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MICORRHYZAL COLONISATION ON ARAUCARIA SEEDLING WITH DIFFERENT DOSES OF NITROGEN, PHOSPHORUS AND POTASSIUM

SUMMARY

Arbuscular mycorrhizal fungi (AMF) favours the growth and development of seedlings of various tree species, with consequent reduction in the use of chemical inputs. The present study aimed to evaluate the effect of nitrogen (N), phosphorus (P) and potassium (K) on diversity and sporulation of AMF species in the root colonisation (RC) of seedlings of *Araucaria angustifolia*. The experiments were performed using different doses of N (fixed P and K), P (fixed N and K) and K (fixed N and P) (mixed in pots whit historical soil from *Araucaria angustifolia* as substrate), with each having four treatments and four replicates. Seedlings were transplanted and two years after, the growth parameters, diversity and spore density, diversity indices (Shannon, Margalef's richness and Pielou equity) and mycorrhizal colonisation were evaluated. A total of 14 species of AMF were identified on roots of *Araucaria angustifolia* species from the genus *Glomus* and *Rhizophagus clarus*, regardless of the doses of fertilisers used. P had the greatest effect on diversity of AMF species, since the average doses favour high RC and a greater number of AMF species.

Keywords: Brazilian pine, Plant nutrition, Mycorrhizal diversity and sporulation

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INTRODUCTION

Araucaria angustifolia are coniferous species native to the southern region of Brazil, demonstrating great economic, ecological and social importance (Dos Santos *et al*., 2016; Zanette *et al*., 2017). Its wood is of excellent quality and highly appreciated by the timber industry (Delucis *et al*., 2013; Hackbarth *et al*., 2017). Hence, in the 1950s its area of distribution was considerably reduced due to the exploitation.

Currently, the remaining areas of *Araucaria angustifolia* provide additional income for several families that benefit from the extraction of the fruit called pinion (Danner, 2012), an important product in the southern region of Brazil (Duarte *et al*., 2006). This suggests the need to improve the productive process of this species from production of seedlings to their implantation and field management, with the aim of reforesting these areas originally populated by the species. However, legal factors preventing its usage as wood, as well as its slow growth, hampers its plantation (Carvalho, 2003).

Araucaria angustifolia have the ability to establish a symbiotic relationship with arbuscular mycorrhizal fungi (AMF), which are associated with host plants, thus favouring a greater capacity of water and nutrient absorption from the soil, as well as promoting a greater growth and survival of plants (Nadeem *et al*., 2014).

The choice of substrate and fertilisation are factors that favour the production of vigorous forest seedlings plants. In addition, the presence of AMF in production of seedlings ensures the successful growth and establishment of certain species, so as to reduce the need for application of chemical inputs (Nouri *et al*., 2014).

Therefore, this study aimed to evaluate the effect of different doses of N, P and K, so as to obtain the appropriate doses that favour the diversity and sporulation of AMF species, in addition to root colonisation (RC) for the adequate growth of seedlings of *Araucaria angustifolia*.

MATERIAL AND METHODS

The present study was conducted in an open field in the Agricultural Sciences sector of the Federal University of Paraná, Curitiba-PR, which is located at 25° 25' 47" S, 49° 16' 19" W and 950 m above sea level.

Soils and roots, which were used for inoculation or growing/cultivating of plants, were collected from pots (volume: 16 L) containing poor ravine soil as substrate, which was collected from an area with history of plantations of *Araucaria angustifolia*. According to granulometry analysis (Embrapa, 2011), the soil was composed of 24% sand, 21% silt and 55% clay. According to chemical analysis (Embrapa, 2011), the soil had a pH of 4.0 ($CaCl_2$) and contained 0.90 mg dm^{-3} P, 4.20 g dm^{-3} carbon (C), 1.60 mmol dm^{-3} calcium (Ca^{+2}), 0.80 mmol dm^{-3} magnesium (Mg^{+2}), 0.08 mmol dm^{-3} K^{+}, 2.90 mmol dm^{-3} aluminium (Al^{+3}) and 11 mmol dm^{-3} H^{++}. The extracted soil was sieved, and 3.6 g kg^{-1} dolomitic

lime was added to increase the pH to 6.0, after which incubation was done for one month.

