

Articles

Native or exotic arbuscular mycorrhizal fungi on angico and brauna seedlings

Fungos micorrízicos arbusculares nativos ou exóticos em mudas de angico e braúna

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ABSTRACT

This study aimed to assess the growth and mycorrhization of greenhouse-grown angico (*Anadenanthera peregrina* (Vell.) Brenan) and brauna (*Melanoxylon brauna* Schott) seedlings based on inoculation with a community of selected or field-collected arbuscular mycorrhizal fungi (AMF), in formulated and commercial substrate. Each species corresponded to one experiment, conducted in a completely randomized design and 2x4 factorial scheme, consisting of two substrates, four inoculation treatments and four repetitions of 12 seedlings each, totaling 384 seedlings per experiment. The substrates used were: DAR - mixture of 30% organic compost, 30% subsoil (clay), 30% sandy soil and 10% rock phosphate; and CS - commercial substrate consisting of expanded vermiculite, peat, charcoal, macro and micronutrients; and four treatments: T1 - control; T2 - fertilized control; T3 - mixture of selected fungi; and T4 - mixture of native fungi. At 100 days after sowing (DAS) for angico and 180 DAS for brauna, seedling height and diameter were measured and leaf area (LA), shoot (SDW) and root dry weight (RDW), number of AMF spores and mycorrhizal colonization were determined. Angico seedlings grown in DAR obtained the highest diameter, height, SDW, RDW and LA values, as well as greater inoculation efficiency, LA, RDW, as well as greater inoculation efficiency and sporulation in this substrate. In both substrates, inoculated angico seedlings were taller and had a larger LA than their noninoculated counterparts. In brauna, the type of substrate only influenced seedling diameter, with DAR promoting greater growth. In DAR substrate, inoculation with selected AMF (T3) resulted in greater seedling height and diameter. In CS, all the seedlings produced in T2 (fertilized and noninoculated) died, whereas the inoculation treatments increased LA, SDW and RDW. It is recommended that angico seedlings grown in DAR be inoculated with native AMF collected in the field. For brauna, further research is needed using other production techniques combined with AMF inoculation.

Keywords: On farm inoculation; *Anadenanthera peregrina*; *Melanoxylon brauna*; Mycorrhiza

RESUMO

O objetivo deste trabalho foi avaliar o crescimento e a micorrização de mudas de angico (*Anadenanthera peregrina* (Vell.) Brenan) e braúna (*Melanoxylon brauna* Schott) em casa de vegetação a partir de inoculante com comunidade de fungos micorrízicos arbusculares (FMAs) selecionados ou coletada em campo, em substrato formulado e comercial. Cada espécie fez parte de um experimento, instalado em delineamento inteiramente casualizado em esquema fatorial 2x4, sendo dois tratamentos de substratos, quatro de inoculação e quatro repetições de 12 mudas cada, totalizando 384 mudas por experimento. Os substratos utilizados foram: RAD - composto pela mistura de 30% de composto orgânico, 30% de subsolo (argiloso), 30% de solo arenoso e 10% de fosfato de rocha; e SC - substrato comercial composto pela mistura de vermiculita expandida, turfa, carvão, macro e micronutrientes; e quatro tratamentos: T1 - controle; T2 - controle adubado; T3 - mistura de fungos selecionados; e T4 - mistura de fungos nativos. Aos 100 dias após a semeadura para angico e 180 dias para braúna, foram realizadas medições de altura e diâmetro do coleto das mudas e determinados: área foliar (AF), matéria seca da parte aérea (MSPA) e sistema radicular (MSSR), número esporos de FMAs e colonização micorrízica. Para o angico, as mudas produzidas no substrato RAD proporcionaram maiores valores de altura, diâmetro, MSPA, AF, MSSR, além de maior eficiência da inoculação e esporulação no substrato. As mudas de angico inoculadas apresentaram maior altura e AF quando comparadas às não inoculadas, em ambos os substratos. Para braúna, o tipo de substrato influenciou apenas o diâmetro, cujo RAD promoveu maior crescimento. No substrato RAD, a inoculação com FMAs selecionados (T3) proporcionou maiores incrementos em altura e diâmetro. No substrato SC todas as mudas produzidas no tratamento T2 (adubado e não inoculado) morreram, enquanto os tratamentos de inoculação promoveram aumentos na produção de AF, MSPA, e MSSR. Recomenda-se a produção de mudas de angico com substrato RAD inoculadas com comunidade de FMAs nativos coletada em campo. Para braúna, sugerem-se estudos utilizando outras técnicas de produção aliadas à inoculação com FMAs.

