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Influence of Arbuscular Mycorrhizae on the Development and Attack of Leaf-Cutting Ants in Clonal Seedlings of *Eucalyptus urophylla x Eucalyptus camaldulensis*

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Authors' contributions

This work was developed with the collaboration of all authors. The authors JCMDO and MDSFP prepared the practical work performed the statistical analysis, writing and submitted the paper. The authors JG, IDSN and GDFSJ collaborated in the statistical analysis and in reviewing the manuscript. The authors AAB collaborated in reviewing the manuscript and in the translation of the paper. All authors read and approved the final manuscript.

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ABSTRACT

The present study aimed to evaluate the effect of the mycorrhizal association in the development and attack of leaf-cutting ants *Atta sexdens* (Linnaeus, 1758) in clonal seedlings of *Eucalyptus urophylla x Eucalyptus camaldulensis* hybrid. The treatments consisted of inoculated and uninoculated seedlings with 100 grams of sand containing spores from arbuscular mycorrhizal fungi (AMF) collected on native soil from four different areas of the amazon: native forest area (NF), permanent preservation area recovered to three years (PPA03), permanent preserved area degraded (PPAD) and permanent preservation area recovered to four years (PPA04). At 45 and 90

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days after inoculation (d.a.i.), height, fresh and dry matter of shoot and root, root development, number of leaves, stem diameter and tolerance to leaf-cutting ants were evaluated. In the first group of plants (45 d.a.i.), mycorrhizal plants presented the higher root volume with the use of APP04, APP03 and NF all with 6.16 mm. For the second group of plants (90 d.a.i.), the highest root volume was also found on mycorrhizal plants with the soil of APP03 and control with 7.16 mm. For the attractiveness test of ants, 16 discs were taken from the non-inoculated seedlings. Regarding the disks of mycorrhizal seedlings with the soil of APP04, only 9 were taken. Although the use of AMF to stimulate plant resistance to attack by leaf-cutting ants is a new study, studies related to the influence of mychorrizas on plant development are quite common and the results presented in this study did not evidence the influence of mychorrizas on plant development. However, it was observed that the early inoculation of the seedlings can reduce the attractiveness of leaf-cutting ants by the seedlings of the hybrid *Eucalyptus urophylla x Eucalyptus camaldulensis*, already in the first 45 d.a.i of the seedlings and the inoculum used can interfere in this process.

Keywords: Amazon soil; attractiveness; AMF; Atta sexdens; mycorrhizae.

1. INTRODUCTION

Cutting ants, represented by the genera *Atta* Fabricius, 1804 and *Acromyrmex* Mayr, 1865 (Hymenoptera: Formicidae), are the most important insect pests of forest crops in the Neotropical regions, and the economic impacts caused by this pest are more significant in *Eucalyptus* plantations. Due to the use of formicidal baits, monitoring of the planting during its production, value paid to third parties for the control of leaf-cutting ants and cost of equipment used, the damages represent up to 30% of the cost of planting and is considered the main entomological problem to be considered for settlement implantation [1,2]. For this reason, methods of control or prevention of ant attack are widely studied [3].

Eucalyptus are used in commercial plantations, mainly due to its high growth rate and multiple uses. They belong to the Myrtaceae family and have more than 730 botanically recognized species, in addition to the large number of hybrids and varieties, but only 20 species are used commercially in the world [4,5]. The last 10 years were marked by a great expansion of areas planted with *Eucalyptus* in tropical regions, especially Brazil, whose area of forests planted with the species of this genus, its hybrids and clones already exceeds the mark of 5.6 million of hectares, representing around 25% of the world total [6].

Several studies have shown that there is a specificity in the attraction of ants to certain plants [7,8,9,10]. This fact may be associated with the resistance of some plants to attack by-leaf cutting ants, probably due to hormonal, genetic, nutritional or chemical factors. Pest insect control by plant resistance has not yet been used on a large scale in the forest sector, however, it is assumed that in the future it may be a very important technique in Integrated Pest Management (IPM) in forest plantations [11]. Cutter ants have the capacity to perceive small quantitative and qualitative distinctions in the components that constitute the leaves of some theoretically resistant species [12]. The most attacked plants are those under water stress [13] and an alternative that can be used to aid in the healthy and rapid development of plants is the use of arbuscular mycorrhizal fungi (AMF) [14].

Soil micro-organisms play an essential role in the productivity of agricultural ecosystems and in the functioning of natural ecosystems, and this role can be beneficial or harmful [15,16]. Among the soil microorganisms with beneficial role are arbuscular mycorrhizal fungi (AMF), which increase the establishment and plant nutrition in most terrestrial ecosystems [17].

Mycorrhizas are mutual symbiotic association of generalized occurrence, between a fungus and a plant. Among the various types of mycorrhizas, the arbuscular is the most widespread in the tropics, consisting of AMFs, of the Glomeromycota phylum [18] and Briophytes, Pteridophytes, Gymnosperms and Angiosperms species.

Several tree species have presented different responses to the root colonization with different species of AMF, and these fungi can provide different services for host plants [19], and can act, for example, as potential agents of biological control, softening the effects or damage caused by phytopathogens and insects, probably by indirect means, by better plant nutrition or increased resistance of the root system. Among the benefits of the arbuscular mycorrhizal association can be cited the best access to soil resources [19], increased aggregation and soil stability [20], increased tolerance of plants to water stress [21] and protection against phytopathogens and insect pests [22], thus contributing to agricultural productivity and sustainability and to the conservation and functionality of natural ecosystems [19].

