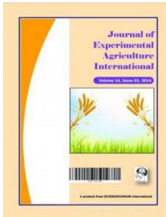

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Influence of Arbuscular Mycorrhizal Fungi and Phosphorus Doses in the Production of *Parkia nitida* (Miquel) in Seedling Nursery in the South of Amazonas

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Authors' contributions

This work was carried out in collaboration between all authors. Author HCT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MACC and MRM guided the research and assisted in writing the final manuscript. Authors LCA, PIAN and ALV assisted in literature research, field activities and laboratory tests. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Little is known about the Arbuscular mycorrhizal Fungi (AMF) inoculation effects on the initial development of *Parkia* sp. and phosphorus nutritional needs. This study aimed to evaluate the effect of the AMF inoculation in different phosphorus doses on initial *Parkia nitida* growth of.

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Study Design: The study was carried out in a completely randomized design in a 5x2 factorial scheme (5 doses of phosphorus in the absence and presence of AMF inoculation). Inoculation was done by applying 5 ml of inoculum soil containing a mix of spores (70 spores/m) of the species *Acaulospora longula, Acaulospora morrowiae, Dentiscutata heterogama* and *Paraglomus occultum*.
Place and Duration of Study: Experiment occurred at the Amazonas Federal Institute of Education, Science and Technology - Campus Lábrea (7°15'02.9"S 64°47'05.5"W) between January 2017 and December 2017.
Methodology: The variables analysed were the Survival Rate (SR), height (h), lap diameter (d), Seedling Quality Index (h/d), leaflet area (LFA), leaflet number (LFN), shoot dry mass (SDM), dry mass of the root (DMR), DMPA/DMR ratio, total dry mass (TDM) and Dickson Quality Index (DQI).
Results: The variables h and h/d were significant (*P*=.05) for the interaction between factors only for the treatment without inoculation. It was not possible to determine the appropriate phosphorus dose for the *Parkia nitida* seedlings production. However, aerial part of the seedlings positively responded to the phosphorus doses increase.
Conclusion: Inoculation did not show significant results in the experimental conditions as well as in the phosphorus interaction.

Keywords: Amazonian wood; degraded areas; dormancy breaking; Faveira-pé-de-arara.

1. INTRODUCTION

One of the greatest difficulties for the Amazonian tree species use in agroforestry systems, afforestation, restoration and recovery of degraded areas, is to obtain seedlings on commercial scale presenting good quality. *Parkia nitida*, popularly known as “Faveira-branca”, is a neotropical tree of great economic and ecological importance [1], a leguminous tree, can be considered a specie with great potential to achieve several of these goals. *Parkia nitida* (Fabaceae Mimosoideae).

The species that occurs in Brazil is frequent in the Amazon and Central Region of the country, in “terra firme” forests and high varzeas of clay soils [2]. It is a large tree that can reach up to 40 m in height, being widely distributed from southern Panama to the central region of the Amazon [1]. Due to the great economic and ecological interest, *Parkia nitida* is recommended for reforestation and recovery of degraded areas [3].

For degraded areas, leguminous tree species are prominent because they present rapid growth and, in association with nitrogen-fixing bacteria, can supply a large amount of this nutrient [4]. In regions such as the Amazon South where the difficulty in acquiring fertilisers and correctives limits regional development, having the possibility of producing vigorous seedlings and with local resources can change such reality. The classes of soils of prominence in the Amazon are classified as Latosols, Argisols, Plinthosols and Spodosols [5]. Soils are generally acidic and nutrient poor, mainly phosphorous (P). Acidity correction is performed by liming, but logistical difficulties and cost make this soil management practically unfeasible in the region. Phosphorus is an integral component of important plant cell compounds, including phosphate-intermediate sugars from respiration and photosynthesis, as well as the phospholipids that make up the cell membrane and nucleic acids [6]. Phosphorus is a critical element for food production systems, manufacturing industries and general economic growth, generating long-term supply insecurity concerns for regional and national economies [7].

