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Differences between *Pseudobombax grandiflorum* and *Bauhinia forficata* in terms of responsiveness and dependence to mycorrhiza

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ABSTRACT: The benefits promoted by arbuscular mycorrhizal fungi (AMF) to forest species seedlings include higher growth rate, better nutrition, and higher survival rates. Inoculation with AMF may facilitate revegetation of degraded lands, although it depends on symbionts and environmental conditions, such as soil P availability. In this sense, the lack of information justifies the carrying out of studies of this nature. We investigated the dependence and responsiveness of two forest species native to the Atlantic Forest, *Pseudobombax grandiflorum* and *Bauhinia forficata*, to different AMF inocula (isolated AMF species, *Dentiscutata heterogama*, DH, *Gigaspora margarita*, GM, *Rhizophagus clarus*, RC, mixed inoculum with these former three AMF species, MI) compared to the uninoculated control (UC), combined with different P doses applied to the substrate (0, 24, 71, 213, and 650 mg kg^{-1}), under greenhouse conditions. We evaluated root colonization, growth, and nutritional variables for *Pseudobombax grandiflorum* and *Bauhinia forficata*, 112 and 116 days after sowing with pre-germinated seeds, respectively. Native forest species exhibited different degrees of mycorrhizal dependence. The highest mycorrhizal dependence of *P. grandiflorum* seedlings was indicated by significant benefits, both growth and nutritional, promoted by innoculation treatments, under fertilization with the intermediate dose of P (213 mg kg^{-1}). In fact, under this P dose, seedlings responded to a maximum increase in biomass in the GM treatment and maximum concentration of P and N in the shoots in the UC and DH treatments, respectively. The lowest mycorrhizal dependence of *B. forficata* seedlings was highlighted by significant growth benefits promoted by inoculation treatments under the lowest doses of P (24 or 71 mg kg^{-1}). Under this P dose, we observed seedlings with maximum value of the root:shoot ratio in most of the types of inoculation and also higher values of biomass and height in the MI treatment. The responsiveness in terms of increase in growth and nutritional variables varied depending on the forest species, the dose of P applied to the substrate, and the AMF type of inoculum used. Root biomass and total dry biomass, mainly, should preferably be included in future studies with the same objective as the present study, as they were more relevant to point out the differences between treatments, in comparison with the variables associated with nutritional variables.

Keywords: mycorrhizal inoculation, phosphorus nutrition, seedlings production, symbiosis, tropical forest species.

INTRODUCTION

One of the major evolutionary strategies for plant survival is the formation of arbuscular mycorrhizal symbiosis (Kamel et al., 2017). The host plant provides carbon photosynthetically fixed to these microorganisms, allowing its growth and life cycle to be completed (Jiang et al., 2017). The fact that the hyphae that grow from these microorganisms, after root colonization, can intercept nutrients that are transferred to the host plant, highlights the importance of this symbiosis in low-fertility soils (Novais et al., 2014). Therefore, seedling inoculation with arbuscular mycorrhizal fungi (AMF) has been used as a strategy to facilitate the revegetation of degraded areas (Vandresen et al., 2007; Dias et al., 2012; Goetten et al., 2016; Oliveira Júnior et al., 2017, 2022) or for agricultural production (Novais et al., 2014; Salloum et al., 2016). In fact, seedlings colonized by AMF show higher growth rate, better nutrition (Goetten et al., 2016) and higher survival rates (Fernandes et al., 2023), when compared with uncolonized seedlings.

The same AMF species can colonize the root system of different plant species, so there is no specificity among symbionts (Janos, 1988; Santos et al., 2013; Moora, 2014; Lekberg and Waller, 2016; Salloum et al., 2016). Nevertheless, this association varies according to the genetic combination between the microorganism and its host (Salloum et al., 2016). A particular plant benefits from mycorrhiza in ways that vary depending on the AMF species, which indicates a variability in the symbiotic efficiency (Novais et al., 2014; Oliveira Júnior et al., 2017). This is a function of the balance between the cost and benefits of the association (Koide, 1991). Environmental conditions are also capable of influencing the efficiency of the symbiosis (Lekberg and Waller, 2016). Soil P availability controls plant response to mycorrhization. In fact, under adequate conditions of available P in the soil, the plant-fungus association can become antagonistic and lose its symbiotic character, since the net benefits arising from mycorrhiza become smaller for the plant, compared to the energy cost it incurs needs to be allocated to maintain such an association (Johnson and Graham, 2013). Thus, under adequate soil P conditions, the plant cannot be responsive to the mycorrhizal association (Janos, 1988, 2007). That means the fungus-plant-environment interaction governs plant response to mycorrhization.

Some plants are moderate or highly dependent on mycorrhiza to grow, even when phosphorus availability in the soil is considered moderate or high, respectively (Oliveira Júnior et al., 2017, 2022). This fact may be due to root architecture traits such as length, volume, forks, and number of tips (Tomazelli et al., 2022), which are influenced by the plant genome (Chen et al., 2013).

Mycorrhizal dependence is defined by phosphorus level in the soil solution needed to "replace" the mycorrhizal benefit, as this is the most limiting nutrient to plant growth in soil at tropical ecosystems (Janos, 1988). A given plant species is said to have high mycorrhizal dependence when the nutritional benefits resulting from the symbiosis increases under conditions of greater P soil availability (Siqueira and Saggin-Júnior, 2001). Plants that benefit from mycorrhizal association, even under adequate P availability in the soil, show mycorrhizal dependence and, therefore, the higher the dose of P at which the plant benefits from mycorrhizal inoculation, the greater its dependence on association with mycorrhizal fungi (Janos, 1988). For this reason, the mycorrhizal dependence of plants must be evaluated under different P levels in the soil, from low to high values (Janos, 2007).

In this study, the definition of Janos (2007) is considered for responsiveness, which is the "vertical magnitude of the effects of mycorrhizas on plant growth referenced to particular phosphorus availabilities, and it may be positive, null or negative". And for, dependence, "to the inability of a plant to grow without mycorrhizas below a particular level of soil phosphorus availability. That is, a plant species that can grow substantially without mycorrhizas at a lower level of phosphorus availability than another plant species is less dependent on mycorrhizas than the other species". The use of native species to revegetate degraded areas and the lack of information about their interaction with mycorrhizal fungi make them targets for mycorrhizal dependency studies (Moora, 2014).

