EFFECT OF MYCORRHIZAL INOCULATION AND SUBSTRATE COMPOSITION ON SEEDLING GROWTH OF TWO ATLANTIC FOREST TREE SPECIES

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Resumo

Fungos micorrízicos e substratos na formação de mudas de duas espécies da Mata Atlântica. A busca por ferramentas biotecnológicas mais sustentáveis de recuperação ambiental cresceu nas últimas décadas, como os inoculantes microbianos. O qual tem sua eficiência ligada ao substrato utilizado para produção de mudas. O presente estudo objetivou avaliar as mudas de *Pseubombax grandiflorum* e *Apuleia leiocarpa* sob à inoculação com fungos micorrízicos arbusculares (FMA) em diferentes substratos e o seu crescimento. Os experimentos foram conduzidos em casa de vegetação com delineamento inteiramente casualizado em esquema fatorial 6 x 2 (seis substratos e dois níveis de inoculação), com 12 tratamentos e seis repetições cada. As espécies apresentaram padrões de crescimento similares. Os melhores resultados foram utilizando-se as composições com esterco bovino e as maiores proporções de Cambissolo e Biomix, todos inoculados Fungos Micorrizicos. O resultado indica relação entre os fatores físicos e químicos do solo (pH, dose ideal de fósforo e teor de nitrogênio) fornecidos pelos substratos, possibilitando a formação de cenário ideal para o melhor desenvolvimento das espécies e da simbiose micorrízica. Os melhores tratamentos para o crescimento de *P. grandiflorum* foram a combinação de 80% de terra do horizonte superficial de um Cambissolo com 20% de Biomix e FMA e a combinação de solo mais fósforo e FMA. Para a espécie *A. leiocarpa* os melhores tratamentos foram a combinação de 80 % de solo com 20% de Biomix e FMA, 70% de solo com 30% de esterco e FMA e solo mais fósforo e FMA.
Palavras-chave: Micorriza, Inóculo, Simbiose, Produção de mudas.

Abstract

The search for more efficient biotechnological tools for forest regeneration has increased over the past decades. Mycorrhizal inoculation is a valuable strategy to promote seedling growth, but its efficiency depends on the substrate used. This study aimed to investigate the effects of arbuscular mycorrhizal fungal inoculation and different types of substrates on the growth of *Pseudobombax grandiflorum* and *Apuleia leiocarpa* seedlings. Experiments were carried out under greenhouse conditions in a completely randomized 6 × 2 factorial design (six substrates and two inoculation levels) with six replications of 12 treatments. Both species showed similar growth patterns. Plants grown in inoculated substrates composed of 70% Cambisol and 30% bovine manure or 80% Cambisol and 20% Biomix showed improved nutritional status. These substrates had good physical and chemical characteristics (pH and P and N levels), which favored plant development and mycorrhizal symbiosis. *P. grandiflorum* seedlings showed enhanced growth in inoculated substrate composed of 80% Cambisol and 20% Biomix and in inoculated Cambisol fertilized with P. For *A. leiocarpa*, the best results were observed for seedlings grown in inoculated substratescomposed of 80% Cambisol and 20% Biomix, 70% Cambisol and 30% bovine manure, or Cambisol fertilized with P.
Keywords: Mycorrhiza; Inoculum, Symbiosis, Seedling production.

INTRODUCTION

Mycorrhiza is a symbiotic relationship between plants and fungi. Commonly found in association with plants, mycorrhizal fungi bring great benefits to their hosts (THIRKELL *et al*., 2016), including enhanced growth, nutrient uptake (in particular of low mobility compounds such as phosphorus), and resistance to biotic and abiotic stresses (Goetten *et al*., 2016; CHAER *et al*., 2011).

Mycorrhizal fungi are used to restore degraded areas because they provide better conditions for plants to grow and compete for environmental resource. They act as root extensions, capturing resources (water and minerals) otherwise not accessible to plant roots, and contribute to soil aggregation by producing the glycoprotein glomalin. These beneficial fungi can also be used as biological indicators: the lower their density and diversity, the higher the degradation (SCORIZA *et al.*, 2016). Because of their benefits, mycorrhizal fungi are an excellent tool for the production of forest seedlings. In particular, arbuscular mycorrhizal fungi stand out for their symbiotic efficiency and easy adaptation to the soil or substrate.

Substrate quality is an important factor for seedling development and establishment of mycorrhizal symbiosis. The substrate can positively or negatively affect nutritional and morphological aspects of young plants depending on its physical and chemical characteristics (Camara et al., 2017). Substrates must be chosen on the basis of plant type and economic factors. In general, substrates should have low density, high porosity, water holding capacity, and cation exchange capacity and be free of pests, pathogens, and weed seeds. The substrate is the main source of resources for developing seedlings and the intermediary between microorganism and host. A better understanding of fungal diversity and plant–soil–fungi relationships are needed.

Thus, the work tested the hypothesis that the change in substrate composition differently influences the quality of seedlings inoculated. The aim of this study was to evaluate the interaction of arbuscular mycorrhizal fungi with different substrates and their effects on the growth of seedlings of two Atlantic Forest species.

