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Applied Soil Ecology 25 (2004) 245–255
Applied Soil Ecology
www.elsevier.com/locate/apsoil

Growth responses of *Araucaria angustifolia* (Araucariaceae) to inoculation with the mycorrhizal fungus *Glomus clarum*

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Received 9 January 2003; accepted 17 September 2003

Abstract

Brazilian pine (*Araucaria angustifolia*, Araucariaceae) is one of the few native gymnosperms from southern Brazil. In Rio Grande do Sul, it occurs mainly in the highlands of the planalto region, as a tree-component of the mixed ombrophyllous forests. The species is valuable for its wood, edible seeds, and ornamental use, and is today listed as a threatened species. This tree-species establishes associations with arbuscular mycorrhizal fungi, and assessing the impact of such association to the species-performance will help us in defining the management procedures for its use in conservation and sustainable purposes. In this study, we tested the nutritional and growth responses of seedlings to inoculation with *Glomus clarum*, a fungal species identified in the rhizosphere of Brazilian pine in natural stands. A greenhouse study was conducted, where plants were either inoculated with *G. clarum* or with a sterilized version of the same inoculum. Plant growth was evaluated over a 21-month period by measuring the total shoot length, and, at the end, by plant dry mass, leaf nutrient content, and root colonization. Seedlings inoculated with *G. clarum* had a high degree of mycorrhizal colonization of their roots (81%). The inoculated seedlings grew significantly more (312% mass increase) than the controls, which had a higher root:shoot ratio than the inoculated seedlings. Leaf concentrations of P, K, Na, and Cu were higher while those of Ca, Mg, Fe, Mn, and B were lower in the inoculated plants. The results indicate a strong response of *A. angustifolia* to mycorrhizas, suggesting a high degree of dependence of the species on these associations.

Keywords: *Araucaria angustifolia*; Araucariaceae; Brazilian pine; Arbuscular mycorrhizal fungi; Mycorrhizas; *Glomus clarum*

1. Introduction

Araucaria angustifolia (Bertol.) Kuntze (Brazilian pine) belongs to the family Araucariaceae. The genus *Araucaria* is presently limited to the southern hemisphere, and *A. angustifolia* and *A. araucana* are the sole representatives in South America. The geographical distribution of *A. angustifolia* includes Argentina and Brazil, where it is concentrated in the southernmost states of Parana, Santa Catarina and Rio Grande do Sul, (Hueck, 1953; Reitz et al., 1988). The species typically occurs in the mixed ombrophyllous forests (IBGE, 1986) on high altitude plateaus, where it is the dominant species of the upper canopy.

Populations have been dramatically reduced because of irrational and uncontrolled exploitation for

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doi:10.1016/j.apsoil.2003.09.009

its valuable wood (Reitz et al., 1988). Brazilian pine is also valued as an ornamental plant and its starchy seeds are an important food source for animals. Much effort at reforestation in southern Brazil is concentrated on fast-growing exotic species of *Pinus* and *Eucalyptus*. *Araucaria angustifolia* is today classified as vulnerable in the IUCN list of threatened species (Hilton-Taylor, 2000), and timber harvest is forbidden by law. However restoring populations will require introducing new individuals on degraded forests and deforested areas. The success of such an action will depend, among many factors, on a profound knowledge of ecological and physiological aspects that are relevant for the natural regeneration and for the establishment of planted individuals. One of these is the ability of seedlings to efficiently harvest nutrients from the soil, and this ability is strongly determined by the mutualistic association between mycorrhizal fungi and tree roots.

Mycorrhizas are widespread associations characterized by a bi-directional transfer of nutrients between the plant and associated fungi, where plants provide sugar to the fungi and these help the plants on the acquisition of mineral nutrients from the soil (Smith and Barker, 2002). Additionally, mycorrhizal fungi also aid in soil-water extraction, increasing drought tolerance of the host (Subramanian et al., 1997; Mathur and Vyas, 2000). These associations are also reported to improve the plant‘s ability to tolerate heavy metal toxicity (Khan, 2001), as well as pathogen (Calvet et al., 1993; Filion et al., 1999; Fusconi et al., 1999) and herbivore (Gehring and Whitham, 1991; Borowicz, 1997) attacks. At the single plant level, these benefits typically result in increased mass production and plant competitive ability.