Previous studies have recorded the importance of inoculating seedlings of Atlantic Forest tree species with the mycorrhizal fungi *Dentiscutata heterogama* (T.H. Nicolson and Gerd.) Sieverd., F.A. Souza and Oehl, *Gigaspora margarita* Becker and Hall, and *Rhizophagus clarus* (T.H. Nicolson and N.C. Schenck) C. Walker and A. Schuessler. *Apuleia leiocarpa* (Vogel) J.F. Macbr seedlings inoculated with a mixture of the aforementioned mycorrhizal fungi species, combined with P doses of 71, 213 and 650 mg kg^{-1}, or with inoculation with *D.* heterogama, combined with P doses of 213 or 650 mg kg^{-1}, showed greater increases in dry biomass of shoots and roots (Oliveira Júnior et al., 2017), this same effect was obtained when *Schinus* terebinthifolius Raddi seedlings were inoculated with the same mixture of mycorrhizal fungi, at the P dose of 213 mg kg^{-1} (Oliveira Júnior et al., 2022). These studies increase the information about the establishment of the mycorrhizal symbiosis and bring useful information to revegetation programs with these species. This practice is fundamental to recover the Brazilian Atlantic Forest which is a key biome for protection, preservation, conservation, and recovery, internationally known (Brancalion et al., 2019) due to the alarming state of devastation, in spite of the high levels of endemism and richness of fauna and flora (Joly et al., 2014). The severity of this scenario has been demonstrated in some abandoned pasture areas subjected to fire, in which the natural regeneration of native Atlantic Forest species was interrupted, and where the formation of ecosystems similar to savannas is observed, whose area of influence can be become even more extensive, in the current scenario of global warming (Sansevero et al., 2020).

This study aimed to evaluate the mycorrhizal dependence and the response of *Pseudobombax grandiflorum* (Cav.) A. Robyns and *Bauhinia forficata* Link. to different AMF inoculums, to assess the combination of host species, AMF inoculum, and P dose that provides maximum growth and nutritional status for the seedlings of both forest species. We hypothesized that *P. grandiflorum* and *B. forficata* seedlings are mycorrhizal dependent and the response of both forest species in terms of biomass gain and nutritional status depends on the AMF inoculum.

MATERIALS AND METHODS

Study species

We carried out experiments with to two native forest species, *Pseudobombax grandiflorum* and *Bauhinia forficata. P. grandiflorum* belongs to Malvaceae and is abundant in fragments of alluvial forests in floodplain areas, being indicated for reforestation of degraded areas by sand mining, in the Atlantic Forest biome (D'Orazio and Catharino, 2013). *B. forficata* belongs to the subfamily Caesalpinioideae from Fabaceae and is found in altered areas such as forest edges in different phytophysiognomies of the Atlantic Rainforest (Silva et al., 2016). The responsiveness and dependence to mycorrhiza of these two native forest species studied had not been previously researched.

The AMF species were *Dentiscutata heterogama* (T.H. Nicolson and Gerd.) Sieverd., F.A. Souza and Oehl (DH), *Gigaspora margarita* Becker and Hall (GM), and *Rhizophagus clarus* (T.H. Nicolson and N.C. Schenck) C. Walker and A. Schuessler (RC). These AMF species promote functional symbiosis in agricultural plant species (Novais et al., 2014) and improve forest species seedlings growth and nutrition under nursery conditions, for revegetation of degraded lands (Goetten et al., 2016; Oliveira Júnior et al., 2017). The species of arbuscular mycorrhizal fungi used were multiplied individually, in pots filled with a substrate based on sterile soil, in which *Uruchloa* sp. was cultivated as the host plant species. Spores density in the inocula of each FMA species were *D. heterogama* (DHET A2 - CNPAB002), 17 spores g^{-1}; *G. margarita* (GMAR A1 - CNPAB001), 35 spores g^{-1}; and *R. clarus* (RCLA A5 – CNPAB005), 26 spores g^{-1}. The inocula were kindly provided by the Johanna Döbereiner Resource Center (Embrapa Agrobiologia, Rio de Janeiro).

Experimental design, sampling, and variables analyses

Experiments were carried out under greenhouse conditions, in a 5 × 5 factorial scheme design (five AMF source and five P doses), and completely randomized design, in Rio de Janeiro State, Brazil. The first factor, AMF source, had five levels, including the three isolated AMF species above mentioned, the mixed inoculum or a mixture of these species, and no inoculation (UC: uninoculated). The second factor was P doses applied to the substrate, which also had five levels (0, 24, 71, 213 and 650 mg kg^{-1} of P). These P doses were determined by the method of remaining P or equilibrium P content of the solution (Alvarez et al., 2000). With this procedure, we aimed to establish doses of P that should be applied to the substrate, ranging from a low to a high dose of the nutrient, with the aim of evaluating the mycorrhizal dependence of the plants (Siqueira and Saggin-Júnior, 2001; Oliveira Júnior et al., 2017, 2022). In the evaluation of mycorrhizal dependence of plants, it is considered the benefit that they present in front of the mycorrhizal inoculation of the seedlings under increasing concentrations of P, which influence the availability of this nutrient in the substrate (Janos, 1988, 2007; Oliveira Júnior et al., 2017). The K concentration was balanced among the different treatments by applying complementary levels of a KCl solution together with the KH_2PO_4 solution.

We employed Mehlich-1 method, which is widely used, to evaluate the P content available in the soil solution for plants, using a mixture of two diluted acids (0.05 mol L^{-1} HCl and 0.0125 mol L^{-1} of H_2SO_4) (Mumbach et al., 2018). The F Test ($p = 0.01$) indicated the available P in the soil presented a significant high correlation with the applied P that was determined by the Mehlich-1 extractor, and the value of the determination coefficient ($R^2 = 0.92$) represented the high percentage of variation explained by the model since the maximum value would be equal to 1.0. The regression technique aims to obtain an equation that adequately models or explains, a fact that is evaluated through the value of the coefficient of determination (R^2), the relationship between one or more explanatory variables and a response variable. The equation obtained by the regression technique is considered adequate when the value of the coefficient of determination is above 0.85 (Oliveira et al., 2017).

Containers used were obtained by joining 700 mL plastic cups and 280 mL plastic tubes (Figure 1) (Oliveira Júnior et al., 2017, 2022) filled with 1 kg of the B horizon of a typical Inceptisol (*Cambissolo Háplico Tb Distrófico*). After being autoclaved twice at 121 °C for two hours (Jesus et al., 2005), this material presented the following chemical attributes: pH(H_2O) (1:2.5), 4.9; P and K extracted by Mehlich^{-1}, 1.89 and 64 mg dm^{-3}, respectively; exchangeable Al, Ca, and Mg (KCl 1 mol L^{-1}), 1.21, 0.46, and 0.21 $cmol_c$ dm^{-3}, respectively (Figure 1).

Subsequently, we applied 943 g of limestone as Minercal, with Relative neutralizing value = 91 %, Neutralizing index = 102 %, CaO + MgO = 52 %, CaO 39 % and MgO 13 % per ton of soil, accordingly to the chemical analysis of the substrate (Oliveira Júnior et al., 2017, 2022). The substrate was then incubated for a period of 60 days and it was considered ready for use.