The fertilisation experiments using different doses of N, P and K were performed in three stages, with each having four treatments and four replications (Table 1). Nutrients were applied in soil homogenisation before filling the pots, except for N fertilisation, which was fractionated twice in the following year after the experiment was installed. A total of 48 *Araucaria angustifolia* seedlings (six months old and 20 cm tall) were transplanted in pots, which were randomly distributed and kept on a plastic cover, so as to avoid possible contact with the ground.

Table 1. Experiments on fertilisation of six-month old *Araucaria angustifolia* seedlings with different doses of N, P and K.

Nutrient	Treatments with different doses of NPK (g dm^{-3})											
	---- Experiment I ----				---- Experiment II ----				---- Experiment III ----			
N	0	0.35	0.70	2.80	1.40				1.40			
P_2O_5	1.15				0	0.29	0.58	2.30	1.15			
K_2O	0.78				0.78				0	0.19	0.39	1.56

N: Urea; P_2O_5: triple superphosphate; K_2O: Potassium chloride.

Two years after transplantation, the height (Alt), stem basal diameter (D) and total fresh matter (MFT) of roots (MFR) and aerial part (MFPA), as well as the total dry matter (MST) of roots (MSR) and aerial part (MSPA), which were obtained after drying in a stove at 60°C forced air circulation (Constantino *et al*., 2019).

A soil sample of 100 g was used for the spore extraction. The soil was liquefied for 10 seconds and sieved in overlapping meshes of 500 and 53 µm (Gerdemann and Nicolson, 1963). Then, 70% sucrose solution was added, and the mixture was centrifuged twice for four minutes (Jenkins, 1964) and sieved again using 250 and 50 µm meshes.

For the purpose of identification, spores were separated into groups according to their morphological characteristics and placed on semi-permanent slides prepared with polyvinyl alcohol, lactic acid, glycerol (Morton *et al*., 1993) and Melzer reagent (Koske and Tessier, 1983). The spores were observed with an optical microscope (400x). The spores were identified according to Invam (2018). AMF communities were characterised by the following diversity indices: 1.) Shannon diversity ($H' = -\Sigma\,[(ni / N)ln(ni / N)]$, where *ni* is number of individuals of each species and *N* is total number of individuals in the community; 2.) Margalef's richness ($D_{Mg} = (S - 1) / lnN$, where *S* is total number of species in the community and *N* is total number of individuals in the community; and 3.) the equity of Pielou ($J = H'/ln(S)$, where *H'* is Shannon diversity and *S* is total number of species in the community. R statistical package was used.

The finest roots were selected and kept in 10% KOH for 24 hours and incubated in a water bath at 80° C for one hour. H_2O_2 was added to clean the

roots, which were washed and stained with a blue ink. They were again incubated in a water bath at 80° C for another 5 minutes and lactoglycerol was added. The mycorrhizal colonisation was counted/evaluated under a stereomicroscope in 1 x 1 cm grid plate (Giovanetti and Mosse, 1980).

Data obtained on growth parameters, AMF spore count and percentage radical colonisation (%Col) were treated by analysis of variance supplemented with Tukey post hoc test at 5% of the probability. Redundancy analysis (RDA) of the AMF species was performed using CANOCO programme, version 4.5, considering the chemical attributes of the soil and the growth parameters as explanatory variables.

RESULTS AND DISCUSSION

According to the chemical analysis of the soil (Table 2), the fertilised samples from the three experiments resulted in highly acidic soils, with average pH of 5.19 (I), 4.86 (II) and 4.61 (III), showing the lowest values when the highest dose of N was applied and in the absence of K. Also, the mean values of Ca^{2+} and Mg^{2+} were found for all the experiments, with the highest values being obtained when the intermediate doses of N, P and K were applied.

Table 2. Chemical attributes of the soil at end of the experiments with two and half years old *Araucaria angustifolia* seedlings following fertilisation with different doses of N, P and K.

