higher than that found in the absolute control (Figure 3).

The results, in general, showed a decrease in the RDW to SDW ratio (RSr) in treatments with inoculation compared with those without inoculation. The highest RSr were found in plants with the highest P rate, without AMF inoculation (2.16) and with inoculation with *G. margarita* (2.64), and in the treatment with a P rate of 0 and inoculated with *R. clarus* (3.36) (Figure 4), denoting higher root system growth.

The best treatments for the RSr were *Dentiscutata heterogama* with 71 mg kg^{-1} of P (DH71) (0.24), *Gigaspora margarita* with 213 mg kg^{-1} of P (GM213) (0.28), *Dentiscutata heterogama* with a P rate of 0 (DH0) (0.34), AMF mix with a P rate of 0 (GRD0) (0.34), *Rhizophagus clarus* with 213 mg kg^{-1} of P (RC213) (0.39), and *Rhizophagus clarus* with 650 mg kg^{-1} of P (RC650) (0.64) (Figure 4).

All factors (fungus species, P rate, and their interaction) were significant at 5 % probability for the variables related to the biomass of the species evaluated (Table 2).

Inoculation with the AMF mix combined with the P rates of 71 to 650 mg kg^{-1} resulted in a higher increase in SDW compared with the treatments of single inoculations at these same rates. An exception was the treatment DH650, which had a similar increase in SDW (Table 2).

Apuleia leiocarpa had no response to inoculation with *G. margarita*, showing increases in RDW and SDW equivalent to those found in the treatments without inoculation (Table 2), except at the rate of 650 mg kg^{-1}, in which the plants showed a significant increase in RDW. Nevertheless, the treatment GM650 had no increase in SDW, indicating that the fungus *G. margarita* was inefficient in increasing SDW, showing more sensitivity to P than the other AMF.

The colonization rate of all treatments with AMF inoculation ranged from 56 % at the lowest P rate to 8 % at the highest P rate. The treatments without inoculation had no mycorrhizal colonization.

The highest increases in seedling height were observed in treatments GM650 (27.2 cm), HD71 (23.5 cm), HD213 (21.8 cm), and RC650 (21.7 cm) (Table 2). Plants inoculated with the AMF mix combined with the P rates of 0 and 24 mg kg^{-1} had high colonization rates; however, their height had little development.

