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RESEARCH PAPER

Arbuscular mycorrhizal fungi and phosphorus affect *Araucaria angustifolia* seedling growth

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Abstract

C. Vilcatoma-Medina, M. A. Dolinski, J. W. Mendoza-Cortez, A. A. Campos de Azeredo, and F. Zanette. 2024. Arbuscular mycorrhizal fungi and phosphorus affect *Araucaria angustifolia* seedling growth. Int. J. Agric. Nat. Resour. 44-56. Symbioses between plants and arbuscular mycorrhizal fungi (AMF) provide many advantages, including a reduced need for fertilization. In this study, the effects of AMF inoculation and phosphorus application on the growth and nutritional status of *Araucaria angustifolia* seedlings were evaluated. The treatments included inoculation with AMF (*Gigaspora margarita*, *Rhizophagus intraradices*, *Acaulospora colombiana*, *Acaulospora scrobiculata*, or *Dentiscutata heterogama*), inoculation with a mixture of AMF in 100 g of 50-year-old araucaria forest soil, application of two doses of P_2O_5 (25 or 100 mg kg^{-1}), and the control. A completely randomized design was used, with seven replicates per treatment. The growth parameters, macronutrient and micro-nutrient contents, root colonization, and mycorrhizal effects on the extracted nutrients were evaluated at 75 and 150 d post germination. Analysis of variance, correlations, and principal component analysis (PCA) were used to evaluate that data. Inoculation with AMF had favorable effects on growth parameters, root colonization, and the extraction of macronutrients and micro-nutrients. In particular, the mixture of AMF from the 50-year-old araucaria forest soil, *R. intraradices*, and *A. colombiana* were beneficial. Similarly, the application of 25 mg of P_2O_5 significantly affected the growth of *A. angustifolia* seedlings compared to other treatments and increased the efficiency of root colonization.

Keywords: Fertilization, nutrient, microorganisms, Parana pine tree.

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Introduction

The tree *Araucaria angustifolia* is endemic to South America and has economic, ecological, and cultural importance (Inza et al., 2018). The species is naturally distributed in the eastern region of Paraná, and southern Brazil has the most notable genetic diversity (Souza et al., 2009; Zanette et al., 2017). Intense exploitation of this species began in the 19th century and reduced its natural range to less than 3% of its initial area (Aquino, 2005; Barbosa et al., 2017).

Although some companies initially reforested using *A. angustifolia*, the introduction of exotic conifers to Brazil led to the discontinuation of the use of this species. Low soil fertility and increased wood production justified this exchange, since *Pinus* species demonstrate great efficiency in the use of nutritional resources at low-fertility sites, despite clear symptoms of deficiency (Hillig et al., 2012; Schneider et al., 2014).

Unlike other conifers, *A. angustifolia* requires high levels of phosphorus (P), nitrogen (N), and potassium (K) (Simões & Couto, 1973), which necessitates fertilization and results in high management costs (Barbosa et al., 2017). A strategy to minimize costs is to establish associations between the root system of *A. angustifolia* seedlings and arbuscular mycorrhizal fungi (AMF), even in the seedling production stage, since this species has been shown to depend on AMF (Vilcatoma-Medina et al., 2018; Moreira-Souza & Cardoso, 2002).

Likewise, for *A. angustifolia* to be cultivated and preserved sustainably, it is necessary to adopt technologies that improve seedling production, especially concerning mineral nutrition. AMF stimulate plant growth by promoting greater absorption of water and nutrients, especially P, (Nadeem et al., 2014; Baum et al., 2015) due to its reduced mobility in the soil. Thus, evaluating the efficiency of P use by AMF, and their impact on *A. angustifolia* seedling nutrition, can provide crucial information that will enable managers to optimize growth and reduce of phosphate fertilization.

The objective of this study was to evaluate the effects of AMF inoculation and phosphate fertilization on the growth and nutritional status of *A. angustifolia* seedlings.

Materials and Methods

This work was carried out in an open field in the Agrarian Sciences Sector of the Federal University of Paraná-UFPR in Curitiba, Paraná (25° 25' 47'' S and 49° 16' 19'' W, 950 m). In August 2017, *A. angustifolia* pine nuts were collected from the same location and sown in 10 L plastic bags in a 1:1 mixture of a commercial substrate (Mecplant® - produced from composted and treated pine bark waste) and washed sand. The sand had the following characteristics: pH ($CaCl_2$) of 5.1; 1.64 g dm^{-3} of organic matter; 206 mg dm^{-3} of P; and 0.74, 8.4, 6.0, and 5.0 mmolc dm^{-3} of K^+, Ca^{+2}, Mg^{+2}, and H^++Al^{+3}, respectively. The day before planting, the pine nuts were submerged in water to evaluate their hydration capacity.

After germination, when seedlings had reached an average height of 5 cm, the following treatments were performed: inoculation (an average concentration of 80 spores per 100 g of material) of five species of AMF (*Gigaspora margarita*, *Rhizophagus intraradices*, *Acaulospora colombiana*, *Acaulospora scrobiculata*, or *Dentiscutata heterogama*); inoculation of 100 g of 50-year-old araucaria forest soil collected from the Experimental Farm of the UFPR in the municipality of Pinhais (in addition to AMF, the soil had the following characteristics: pH ($CaCl_2$) of 4.8; 1.21 g dm^{-3} of organic matter; 4.5 mg dm^{-3} of P; and 0.24, 8.9, 4.7, and 10.2 mmolc dm^{-3} of K^+, Ca^{+2}, Mg^{+2}, and H^++Al^{+3}, respectively); application of two doses of P (25 or 100 mg kg^{-1} of P_2O_5) using simple superphosphate, and a control (no inoculation or P fertilization).

The treatments were randomly applied to the experimental units (plastic bags), following a completely randomized design with seven treatment repetitions. Evaluations were carried out at 75 and 150 d post germination.

The five species of AMF were propagated on *Brachiaria decumbens* in 5 L pots in the greenhouse of the Department of Phytotechnics and Plant Health at the UFPR. The substrate used was a 1:1 mixture of washed sand and medium-textured vermiculite that was previously sterilized in an autoclave at 120 °C for two periods of 1 h at 24-hour intervals.

After 75 and 150 d of growth, the following parameters were measured: height (H); basal stem diameter (D); root length; fresh (MFPA) mass and dry (MSPA) mass of the aerial part of a seedling, comprising the aciculum (narrow, awl-shaped, lanceolate leaves) and trunk; and the fresh (MFR) mass and dry (MSR) mass of the roots. Plant material was oven-dried at 60 °C to obtain dry mass.