Soil attribute	Treatments with different doses of NPK (g dm^{-3})											
	------ Experiment I (N) ------				------ Experiment II (P) ------				------ Experiment III (K) ------			
	0	0.35	0.70	2.80	0	0.29	0.58	2.30	0	0.19	0.39	1.56
pH ($CaCl_2$)	5.53a*	5.60a	5.33a	4.33b	4.78a	4.98a	4.90a	4.78a	4.40b	4.68ab	4.88a	4.48b
Ca^{2+} (cmol dm^{-3})	4.18ab	4.33a	4.33a	3.63b	3.65b	3.95b	4.13b	4.75a	3.55b	4.10ab	4.33a	3.68ab
Mg^{2+} (cmol dm^{-3})	3.25a	3.33a	3.15a	2.55b	3.13a	3.15a	3.25a	3.15a	2.78b	3.13a	3.20a	2.60b
K^{+} (cmol dm^{3})	0.50a	0.48a	0.42a	0.21b	0.41b	0.38b	0.45a	0.33b	0.07b	0.11b	0.15b	0.69a
Al^{3+}(cmol dm^{-3})	0.00b	0.00b	0.03b	1.10a	0.35a	0.20a	0.20a	0.30a	0.83a	0.30ab	0.18b	0.53ab
$H+Al^{3+}$ (cmol dm^{-3})	5.58ab	4.30b	4.80b	8.08a	6.20a	5.80a	5.60a	6.75a	7.68a	6.48ab	6.05b	7.38ab
T (cmol dm^{-3})	13.50a	12.4a	12.7a	14.4a	13.3b	13.2b	13.4b	14.98a	14.0a	13.81a	13.72a	14.34a
SB (cmol dm^{-3})	7.92a	8.13a	7.90a	6.39b	7.18a	7.48a	7.83a	8.23a	6.39b	7.33ab	7.67a	6.96ab
m (%)	0.00b	0.00b	0.25b	14.7a	4.75a	2.75a	2.50a	3.50a	11.7a	4.00ab	2.00b	7.25ab
P (mg dm^{-3})	29.90a	35.3a	37.9a	34.7a	1.43b	7.53b	16.3b	135.5a	34.8b	43.63b	42.7b	50.05a
C (g dm^{-3})	6.73a	5.08a	10.5a	10.0a	4.30a	4.68a	5.75a	2.98a	8.90a	8.65a	8.35a	9.45a

Where: pH ($CaCl_2$ 0,01 mol L^{-1}); Ca^{2+}, Mg^{2+}, Al^{3+} (extracted with KCl 1 mol L^{-1}); $H+Al^{3+}$ (extraction by calcium acetate 0.5 mol L^{-1}); K^{+} and P (Mehlich-1 extraction); Cation exchange capacity (T); Base saturation (SB) and Al^{3+} saturation (m).

* Averages with same letter on the line do not differ from each other by Tukey's test at 5% of the probability.

In samples from the first two experiments (I and II), the final availability of K was medium to high and, in the third experiment, application of the highest dose of K resulted in a high value for this nutrient (0.69 cmol dm^{-3}). Furthermore, in general, the presence of Al^{3+} in experiments II and III was similar, varying within the range of 0.18–0.83 cmol dm^{-3}; however, in experiment I, as the dose of N increased, the concentration of this nutrient was in the ranged of 0.0–1.10 cmol dm^{-3}.

For P, application of moderate doses of N and K in experiments I and III, respectively, resulted in average values for this nutrient; whereas, in experiment II, a high value was obtained (135.58 mg dm^{-3} P) when the highest dose of P was applied. On the other hand, regardless of the experiments, there were no significant differences in the concentration of C with respect to application of the different doses of N, P and K.

At least 14 AMF species were identified in the different experiments (Table 3). Dominant species on *Araucaria angustifolia* seedlings roots were from the genus *Glomus* and *Rhizophagus clarus*, regardless of the application of N, P and K (during cultivation).