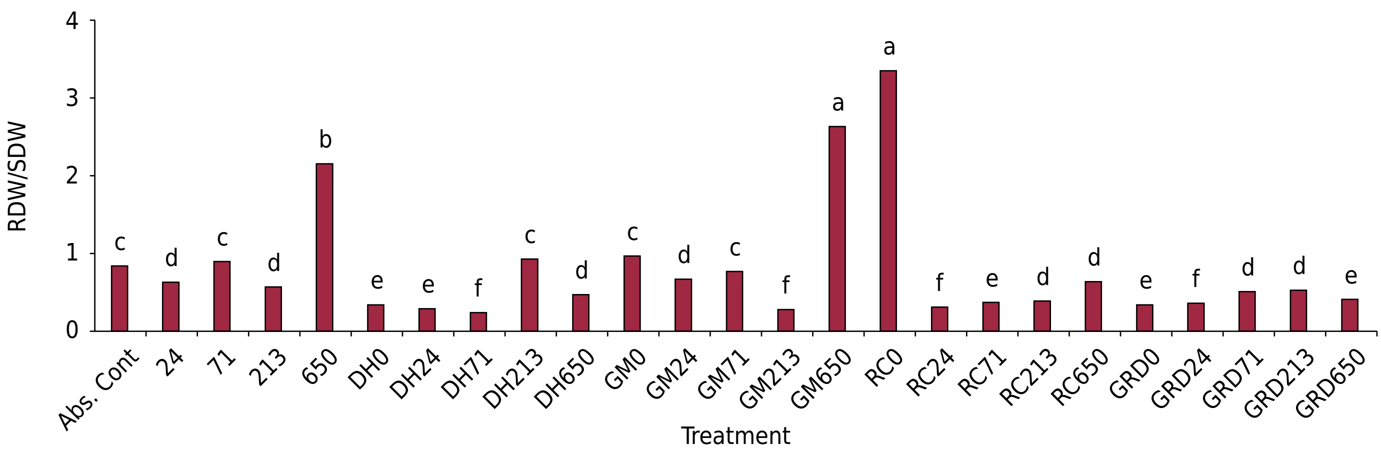

Figure 4. Comparative analysis of the ratios of RDW (root dry weight) to SDW (shoot dry weight) of *Apuleia leiocarpa* seedlings with the highest biomass increases due to inoculation with the AMF (arbuscular mycorrhizal fungi), *Dentiscutata heterogama* (DH), *Gigaspora margarita* (GM), *Rhizophagus clarus* (RC), and AMF mix of these three species (GRD) and phosphorus rates (0, 24, 71, 213, and 650 mg kg^{-1}). Averages followed by different lowercase letters in the columns indicate significant difference between treatments by the Scott Knott test (p=0.05).

Seedling stem diameters ranged from 5.99 mm (control treatment) to 7.71 mm (DH650), with no significant differences depending on the treatments (Table 2).

Nutritional benefit

The plants of treatments inoculated with AMF had greater nutritional benefits compared with those of treatments without inoculation. These benefits were evident for P nutrition, with differences found only in the plant shoots (Table 3). No significant differences were observed in N and K contents in either roots or shoots.

Table 2. Growth of *Apuleia leiocarpa* seedlings inoculated with different arbuscular mycorrhizal fungi (AMF), combined with five phosphorus rates

Treatment	RDW	SDW	TDW	RSr	D	Mycorrhizal	Height
	g				mm	%	cm
0	0.16 eB	0.20 dB	0.36 fB	0.84 cB	5.99 a	-	16.00 b
24	0.15 eB	0.23 cB	0.38 fC	0.63 dA	6.02 a	-	19.23 b
71	0.20 eB	0.22 cC	0.42 eC	0.90 cA	6.00 a	-	18.50 b
213	0.16 dB	0.27 cC	0.43 eC	0.57 dB	6.40 a	-	19.20 b
650	0.44 bB	0.21 dB	0.65 cC	2.16 bA	6.58 a	-	18.21 b
DH0	0.17 eB	0.50 bA	0.67 cA	0.34 eB	6.21 a	55 a	18.00 b
DH24	0.15 eB	0.50 bA	0.65 cB	0.29 eB	6.75 a	16 c	19.01 b
DH71	0.17 eB	0.72 bB	0.89 bB	0.24 fC	7.42 a	33 b	23.50 a
DH213	0.47 bA	0.51 bB	0.99 bB	0.93 cA	7.05 a	55 a	21.80 a
DH650	0.60 aA	1.28 aA	1.88 aA	0.47 dB	7.71 a	30 b	19.81 b
GM0	0.16 eB	0.16 dB	0.32 fB	0.97 cB	6.22 a	14 c	20.06 b
GM24	0.14 eB	0.21 cB	0.35 fC	0.67 dA	6.48 a	13 c	19.30 b
GM71	0.16 eB	0.21 dC	0.37 fC	0.77 cB	7.04 a	12 c	19.03 b
GM213	0.08 eC	0.29 cC	0.37 fC	0.28 fC	6.20 a	8 c	18.17 b
GM650	0.65 aA	0.24 cB	0.89 bB	2.64 aA	7.57 a	0	27.20 a
RC0	0.58 aA	0.17 dB	0.75 bA	3.36 aA	6.94 a	52 a	19.33 b
RC24	0.20 eA	0.65 bA	0.85 bA	0.31 fB	6.93 a	41 a	16.50 b
RC71	0.21 cB	0.57 bB	0.78 bB	0.37 eC	6.89 a	47 a	16.66 b
RC213	0.17 eB	0.45 bB	0.63 dC	0.39 dB	6.39 a	44 a	19.70 b
RC650	0.18 eB	0.28 cB	0.46 eC	0.64 dB	6.14 a	24 b	21.70 b
GRD0	0.17 eB	0.49 bA	0.66 cA	0.34 eB	6.85 a	56 a	20.01 b
GRD24	0.18 dB	0.51 bA	0.69 cB	0.36 fB	6.21 a	27 b	19.22 b
GRD71	0.54 bA	1.05 aA	1.59 aA	0.51 dB	7.70 a	50 a	19.40 b
GRD213	0.47 bA	0.88 aA	1.35 aA	0.53 dB	7.65 a	48 a	19.05 b
GRD650	0.48 bB	1.15 aA	1.63 aA	0.41 eB	7.69 a	28 b	19.71 b
Test F (rate)	49.65*	16.21*	38.28*	26.87*			
Test F (fungus)	12.79*	71.66*	85.73*	23.29*			
Interaction	23.18*	7.89*	13.37*	24.07*			
CV rate	59.26	70.22	54.55	98.36			
CV fungus	66.77	53.83	46.35	99.52			
CV (%)	30.61	36.42	25.25	47.33	8.71	38.27	15.74

