

Original Research Article

Arbuscular mycorrhizal fungi and phosphorus doses in the production of *Parkia nitida* (Miquel) in nursery seedlings in the south of Amazonas

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. Experiment occurred at the Amazonas Federal Institute of Education, Science and Technology - Campus Lábrea (7°15'02.9"S 64°47'05.5"W), being 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/mL) of the species *Acaulospora longula*, *Acaulospora morrowiae*, *Dentiscutata heterogama* and *Paraglomus occultum*. The variables analyzed were the Survival Rate (SR), height (h), lap diameter (d), Seedling Quality Index (h/d), leaflet area (LFA), leaflet number (LFN), dry mass of the aerial part (DMAP), dry mass of the root (DMR), DMPA/DMR ratio, total dry mass (TDM) and Dickson Quality Index (DQI). The variables h and h/d were significant 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. Inoculation did not show significant results in the experimental conditions as well as in the phosphorus interaction.

Keywords: *Faveira-pé-de-arara*. Degraded areas. Amazonian Wood. Dormancy breaking.

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*, a leguminous tree, can be considered a species with great potential to achieve several of these goals. *Parkia nitida* (Fabaceae Mimosoideae), popularly known as "Faveira-branca", is a neotropical tree of great economic and ecological importance [1].

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 40m in height, being widely distributed from southern Panama to the central region of the Amazon. Due to the great economic and ecological interest, *Parkia nitida* is recommended for reforestation and recovery of degraded areas [3].

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

38 Arbuscular mycorrhizal fungi belong to Glomeromycota phylum and are associated with the majority
39 of vascular plants [4]. When associated with plants roots, they increase their exploitation area,
40 influencing their capacity to absorb water and nutrients, especially those with low mobility such as
41 phosphorus [8], promoting greater resistance to biotic and abiotic stress.

42 The quality of seedlings produced is a major factor for the success of forest stands [9]. The benefits of
43 symbiosis for seedlings depend on the specific combination between fungal isolate and cultivated
44 plant [10]. It is widely accepted that seedlings mycorrhization favors its nursery initial growth and its
45 field adaptation and establishment [11], especially the perennial, like the fruit trees [12]. Mycorrhizal
46 inoculation is generally more efficient than the use of high fertility substrate to promote plant growth
47 and reduce stress after transplant and may also act as biological control.

48 As a result, the use of mycorrhizal fungi is an alternative for the optimization of fruit tree seedlings,
49 since it allows seedling formation time reduction, increasing nursery productivity, rotation in the
50 occupation of infrastructure and the efficiency of specialized staff [13]. However, little is known about
51 *Parkia nitida* nutritional requirements and ecological relationships, such as their ability to form
52 symbioses with fungi from Glomeromycota phylum [11].

53 In the Amazonian environment P has low availability, since both the forest and the soil are drains. The
54 tropical forest has 54.5 kg ha⁻¹ of P immobilized in its biomass, with cycling values of the order of 17

kg ha⁻¹ [14]; this balance is also derived from interactions and biological activities and these are essential for the maintenance of the climactic situation of the vegetation [15]. One of the biological factors directly linked to this success are the Arbuscular Mycorrhizal Fungi (AMF). Therefore, strategies aimed at maximizing fertilization are recommended with the use of arbuscular mycorrhizal fungi. Typically, higher concentrations of P in soil inhibit fungal colonization in the roots and lower concentrations, instead, favor colonization [16]. Thus, the highest values of colonization 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.

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. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

The study was conducted in southern Amazonas, Lábrea municipality, which has a total area of 6.000 ha [17]. 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 [18], previously sieved with a 4mm mesh to homogenize.

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 [19] 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: 2,24 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 neutralization of 92.54% following literature recommendations [8]. After this procedure, substrate was homogenized. In the fertilization, granulated super-phosphate (P₂O₅) was used in 5 concentrations as follows: 0, 20, 40, 80 and 160 mg of P₂O₅ L⁻¹ substrate, according to literature [20].

85 Arbuscular mycorrhizal fungi spores samples were obtained in soils collected in a Brazil-nut tree
 86 *Bertholletia excelsa* dominated forest fragment, located at the Transamazônia Highway (7 ° 28'15.3
 87 "S 64 ° 39'26.3" W). Arbuscular mycorrhizal fungi spores were extracted through wet sieving method
 88 [21]. Spores were conditioned in sterilized soil in 3 kg culture pots, aiming to its multiplication for an 8
 89 month period [22].