Table 3. Average number of mycorrhizal spores obtained from the experiments with two and half years old *Araucaria angustifolia* seedlings following fertilisation with different doses of N, P and K.

AMF species	Treatments with different doses of NPK (g dm^{-3})											
	------ Experiment I (N) ------				------ Experiment II (P) ------				------ Experiment III (K) ------			
	0	0.35	0.70	2.80	0	0.29	0.58	2.30	0	0.19	0.39	1.56
Acaulospora tuberculata	10a*	9a	6a	2a	9a	12a	14a	14a	2a	5a	6a	4a
Acaulospora scrobiculata	66a	49a	63a	33a	48b	89a	88a	54ab	51a	97a	86a	110a
Acaulospora spinosa	182a	81a	137a	13a	41a	44a	54a	52a	27a	34a	78a	58a
Gigaspora sp.	13a	10ab	6ab	5b	18a	21a	19a	14a	4b	6ab	10a	6ab
Dentiscutata heterogama	9a	5a	4a	1a	14a	16a	13a	13a	1ab	3a	1ab	0b
Ambispora leptoticha	31a	30a	28a	11a	13a	13a	18a	11a	12a	39a	32a	34a
Entrophospora infrequens	0a	0a	1a	0a	8a	10a	9a	6a	0a	0a	0a	0a
Glomus spinuliferum	41b	25b	33b	106a	106a	116a	95a	107a	57a	48a	39a	32a
Glomus macrocarpum	73b	93b	90b	184a	135a	133a	134a	152a	154a	145a	174a	122a
Glomus sp.1	35a	46a	58a	74a	60a	84a	64a	66a	64a	84a	95a	81a
Glomus sp.2	58b	57b	80ab	119a	148a	241a	177a	140a	141a	158a	165a	119a
Glomus sp.3	92a	74a	84a	83a	194a	257a	206a	166a	81b	119ab	152ab	156a
Glomus sp.4	44a	56a	57a	20a	123a	102a	89a	86a	19a	29[a]	29a	34a
Rhizophagus clarus	901a	300b	229b	238b	158b	441ab	618a	345ab	232a	316a	411a	274a
Total Spores	1552a	832b	874b	887b	1070a	1576a	1596a	1223a	841a	1083a	1276a	1029a

* Averages with same letter on the line do not differ from each other by Tukey's test at 5% probability.

There was a greater presence of fungi from the genus *Glomus*, which had numerous populations in experiment II (general average of 59%), which increased until the application of the average doses of P (from 0 to 0.58 g dm^{-3}). The constant application of P and N in experiment I as well as K in experiment II at different concentrations resulted in almost similar populations, with averages of 44% and 55%, respectively. The species *G. macrocarpum*, *G.* sp.2 and *G.* sp.3 dominated in increasing order (Table 3).

A significant number of *Rhizophagus clarus* species was also found, with the highest average observed in experiment I (417 individuals, representing 37% of the population) and being almost similar when compared to experiment II (390 individuals). There was a greater number of individuals of this species in the

absence of N (901 individuals) and when intermediate doses of P were applied (441 and 618 individuals).

A greater number of individuals of the genus *Acaulospora* sp. in experiment I was obtained (15% of the total population) when N was not applied (258 individuals). Larger populations of this genus (*Acaulospora)* were obtained when intermediate amounts of N, P and K were applied in each experiment. Similar results were observed for *R. clarus*. *A. scrobiculata* and *A. spinosa* were the most representatives, highlighting that these species had 89 and 88 individuals when intermediate doses were applied in experiment II and when N was not applied in the experiment I, with an average of 182 spores, which is different from those of the rest.

In all treatments, *Gigaspora* sp. and *D. heterogama* species presented a general average of 1% of the total population and both stood out in experiment II; however, this did not differ significantly when the different doses of P was applied, obtaining averages of 18 *Gigaspora* sp. and 14 *D. heterogama* spores. In contrast, the species *A. leptoticha* obtained a general average of 3% of the total of individuals in experiments I and III, presenting the highest values, regardless of the doses of N and K applied, with 25 and 29 spores obtained, respectively.