The C and N contents of different parts of *A. angustifolia* seedlings were determined by dry combustion with a nonmetallic element analyzer (Elementar, Vario EL III) using approximately 15 mg plant material per sample. Other elements were analyzed according to methodology adapted from Martins and Reissmann (2007). Samples of approximately 0.5 g of plant material were incinerated in porcelain crucibles in a muffle furnace at 500 °C for 4 h. Next, 10 mL of 3 mol L^{-1} HCl was added to the crucibles, which remained on a heating plate for 70 min at 70 °C. After this period, the digestion solutions were filtered using filter paper with a pore diameter of 8 μm, and the extracts were collected in 50 mL volumetric flasks. Deionized water was used to measure the volumetric flasks. In the extracts, the concentrations of P, K, Ca, Mg, Fe, Cu, Mn, and Zn were determined with an inductively coupled optical plasma emission spectrometer (ICP–OES, 720-ES series) (Varian, Palo Alto, CA, USA)

To evaluate root colonization by AMF, the finest plants were kept in 10% KOH for 24 h after being immersed in a water bath at 80 °C for 1 h. For clarification, H_2O_2 was added, and then the samples were washed, stained with blue ink, and immersed in a water bath for 5 min before lactoglycerol was added. To assess mycorrhizal colonization, the roots were placed in a 1×1 cm plate and counted using a stereomicroscope, according to Giovanetti and Mosse (1980). At the end of the experiment, the mycorrhizal effect (*EM*) was calculated for each element or extracted nutrient as follows:

$$EM = \frac{(\quad)_{150} xMS150 - (\quad)_{75} xMS75}{(\quad)_{150} xMS150} x100$$

using the nutrient extraction concentration ( ), dry mass at 150 days (MS150) and dry mass at 75 days (MS75).

Differences in growth parameters, macronutrient and micronutrient contents, and root colonization were evaluated using analysis of variance (ANOVA). When there was a significant effect of treatment, mean comparisons were performed using Tukey tests ($P \leq 0.05$). Correlations and principal component analysis (PCA) were also performed. Statistical analyses were done using R software (R Core Team, 2018).

Results

Table 1 shows the growth parameters, fresh mass, and dry mass of the *A. angustifolia* seedlings 75 and 150 d post germination. At 75 d post germination, inoculation with *R. intraradices* and the lower P dose (25 P_2O_5) yielded the highest and lowest averages, respectively, compared to other treatments. At 150 d post germination (Table 1), there were significant differences among the treatments, and contrary to the initial assessment, seedlings that received the lower dose of P had the highest averages. Inoculation with AMF from the 50-year-old araucaria forest soil or *R. intraradices* produced slightly more prominent effects. In contrast, the lowest values were observed in the control group, regardless of the treatment.

Table 1. Averages of the araucaria seedling growth parameters, as a function of inoculation with different AMF species (GM: *Gigaspora margarita*; RI: *Rhizophagus intraradices*; AC: *Acaulospora colombiana*; ASC: *Acaulospora scrobiculata*; DH: *Dentiscutata heterogama*), 50-year-old araucaria forest soil (F50), phosphorus dose (25 and 100 mg kg^{-1} - 25P_2O_5 and 100P_2O_5), and control (T), evaluated at 75 and 150 days after germination.

Treatments	Height		Root length		Diameter		Needles				Trunk				Root			
							Fresh mass		Dry mass		Fresh mass		Dry mass		Fresh mass		Dry mass	
	75	150	75	150	75	150	75	150	75	150	75	150	75	150	75	150	75	150
	cm				mm		g plant^{-1}											
GM	22,00a* ± 4,32	28,57ab* ± 3,39	20,40a ± 4,75	27,50ab ± 3,24	3,94ab ± 0,53	4,77a ± 1,08	4,20ab ± 0,65	7,59de ± 1,08	1,13a ± 0,28	2,40cd ± 0,43	3,30ab ± 0,43	6,12d ± 1,08	0,68abc ± 0,11	2,25cd ± 0,43	2,40a ± 0,43	4,45b ± 1,07	0,52a ± 0,09	1,43c ± 0,43
RI	25,71a ± 4,34	30,14ab ± 4,32	20,10a ± 4,54	29,03a ± 3,24	4,07ab ± 0,48	4,71a± 1,08	4,60a ± 0,65	9,14cd ± 1,08	1,23a ± 0,28	2,88c ± 0,43	3,70a ± 0,43	5,82d ± 1,08	0,81a ± 0,11	1,79cd ± 0,43	2,40a ± 0,43	4,53b ± 1,30	0,54a ± 0,09	1,47bc ± 0,43
AC	23,29a ± 4,40	30,29ab ± 4,95	20,00a ± 4,54	27,50ab ± 3,24	3,96ab ± 0,45	4,79a ± 1,08	3,80abc ± 0,65	6,30e ± 1,07	1,00a ± 0,28	2,44cd ± 0,22	3,50ab ± 0,43	6,30cd ± 1,07	0,74a ± 0,11	2,27cd ± 0,43	2,10ab ± 0,43	5,68ab ± 1,08	0,47ab ± 0,09	2,15abc ± 0,43
ASC	20,43a ± 4,17	28,43ab ± 3,09	20,50a ± 4,54	30,00a ± 3,24	3,6ab ± 0,43	4,61a ± 1,08	3,00c ± 0,54	6,42e ± 1,08	0,79a ± 0,22	2,22cd ± 0,43	2,40c ± 0,43	5,28d ± 1,07	0,54bc ± 0,11	1,94cd ± 0,43	2,00ab ± 0,43	5,57ab ± 1,08	0,42ab ± 0,09	1,86abc ± 0,43
DH	21,00a ± 4,32	27,00b± 2,16	19,70a ± 4,75	26,00ab± 3,24	3,87ab ± 0,48	4,67a ± 1,08	3,80abc ± 0,65	6,52e ± 0,86	1,02a ± 0,26	2,22cd ± 0,43	2,70bc ± 0,43	5,13d ± 1,08	0,55bc ± 0,11	1,73d ± 0,43	2,20ab ± 0,43	4,38b ± 1,07	0,45ab ± 0,09	1,46bc ± 0,43
F50	22,43a ± 4,32	30,29ab ± 4,95	20,80a ± 4,96	26,51ab ± 3,24	3,74ab ± 0,52	5,04a ± 1,08	4,20ab ± 0,65	9,48bc ± 1,08	1,14a ± 0,30	3,02bc ± 0,43	3,30ab ± 0,65	8,03bc ± 1,14	0,72ab ± 0,11	2,55bc ± 0,43	2,60a ± 0,43	4,37b ± 1,08	0,55a ± 0,09	1,45bc ± 0,43
25 P_2O_5	19,57a ± 3,39	34,67a ± 4,32	20,10a ± 4,54	30,50a ± 4,32	3,39b ± 0,30	5,88a ± 1,08	3,30bc ± 0,65	14,08a ± 1,08	0,90a ± 0,22	4,57a ± 0,86	2,40c ± 0,43	10,03a ± 1,13	0,51c ± 0,11	3,18ab ± 0,22	1,60b ± 0,43	7,38a ± 1,07	0,35b ± 0,09	2,41a ± 0,45
100 P_2O_5	23,29a ± 4,30	30,86ab ± 4,32	18,80a ± 4,97	27,04ab± 3,24	4,22a ± 0,48	5,33a ± 1,08	4,30ab ± 0,65	11,30b ± 1,08	1,12a ± 0,26	3,87ab ± 0,65	3,20abc ± 0,54	9,52ab ± 1,12	0,63abc ± 0,11	3,33a ± 0,64	2,30ab ± 0,43	6,87a ± 1,08	0,48ab ± 0,09	2,20ab± 0,43
T	23,86a ± 4,23	27,57b ± 4,32	20,40a ± 5,18	22,00b ± 4,32	4,04ab ± 0,52	4,84a ± 1,08	4,20ab ± 0,65	4,21f ± 1,08	1,18a ± 0,28	1,69d ± 0,43	3,60a ± 0,54	5,33d ± 1,08	0,80a ± 0,11	2,01cd ± 0,43	2,30ab ± 0,43	6,50a ± 1,08	0,51a ± 0,11	1,85abc ± 0,43