Average density of spores in the inocula of each FMA species were *D. heterogama* (DHET A2 - CNPAB002), 17 spores g^{-1}; *G. margarita* (GMAR A1 - CNPAB001), 35 spores g^{-1}; and *R. clarus* (RCLA A5 – CNPAB005), 26 spores g^{-1}. Thus, we applied to the substrate near the seedling roots 1.76, 0.86, and 1.15 g of inocula respective to the species *D. heterogama*, *G. margarita* and *R. clarus*, respectively, aiming to provide approximately 30 spores g^{-1} per container. The inocula of each AMF species also consisted of hyphae and fragments of colonized roots. All containers received 10 mL of a filtrate with no AMF propagules, which was obtained from the inoculums of the three AMF species, to standardize the treatments when considering the other components of the inoculum microbiota (Souza et al., 2009; Oliveira Júnior et al., 2017, 2022). This filtrate was prepared by processing approximately 40 g of the inoculum with 400 mL of distilled water in a blender, followed by sieving (0.053 mm) and passing it twice through filter paper to ensure the exclusion of AMF propagules (Souza et al., 2009). After adding the filtrate, the vessels were irrigated to facilitate the diffusion of the filtrate through the pores of the substrate.

Seeds of *P. grandiflorum* and *B. forficata* were obtained from Fazenda Modelo of the Rio de Janeiro municipality, state of Rio de Janeiro. In laboratory, the seeds were disinfected on the surface and scarified with H_2O_2 30 % for 2 min and germinated with filter paper and cotton at 28 °C for 5 days, in petri dishes (Oliveira Júnior et al., 2022). Each container received three pre-germinated seeds. After 15 days, we performed the thinning to maintain only the most vigorous seedling per container. From there, we fertilized the soil of each container with 100 mL of a nutrient solution (Bertrand et al., 2000) and 150 mg of N as NH_4NO_3. The fertilization was applied monthly and the plants were irrigated daily by an automatic sprinkler system to maintain the substrate's field capacity at around 70 % of humidity (Oliveira Júnior et al., 2017, 2022).

P. grandiflorum and *B. forficata* were collected 112 and 116 days after sowing with pre-germinated seeds, respectively. The following variables were measured by means of six replicates per treatment: plant height (H), stem diameter (D), shoot and root dry weights (SDM and RDM, respectively), P and N contents in shoots. We estimated the total dry biomass by the sum of shoot and root dry weights, and also the root dry weight:shoot dry weight ratio (root:shoot ratio). The dry biomass of the aerial part of the seedlings was ground in an electric mill and stored in plastic pots. Subsequently, aliquots of this material were subjected to sulfur digestion to evaluate the N and P contents, which were determined by the Kjeldahl method and colorimetry, respectively (Silva, 1999).

Half gram of thin roots was separated from three replications and kept in a 50 % ethanol solution until going through further bleaching and staining (Koske and Gemma, 1989; Grace and Stribley, 1991). Subsequently, we evaluated the percentage rate of the root colonization by AMF (R) by the gridline intersection method (Giovannetti and Mosse, 1980). There is an interval between a minimum and a maximum dose of available P in the soil in which the plant is capable of benefiting from mycorrhizal symbiosis that is, responding to this symbiosis with increases in total dry biomass, compared to the uninoculated control. Below the minimum dose or above the maximum dose of P in the soil, mycorrhiza does not provide increases in total dry biomass for the plant in comparison to the uninoculated one. This interval, in which the curve representing the

Figure 1. Tubes used for seedling production of *Pseudobombax grandiflorum* and *Bauhinia forficata* (a) showing in detail the joining of 700 mL plastic cups and 280 mL plastic tubes (b).

response in total dry biomass of the inoculated plant intersect with the response of the non-inoculated plant, is called mutualism range. Based on this, the mycorrhizal benefit can be estimated through the difference between the integral of the curve of the plant with inoculation and the integral of the curve of the plant without inoculation, in the mutualism range, in a polynomial regression (Saggin Júnior and Siqueira, 1995; Oliveira Júnior et al., 2017, 2022).

Phosphorus benefit for plants was calculated by the integral of the area of the response curve of plants without inoculation, in terms of increase in total dry biomass, comprised between the minimum and maximum doses of P applied to the soil, in a polynomial regression (Saggin Júnior and Siqueira, 1995; Oliveira Júnior et al., 2017, 2022). Next, the value of symbiotic efficiency was calculated by multiplying the value 100 % and the mycorrhizal benefit/phosphorus benefit ratio (Saggin Júnior and Siqueira, 1995; Oliveira Júnior et al., 2017, 2022).

Statistical analyses

Data were submitted to the Box-Cox transformation. Further, the data were evaluated by Analysis of Variance and Scott-Knott's test ($p<0.05$). We also applied regression analysis to the quantitative factors. These statistical analyzes were performed using version 5.0 of the software SISVAR. With the aid of version 2.17c of the software Paleontological Statistics (PAST), we performed the principal components analysis aiming to identify associations among the treatments and seedlings attributes.

RESULTS

Pseudobombax grandiflorum

Root colonization with all AMF inoculation treatments decreased with increasing P doses in the substrate and was drastically reduced at the highest P dose (650 mg kg^{-1}), with values between 6 and 11 %, in contrast to values between 44 and 60 % when P fertilization has not been carried out (Figure 2a). Linear models could be adjusted to the response of the seedlings to *G. margarita, R. clarus*, and mixed inoculum, showing a linear reduction with increasing P doses.

The highest value of root colonization by AMF (approximately 60 %) was verified in the absence of P application when seedlings were inoculated with mixed inoculum, followed by the P dose of 213 mg kg^{-1} in the inoculation with *D. heterogama*, at the lowest P dose (24 mg kg^{-1}) in the inoculation with *G. margarita*, and at intermediate P doses (between 71 and 213 mg kg^{-1}) in the inoculation with *R. clarus* (approximately 40 %) (Figure 2a).

Plant biomass (shoot, root, and total dry biomass) increased linearly with increasing doses of P in the absence of AMF inoculation and with inoculation with *R. clarus* (root and total dry weight) or *D. heterogama* (shoot dry weight) (Figures 2b, 2c, and 2d). In general, the maximum value of biomass accumulation was registered between the P dose of 213 and 650 mg kg^{-1} when the seedlings were inoculated either with *G. margarita*; and at the higher dose of P (650 mg kg^{-1}) when the seedlings were inoculated with *R. clarus* (root and total dry weight).

Adjusted model predicted that the best seedlings biomass (shoot, root, and total dry biomass) response to the inoculation would be between P dose of 200 and 400 mg kg^{-1}, decreasing at higher doses. Estimated maximum efficiency doses (EMED) of P for shoot, root, and total dry biomass accumulation in response to the AMF inoculation (*G. margarita*) were 340, 309, and 362 mg kg^{-1}, respectively, which would reflect in 8.10, 10.21, and 19.44 g $plant^{-1}$ of shoot, root and total dry biomass, respectively. The lowest values of shoot, root, and total dry biomass were observed for the uninoculated control, however the values of these variables increased linearly as the P concentration in the substrate increased, if compared with the inoculated treatments (Figures 2b, 2c, and 2d).