Arbuscular mycorrhizas are the most ancient (Redecker et al., 2000) and widespread mycorrhizal associations. The arbuscular mycorrhizal (AM) fungi were recently placed in a new monophyletic phylum, the Glomeromycota, encompassing three orders (Schussler et al., 2001) and five families (Morton and Redecker, 2001). Associations with these fungi are widespread among tropical trees, shrubs and herbs (Harley and Smith, 1983), including members of the Araucariaceae (Smith and Read, 1997).

Mycorrhizal associations in *A. angustifolia* were first described by Milanez and Monteiro (1950), and a more detailed analysis of the AM structures present in the species was provided by Breuninger et al. (2000). Many different species of AM fungi, distributed among the genera *Glomus*, *Acaulospora*, *Entrophosphora* and *Scutellospora*, have been found in the rhizosphere of Brazilian pine plants (Breuninger et al., 2000). However, the growth responses of Brazilian pine to such associations, as well as the degree of mycorrhizal dependency of the species, are mostly unknown. Muchovej et al. (1992) inoculated seedlings with both ectomycorrhizal (*Rhizopogon nigrescens* and *Pisolithus tinctorius*) and AM (*Acaulospora scrobiculata* and *Glomus mosseae*) fungi, and, as expected, root colonization only took place for the AM fungi. However, no growth enhancement was induced by such colonization, which was attributed by the authors to the short duration of the experiment (5 months) and to the small size of the pots. Some characteristics of the species, such as poorly developed root hairs, a coarse root system and large seed size (around 7 g each), are considered to be indicators of a high degree of mycorrhizal dependency (Janos, 1980; Pope et al., 1982; Bowen, 1984; Haselwandter and Bowen, 1996).

The much slower initial growth of the *A. angustifolia* when compared to species of *Pinus* and *Eucalyptus* has resulted in extensive areas previously occupied by Brazilian pine to be planted with these non-native species. If Brazilian pine does have a great dependency on mycorrhizal associations, its success in reforestation programs may greatly depend on assuring the colonization of seedlings, which could increase the competitive ability of seedlings, potentially increasing their initial growth rate. Thus assessing the species-response to colonization by different species of AM fungi is essential in determining the effectiveness of such associations and in suggesting plantation and management procedures. In this study, a greenhouse experiment was conducted in order to quantify the species-response to inoculation with an AM fungus naturally present in the rhizosphere.

2. Materials and methods

2.1. Growth conditions

The experiment was conducted in a glass greenhouse (Soil Department, Federal University of Rio

Grande do Sul, Porto Alegre, Brazil), with no temperature and humidity control. Pine seeds were collected from the ground in a natural forest (São Francisco National Forest, São Francisco de Paula, RS, Brazil) in July 1998. The seeds were disinfected with a 2.5% solution of sodium hypochloride (NaClO) for 20 min, and then thoroughly rinsed in distilled water. The upper third (radicle-protruding region) of the seed had its external integuments removed to accelerate the germination process (Áquila and Ferreira, 1984). Germination took place in plastic trays lined with wet paper under laboratory conditions. Germinated seeds (radicle length between 1.5 and 3.0 cm) were transplanted to 1.5 l plastic bottles, wrapped in aluminum foil and filled with a soil mixture containing peat and washed sand (1:1, v/v).

2.2. Experimental design

The growing plants were submitted to one of the two treatments: inoculation of the soil mixture with an AM fungus, *Glomus clarum* Nicolson and Schenck, and non-inoculated control conditions. The inoculum was produced in the Department of Horticulture of the Federal University of Rio Grande do Sul (Porto Alegre, Brazil), from rhizosphere soil and roots of pre-colonized oat plants. The chosen species of mycorrhizal fungus was found to be naturally associated with the rhizosphere of Brazilian pine in forests located in upland forests of Rio Grande do Sul and São Paulo (unpublished results). Its choice was based on the fact that *G. clarum* was the only one that was both available in sufficient amounts for the proper conduction of the experiment and occurred in natural association with Brazilian pine. Treatments were randomly assigned to the experimental units (pots), following a completely randomized design, with seven replicates for each treatment. Prior to the inoculation procedure, the soil mixture was sterilized by autoclave at 120 °C for three 1 h periods, at 24 h intervals. Each pot was filled with soil mixture and 17 g of soil with inoculum (approximately 80 spores). For the control group, this inoculum was previously steam-sterilized, following the procedures described above. Pots were irrigated on a weekly basis with distilled water applied on the soil surface. One experimental unit was lost from the control group, so that the experiment was conducted with unequal replication of the treatments. After 54 weeks (September 1999), plants were moved to 8 l pots, after noticing that shoot growth had mostly stopped for both plant groups. These larger pots were filled with the same steam-sterilized mixture initially used, and the original soil in each pot was also transferred to the new ones. Physical and chemical characteristics of this soil mixture are shown in Table 1.