Palavras-chave: Inoculante *on farm*; *Anadenanthera peregrina*; *Melanoxylon brauna*; Micorrizas

1 INTRODUCTION

The use of native species in forest restoration projects should be encouraged, with forest nurseries responsible for supplying seedlings of native species with ecosystem functions similar to those of the original plant coverage in the region (Oliveira Neto; Fonseca; Carvalho, 2015). Species not submitted to genetic selection and for which there is little information on initial growth are generally produced in smaller numbers by forest nurseries, as is the case of *Anadenanthera peregrina* (angico) and *Melanoxylon brauna* (brauna). These arboreal species belong to the family Fabaceae and occur in the Atlantic Forest biome. They have timber and ecological value, are subject to predatory exploitation and lack seedling production technologies (Francisco *et al.*, 2017).

Inoculation with plant growth-promoting microorganisms, particularly arbuscular mycorrhizal fungi (AMF), is a seedling production technique that can be enhanced to improve the availability of seedlings of native species (Camara *et al.*, 2017). AMF belong to the phylum Glomeromycota, whose symbiosis with plant roots increases the surface area for water and nutrient uptake, improves water balance and tolerance to root diseases, Al and heavy metal toxicity, increases seedling growth and vigor during development and transplantation to the field, and stimulates sustainable agriculture by using fewer industrialized pesticides (Nouh; Abdel-Azeem, 2020; Moreira; Siqueira, 2006). There are reports that seedlings of trees species with mycorrhizae have high survival rates in areas degraded by mining processes (Mello *et al.*, 2011). Degraded soils generally exhibit severe nutrient deficiencies, such as N and P, as well as compromised physical attributes. Arbuscular mycorrhizae increase nutrient absorption and contribute to better soil fertility and structure, thus improving the establishment of new species in a given location (Fall *et al.*, 2022).

However, AMF species differ in terms of promoting growth in tree species because although symbioses are purportedly not host specific (Helgason *et al.*, 2007), there is functional compatibility between symbionts (Silva *et al.*, 2004). This means that inoculants with different compositions of AMF species have different levels of efficiency in the beneficial responses of plants. Inoculants can be multiplied from native AMF communities that occur in the field or selected species maintained in germplasms (Rodrigues; Barroso; Figueiredo, 2018). Evaluating these types of inoculants for each tree species ensures efficient mycorrhization of seedlings in nurseries.

One of the factors that can interfere in seedling mycorrhization is the substrate, whose chemical and physical characteristics may influence AMF colonization and growth-promoting efficiency (Dalanhol *et al.*, 2016). As such, the use of suitable substrate combined with AMF inoculation contributes to better quality seedlings. A suitable substrate should contain enough of all the nutrients that plants need without inhibiting mycorrhizal association, be homogeneous, have good water and nutrient

absorption capacity, enable soil aeration, and be free of pathogens and toxic substances (Hartmann *et al.*, 2011; Wendling; Gatto, 2002).

As such, the present study aimed to assess the performance of AMF inoculants composed of communities of native or selected species in promoting growth and nutrition in angico and brauna seedlings grown in different substrates.

2 MATERIALS AND METHODS

The production experiments for angico (*Anadenanthera peregrina*) and brauna (*Melanoxylon brauna*) seedlings inoculated with AMF were conducted in a greenhouse belonging to the Forestry Institute of the Rural Federal University of Rio de Janeiro in Seropédica, Rio de Janeiro state (RJ), Brazil (22°45'27,01"S and 43°41'46,89"W), from April to October 2017.

