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GROWTH AND NUTRITION OF EUCALYPT ROOTED CUTTINGS PROMOTED BY ECTOMYCORRHIZAL FUNGI IN COMMERCIAL NURSERIES

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ABSTRACT

Ectomycorrhizal fungi (EMF) may improve the adaptation of eucalypts saplings to field conditions and allow more efficient fertilizer use. The effectiveness of EMF inoculum application in promoting fungal colonization, plant growth, nutrient uptake, and the quality of rooted cuttings was evaluated for *Eucalyptus urophylla* under commercial nursery conditions. For inoculated treatments, fertilization of the sapling substrate was reduced by 50 %. The experiment was carried out in a completely randomized design in a 4 × 4 factorial arrangement, wherein the factors were inoculum application rates of 0 (control), 5, 10, and 15 gel beads of calcium alginate containing the vegetative mycelium of *Amanita muscaria*, *Elaphomyces antracinus*, *Pisolithus microcarpus*, and *Scleroderma areolatum*, plus a non-inoculated treatment without fertilization reduction in the substrate (commercial). Ectomycorrhizal fungi increased plant growth and fungal colonization as well as N and K uptake evenly. The best plant growth and fungal colonization were observed for the highest application rate. The greatest growth and fungal colonization and contents of P, N, and K were observed at

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the 10-bead rate. Plant inoculation with *Amanita muscaria*, *Elaphomyces anthracinus*, and *Scleroderma areolatum* increased P concentrations and contents in a differential manner. The Dickson Quality Index was not affected by the type of fungi or by inoculum application rates. Eucalypt rooted cuttings inoculated with ectomycorrhizal fungi and under half the amount of commercial fertilization had P, N, and K concentrations and contents greater than or equal to those of commercial plants and have high enough quality to be transplanted after 90 days.

Keywords: symbiosis, plant nutrition, cuttings production, phosphorus, nitrogen, potassium.

RESUMO: *CRESCIMENTO E NUTRIÇÃO DE MUDAS CLONAIS DE EUCALIPTO PROMOVIDOS POR FUNGOS ECTOMICORRÍZICOS EM VIVEIROS COMERCIAIS*

A utilização dos fungos ectomicorrízicos (FEM) em eucalipto pode melhorar a adaptação das mudas no campo e permitir um uso mais eficiente de fertilizantes. A eficiência de doses de inoculante de FEM em promover a colonização ectomicorrízica, o crescimento, a absorção de nutrientes e a qualidade de mudas clonais de Eucalyptus urophylla foi avaliada em condições de viveiro comercial. Para os tratamentos com doses de inoculante, a adubação de substrato de produção das mudas foi reduzida em 50 %. O experimento foi realizado em delineamento inteiramente casualizado em esquema fatorial 4 × 4, sendo: as doses 0 (controle), 5, 10 e 15 esferas de gel de alginato de cálcio contendo micélio vegetativo de Amanita muscaria, Elaphomyces antracinus, Pisolithus microcarpus e Scleroderma areolatum, mais um tratamento não inoculado sem redução da fertilização de substrato (Comercial). Os FEM aumentaram o crescimento, a colonização e a absorção de N e K de forma igual. O melhor crescimento e colonização foram observados na maior dose de inoculante; e os maiores teores e conteúdos de P, N e K, na dose de 10 esferas. A inoculação das mudas clonais com Amanita muscaria, Elaphomyces anthracinus e Scleroderma areolatum aumenta de forma diferenciada os teores e conteúdos de P. O índice de qualidade de Dickson não foi influenciado pelos fungos e nem pelas doses de inóculo. As mudas clonais de eucalipto inoculadas pelos FEM e crescidas com a metade da adubação de substrato apresentaram teores e conteúdos de P e N e teor de K maiores ou iguais àquelas do Comercial e qualidade suficiente para o transplantio aos 90 dias.

Palavras-chave: simbiose, nutrição de plantas, produção de mudas, fósforo, nitrogênio, potássio.

INTRODUCTION

Ectomycorrhizal symbiosis is common in Brazilian large-scale eucalypts plantations. However, it is rare in forest plant nurseries due to high P and N fertilizations (Soares et al., 1990) and the use of inert substrates such as vermiculite, carbonized rice husk, and coconut fiber, which are devoid of ectomycorrhizal fungi (EMF). Despite decades of studies, fungal inoculation procedures in commercial plantations have not been established for Brazilian conditions. The lack of suitable ectomycorrhizal inocula on the market is one of the main factors that contributes to this situation (Rossi et al., 2007).