MATERIAL AND METHODS

Experimental design

Pseudobombax grandiflorum (Cav.) A. Robyns and *Apuleia leiocarpa* (Vogel) J.F.Macbr were grown under greenhouse conditions in two experimental periods, from December to April (124 days) and April to August (118 days) 2016. A completely randomized 6 × 2 factorial design (six substrates and two levels of inoculation) with six repetitions of 12 treatments was used. Inoculation levels consisted of inoculation or non-inoculation of substrates with arbuscular mycorrhizal fungi. Substrates in treatments T_2, T_4, T_6, T_8, T_{10}, and T_{12} were inoculated with mycorrhizal fungi, whereas substrates in treatments T_1, T_3, T_5, T_7, T_9, and T_{11} were not inoculated.

Substrates

The composition and chemical characteristics of the six substrates used in the experiment are shown in Table 1. The soil was classified as typical dystrophic Cambisol (Cambissolo Háplico). Soil samples were collected from the 10 cm depth. Substrate 6 (composed of Cambisol fertilized with phosphorous) was used in a prior evaluation of mycorrhizal dependence of plant species.

Table 1. Composition and chemical properties of substrates used in the experiments.
Tabela 1. Análise química dos substratos utilizados nos experimentos com as espécies *P. grandiflorum* e *A. leiocarpa*.

Treatments	Substrate	Composition	pH in H_2O	Ca	Mg	Al	P	K	N	S	CEC	V
				(cmol$_c$ dm^{-3})			(mg kg^{-1})		(g kg^{-1})	cmol$_c$ dm^{-3}		(%)
T_1 and T_2	1	60% Cambisol, 10% Biomix, 10% sand, 20% vermiculite	7.08	3.0	12.11	0	14.35	35.41	1.1	15.23	16.34	93
T_3 and T_4	2	80% Cambisol, 20% Biomix	6.32	7.96	0.92	0	57.81	43.69	1.4	8.99	11.38	79
T_5 and T_6	3	50% Cambisol, 30% Biomix, 10% sand, 10% vermiculite	7.13	9.75	2.79	0	95.46	22.71	1.7	12.6	13.72	92
T_7 and T_8	4	60% Cambisol, 40% Biomix	6.81	11.17	2.19	0	83.46	77.48	1.7	13.56	15.99	85
T_9 and T_{10}	5	70% Cambisol, 30% bovine manure	6.61	5.82	1.97	0	89.39	84.56	3.1	8.01	10.34	78
T_{11} and T_{12}	6	Cambisol fertilized with phosphorus	5.26	1.75	0.48	0.3	401.86	161.64	1.1	2.64	7.14	37

S, sum of bases (Ca, Mg, and K); CEC, cation exchange capacity at pH 7.0, calculated by the formula CEC = S + (H + Al); V, base saturation, calculated by the formula V% = (100 × S)/T.

Seed preparation

A. leiocarpa and *P. grandiflorum* seeds were disinfected with 30% H_2O_2 for 2 min, placed in Petri dishes containing filter paper and cotton, and incubated in a BOD germination chamber at 28 °C under constant light for 5 days until reaching 1 cm height.

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Fungal inoculum

A mixture of three arbuscular mycorrhizal fungal species was used: *Dentiscutata heterogama* (DHET A2, CNPAB002), *Gigaspora margarita* (GMAR A1, CNPAB001), and *Rhizophagus clarus* (RCLA A5, CNPAB005). Each starting container received 1 g of inoculum containing 17, 35, and 26 spores of *D. heterogama*, *G. margarita*, and *R. clarus*, respectively.

Uninoculated treatments received 100 mL of non-mycorrhizal microbiota as a control. Non-mycorrhizal microbiota was separated from 100 g of fungal inoculum by suspension in 1 L of distilled water, followed by filtering through a 50 mm filter paper.

Experiments

Starting containers were constructed using 280 mL plastic vials and 700 mL disposable plastic cups. Containers were filled with 1 kg of substrate. Prior to the experiment, *A. leiocarpa* seeds were scarified by immersion in sulfuric acid for 15 min to overcome dormancy. Plants were watered daily to 70% of the field capacity by spray irrigation. *P. grandiflorum* was evaluated for 124 days and *A. leiocarpa* for 118 days.

Sampling procedures

After the experimental period, seedlings were collected and separated into shoots and roots. From three replicates, 0.5 g of fine roots were used for evaluation of mycorrhizal colonization (KOSKE and GEMMA, 1989). Fungal colonization was evaluated by the gridline intersect method, according to Giovannetti and Mosse (1980). Root samples from the other three replicates were dried in a forced-air oven (65 °C, 72 h) and weighed for determination of root dry weight (RDW). Samples from all replicates were used to determine shoot dry weight (SDW). After SDW determination, shoots were crushed and subjected to sulfuric acid digestion for determination of N, P, and K. P levels were assessed by colorimetric analysis, K by flame photometry (TEDESCO, *et al.* 1995), and N by the modified Kjeldahl method.

Variables

The variables evaluated were SDW, RDW, total dry weight (TDW), height (H), diameter (D), and mycorrhizal colonization percentage. Plant height was measured from the collet to the terminal bud. The following indices were determined to assess seedling quality: H/D, H/SDW, SDW/RDW, and the Dickson quality index (DQI) (DICKSON *et al.*, 1960).