The two AMF inoculants used were soil inocula, containing spores and other unquantified propagules, such as hyphae and colonized roots. Soil inoculum is multiplied on live plants in a greenhouse, using signal grass as host. The first inoculant consisted of a mixture of selected fungal species, containing an equivalent number of spores of the following species: *Acaulospora foveata* Trappe & Janos (1982), *Acaulospora mellea* Spain & N.C. Schenck (1984), *Acaulospora morrowiae* Spain & N.C. Schenck (1984), *Acaulospora scrobiculata* Trappe (1977), *Dentisculata heterogama* (T.H. Nicolson & Gerd.) Sieverd., F.A. Souza & Oehl (2008), *Gigaspora candida* Bhattacharjee, Mukerji, J.P. Teware & Skoropad (1982), *Glomus formosanum* C.G. Wu & Z.C. Chen (1986), *Rhizophagus clarus* (T.H. Nicolson & N.C. Schenck) C. Walker & Schuessler (2010), *Scutellospora calospora* (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders (1986) and *Scutellospora pellucida* (T.H. Nicolson & N.C. Schenck) C. Walker & F.E. Sanders (1986). The inoculant was supplied by the Johanna Döbereiner Biological Resources Center (CRB-JD) of Embrapa Agrobiologia, located in the municipality of Seropédica (RJ). It was applied to the substrate near the seedling roots, at a dose of 100 spores of the fungal species mixture per seedling, corresponding to 7.2 g of soil inoculum per seedling, and designated the "selected AMF community" in this study.

The second inoculant was a mixture of native AMF collected in the field and multiplied via the on-farm method at the Viveiro Lua Nova nursery in the municipality of Miguel Pereira (RJ) (22°25'52,53"S 43°29'21,40"W), containing spores from the following species: *Acaulospora mellea*, *Acaulospora scrobiculata*, *Claroideoglossum etunicatum* (W.N.Becker & Gerd.) C.Walker & A.Schüßler (2010), *Glomus macrocarpum* Tul. & C.Tul. (1845), *Gigaspora* sp. and *Glomus* sp. It was also applied to the substrate close to the seedling roots at a dose of 100 spores of the fungal species mixture per seedling, corresponding to 18.14 g of soil inoculum per seedling, and referred to as the "native AMF community" in this study.

The native community was collected by Viveiro Lua Nova in June 2016 in the rhizosphere of a *Brachiaria* sp. pasture (22°25'55"S, 43°29'24"W, altitude of 739 m) located on nursery property, where the climate is classified as Cfb (wet temperate) according to Köppen's classification, with a temperate summer. Average annual temperature and rainfall are 15.7 to 27.7 °C and 2,250 mm (Alvares *et al.*, 2013). It was multiplied between June and December 2016 using the on-farm method, in a brick seedbed containing river sand as substrate and planted with *Brachiaria decumbens* Stapf. Seeds, which were washed with detergent and disinfected with 5% sodium hypochlorite before sowing. The bed was inoculated with the native material at a ratio of 10 L of propagule source per square meter.

Two different substrates were also used in the experiments. The first, referred to here as DAR (degraded area recovery) and recommended by Franco *et al.* (1992) for seedling production aimed at recovering degraded soils, contained 30% organic compost, comprising elephant grass and wheat bran mixture cured for around one year, 30% subsoil (clay), 30% sandy soil and 10% rock phosphate (proportions based on volume). The second substrate, called CS (commercial substrate), contained expanded vermiculite, peat, charcoal, and macro and micronutrients. The results of the chemical and physical analyses of the substrates are presented in Table 2. Physical analyses, such as apparent density, macroporosity, microporosity, total porosity and water-holding capacity (WHC) were performed in accordance with MAPA (2007) and Silva (1998).