2.3. Growth measurements

On a weekly or biweekly basis, plant height and total shoot length (height plus length of lateral branches) were measured 76 weeks after the beginning of the experiment (22 weeks after transplantation), the experiment ended and the plants were harvested for additional growth analysis. Basal stem diameter was measured, lateral branches and branch whorls were

Table 1
Chemical characteristics of the soil mixture used in the experiment and of a soil of natural occurrence of *A. angustifolia*

Soil parameter	Experimental soil	Natural soil[a]
Cation exchange capacity ($cmol\,l^{-1}$)	11.1	19.63
Base saturation (%)	93.0	51.50
Al saturation (%)	0	5.35
Al + H ($cmol\,l^{-1}$)	0.8	9.25
Clay (%)	7	27.85
pH (in water)	7.7	4.65
Organic matter (%)	3.2	5.33
N (%)	0.09	0.15
P ($mg\,l^{-1}$)	39	2.35
K ($mg\,l^{-1}$)	75	132.25
Al ($cmol\,l^{-1}$)	0	1.05
Ca ($cmol\,l^{-1}$)	9.5	–
Mg ($cmol\,l^{-1}$)	0.6	–
S ($mg\,l^{-1}$)	21.0	–
Zn ($mg\,l^{-1}$)	4.9	–
Cu ($mg\,l^{-1}$)	0.5	–
B ($mg\,l^{-1}$)	1.2	–
Mn ($mg\,l^{-1}$)	4	–

Clay content was determined by density analysis. pH was measured in water solution (1:1, v/v). Organic matter (OM) was obtained by multiplying the organic carbon (measured by humid digestion) content by 1.72. Determination of P and K was based on the Mehlich I method. Exchangeable Ca, Mg. Al, and Mn was extracted with KCl $1\,mol\,l^{-1}$, S–SO_4 with $CaHPO_4$ $500\,mg\,l^{-1}$ and B with hot water. Soil N was estimated from N = OM/34.4. Analyses were performed by the Analyses Lab of the Soil Department of the Federal University of Rio Grande do Sul.

[a] Data from Duarte et al. (2002).

counted, length of the main root was measured, and shoot and root structures were oven-dried at 60 °C. A sample of 20 mature leaves had their area measured before drying for calculation of specific leaf mass.

2.4. Mycorrhizal colonization

Approximately 1 g of fresh lateral roots were excised from each plant in order to quantify the degree of mycorrhizal colonization (the dry weight of such roots was estimated and added to the root mass). These sampled roots were first order lateral roots emerging from the upper half of the main tap root. The roots were kept in FAA (ethanol 50%, acetic acid and formaldehyde 35% in the proportion of 9:0.5:0.5, v/v/v) for 3 days and then transferred to an ethanol solution (70%) until clearing and staining procedures were done. The technique described by Phillips and Hayman (1970) was adapted for *A. angustifolia*, because the original root clearing procedure, which included heating the roots at 90 °C for about 1 h in 10% KOH, was not successful in clearing the roots, and going beyond this time-period resulted in excessive damage to the root tissues. The adapted technique included the following procedures: (1) autoclaving of the roots in a 10% solution of KOH for a period of 20 min, followed by water-rinsing, (2) immersion of the cooled roots in alkaline H_2O_2 for 20 min, followed by another water-rinsing, (3) root immersion in a 10% solution of HCl for 10 min; and (4) staining of the root samples with Trypan Blue in lactoglycerol (0.05%) for a 1 h period. Quantification followed the grid-intersection method (Giovannetti and Mosse, 1980), using a 1.27 cm^2 grid area.