Several studies have shown that soil symbionts can alter interactions between plants and other above-ground organisms, for example, attracting or repelling pollinators and herbivores [23] and changing the adequacy of these bodies [24].

Eucalyptus plants have a good association of its root system with AMF and ectomycorrhizal fungi (ECM), with a greater presence of AMF in young individuals and ECM in adults [25]. The association of AMF with plants has several benefits, like increased water and nutrients absorption and firmness of aggregates in the soil facilitating the stability of the plant [26]. The understanding of the efficacy of such symbiosis can help to maximize the production process of mycorrhizal *Eucalyptus* seedlings, providing the best advance of clones in the nursery stage and a better establishment in the field [14]. Thus, the present study aimed to evaluate the effect of the mycorrhizal association, in the development and attack of leaf-cutting ants *Atta sexdens* (Linnaeus, 1758) in clonal seedlings of *Eucalyptus urophylla x Eucalyptus camaldulensis* hybrid.

2. MATERIALS AND METHODS

2.1 Study Area

The experiment was conducted in two stages. The first one was performed in a greenhouse, with a controlled temperature of approximately 28° C, located at Universidade do Estado do Mato Grosso (UNEMAT) - Alta Floresta *Campus*. The second stage was carried out in the field and consisted in the selection of ants in different places of the city Fig. 1 and assembly of the experiment of ant attractiveness by the leaves obtained from the experiment set up in the first stage.

Both steps were carried out in the municipality of Alta Floresta, northern Mato Grosso state, Brazil Fig. 2. Alta Floresta (09º 52 '32"S 56º05'10"W) is located 830 km from the capital, Cuiabá, with a territorial extension of 8.947 km^2, with an average temperature of 29º C and estimated population at 49.233 inhabitants [27].

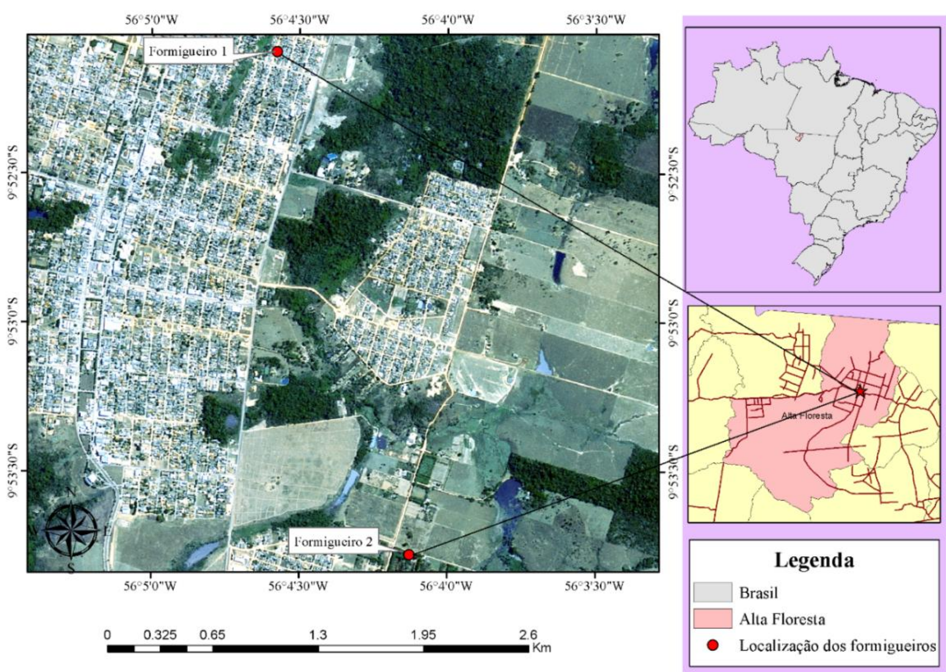

Fig. 1. Location map of the ants where the tests of attractiveness of leaf-cutting ants were conducted

Source: the author (2020)