The treatments consist of letters representing the arbuscular mycorrhizal fungi species (DH: *Dentiscutata heterogama*, GM: *Gigaspora margarita*, RC: *Rhizophagus clarus*, and GRD: DH+GM+RC), followed by numbers representing the P rates (0, 24, 71, 213, and 650 mg kg^{-1}); the numbers without letters represent the P rates with absence of fungi; the rate 0 represents the absolute control. RDW: root dry weight; SDW: shoot dry weight; TDW: total dry weight; RSr: root to shoot ratio; D: stem diameter at the soil surface. Averages followed by different lowercase letters in the columns indicate significant difference between treatments by the Scott Knott test (p=0.05). Averages followed by different uppercase letters in the columns indicate significant difference between treatments with the same P rate and different fungi species by the Scott Knott test (p=0.05). *: significant at 5 %.

Table 3. Interaction between phosphorus rates and different arbuscular mycorrhizal fungi (AMF) on nutritional characteristics of *Apuleia leiocarpa*

Treatment	Root			Shoot		
	N	P	K	N	P	K
	%					
0	0.27 a	0.27 a	1.92 a	0.75 a	0.13 b	1.35 a
24	0.33 a	0.25 a	2.18 a	0.87 a	0.24 b	1.34 a
71	0.24 a	0.24 a	2.03 a	0.86 a	0.29 b	1.32 a
213	0.50 a	0.27 a	2.61 a	0.86 a	0.30 b	1.40 a
650	0.41 a	0.27 a	2.25 a	0.92 a	0.25 b	1.39 a
DH0	0.27 a	0.28 a	2.26 a	0.75 a	0.34 a	1.33 a
DH24	0.24 a	0.25 a	2.25 a	0.75 a	0.41 a	1.38 a
DH71	0.32 a	0.29 a	2.47 a	0.67 a	0.46 a	1.49 a
DH213	0.31 a	0.24 a	2.07 a	0.77 a	0.50 a	1.35 a
DH650	0.34 a	0.34 a	2.28 a	0.71 a	0.42 a	1.40 a
GM0	0.35 a	0.31 a	1.53 a	0.89 a	0.37 a	1.37 a
GM24	0.40 a	0.35 a	2.52 a	0.90 a	0.37 a	1.31 a
GM71	0.34 a	0.36 a	1.83 a	0.94 a	0.45 a	1.33 a
GM213	0.38 a	0.33 a	2.22 a	0.83 a	0.22 b	1.34 a
GM650	0.34 a	0.29 a	1.96 a	0.87 a	0.43 a	1.42 a
RC0	0.46 a	0.40 a	3.53 a	0.74 a	0.27 b	1.33 a
RC24	0.36 a	0.27 a	2.31 a	0.88 a	0.49 a	1.37 a
RC71	0.35 a	0.30 a	2.72 a	0.79 a	0.27 b	1.31 a
RC213	0.33 a	0.30 a	2.16 a	0.77 a	0.63 a	1.36 a
RC650	0.30 a	0.32 a	2.14 a	0.93 a	0.49 a	1.31 a
GRD0	0.33 a	0.32 a	2.89 a	0.95 a	0.33 b	1.41 a
GRD24	0.37 a	0.40 a	3.45 a	1.02 a	0.39 a	1.34 a
GRD71	0.37 a	0.51 a	2.34 a	1.19 a	0.38 a	1.29 a
GRD213	0.38 a	0.53 a	1.86 a	0.89 a	0.50 a	1.36 a
GRD650	0.47 a	0.67 a	2.49 a	0.81 a	0.52 a	1.33 a
CV (%)	32.46	40.62	25.47	32.48	62.51	28.35

