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Selection of mycorrhizal fungi for the initial growth of *Albizia polycephala*

Renata Soares dos Santos[1], Rafael Nogueira Scoriza[1], Eliane Maria Ribeiro da Silva[2], Orivaldo José Saggin Júnior[2]

[1] Universidade Federal Rural do Rio de Janeiro, BR-465, Km 7, CEP 23897-000, Seropédica-RJ, Brasil. E-mail: renatalm2@gmail.com; rafaelscoriza@gmail.com
[2] Embrapa Agrobiologia, Rodovia BR-465, km 7, Ecologia, CEP 23891-000, Seropédica-RJ, Brasil. E-mail: eliane.silva@embrapa.br; orivaldo.saggin@embrapa.br

ABSTRACT

The use of inoculant mycorrhizal fungi brings several benefits to plants and may contribute to the production of high-quality and low-cost forest seedlings. Thus, the aim of this study was to evaluate the contribution of different species of mycorrhizal fungi to the initial growth of seedlings of *Albizia polycephala*. The experiment was evaluated every two weeks for a total period of 135 days. A randomized block design was established with nine replications and six treatments where there was one control (absence of inoculants) and five treatments with different species of AMF: *Gigaspora margarita*, *Dentiscutata heterogama*, *Scutellospora calospora*, *Claroideoglomus etunicatum* and *Acaulospora colombiana*. All AMF inoculants promoted growth in seedlings of *Albizia polycephala*, but the most effective were *Acaulospora colombiana*, mainly due to superiority in diameter, size of the largest leaf and weight of dry matter, which reflected similar results regarding mycorrhizal efficiency. Inoculation with *Scutellospora calospora* is a secondary alternative because this also favors the growth of *A. polycephala* and contributes to the increase in the content of leaf phosphorus.

Key words: white angico, inoculant, forest seedlings

Seleção de fungos micorrízicos arbusculares para o crescimento inicial em Albizia polycephala

RESUMO

O uso de inoculantes de fungos micorrízicos traz diversos benefícios às plantas, podendo contribuir para a produção de mudas florestais de qualidade e baixo custo. Com isso, o objetivo deste trabalho foi avaliar a contribuição de diferentes espécies de fungos micorrízicos arbusculares para o crescimento inicial de mudas de *Albizia polycephala*. O experimento foi avaliado quinzenalmente por um período total de 135 dias, conduzido em blocos casualizados, com nove repetições e seis tratamentos, sendo uma testemunha (sem inoculação) e cinco espécies de FMAs: *Gigaspora margarita*, *Dentiscutata heterogama*, *Scutellospora calospora*, *Claroideoglomus etunicatum* e *Acaulospora colombiana*. Todos os inoculantes de FMA promoveram crescimento nas mudas de *Albizia polycephala*, porém o mais eficiente foi o de *Acaulospora colombiana*, principalmente pela superioridade no diâmetro, tamanho da maior folha e peso da matéria seca, que refletiram em resultado semelhante para a eficiência micorrízica. A inoculação com *Scutellospora calospora* é uma alternativa secundária, pois também favorece o crescimento de *A. polycephala*, além de contribuir para o aumento no teor de fósforo foliar.

Palavras-chave: angico-branco, inoculante, mudas florestais

Introduction

Preservation and restoration of forests are a growing concern that demands production of seedlings of low cost, high quality and able to survive in low-fertility soils (Scabora et al., 2011). The use of arbuscular mycorrhizal fungi (AMF) facilitates this demand. The correct selection and inoculation of species with beneficial functions can contribute to vegetation establishment on degraded soils by accelerating its development, making plants more tolerant to stress incurred by transplantation and promoting the restoration of nutrient cycling (Balota et al., 2011; Machineski et al., 2011; Angelini et al., 2013).

The mutual symbiosis between mycorrhizal fungi and plant promotes a significant increase of volume of explored soil, facilitating the absorption of water and nutrients, especially those with slow movement such as phosphorus (Smith and Read, 2008;. Balota et al, 2011), resulting in higher growth rates, survival and biomass allocation (Cavalcante et al., 2008). Other benefit of inoculation with AMF is the maintenance of plant growth even under conditions of stress, toxicity and soil acidity, fungal pathogens and certain pests (Smith & Read, 2008; Stürmer & Siqueira, 2013; Faria et al., 2013).