* Means followed by the same letter in the column not different from each other with Tukey's test (P≤0.05).

Table 2. Macro and micronutrients present in the needles, trunk, and roots of araucaria seedlings, as a function of inoculation with different AMF species (GM: *Gigaspora margarita*; RI: *Rhizophagus intraradices*; AC: *Acaulospora colombiana*; ASC: *Acaulospora scrobiculata*; DH: *Dentiscutata heterogama*), 50-year-old araucaria forest soil (F50), phosphorus dose (25 and 100 mg kg^{-1} - 25P_2O_5 and 100P_2O_5), and control (T), evaluated at 75 and 150 days after germination.

Treatments	C (%)						N (%)					
	Needles		Trunk		Root		Needles		Trunk		Root	
	75	150	75	150	75	150	75	150	75	150	75	150
GM	43a* ± 6,48	41a ± 4,32	41a ± 6,48	43a ± 4,32	40a ± 6,48	42ab ± 4,32	1,52b ± 0,43	1,34ab ± 0,45	0,95b ± 0,32	0,76ab ± 0,1	1,02a ± 0,43	0,39ab ± 0,13
RI	44a ± 6,48	43a ± 4,32	42a ± 6,48	42a ± 4,32	39a ± 6,48	44a ± 4,32	1,51b ± 0,43	1,94a ± 0,42	0,91b ± 0,32	0,85a ± 0,1	0,82a ± 0,32	0,50ab ± 0,13
AC	43a ± 6,48	44a ± 4,32	42a ± 6,48	44a ± 4,32	42a ± 6,48	42a ± 4,32	1,54b ± 0,43	0,96b ± 0,45	0,93b ± 0,32	0,48c ± 0,2	0,82a ± 0,32	0,40ab ± 0,13
ASC	45a ± 6,48	44a ± 4,32	44a ± 6,48	43a ± 4,32	44a ± 6,48	38abc ± 4,32	1,77b ± 0,43	1,31ab ± 0,45	0,91b ± 0,32	0,53bc ± 0,1	1,12a ± 0,43	0,35b ± 0,13
DH	44a ± 6,48	42a ± 4,32	43a ± 6,48	43a ± 4,32	41a ± 6,48	33c ± 4,32	1,65b ± 0,43	1,50ab ± 0,40	0,83b ± 0,22	0,76ab ± 0,2	1,15a ± 0,43	0,46ab ± 0,13
F50	44a ± 6,48	43a ± 4,32	42a ± 6,48	43a ± 4,32	42a ± 6,48	34bc ± 4,32	1,59b ± 0,43	1,46ab ± 0,42	0,97b ± 0,32	0,87a ± 0,1	1,01a ± 0,42	0,61a ± 0,13
25 P_2O_5	44a ± 6,48	42a ± 4,32	44a ± 6,48	41a ± 4,32	43a ± 6,48	42a ± 4,32	1,70b ± 0,43	1,25ab ± 0,42	0,78b ± 0,22	0,58bc ± 0,1	0,90a ± 0,32	0,53ab ± 0,13
100 P_2O_5	45a ± 6,48	42a ± 4,32	42a ± 6,48	43a ± 4,32	41a ± 6,48	42a ± 4,32	3,20a ± 0,65	1,16b ± 0,42	1,86a ± 0,54	0,66abc ± 0,1	1,29a ± 0,43	0,46ab ± 0,13
T	44a ± 6,48	44a ± 4,32	43a ± 6,48	43a ± 4,32	43a ± 6,48	41ab ± 4,32	1,46b ± 0,32	0,85b ± 0,31	0,97b ± 0,43	0,57bc ± 0,1	0,80a ± 0,32	0,31b ± 0,13

Treatments	P (mg kg^{-1})						K (mg kg^{-1})					
	Needles		Trunk		Root		Needles		Trunk		Root	
	75	150	75	150	75	150	75	150	75	150	75	150
GM	3,07ab ± 0,65	4,15bc ± 1,08	3,12b ± 0,65	2,53bcd ± 0,86	4,63b ± 1,08	2,07a ± 0,65	18a ± 4,32	13a ± 2,16	19a ± 4,32	11abc ± 2,16	11a ± 2,16	6,14ab ± 2,16
RI	2,76ab ± 0,65	6,62a ± 1,08	2,77b ± 0,66	5,06a ± 1,08	3,57b ± 1,08	2,98a ± 0,65	16a ± 4,32	13a ± 2,16	18a ± 4,32	12ab ± 2,16	10a ± 2,16	7,29a ± 2,16
AC	3,16ab ± 0,65	2,78cd ± 1,08	2,73b ± 0,65	1,83d ± 0,52	3,61b ± 1,08	1,98a ± 0,65	18a ± 4,32	15a ± 2,16	18a ± 4,32	11abc ± 2,16	10a ± 2,16	5,72ab ± 2,16
ASC	3,34ab ± 0,65	3,91bcd± 1,08	2,55b ± 0,65	1,93d ± 0,65	5,18ab ± 1,08	0,82b ± 0,39	16a ± 4,32	14a ± 2,16	14a ± 4,32	8,18c ± 2,16	12a ± 2,16	2,94b ± 1,08
DH	3,39ab± 0,65	4,41bc ± 1,08	3,22b ± 0,65	3,71b ± 0,86	5,00ab ± 1,08	2,31a ± 0,65	16a ± 4,32	13a ± 2,16	19a ± 4,32	12ab ± 2,16	12a ± 2,16	6,52a ± 2,16
F50	2,75ab ± 0,65	3,97bcd ± 1,08	3,81b ± 0,65	3,41bc ± 0,65	4,21b ± 1,08	2,23a ± 0,65	16a ± 4,32	14a ± 2,16	19a ± 4,32	14a ± 2,16	10a ± 2,16	6,94a ± 2,16
25 P_2O_5	3,16ab ± 0,65	5,51ab± 1,08	3,34b ± 0,65	3,51bc ± 0,65	5,21ab ± 1,08	2,32a ± 0,65	16a ± 4,32	14a ± 2,16	18a ± 4,32	13ab ± 2,16	12a ± 2,16	6,64a ± 2,16
100 P_2O_5	3,79a ± 0,65	3,64cd ± 1,08	6,56a ± 1,51	3,10bcd ± 0,65	6,51a ± 1,08	2,47a ± 0,65	14a ± 4,32	14a ± 2,16	18a ± 4,32	11abc ± 2,16	12a ± 2,16	6,35ab ± 2,16
T	2,64b ± 0,65	2,30d ± 0,65	2,50b ± 0,65	2,31cd ± 0,86	3,62b ± 1,08	2,02a ± 0,65	16a ± 4,32	12a ± 2,16	17a ± 4,32	10bc ± 2,16	11a ± 2,16	6,14ab ± 2,16