Data analysis

Data were first Box–Cox transformed to meet normality assumptions via the Shapiro–Wilk test. Transformed data were subjected to analysis of variance. Means were compared using the Scott–Knott test ($p < 0.05$). Statistical analyses were carried out using Sisvar (FERREIRA, 2011). Principal component analysis (PCA) was performed based on the correlation matrix using the R program with the vegan package. The number of extracted components was chosen on the basis of the cumulative variance (minimum of 70%).

RESULTS

Seedling response to inoculation

Inoculation increased SDW and TDW in both species, regardless of the substrate used. TDW was 300% higher in *P. grandiflorum* grown in inoculated Cambisol fertilized with P (T_{12}) than in uninoculated substrate (T_{11}) (Table 2). For *A. leiocarpa* grown in P-fertilized Cambisol, inoculation promoted a 400% increase in TDW. Even in the presence of high P concentrations (650 mg kg^{-1} soil), uninoculated plants did not increase in height, diameter, or SDW (T_{11}), evidence of the plants' dependence on mycorrhizal fungi (Table 2)

The T_4 treatment (80% Cambisol + 20% Biomix + mycorrhizal inoculum) resulted in the highest biomass gain for *P. grandiflorum*. For, *A. leiocarpa* the highest biomass gain was obtained with T_{12} (Cambisol + P + mycorrhizal inoculum) but the highest TDW value was obtained with T_{10} (70% Cambisol + 30% bovine manure + mycorrhizal inoculum) (Table 3).

Mycorrhizal colonization in *P. grandiflorum* ranged from 11% in T_{10} (70% Cambisol + 30% bovine manure) to 24% in T_{12} (Cambisol + P), varying according to the concentration of P in the soil (Table 2). In *A. leiocarpa*, mycorrhizal colonization varied from 18% in T_{10} to 33% in T_{12} (Table 3).

P. grandiflorum seedling height was highest in T_8 and T_9 (70% Cambisol + 30% manure, with and without inoculation), whereas *A. leiocarpa* height was highest in T_3 and T_4 (80% Cambisol + 20% Biomix, with and without inoculation) and T_{11} and T_{12} (Cambisol + P, with and without inoculation) (Table 3).

P. grandiflorum diameter was largest in T_8 and T_9 (Table 2). *A. leiocarpa*, in contrast, showed no variation in diameter between treatments (Table 3).

Table 2. Effects of substrate and mycorrhizal fungal inoculation on *Pseudobombax grandiflorum* seedling growth.
Tabela 2. Efeito de diferentes tipos de substrato e da inoculação com fungos micorrízicos no crescimento da espécie *P. grandiflorum*.

Treatment	RDW (g)	SDW (g)	TDW (g)	Diameter (mm)	Height (cm)	M (%)
T_1	0.48 c	0.67 c	1.15 c	5.09 c	8.35 c	
T_2	0.64 c	0.91 b	1.55 b	5.06 c	7.33 c	18 a
T_3	0.57 c	0.63 c	1.20 c	6.47 b	9.70 c	
T_4	1.31 a	1.31 a	2.62 a	6.85 b	11.03 b	20 a
T_5	0.42 c	0.59 c	1.01 c	5.01 c	7.86 c	
T_6	0.51 c	0.84 b	1.35 c	4.92 c	7.34 c	17 a
T_7	0.56 c	0.80 b	1.36 c	3.95 c	6.21 c	
T_8	0.88 b	0.93 b	1.81 b	4.77 c	7.51 c	13 b
T_9	0.11 d	0.52 d	0.63 d	7.56 a	13.72 a	
T_{10}	0.85 b	0.78 b	1.63 b	8.27 a	14.83 a	11 b
T_{11}	0.26 d	0.38 d	0.64 d	6.04 b	10.38 b	
T_{12}	0.71 c	1.23 a	1.94 b	6.41 b	10.63 b	24 a
F-test						
Substrate	0	0	0			
Fungi	0	0	0			
Interaction	0.01	0.02	0.02			
CV (%)	50.64	19.24	26.3	15.34	20.33	21.77

Means in a column followed by different letters differ significantly by the Scott–Knott test ($p < 0.05$). T_1, 60% Cambisol + 10% Biomix + 10% sand + 20% vermiculite, uninoculated; T_2, 60% Cambisol + 10% Biomix + 10% sand + 20% vermiculite + mycorrhizal inoculum; T_3, 80% Cambisol + 20% Biomix, uninoculated; T_4, 80% Cambisol + 20% Biomix + mycorrhizal inoculum; T_5, 50% Cambisol + 30% Biomix + 10% sand + 10% vermiculite, uninoculated; T_6, 50% Cambisol + 30% Biomix + 10% sand + 10% vermiculite + mycorrhizal inoculum; T_7, 60% Cambisol + 40% Biomix, uninoculated; T_8, 60% Cambisol + 40% Biomix + mycorrhizal inoculum; T_9, 70% Cambisol + 30% bovine manure, uninoculated; T_{10}, 70% Cambisol + 30% bovine manure + mycorrhizal inoculum; T11, 100% Cambisol fertilized with phosphorus, uninoculated; T_{12}, 100% Cambisol fertilized with phosphorus + mycorrhizal inoculum; RDW, root dry weight; SDW, shoot dry weight; TDW, total dry weight; M, mycorrhizal colonization; CV, coefficient of variation.