Among the forest species that have potential use in forestry practices, *Albizia polycephala* (Benth.) Killip ex Record.), popularly known as white angico, belongs to the climax ecological group, that demand light and have anemochoric dispersion (Alvarenga et al., 2006). It belongs to the family Fabaceae and is an endemic species with large natural occurrence in Caatinga, Atlantic rainforest and Cerrado throughout 15 Brazilian states (Carvalho, 2006). It occurs inside primary forests as well as in secondary associations ("capoeira" and "capoeirões") of rainforests and seasonal forests, occupying the forest canopy and occurring in small populations with discontinuous distribution (Carvalho, 2006; Caiafa & Martins, 2010). It may reach high frequency, density and relative dominance in natural formations of Atlantic rainforest (Batista et al., 2012).

There is little information on the initial growth of *Albizia polycephala,* and nothing is known on the effect of inoculation with micro-organisms on the growth of this species. However, Carvalho (2006) reports the association with bacteria of the genus *Rhizobium* which produces abundant quantities of nodules, leading us to believe that inoculation with mycorrhizal fungi can favor the growth of this legume. Thus, the aim of the present study was to evaluate the contribution of different species of arbuscular mycorrhizal fungi to the initial growth of seedlings of *Albizia polycephala,* allowing for the selection of the most efficient plant-fungus symbiosis.

Material and Methods

Seed germination and seedling inoculation with AMF

The experiment was conducted in the laboratory of Mycorrhizae, Embrapa Agrobiology. Seeds of *Albizia polycephala,* commercially purchased, were superficially disinfected by immersion in 2% sodium hypochlorite for 3 minutes. Then, mechanical scarification was performed using sandpaper (# 100) to facilitate the absorption of water by the seed. Seeds were placed in trays containing a mixture of sand and autoclaved vermiculite in 2: 1 proportion (vv^{-1}). Seedlings that presented the first pair of leaves were transplanted to 700 ml plastic containers with 380 cm^3 PVC plastic tube attached to its bottom. Physical and chemical characteristics of the soil used are shown in Table 1.

Inoculation with mycorrhizal fungi from the collection of the own laboratory was performed at the time of transplanting seedlings, using inoculum soil with at least 100 spores, which may also contain fragments of roots and hyphae. The experiment was conducted in a completely randomized block design with nine replications and six treatments where there was one control (without inoculation) and five treatments corresponding to different species of AMF: *Gigaspora margarita* W.N. Becker & I.R. Hall, *Dentiscutata heterogama* (T.H. Nicolson & Gerd.) Sieverd., F.A. Souza & Oehl, *Scutellospora calospora* (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders, *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüßler e *Acaulospora colombiana* (Spain & N.C. Schenck) Kaonongbua, J.B. Morton & Bever.

Table 1. Chemical and physical characteristics of the soil used in the evaluation of the initial growth of *Albizia polycephala* inoculated with arbuscular mycorrhizal fungi

Chemical characteristics								
pH	P	K	Al	H+Al	Ca	Mg	C	N
(H_2O)	mg L^{-1}			cmol$_c$ dm^{-3}			g kg^{-1}	
4.50	4.19	34.30	0.33	2.87	0.70	0.30	2.80	0.40
Physical characteristics								
Total clay		Total sand		Fine sand		Coarse sand		Silty
%								
68.3		17.8		4.3		13.5		13.9

Realization of the experiment and evaluation of the initial growth of seedlings of *Albizia polycephala*

Seven days after the setting the experiment, 15 ml of a filtrate without the presence of AMF structures were applied to each pot in order to standardize the microbial community among treatments. For its production, we used 5 g of each inoculum per 1 L of water. These were stirred in a blender and sieved in a 0.053 mm mesh. The sieved liquid was put in filter paper. In the fortnightly evaluations, when symptoms of nutritional deficiency were noted, 10 ml of nutrient solution were applied per pot. This solution consists of 0.5 mL of KH_2PO_4 0.006 mol L^{-1} solution, 1.5 mL of KCl 1 mol L^{-1} solution, 1.5 mL of $(NH_4)_2SO_4$ 0.5 mol L^{-1} solution, 1.5 mL of $Ca(NO_3)_2.4H_2O$ 1 mol L^{-1} solution, 1 mL of $MgSO_4.7H2O$ 0.6 mol L^{-1} solution, 0.5 mL of $C_{10}H_{12}FeN_2NaO_8$ 0.06 mol L^{-1} solution and 0.5 mL of the mixture between H_3BO_3 0.03 mol L^{-1}, $MnCl_2.4H_2O$ 0.004 mol L^{-1}, $ZnSO_4.7H_2O$ 0.0005 mol L^{-1}, $CuSO_4.5H_2O$ 0.002 mol L^{-1} and $Na_2MoO_4.2H_2O$ 0.00004 mol L^{-1}, filled up to 1 liter of distilled water.