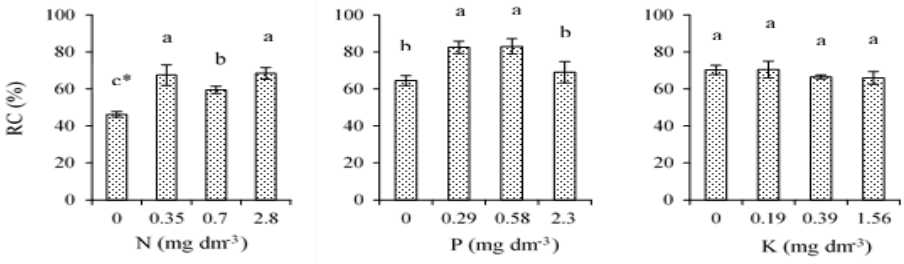
The species *Entrophospora infrequens* had a greater presence in experiment II, with an average of 1% of the population (eight individuals), regardless of the doses of P applied. In general, the AMF population was higher in experiment II, with an average of 1,366 individuals, which increased until the application of intermediate doses of P (from 0 to 0.58 g dm^{-3}). In the case of the other treatments, there was no significant difference between the different doses evaluated and they presented averages of 1,036 (experiment I) and 1,057 (experiment III) spores.

Results of the comparisons between the Shannon diversity index values, Margalef's richness and Pielou equity of the experiments are shown in table 4. The highest values of all 3 indices occurred in experiment II, with minimal differences between the applied doses of P.

Table 4. Mycorrhizal diversity index of two and half years old *Araucaria angustifolia* seedlings following fertilisation with different doses of N, P and K.

Species richness (RE)/Index	Treatments with different doses of NPK (g dm^{-3})											
	----- Experiment I (N) -----				----- Experiment II (P) -----				----- Experiment III (K) -----			
	0	0.35	0.70	2.80	0	0.29	0.58	2.30	0	0.19	0.39	1.56
RE	13	13	14	13	14	14	14	14	13	14	13	12
Shannon	1.60	2.10	2.20	2.00	2.28	2.14	2.03	2.18	2.03	2.10	2.05	2.12
Margalef	1.63	1.79	1.92	1.77	1.86	1.77	1.76	1.83	1.78	1.86	1.68	1.59
Pielou	0.62	0.82	0.83	0.78	0.86	0.81	0.77	0.83	0.79	0.80	0.80	0.85

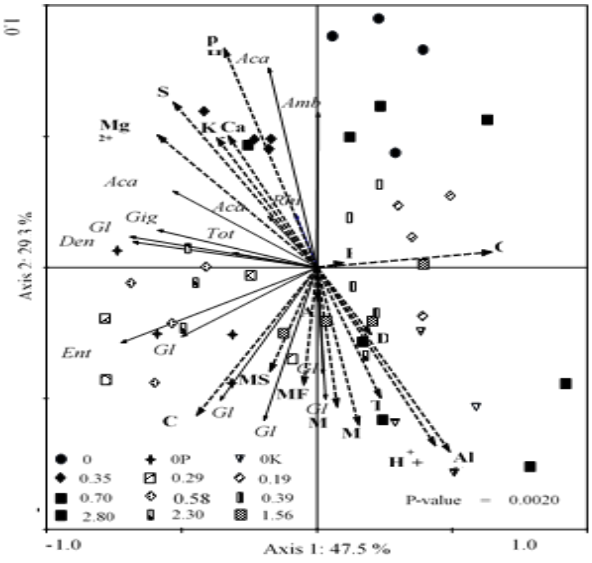
According to percentage RC of araucaria seedlings, the highest values were obtained when intermediate doses of P were applied (0.29 and 0.58 g dm^{-3}), reaching 83% (I experiment); whereas, its lowest value (46%) was obtained in the absence of N (Figure 1).



* Averages with same letter do not differ from each other by Tukey's test at 5% probability.

Figure 1. Root colonisation (RC) of two and half years old *Araucaria angustifolia* seedlings following fertilisation with different doses of N, P and K.