Treatments	Ca (mg kg^{-1})					
	Needles		Trunk		Root	
	75	150	75	150	75	150
GM	8,72a ± 1,08	7,58b ± 2,16	4,03abc ± 0,86	2,86b± 1,08	2,38bc ± 0,54	1,96ab ± 0,65
RI	6,80bc ± 1,08	6,93b ± 2,16	3,94abc ± 0,86	3,37b± 1,08	1,91c ± 0,43	2,18a ± 0,65
AC	8,62ab ± 1,08	7,85b ± 2,16	3,71bc ± 0,86	3,64b ± 1,08	1,97c ± 0,43	1,60ab ± 0,65
ASC	5,69c ± 1,08	7,40b ± 2,16	2,94c ± 0,86	2,83b ± 1,08	2,24bc ± 0,43	0,95b ± 0,52
DH	8,88a ± 1,08	9,27b ± 2,16	3,72bc ± 0,86	3,85b ± 1,12	2,86ab ± 0,54	2,20a ± 0,65
F50	7,80ab ± 1,08	8,51b ± 2,16	3,88abc ± 0,86	3,29b ± 1,08	2,37bc ± 0,43	2,09a ± 0,65
25 P_2O_5	8,07ab ± 1,08	8,28b ± 2,16	4,74ab ± 0,86	4,08b ± 1,08	2,25bc ± 0,43	2,22a ± 0,65
100 P_2O_5	9,22a ± 1,08	15,57a ± 2,16	5,31a ± 0,86	7,68a ± 1,08	3,40a ± 0,54	2,36a ± 0,65
T	6,79bc ± 1,08	7,43b ± 2,16	3,60bc ± 0,86	2,82b ± 1,08	1,95c ± 0,43	1,64ab ± 0,65

Treatments	Mg (mg kg^{-1})						Fe (mg kg^{-1})					
GM	3,86a ± 1,08	4,70a ± 2,2	4,61a ± 1,08	2,36abc ± 0,65	2,23ab ± 0,65	1,30ab± 0,43	183b ± 64,80	180ab± 64,8	46ab ± 8,64	49ab ± 10,80	903a ± 108	854ab ± 108
RI	3,30a ± 1,08	4,46a ± 2,2	5,17a ± 1,08	2,65ab ± 0,65	1,98ab ± 0,65	1,39a ± 0,43	92c ± 34,64	132ab ± 52,0	52a ± 8,64	45ab ± 10,80	753ab ± 108	627cd ± 108
AC	3,96a ± 1,08	4,36a ± 2,2	3,97ab ± 1,08	2,07abc ± 0,65	1,89ab ± 0,65	1,17ab ± 0,43	118bc ± 43,2	192a ± 64,8	44ab ± 8,64	59a ± 10,80	351c ± 108	549cd ± 108
ASC	3,12a ± 1,08	4,38a ± 2,2	2,38b ± 0,82	1,29c ± 0,52	1,89ab± 0,65	0,63b ± 0,22	114bc ± 34,6	168ab ± 64,8	40ab ± 8,64	40b ± 10,80	609b ± 108	676bc ± 108
DH	4,18a ± 1,08	4,50a ± 2,2	5,31a ± 1,08	2,89a ± 0,65	2,72a ± 0,65	1,56a ± 0,43	530a ± 108,01	191a ± 64,8	39ab ± 8,64	49ab ± 10,80	676b ± 108	629cd ± 108
F50	3,29a ± 1,08	4,65a ± 2,2	4,10ab± 1,08	2,57ab± 0,65	1,90ab ± 0,43	1,40a ± 0,43	74c ± 34,64	173ab ± 64,8	48ab ± 8,64	47ab ± 10,80	646b ± 108	679bc ± 108
25 P_2O_5	3,51a ± 1,08	3,76a ± 1,7	4,17a ± 1,08	2,64ab ± 0,65	2,41ab± 0,65	1,34a ± 0,43	89c ± 34,64	85b ± 43,2	49ab ± 8,64	43ab ± 10,80	382c ± 108	999a ± 108
100 P_2O_5	3,05a ± 1,08	3,57a ± 1,1	4,37a ± 1,08	2,28abc ± 0,65	2,00ab ± 0,43	1,09ab ± 0,22	59c ± 27,08	116ab± 34,6	34b ± 8,64	59a ± 10,80	655b ± 108	608cd± 108
T	3,14a ± 1,08	3,50a ± 1,1	3,53ab ± 0,86	1,75bc ± 0,65	1,62b ± 0,43	1,39a ± 0,43	74c ± 34,64	94b ± 34,6	35b ± 8,64	45ab ± 10,80	394c ± 108	486d ± 108