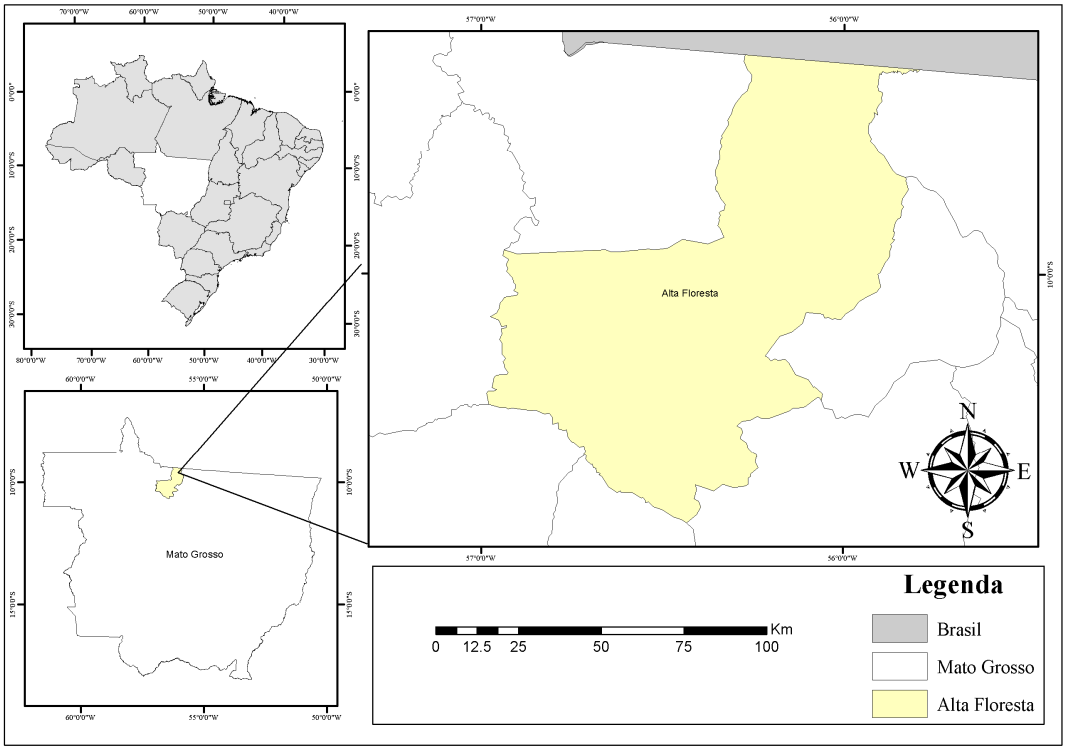

Fig. 2. Geographic location of the study area, municipality of Alta Floresta-MT
Source: the author (2020)

The annual average temperature is 26º C and the average annual precipitation is 2.750 mm, concentrated basically from January to March. The climate is Aw, tropical, with dry season in the winter according to Köppen-Geiger classification and the soil is a yellow and red-yellow soil, with small percentages of Latosol [28].

2.2 Fungal Inoculum

The fungal inoculum used in the experiment during the transplanting of the seedlings was obtained after multiplication, in trap culture, of AMF spores collected from soil of different areas: native forest area (NF), a permanent preservation area recovered to three years (PPA03), a permanent preserved area degraded (PPAD), a permanent preservation area recovered to four years (PPA04) and the control, where there was no incorporation with the fungal inoculum. About 200g of each soil sample collected (NF, PPAD, PPA03 and PPA04) was used to produce trap culture. Multiplication and production of the fungal inoculum in trap culture was done according to proposed methodology [29]. The soil samples were placed in pots (2 kg) containing sterilized sand. Approximately 100 seeds of *Brachiaria decumbens* (used as a plant trap) in a total of 40 pots (4 samples of the different soils used x 10 repetitions). A completely randomized design was used.

In the initial three months of the establishment of the trap culture, the plants were irrigated daily. After that period, the plants were subjected to water stress of seven days without water, with the objective of favor the mycorrhizal sporulation of plants. After that period, the aerial part was separated from the root system and the soil (containing the spores, hyphae and roots colonized by the AMFs) was sieved, packed in plastic bags and stored at 4°C until use as a fungal inoculum. 100 g of this inoculum were incorporated into the soil during the transplanting of seedlings to polyethylene bags (13x15 cm).

2.3 Plant Material and Growing Conditions

The plant material used for the experiment was 60 seedlings of the clone VM01 of the *Eucalyptus urophylla* x *Eucalyptus camaldulensis* hybrid obtained from the Flora Sinop® nursery, located in the municipality of Sinop, in the State of Mato Grosso, approximately 320 km of Alta Floresta.

The soil used for the experiment was the Red-Yellow Latosol containing adequate P (phosphorus) availability Table 1 and was collected in a native forest area at depth of 0-20 cm. The analysis of the chemical properties of the soil used to conduct the experiment was carried out according to the methodology proposed by EMPRAPA [30]. The inoculated roots received 100 g of soil containing mycelium and AMF spores obtained from traps (as described in item 2.2) and for control there was no incorporation of the fungi. Each polyethylene bag received only one seedling and for irrigation a water depth of approximately 6 mm was daily used. After inoculation, seedlings were maintained in a greenhouse for 45 and 90 days, with 30 seedlings for each period. The treatments related to the use of mycorrhizas were arranged in a completely randomized design, composed of 5 treatments with 6 replicates each.

After 45 days in the greenhouse, the parameters evaluated were survival, height, root development by root volume (by the water displacement method), number of healthy leaves, fresh and dry matter of shoot and root (by means of weighing and drying in greenhouse) and stem diameter (with the use of digital caliper) of 30 seedlings. The same procedures, both the evaluation of the analyzed parameters, were performed with the rest of the seedlings after 90 days of transplantation.

2.4 Attractiveness Test

The experiment was carried out under field conditions and consisted primarily in the removal of 20 foliar discs of 1 cm in diameter from each plants of all replicates with the use of drillers. After 45 days in a greenhouse, leaf discs were collected from part of the plants obtained in the previous item (item 2.2) to perform the attractiveness test. The same procedure was performed with the rest of the plants after 90 days in the greenhouse.

The treatments related to the use of mycorrhizas were arranged in a completely randomized design, composed of 2 groups of 5 treatments with 6 replicates each. The factorial scheme of discs collecting for the attractiveness test was 5x5x4 (five discs, five treatments, 4 plates), resulting in 100 discs collected for each anthill.