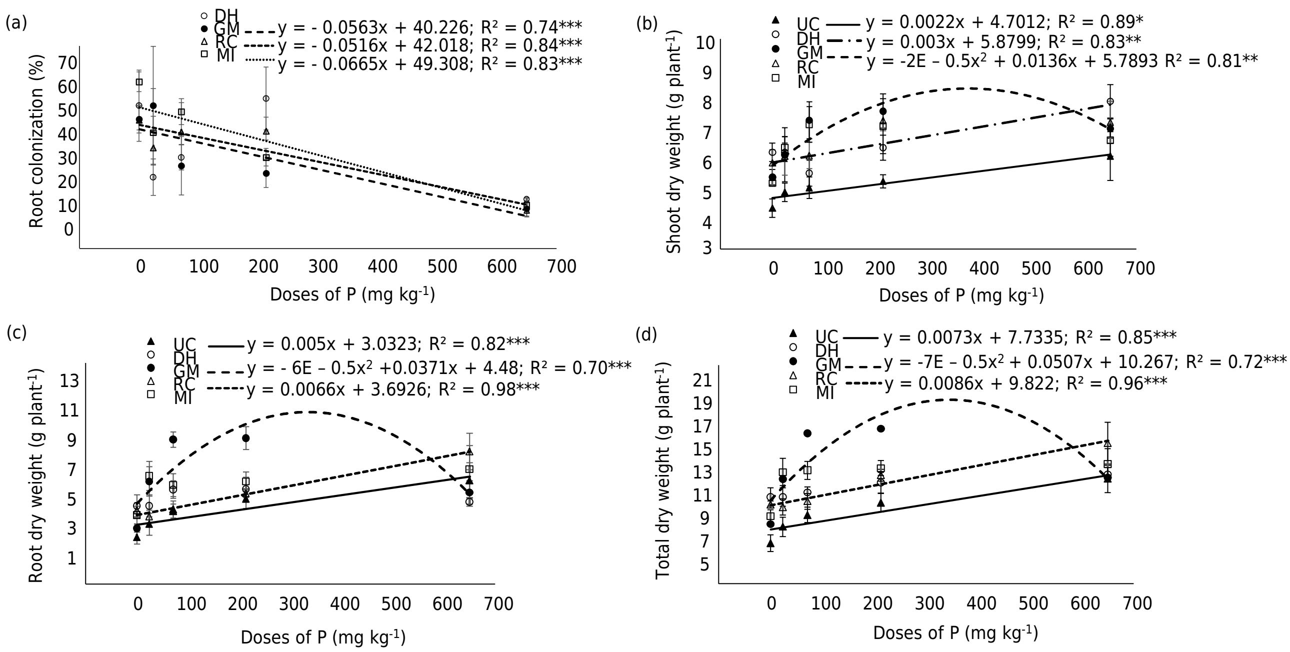

Figure 2. Root colonization (a), shoot (b), root (c) and total (d) dry weight of *Pseudobombax grandiflorum* seedlings inoculated with the individual different species of arbuscular mycorrhizal fungi (DH: *Dentiscutata heterogama*, GM: *Gigaspora margarita*, RC: *Rhizophagus clarus*) or mixed inoculum (MI), and uninoculated control (UC) in a substrate fertilized with 0, 24, 71, 213, and 650 mg kg^{-1} of P. Plants were sampled 112 days after sowing. (*) p≤0.05; (**) p≤0,01 (***); p≤0.001.

When analyzing the root:shoot ratio, models could be fit only for the uninoculated control and the inoculation with *R. clarus*, which showed a linear increase of this variable as the P doses increased. The maximum value of root:shoot ratio was obtained between 71 and 213 mg kg^{-1} doses of P when seedlings were inoculated with *G. margarita*, followed by 71 mg kg^{-1} with *D. heterogama* inoculation, at the highest P dose (650 mg kg^{-1}) in the absence of AMF inoculation, and with *R. clarus* or mixed inoculation (Figure 3a).

When the seedlings were inoculated with the mixed inoculum, the stem diameter response followed a positive linear model. There was a trend towards higher diameter values were registered with the application of a P dose of 71 mg kg^{-1} in the absence of AMF inoculation or with *G. margarita* inoculation, followed by the highest dose of P (650 mg kg^{-1}) when the seedlings were inoculated with mixed inoculum, *D. heterogama* or *R. clarus* (Figure 3b).

In general, no clear responses were observed for shoot N and P contents, except for a few treatments (Figures 3c and 3d). Shoot N content was maximum at intermediate doses for seedlings inoculated with *D. heterogama*, mainly around P dose of 213 mg kg^{-1} (Figure 3c). Maximum values of shoot N content were observed with P dose of 71 mg kg^{-1} inocullated with *R. clarus*, of 650 mg kg^{-1} with *G. margarita* inoculation, and 213 and 650 mg kg^{-1} in the absence of AMF inoculation or when the seedlings were inoculated with mixed inoculum.

Shoot P content was maximum at P dose of 213 mg kg^{-1} for the uninoculated control (Figure 3d). The maximum values of shoot P content were observed with P dose of 24 mg kg^{-1} with *G. margarita* inoculation, 650 mg kg^{-1} with *R. clarus* inoculation, of 213 mg kg^{-1} with the mixed inoculum, and of 650 with *D. heterogama* inoculation.

Bauhinia forficata

Root colonization decreased with P doses for all treatments, with drastic reductions at the highest P dose (650 mg kg^{-1}) in plants inoculated with *G. margarita* and *D. heterogama* (0 and 2 %, respectively) (Figure 4a), although no model has fitted for this last inoculation treatment.