Table 3. Effects of substrate and mycorrhizal fungal inoculation on *Apuleia leiocarpa* seedling growth.
Tabela 3. Efeito de diferentes tipos de substrato e da inoculação com fungos micorrízicos no crescimento da espécie *A. leiocarpa*.

Treatment	RDW (g)	SDW (g)	TDW (g)	Diameter (mm)	Height (cm)	M (%)
T_1	0.23 c	0.33 c	0.57 b	1.39 a	7.01 b	
T_2	0.26 b	0.26 c	0.52 c	1.32 a	7.15 b	24 b
T_3	0.16 d	0.27 b	0.43 d	1.37 a	9.48 a	
T_4	0.22 c	0.66 a	0.88 a	1.58 a	11.36 a	36 a
T_5	0.22 c	0.39 b	0.61 b	1.30 a	6.35 b	
T_6	0.31 b	0.43 a	0.75 b	1.26 a	6.46 b	25 b
T_7	0.15 c	0.33 c	0.48 d	1.29 a	5.15 b	
T_8	0.28 b	0.47 b	0.75 b	1.31 a	6.41 b	21 b
T_9	0.14 d	0.23 c	0.37 d	1.36 a	6.76 b	
T_{10}	0.23 c	0.68 a	0.91 a	1.40 a	6.78 b	18 b
T_{11}	0.09 d	0.15 d	0.24 d	1.30 a	9.56 a	
T_{12}	0.45 a	0.58 a	1.03 a	1.53 a	10.16 a	33 a
F-test						
Substrate	0.35	0	0.002			
Fungi	0	0	0			
Interaction	0	0.16	0			
CV (%)	39.16	41.07	36.33	14.29	21.88	24.15

Means in a column followed by different letters differ significantly by the Scott–Knott test ($p < 0.05$). T_1, 60% Cambisol + 10% Biomix + 10% sand + 20% vermiculite, uninoculated; T_2, 60% Cambisol + 10% Biomix + 10% sand + 20% vermiculite + mycorrhizal inoculum; T_3, 80% Cambisol + 20% Biomix, uninoculated; T_4, 80% Cambisol + 20% Biomix + mycorrhizal inoculum; T_5, 50% Cambisol + 30% Biomix + 10% sand + 10% vermiculite, uninoculated; T_6, 50% Cambisol + 30% Biomix + 10% sand + 10% vermiculite + mycorrhizal inoculum; T_7, 60% Cambisol + 40% Biomix, uninoculated; T_8, 60% Cambisol + 40% Biomix + mycorrhizal inoculum; T_9, 70% Cambisol + 30% bovine manure, uninoculated; T_{10}, 70% Cambisol + 30% bovine manure + mycorrhizal inoculum; T11, 100% Cambisol fertilized with phosphorus, uninoculated; T_{12}, 100% Cambisol fertilized with phosphorus + mycorrhizal inoculum; RDW, root dry weight; SDW, shoot dry weight; TDW, total dry weight; M, mycorrhizal colonization; CV, coefficient of variation.

Seedling quality

There was no significant difference in H/D index between treatments for *P. grandiflorum* (Table 4). For *A. leiocarpa*, significantly higher H/D indices were obtained in T_3 and T_4 (uninoculated and inoculated substratescomposed of 80% Cambisol + 20% Biomix) and T_{10} and T_{11} (uninoculated and inoculated Cambisol fertilized with P) (Table 5).

P. grandiflorum seedlings showed a four times higher SDW/RDW index when grown in uninoculated Cambisol and bovine manure (T_9) (Table 4). This substrate affected plant development by promoting shoot growth to the detriment of root growth. In contrast, *A. leiocarpa* seedlings showed altered development in inoculated substrate composed of 80% Cambisol and 20% Biomix (T_4), with an SDW/RDW index three times higher than that obtained in inoculated substrate composed of 60% Cambisol, 10% Biomix, 20% sand, and 10% vermiculite (T_2).

The highest DQI for *P. grandiflorum* were obtained in T4 (80% Cambisol + 20% Biomix + mycorrhizal inoculum) (1.01), T8 (60% Cambisol + 40% Biomix + mycorrhizal inoculum) (0.69), T_{10} (70% Cambisol + 30% bovine manure + mycorrhizal inoculum) (0.60), and T_{12} (Cambisol fertilized with phosphorus + mycorrhizal inoculum) (0.57) (Table 4). DQI values for *A. leiocarpa* were generally low; the best DQI were observed in T_6 (50% Cambisol + 30% Biomix + 10% sand + 10% vermiculite + mycorrhizal inoculum) (0.12), T_{10} (70% Cambisol + 30% bovine manure + mycorrhizal inoculum) (0.12), and T12 (Cambisol fertilized with phosphorus + mycorrhizal inoculum) (0.13) (Table 5).