Arbuscular mycorrhizal fungi (AMF) belong to Glomeromycota phylum and are associated with the majority of vascular plants [4]. According to Ribeiro et al. [8] AMF are ecologically significant because they contribute to relationships in and on the roots of the plant in a symbiotic association. When associated with plants roots, they increase their exploitation area, influencing their capacity to absorb water and nutrients, especially those with low mobility such as phosphorus [9], promoting greater resistance to biotic and abiotic stress, therefore, the plant provides AMF with soluble carbon sources and AMD provides the plant with an increased capacity to absorb more water and nutrients from the soil. Therefore, according to Kapulnik et al. [10] AMF increases plant resistance to stress, enhancing productivity and sustainability of crops, especially under prolonged stress conditions, like drought.

The quality of seedlings produced is a major factor for the success of forest stands [11]. The benefits of symbiosis for seedlings depend on the specific combination between fungal isolate and cultivated plant [12]. It is widely accepted that seedlings mycorrhization favors its nursery initial growth and its field adaptation and establishment [13], especially the perennial, like the fruit trees [14]. Mycorrhizal inoculation is generally more efficient than the use of high fertility substrate to promote plant growth and reduce stress after transplant and may also act as biological control.

As a result, the use of mycorrhizal fungi is an alternative for the optimisation of fruit tree seedlings, since it allows seedling formation time reduction, increasing nursery productivity, rotation in the occupation of infrastructure and the efficiency of specialised staff [15]. However, little is known about *Parkia nitida* nutritional requirements and ecological relationships, such as their ability to form symbioses with fungi from Glomeromycota phylum [13].

In the Amazonian environment P has low availability, since both the forest and the soil are drains. The tropical forest has 54.5 kg ha^{-1} of P immobilised in its biomass, with cycling values of the order of 17 kg ha^{-1} [16]; this balance is also derived from interactions and biological activities and these are essential for the maintenance of the climactic situation of the vegetation [17]. One of the biological factors directly linked to this success are the Arbuscular Mycorrhizal Fungi (AMF). This is in line with the research by Ribeiro et al. [8] where AMF application increased leaf and maize ear ratio generally and a decrease in stem ratio. Therefore, strategies aimed at maximising fertilisation are recommended with the use of arbuscular mycorrhizal fungi. Typically, higher concentrations of P in soil inhibit fungal colonisation in the roots and lower concentrations, instead, favour colonisation according to Smith et al. [18]. Thus, the highest values of colonisation by fungi are found in the roots submitted to soils of low concentration of P in the applied nutrient solution, in comparison to the treatment with high level of P.

Considering the logistical difficulties of southern Amazonas, the study sought to present a new, efficient and inexpensive way of producing seedlings in nursery, specifically *Parkia nitida*, which represents, besides a cheap solution, also has great potential for the recovery of degraded areas. Therefore, this study aimed to evaluate the effect of inoculation with Arbuscular Mycorrhizal Fungi in different doses of phosphorus on the initial growth of *Parkia nitida* in southern Amazonas.

2. MATERIALS AND METHODS

The study was conducted in southern Amazonas, Lábrea municipality, which has a total area of 6,000 ha [19]. Experimental conduction occurred at the Amazonas Federal Institute of Education, Science and Technology - Campus Lábrea (7 ° 15'02.9 "S 64 ° 47'05.5" W).

Seedlings substrate was obtained through the mixture of soil, sand and semi-composite sawdust in the ratio of 5:3:2 (v/v) trait adapted from Santos et al. [20], previously sieved with a 4 mm mesh to homogenise. Sand originated from the Purus River and the sawdust was obtained from a local logging.