Table 1 – Chemical and physical characteristics of the substrates (dry basis sample) before experiment setup

Substrate	N	P	K	Ca	Mg	S	pH	EC dS.m ⁻¹	AD g.cm ⁻³	Porosity (%)			WHC _{10cm} mL.50 cm ⁻³
										Total	Micro	Macro	
DAR	4.9	1.0	1.8	1.3	3.2	1.7	5.7	1.2	1.19	49.6	39.2	10.4	19.6
CS	6.3	3.2	2.8	1.5	6.0	8.9	6.1	2.1	0.34	76.5	55.0	20.6	28.0

Source: Authors (2021)

In where: N = Kjeldahl; P = Colorimetry; K = Flame photometry; Ca and Mg = Atomic absorption spectrometry; S = Turbidimetry; pH = Potentiometry; EC = Electrical conductivity; AD = apparent density; Total = total porosity percentage; Micro = microporosity percentage (pore diameter of 50 to 0.2 μm); Macro = Macroporosity percentage (pore diameter greater than 50 μm); WHC_{10 cm} = water-holding capacity at a tension of 10 cm; DAR = degraded area recovery substrate; SC = commercial substrate.

Both substrates were autoclaved at 120°C and a pressure of 1 kgf.cm⁻² for one hour over two consecutive days. In order to stabilize manganese content, the substrates were stored in a warehouse with no temperature control for two weeks to air dry.

Each tree species represented one experiment. Both experiments were conducted in a completely randomized design and 2x4 factorial scheme, consisting of two substrates, four inoculation treatments and four repetitions of 12 seedlings each, totaling 384 seedlings per experiment. The inoculation treatments were as follows: T1: noninoculated control; T2: noninoculated control fertilized according to the recommendations of Freire *et al.* (2013) for producing leguminous tree seedlings; T3: inoculated with selected fungi; T4: inoculated with native fungi.

Seeds were sown directly into 280 cm³ tubes, with three seeds per tube, and the amount of inoculum corresponding to each treatment was added, along with a thin layer of substrate. Thinning was performed after emergence, leaving only the most vigorous plant per tube.

The spacing between tubes was increased as the plants grew to prevent competition for light and water and etiolation.

At 100 DAS for angico and 180 DAS for brauna, seedling height and diameter were measured, and the shoots and roots removed. The leaves were removed from the shoots and leaf area (LA) measured using an LI-3600 leaf area meter.

From each seedling, 50 cm³ of substrate was removed from which AMF spores were extracted via the wet sieving method (Gerdemann; Nicolson, 1963), using 38 µm-mesh sieves, followed by centrifugation in water and sucrose. Spores of each sample were transferred to a Petri dish and counted under a stereoscopic microscope.

In order to assess mycorrhizal colonization, 0.5 g of root was removed from each seedling. The root samples were washed, cleared and stained as described by Giovannetti and Mosse (1980), then placed in a Petri dish with a grid of ½ inch squares and observed under a stereoscopic microscope. For each sample, 100 root segments were observed to determine the presence or absence of colonization. The number of grid segments showing colonization was converted into a percentage based on the total number of segments analyzed.

The root system was washed and the roots and shoots were stored separately in paper bags, dried in a forced-air oven at 65°C until constant mass, and shoot (SDW) and root dry weight (RDW) determined.

Inoculation efficiency was established using the following Equation (1):

$$IE(\%) = (DWT_i - DWT_t) / DWT_t \cdot 100 \quad (1)$$

where: IE = Inoculation efficiency (%); DWT_i = dry weight of the inoculated treatment; DWT_t = Control with no inoculation or fertilization

Prior to analysis of variance (ANOVA), the normality and homogeneity of variance of the data were analyzed via the Lilliefors, Cochran and Bartlett's tests, respectively, in R software (R CORE TEAM, 2015). Transformation was not necessary. The data were then submitted to ANOVA and the Scott-Knott test at 95% significance in Sisvar software (Ferreira, 2011).

2 RESULTS AND DISCUSSIONS

2.1 Angico experiment

The heights of the *Anadenanthera peregrina* seedlings in both substrates (Table 2) were similar to those obtained by Vieira, Webber and Scaramuzza (2017) for angico seedlings grown in 180 days in substrate containing different proportions of calcium and magnesium, and by Fonseca, Terra and Souza (2017) for seedlings of the same species after 90 days in substrate with varying amounts of rice husk ash.