Table 4. Quality parameters of *Pseudobombax grandiflorum* seedlings grown in inoculated and uninoculated substrates.
Tabela 4. Índice de qualidade das mudas de *P. grandiflorum* produzidas em diferentes tipos de substrato e inoculadas com fungos micorrízicos.

Treatment	Index			
	H/D	H/SDW	SDW/RDW	DQI
T_1	1.64 a	12.39 c	1.40 b	0.38 c
T_2	1.45 a	8.09 d	1.41 b	0.54 c
T_3	1.50 a	15.34 b	1.12 b	0.46 c
T_4	1.61 a	8.44 d	0.99 b	1.01 a
T_5	1.57 a	13.32 c	1.42 b	0.34 c
T_6	1.49 a	8.76 d	1.65 b	0.43 c
T_7	1.72 a	12.91 c	1.43 b	0.43 c
T_8	1.57 a	8.04 d	1.06 b	0.69 b
T_9	1.81 a	26.46 a	4.56 a	0.10 d
T_{10}	1.79 a	19.09 b	0.91 b	0.60 b
T_{11}	1.57 a	16.16 b	1.48 b	0.21 d
T_{12}	1.66 a	8.65 d	1.73 b	0.57 b
CV (%)	6.84	40.54	57.89	46.63

Means in a column followed by different letters differ significantly by the Scott–Knott test ($p < 0.05$). T_1, 60% Cambisol + 10% Biomix + 10% sand + 20% vermiculite, uninoculated; T_2, 60% Cambisol + 10% Biomix + 10% sand + 20% vermiculite + mycorrhizal inoculum; T_3, 80% Cambisol + 20% Biomix, uninoculated; T_4, 80% Cambisol + 20% Biomix + mycorrhizal inoculum; T_5, 50% Cambisol + 30% Biomix + 10% sand + 10% vermiculite, uninoculated; T_6, 50% Cambisol + 30% Biomix + 10% sand + 10% vermiculite + mycorrhizal inoculum; T_7, 60% Cambisol + 40% Biomix, uninoculated; T_8, 60% Cambisol + 40% Biomix + mycorrhizal inoculum; T_9, 70% Cambisol + 30% bovine manure, uninoculated; T_{10}, 70% Cambisol + 30% bovine manure + mycorrhizal inoculum; T11, Cambisol fertilized with phosphorus, uninoculated; T_{12}, Cambisol fertilized with phosphorus + mycorrhizal inoculum; CV, coefficient of variation; H/D, ratio of plant height to diameter; H/SDW, ratio of plant height to shoot dry weight; SDW/RDW, ratio of shoot dry weight to root dry weight; DQI, Dickson quality index, calculated according to Dickson (1960).

Table 5. Quality parameters of *Apuleia leiocarpa* seedlings grown in inoculated and uninoculated substrates.
Tabela 5. Índice de qualidade das mudas de *A. leiocarpa* produzidas em diferentes tipos de substrato e inoculadas com fungos micorrízicos.

Treatment	Index							
	H/D		H/SDW		SDW/RDW		DQI	
T_1	5.04	b	21.24	b	1.43	b	0.09	b
T_2	5.42	b	27.50	b	1.00	c	0.08	b
T_3	6.92	a	35.11	b	1.69	b	0.05	c
T_4	7.19	a	17.21	c	3.00	a	0.09	b
T_5	4.88	b	16.28	c	1.77	b	0.09	b
T_6	5.13	b	15.02	c	1.39	b	0.12	a
T_7	3.99	b	15.61	c	2.20	b	0.08	b
T_8	4.89	b	13.64	c	1.68	b	0.11	B
T_9	4.97	b	29.39	b	1.64	b	0.06	c
T_{10}	4.84	b	9.97	d	2.96	a	0.12	a
T_{11}	7.35	a	63.73	a	1.67	b	0.03	c
T_{12}	6.64	a	17.52	c	1.29	b	0.13	a
CV (%)	19.8		62.16		34.19		35.67	

Means in a column followed by different letters differ significantly by the Scott–Knott test ($p < 0.05$). T_1, 60% Cambisol + 10% Biomix + 10% sand + 20% vermiculite, uninoculated; T_2, 60% Cambisol + 10% Biomix + 10% sand + 20% vermiculite + mycorrhizal inoculum; T_3, 80% Cambisol + 20% Biomix, uninoculated; T_4, 80% Cambisol + 20% Biomix + mycorrhizal inoculum; T_5, 50% Cambisol + 30% Biomix + 10% sand + 10% vermiculite, uninoculated; T_6, 50% Cambisol + 30% Biomix + 10% sand + 10% vermiculite + mycorrhizal inoculum; T_7, 60% Cambisol + 40% Biomix, uninoculated; T_8, 60% Cambisol + 40% Biomix + mycorrhizal inoculum; T_9, 70% Cambisol + 30% bovine manure, uninoculated; T_{10}, 70% Cambisol + 30% bovine manure + mycorrhizal inoculum; T11, Cambisol fertilized with phosphorus, uninoculated; T_{12}, Cambisol fertilized with phosphorus + mycorrhizal inoculum; CV, coefficient of variation; H/D, ratio of plant height to diameter; H/SDW, ratio of plant height to shoot dry weight; SDW/RDW, ratio of shoot dry weight to root dry weight; DQI, Dickson quality index, calculated according to Dickson (1960).