Results of the RDA on effects of the final chemical analysis of the soil, growth responses and percentage colonisation of AMF species in two and half years old araucaria seedlings, relative to different doses of N, P and K (Figure 2), shows that separation of most of the experimental treatments occurs in presence of cations (Ca^{2+}, K^{+}, Mg^{2+} and SB) and at the pH in axis 1 ($p < 0.05$), which explains 47.5% of all data variability. Therefore, this axis was responsible for the grouping of treatments with moderate doses of N, together with the presence of most of the AMF observed in the samples (*Aca Tub, Aca Scr, Aca Spi, Gig Sp., Den Het, Amb Lep, Gl* 4 *and Rhi Cla*). On the other hand, axis 2 explains 29.3% of the variability, mainly in the *Glomus* genus (*Gl Spi*, *Gl M*, *Gl* 1 and *Gl* 2), influencing the seedling growth responses (MFR, MSR, MFT, MST, Alt and D) and percentage colonisation (%Col). In the case of the cations (T, $H^{+}+Al^{3+}$ and $Al^{3+)}$ and C present in the samples, they did not have the least influence on the AMF species community.



Variables	Redundancies	
	Axis 1	Axis 2
pH $Cacl_2$	-0.2524	0.7302
Ca^{2+} (cmol dm^{-3})	-0.2411	0.4396
Mg^{2+} (cmol dm^{-3})	-0.4337	0.4414
K^{+} (cmol dm^{-3})	-0.2717	0.4325
Al^{3+} (cmol dm^{-3})	0.3619	-0.6137
$H^{+}+Al^{3+}$ (cmol dm^{-3})	0.3228	-0.5926
T (cmol dm^{-3})	0.1738	-0.4344
SB (%)	-0.3901	0.5512
P (cmol dm^{-3})	0.0554	0.0110
C (g dm^{-3})	0.4759	0.0521
Alt (cm)	0.0021	-0.1132
D (mm)	0.1472	-0.2236
MFR (g)	-0.0360	-0.3915
MSR (g)	-0.1285	-0.3477
MFT (g)	0.0948	-0.4957
MST (g)	0.0551	-0.4651
Col (%)	-0.3275	-0.4930

Figure 2. Redundancy analysis (RDA) of the AMF species in relation to chemical analysis of the soils and growth responses of two and a half years old *Araucaria angustifolia* seedlings, depending on fertilisation with different doses of N, P and K. *Aca Tub*: *Acaulospora tuberculata*; *Aca Scr: Acaulospora scrobiculata; Aca Spi: Acaulospora spinosa; Gig Sp: Gigaspora sp.; Den Het: Dentiscutata heterogama; Amb Lep: Ambispora leptoticha; Ent Inf: Entrophospora infrequens; Gl Spi: Glomus spinuliferum; Gl M: Glomus macrocarpum; Gl 1: Glomus sp.1; Gl 2: Glomus sp.2; Gl 3: Glomus sp.3; Gl 4: Glomus sp. 4; and Rhi Cla: Rhizophagus clarus.*

There is a difference in the diversity of AMF in seedlings from nurseries in relation to those observed in native areas, or in replanted areas of araucaria that were studied in various regions of São Paulo (SP), Rio Grande do Sul (RS) and Paraná (PR). In this sense, in SP, an average of 43 species was obtained (Bonfim *et al*., 2016; Moreira *et al*., 2007a; Moreira *et al.*, 2009); however, in RS, an average of 12 species was found (Breuninger *et al*., 2000; Zandavalli *et al*., 2008), while in the case of PR, Vilcatoma-Medina *et al*. (2018) obtained an average of eight species of *Araucaria angustifolia* seedlings of different ages. The application of different doses of N, P and K in araucaria seedlings influenced the presence of the 14 species, stimulating symbiosis (both to promote the multiplication of microorganisms and optimal growth of plants), especially when moderate doses of P were applied.