Table 2 – Collective height and diameter of *Anadenanthera peregrina* seedlings produced in two types of substrates under different inoculation treatments, at 100 days after sowing

Inoculation treatment	Height (cm)		Diameter (mm)	
	DAR	CS	DAR	CS
T1 Control with no inoculation (NI)	45.7 Ba	32.0 Bb	2.09 Aa	1.61 Bb
T2 Control fertilized NI	45.4 Ba	34.4 Ab	2.16 Aa	1.47 Cb
T3 selected AMF community	45.9 Ba	34.0 Ab	2.08 Aa	1.77 Ab
T4 native AMF community	49.3 Aa	34.8 Ab	1.91 Ba	1.78 Ab
CV(%)	9.35		14.55	

Source: Authors (2021)

In where: DAR = degraded area recovery substrate; CS = commercial substrate; NI = no inoculation; AMF = arbuscular mycorrhizal fungi. Means followed by the same uppercase letter in the column and lowercase in the row, for each variable, do not differ according to the Scott-Knott test ($P < 0.05$).

In general, seedlings grown in DAR obtained significantly higher collective height and width than those in CS (Table 2). The data in Table 1 indicate greater total porosity for CS than DAR. According to Abreu *et al.* (2017), the greater porosity of commercial substrates may result in increased nutrient leaching during seedling development. Since mineral-based topdressing was not applied, the nutrients in CS may have leached more rapidly than in the DAR substrate, preventing the angico seedlings from receiving adequate nutrition for growth, despite the higher initial nutrient levels in the former.

The smallest seedlings were those grown in CS in the control treatment without inoculation (T1), thus demonstrating the benefit of inoculation in the production of tree seedlings. Regarding collective seedling diameter, the highest values were recorded in the AMF inoculation treatments (Table 2). Similar results were reported by Silva *et al.* (2017), who evaluated the production of Australian red cedar seedlings (*Toona ciliata* M. Roem var. *australis*) and found that those inoculated with AMF exhibited greater collective height and diameter values than their noninoculated counterparts.

In the present study, DAR-grown seedlings inoculated with native AMF exhibited a smaller collective diameter than those in the other inoculation treatments, indicating some etiolation despite the precaution of increasing seedling spacing during the experiment. Although height and diameter are easily measured, it is recommended that seedling growth analysis consider other variables. The finding that DAR produced larger seedlings than CS was confirmed on analysis of leaf area (LA) and shoot (SDW) and root dry weight (RDW) (Table 3).

Table 3 – Shoot (SDW) and root dry weight (RDW) and leaf area (LA), of *Anadenanthera peregrina* seedlings produced in two types of substrate under different inoculation treatments, at 100 days after sowing

Inoculation treatment	SDW (g)		LA (cm ²)		RDW (g)	
	DAR	CS	DAR	CS	DAR	CS
T1 Control with no inoculation (NI)	0.34 Aa	0.27 Ab	0.62 Ca	0.18 Bb	0.86 Aa	0.47 Ab
T2 Control fertilized NI	0.48 Aa	0.18 Ab	0.48 Da	0.11 Bb	0.73 Aa	0.39 Ab
T3 selected AMF community	0.84 Aa	0.45 Ab	3.34 Ba	1.08 Ab	1.05 Aa	0.66 Ab
T4 native AMF community	0.83 Aa	0.29 Ab	5.71 Aa	1.34 Ab	1.16 Aa	0.87 Ab
CV(%)	37.59		40.47		39.69	

Source: Authors (2021)

In where: SDW = shoot dry weight; LA = leaf area; RDW = root dry weight; DAR = degraded area recovery substrate; CS = commercial substrate; NI = no inoculation; AMF = arbuscular mycorrhizal fungi. Means followed by the same uppercase letter in the column and lowercase in the row, for each variable, do not differ according to the Scott-Knott test ($P < 0.05$).

Within each substrate, there was no significant difference between inoculation treatments for SDW and RDW. This corroborates the findings of Andrade *et al.* (2019),

who reported no significant difference in RDW in *Schizolobium amazonicum* seedlings produced with different combinations of *Rhizogloium clarus*, *Claroideogloium etunicatum* and *Gigaspora margarita*. Root dry weight is an important growth parameter because the roots must be large enough to ensure an adequate water and nutrient supply after planting (Caldeira *et al.*, 2012), resulting in better survival in the field, where stressful conditions are common.