PCA of *P. grandiflorum* data (Figure 1) identified two principal components, which together explained 90.83% of the variance in the dataset. Principal component 1 (PC1) was highly correlated with biomass parameters (RDW and SDW), whereas principal component 2 (PC2) was strongly correlated with growth parameters (height and diameter). Treatments could be divided into two groups according to their position in the PCA biplot (Figure 1): inoculated treatments are located on the right-hand side of the graph, whereas uninoculated treatments are plotted on the left-hand side. It can also be seen that the best treatments for *P. grandiflorum* were T_4 (80% Cambisol + 20% Biomix + mycorrhizal inoculum) and T_{12} (Cambisol fertilized with P + mycorrhizal inoculum), confirming the beneficial effect of mycorrhizal inoculation.

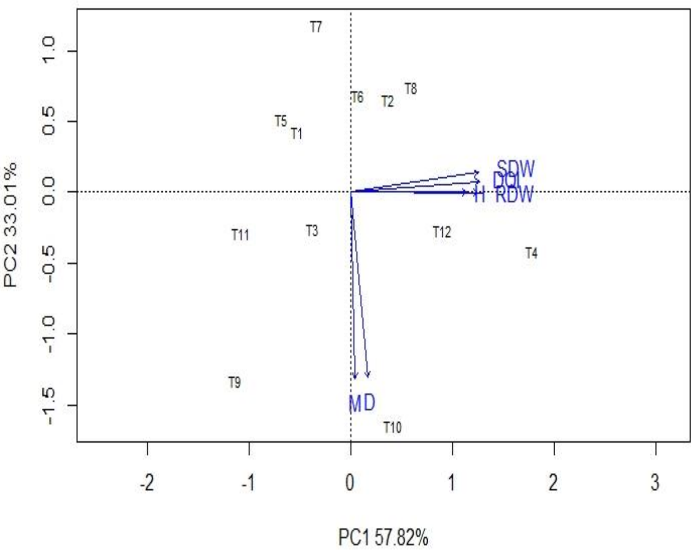

Figure 1. Principal component analysis of biomass and growth data of *Pseudobombax grandiflorum* seedlings grown in inoculated and uninoculated substrates.

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Figura 1. Análise de Componentes Principais das variáveis de biomassa e crescimento mensuradas em experimento com a espécie *P. grandiflorum*, discriminando tratamentos com e sem inoculação.

Data are the mean of six replicate determinations. D, diameter; H, height; SDW, shoot dry weight; RDW, root dry weight; DQI, Dickson quality index; M, mycorrhizal colonization; T_1, 60% Cambisol + 10% Biomix + 10% sand + 20% vermiculite, uninoculated; T_2, 60% Cambisol + 10% Biomix + 10% sand + 20% vermiculite + mycorrhizal inoculum; T_3, 80% Cambisol + 20% Biomix, uninoculated; T_4, 80% Cambisol + 20% Biomix + mycorrhizal inoculum; T_5, 50% Cambisol + 30% Biomix + 10% sand + 10% vermiculite, uninoculated; T_6, 50% Cambisol + 30% Biomix + 10% sand + 10% vermiculite + mycorrhizal inoculum; T_7, 60% Cambisol + 40% Biomix, uninoculated; T_8, 60% Cambisol + 40% Biomix + mycorrhizal inoculum; T_9, 70% Cambisol + 30% bovine manure, uninoculated; T_{10}, 70% Cambisol + 30% bovine manure + mycorrhizal inoculum; T11, Cambisol fertilized with phosphorus, uninoculated; T_{12}, Cambisol fertilized with phosphorus + mycorrhizal inoculum.

PCA of *A. leiocarpa* data (Figure 2) extracted two principal components, which accounted for 86.81% of the total variance. Uninoculated treatments also appeared to the left and inoculated treatments to the right of the biplot. The best treatments for *A. leiocarpa* were T_4 (80% Cambisol + 20% Biomix + mycorrhizal inoculum), T_{10} (70% Cambisol + 30% bovine manure + mycorrhizal inoculum), and T_{12} (Cambisol fertilized with P + mycorrhizal inoculum).