The leaf discs were removed with a stainless steel perforator, without direct contact between the hands and the discs in order to avoid contamination, since the ants, which are sensorial insects, could refuse or prefer these discs due to some substance that could be transmitted by the touch of the hands to vegetable leaves. The total withdrawn was 100 leaf discs for each anthill of ants (*Atta sexdens*). The anthill co-ordinates were: anthill 1-9°52'05.644"S 56°04'34.758"W and anthill 2- 09º53'46.060"S 56º04'07.764"W Fig. 1.

The disks were stored in round plastic plates. Each treatment was divided into four plates, each with five leaf discs Fig. 3A, totaling 20 plates, which were randomly distributed along the ant path Fig. 3B).

The attractiveness test was performed as described by [31], however, it was used plastic plates with a diameter of approximately 7 cm. The plates were washed with neutral soap and arranged approximately 5 cm away from the active trail of each anthill. The treatments were made available to the ants in a time interval of 90 minutes or until the last sample of the treatments had been loaded. The test was performed in the period between 5:30 p.m. to 7:00 p.m., as this is one of the periods that the ants are more active. The entire conduct of the attractiveness test was monitored to prevent the leaves from being foraged by other insects.

2.5 Data Analysis

The results were submitted to analysis of variance and the averages were compared by Tukey test at the level of 5% of probability, performed with the Sisvar® statistical analysis software [32].

Table 1. Chemical properties of the soil used in the experiment

Depth	pH H_2O	P	K	Ca	Mg	Al+H	T	V
cm		mg/dm³	---------------------		cmol$_c$/dm³	--------------------		(%)
0-20	5,9	2,0	0,34	6,96	1,07	2,48	10,9	77,1

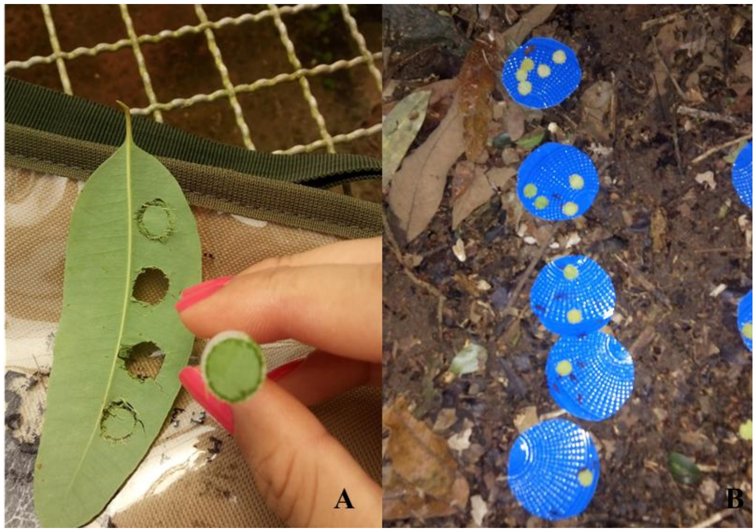

Fig. 3. (A) Removal of the *Eucalyptus* leaf discs. (B) Plastic plates containing the discs distributed along the ants' path

3. RESULTS AND DISCUSSION

3.1 Quality of Seedlings

The study aimed to evaluate whether arbuscular mycorrhizal fungi from different types of amazonian soil use (native forest area-NF, a permanent preservation area recovered to three years - PPA03, a permanent preserved area degraded-PPAD, a permanent preservation area recovered to four years-PPA04) are able to influence the development and attack of leaf-cutting ants *Atta sexdens* (Linnaeus, 1758) in clonal seedlings of *Eucalyptus urophylla x Eucalyptus camaldulensis* hybrid, but there are few studies on AMF in soils of the Amazon and on the potential use of inoculants isolated from these soils in the production of eucalyptus seedlings.

The Table 2 shows the results of the morphological parameters of the hybrid *E. urophylla* x *E. camaldulensis*, after inoculation with the AMF. There were no significant differences between the four types of mycorrhizae from Amazonian soils for the variables of height, stem diameter, number of leaves, root volume, fresh and dry weight of shoot and root. There was a statistical difference between the treatments only for the variables fresh weight and dry weight of the roots when they were evaluated after 90 days of plant inoculation. It is noted that despite the statistical difference observed through the analysis of variance, all means were followed by the same letter by the Tukey test, which can be justified by the F value being very close to 0.05 and the test of means used to be one of the most sensitive and accurate. Thus, for this study, when morphological variables are observed, we cannot affirm that mycorrhizas are capable of providing the highest growth of the clonal seedlings of the hybrid of *Eucalyptus urophylla x Eucalyptus camaldulensis* considering the inoculation time of 45 days. When the seedlings were inoculated for 90 days until the analysis of the growth variables analyzed, there was a modest increase in root biomass production. Thus, the inoculation period of the seedlings seems to be a factor to be considered when it seeks to verify the effects of mycorrhizas on the development of the seedlings of the clone studied in this work.

This was also observed by Lima and Sousa [33], that reported no significant effect of the inoculation of AMF with *Eucalyptus grandis* x *Eucalyptus urophylla* clones. In the study of [34], related to *Eucalyptus grandis* seedlings inoculated with AMF on Alic Red-Yellow Latosol, it was shown that there was also no difference between inoculated and non-inoculated seedlings.

The study of Lima and Sousa [33], pointed out that the association of the clone of the *Eucalyptus* spp. species (clone 2361) with the arbuscular mycorrhizal fungus (*Entrophospora infrequens*), resulted in a significant increase of 107.3% and 120.6% in the production of dry biomass of shoot and root, respectively.