For both substrates the highest LA values were recorded in the inoculated treatments (T3 and T4). Similar results were obtained by Brito, Tellechea, Heitor, Freitas and Martins (2017) in mycorrhized *Schizolobium amazonicum* seedlings, with LA generally related to plant growth and development.

In regard to root mycorrhizal colonization and AMF sporulation in the substrate, the results show no difference between the two inoculants for mycorrhizal promotion, with superior performance in both inoculated treatments when compared to their noninoculated counterparts (Table 4). With respect to the substrates, both were efficient at maintaining arbuscular mycorrhizal symbiosis, but inoculation efficiency was two to three times greater for DAR in relation to CS. This was expected because the CS contains more nutrients, especially P, whose high soil content tends to reduce symbiosis functionality (Smith *et al.*, 2011).

Table 4 – Mycorrhizal root colonization, number of spores and inoculation efficiency in *Anadenanthera peregrina* seedlings produced in two types of substrate under different inoculation treatments, at 100 days after sowing

Inoculation treatment	Mycorrhizal colonization (%)		Number of spores in 50 cm ³ of substrate		Inoculation efficiency (%)	
	DAR	CS	DAR	CS	DAR	CS
	T1 Control with no inoculation (NI)	1 Ba	0 Ba	114 Ba	13 Bb	
T2 Control fertilized NI	1 Ba	9 Ba	63 Ba	21 Bb		
T3 selected AMF community	79 Aa	79 Aa	574 Aa	327 Ab	187	88
T4 native AMF community	82 Aa	81 Aa	602 Aa	331 Ab	181	58
CV(%)	31.85		29.19			

Source: Authors (2021)

In where: DAR = degraded area recovery substrate; CS = commercial substrate; NI = no inoculation; AMF = arbuscular mycorrhizal fungi. Means followed by the same uppercase letter in the column and lowercase in the row, for each variable, do not differ according to the Scott-Knott test ($P < 0.05$).

The inoculants showed similar inoculation efficiency. Although AMF are not specific, the effects of inoculation on the host plant depend on the combination of fungal species, plant and environment (Nouh; Abdel-Azeem, 2020). In this respect, the similarity between inoculants can be justified by the presence of shared AMF. Additionally, Pouyú-Rojas, Siqueira and Donizetti Santos (2006) found that fungi have different degrees of symbiotic efficiency, promoting growth in a few or many plants, that is, being generalists or not. The presence of *Brachiaria* sp. plants in the area where the native inoculum was collected and during its on-farm multiplication may have selected and multiplied generalist AMF species, resulting in the optimal performance of this inoculant when compared to that comprised of native species. Additionally, given that angico occupies the early stages of ecological succession, and maintains high colonization rates to supply the intense flow of nutrients required for initial growth (Zangaro *et al.*, 2007), the species is likely highly susceptible to mycorrhizae. Pouyú-Rojas, Siqueira and Donizetti Santos (2006) found that plants have different levels of mycorrhizal susceptibility and can form associations with a few or many AMF species.

Despite not being inoculated, samples in the control treatments (T1 and T2) exhibited spores and mycorrhizal colonization. Although this mycorrhizal contamination was low and did not affect the results, it likely occurred through spores carried in the irrigation water or because spores made inviable by autoclaving were counted, since the experimental period was not long enough to allow the decomposition of dead spores. The small percentage of colonized roots in these treatments indicates low contamination by viable sources of propagules.

2.2 Brauna experiment

All the brauna seedlings grown in CS and treatment T2 (not inoculated or fertilized) died within 100 DAS. The most plausible hypothesis to explain brauna mortality in this treatment is that the species is sensitive to high substrate saline content. As shown in Table 1, the electrical conductivity values recorded for DAR and

CS are considered extremely high (Cavins *et al.*, 2000). However, adding fertilizer may have further increased the level of water-soluble electrolytes in CS, so that despite the porosity and leaching capacity of this substrate, the saline level may have harmed the brauna plants.