After 30 days of transplantation, fortnightly evaluations of height (using ruler with scale in cm), diameter at the plant neck height (using digital caliper with scale in millimeters) and length of the longest leaf (using ruler with scale in centimeters)

were carried out for a period of four months. At the end of the four months, the weight of the dry matter of shoot (aerial parts of the plant) (WDMS), weight of the dry matter of roots (WDMR), weight of the dry matter of nodules, shoot/root ratio, leaf phosphorus content, root colonization rate, spore density and symbiotic efficiency were evaluated.

Spores were counted from a sample of 50 g of soil per pot. For extraction, the soil sample was moistened and soil aggregates were manually undone. After stirring, the suspension was left still for 30 seconds. This suspension was subjected to the set of superposed sieves (0.42 mm sieve over the 0.053 mm sieve). This process was repeated until, when adding water to the suspension, this resulted to be translucent. The sample was then centrifuged at 3000 rpm for 3 min, and the supernatant was discarded. An amount of 45% sucrose solution was added, stirring the suspension and submitting it to a new centrifugation at 2000 rpm for 2 min. The material was poured into the 0.053 mm sieve, removing excess sucrose solution in tap water and the sample of spores was stored in refrigerator. For the counting, spores were placed in a fluted Petri dish and observed under stereomicroscope.

In order to evaluate root colonization, roots were initially washed and 0.5 g of fine roots were randomly collected per sample. These were individually put in test tubes, subjected in sequence to 2.5% potassium hydroxide for 24 h, washed in tap water, 1% hydrochloric acid for 24 h, and methyl blue dye in 0.05% acidified glycerol for 24 h. The evaluation of the percentage of fungal structures in the root was performed using the method of interception in checkered plate, where the samples were placed in a Petri dish with a grid of ½ inch and observed under stereoscopic microscope. One hundred root segments were observed crossing the grid lines in each sample, checking the presence or absence of colonization. The total number of segments with presence of colonization was converted into a percentage based on the total observed segments.

Symbiotic efficiency (SE) in percentage for each inoculant of AMF was calculated for the shoot and root, based on the weight of dry matter (Angelini et al., 2013).

Data analysis

Data were analyzed for homogeneity of variances of errors with Cochran & Barttlet test, and for normality with Lilliefors test. When defined as parametric, the evaluation of the contribution of each fungus to the growth was carried out with Dunnett test, at 5%. In order to compare the data between the AMF species, data were submitted to analysis of variance with the Scott-Knott test at 5% of probability. Exceptionally, the weight of nodules was analyzed using the Friedman test. Pearson correlation analysis at 5% was used to test the relation between dry weight of shoots and roots.

Results and Discussion

The species of mycorrhizal fungi used in this study contributed differently to the initial growth of the forest legume *Albizia polycephala*. With the exception of growth in height, only the species *S. calospora* contributed to all other evaluated characteristics of seedling growth. Besides this species of AMF, only *A. colombiana* contributed to the weight of nodules and *G. margarita* to foliar phosphorus content (Table 2).

As noted, it is not appropriate to assume that the presence of the fungus will always stimulate plant growth. It must be considered that there are differences in genetic compatibility between AMF species (Machineski et al., 2009) with a particular host plant, what may determine its dependence on mycorrhizae and contribute to its adaptation to the local (Balota et al., 2011; Mello et al., 2012, Faria et al., 2013). Furthermore, the adaptation of the fungus to original soil conditions may impair its performance as symbiont where this was applied (Pánková et al., 2014). Thus, this knowledge on mycorrhizal condition, ability to form symbioses and to respond to inoculation with mycorrhizal fungi is helpful to the production of seedlings of this species, ensuring the success of its use in reforestation (Scabora et al., 2011; Moora, 2014).