Soil and climatic factors can affect AMF population and nutrient absorption, which can affect seedling growth (Helgason and Fitter, 2009; Tahat and Sijam, 2012; Toljander *et al*., 2008). Araucaria cultivation grows well in soils with a moderately acidic pH. In a previous study, it was shown that when the acidity of the substrate was corrected and moderate doses of P were used, 11 AMF species were identified (Moreira *et al*., 2012). In the present study, application of the different doses of N and K may have influenced the structure of the substrate and contributed to a greater diversity of species and high sporulation.

A strong correlation between P and K and AMF is evident, such that the deficiency of these nutrients can induce the accumulation of K, preventing the transfer of polyphosphates to the plant (García *et al.*, 2014). Although AMF symbiosis improves the absorption of P and N by the host, these nutrients in turn influence the RC of AMF and its symbiotic performance (Nouri *et al*., 2014).

In the present study, fungi of the genera Glomus and Acaulosora were dominant in araucaria seedlings, as well as in most studies performed in different forests in Brazil (Moreira *et al*., 2007a; Bonfim *et al*., 2016; Moreira *et al*., 2016). These genera produce more spores and require less time to reproduce in the same environment when compared to the rest of AMF (Hart *et al*., 2002; Piotrowski *et al*., 2004; Suresh *et al*., 2010).

The species from genus *Glomus* were dominate until the application of moderate doses of P, as reported by Moreira and Cardoso (2002), Moreira *et al*. (2012) and, in *Acacia mearnsii* seedlings (Mello *et al*., 2008), since high concentrations of P inhibit symbiosis with *G. intraradices* (Breuillin *et al*., 2010).

The importance of the presence of the *Glomus* is confirmed by inoculation with *G. intraradices* and *G. clarum*, which influence the growth parameters of seedlings of *A. angustifolia* (Moreira-Souza and Cardoso, 2002). *G. macrocarpum* was confirmed as one of the most important species in young plantations of *Eucalyptus urophylla* (Santos *et al.*, 2017), especially in the moderate presence of N and P, as corroborated in this study on seedlings of *A. angustifolia*. *G. clarum* is also beneficial for annual crops, while it favours plant growth and improves the efficiency of P and N absorption (Cely *et al*., 2016).

Rhizophagus clarus is among the most common species of the ecosystem (Lee and Eom, 2015). It was the second most abundant in araucaria seedlings and its population decreased by high application of P. Ferreira *et al.* (2015) reported a reduction of up to 70% of *R. clarus* in the presence of excess P, while *R. irregularis* was found to be be tolerant to high levels of P in the cultivation of *Eucalyptus marginata* (Kariman *et al*., 2014).

Excess fertilisation with P affects sporulation and consequently reduces the number of individuals of *Acaulospora* genus (Lin *et al*., 2012; Moreira *et al.*, 2012), which was also observed in the present study. In the treatments that had medium-high levels of P, there were greater number of individuals of *Aaulospora* compared to those obtained by treatment with an average P content of 13 mg dm^{-3} (Moreira *et al.*, 2007a). In the samples with average levels of P (0.29 to 0.58

g dm^{-3}) (experiment II), the number of individuals of the species *A. scrobiculata* was 68% higher when compared to araucaria seedlings of different ages with high levels of P (Vilcatoma-Medina *et al*., 2018).

Gigaspora (Gigaspora sp. and *D. heterogama*) was affected by the increase in the doses of nutrients, especially N. A similar result was obtained by Johnson *et al*. (2003) for soils enriched with N and in sandy soils with medium pH (Lekberg *et al.,* 2007). The species *A. leptoticha* and *E. infrequens* were present in different soils of Brazilian forests with acidic reaction, moderate Al^{3+} and low K content (Costa *et al.,* 2016; Silva *et al.,* 2007), which were also recorded in this study. Thus, they are considered to be important in the production of Araucaria seedlings.

Regarding the analysis of AMF diversity index, experiment II had a greater prominence, probably due to the chemical properties of the substrates at end of the experiment, as in the case of the P, which presented different and low concentrations compared to the rest of the experiments, which were always constant and high, as well as for pH and organic matter. Diversity and richness of AMF in a native araucaria forest was greater when compared to those of a replanted forest (Moreira *et al.* 2007b).