Table 2. Stem diameter, height, number of healthy leaves, shoot fresh matter, root fresh matter, root volume, shoot dry matter and root dry matter of seedlings of the hybrid *Europhylla* x *Ecamaldulensis*, at 45 and 90 d.a.i

	Treatment**	Stem Diameter (mm)	F	Height (cm)	F	Number of Healthy Leaves	F	Shoot Fresh Matter (g)	F	Root Fresh Matter (g)	F	Root Volume (cm³)	F	Shoot Dry Matter (g)	F	Root Dry Matter (g)	F
45 d.a.i	NF	4.58 a	0.738	55.16 a	2.369	34.83 a	1.486 ns	11.23 a	1.601	6.78 a	0.689	6.16 a	1.323	5.24 a	0.897	1.39 a	0.502
	PPA03	4.92 a	ns	55.50 a	ns	42.16 a		14.10 a	ns	6.78 a	ns	4.16 a	ns	5.86 a	ns	1.18 a	ns
	PPAD	4.62 a		53.33 a		38.83 a		12.12 a		6.20 a		4.66 a		5.00 a		1.11 a	
	PPA04	4.40 a		57.50 a		30.66 a		12.42 a		7.62 a		6.16 a		5.08 a		1.26 a	
	Control	4.61 a		46.17 a		45.83 a		13.48 a		6.86 a		4.33 a		5.66 a		1.28 a	
90 d.a.i	NF	6.18 a	1.742	61.00a	0.704	26.66 a	1.075 ns	18.32 a	2.044	16.25 a	0.046 *	7.00 a	0.234	7.47 a	1.686	3.76 a	0.050
	PPA03	4.64 a	ns	55.33 a	ns	40.33 a		17.91 a	ns	16.61 a		7.16 a	ns	8.35 a	ns	3.77 a	*
	PPAD	6.21 a		57.00 a		31.50 a		17.46 a		15.96 a		6.33 a		7.96 a		3.74 a	
	PPA04	5.44 a		56.66 a		40.33 a		18.06 a		15.90 a		6.50 a		7.60 a		3.58 a	
	Control	5.32 a		59.33 a		31.33 a		21.27 a		16.31 a		7.16 a		9.19 a		3.70 a	

*Averages followed by the same letter in the column do not differ statistically from each other by the Tukey test (P = .05) for each period evaluated. *: significant at 5% probability level by the Tukey test. ns: not significant. NF-native forest; PPA03-permanent preservation area recovered to 03 years; PPAD-permanent preservation area degraded; PPA04 - permanent preservation area recovered to 04 years; and control with soil of origin and without inoculum.**n=6*

The positive or negative impacts of AMF inoculation in growth of the different species depends on factors such as: forest species, type of AMF, substrate, nutrient availability and soil Ph. [35] The same authors observed that seedlings of *Lecointea amazonica* Ducke and *Sclerolobium paniculatum* Vogel inoculated with native fungi showed no significant difference between treatments for height, diameter and dry matter of shoot and root.

Analyzing each variable in relation to period of analysis, it can be verified that the AMF inoculation did not cause significant differences in the shoot dry weight between the treatments Fig. 4. According to [33], the inoculation with the fungal species *Acaulospora* sp. and *Glomus etunicatum* increased the dry biomass production of shoots of *E. grandis* clone by 56.4% and 45.9%, respectively. The inoculation with AMF increased the dry biomass production of the shoot by 63.1%. In the *Eucalyptus* clone 5204, all AMF species influenced biomass production in the shoot. Analyzing the use of mycorrhizal fungi [26], it was observed that there was no corresponding increase in shoot dry matter when compared to control (uninoculated seedlings).

It was also observed that the plants with best response to mycorrhization in relation to root dry weight were those grown in the soil of Native Forest (NF), presenting 1.39 g with 45 d.a.i. At 90 d.a.i., inoculated plants grown in NF, PPA03 and control, presented close averages of 3.70 g. In the work of [36], it was observed that the inoculation with the AMF increased shoot and root dry weight of *Eucalyptus* when compared to the control. It was analyzed the effect of AMF on the development and survival of seedlings of different plant species, mainly forest species, and it was found that there was an increment root fresh matter by 251 and 223% when compared to the control [37]. When analyzing *Eucalyptus* spp. Seedlings, AMF increased the area of root absorption of the colonized plants, thus potentializing the use of water and nutrients [38].

Regarding the stem diameter and height, it is observed that the PPA03 resulted in the largest stem diameter the first time period (4.9 mm) and the PPAD in the second time period (6.21 mm).

The use of AMF in the *Eucalyptus grandis* x *Eucalyptus urophylla* clones did not promoted a significant difference on the stem diameter of the clones [23]. Height growth was in similar for both periods. The PPA04 resulted in the highest mean of height in the first time and the NF in the second time with 57.5 and 61 cm, respectively.