The evaluation of different mycorrhizal fungi contribution in a forest species, regardless of its efficiency for each feature, is a perspective rarely explored in current research. However, it is important to note that all these features may be crucial for the establishment of seedlings and their survival in field. Thus, the present evaluation may be relevant to the proper selection and inoculation of mycorrhizal fungi with beneficial functions (Soares & Carneiro, 2010; Angelini et al., 2013).

Inoculation with different species resulted in changes in seedlings' development along their growth in greenhouse. After forty-five days transplantation, the seedlings inoculated with *S. calospora* and *A. colombiana* showed larger leaf size than seedlings with other inoculants. From the third evaluation onwards, it was observed that the same species of AMF and *D. heterogama* resulted in seedlings with values of diameter and size of the largest leaf superior to the control. In the last evaluation, the 135th day, it was observed that the inoculation with *A. colombiana* results in seedlings with larger diameter and size of the largest leaf. Still, all other inoculants of AMF were equal among themselves, but all higher than the control (Table 3).

Table 2. Contribution of arbuscular mycorrhizal fungi to the initial growth of *Albizia polycephala*

AMF species	Diameter	Height	Size of the largest leaf	WDMS	WDMR	Weight of nodules	Leaf phosphorus
S. calospora	+	0	+	+	+	+	+
A. colombiana	+	0	+	+	+	+	0
C. etunicatum	+	0	+	+	+	0	0
D. heterogama	+	0	+	+	+	0	0
G. margarita	+	0	+	+	0	0	+

Feature improvement (+) or absence of difference (0) in the control according to Dunnett test at 5%. WDMS = weight of the dry matter of shoot; WDMR = weight of the dry matter of roots.

Table 3. Diameter at the plant neck, height and size of the largest leaf seedlings of *Albizia polycephala* inoculated with arbuscular mycorrhizal fungi

Inoculant	30	45	60	75	90	105	120	135
	Days							
	Neck diameter (mm)							
S. calospora	1.5 a[(1)]	1.9 a	2.1 a	2.3 b	2.4 b	2.5 b	2.5 b	2.6 b
A. colombiana	1.6 a	2.0 a	2.4 a	2.7 a	2.8 a	2.9 a	3.0 a	3.0 a
C. etunicatum	1.4 a	1.7 a	1.8 b	2.1 c	2.2 c	2.3 b	2.4 b	2.4 b
D. heterogama	1.4 a	1.8 a	2.0 a	2.4 b	2.4 b	2.4 b	2.4 b	2.4 b
G. margarita	1.4 a	1.6 a	1.5 b	1.8 c	1.9 c	2.1 c	2.1 c	2.3 b
Control	1.3 a	1.7 a	1.5 b	1.7 c	1.7 c	1.8 c	1.8 d	1.8 c
CV	18.93	21.34	21.03	17.76	15.75	14.73	13.20	11.29
	Height (cm)							
S. calospora	6.2 a	6.9 a	7.2 a	7.2 a	7.7 a	7.7 a	7.8 a	7.8 a
A. colombiana	6.4 a	7.6 a	8.1 a	8.4 a	8.7 a	8.7 a	8.8 a	8.8 a
C. etunicatum	6.0 a	6.7 a	7.5 a	7.9 a	8.3 a	8.3 a	8.5 a	8.5 a
D. heterogama	4.9 a	5.9 a	6.3 a	6.4 a	6.7 a	6.7 a	6.9 a	6.9 a
G. margarita	5.1 a	5.9 a	7.1 a	7.6 a	8.2 a	8.5 a	8.6 a	8.6 a
Control	6.1 a	6.7 a	7.1 a	7.4 a	7.9 a	8.1 a	8.2 a	8.2 a
CV	34.40	33.59	31.43	29.87	28.05	26.79	26.66	26.63
	Size of the largest leaf (cm)							
S. calospora	4.1 a	4.7 a	5.1 a	4.9 b	5.0 b	5.2 b	5.0 b	5.2 b
A. colombiana	4.1 a	5.6 a	6.4 a	6.7 a	6.5 a	6.5 a	6.4 a	6.5 a
C. etunicatum	3.8 a	4.1 b	4.4 b	4.8 b	4.8 b	4.9 b	4.9 b	5.0 b
D. heterogama	3.8 a	4.1 b	4.8 a	4.7 b	5.4 b	5.3 b	5.4 b	5.5 b
G. margarita	3.2 a	3.6 b	3.6 b	4.2 b	4.7 b	4.9 b	5.1 b	5.2 b
Control	3.1 a	3.1 b	3.2 b	3.2 c	3.1 c	3.1 c	2.9 c	2.7 c
CV	26.41	24.11	24.79	23.63	20.70	19.43	16.94	16.08