The soil used was classified according to the Brazilian Soil Classification System [21] as Flavic Type 2 Neosol (average texture = 35% clay, 50% silt and 15% Sand) with the following analytical results: pH 4.2; K: 17.10 $mg.dm^{-3}$; P: 1.07 $mg.dm^{-3}$; Ca: 0.25 $cmol.dm^{-3}$; Mg: 0.09 $cmol.dm^{-3}$; Al: 3.20 $cmol.dm^{-3}$; H+Al: 18.73 $cmol.dm^{-3}$; SB: 0.38 $cmolc.dm^{-3}$; t: 3.58 $cmolc.dm^{-3}$; T: 19.11 $cmolc.dm^{-3}$; V: 2.01 %, m: 89.39 %; O.M: 2.58 $dag.kg^{-1}$; P-rem: 8.31 $mg.L^{-1}$; Zn: 0.10 $mg.dm^{-3}$; Fe: 227.22 $mg.dm^{-3}$; Mn: 0.91 $mg.dm^{-3}$; Cu: 0.23 $mg.dm^{-3}$; B: 0.12 $mg.dm^{-3}$; S: 224 $mg.dm^{-3}$ and the extractor was Mehlich 1 (P – Na – K – Fe – Zn – Mn – Cu).

The correction was performed considering raising saturation to 50% using dolomitic limestone with relative total neutralisation of 92.54% following literature recommendations [8]. After this procedure, substrate was homogenised. In the fertilisation, granulated super-phosphate (P_2O_5) was used in 5 concentrations as follows: 0, 20, 40, 80 and 160 mg of P_2O_5 L^{-1} substrate, according to literature [22].

Arbuscular mycorrhizal fungi spores samples were obtained in soils collected in a Brazil-nut tree *Bertholletia excelsa* dominated forest fragment, located at the Transamazônia Highway (7 ° 28'15.3 "S 64 ° 39'26.3" W). Arbuscular mycorrhizal fungi spores were extracted through wet sieving method [23]. Spores were conditioned in sterilised soil in 3 kg culture pots, aiming to its multiplication for an 8 month period [24].

Seed dormancy breaking was adapted from Brito et al. [25]. Mechanical scarification was performed using abrasive rotary grinding wheel coupled to a bench-mounted hand drill with a transverse insertion in the seed. This procedure results in a small removal of part of the tegument laterally related to the thread until reaching the primary endosperm. Subsequently, they were soaked in water for 24 hours for the activation of germination enzymes [26,27].

The sowing occurred in two 200 and three 128 cell trays, filled with sand less than four millimeters in size and covered with black shading screen with 50% luminosity at a height of 10 cm in relation to the tray surface. Irrigation took place over 10 days at intervals of 10 hours each day, after which the seedlings were immersed in water to facilitate the trays removal, avoiding roots damage. These were selected and pruned, leaving only five centimeters in roots length.

Germination of *Parkia nitida* is epigeal type and phanerocotiledonar [28]. The substrate used for this stage was adequate, as observed for arboreal legume [29], the shading maintained adequate moisture and solar incidence. A total of 494 seedlings were obtained, representing 63% of the seeds used. There was no significant difference between the trays types. Germination rate for 10 days was close to values obtained by the regression model observed by Cruz et al. [28] using a similar method of scarification for dormancy breaking. Seedlings present desirable phenotypic characteristics. Propagation of nursery seedlings exceeds the direct germination rates previously observed [30] even for high rates.

A total of 300 plastic bags (18x25 cm) were prepared with 1 L of substrate for seedlings. Sixty seedlings plastic bags were fertilised for each dose of P_2O_5, half of which was inoculated with Arbuscular Mycorrhizal Fungi and the other half remained inoculum-free.

Transplanting was done to 6 cm depth pits and the roots of the seedlings were pruned to 5 cm for standardisation. Inoculation was carried out in the pit, trait adapted from the research by Meir et al. [31] through the application of 5 ml inoculum soil (colonised roots, pieces of hyphae, spores and soil), containing a mixture of *Acaulospora longula, Acaulospora morrowiae, Dentiscutata heterogama e Paraglomus occultum* spores (70 spores.ml^{-1}).