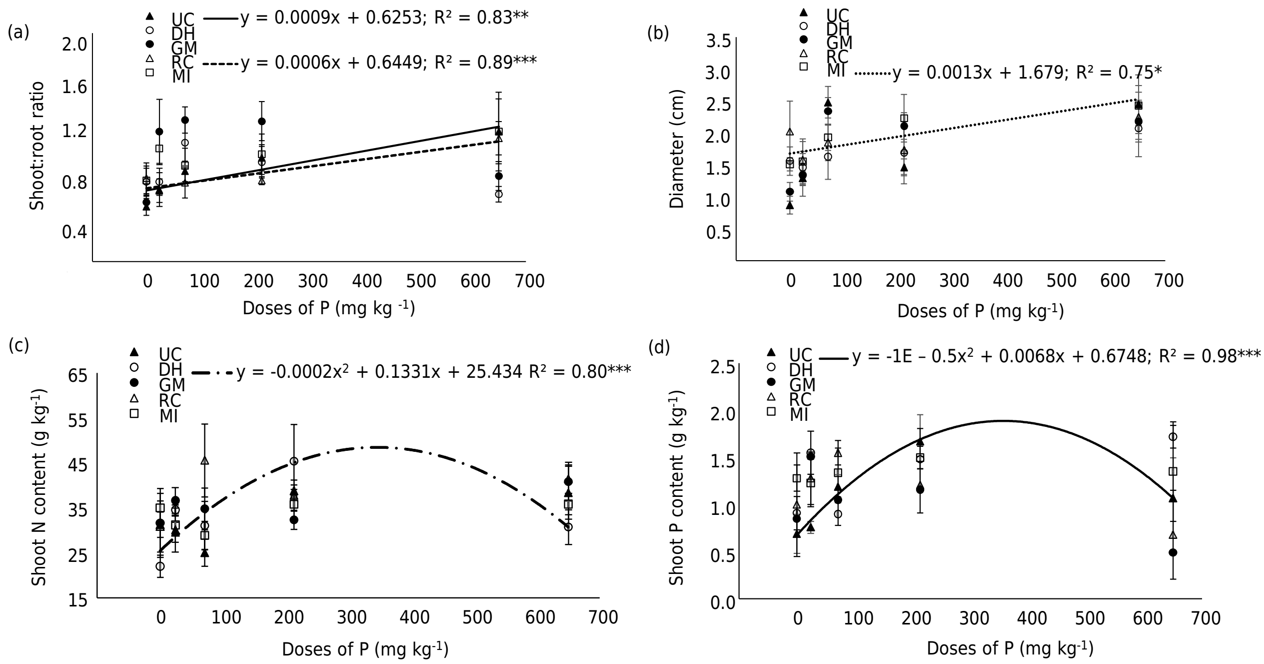

Figure 3. Root:shoot ratio (a), diameter (b), N (c), and P (d) contents in shoot of *Pseudobombax grandiflorum* seedlings inoculated with the individual different species of arbuscular mycorrhizal fungi (DH: *Dentiscutata heterogama*, GM: *Gigaspora margarita*, RC: *Rhizophagus clarus*) or mixed inoculum (MI), and uninoculated control (UC) in a substrate fertilized with 0, 24, 71, 213, and 650 mg kg^{-1} of P. Plants were sampled 112 days after sowing. (*) p≤0.05; (**) p≤0.01 (***); p≤0.001.

Average root colonization was 22 and 27 % for *R. clarus* and the mixed inoculum at the highest P dose, respectively (Figure 4a). In the case of *R. clarus*, the model shows the lowest colonization values at intermediate P doses. The highest value of root colonization by AMF (approximately 60 %) was verified at a dose of 71 mg kg^{-1} with *D. heterogama* inoculation, followed by this same P dose with mixed inoculum, at a dose of 24 mg kg^{-1} in the treatment with *R. clarus*, and in the absence of P in the inoculation treatment with *G. margarita* (approximately 35 %).

In general, the maximum value of plant biomass (shoot, root, and total dry biomass) accumulation was registered between the P dose of 213 and 650 mg kg^{-1} for practically all AMF inoculation treatments, whose quadratic model presented the best fit (Figures 4b, 4c, and 4d). The highest value of shoot dry weight was observed in seedlings inoculated with *D. heterogama* (Figure 4b), whose EMED of P for shoot biomass accumulation in response to the AMF inoculation would be the P application of 410 mg kg^{-1}, which would influence the production of 3.80 g plant^{-1} of shoot dry weight, with seedling inoculation. On the other hand, the maximum root and total dry biomass was achieved with the inoculation of the mixed inoculum, whose EMED of P were 392.5 and 396 mg kg^{-1}, respectively, which would reflect on root and total dry biomass of 7.62 and 10.53 g plant^{-1}, respectively, with the AMF mixed inoculum.

No clear pattern was observed for root:shoot ratio (Figure 5a). However, the maximum it seemed that maximum values of root:shoot ratio were verified at the lowest dose of P (24 mg kg^{-1}) when the seedlings were inoculated with *G. margarita* or *R. clarus*; followed by the absence of P application to the substrate when the seedlings did not receive AMF inoculation; at 24 mg kg^{-1} when the seedlings received the *D. heterogama* inoculum; and at the doses of 24 and 213 mg kg^{-1} when the seedlings were inoculated with the mixed inoculum.

In terms of diameter, there was no variation among the different treatments of AMF inoculation and the absence of AMF inoculation, due to the overlap among the response curves for this variable, considering all the treatments (Figure 5b). Suitable models for the mixed inoculum, *R. clarus* and the uninoculated control estimated that the P dose of 213 mg kg^{-1} would provide seedlings with maximum values of stem diameter (Figure 5b).

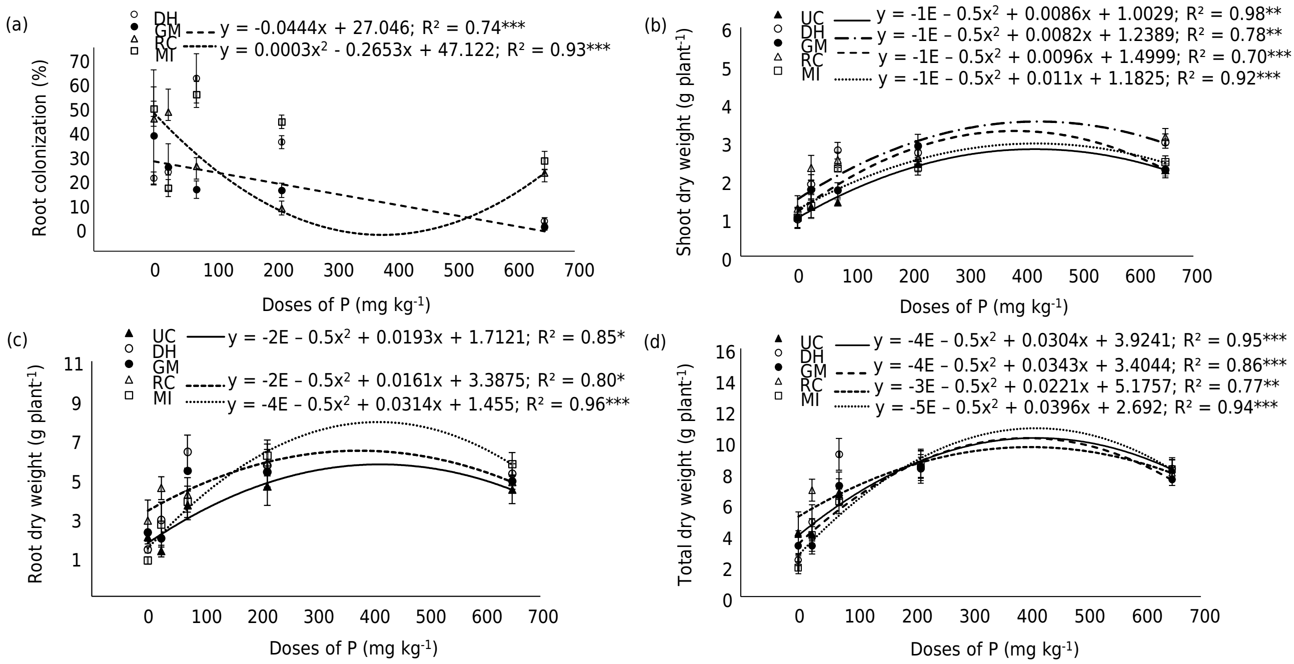

Figure 4. Root colonization (a), shoot (b), root (c) and total (d) dry weight of *Bauhinia forficata* seedlings inoculated with the individual different species of arbuscular mycorrhizal fungi (DH: *Dentiscutata heterogama*, GM: *Gigaspora margarita*, RC: *Rhizophagus clarus*) or mixed inoculum (MI), and uninoculated control (UC) in a substrate fertilized with 0, 24, 71, 213, and 650 mg kg^{-1} of P. Plants were sampled 112 days after sowing. (*) $p \leq 0.05$; (**) $p \leq 0.01$ (***); $p \leq 0.001$.