The highest collective brauna seedling diameters were recorded in DAR (Table 5), showing the same tendency observed for angico seedlings, despite initial analysis indicating smaller amounts of N, P, K, Ca, Mg and S than in CS (Table 1). However, seedling height did not differ between the two substrates (Table 5). This corroborates the hypothesis that slow-growing species are less sensitive to variations in soil fertility and that very high levels at certain growth stages may compromise their development (Siqueira *et al.*, 1998).

Table 5 – Collective height and diameter of *Melanoxylon brauna* seedlings produced in two types of substrate under different inoculation treatments, at 180 days after sowing

Inoculation treatment	Height (cm)		Diameter (mm)	
	DAR	CS	DAR	CS
T1 Control with no inoculation (NI)	15.6 Ba	15.5 Aa	1.69 Ba	1.48 Ab
T2 Control fertilized NI	15.7 Ba		1.68 Ba	
T3 selected AMF community	19.4 Aa	16.0 Aa	2.05 Aa	1.49 Ab
T4 native AMF community	15.5 Ba	15.4 Aa	1.71 Ba	1.45 Ab
CV(%)	18.79		14.85	

Source: Authors (2021)

In where: DAR = degraded area recovery substrate; CS = commercial substrate; NI = no inoculation; AMF = arbuscular mycorrhizal fungi. Means followed by the same uppercase letter in the column and lowercase in the row, for each variable, do not differ according to the Scott-Knott test ($P < 0.05$).

An effect of inoculation treatments on collective seedling height and diameter was only observed in DAR, whereby inoculation with the selected AMF community produced higher values when compared to the remaining treatments. However, these results did not carry through to the variables LA, SDW and RDW (Table 6).

As such, there was no difference between substrates for these variables (Table 6), with the primary differences being between inoculation treatments (Table 6).

Both inoculants produced higher RDW values when compared to the noninoculated treatments. There was no significant difference between inoculation treatments for SDW or LA in the DAR substrate, while in CS, both inoculants improved SDW, but the greatest LA increase was obtained in the treatment involving inoculation with the native AMF community (T4).

Table 6 – Shoot (SDW) and root dry weight (RDW) and leaf area (LA) of *Melanoxylon brauna* seedlings produced in two types of substrate under different inoculation treatments at 180 days after sowing

Inoculation treatment	SDW (g)		LA (cm ²)		RDW (g)	
	DAR	CS	DAR	CS	DAR	CS
T1 Control with no inoculation (NI)	0.41 Aa	0.31 Ba	2.52 Aa	2.04 Ba	0.23 Ca	0.21 Ba
T2 Control fertilized NI	0.42 Aa		2.46 Aa		0.21 Ca	
T3 selected AMF community	0.43 Aa	0.42 Aa	2.21 Aa	3.71 Aa	0.34 Ba	0.57 Aa
T4 native AMF community	0.40 Aa	0.38 Aa	2.45 Aa	3.64 Aa	0.50 Aa	0.48 Aa
CV(%)	18.14		46.36		38.02	

Source: Authors (2021)

In where: SDW = shoot dry weight; LA = leaf area; RDW = root dry weight; DAR = degraded area recovery substrate; CS = commercial substrate; NI = no inoculation; AMF = arbuscular mycorrhizal fungi. Means followed by the same uppercase letter in the column and lowercase in the row, for each variable, do not differ according to the Scott-Knott test ($P < 0.05$).

Based on these findings that brauna growth in response to inoculation varies according to the variable analyzed (Tables 6 and 7), unlike angico, which showed a uniform trend throughout the experiment (Tables 3 and 4), it can be inferred that the effect of the mycorrhizae on brauna seedlings was not directly related to nutritional improvement, with mycorrhizal benefits including overcoming stress, such as that caused by the salinity or pathogens present in CS. This is evident in the poor root system formation observed for brauna seedlings, which compromised good substrate aggregation, a vital characteristic in adequate seedling development for transplantation to the field. The root volume of seedlings in all the treatments was unable to provide satisfactory aggregation, which, in the case of commercial seedlings, implies a longer production cycle and consequently higher production costs (Lisboa *et al.*, 2012).