The substrate used to produce the seedlings showed to be permeable and slightly hard. The permeability was due to the introduction of the sand trace and the hardness was related to the clay type of the soil used. Normally, the substrates used in the production of seedlings (coffee and fruit) have 30% of manure as an increase to organic matter. Proportion adaptation [32] of 20% of organic matter was used, in this case from sawdust that did not undergo chemical and/or physical characterisation, which makes inferences about the degree of natural degradation impossible and it can acidify the substrate besides having tannins that can affect the symbiosis. However, the production of seedlings in a conventional way largely uses sawdust and, in the Amazon region, closer to the cities, it is a co-product with great availability, which does not occur with animal manures.

The effect of AMF in interaction with the dose of P_2O_5 was evaluated by the inoculation of half of the seedlings, while the other half was not inoculated. In order to evaluate the different concentrations of phosphorus in the mycorrhizal process, the work was carried out based on previously experiment by Alves et al. [11], with 5 treatments in relation to the P, as follows: T1 – control, without addition of P_2O_5; T2 - addition of 20 mg.L^{-1} P_2O_5 content; T3 - addition of 40 mg.L^{-1} P_2O_5 content; T4 - addition of 80 mg.L^{-1} P_2O_5 content, and T5 - addition of 160 mg.L^{-1} P_2O_5 content.

Experimental design was done in randomized blocks, with factorial scheme 5x2, in the presence and absence of AMF, with five variations in the phosphorus dose with 30. Each plot was constituted by one seedling with thirty repetitions.

Data were collected by evaluating the height (h), leaf area (LA), shoot dry mass (SDM), dry mass of the root (DMR) and total dry mass (TDM) [33].

At the end of the experiment, aerial part and the root system were separated to determine the dry matter biomass (n=60). Samples were packed in paper bags and then dried at 70ºC/48h (forced air circulation drying oven). To obtain the SDM and DMR, samples were taken from the oven, placed in desiccator to reach room temperature and then weighed in an analytical balance (0.001 g accuracy). TDM seedling was obtained by the sum of the root and aerial part dry matter weights.

The following variables were observed to analyse the seedlings development: i) length of the aerial part or height (h): graduated ruler, being taken as standard the terminal bud - apical meristem; ii) Collar diameter (d): Measurement above 1 cm of the node formed just above the soil surface of the vessel, with the aid of a digital caliper; iii) relation of height and diameter of the collar (h/d): ratio between the height of the seedling and its diameter of the collar allowed to obtain a quality index of seedlings; iv) shoot dry mass part and dry mass of the root ratio (SDM/DMR): quality index of seedlings; v) Survival rate (SR): survival percentage; vi) Dickson quality index (IQD) [34]: determined as a function of aerial part (h), collar diameter (d), shoot dry mass (SDM) and dry mass of the root (DMR).

Data from the last collection were used (153 days after transplanting). Observations of the last collection were submitted to the individual and joint analysis of variance (factorial 5x2), in which the effects of systems were evaluated by the F-test and the effects of doses by the Tukey's test. At the same time, the significance of the model ($P<0.05$) and the coefficient of determination (R^2). Data was submitted to statistical tests with the aid of Sisvar software.

3. RESULTS AND DISCUSSION

In the production of Parkia seedlings commercially, soil sterilisation was not performed, which facilitates root colonisation by AMF, which may interfere with the inoculation results.

P doses had a significant effect in the absence of AMF (Fig. 1), the peak dose was 98.67 mg L^{-1} of P, and this enabled a larger increase in h.

The curve behavior observed (Fig. 2) resembles the one described previously by Shimizu et al. [27] in the treatment without fungus that involved phosphate fertilisation, by the curvilinear behavior, the elevation of the dose of phosphorus above the maximum point does not provide significant increases for the variables h, which meets the maximum law [35].