Treatments	Cu (mg kg^{-1})						Mn (mg kg^{-1})					
GM	3,45b ± 0,65	3,17ab ± 1,08	3,53b ± 0,65	2,63ab ± 1,08	4,81bcd ± 0,86	10b ± 4,32	171abc± 64,8	192bc± 43	47bc ± 10,8	55b ± 10,8	47a ± 10,8	64bc ± 10,8
RI	3,15b ± 0,54	3,77a ± 1,08	3,24bc ± 0,65	3,25a ± 1,08	4,22d ± 0,73	9,35bc ± 4,32	99bc ± 43,2	141c ± 43	35cd ± 10,8	27d ± 8,6	39ab ± 10,8	51c ± 10,8
AC	3,33b ± 0,65	3,26ab ± 1,08	3,46b± 0,65	3,20a ± 1,08	3,90d ± 0,65	4,27cd ± 2,16	146abc ± 43,2	149c ± 43	33cd ± 10,8	43bcd ± 10,8	28b ± 8,6	48c ± 10,8
ASC	3,76ab ± 0,65	2,92ab ± 1,08	3,53b ± 0,65	2,00ab ± 0,87	5,62abc ± 0,86	3,57d ± 1,94	104bc ± 43,2	152c ± 43	24d ± 10,8	34cd ± 10,8	41ab ± 10,8	43c ± 10,8
DH	4,65a ± 0,65	3,30ab ± 1,08	5,42a ± 0,65	3,01ab ± 1,08	6,41a ± 0,86	19a ± 4,32	167abc± 64,8	189bc± 43	38bcd ± 10,8	34cd ± 10,8	49a ± 10,8	124a ± 21,6
F50	3,68ab ± 0,65	3,42ab ± 1,08	5,36a ± 0,65	3,08ab ± 1,08	6,16ab ± 0,86	17a ± 2,16	94c± 43,2	141c ± 43	34cd ± 10,8	29d ± 10,8	45ab ± 10,8	82b ± 21,6
25 P_2O_5	2,90b± 0,65	1,98b ± 0,87	2,86bc ± 0,65	2,20ab ± 0,87	4,05d ± 0,86	8,84bcd ± 2,16	188ab ± 64,8	266b ± 65	57b ± 10,8	51bc ± 10,8	39ab ± 10,8	62bc ± 21,6
100 P_2O_5	2,91b ± 0,65	2,76ab ± 1,08	2,34c ± 0,43	1,54b ± 0,61	4,42cd ± 0,65	7,00bcd ± 3,46	201a ± 64,8	464a ± 86	87a ± 15,1	83a ± 6,1	55a ± 10,8	50c ± 10,8
T	2,77b ± 0,65	1,88b ± 0,87	3,28bc± 0,65	1,94ab ± 0,77	5,62abc ± 0,86	3,99cd ± 1,08	155abc± 43,2	137c ± 43	47bc ± 10,8	43bcd ± 10,8	41ab ± 10,8	47c ± 10,8

Treatments	Zn (mg kg^{-1})					
GM	28a ± 8,64	22bcd ± 6,48	16a ± 4,32	19abc ± 6,48	18a ± 4,32	18ab ± 6,48
RI	24abc ± 6,48	27ab ± 6,48	14a ± 3,90	26a ± 6,48	16a ± 4,32	21a ± 6,48
AC	26ab ± 6,48	15cd ± 6,48	16a ± 4,32	11c ± 6,48	20a ± 4,32	9,2bc ± 3,46
ASC	19abc ± 4,32	18bcd ± 6,48	11a ± 3,46	17abc ± 6,48	17a ± 4,32	7,0c ± 3,46
DH	21abc ± 6,48	25abc ± 6,48	17a ± 4,32	24ab ± 6,48	23a ± 6,48	19a ± 6,48
F50	15c ± 4,32	35a ± 8,65	15a ± 4,32	21abc ± 6,48	22a ± 6,43	20a ± 6,48
25 P_2O_5	17bc± 4,32	20bcd ± 6,48	14a ± 3,46	18abc ± 6,48	17a ± 4,32	15abc ± 4,32
100 P_2O_5	16c ± 4,32	14d ± 4,32	13a ± 3,46	14bc ± 6,48	19a ± 4,32	15abc ± 4,32
T	18bc ± 4,32	12d ± 4,32	17a ± 4,32	11c ± 4,32	17a ± 5,20	23a ± 6,48

* Means followed by the same letter in the column not different from each other with Tukey's test (P≤0.05).

Table 2 presents the concentrations of macronutrients and micronutrients in the needles, trunks, and roots of the *A. angustifolia* seedlings at 75 and 150 d post germination. At 75 d post germination, the highest C concentration occurred in seedlings that were inoculated with *A. scrobiculata*, while the highest concentrations of N, Ca, and P, were associated with the higher P dose (100 P_2O_5). The highest K and Mg averages occurred in seedlings inoculated with *G. margarita* and *D. heterogama*, respectively. Significant differences in micronutrient concentrations among treatments were observed 75 d post inoculation; higher averages occurred in seedlings inoculated with *D. heterogama*. It is also worth mentioning that seedlings receiving the higher P dose (100 P_2O_5) had the highest Mn average.

At 150 d post germination (Table 2), the needles, trunks, and roots of the *A. angustifolia* seedlings inoculated with *R. intraradices* had the highest macronutrient averages. This treatment was followed by inoculation with *Acaulospora*, inoculation with soil from the 50-year-old araucaria forest, addition of the higher dose of P, and inoculation with *D. heterogama*, in which the *A. angustifolia* seedlings had high concentrations of C, N, Ca, and Mg, respectively. There were significant differences micronutrients between the treatments, and seedlings that were inoculated with *R. intraradices* stood out for concentrations of Cu and Zn. Moreover, inoculation with *A. colombiana* or the addition of the higher P dose of affected the concentration of Fe. A similar pattern for Mn in the aerial parts and roots of *A. angustifolia*

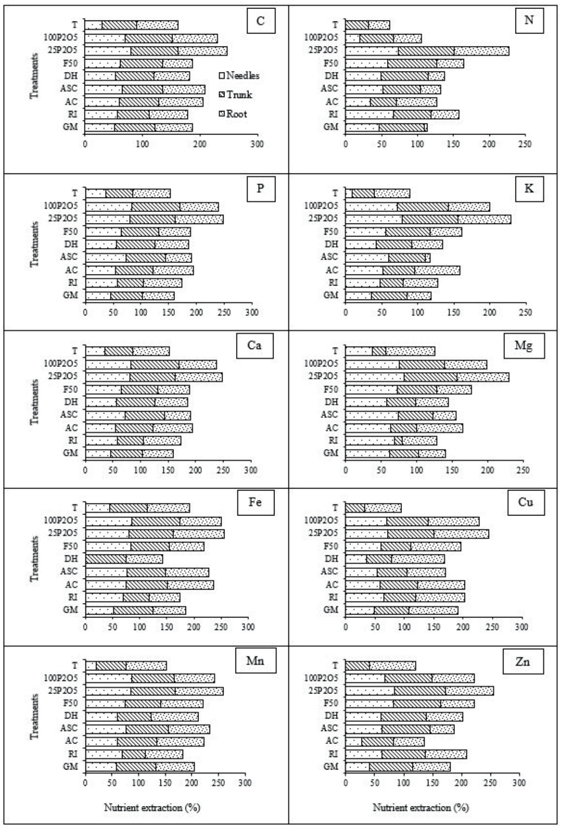

Figure 1. Extraction of macro and micronutrients in the needles, trunk, and roots of araucaria seedlings, as a function of inoculation with different AMF species (GM: *Gigaspora margarita*; RI: *Rhizophagus intraradices*; AC: *Acaulospora colombiana*; ASC: *Acaulospora scrobiculata*; DH: *Heterogama dentiscutata*), 50-year-old araucaria forest soil (F50), phosphorus dose (25 and 100 mg kg^{-1} - $25P_2O_5$ and $100P_2O_5$) and control (T), evaluated at 150 days after germination.