90 Seed dormancy breaking was adapted from literature [23]. Mechanical scarification was performed
 91 using abrasive rotary grinding wheel coupled to a bench-mounted hand drill with a transverse
 92 insertion in the seed. This procedure results in a small removal of part of the tegument laterally related
 93 to the thread until reaching the primary endosperm. Subsequently, they were soaked in water for 24
 94 hours for the activation of germination enzymes [24;25].

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

100 Germination of *Parkia nitida* is epigeal type and phanerocotyledonar [26]. The substrate used for this
 101 stage was adequate, as observed for arboreal legume [27], the shading maintained adequate
 102 moisture and solar incidence. A total of 494 seedlings were obtained, representing 63% of the seeds
 103 used. There was no significant difference between the trays types. Germination rate for 10 days was
 104 close to values obtained by the regression model [26] using a similar method of scarification for
 105 dormancy breaking. Seedlings present desirable phenotypic characteristics. However, germination
 106 time could be expanded to a better seed utilization. Propagation of nursery seedlings exceeds the
 107 direct germination rates previously observed [28] even for high rates.

108
 109 A total of 300 plastic bags (18x25 cm) were prepared with 1L of substrate for seedlings. Sixty
 110 seedlings plastic bags were fertilized for each dose of P₂O₅, half of which was inoculated with
 111 Arbuscular Mycorrhizal Fungi and the other half remained inoculum-free.

112 Transplanting was done to 6cm depth pits and the roots of the seedlings were pruned to 5cm for
 113 standardization. Inoculation was carried out in the pit through the application of 5 mL inoculum soil
 114 (colonized roots, pieces of hyphae, spores and soil), containing a mixture of *Acaulospora longula*,
 115 *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 [29] of 20% of organic matter was used, in this case from sawdust that did not undergo chemical and/or physical characterization, 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 [11], with 5 treatments in relation to the P, as follows: T1 – control, without addition of P_2O_5 ; T2 - addition of $20 \text{ mg.L}^{-1} P_2O_5$ content; T3 - addition of $40 \text{ mg.L}^{-1} P_2O_5$ content; T4 - addition of $80 \text{ mg.L}^{-1} P_2O_5$ content, and T5 - addition of $160 \text{ mg.L}^{-1} P_2O_5$ 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. Each plot was constituted by one seedling.

Data were collected by evaluating the height (h), leaf area (LA), dry mass of the aerial part (DMAP), dry mass of the root (DMR) and total dry mass (TDM) [30].

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 $70^\circ\text{C}/48\text{h}$ (forced air circulation drying oven). To obtain the DMAP 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 analyze 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) dry mass of the aerial part and dry mass of the root ratio (DMPA/DMR): quality index of seedlings; v) Survival rate (SR): survival percentage; vi) Dickson quality index (IQD) [31]: determined as a function of aerial part (h), collar diameter (d), dry mass of the aerial part (DMPA) 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 sterilization was not performed, which facilitates root colonization by AMF, which may interfere with the inoculation results.

Also, 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.