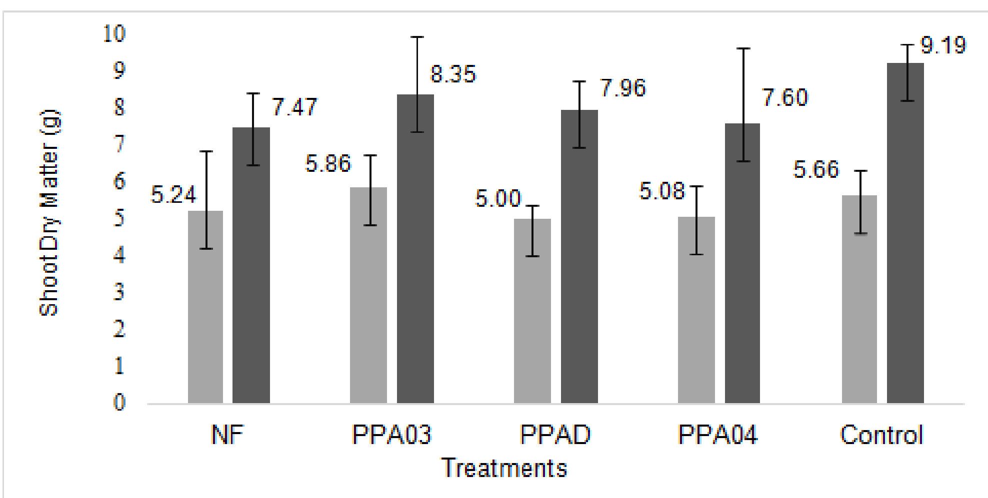

Fig. 4. Shoot dry weight (g) after 45 (■) and 90 (■) inoculation d.a.i of the seedlings with the AMF obtained from different types of soil use (-native forest, PPA03-permanent preservation area recovered to 03 years old, PPAD-permanent preservation area degraded, PPA04-permanent preservation area recovered to 04 years, and control with soil of origin and without inoculum). n=6

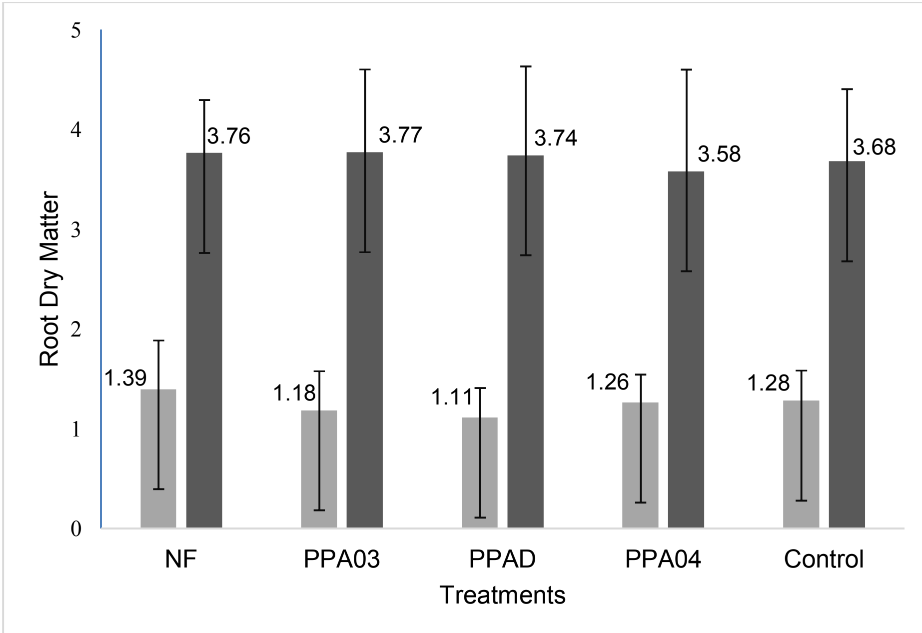

Fig. 5. Comparison of root dry matter (g) after 45 (■) and 90 (■) d.a.i of the seedlings with the AMF obtained from different types of soil use (NF-native forest, PPA03-permanent preservation area recovered to 03 years old, PPAD-permanent preservation area degraded, PPA04-permanent preservation area recovered to 04 years, and control with soil of origin and without inoculum). n=6

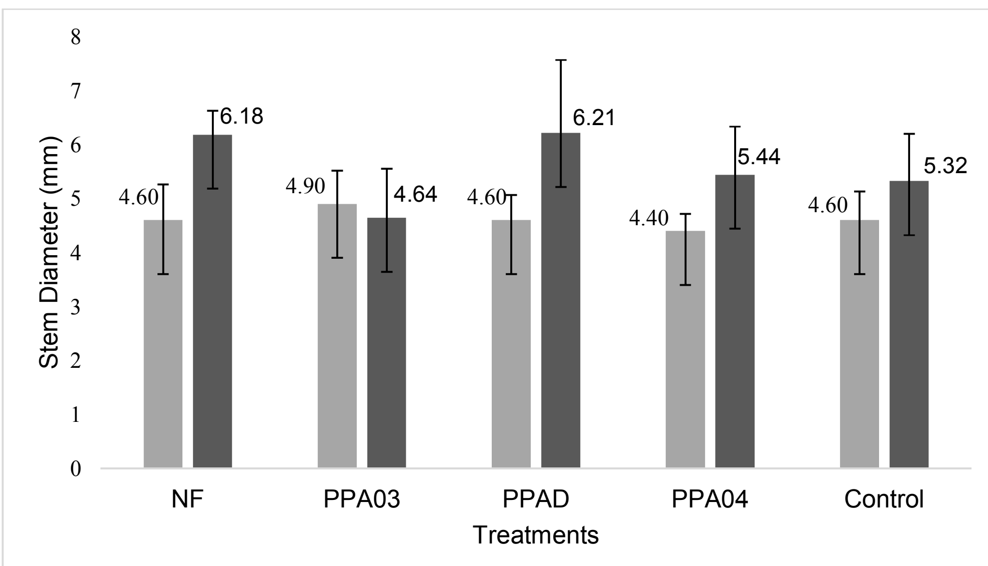

Fig. 6. Comparison of stem diameter (mm) after 45 (■) and 90 (■) d.a.i of the seedlings with the AMF obtained from different types of soil use (NF-native forest, PPA03-permanent preservation area recovered to 03 years old, PPAD-permanent preservation area degraded, PPA04-permanent preservation area recovered to 04 years, and control with soil of origin and without inoculum). n=6