* Means followed by the same letter not different from each other with Tukey's test ($P \leq 0.05$).

seedlings was observed with the addition of the higher P dose or inoculation with *D. heterogama*, respectively. Figure 1 shows the final percentages of the elements extracted from each plant part, highlighting the effect of the lower dose of P in the aerial parts of seedlings, while Fe, Cu, and Mn had very high values in the roots.

Among the AMF that were inoculated, the *Acaulospora* genus stood out for the concentrations of C, K, Ca, Fe, Mn, and Mg that were extracted from the seedlings. The species *A. colombiana* was important for the first five elements, and *A. scrobiculata* was important for the last element, with higher percentages occurred in the aerial parts of seedlings. The seedlings inoculated with *R. intraradices* or 50-year-old araucaria forest soil excelled in extracting the main macronutrients, such as N and P, especially in their aerial parts, and in extracting the microelements Cu and Zn in the root and aerial parts, respectively. Additionally, it is worth mentioning that seedlings in the control treatment presented the lowest extraction percentages, regardless of the nutrient evaluated.

Figure 2 illustrates root colonization of the seedlings at the two evaluation periods. During the initial assessment, seedlings inoculated with, *R. intraradices* and *A. colombiana* exhibited the highest root colonization percentages (48%). Conversely, at 150 d, higher values were recorded for most AMF inoculations and P additions, with notable prominence of seedlings inoculated with AMF from the 50-year-old araucaria forest soil (68% root colonization).

According to the correlation matrix between the treatments and the percentage of colonization, growth parameters, and presence of macronutrients and micronutrients (Figure 3) for the two evaluation periods (Figure 3A), there were positive correlations with MFPA, MSPA, MFR, MSR, H, and D at 75 d. Additionally, N, P, K, and Ca were positively correlated with Mn, both in the aerial parts and roots. Cu was positively correlated with Fe and Zn, both in the aerial parts and roots, while root colonization was slightly positively correlated with N, Ca, and Mg in the aerial parts and roots. The positive correlation between the growth parameters at 150 d (Figure 3B) was similar to that found at 75 d. Macronutrients and micronutrients were positively correlated in the aerial parts and roots. Root colonization was positively correlated with N, Cu, and Fe in the aerial parts and roots, with P and K in the aerial parts, and with root length.

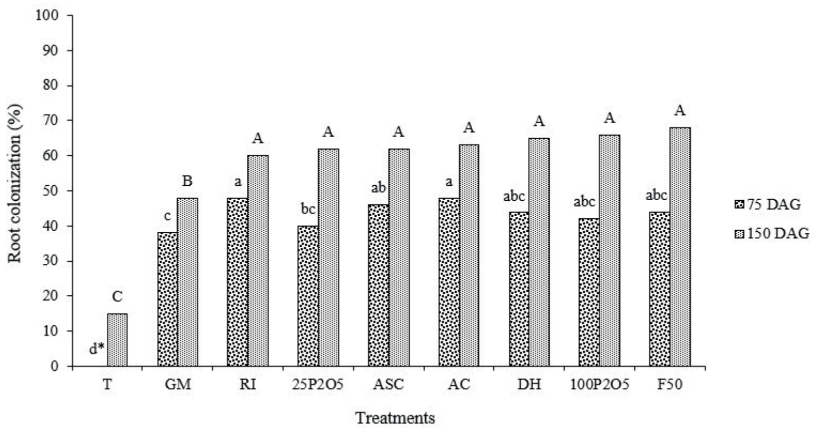

Figure 2. Root colonization of araucaria seedlings, as a function of inoculation with different AMF species (GM: *Gigaspora margarita*; RI: *Rhizophagus intraradices*; AC: *Acaulospora colombiana*; ASC: *Acaulospora scrobiculata*; DH: *Heterogama dentiscutata*), 50-year-old araucaria forest soil (F50), phosphorus dose (25 and 100 mg kg^{-1} - $25P_2O_5$ and $100P_2O_5$) and control (T), evaluated at 75 and 150 days after germination (DAG).

* Means followed by the same letter not different from each other with Tukey's test (P≤0.05).

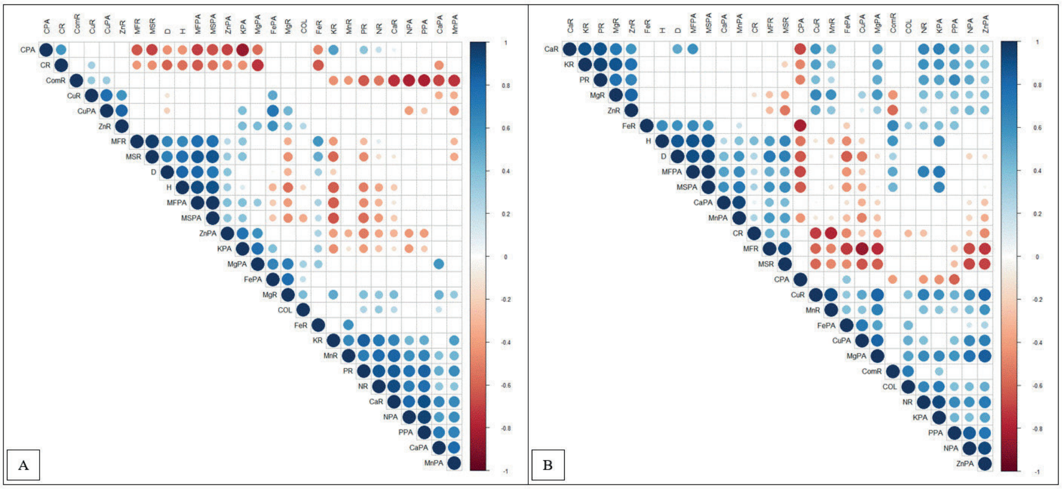

Figure 3. Correlation matrix of the averages of the treatments with the colonization percentage (COL), growth parameters (H: height; D: diameter; ComR: root length; MFPA: fresh mass of the aerial part; MSPA: dry mass of the aerial part; MFR: fresh mass of roots; MSR: dry mass of the root) and content of macronutrients (C, N, P, K, Ca, Mg and S) and micronutrients (Fe, Zn, Mn, and Cu) in the aerial part (PA) and root (R) in araucaria seedlings, as a function of inoculation with different species of AMF (GM: *Gigaspora margarita*; RI: *Rhizophagus intraradices*; AC: *Acaulospora colombiana*; ASC: *Acaulospora scrobiculata*; DH: *Heterogama dentiscutata*), 50-year-old araucaria forest soil, phosphorus dose (25 and 100 mg kg^{-1} - 25P_2O_5 and 100P_2O_5) and control, evaluated at 75 (A) and 150 (B) days after germination.