The treatments consist of letters representing the arbuscular mycorrhizal fungi species (DH: *Dentiscutata heterogama*, GM: *Gigaspora margarita*, RC: *Rhizophagus clarus*, and GRD: DH+GM+RC), followed by numbers representing the P rates (0, 24, 71, 213, and 650 mg kg^{-1}); the numbers without letters represent the P rates with absence of fungi; the P rate 0 represents the absolute control. Averages followed by different lowercase letters in the columns indicate significant difference between treatments by the Scott Knott test (p=0.05).

DISCUSSION

The forest species *A. leiocarpa*, which is native to the Brazilian Atlantic Forest, showed greater gains in biomass when in symbiosis with arbuscular mycorrhizal fungi (AMF). The results showed that *A. leiocarpa* has different responses to inoculation depending on the AMF species, as also reported for other plant species (Saggin-Júnior et al., 1994; Siqueira and Saggin-Júnior, 2001; Jansa et al., 2007). The response to the increase in P rates was linear and positive for the plants inoculated with the fungi *D. heterogama* and *G. margarita*, and linear and negative for those inoculated with *Rhizophagus clarus*, indicating an ecological difference between these fungi species. Moreover, *A. leiocarpa* had a different response to inoculation with the AMF mix, whose data fitted a positive square root model with increasing P rates and showed a significantly higher biomass increase at the lowest rates, indicating synergism between the fungi. Moreover, the fungi probably colonize the roots according to their preferential P rates, thus, better supplying the plant needs under different conditions than species applied singly (Figure 2).

Apuleia leiocarpa can be classified as a species of high mycorrhizal dependency, considering its significant responses to inoculation with *D. heterogama* and the AMF mix, and the high mycorrhizal and relative benefits obtained from inoculation with these fungi (Janos, 1988; Siqueira and Saggin-Júnior, 2001).

The higher shoot growth of inoculated *A. leiocarpa* resulted in a lower ratio of root (RDW) to shoot dry weight (SDW) (Zangaro et al., 2005). Similar results were found in all treatments with P rates and without inoculation, and those inoculated with *G. margarita*, exhibiting an increase in the RDW to SDW ratio (RSr) with increasing P rates, except in the treatment inoculated with *R. clarus* and a P rate of 0 (CR0). This result indicates that this fungus induces the root system development of its host under low P availability. Pereira et al. (1996) evaluated inoculation with the fungus *Glomus etunicatum* (Becker and Gerdemann) in the forest species *Acacia mangium* (L.), *Senna macranthera* (Collad.) I and B, *Senna multijuga* (L.C. Rich) I and B, and *Anadenanthera peregrina* (L.) Speg. and found different responses of these plant species, reporting increases in the RSr at the lowest P rate only for *Senna macranthera* (Collad.) I and B., *Senna multijuga* (L.C. Rich) I. and B., and *Anadenanthera peregrina* (L.) Speg.

Height increases were not related to increases in plant biomass, since the plants of most of the treatments had similar heights, unlike the results reported in previous studies (Saggin-Júnior et al., 1994; Pouyú-Rojas et al., 2000).

Another positive effect of mycorrhizal inoculation was improvement in P nutrition, denoting the importance of inoculation for P uptake in *A. leiocarpa*. This improvement was observed in plants inoculated singly or with the mix of the three fungi species. Inoculation with AMF favored P content in the shoots, regardless of the P rates. For example, the P content of the AMF mix inoculation treatment was 6.79 times greater than the P of the plants without inoculation combined with the P rate of 0. Similar results were found by Moreira-Souza and Cardoso (2002), who evaluated the species *Araucaria angustifolia* inoculated with the fungus *Glomus intraradices*, *Gigaspora rosea*, and a native AMF mix, and they found increases in the amount of P taken up and accumulated with increasing P rates. According to these results, the main effect of the fungi for plant nutrition is improvement in uptake of low mobility nutrients, especially P, Cu, and Zn (Habte, 2000).