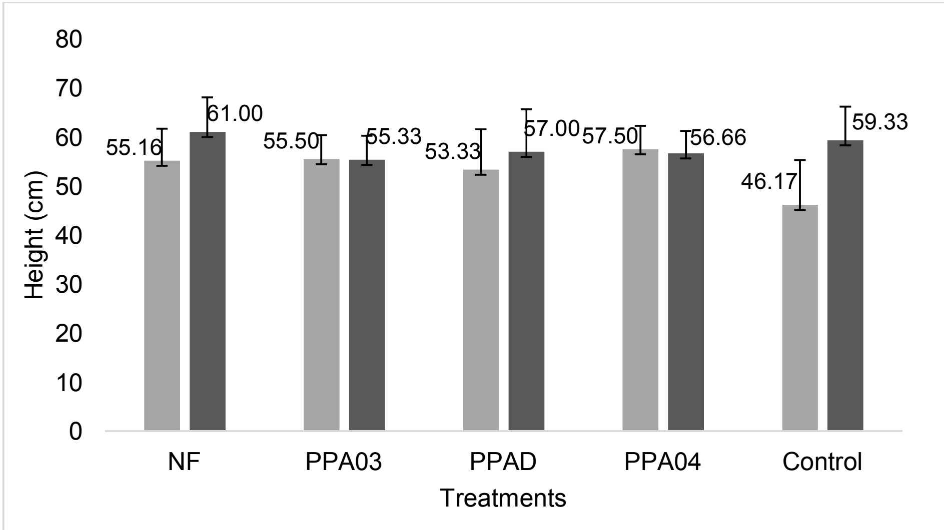

Fig. 7. Comparison of height (cm) after 45 ( ) and 90 ( ) d.a.i of the seedlings with the AMF obtained from different types of soil use (NF-native forest, PPA03-permanent preservation area recovered to 03 years old, PPAD-permanent preservation area degraded, PPA04-permanent preservation area recovered to 04 years, and control with soil of origin and without inoculum). n=6

It was observed that the *Caryocar brasiliense* A.St.-Hil. and *Malpighia emarginata* DC. plants inoculated with AMF presented higher growth and stem diameter when compared to the control [39] and the height of mycorrhizal plants, with four months old, were identical or higher than the plants without mycorrhizae at six months old. The influence of inoculation on *Eucalyptus grandis* seedlings was explained by [38], and seedlings that were cultivated in sandy soil and later inoculated with mycorrhizal fungi presented the best responses than uninoculated seedlings. Inoculated plants presented higher height, stem diameter and survival rate, thus demonstrating the improvement of resistance to adverse environments. It has been reported that inoculation of *Pinus* spp. with mycorrhizal fungi increased shoot and root dry matter, height and stem diameter [40]. Regarding *Eucalyptus* spp., the AMF increased height and stem diameter, but there was no significant difference in relation to the dry matter of the seedlings [13].

Considering the benefits that FMAs have on plants, the selection of the most efficient isolates in promoting growth and increasing plant tolerance to pest attack, such as leaf-cutting ants, can serve as a reference for future prospects in amazon soils for the use of these AMF in the production of quality seedlings of the hybrid *Eucalyptus urophylla x Eucalyptus camaldulensis*. The result benefit seedling producers of this hybrid, as well as researchers, for the adjustment of practices that benefit the establishment of new Plantations.

3.2 Attractiveness of Leaf-Cutting Ants

There was no significant difference between the averages for the ant attractiveness test in relation to the inoculated plants Table 3.

Table 3. Attractiveness test of leaf-cutting ants to the hybrid *Eucalyptus urophylla* x *Eucalyptus camaldulensis*

Treatment	Test of Averages
PPA04	16.33 a
NF	16.67 a
PPAD	17.00 a
PPA03	18.00 a
Control	18.67 a

Averages followed by the same letter in the column do not differ statistically from each other by the Tukey test (P = .05). NF-native forest; PPA03-permanent preservation area recovered to 03 years; PPAD-permanent preservation area degraded; PPA04-permanent preservation area recovered to 04 years; and control with soil of origin and without inoculum

As can be seen in Table 4, in the group of plants in which the attractiveness test was carried out

45 days after seedling inoculation, It was possible to verify that the species with the PPA04 inoculation were the least carried by the ants and the control was the most attacked. The use of AMF to stimulate the resistance of the plant to attack of leaf-cutter ants is a new study, however the control of ants by plants resistance has already been studied by several researchers. According to the variety of species studied, the reaction of the ants may be different and was suggested that the different types of compounds present in the leaves can influence this preference of attack by the leaf-cutting ants [41]. It was also reported that leaf discs of *Eucalyptus grandis* were less preferred by *Atta sexdens* ants compared to 41 other native and exotic forest species under laboratory conditions. According to [42], when they performed an evaluation on the preference of *Atta sexdens* and *Atta laevigata* (Smith, 1858) by leaf discs of *Eucalyptus* spp. species under laboratory conditions, it was possible to conclude that when the number of provenances is higher, the clarity of differences between them is better. Among the treatments, no species tested showed immunity, thus they were all taken in some way, pointing out that, under field conditions, even plants with more resistant genes can undergo some kind of damage.