The PCA based on the order between the growth parameters and the percentage of root colonization in the AMF, 50-year-old araucaria forest soil, P application, and control treatments is presented in Figure 4, with perpendicular Axes 1 and 2. At 75 d post germination (Figure 4A), the effects of most of the treatments on the evaluated parameters were verified. Axes 1 and 2 represent 64.1 and 17.5% of the variability, respectively, which explains an accumulation of 81.6% of the total variability in the data. Growth (MFPA, MSPA, MFR, MSR, and H) was favorable in the presence of *G. margarita* and *R. intraradices*; so was root length in 50-year-old araucaria forest soil and root colonization in the *D. heterogama* and *A. colombiana* treatments. On the other hand, the growth parameters in response to *A. scrobiculata*, P application, and control treatments differed greatly.

At 150 d post germination (Figure 4B), the differences among the treatments were also confirmed, particularly among the seedlings inoculated with AMF, the seedlings receiving P application, and the control seedlings. Axes 1 and 2 account for 63.7 and 22.8% of the variability, respectively, which explains 86.5% of the total variability in the data. Growth (MFPA, MSPA, MFR, MSR, and D) was favorable in the *A. colombiana* and P treatments, especially with the application of 100 mg kg^{-1} P_2O_5. Similarly, the length of the roots and the percentage of colonization were increased in the 50-year-old araucaria forest soil treatment. It should be noted that seedlings in the control treatment were very different.

Discussion

The inoculation of AMF and the application of P benefitted *A. angustifolia* seedlings, in contrast to the control plants, in terms of growth parameters, macronutrient and micronutrient concentrations, and root colonization. Previous studies corroborate the benefits of mycorrhizal association during adulthood (Moreira-Souza et al., 2003; Zandavalli et al., 2008; Moreira et al., 2009; Moreira et al., 2016; Tyagi et al., 2017), however, few studies have addressed AMF inoculation in *A. angustifolia* seedlings (Moreira-Souza et al., 2002; Zandavalli et al., 2004; Moreira et al., 2012) in terms of producing seedlings with defined quality standards.

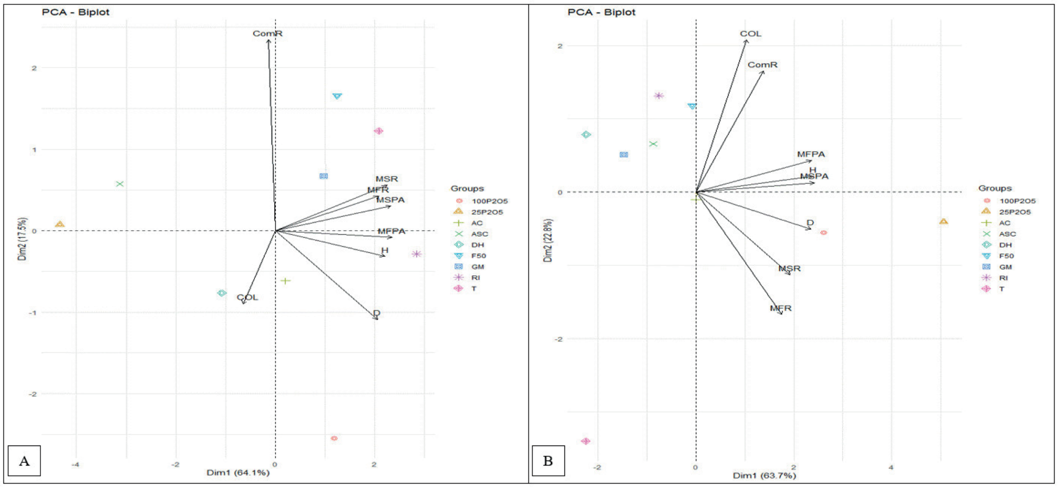

Figure 4. Principal component analysis (PCA) diagram for growth parameters (H: height; D: diameter; ComR: root length; MFPA: fresh mass of the aerial part; MSPA: dry mass of the aerial part; MFR: fresh root mass; MSR: root dry mass) and colonization percentage (COL) of araucaria seedlings, as a function of inoculation with AMF species (GM: *Gigaspora margarita*; RI: *Rhizophagus intraradices*; AC: *Acaulospora colombiana*; ASC: *Acaulospora scrobiculata*; DH: *Heterogama dentiscutata*), 50-year-old araucaria forest soil (F50), phosphorus dose (25 and 100 mg kg^{-1} - $25P_2O_5$ and $100P_2O_5$) and control (T), evaluated at 75 (A) and 150 (B) days after germination.

Previous studies have reported favorable effects of inoculation with *Glomus intraradices*, *Gigaspora rosea*, *Glomus clarum* (currently known as *Rhizophagus clarus*), and species found in 50-year-old araucaria forest soil on the growth of *A. angustifolia* seedlings. In the present study, inoculation of a mixture of AMF in the 50-year-old araucaria forest soil, *R. intraradices*, and *A. colombiana* stood out in order of importance. However, results were obtained for the other species.

At 75 d post germination, inoculation with *R. intraradices* stood out from the treatments with other AMF. A similar result occurred in *Poncirus trifoliata* and *Catharanthus roseu* plants, which exhibited considerable root colonization, height gain, and nutrient absorption (Wang et al., 2016; Monnerat et al., 2018), which confirms that *R. intraradices* promotes favorable responses in a short period of time in *A. angustifolia*.

In the genus *Acaulospora*, the species *A. colombiana* favorably influenced seedling growth; seedlings exhibited high mycorrhizal colonization and concentrations of macronutrients and micronutrients, especially in the aerial parts of the plant. A similar result occurred in seedlings of Australian cedar (Silva et al., 2017) and soursop (Gomes Júnior et al., 2018) when they were cultivated in a substrate with low P availability or in organic compost, respectively, demonstrating in the latter case that AMF respond favorably in the presence of organic matter.