2.5. Leaf nutrients

Due to the large amount of plant material required, mature leaves from all plants of a given treatment made up a compound sample for nutrient analysis. All analyses were performed by the Analysis Lab of the Soil Department (Federal University of Rio Grande do Sul) and are described in Tedesco et al. (1995). Briefly, N, P, K, Ca and Mg were extracted with H_2O_2 and H_2SO_4, and micronutrients (Zn, Cu, Fe, Mn, B), Na and S underwent digestion with HNO_3–$HClO_4$, except for B, which was extracted with H_2SO_4 after burning the leaf material in a muffle furnace. N was determined by semi-micro-Kjeldahl analysis, P, Ca, Mg, micronutrients and Na were determined spectrophotometrically and K and S by flame spectrophotometry.

2.6. Statistical analysis

Data collected were submitted to variance analysis (ANOVA). When data did not meet the normality and homoscedasticity requirements, they were either log transformed ($\log[X + 1]$, for leaf dry mass) or submitted to non-parametric ANOVA (stem basal diameter and root length ratio). The statistical package SAS, version 6.11 (SAS Institute), was used in all analysis.

3. Results

All plants inoculated with *G. clarum* had root colonization by mycorrhiza. The fraction of the total lateral root length examined that was colonized by mycorrhizal fungi averaged 81%. None of the plants from the control group were colonized by fungi.

Differences in total shoot length (Fig. 1) between the two treatments became apparent after 30 week of plant growth. After 36 weeks, growth stopped in both the groups and the difference between them remained stable but statistically significant ($P = 0.04$). After the transplant, the inoculated plants accelerated shoot growth considerably, while the control plants ceased growth and displayed progressive browning and necrosis of the leaves. From the 65th week onwards, differences between the two treatments became highly significant ($P < 0.01$). By the end of the experiment, the total shoot length of the inoculated group was 3.6 times greater than that of the control.

In addition to a greater shoot length, the inoculated plants also had much larger mass accumulation in roots, stem and leaves (Fig. 2). However, this mass increase was more pronounced for the above-ground structures, resulting in a larger root:shoot mass ratio in the control than in the inoculated plants (Fig. 3). At the root level, mass allocation to lateral roots, as opposed to the main tap root (Fig. 3), also tended to be greater in the control plants ($P = 0.08$).

Except for the length of the tap root, which was limited in both cases by the pot depth, all the additional

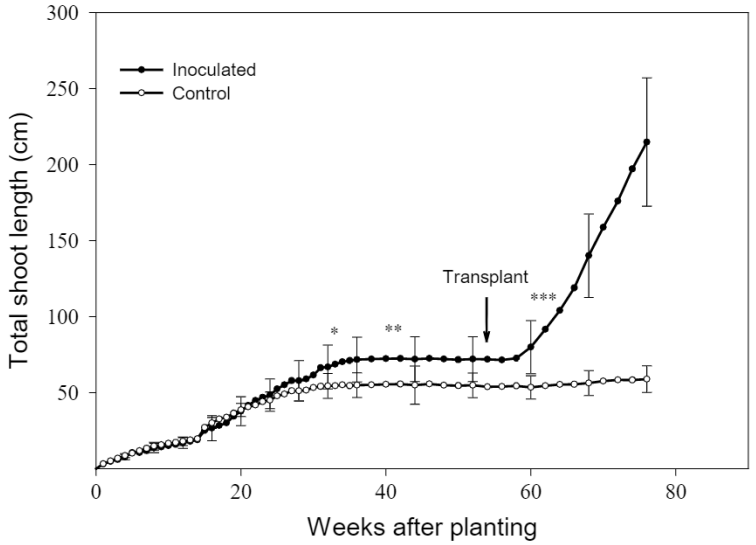

Fig. 1. Increases in total shoot length during the course of the experiment. Vertical bars are the standard errors of the mean. Asterisks indicate the period of time after which differences between treatments turned significant. (*): $P \leq 0.10$, (**): $P \leq 0.05$ and (***): $P \leq 0.01$.