When comparing all the treatments, the inoculation with *D. heterogama* could produce seedlings with maximum values of diameter, mainly at the P dose of 71 mg kg^{-1} (Figure 5b). Maximum values of height were obtained with the P dose of 71 mg kg^{-1} in the inoculation with mixed inoculum, of 71 or 650 mg kg^{-1} when seedlings were inoculated with *R. clarus*, of 650 mg kg^{-1} in the absence of AMF inoculation and with *G. margarita* inoculation (Figure 5c).

Shoot P content linearly increased with increasing P doses and were maximum at the highest P dose (650 mg kg^{-1}), regardless the inoculation treatment or absence of AMF inoculation (Figure 5d). The mixed inoculum provided maximum shoot P content, even when root colonization was low (27 %).

Table 1. Response of forest species seedlings to the inoculation with the individual different species of arbuscular mycorrhizal fungi (*Dentiscutata heterogama*, *Gigaspora margarita*, *Rhizophagus clarus*) or mixed inoculum, based on the total dry biomass of the plant[(1)]

Mycorrhizal inoculum	*Pseudobombax grandiflorum*			*Bauhinia forficata*		
	MB[(2)]	PB[(3)]	SE[(4)]	MB[(2)]	PB[(3)]	SE[(4)]
	Area (dimensionless)		%	Area (dimensionless)		%
Dentiscutata heterogama	3.330	3.054	109	2.827	3.530	80
Gigaspora margarita	4.674	3.054	153	3.948	3.530	112
Rhizophagus clarus	3.291	3.054	108	2.716	3.530	77
Mixed inoculum	4.174	3.054	137	3.988	3.530	113

[(1)] Average of four replications. [(2)] MB: Mycorrhizal Benefit estimated by subtraction of the area under the curve of not-inoculated treatments from the area under the curve of the less inoculated treatments. [(3)] PB: Phosphorus Benefit estimated by calculation of integral defined by the curve of not-inoculated treatments. [(4)] SE: Symbiotic Efficiency estimated by the relationship between the mycorrhizal benefit and the phosphorus benefit.

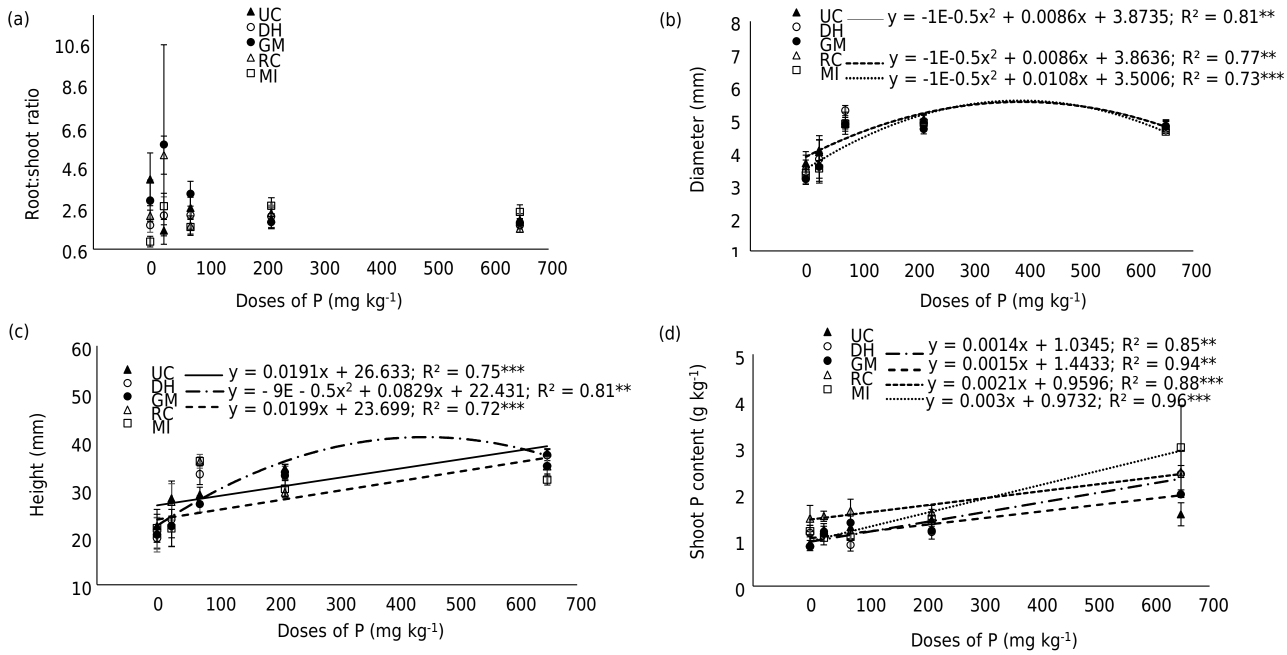

Figure 5. Root:shoot ratio (a), diameter (b), height (c), and P contents in shoot (d) of *Bauhinia forficata* seedlings inoculated with the individual different species of arbuscular mycorrhizal fungi (DH: *Dentiscutata heterogama*, GM: *Gigaspora margarita*, RC: *Rhizophagus clarus*) or mixed inoculum (MI), and uninoculated control (UC) in a substrate fertilized with 0, 24, 71, 213, and 650 mg kg^{-1} of P. Plants were sampled 112 days after sowing. (*) p≤0.05; (**) p≤0.01 (***); p≤0.001.

Mycorrhizal benefit

Mycorrhizal benefit varied according to the mycorrhizal inoculum for both species of plant. Treatments that provided higher benefits for *P. grandiflorum* and *B. forficata* seedlings were the inoculation with *G. margarita* and with the mixed inoculum, as shown by their higher symbiotic efficiencies, when comparing with the control (Table 1). Inoculation with *D. heterogama* and with *R. clarus* did not satisfactorily benefit the seedlings of *B. forficata*, since such treatments provided 80 and 77 % of biomass, respectively, in comparison with the uninoculated control.

Principal Component Anaylsis

From the relationship between Principal Components 1 and 2, it was verified the treatments were spatially distributed along an increasing gradient of P doses (0, 24, 71, 213 and 650 mg kg^{-1}) that were applied to the substrate, from left to right, for seedlings of both forest species (Figures 6a and 6b). The treatments characterized by the absence of P fertilization or with the lowest P dose applied to the substrate (24 mg kg^{-1}), regardless of the absence or presence of mycorrhizal inoculation, were arranged to the left of Principal Component 1 (negative eigenvectors), for both forest species (Figures 6a and 6b).