Similarly, moderately favorable root colonization and nutrient absorption responses were observed in the *D. heterogama* treatment. When this species was inoculated in vine seedlings of the cultivar 'Paulsen' (in sandy soil contaminated with cupric fungicides) and in seedlings of *Diospyros kaki* (in a sandy substrate with high concentrations of P), it was very effective (Rosa et al., 2016; Machineski et al., 2018). These results confirm that *D. heterogama* adapts better to sandy soils.

Growth and root colonization were not improved following *G. margarita* inoculation. This is probably because the substrate had good organic matter availability and moderate acidity. Moreira-Souza and Cardoso (2002) showed that under low P availability, *G. margarita* shows greater mycorrhizal efficiency. Similarly, Budi et al. (2012) verified the positive effects of the inoculation of *G. margarita* in cinnamon (*Melia azedarach*) plants cultivated on a substrate with a low P content and high aluminum concentration.

The inoculation with the soil from the 50-year-old araucaria forest yielded one of the best results compared to other inoculation treatments. This soil, in addition to exerting a positive effect on the concentration of both macronutrients and micronutrients in *A. angustifolia* seedlings, also corrected the pH of the substrate. This may have improved the diversity of the AMF that were present, which was demonstrated by Moreira-Souza and Cardoso (2002) and Moreira et al. (2012). In contrast, low nutrient availability in the substrate may also affect the mixture of AMF, a finding corroborated by Camara et al. (2017) in seedlings of saguaraji (*Colubrina glandulosa*). It is worth mentioning that a high P content in the substrate can affect the behavior of AMF (Collins & Foster, 2009), yielding unfavorable outcomes, as observed in guabiroba seedlings (*Campomanesia xanthocarpa*) (Dalanhol et al., 2017) and Asian pear plantations (*Pyrus pyrifolia* var. *culta*) (Yoshimura et al., 2013).

Compared with the control treatment, inoculation with AMF promoted greater dry matter accumulation in the aerial parts of the *A. angustifolia* seedlings but not in the roots (except for inoculation with *A. colombiana*). This result corroborates the findings of Zandavalli et al. (2004), who inoculated *G. clarum* in the soil of naturally occurring *A. angustifolia* and differs from the findings of Moreira-Souza and Cardoso (2002), who inoculated *G. rosea*, *G. intraradices*, and a mixture of AMF from 50-year-old araucaria forest soil in a substrate with an acidic pH, a high organic matter content, and different P concentrations. According to Wu et al. (2012) and Wu et al. (2013), AMF improve and increase root length, volume, and number of lateral roots, as was found in the inoculated seedlings in this study.

At the end of the experiment, the concentrations of macronutrients and micronutrients in the shoots and roots of the *A. angustifolia* seedlings were generally higher in seedlings inoculated with AMF than in the control seedlings. Similarly, Moreira-Souza and Cardoso (2002) and Zandavalli et al. (2004) reported that the nutrient concentrations in leaves were similar to those in one-year-old *A. angustifolia* seedlings, as were the concentrations of these nutrients in soursop seedlings (Gomes Júnior et al., 2018), jatropha (Saboya et al., 2012), and coffee (França et al., 2016).

At 75 d post germination, the application of P had promoted favorable responses; at 150 d, the lower dose of P had promoted greater height, root length, and efficiency in the extraction of macronutrients and micronutrients, in the aerial parts and roots, which differed from the other treatments. Moreover, in the treatment with the higher dose of P, at 75 d post germination, the first root whorl formed in the seedlings. According to Savoya et al. (2012), P is the most essential nutrient in the early stages of seedling growth because it stimulates the formation of roots, and its absorption benefits from the presence of AMF.

Strong correlations occurred between colonization percentage and macronutrients in the aerial parts and roots. This is because AMF increase the presence of these nutrients in the substrate and promote plant absorption. On the other hand, when macronutrients are applied in excess, they can reduce AMF colonization and root biomass, as was found in apple grafts (Berdeni et al., 2018).

According to the PCA, at the end of the experiment, the presence of AMF and the application of P positively influenced root colonization and growth parameters, respectively. In a study by Ndoye et al. (2012) with *Acacia senegal* seedlings, the inoculation of different AMF in association with beneficial bacteria had a positive effect on these parameters and nutrient concentrations in the aerial parts of the seedlings.

Conclusions

AMF inoculation had favorable effects on growth parameters, root colonization, and the extraction of macronutrients and micronutrients. In particular, the mixture of AMF from 50-year-old araucaria forest soil, *R. intraradices*, and *A. colombiana* were beneficial. The application of 25 mg of P_2O_5 had a significant effect on the growth parameters of the *A. angustifolia* seedlings in relation to those in the other treatments and promoted efficient root colonization.

Resumen

C. Vilcatoma-Medina, M.A. Dolinski, J.W. Mendoza-Cortez, A.A. Campos de Azeredo, y F. Zanette. 2024. Hongos micorrízicos arbusculares y fósforo sobre el crecimiento de plántulas de *Araucaria angustifolia*. Int. J. Agric. Nat. Resour. 44-56. La simbiosis entre plantas y hongos micorrízicos arbusculares (FMA) tiene muchas ventajas, entre ellas la reducción de la fertilización. El presente estudio tuvo como objetivo evaluar el efecto de la inoculación de especies de FMA y la aplicación de fósforo sobre el crecimiento y estado nutricional de plántulas de *Araucaria angustifolia*. Los tratamientos que incluyeron la inoculación de especies de FMA (*Gigaspora margarita*, *Rhizophagus intraradices*, *Acaulospora colombiana*, *Acaulospora scrobiculata* y *Dentiscutata heterogama*), aplicación de 100 g de un suelo de bosque de araucaria de 50 años, dos dosis de P_2O_5 (25 y 100 mg kg^{-1}) y un testigo, se distribuyeron en un diseño completamente al azar, con siete repeticiones por cada tratamiento. Se evaluaron a los 75 y 150 días después de la germinación los parámetros de crecimiento, el contenido de macro y micronutrientes, la colonización radicular y el efecto micorrízico de los nutrientes extraídos. Los datos obtenidos fueron sometidos al análisis de varianza, correlación y componentes principales (PCA). La inoculación de especies de FMA brindaron respuestas favorables sobre los parámetros de crecimiento, colonización radicular y sobre la extracción de macro y micronutrientes, destacando la mezcla con especies de FMA del suelo proveniente del bosque de araucaria de 50 años y las especies *Rhizophagus intraradices* y *Acaulospora colombiana*. Así mismo, la aplicación de 25 mg de P_2O_5 influyo significativamente sobre los parámetros de crecimiento de las plántulas de *Araucaria angustifolia* en relación con los otros tratamientos, además de incrementar la eficiencia en la colonización radicular.

Palabras clave: Fertilización, microorganismos, nutriente, pino de Paraná.

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