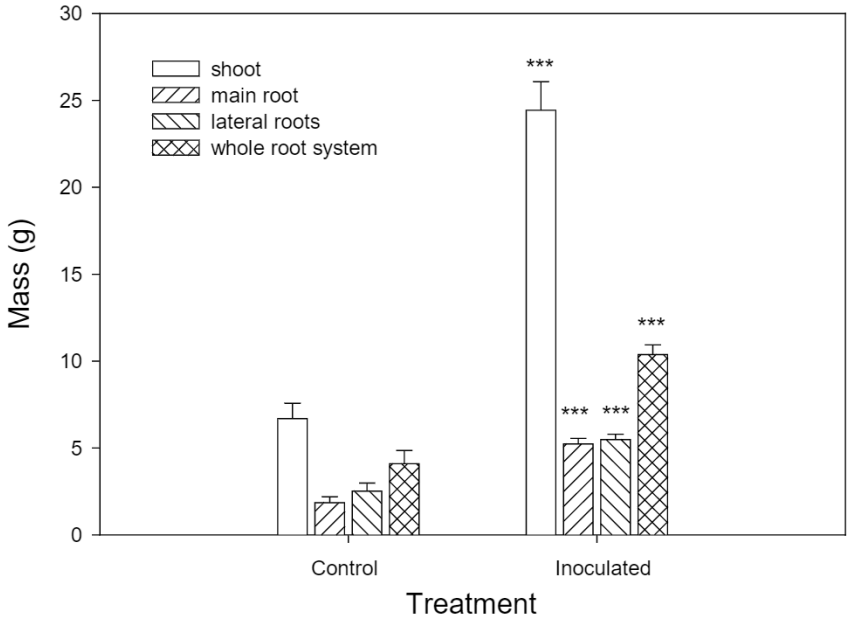

Fig. 2. Mass accumulated in different plant parts by the end of the experiment. Vertical bars are the standard errors of the mean. Values of shoot mass were log-transformed prior to statistical analysis. Asterisks indicate the level of significance of the difference between treatment means. (***): $P \leq 0.01$.

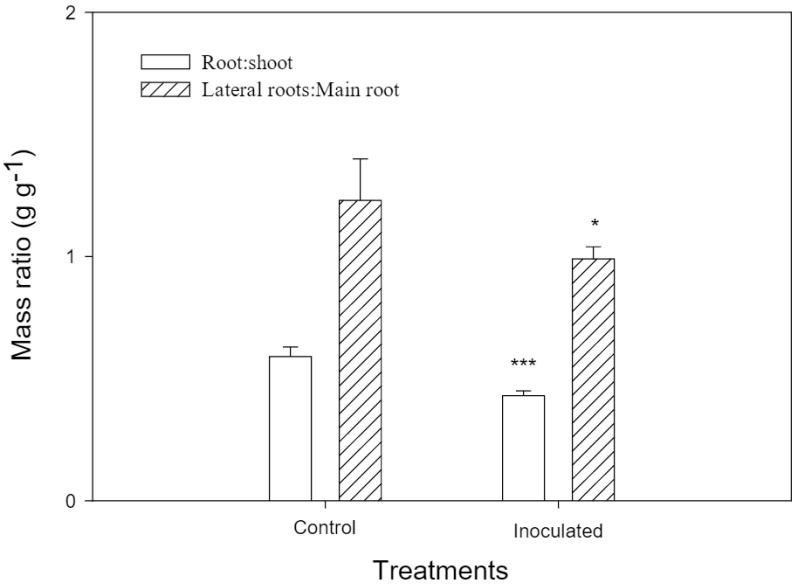

Fig. 3. Mass ratios between different plant parts by the end of the experiment. Vertical bars are the standard errors of the mean. Asterisks indicate the level of significance of the difference between treatment means. (***): $P \leq 0.01$ and (*): $P \leq 0.10$.

growth parameters evaluated in this experiment confirmed the much greater growth attained by the plants which were colonized by the mycorrhizal fungus (Table 2).

Among the macronutrients, the concentrations of N, and S in the leaves did not differ much between the two treatments, while K- and particularly P-concentrations were apparently greater in the inoculated than in the control plants. The concentrations of Ca and Mg in the leaves of the inoculated plants, on the other hand, were substantially lesser than in the leaves of control plants (Table 3). The micronutrients Cu and Na were the only ones to appear in greater concentrations in the inoculated plants, while Fe, B, and particularly Mn were present in much smaller concentrations in these plants.