Ectomycorrhizal fungi bring benefits to plants as they enhance growth, nutrient uptake, and biotic and abiotic stress tolerance (Grazziotti et al., 2003; Aggangan et al., 2010; Sousa et al., 2012; Targhetta et al., 2013; Fernandes et al., 2014). Ectomycorrhizal fungi used in commercial eucalypts plantations have been considered an alternative for improving fertilizer uptake, making saplings more resistant to pathogens, and increasing survival performance after transplanting (Chen et al., 2006a; Souza et al., 2012), especially in low fertility soils.

Ectomycorrhizal fungi inoculation studies show that results vary according to the host plant, inoculum type, fertilizer rates, and fungal isolate or EMF species (Garbaye, 1990; Oliveira et al., 2006). Chinese studies on eucalypts seedlings demonstrated that colonization intensity differs among 15 spore collections of *Scleroderma*, but only two of them form mycorrhizae over more than 50 % of fine roots in *Eucalyptus urophylla* and four in *Eucalyptus globulus* (Chen et al., 2006c). The authors observed a total dry matter increase up to 1.6 times for *E. urophylla* and two times for *E. globulus* compared to non-inoculated seedlings. *Chondrogaster angustisporus* and *Pisolithus microcarpus* vegetative inoculum in *Eucalyptus dunnii* increased the percent colonized root length, P uptake and shoot dry mass (SDM) of colonized plants (Souza et al., 2004). *E. dunnii* inoculated with *Pisolithus* sp., grown in a mixture with vermiculite-peat culture medium, had greater colonization at the highest inoculum rate (10 % in sapling substrate), whereas shoot height, diameter, and SDM increases were observed as of a 3 % inoculum rate and greater P concentrations as of 1 % (Alves et al., 2001).

The vast majority of studies assess the effects of EMF inoculation under controlled conditions,

especially in greenhouses (Courty et al., 2010) and with seminal saplings (Alves et al., 2001; Souza et al., 2004; Chen et al., 2006c). The development of this biotechnology for routine inoculation in commercial nurseries may increase the sustainability of planted forests in Brazil, making the already competitive forestry sector even more competitive. This may also reduce dependence on limited sources of phosphate fertilizers, which could be targeted to food production. The objective of this study was to evaluate the efficiency of application rates of ectomycorrhizal fungal inocula on promoting mycorrhizal colonization, plant growth, nutrient uptake, and quality of *Eucalyptus urophylla* rooted cuttings under commercial nursery conditions.

MATERIAL AND METHODS

Nursery description

The study was carried out in a commercial nursery of eucalypts saplings located in Itamarandiba, MG, Brazil. The average annual temperature is 20.1 °C, the average minimum is 15 °C and the average maximum is 26.1 °C; the average temperature of the warmest month is 23.8 °C. Average annual pluvial precipitation is 1081.1 mm, and the rainy season occurs from October to March and represents 89 % of the total rainfall for the year (INMET, 2009). The climate is high-altitude tropical - *Cfa* (Köppen, 1918).

Experimental design

The experiment was carried out in a completely randomized design in a 4 × 4 factorial arrangement plus an additional treatment, with four replications. The experimental plot was composed of 40 saplings of the natural hybrid of *Eucalyptus urophylla* S.T. Blake; the 18 central plants were considered for sampling. Factors were application rate and EMF species, with four variations each: zero (control), 5, 10, and 15 gel beads of calcium alginate with vegetative mycelia of *Amanita muscaria* (L.) Lam., *Scleroderma areolatum* Ehrenb., *Pisolithus microcarpus* (Cooke & Massee) G. Cunn., and *Elaphomyces anthracinus* Vittad. (*Cenococcum geophilum* Fr.). For the inoculated treatments, commercial fertilization in the substrate was reduced by 50 % to prevent an inhibitory effect on ectomycorrhizal colonization. The additional treatment was non-inoculated rooted cuttings without substrate fertilization reduction (commercial). The commercial treatment was included so we could compare the results from ECM application to what has been successfully done for many years in reforestation companies.

Fungal isolates and inoculum production

The fungal isolates used were UFSC-Am161 of *A. muscaria*, UFSC-Sc129 of *S. areolatum*, ITA-06 of *P. microcarpus*, and Amance of *E. anthracinus*. The first three were sampled from eucalypts plantations. *A. muscaria* (UFSC-Am161) was obtained from a *Eucalyptus viminalis* plantation and *S. areolatum* (UFSC-Sc129) from *E. dunnii*, both from the state of Santa Catarina, Brazil. *P. microcarpus* (ITA-06) was obtained in eucalypts plantations from the State of Minas Gerais, Brazil, and *E. anthracinus* (Amance) was obtained from a *Fagus sylvatica* plantation in France, which is generally considered dominant in natural ecosystems.