The colonization rate varied depending on the AMF species and P rates. However, the colonization of all fungi species tended to be inverse to the P rates, decreasing with increasing P rates. This result was probably because available P content is one of the main limiting factors for symbiosis (Smith and Read, 1997; Rocha et al., 2006; Parniske, 2008; Renuka et al., 2012).

The response models vary depending on the plant species since the increase in *A. leiocarpa* growth showed a linear fit to the fungi inoculations, whereas Saggin-Júnior et al. (1994) reported a square root fit for coffee seedlings inoculated with *G. margarita* and *R. clarus*, and negative responses from high P rates.

Functional specificity among fungi species is a possible hypothesis since inoculations with the AMF mix promote additional growth in the host plant compared with inoculations with a single fungus. Arbuscular mycorrhizal fungi show different efficiencies with the use of P, even among isolates of the same species, and different AMF species have different strategies for soil utilization and root colonization, indicating the complementarity and benefit of greater richness of species in the system (Maherali and Klironomos, 2007). This is a favorable factor for use of the AMF mix, since plants inoculated with the AMF mix were able to adapt to different environmental conditions. Studies show that greater richness and complementarity are related to greater productivities of plant communities.

These results have important implications for studies on plant-AMF interactions. For example, studies on mycorrhizal dependency must consider inoculations with different AMF species to avoid underestimating the response and dependency of the plant species under evaluation. The *A. leiocarpa* responses (positive or negative) to increasing P rates varied with the AMF species, indicating that the choice of the fungus species for estimating mycorrhizal dependency can lead to different conclusions. The plants that were not inoculated had very small responses to an increase in the P rates, also denoting a high dependency of *A. leiocarpa* on mycorrhizae for uptake of this nutrient, and implying a very high Janos' T value. Other authors have reported varied responses of this plant to AMF species (van der Heidjen et al., 1998, Siqueira and Saggin-Júnior, 2001); different responses to inoculations with the fungi *R. clarus* and *D. heterogama* were found.

Results indicate that inoculation with the AMF mix was the best treatment, to which plants showed significant responses when combined with low and intermediate P rates, and thus, it can be recommended for producing *A. leiocarpa* seedlings. The positive responses to lower P rates mean a decrease in fertilization costs. Other studies reported the benefits of co-inoculation of AMF, such as greater tolerance to fungicides (Schreiner and Bethlenfalvay, 1997) and functional complementarity (Jansa et al., 2007), which were not assessed in this study.

Another implication is that inoculation with the AMF mix may benefit the plant by increasing functional redundancy (Jansa et al., 2007), since different species may have different responses to environmental conditions after planting. AMF may have different soil use strategies; thus, a fungi community can benefit the plant in different conditions. The diversity in a community of mycorrhizal species with different functional responses may contribute to increase the productivity of plant communities (Maherali and Klironomos, 2007).

From the ecological point of view, these results are relevant to establishing species in a field, but raise questions about the role of soil community compositions on the plant growth of forest communities, especially in the Atlantic Forest.

All these characteristics must be considered to choose forest species and mycorrhizal fungi for recovery of degraded areas, obtaining the most appropriate and fast growing seedlings and, consequently, decreasing costs. Thus, the selection of symbionts with greater compatibility tends to facilitate the recovery of degraded areas, reduce chemical inputs, and improve inoculant production conditions by working with a smaller number of plant species and mycorrhizal fungi of greater efficiency, generating good quality seedlings.

CONCLUSIONS

The *A. leiocarpa* seedlings showed high mycorrhizal dependency, and seedling response to inoculation with arbuscular mycorrhizal fungi (AMF) was dependent on the fungal species colonizing their roots. *Apuleia leiocarpa* was highly responsive to inoculations with *Dentiscutata heterogama* and the AMF mix (*Dentiscutata heterogama*, *Gigaspora margarita* and *Rhizophagus clarus*).