[(1)] Equal letters in the column do not differ according to the Scott-Knott test at 5%

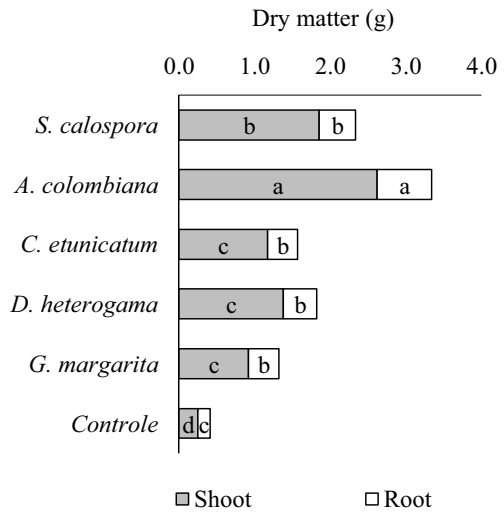
Some authors have found a positive influence on the increase in diameter in genipap seedlings (*Genipa American*) and *Acacia mangium* inoculated with AMF (Soares et al., 2012; Angelini et al., 2013) and differences in diameters throughout the experiment depending on the species of AMF used (Mello et al., 2012.). These variations may be related to soil conditions, degree of mycorrhizal dependency of the plant, compatibility between species and capacity of colonization in AMF species (Machineski et al., 2009).

The height of the seedlings of *Albizia polycephala* did not differ between treatments throughout the evaluations (Table 3). This result is unexpected because each species of AMF carries different amounts of P, causing different effects on their growth, as observed by other authors (Faria et al., 1995; Machineski et al., 2009; Mello et al., 2012; Shukla et al., 2012; Angelini et al., 2013)

The change in size of the largest leaf observed between treatments may reflect a differentiated absorption of nutrients between AMF species, since mycorrhizal fungi cause several benefits to growth, just as a bio-fertilizer, helping to balance plant nutrition (Soares & Carneiro, 2010) and conferring greater vigor (Stürmer & Siqueira, 2013). The evaluation of this feature of the plan is important because it is related to the establishment and increased survival in field (Souza et al., 2006; Soares & Carneiro, 2010; Angelini et al., 2013), because larger leaves are directly related to greater photosynthetic potential.

Regarding the weight of the dry matter of shoot (WDMS), the inoculant *A. colombiana* led to better results than the other treatments. As for the weight of the dry matter of the root (WDMR), this was superior in the treatments where the inoculated species were *A. colombiana* and *S. calospora* when compared to the control (Figure 1).

There was a high correlation between the weight of shoots and roots ($r = 0.97$; $p < 0.01$), considering all treatments. This result indicates that a greater root development, promoted by colonization with mycorrhizal fungi, causes greater absorption of nutrients and water (Smith & Read, 2008; Balota et al., 2011) and consequently, allocation of biomass to the shoot (Cavalcante et al., 2008).



Equal letters for shoot or root part do not differ according to the Scott-Knott test at 5%

Figure 1. Dry weight of shoot and root in seedlings of *Albizia polycephala* inoculated with different species of mycorrhizal fungi

AMF inoculation in seedlings of other Albizia genera has been tested in relation to weight of dry matter. Inoculation of *A. lebbeck* with *C. etunicatum* resulted in superior dry matter of shoot and root when contrasted to the control (Faria et al., 1995). Seedlings of *A. morrowiae* inoculated with *A. colombiana* had lower values of WDMR than other species of inoculants (Angelini et al., 2013). As stated earlier, these results confirm the existence of the intimate relationship species-host in this symbiosis.