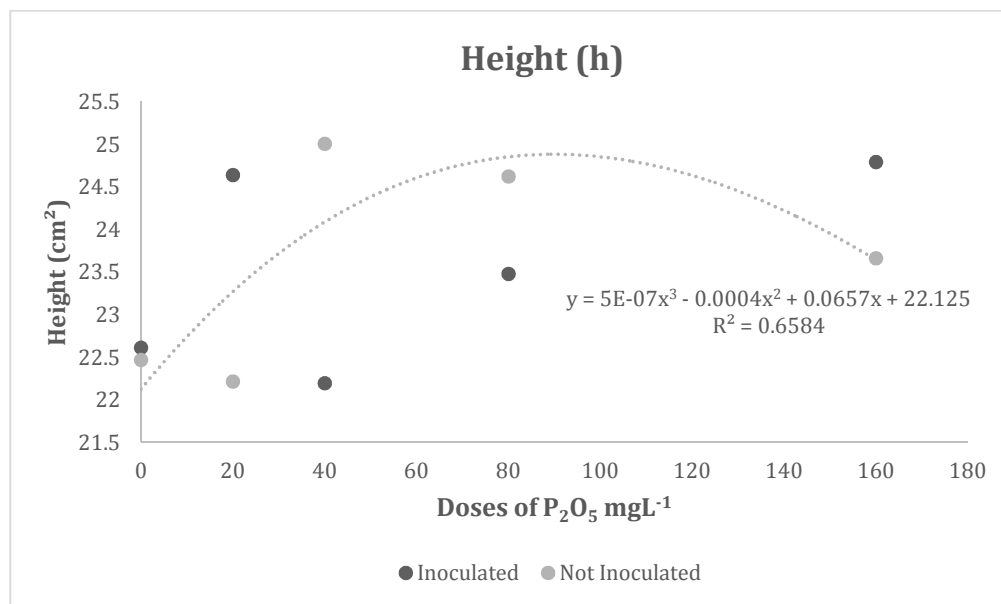


Fig. 1 Height (h) of *Parkia nitida* seedlings as a function of arbuscular mycorrhizal fungi (FMA) and phosphate fertilization, 153 days after transplanting

The curve behavior observed (Fig. 2) resembles the one described previously [25] in the treatment without fungus that involved phosphate fertilization, 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 [32].

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 [33]. 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 [34]. This index only underwent significant interaction of the following sources of variation: doses of phosphorus in the absence of fungus.

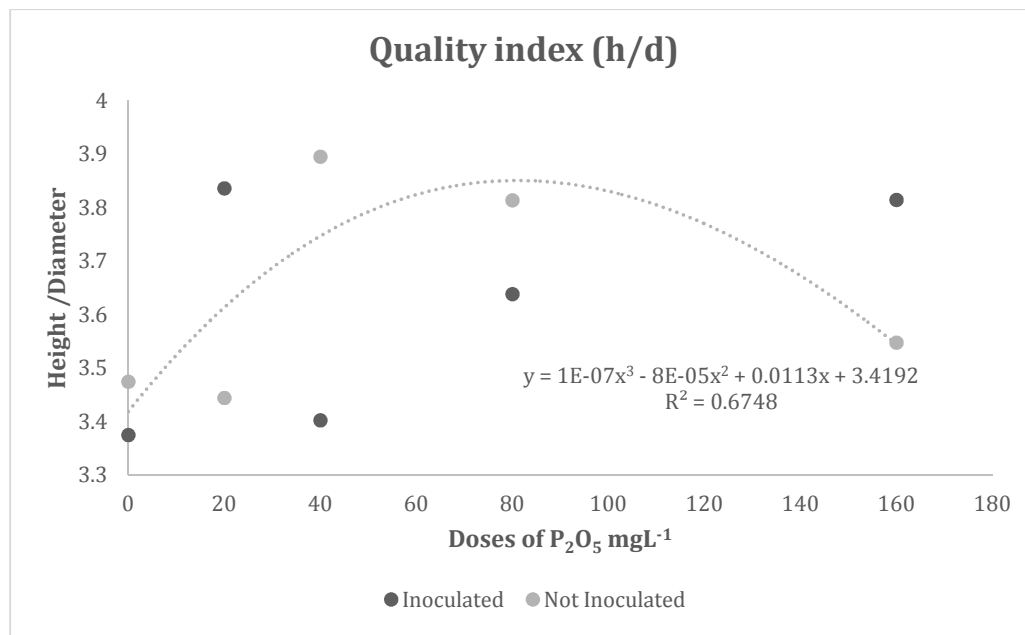


Fig. 2 Quality index of height per diameter (h/d) of *Parkia nitida* seedlings as a function of arbuscular mycorrhizal fungi (AMF) and phosphate fertilization, 153 days after transplanting

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 was also observed elsewhere [35], since phosphorus is linked to the photosynthetic capacity of the plants, so it may be positively influencing.