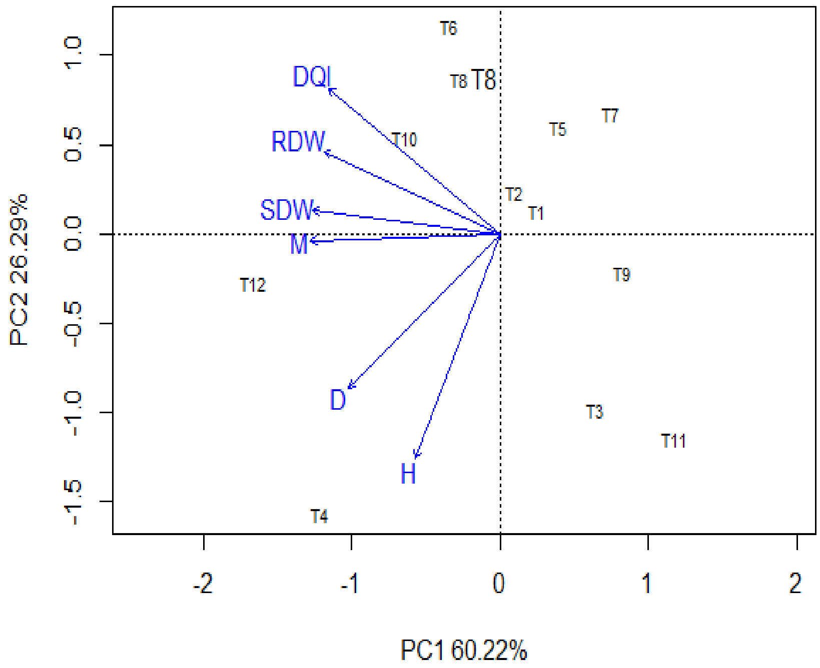

Figure 2. Principal component analysis of biomass and growth data of *Apuleia leiocarpa* seedlings grown in inoculated and uninoculated substrates.

Figura 2. Análise de Componentes Principais das variáveis de biomassa e crescimento mensuradas em experimento com a espécie *A. leiocarpa*.

Data are the mean of six replicate determinations. D, diameter; H, height; SDW, shoot dry weight; RDW, root dry weight; DQI, Dickson quality index; M, mycorrhizal colonization; T_1, 60% Cambisol + 10% Biomix + 10% sand + 20% vermiculite, uninoculated; T_2, 60% Cambisol + 10% Biomix + 10% sand + 20% vermiculite + mycorrhizal inoculum; T_3, 80% Cambisol + 20% Biomix, uninoculated; T_4, 80% Cambisol + 20% Biomix + mycorrhizal inoculum; T_5, 50% Cambisol + 30% Biomix + 10% sand + 10% vermiculite, uninoculated; T_6, 50% Cambisol + 30% Biomix + 10% sand + 10% vermiculite + mycorrhizal inoculum; T_7, 60% Cambisol + 40% Biomix, uninoculated; T_8, 60% Cambisol + 40% Biomix + mycorrhizal inoculum; T_9, 70% Cambisol + 30% bovine manure, uninoculated; T_{10}, 70% Cambisol + 30% bovine manure + mycorrhizal inoculum; T11, Cambisol fertilized with phosphorus, uninoculated; T_{12}, Cambisol fertilized with phosphorus + mycorrhizal inoculum.

Note in Figures 1 and 2 that plant height and diameter show no correlation with mycorrhizal inoculation for *P. grandiflorum* but a weak association for *A. leiocarpa*. Such discrepancy stems from ecological factors and differences in nutrient requirements between species.

Nutritional status

K content was not influenced by mycorrhizal inoculation; no differences were observed between treatments. N levels were significantly higher in T_{10} for both species, as bovine manure contains high levels of this nutrient. Mycorrhizal inoculation increased P levels, regardless of substrate. The highest P concentration was observed in T_{10} (Table 6), showing that mycorrhizal inoculation increases P uptake by plants (OLIVEIRA JÚNIOR *et al.*, 2017).

Table 6. Interaction effects between substrate and mycorrhizal inoculation on the nutritional status of *Pseudobombax grandiflorum* and *Apuleia leiocarpa* seedlings.
Tabela 6. Efeito da interação entre os substratos utilizados e os fungos micorrízicos no estado nutricional das espécies *P. grandiflorum* e *A. leiocarpa*.

Treatment	*P. grandiflorum*						*A. leiocarpa*					
	N (%)		P (%)		K (%)		N (%)		P (%)		K (%)	
T_1	0.42	b	0.19	b	0.69	a	0.32	b	0.09	b	0.58	a
T_2	0.58	b	0.23	a	0.91	a	0.47	b	0.09	b	0.80	a
T_3	0.33	b	0.17	b	0.62	a	0.23	b	0.06	b	0.37	a
T_4	0.56	b	0.24	a	0.48	a	0.46	b	0.13	a	0.51	a
T_5	0.39	b	0.15	b	0.64	a	0.29	b	0.07	b	0.53	a
T_6	0.51	b	0.25	a	0.55	a	0.41	b	0.13	a	0.44	a
T_7	0.35	b	0.18	b	0.54	a	0.39	b	0.07	b	0.44	a
T_8	0.50	b	0.26	a	0.50	a	0.24	b	0.13	a	0.40	a
T_9	0.46	b	0.14	b	0.56	a	0.35	b	0.04	b	0.45	a
T_{10}	0.91	a	0.31	a	0.86	a	0.69	a	0.14	a	0.75	a
T_{11}	0.33	b	0.14	b	0.58	a	0.19	b	0.04	b	0.48	a
T_{12}	0.38	b	0.18	b	0.59	a	0.27	b	0.12	a	0.48	a
CV (%)	32.55		25.15		51.02		38.57		39.22		61.68	