Table 2
Additional growth parameters measured in inoculated and control plants of *A. angustifolia* after 76 weeks of growth

Growth parameter	Inoculated plants	Control plants
Length of the main root (cm)	44.78 ± 3.05[ns]	35.44 ± 4.94
Basal stem diameter (mm)	9.49 ± 0.23***	5.97 ± 0.76
Number of branch whorls	3.00 ± 0.30**	1.60 ± 0.24
Number of branches	7.57 ± 0.71**	3.80 ± 0.37
Mass of a mature leaf (mg)	10.30 ± 0.40**	7.85 ± 0.95
Area of a mature leaf (cm^2)	0.821± 0.027***	0.552 ± 0.048
Specific leaf mass ($g\,m^{-2}$)	125.79 ± 4.08*	139.71 ± 6.24

Values are mean ± standard error. Values for number of branches and leaf mass were log-transformed prior to statistical analysis. Asterisks indicate significant difference between means.

* $P \leq 0.10$.

** $P \leq 0.05$.

*** $P \leq 0.01$.

[ns] No significant difference.

Table 3
Nutrient concentration in leaves of *A. angustifolia*, measured on a compound sample made up of all experimental units within each treatment, including reference values from other sources

Nutrient	Concentration (% or mg kg^{-1})		
	Control	Inoculated	Others[a]
N	1.4	1.5	**0.8**–1.7
P	0.06	0.16	0.1–**0.2**
K	1.0	1.5	**0.7**–1.7
Ca	2.6	1.4	0.2–**0.8**
Mg	0.21	0.11	**0.2**–0.3
S	0.06	0.09	**0.06**
Cu	2.2	5.3	5.0–**9.3**
Zn	13	12	12–**63**
Fe	149	89	47–**165**
Mn	171	78	**59**–1129
Na	71	294	**10**
B	41	22	8–**42**

Values are expressed in % (N, P, K, Ca, Mg and S) or mg kg^{-1} (Cu, Zn, Fe, Mn, Na and B).

[a] Range of concentration values based on data from mature leaves of adult trees, compiled by La Torraca et al. (1983), and from shoots of one-year-old plants (bold-face) grown in nutrient solution (Simões, 1973).

4. Discussion

Benefits of mycorrhizal associations for many tree species have already been documented (e.g. Kormanik et al., 1981, 1982; Jones et al., 1998; Mason et al., 2000; Mathur and Vyas, 2000; Azcón-Aguiar et al., 2003). Natural levels of root colonization were found to be around 60% in adult trees of *A. angustifolia* in two forest sites located on the high altitude plateaus of Rio Grande do Sul (Breuninger et al., 2000), but up to this date there was no study demonstrating the benefits of mycorrhiza for *A. angustifolia*. *Glomus clarum* has proven to be a very efficient root colonizer, and such colonization resulted in major growth enhancement of young plants of *A. angustifolia*. We suggest that, given the major response of the species to mycorrhizal inoculation, that its dependency (sensu Pope et al., 1982) on this association is high under natural conditions.

The significant and pronounced growth response exhibited by the inoculated plants took a long time to occur (more than 30 weeks). This major delay is probably related to the slow growth of the species. Brazilian pine seeds are well-known for their size and nutritional value (Bobbio et al., 1978; Ferreira, 1981), and the growth of seedlings slows when seed reserves are exhausted. Increases in the fungal mass are greatly dependent on the availability of plant carbohydrates. Photosynthetic rates of slow-growing plants are typically low (Chapin, 1980), which probably results in a longer period of time required for the hyphal mass to be large enough to benefit nutrient acquisition for these plants. For species with a high level of dependency on mycorrhizal associations, such a delay may result in reduced competitive ability for soil resources during the early stages of development. The disadvantage might be at least partly offset by a high availability of seed reserves. Seed storage in Brazilian pine might serve not only for boosting initial growth and establishment but also to sustain the mycorrhizal fungus with carbohydrates, avoiding a major and dangerous delay in the colonization process. However, the role of seed reserves on the establishment of mycorrhizal associations in Brazilian pine seedlings still requires investigation.