Inoculation of *Apuleia leiocarpa* with the AMF mix under greenhouse conditions resulted in the greatest growth; thus, it is recommended for production of *A. leiocarpa* seedlings. The best treatment was inoculation with the AMF mix combined with the P rate of 71 mg kg^{-1}.

REFERENCES

Abbott LK, Robson AD. The role of vesicular arbuscular mycorrhizal fungi in agriculture and the selection of fungi for inoculation. Aust J Agric Res. 1982;33:389-408. https://doi.org/10.1071/AR9820389

Alvarez V VH, Novais RF, Dias LE, Oliveira JA. Determinação e uso do fósforo remanescente. Bol Inf Inf Soc Bras Cienc Solo. 2000;25:27-32.

Bertrand H, Plassard C, Pinochet X, Touraine B, Normand P, Cleyet-Marel JC. Stimulation of the ionic transport system in *Brassica napus* by a plant growth-promoting rhizobacterium (*Achromobacter* sp.). Can J Microbiol. 2000;46:229-36. https://doi.org/10.1139/w99-137

Bever JD. Host-specificity of AM fungal population growth rates can generate feedback on plant growth. Plant Soil. 2002;244:281-90. https://doi.org/10.1023/A:1020221609080

Chaer GM, Resende AS, Campello EFC, Faria SM, Boddey RM. Nitrogen-fixing legume tree species for the reclamation of severely degraded lands in Brazil. Tree Physiol. 2011;31:139-49. https://doi.org/ 10.1093/treephys/tpq116

Carvalho PER. Grápia: Taxonomia e nomenclatura. Colombo: Embrapa Floresta; 2003. (Circular técnica, 77).

Ferreira DF. Programa de análises estatísticas (Statistical Analysis Software) e planejamento de experimentos. SISVAR 5.0 (Build 67) [programa de computador]. Lavras: DEX-UFLA; 2003. Disponível em: http://www.dex.ufla.br/~danielff/softwares.htm.

Franco AA, Faria SM. The contribution of N_2-fixing tree legumes to land reclamation and sustainability in the tropics. Soil Biol Biochem. 1997;29:897-903. https://doi.org/10.1016/S0038-0717(96)00229-5

Grace C, Stribley DP. A safer procedure for routine staining of vesicular-arbuscular mycorrhizal fungi. Mycol Res. 1991;95:1160-2. https://doi.org/10.1016/S0953-7562(09)80005-1

Giovanetti M, Mosse B. An evaluation of techniques to measure vesicular-arbuscular mycorrhizal infection roots. New Phytol. 1980;84:489-500. https://doi.org/10.1111/j.1469-8137.1980.tb04556.x

Habte M. Mycorrhizal fungi and plant nutrition. In: Silva JA, Uchida R, editors. Plant nutrient management in Hawaii's soils, approaches for tropical and subtropical agriculture. Hawaii: University of Hawaii at Manoa; 2000. p.127-31

Janos DP. Mycorrhiza applications in tropical forestry: are temperate-zone approaches appropriate? In: NG FSP, editor. Trees and mycorrhiza. Kuala Lumpur: Forest Research Institute; 1988. p.133-88.

Jansa J, Smith FA, Smith SE. Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi? New Phytol. 2007;177:779-89. https://doi.org/10.1111/j.1469-8137.2007.02294.x

Koide RT. Nutrient supply, nutrient demand and plant response to mycorrhizal infection. New Phytol. 1991;117:365-86. https://doi.org/10.1111/j.1469-8137.1991.tb00001.x

Koske RE, Gemma JN. A modified procedure for staining roots to detect VA mycorrhizas. Mycol Res. 1989;92:486-8. https://doi.org/10.1016/S0953-7562(89)80195-9

Maherali H, Klironomos JN. Influence of phylogeny on fungal community assembly and ecosystem functioning. Science. 2007;316:1746-8. https://doi.org/10.1126/science.1143082

Moreira-Souza M, Cardoso EJBN. Dependência micorrízica de *Araucaria angustifolia* (Bert.) O. Ktze. sob doses de fósforo. Rev Bras Cienc Solo. 2002;26:905-12. https://doi.org/10.1590/S0100-06832002000400007

Newman EI. A method of estimating the total length of root in a sample. J Appl Ecol. 1966;3:139-45. https://doi.org/10.2307/2401670

Parniske M. Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nature Rev Microbiol. 2008;6:763-75. https://doi.org/10.1038/nrmicro1987

Pereira EG, Siqueira JO, Curi N, Moreira FMS, Purcino AAC. Efeitos da micorriza e do suprimento de fósforo na atividade enzimática e na resposta de espécies arbóreas ao nitrogênio. Rev Bras Fisiol Veg. 1996;8:59-65.