In contrast, the treatments in which the two highest doses of P were applied to the substrate (213 and 650 mg kg^{-1}), regardless of the absence or presence of mycorrhizal inoculation, were located to the right of Principal Component 1 (positive eigenvectors), in the case of both forest species. Principal Component 1 explained most of the variability of the results (Figures 6a and 6b).

Pseudobombax grandiflorum seedlings at the P doses of 71 and 213 mg kg^{-1} and with *G. margarita* inoculation were associated with higher mean values of shoot, root, total dry biomass, and root:shoot ratio (Figure 6a). The highest mean value of height, stem diameter, and shoot N content were associated with seedlings produced at the 213 mg kg^{-1} of P in the absence of mycorrhizal inoculation or in the presence of *D. heterogama*, and at the highest P dose (650 mg kg^{-1}) in the presence of *G. margarita*, *R. clarus*, or mixed inoculum. Root colonization and P shoot content in *Pseudobombax grandiflorum* seedlings were not specifically associated with any treatment.

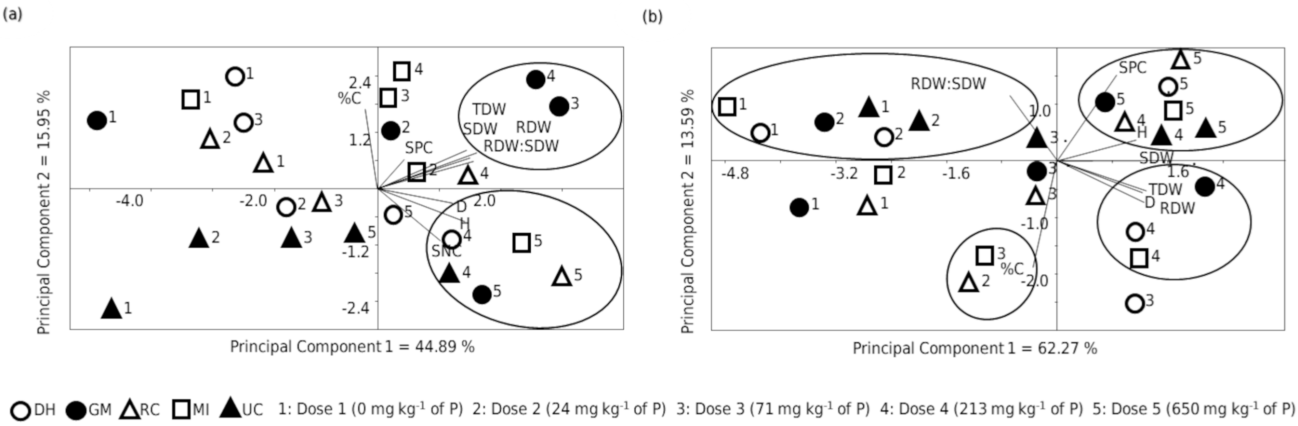

Figure 6. Ordering diagrams resulting from multivariate analysis of principal components for *Pseudobombax grandiflorum* (a) and *Bauhinia forficata* (b) seedlings inoculated with the individual different species of arbuscular mycorrhizal fungi (DH: *Dentiscutata heterogama*, GM: *Gigaspora margarita*, RC: *Rhizophagus clarus*) or mixed inoculum (MI), and uninoculated control (UC) in a substrate fertilized with 0, 24, 71, 213, and 650 mg kg^{-1} of P. %C: root colonization; H: height; D: diameter; SDW, RDW, TDW: shoot, root, and total dry biomass, respectively; RDW:SDW: root:shoot ratio; SNC and SPC: shoot concentration of N and P, respectively.ed with 0, 24, 71, 213, and 650 mg kg^{-1} of P. Plants were sampled 112 days after sowing. (*) p≤0.05; (**) p≤0.01 (***); p≤0.001.

Seedlings of *Bauhinia forficata* with the application of 213 mg kg^{-1} of P, in the presence of *D. heterogama*, *G. margarita*, or mixed inoculum were associated with higher mean values of diameter, root, and total dry biomass (Figure 6b). On the other hand, seedlings of this forest species at the highest P dose (650 mg kg^{-1}) were associated with higher mean values of height and shoot P contents regardless of the absence or presence of the mycorrhizal inoculation (*D. heterogama*, *G. margarita*, *R. clarus*, mixed inoculum), besides the P dose of 213 mg kg^{-1} in the absence of inoculation or in the presence of inoculation with *R. clarus*.

Seedlings of *Bauhinia forficata* at lower P doses (24 and 71 mg kg^{-1}) in the presence of *R. clarus* and mixed inoculum, respectively, were associated with higher mean values of root colonization (Figure 6b). The highest mean root:shoot ratio values were associated to the seedlings that were produced in the absence of phosphate fertilization, or with the addition of the lowest dose of P (24 mg kg^{-1}) in the absence of mycorrhizal inoculation and/or with inoculation with *D. heterogama*, *G. margarita* and mixed inoculum.

Mainly root and total dry biomass presented the highest values of correlation coefficient (greater than or equal to 0.95) with Principal Component 1 (0.97 and 0.95, respectively), followed by diameter (0.9316), shoot dry weight (0.91) and height (0.91). The variables root:shoot ratio, root colonization, and P shoot content presented insignificant correlation coefficient (<0.70) with Principal Components 1 and 2.

DISCUSSION

Treatment spatial arrangements applied to seedlings of both forest species, along an increasing gradient of P doses that were applied to the substrate, shown by the principal component analysis the relevant impact of P dose on the variables studied. This pattern recorded for the seedlings of both forest species was reinforced by the opposite spatial arrangement occupied by the group formed by the absence of P fertilization or with the lowest P dose applied to the substrate (24 mg kg^{-1}), in comparison with the arrangement of the two highest doses of P (213 and 650 mg kg^{-1}), using Principal Component 1 as a reference, which explained most of the result variability. Increased values of shoot, root, and total dry biomass in the uninoculated control as the P concentration in the substrate increased, highlighted the strong positive seedlings response to increasing P doses.

Root and total dry biomass were considered the most important for the discussion of the results of this study, due to the highest influence on Principal Component 1 (correlation coefficient greater than or equal to 0.95). Therefore, these variables should preferably be included in future studies with the same objective. However, it should also be highlighted the importance of discussing the results regarding the variables associated to total dry biomass and root dry weight, which are shoot dry weight, the percentage of mycorrhizal colonization and the root:shoot ratio.

In general, the biomass values (shoot, root, and total dry biomass) increased as the P doses increased, for both forest species. This pattern of increasing biomass due to the substrate fertilization with P, both in inoculated and uninoculated control, has been reported (Oliveira Júnior et al., 2017). However, this result depends on the plant species, as it was not observed for all Brazilian native forest species in a study under nursery conditions (Goetten et al., 2016). This wide variation in the pattern of plant response to mycorrhization emphasizes the complexity of the results that can be obtained, which vary depending on three factors: environment, host plant and mycorrhizal fungus.