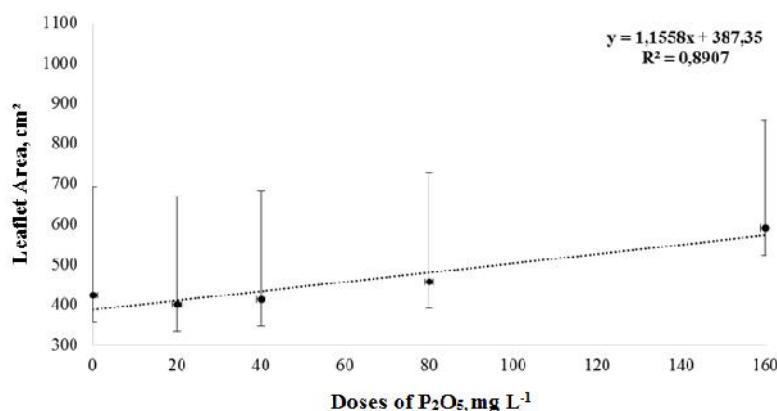


Fig. 3 Influence of phosphate fertilization on the total leaflet area of *Parkia nitida* seedlings at 153 days after transplanting.

Another verified index was DMAP/DMR, which averaged 3.16, higher than the 2.0, that is established as reference to this index [36], 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 DMAP (Fig. 4). The LA is closely related to DMAP and TDM, since the gain was in both area and mass.

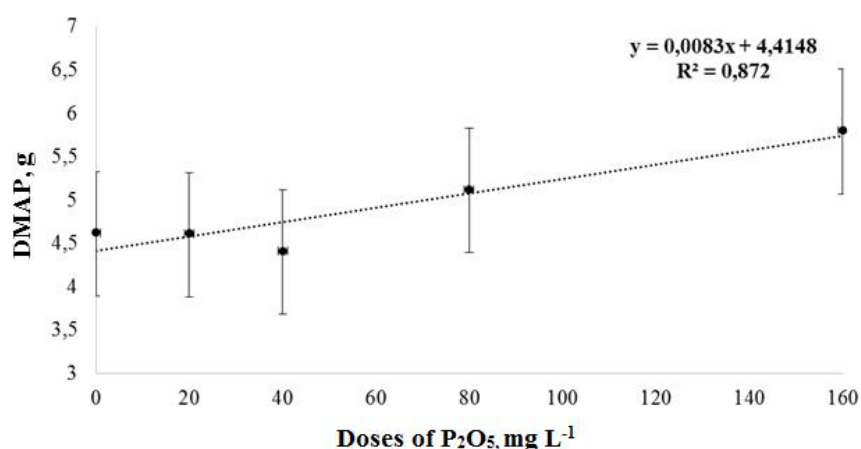


Fig. 4 Influence of the phosphate fertilization on the dry mass of the aerial part of the *Parkia nitida* seedlings at 153 days after transplanting.

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 DMAP showing this interference. On average, the DMR was 1.57 g, corresponding to less than half of the DMAP. It is

verified in the literature that the DMR is directly linked to the availability of P. In the experimental conditions, the plant priorities 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.

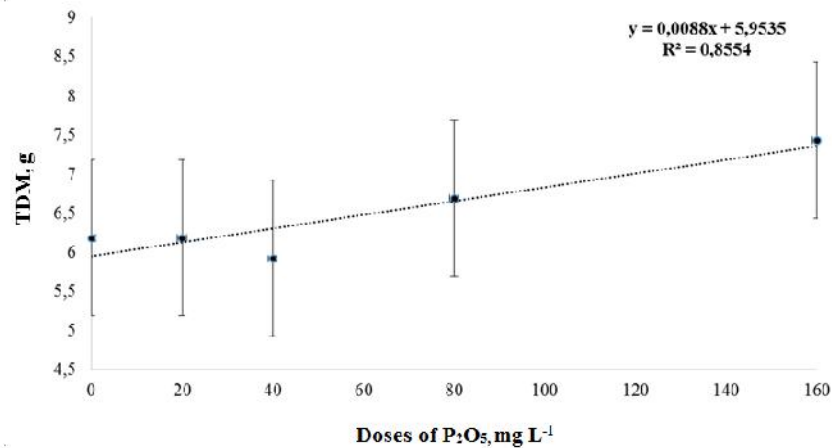


Fig. 5 Influence of phosphate fertilization on total dry mass of *Parkia nitida* seedlings at 153 days after transplanting.

The behaviour of *Parkia nitida* related to doses of P is increasing and linear for the variables LA, DMAP 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 [37].

The interactions in the treatments that obtained significance were those that did not undergo inoculation by inoculum soil. 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 fertilization for better development of the plant.

4. CONCLUSION

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

229 The seedlings of *Parkia nitida* showed linear growth, in the biomass and in the leaflet area, with
230 increasing doses of phosphorus.

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