The degree of root colonization is controlled by the level of phosphorus in the host tissues (Koske and Gemma, 1992). A high availability of phosphorus to the plants is expected to reduce root colonization by mycorrhizal fungi (Brandon and Shelton, 1997; Treseder and Vitousek, 2001). In our experiment, plants were grown in a soil mixture containing a high level of P, contrasting with the typically low availability in which plants are naturally exposed (Table 1). Based on their review of the mycorrhizal relations in trees, Haselwandter and Bowen (1996) concluded that a high dependency on mycorrhiza is typically related to plants with coarse root systems and that these plants are usually responsive to mycorrhizal inoculation even at high levels of phosphorus availability. The results of the present study corroborate their conclusions and provide further evidence of the high degree of mycorrhizal dependency of Brazilian pine. However, conducting a similar experiment under more natural soil conditions would greatly contribute to a better characterization of the importance of mycorrhiza for the species.

Compared to the controls, inoculated plants invested proportionally less mass in root than in shoot structures, reflecting the higher availability of soil resources due to the greater surface area for nutrient uptake provided by the mycorrhizal association.

Similar results were also reported in other studies (e.g. Allsopp and Stock, 1992; Wright et al., 1998). Even though colonization by mycorrhizal fungi typically results in more numerous lateral roots in the host, these are much shorter than in non-colonized root systems (Berta et al., 1992; Tisserant et al., 1996). The lower mass investment in lateral roots reported in this study may reflect this morphological change induced by the association and suggest that extending the lateral roots might not be very important for nutrient foraging when hyphal extensions are also present.

An adequate balance between the offer and demand of nutrients favors the maintenance of adequate contents of leaf nutrients and, by consequence, the proper functioning of cells. As a consequence, in plants that efficiently adjust their size to the amount of available soil resources, leaf content of a given nutrient might not be a good indicator of deficiency of that nutrient, as it might not vary significantly across a range of soil nutrient availability. Leaves of control plants had either the same, greater or lower nutrient contents than the inoculated ones.

Plant growth is very responsive to the availability of nitrogen, such that the balance between offer and demand is rapidly established and N-concentration remains quite stable (Chapin, 1980). Many authors have suggested that mycorrhizas can result in increasing absorption of this element (Buwalda et al., 1983; Ikram et al., 1992; Marschner and Dell, 1994), but given the little variation in N-concentration of the two groups of plants, we cannot reach any conclusion in this respect. Other nutrients (K, S, Zn, Fe, Mn, and B) also did not show much variation between treatments and/or were within the reported range of concentration values reported for the species (Simões, 1973; La Torraca et al., 1983), in both groups of plants (Table 3).

The concentration of P, on the other hand, was almost three times greater in the inoculated plants than in the control ones, where the measured concentrations were below values reported for Brazilian pine and the species in general (Epstein, 1975), indicating that in the absence of mycorrhizal associations, plants were not able to harvest enough P from the soil and maintain adequate levels in their tissues, despite the major reduction in plant growth. In the latter, the benefits of the association are very clear and in accordance with the results of many studies (e.g. Buwalda et al., 1983; Marschner and Dell, 1994; Moyersoen et al., 1998) and to the expected benefits of the association in terms of absorption of this highly immobile nutrient.

The increases in Cu- and Na-concentrations promoted by the mycorrhizal association was noticeable. Inoculation brought Cu-concentration within the normal range. Considering the high immobility of this element in the soil (Haselwandter and Bowen, 1996), it is not surprising to find beneficial effects of mycorrhizal association in situations where the availability of the nutrient is low. In the specific case of Na, Ojala et al. (1983) have also reported increased absorption of this element in mycorrhizal plants of *Allium cepa* grown on different levels of salinity. The physiological significance of the extremely high values of Na-concentration measured in this study deserves future attention. Other nutrients were present in lower concentrations in the leaves of the inoculated plants when compared to the control plants. In the case of Ca, such reduction brought leaf levels closer to expected values, but still much above the expected range. For Mg, the opposite occurred. The surprisingly high values of Ca could be related to the very high levels of Ca in the soil mixture, together with a high capacity for Ca storage in the leaves. Recently, Mastroberti and Mariath (2003) characterized the compartmented cells in the mesophyll of *Araucaria angustifolia* and determined the pectic nature of the partitions in the lumen of these cells. The high affinity of pectins with Ca could confer the species a high capacity for Ca storage. The levels of Fe and Mn in inoculated plants were also closer to the normal.