For *Pseudobombax*, the root:shoot ratio increased as the P doses increased with the uninoculated control and with the inoculation with *R. clarus*, but no clear pattern was observed for *Bauhinia* root:shoot ratio. However, the maximum value of this variable was obtained in intermediate P doses (71 and 213 mg kg^{-1}), when *Pseudobombax* seedlings were inoculated with *G. margarita*, and at the lowest dose of P (24 mg kg^{-1}), when the *Bauhinia* seedlings received either *G. margarita* or *R. clarus* inoculum.

The maximum value of the root:shoot ratio in *Bauhinia* seedlings that received the lowest dose of P can be explained by the fact the plants invest in root growth and extension, to compensate for the low availability of P in the rhizosphere. Changes in root growth can increase the efficiency in the use of P by plants, which is generally not uniformly distributed in the soil, whose availability decreases rapidly in the rhizosphere as this nutrient is absorbed (Bhattacharya, 2019). For this reason, the increase in the root:shoot ratio with increasing P doses, as well as the maximum value obtained for this variable when *Pseudobombax* seedlings were inoculated with intermediate P doses, was not expected.

In relation to *Pseudobombax grandiflorum* seedlings, inoculation with *G. margarita,* together with the application of doses of 71 or 213 mg kg^{-1} of P, provided increases in plant biomass (shoot, root and total dry biomass) and root:shoot ratio. Therefore, the most recommended treatment would be the inoculation of seedlings of this forest species with *G. margarita* in addition to 71 mg kg^{-1} of P, to relieve the cost of producing these seedlings.

In *Bauhinia forficata* seedlings, inoculation with *G. margarita*, *D. heterogama*, or mixed inoculum together with the application of 213 mg kg^{-1} of P, promoted an increase in plant biomass (root and total dry biomass). However, we recommend seedlings inoculation of this forest species with *G. margarita*, *D. heterogama*, or mixed inoculum in combination with to the dose of 24 mg kg^{-1} P, which provided an increase in the root:shoot ratio, with the objective of reducing the cost of seedling production.

The rate of root colonization and response of plants varied according to the AMF species (Jansa et al., 2007). Nevertheless, the rate of root colonization decreased proportionally as increased the P doses added to the substratum, regardless the AMF inoculation treatment, for both forest species. The decrease in mycorrhizal colonization as a function of increasing the P dose added to the substrate is a response commonly observed in different studies (Oliveira Júnior et al., 2017). The reason for this pattern would be that, under adequate conditions of P availability, plants would decrease the energy demand to sustain the mycorrhizal association (Koide, 1991). Thus, one of the principal limiting factors for symbiosis is the soil P availability for plants (Oliveira Júnior et al., 2017).

The nature of the *P. grandiflorum* biomass response to the inoculation varied with the different AMF inoculum. Depending on the AMF species colonizing the roots, *P. grandiflorum* can be classified as of low or high responsiveness, which is the case when it is inoculated with *D. heterogama* or *G. margarita*, respectively. This highlights the need of testing different AMF species to assess responsiveness to mycorrhizal inoculationin order of not underestimate the plant response. A similar response to AMF inoculation was observed for *Apuleia leiocarpa* (Oliveira Júnior et al., 2017), and this variation in the plant response has been previously observed for several forest species in interaction with a single species of mycorrhizal fungi (Siqueira and Saggin-Júnior, 2001).

A high mycorrhizal dependence of *P. grandiflorum* was characterized by the benefit generated by the AMF inoculation even at higher P doses, and its high responsiveness was emphasized by the large difference between the biomasses of inoculated plants and uninoculated control. Using inoculation with *G. margarita* combined with low to intermediate P doses could be beneficial. In the case of the mixed inoculum, it is important to highlight each species has a distinct strategy: for example, species of the family Gigasporaceae are known for extensively colonizing the soil, while Glomeraceae has most of its hyphae colonizing the roots (Maherali and Klironomos, 2007). These characteristics point to a complementary or functional specificity, indicating the advantage of having more species in the system (Oliveira Júnior et al., 2017). For this reason, we argue that using the mixed inoculum could bring additional benefits in the field. In fact, both greater species richness and greater species complementarity are related to plant communities with higher productivity (Santos et al., 2013).

Species *B. forficata* responded to inoculation mainly at the P dose of 24 mg kg^{-1}. On the other hand, there was no significant difference between the inoculated and non-inoculated treatments at the highest dose of P (650 mg kg^{-1} P), which characterized not mycorrhizal dependence of this species. However, different results have been reported when speaking of the association of *B. forficata* with AMF. *B. forficata* seedlings showed a very high rate of root colonization by native AMF (>80 %) and the response to colonization (difference between total dry biomass of plants inoculated and uninoculated control) was considered average (between 40-59 %) under greenhouse conditions, but there was no root colonization under field conditions (Zangaro et al., 2002). In contrast, the inoculation of *B. forficata* seedlings with native AMF did not influence the increase in total biomass, when compared with uninoculated control, under nursery conditions (Vandresen et al., 2007).

This divergence of results for the same plant species is because the effect of mycorrhizal inoculation also depends on other factors, such as not only the AMF species, but also the environmental conditions, which influence the hydrobalance, nutrient acquisition, and carbon between the symbionts involved (Lekberg and Waller, 2016), which may vary when comparing different studies. Effects of association between symbionts are not yet fully understood, since there may be a wide variation in terms of the benefits generated by AMF to the host plant, as well as in the ability to obtain photoassimilates by AMF from the host plant (Saggin Júnior and Siqueira, 1995).

Some studies have suggested a lesser importance of plant species for the establishment of a fungal community and have emphasized the relevance of other characteristics such as: environmental conditions, interactions between different AMF species in the rhizosphere, or their random distribution in nature (Lekberg and Waller, 2016). Thus, the composition of the AMF community is extremely important for establishing a sustainable plant community, as plant community dynamics are driven by competition or resource sharing. This process is since plant species may differ in their response to mycorrhization, depending on the AMF species, and positive effects or absence of differences can be observed in comparison with non-inoculation. Thus, any change in the composition of the AMF community can have direct effects on plant biodiversity in the environment (van der Heijden et al., 1998).

CONCLUSIONS

Considering Janos' (2007) definition for mycorrhizal responsiveness and dependence, *P. grandiflorum* is responsive and dependent on arbuscular mycorrhizal fungi, while *B. forficata* is only responsive. *P. grandiflorum* seedlings reached maximal values of biomass with the *G. margarita* inoculation under intermediate P dose (213 mg kg^{-1}). In contrast, *B. forficata* seedlings obtained increase in root:shoot ratio, which can reduce seedling production cost, when inoculated with the mixed inoculum under low P dose (24 mg kg^{-1}). According to the analysis of main components the biomass variables, especially root and total dry biomass, in addition to the diameter, shoot dry weight and height were more relevant and thus should preferably be included in future studies with the same objective, than the variables correlated with the nutritional status.

DATA AVAILABILITY

The data will be provided upon request.

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