Research related to plant species resistance to leaf-cutting ants is also related to the ability to produce secondary compounds, as a method of plant defense evolution. Eucalyptus plants have an efficient method to protect themselves from herbivorous attacks and despite this, even with all studies on plant resistance through non-preference, there is little information on planting that use this practice as a possible form of ants control [43].

It was observed that depending on the species, clone or eucalyptus hybrid, the age has influence on the leaf carrying preference for *Atta sexdens* [11]. In such studies the preferred specimens when the plants were two years old were the clones VM01 and the hybrid *Eucalyptus urograndis*, but at three years old they were least preferred to the by the *Atta sexdens*. A test was carried out comparing the different inoculation times of *Eucalyptus* grown in greenhouse inoculated with AMF spores collected from native Amazonian soil in different areas and their influence on the attractiveness of leaf-cutting ants. We observed that at 45 d.a.i. there was a higher preference of leaf discs from non-inoculated plants Table 4.

Table 4. Different inoculation periods of the hybrid *Eucalyptus urophylla* x *Eucalyptus camaldulensis* and their influence on the attractiveness of leaf-cutting ants

Treatments	Anthill 1 45 d.a.i	Anthill 2 90 d.a.i
PPA04	2.25 a	5.0 a
NF	2.50 ab	5.0 a
PPAD	3.0 abc	5.0 a
PPA03	3.5 bc	5.0 a
Control	4.0 c	5.0 a

Averages followed by the same letter in the column do not differ statistically from each other by the Tukey test (P = .05). NF-native forest; PPA03 - permanent preservation area recovered to 03 years; PPAD-permanent preservation area degraded; PPA04-permanent preservation area recovered to 04 years; and control with soil of origin and without inoculums

However, for the anthill 2 there was no significant variation between the treatments, observing that all the leaf discs were carried 90 d.a.i. This factor can be attributed to the stress conditions that the plants were submitted during growth in the greenhouse, which could be related to the size of the plastic bag in which the seedlings were planted, thus causing a water and nutritional stress. According to [44], in a feed preference test of *Atta sexdens* with plants submitted to water stress and plants with normal watering, a mean acceptability index can be evidenced where about 86% of the leaf area removed came from the plants with irrigation suspension. It was also noted the preference of *Atta laevigata* for wilted leaves under natural conditions [45] and the preference of *Atta colombica* (Guérin-Méneville, 1844) by plants of *Piper marginatum* Jacq. subjected to water stress, under laboratory conditions [46].

For leaf-cutting ants, the amount of water present in the collected leaves is not the most important factor, since the fungi that associate with them have their activity improved by increasing the carbon content in the wilted leaves as a result of the stressful stimulus [46]. It is known that AMF use carbon from photosynthesis of plants, that associated symbiotically with them as a source of energy and to produce their biomass [47], which further supports the results obtained in this work, that leaf-cutting ants presented less attractiveness by plants inoculated by AMF at 45 d.a.i Table 4.

There was no statistical difference between the plants inoculated with the different sources of inoculum and the control at 90 d.a.i., that can be explained by the increased nutrient demand through the growth of seedlings and the limitation caused by the size of the container in which the seedlings were, which may have led to a decrease of nutrients in the soil, as observed in other studies [48]. Mycorrhizal symbiosis favors the development of plants and the absorption of nitrogen (N), phosphorous (P), and potassium (K) in *Eucalyptus* seedlings [33], but when the plants are cultivated for relatively long periods of time, it may be observed an exhaustion of the nutrient sources available in the substrate and this relationship may shift from symbiotic to parasitic [49,47].

The nutritional reduction factor of the studied plants may have influenced the last two tests of resistance of plants to the attractiveness of the *Atta sexdens*, because a stressed plant is preferable to the attack of leaf-cutting ants. Stress, water or nutritional conditions can affect the defense mechanisms of the plant, because the perennial woody species invest energy in the production of defense compounds, that are used to protect the plant against attacks of diseases and herbivores, so with the deficiency of nutrients or water the plant restricts the production of these compounds [50]. Any environmental, biotic or abiotic factors can become a stressor for the plant if its dosage is too high or too low [51]. Thus, abiotic conditions such as nutrient, water and light deficits can be considered stressing factors for plants [52]. The leaves submitted to stress are more used because they have lower efficiency in producing chemical defensive compounds [53].

4. CONCLUSION

Under the conditions of this study, the inoculation of mycorrhizal fungi with hybrid plants of *Eucalyptus urophylla* x *Eucalyptus camaldulensis*, did not influence its development. Regarding the attractiveness test of leaf-cutting ants, it can be observed that the use of mycorrhizal fungi to induce plant resistance did not promoted significant responses in relation to inoculated plants with 90 days, but with 45 days of inoculation it was noticed that the seedlings of the PPA04 had their leaf discs less carried by leaf-cutting ants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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