Pouyú-Rojas E, Siqueira JO. Micorriza arbuscular e fertilização do solo no desenvolvimento pós-transplante de mudas de sete espécies florestais. Pesq Agropec Bras. 2000;35:103-14. https://doi.org/10.1590/S0100-204X2000000100013

Renuka G, Rao MS, Kumar VP, Ramesh M, Reddy SR. Arbuscular mycorrhizal dependency of *Acacia melanoxylon*. Proc Nat Acad Sci, India Section B: Biol Sci. 2012;82:441-6. https://doi.org/10.1007/s40011-012-0025-1

Rocha FS, Saggin Júnior OJ, Silva EMR, Lima WL. Dependência e resposta de mudas de cedro a fungos micorrízicos arbusculares. Pesq Agropec Bras. 2006;41:77-84. https://doi.org/10.1590/S0100-204X2006000100011

Rogers JB, Christie P, Laidlaw AS. Some evidence of host specificity in arbuscular mycorrhizas. Pedosphere. 1994;4:377-81.

Saggin Júnior OJ, Siqueira JO. Avaliação da eficiência simbiótica de fungos endomicorrízicos para o cafeeiro. Rev Bras Cienc Solo. 1995;19:221-8.

Saggin-Júnior OJ, Siqueira JO, Guimarães PTG, Oliveira E. Interação fungos micorrízicos versus superfosfato e seus efeitos no crescimento e teores de nutrientes do cafeeiro em solo não fumigado. Rev Bras Cienc Solo. 1994;18:27-36.

Sanders IR, Clapp JP, Wiemken A. The genetic diversity of arbuscular mycorrhizal fungi in natural ecosystems- a key to understanding the ecology and functioning of the mycorrhizal symbiosis. New Phytol. 1996;133:123-34. https://doi.org/10.1111/j.1469-8137.1996.tb04348.x

Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Oliveira JB, Coelho MR, Lumbreras JF, Cunha TJF. Sistema brasileiro de classificação de solos. 3a ed. Rio de Janeiro: Embrapa Solos; 2013.

Schreiner RP, Bethlenfalvay GJ. Mycorrhizae, biocides, and biocontrol. 3. Effects of three different fungicides on developmental stages of three AM fungi. Biol Fertil Soils. 1997;24:18-26. https://doi.org/10.1007/BF01420215

Silva FC, organizador. Manual de análises químicas de solos, plantas e fertilizantes. Brasília, DF: Embrapa Solos; 1999.

Sieverding E, Friedrichsen J, Suden W. Vesicular-arbuscular mycorrhiza management in tropical agrosystems. Eschborn: Deutsche Gesellschaft für Technische Zusammenarbeit; 1991.

Siqueira JO, Saggin-Júnior OJ. Dependency on arbuscular mycorrhizal fungi and responsiveness of some Brazilian native woody species. Mycorrhiza. 2001;11:245-55. https://doi.org/10.1007/s005720100129

Smith ES, Read JD. Mycorrhizal symbiosis. 2nd ed. New York: Academic Press; 1997.

van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature. 1998;396:69-72. https://doi.org/10.1038/23932

Wang B, Qiu YL. Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza. 2006;16:299-363. https://doi.org/10.1007/s00572-005-0033-6

Zangaro W, Nishidate FR, Camargo FRS, Romagnoli GG, Vandressen J. Relationships among arbuscular mycorrhizas, root morphology and seedling growth of tropical native woody species in southern Brazil. J Trop Ecol. 2005;21:529-40. https://doi.org/10.1017/S0266467405002555