The highest *A. angustifolia* seedlings occurred in experiment II (Constantino *et al.* 2019). In the presence of P, an average height of 79 cm was obtained, which is 34% higher when compared to its absence. When moderate and constant doses of P were applied in the different concentrations of N and K, these stood out with average heights of 74 cm and 73 cm, respectively.

When comparing the height and diameter of the three experiments, our results were 50% smaller and 30% thinner than those reported by Zandavalli *et al*. (2004) in two-year-old seedlings. Moreover, Zandavalli *et al*. (2004) inoculated *A. angustifolia* with *G. clarum* in a sterilised natural soil that had a good amount of P, K, organic matter and low content of Al^{3+} (in comparison with the soil used in the present study). When AMF species were inoculated together with the application of moderate doses of P on seedlings of *Khaya senegalensis* and *Acacia mangium*, the growth parameters improved considerably (Jeyanny *et al.* 2011; Jeyanny *et al.*, 2013).

In two-year-old *A. angustifolia* seedlings, the variables MFPA (341 g) and MFR (256 g) were highlighted in the presence of moderate doses of P and N (Constantino *et al*., 2019), which were higher than those found by Zandavalli *et al.* (2004). In a one-and-half-year-old *A. angustifolia* seedlings, MSPA and MSR occurred similarly to those verified for MFPA (140 g) and MFR (122 g), which were twice as high as those found by Moreira-Souza and Cardoso (2002). The presence of cations, such as Al^{3+}, which are predominant in acid soils, can inhibit the absorption of nutrients, while certain species of AMF can facilitate their availability in the soil (Meharg, 2003). This fact was verified in this study, where the final presence of this element did not interfere in the substrates, since its effect was moderately considerable, due to the contribution of the nutrients that facilitated the reproduction of the fungi, as well as due to the fact that it was

corroborated in the seedlings of araucaria of different ages (Vilcatoma-Medina *et al.*, 2018) and in *Tectona grandis* seedlings (Rodrigues., *et al* 2018).

The highest values for mycorrhizal colonisation of *A. angustifolia* seedlings were evident in the moderate concentrations of P (experiment II), which were three times higher than those observed in one-year-old seedlings fertilised with P (Moreira-Souza and Cardoso, 2002; Moreira *et al.*, 2012), as well as compared to those in native, replanted and burned forests of *A. angustifolia* (Moreira *et al*., 2006; Moreira *et al.*, 2007a; Moreira *et al.*, 2016), which is similar to those presented in seedlings aged 2 and 5 years (Vilcatoma-Medina *et al.*, 2018; Zandavalli *et al.*, 2004) and to those of the forests of *A. araucana* (Diehl *et al.*, 2008; Diehl and Fontella, 2010); and much higher than those of *Eucalyptus* sp. plantations (Lima *et al*., 2013).

In the present study, the importance of fertilisation on *A.angustifolia* seedlings in the presence of AMF followed the order: P > N > K. This result is probably due to the fundamental role that P plays in the composition of organic molecules, such as nucleic acids, ATP and phospholipids (Campos, *et al.*, 2018). Therefore, the more limiting phosphorous is, the greater the effect it will have on the behaviour of AMFs. It was also found that the application of moderate doses of P and N and low K resulted in a significant number of AMF.

As already seen in the present study, the population of AMF species were favourably influenced by different concentrations of nutrients and consequently on the growth of *A. angustifolia* seedlings. This result was similar to that observed by Fuentes-Ramírez *et al.* (2018), in which AMF species increased significantly after burning in *Araucaria araucana* plantations, with incorporation of nutrients due to the mineralisation of organic matter and contribution of ash.

CONCLUSIONS

Araucaria angustifolia seedlings are inhabited (or colonised) by a diversity of AMFs, with emphasis on the species from the genus *Glomus* and *Rhizophagus clarus*, regardless of the fertilisation regime carried true its cultivation.

Phosphorus is the element that has the greatest effect on AMF diversity, such that when applied in medium doses, it favours radical colonisation and a highest number of AMF species.

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