Fungi were grown in a liquid PGKM (Kuek, 1996) culture medium under submerged conditions (Rossi et al., 2007). After cultivation, mycelium was homogenized at 3,600 rpm and inserted into 4 mm gel beads of calcium alginate. Inocula were produced at the Laboratory of Bioprocesses of the Universidade Federal de Santa Catarina, where they went through feasibility tests. After 100 % viability, 50 beads of each inoculum were sent to the Universidade Federal dos Vales do Jequitinhonha e Mucuri for plant experiments.

Substrate production for rooted cuttings

The substrate used for all treatments was composed of a mixture of vermiculite, carbonized rice husk, and coconut fiber at a 2:1:1 (v:v:v) ratio. This substrate is relatively inert in terms of readily available nutrients for plants. Routine nursery fertilization was used for the commercial treatment and consisted of 205 mg dm^{-3} N and 456.4 mg dm^{-3} P (11.9N-60.8P-00K, MAP); 95 mg dm^{-3} N, 13.1 mg dm^{-3} P, and 41.5 mg dm^{-3} K [19N-06P-10K slow-release (3 months), Osmocote®]; 41 mg dm^{-3} Mg (Magnesium sulfate heptahydrate - with 9 % Mg); 360 mg dm^{-3} K (Potassium chloride - 62 % K); 143 mg dm^{-3} Ca (Calcium chloride - 27 % Ca); 1.56 mg dm^{-3} Fe (Fe chelate for fertirrigation Ferrilene® - 6 % Fe); 2 mg dm^{-3} B (Boric acid - 17 % B); 1 mg dm^{-3} Cu (Copper sulfate - 35 % Cu); 5 mg dm^{-3} Mn (Manganese sulfate - 30 % Mn); and 1 mg dm^{-3} Zn (Zinc sulfate heptahydrate - 20 % Zn). For inoculated treatments, including the control, these amounts of substrate fertilizers were reduced by 50 %.

Chemical composition of the substrate with half the amount of fertilizer was analyzed according to Embrapa (2011): pH(H_2O) 7.3; Carbon organic 132.3 g kg^{-1}; N 6.1 g kg^{-1}; P 372.4 mg dm^{-3}; K 1.85 mg dm^{-3}; Ca^{2+} 5.7 $cmol_c$ dm^{-3}; Mg^{2+} 1.3 $cmol_c$ dm^{-3}; Al^{3+} 0.1 mg dm^{-3}; effective cation-exchange capacity 11.8 $cmol_c$ dm^{-3}; cation-exchange capacity at pH 7 12.9 $cmol_c$ dm^{-3}; Al saturation 1 %; and base saturation 91 %.

Inoculation, planting of mini-cuttings, and experimental maintenance fertilization

Tubes with a volume of 55 cm^3 received substrates and were subjected to vibration for compression. Then the substrate of 0.02 m upper layer was

removed. For inoculated treatments, alginate beads with mycelium were applied on the substrate according to the pre-established rates, and then the tubes were filled. Because the compression, the complete amount of substrate was approximately 77 cm^3 substrate (13,000 tubes were filled up with 1.0 m^3 substrate). Thus, each rooted cutting was fertilized with 36 mg P for the commercial treatment and 18 mg P in the inoculated treatments, in which fertilization in the substrates was reduced by 50 %.

Subsequently, 0.06 to 0.08 m eucalypt mini-cuttings with two pairs of leaves were planted and immediately placed in a greenhouse and kept under intermittent mist irrigation, where they remained for 30 days. After 15 days under greenhouse conditions, the cuttings were fertirrigated by a hand sprinkler at seven-day intervals with 2 L m^{-2} fertilizer growing solution composed of 0.75 g L^{-1} calcium nitrate with 19 % Ca and 15 % N; 0.9 g L^{-1} N6-P12-K36 Kristalon®; 2.5 g L^{-1} ammonium sulfate - 20 % N; 25 mg L^{-1} Ferrilene® - 6 %; 8.5 mg L^{-1} boric acid - 17 % B; 1.2 mg L^{-1} copper sulfate - 35 % Cu; 0.7 mg L^{-1} zinc sulfate heptahydrate - 20 % Zn; 0.2 mg L^{-1} sodium molybdate - 39 % Mo; and 6.5 mg L^{-1} manganese sulfate - 30 % Mn. After this period, saplings underwent an acclimatization stage, in which they remained for 10 days under shade and, up to 68 days, receiving two weekly fertirrigations with two manual waterings of 2 L m^{-2} of solution fertilizer growing solution as described above. Subsequently, they received three fertirrigations in one week with a hardening fertilizer solution composed of 0.375 g L^{-1} calcium nitrate 19 % Ca and 15 % N; 0.45 g L^{-1} N6-P12-K36 Kristalon®; 1 g L^{-1} potassium chloride - 62 % K; 25 mg L^{-1} Ferrilene® - 6 %; 8.5 mg L^{-1} boric acid - 17 % B; 1.2 mg L^{-1} copper sulfate - 35 % Cu; 0.7 mg L^{-1} zinc sulfate heptahydrate - 20 % Zn; 0.2 mg L^{-1} sodium molybdate - 39 % Mo; and 6.5 mg L^{-1} manganese sulphate - 30 % Mn.