These results suggest the need for further research on brauna seedling production, using containers with a smaller volumetric capacity, other substrates and even alternative mycorrhizal fungi or other potentially beneficial microorganisms isolated from brauna plants in their natural environment.

Although inoculation promoted greater root mycorrhizal colonization and sporulation in the substrate for brauna seedlings (Table 7), these effects were considered low (Carneiro *et al.*, 1998). This suggests that the brauna plants and AMF communities present in both inoculants did not form a highly effective symbiosis. This pattern may be related to the possibility of brauna being highly exclusive to certain AMF species (Pouyú-Rojas; Siqueira; Donizetti Santos, 2006) or because these slow-growing plants naturally require only low AMF colonization to supply their nutritional needs. According to Kiriachek *et al.*, (2009), both soil P availability and plants' genetic load of mycorrhizal dependence are involved in root colonization signalling and control mechanisms.

The 8 to 18-fold greater inoculation efficiency in CS when compared to the DAR substrate and the opposite behavior to that observed for angico (Table 4) reinforces the hypothesis that brauna used mycorrhizal symbiosis to overcome the stress caused by CS.

Table 7 – Mycorrhizal colonization (%), number of spores and inoculation efficiency (%) of *Melanoxylon brauna* seedlings produced in two types of substrate under different inoculation treatments at 180 days after sowing

Inoculation treatment	Mycorrhizal colonization (%)		Number of spores in 50 cm ⁻³ of substrate		Inoculation efficiency (%)	
	DAR	CS	DAR	CS	DAR	CS
T1 Control with no inoculation (NI)	0 Ba	0 Ba	1 Ba	0 Ba		
T2 Control fertilized NI	0 Ba		2 Ba			
T3 selected AMF community	20 Aa	12 Aa	39 Aa	34 Aa	4	73
T4 native AMF community	28 Aa	20 Aa	48 Aa	51 Aa	7	57
CV(%)	38.25		46.72			

Source: Authors (2021)

In where: DAR = degraded area recovery substrate; CS = commercial substrate; NI = no inoculation; AMF = arbuscular mycorrhizal fungi. Means followed by the same uppercase letter in the column and lowercase in the row, for each variable, do not differ according to the Scott-Knott test ($P < 0.05$).

Producing seedlings of slow-growing tree species may require other techniques that could not be used in the present study and that more closely simulate the natural development conditions of these plants in forests. Generally classified as climax species, the seedlings of slow-growing trees naturally develop under the forest canopy and only become part of it when clearings open (Silva *et al.*, 2017). These same authors also report that the development time of these seedlings is naturally far longer than the 180 days used in the present study. Moreover, during their time in the seedling bank, these seedlings likely survive with little sunlight and in soil with low fertility at depth, but rich in organic matter decomposing on the surface. As such, they may have been selected for their low level of colonization, consistent with their photosynthetic capacity, but despite being little colonized, they may be highly dependent on this association to form part of the mycorrhizal network of plants in the forest canopy, sharing nutrients, photosynthates and other benefits (Salles; Lima; Costa, 2017).

3 CONCLUSIONS

The results indicate that the native AMF communities studied here and multiplied in an on-farm system are promising for inoculation of angico seedlings.

Although inoculation provided adequate growth for angico seedlings, the highest diameter, height, LA, SDW and RDW values were obtained in the DAR substrate.

Inoculants containing selected and native AMF showed high inoculation efficiency in angico seedlings, with the best results obtained in DAR.

The techniques presented here were not efficient in producing good quality brauna seedlings, suggesting the need for further research with this species.

Brauna did not adapt to the fertilized commercial substrate without AMF, indicating that AMF helped the plants overcome some of the stress caused by this substrate.

The promotion of mycorrhization in brauna and angico, measured by mycorrhizal root colonization and sporulation in the substrate, did not differ between inoculants containing selected native AMF, in either substrate.

The selected and native AMF communities exhibited low inoculation efficiency for brauna, requiring additional studies with other AMF or potentially beneficial microorganisms isolated from brauna plants in their natural environment.

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