The evaluation of symbiotic efficiency of shoot and root, which considers the weight of inoculated and non-inoculated

Table 4. Characteristics of seedlings of *Albizia polycephala* and their association with the different species of AMF inoculants

Inoculant	Symbiotic efficiency in the shoot	Symbiotic efficiency in the root	Root/shoot ratio	Phosphorus foliole (g kg^{-1})	Colonization (%)	Spores (g^{-1} soil)	Nodules (g)
S. calospora	210 b[(1)]	1053 b	3.89 a	1.77 a	28.5 a	0.96 c	0.081 A
A. colombiana	356 a	1593 a	3.63 a	1.11 b	26.5 a	12.3 b	0.085 A
C. etunicatum	148 b	631 b	2.98 a	1.06 b	22.0 a	51.2 a	0.004 A
D. heterogama	179 b	821 b	3.15 a	1.54 a	29.0 a	6.2 c	0.027 A
G. margarita	153 b	516 b	2.30 b	2.51 a	27.8 a	2.76 c	0.016 A
Control	-	-	1.52 b	0.56 b	0 b	0 c	0.000 A
CV	27.14	54.87	40.57	62.90	49.84	64.93	31.10

[(1)]Lowercase and uppercase letters in the same column do not differ according to the Scott-Knott and Friedman test at 5%, respectively

plants, confirmed that the fungus *A. colombiana* had superior results than the others (Table 4). As positive symbiotic efficiency was found in all species tested, it can be said that they promoted the growth of *Albizia polycephala*, what confirms and supports the discussion based on the Table 2. Mycorrhizal efficiency in different AMF species results in different growth responses in seedlings and, even when there is no specificity, the symbiosis is determined by edaphoclimatic factors and aspects of the fungus - plant relationship (Soares et al., 2012).

Mycorrhizal seedlings had higher root/shoot ratios with the exception of seedlings inoculated with *G. margarita,* which did not differ from control (Table 4). Greater ratios have been observed in plants with mycorrhizae in other studies (Pereira et al., 1996). This variation is due to the allocation of biomass, which can not be affected by mycorrhizal colonization (Vandresen et al., 2007). The fact that *G. margarita* led to root/shoot ratio that were not superior then control is explained by the its lack of influence on root growth (Table 2).

With respect to the phosphorus content in the leaflet, highest values were observed in seedlings inoculated with *S. calospora*, *G. margarita* and *D. heterogama* (Table 4), although the latter did not contribute to the improvement of this trait in the plant (Table 2). The highest values of leaf phosphorus generally contribute to the growth of plants with mycorrhizae (Cavalcante et al., 2008). This was true for inoculation with *S. calospora* if observed the number of favored characteristics (Table 2), but false for the other inoculants. The lack of clarity in this regard comes from nutritional imbalance that can occur in the early stage of seedling growth, where the fungus species is more favored (by assimilates) than the plant (for all nutrients needed), resulting in low growth (França et al., 2014).

The evaluation of mycorrhizal colonization rate showed that the different inoculants did not promote statistical different results, peaking at 29% with *D. heterogama* (Table 4). According Machineski et al. (2009) differences in colonization among AMF species are expected and result in different benefits to the plant. However root colonization depends on several factors, such as forest species, nutrition, substrate, AMF species and soil pH (Mello et al., 2012). Thus, there is a difficulty in finding a single explanation for the results observed in this study.

Nodules were found only in plants inoculated with AMF, even when there was no difference in its weight between treatments (Table 4). The presence of nodules in legumes is common (Angelini et al., 2013), as for example, these were observed in high abundance for *A. polycephala* by Carvalho (2006). Generally, plants colonized with AMF are able to absorb more P and provide a greater amount of carbohydrates to rhizobia, stimulating nodulation (Pereira et al., 1996). This dual symbiosis may favor plant growth, and this could mean less expenses with fertilizers (Dias et al., 2012).

Regarding the number of mycorrhizal fungi spores at the end of the experiment, the treatment with *C. etunicatum*, with 51 spores g^{-1}, was superior to the others in this parameter. Spores were not observed in the control (Table 4). The high sporulation found in the species *C. etunicatum* indicates a good functional compatibility of symbionts (Mello et al., 2012), although this may not necessarily result in higher growth (Machineski et al., 2009). In fact, some plant features were favored by this inoculant (Table 2), and these results may be related to the reproductive behavior of this species, which produces large numbers of small spores.

Conclusions

The initial growth of seedlings of *Albizia polycephala* is most efficient when the seedlings are inoculated with the mycorrhizal fungus *Acaulospora colombiana*.

Inoculation with *Scutellospora calospora* is a secondary alternative because this also favors the growth of *A. polycephala* and contributes to the increase in the content of leaf phosphorus.

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