The leaf symptoms displayed by the control plants, particularly after transplantation, indicated a nutritional imbalance. Steam sterilization, commonly adopted in mycrorrhizal inoculation studies, can result in increased levels of the reduced forms of ions, such as Fe and, especially, Mn, increasing the availability of these elements to the plants. Lopes and Wollum (1976) measured a 5.5-fold increase in the availability of Mn due to this procedure. Toxic levels of Mn are typically associated with leaf chlorosis and necrosis and reduced growth (Clark, 1963; Martin et al., 1963; Lopes and Wollum, 1976; Ezeta and Santos, 1981). In *Theobroma cacao* (Ezeta and Santos, 1981), plant colonization by different mycorrhizal fungi resulted in reduced levels of Mn in the leaf tissues, and the authors suggested that when the fungi are associated with roots, they reduce absorption by root cells of

potentially toxic elements by storing them in their hyphae. Besides, the potential for Mn reduction in the rhizosphere of colonized plants is small, reducing the availability of this ion (Kothari et al., 1991). The values of Mn in the control plants were well below the highest reported values for leaves of mature trees, but still much higher than the shoot concentration measured by Simões (1973) in young plants. Thus, the possibility of Mn toxicity in the control plants cannot be discarded. It is possible that the exposure of roots to a much greater volume of sterilized soil caused the control plants to stop growth and express the toxicity effects of Mn. Despite being exposed to the same soil, the inoculated plants were probably protected from such injury. On the other hand, Simões (1973) has described similar leaf symptoms for young plants growing under phosphorus deprivation, suggesting that limitations on the absorption of this nutrient could also be mediating the leaf symptoms observed on the non-inoculated plants.

The distinct behavior shown by both treatments after transplant can also be associated with greater tolerance for transplantation those plants that established the mycorrhizal association. Brazilian pine is traditionally sown directly in the field due to a high degree of plant failure that occurs when transplantation takes place. The potential benefit of pre-inoculating plants with mycorrhizal spores in the nursery in order to increase transplant survival needs to be investigated. In this case, the time it takes for plants to express the benefits of such association must be taken into account while determining the timing of transplantation.

Extrapolation of the results obtained in this pot experiment to natural conditions must be made with caution, specially regarding the importance of *Glomus clarum* for the success of Brazilian pine in the field and the species response to mycorrhizal colonization under natural soil conditions. While this study leaves no doubt on the responsiveness of the species to mycorrhiza, it cannot lead to the conclusion that natural colonization with *G. clarum* will benefit the species under field conditions. Hepper et al. (1988) reported a high degree of competition among species of *Glomus*, to the point of total exclusion of one species by another. On these competitive relationships, *G. clarum* may or may not be one of the winners. Daft and Hogart (1983) described this *Glomus* species and three others as adopting a ruderal class of strategy because of their rapid colonization of the host root system and their profuse sporulation. They also reported *G. clarum* as being a good competitor when mixed with the others and the one generating the greatest increase in onion growth when inoculated alone. Also Schmitz et al. (2001) found *G. clarum* to be more effective than *G. etunicatum* in promoting the growth of *Poncirus trifoliata*. These isolated results give us at least some indication that the chosen fungal species might be effective in the field as well.

5. Conclusions

In conclusion, given the unquestionable benefits of the mycorrhizal associations to the species, they cannot be ignored in any program aimed at the management of existing populations or the reintroduction of the species in deforested areas. The presence of AM fungi must be ensured in areas where the species is to be reintroduced by seed sowing, and, if transplantation takes place, plants must be precolonized by mycorrhizal fungi. Finally, an effort also needs to be made in order to identify other species of mycorrhizal fungi that are efficient in colonizing and providing benefits to Brazilian pine.

Acknowledgements

We thank the Plant Anatomy Lab of the Federal University of Rio Grande do Sul for technical assistance, the Department of Soils from the same University for providing greenhouse space, and the National Council for Scientific and Technological Development (CNPq/Brazil), for the graduate fellowships awarded to the first author.

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