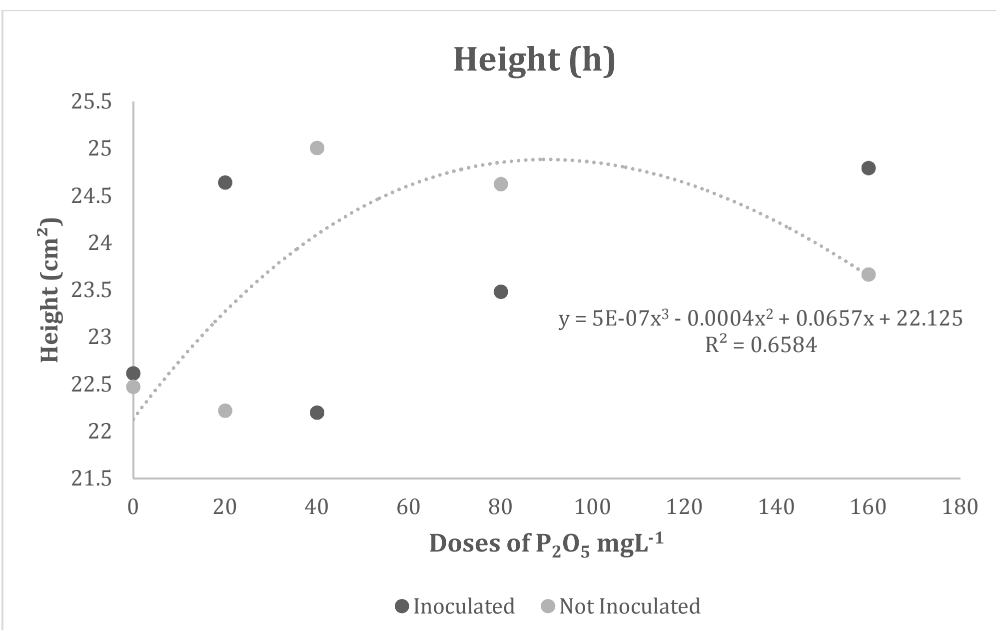

Fig. 1. Height (h) of *Parkia nitida* seedlings as a function of arbuscular mycorrhizal fungi (FMA) and phosphate fertilisation, 153 days after transplanting

At 153 days after transplanting, the mean height of the seedlings was 23.6 cm and the mean collar diameter was 6.5 mm, the height for forest species should be superior to 50 cm [36]. Observing this data, it is possible that the maintenance of the seedlings for a longer period in the greenhouse allows positive increases in its quality.

The seedling quality index that relates height per diameter showed an average of 3.6 (Fig. 2), showing that there was a higher growth rate in height in relation to the diameter.

In order to take the seedlings to field planting, a balanced development is necessary, as observed in Campos and Uchida [37]. This index only underwent significant interaction of the following sources of variation: doses of phosphorus in the absence of fungus.

The variables h and h/d presented significance for the interaction of factors (dose of phosphorus per fungus). However, this occurred only for treatments in the absence of AMF, evidencing that further studies with other fungal species are necessary to determine the ability or not of mycorrhizae of the *Parkia nitida* plant and its effects on the development of the species.

The leaflet area (LA) was significantly higher at higher P rates. A linear increase with a coefficient of determination of 89.07% was observed (Fig. 3). This behavior of increment in the leaflet area by phosphorus dose is in line with Rodrigues et al. [38], since phosphorus is linked to the photosynthetic capacity of the plants, so it may be positively influencing.

Another verified index was SDM/DMR, which averaged 3.16, higher than the 2.0, that is established as reference to this index consistent with the finding of research by Gomes et al. [39], however, studies directed to *Parkia nitida* are still necessary to determine the specific value. It is possible that the roots do not have adequate photoassimilated resources for their development.

The development of the leaflets in the area by increment in the dose of P reflected in the SDM (Fig. 4). The LA is closely related to SDM and TDM, since the gain was in both area and mass.