Means in a column followed by different letters differ significantly by the Scott–Knott test ($p < 0.05$). T_1, 60% Cambisol + 10% Biomix + 10% sand + 20% vermiculite, uninoculated; T_2, 60% Cambisol + 10% Biomix + 10% sand + 20% vermiculite + mycorrhizal inoculum; T_3, 80% Cambisol + 20% Biomix, uninoculated; T_4, 80% Cambisol + 20% Biomix + mycorrhizal inoculum; T_5, 50% Cambisol + 30% Biomix + 10% sand + 10% vermiculite, uninoculated. T_6, 50% Cambisol + 30% Biomix + 10% sand + 10% vermiculite + mycorrhizal inoculum; T_7, 60% Cambisol + 40% Biomix, uninoculated. T_8, 60% Cambisol + 40% Biomix + mycorrhizal inoculum; T_9, 70% Cambisol + 30% bovine manure, uninoculated; T_{10}, 70% Cambisol + 30% bovine manure + mycorrhiza; T11, 100% Cambisol fertilized with phosphorus, uninoculated. T_{12}, 100% Cambisol fertilized with phosphorus + mycorrhizal inoculum; CV, coefficient of variation; N, nitrogen; P, phosphorus; K, potassium.

DISCUSSION

Seedling response to inoculation

The main and interaction effects of substrate and mycorrhizal inoculation on growth parameters were significant for both species, showing their importance for seedling development. The difference in colonization percentage observed in *A. leiocarpa* corroborates the findings of Oliveira Júnior *et al.* (2017), who showed that the species is dependent of mycorrhiza and is capable of forming a strong symbiosis that is highly resistant to changes in environmental conditions. For, *A. leiocarpa* the results suggest that the high levels of N in bovine manure favored plant growth.

Overall, the best results were obtained using Cambisol, bovine manure, and mycorrhizal fungi (T_{10}) and 80% Cambisol, 20% Biomix, and mycorrhizal fungi (T_4). These results were likely due to the physical and chemical characteristics (pH, N and P levels) of the substrates, which contributed to seedling growth and mycorrhizal symbiosis. In general, inoculated plants had higher heights, but no relationship was found between plant height and mycorrhizal colonization.

Seedling quality

Treatments T_3, T_4, T_{10} and T_{11}promoted plant growth and resulted in improved seedling quality, according to criteria proposed by Caldeira *et al.* (2008). The authors also recommended maintaining an H/D ratio below 10 during seedling development; all *P. grandiflorum* and *A. leiocarpa* seedlings were within the recommended range of H/D ratio. This parameter is known to be influenced by inherent species traits, seedling age, substrate composition, pot volume, and nursery practices.

The highest H/SDW index for *P. grandiflorum* was observed in T_9 (70% Cambisol + 30% bovine manure, uninoculated), indicating reduced biomass gain, lignification, and quality (Table 4).

A. leiocarpa seedlings grown in uninoculated Cambisol fertilized with P (T_{11}) had a very high H/SDW index and showed signs of etiolation. Mycorrhizal inoculation (T_{12}) decreased the H/SDW index by a factor of 4, indicating that mycorrhizal fungi contributed to nutrient uptake and healthy growth (Table 5). Gomes *et al.* (2013)

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considered H/SDW values below 2 to be indicative of greater survival capacity, regardless of tree species, because the H/SDW index is inversely proportional to lignification. High SDW/RDW ratios indicate a growth imbalance that may result in low resistance to environmental conditions, deficient water absorption, and decreased survival (GOMES *et al.*, 2013). Based on important morphological characteristics for seedling quality, the DQI provides a balanced measure of plant development, robustness, and biomass distribution. The index is affected by species, substrate composition, production and management practices, type of container, and plant age (CALDEIRA *et al.*, 2008). The results are evidence of the beneficial effects of mycorrhizal fungi on the development and quality of the studied plants. Tavares *et al.* (2016) reported positive results with mycorrhizal inoculation of *Acacia mangium* Willd for seedling production in non-sterilized Cerrado soil, concluding that beneficial fungi improved seedling growth, nutrition, and quality

Nutritional status

It is well established that mycorrhizal fungi enhance mineral nutrition in plants. Dias *et al.* (2012) evaluated the effects of inoculation with mycorrhizal fungi and rhizobia on the growth and nutrition of *Anadenanthera macrocarpa* progeny. The authors observed that co-inoculation increased absorption of N and P by seedlings compared with uninoculated plants, producing quality seedlings at a lower cost.

In the present study, the combination of Cambisol, bovine manure, and mycorrhizal fungi (T_{10}) created a favorable condition for nutrient absorption, particularly for N. Bovine manure can contribute to the physical characteristics of the substrate by increasing aggregate stability and porosity as well as enhancing nutrient supply and moisture retention (OGBONNA *et al.*, 2012).

CONCLUSIONS

P. grandiflorum and *A. leiocarpa* seedlings responded differently to different substrates. *P. grandiflorum* seedlings showed improved growth and quality in T_4 (80% Cambisol + 20% Biomix + mycorrhizal inoculum) and T_{12} (Cambisol fertilized with P + mycorrhizal inoculum).

For *A. leiocarpa*, the best treatments were T_4 (80% Cambisol + 20% Biomix + mycorrhizal inoculum), T_{10} (70% Cambisol + 30% bovine manure + mycorrhizal inoculum), and T_{12} (Cambisol fertilized with P + mycorrhizal inoculum). Mycorrhizal inoculation was beneficial for seedling growth and quality.

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