The total dry mass (TDM) (Fig. 5), being a composition of variables, was influenced by the dry mass of the root (DMR). The TDM had a lower coefficient of determination than that of the SDM showing this interference. On average, the DMR was 1.57 g, corresponding to less than half of the SDM. It is verified in the literature that the DMR is directly linked to the availability of P. In the experimental conditions, the result of this study indicated that the plant priories the development of the aerial part and it is possible that higher doses of phosphorus improve the indexes of quality like the DQI, which presented average of 0.96.

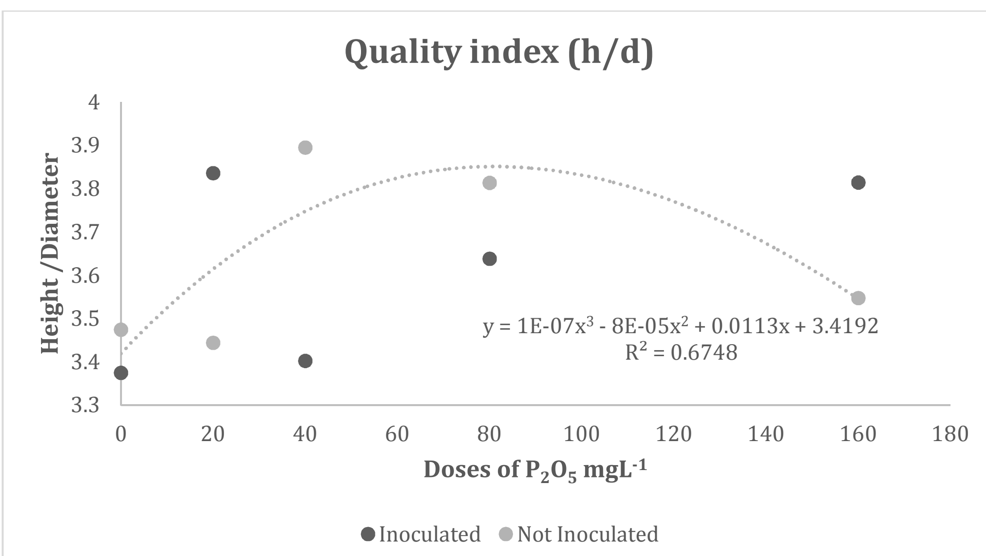

Fig. 2. Quality index of height per diameter (h/d) of *Parkia nitida* seedlings as a function of arbuscular mycorrhizal fungi (AMF) and phosphate fertilisation, 153 days after transplanting

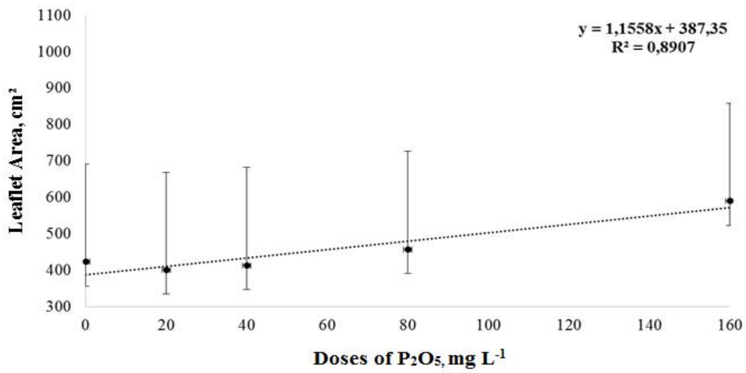

Fig. 3. Influence of phosphate fertilisation on the total leaflet area of *Parkia nitida* seedlings at 153 days after transplanting

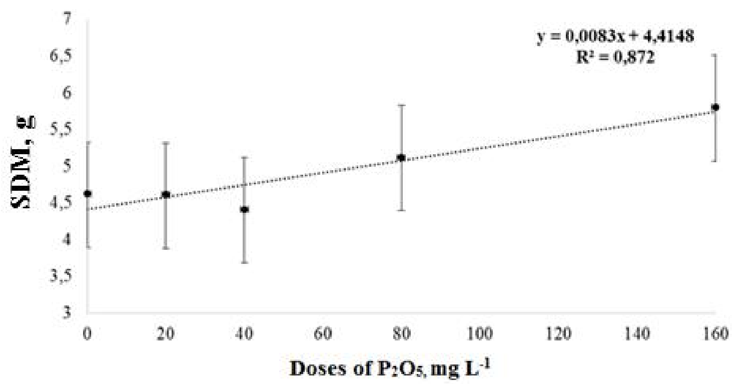

Fig. 4. Influence of the phosphate fertilisation on the shoot dry mass part of the *Parkia nitida* seedlings at 153 days after transplanting

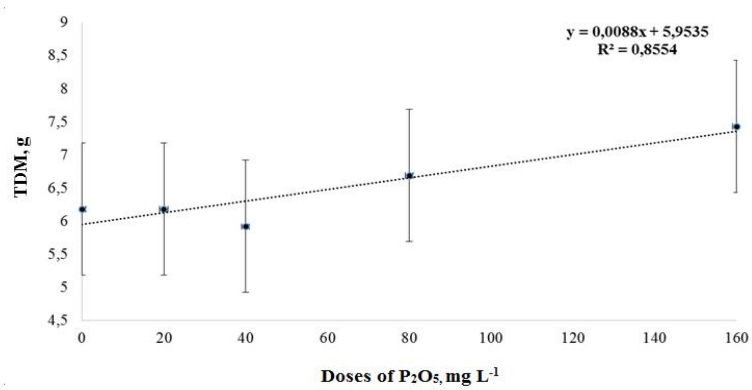

Fig. 5. Influence of phosphate fertilisation on total dry mass of *Parkia nitida* seedlings at 153 days after transplanting

The behaviour of *Parkia nitida* related to doses of P is increasing and linear for the variables LA, SDM and TDM, which evidences the necessity of application of P for better results in the seedlings production.

Parkia nitida presents desirable characteristics for propagation in nursery, with emphasis on its physiological resistance. The seedling survival rate (SR) was 100%, demonstrating that root pruning at transplant did not interfere with survival [40].

The interactions in the treatments that obtained significance were those that did not undergo inoculation by inoculum soil. According to the research by Kapulnik et al. [10], the ability to respond noticeably to AMF inoculation depends not only on plant genotype but also on the identity of the AMF inoculated. Seedlings used in this work come from seeds and, therefore, may have varied genotypes. It is possible that the genetic variability of *Parkia nitida* seeds is high. This is a desirable feature for species destined for floristic recovery and recomposition. For degraded areas, the lower the genotype/ environment interaction, the greater the possibility of success.

For commercial production of “Faveira-branca” seedlings it is recommended the phosphate fertilisation for better development of the plant.

AMF form a symbiotic mutualistic association with the roots of most tropical plants and extend the area of exploitation in the soil favoring the absorption of water and nutrients, mainly phosphorus. Although this association is well studied, little is known about the effects of FMA inoculation on the initial development of Parkia genus plants and mainly on their nutritional need for phosphorus. In regions such as the South of the Amazon where the difficulty in acquiring fertilisers and correctives limits regional development, having the possibility of producing vigorous seedlings and with local resources can change this reality. Therefore, this manuscript hopes to contribute to new research that will help reduce these limitations and also, contribute to studies related to the use of FMA specifically in the development of *Parkia nitida*.

4. CONCLUSION

The species *Parkia nitida* (Miquel) presented a small effect of inoculation with arbuscular mycorrhizal fungi on initial development in the nursery.

The seedlings of *Parkia nitida* showed linear growth, in the biomass and in the leaflet area, with increasing doses of phosphorus, reaching peak at 98.67 mg L^{-1} of P.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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