Considering that all growth (12 applications) and hardening (3 applications) solutions applied within the tube upper area (27-mm inner diameter) infiltrated, fertirrigations provided 0.7 mg P per sapling. In addition, cuttings did not receive any phytosanitary treatment.

Sampling and evaluations

Sapling shoot height (SH) and root collar diameter (CD) were evaluated at 90 days; after that, they were cut close to upper edge of the tube separating shoots from roots. Roots were washed under tap water to remove substrate; then, composite root samples of each plot were obtained, cut into lengths of approximately 0.02 m, and stored in formaldehyde-acetone-ethanol at 9:1:1 (v:v:v) ratio for later evaluation of ectomycorrhizal colonization (Giovanetti and Mosse 1980). The remaining roots and shoots were dried to constant weight in a forced-air oven at 65 °C for measurement of shoot dry matter (SDM) and root dry matter (RDM). From these data, the Dickson Quality Index (DQI) (Dickson et al., 1960) was determined through the formula: DQI = (SDM/RDM)/[(SH/CD) + (SDM/RDM)].

After weighing SDM, leaves were detached and weighed to determine leaf dry matter (LDM). Then they were ground in a Willey type mill and digested in nitric-perchloric acid at a 2:1 (v:v) ratio. After that, P concentration was determined by colorimetry and K concentration by photometry (Malavolta et al., 1997). In addition, N concentration was determined by the Kjeldahl method (distillation) after sulfuric digestion. Therefore, leaf N, P, and K contents were calculated based on the leaf dry matter (LDM) of each plant.

Statistics

Shoot height, root collar diameter, and colonized root length percentage data were transformed using *ln* (x + 2) since they did not follow normal distribution and, or, were lower than 30 %. In the factorial, mean values were compared by the Tukey test ($p<0.05$) only when the fungal effect was observed. When the fungus × application rate interaction was significant, regression was performed. For comparison between each inoculated treatment (with substrate fertilization reduction) and the commercial treatment (without substrate fertilization reduction) the Dunnett test ($p<0.05$) was used. Correlation analyses were performed among all variables, considering a 5 % level of probability by the "t" test as significant.

RESULTS

Inoculation effect

In plants grown under 50 % of fertilizer, the CD, SH, SDM, and EMF colonization was influenced by the inoculum rate and not by the fungal species (Table 1, Figure 1). In contrast, RDM was influenced by the fungal species and not by the application rate (Tables 1 and 2). The highest RDM was observed for *S. areolatum* (464 mg per cutting) inoculated plants, which were 1.3 times higher than *P. microcarpus* inoculated ones (368 mg per cutting). DQI was not affected either by the type of fungi or by inoculum application rates (Tables 1 and 2).

Root collar diameter and SH showed linear behavior with increasing inoculum application rates, reaching 2.78 mm for CD and 0.25 m for SH (Figures 1a, 1b) at the highest rate (15 beads). At this rate, CD was 1.1 times higher than for non-inoculated plants (Figure 1a), and SH was 1.2 times higher (Figure 1b). Although the SDM had been influenced by the inoculum application

Table 1. Summary of ANOVA table for all variables analyzed

Variable	Rate (R)	Fungi (F)	R × F	Commercial × factorial design	CV	MSR
					%	
Collar diameter	**	ns	ns	**	2.58	0.0016
Shoot height	**	ns	ns	**	1.75	0.0032
Shoot dry matter	*	ns	ns	**	15.93	17,475.66
Root dry matter	ns	**	ns	**	17.66	5,895.76
Colonized root length	**	ns	ns	**	14.68	0.0978
Dickson Quality Index	ns	ns	ns	ns	27.35	0.0001
P leaf concentration	**	**	**	**	8.54	0.0058
P leaf content	**	*	**	ns	29.41	0.0172
N leaf concentration	**	ns	ns	ns	20.63	11.0877
N leaf content	**	ns	ns	**	34.43	8.1055
K leaf concentration	**	ns	ns	*	14.97	12.5376
K leaf content	**	ns	ns	**	24.98	9.2611

ns: not significant; * and **: significant at 5 and 1 % by the "F" test, respectively. CV: coefficient of variation; MSR: mean square of residuals.

Table 2. Root dry matter and Dickson Quality Index for *Eucalyptus urophylla* rooted cuttings that received increasing inoculum rates of *Amanita muscaria*, *Elaphomyces anthracinus*, *Pisolithus microcarpus*, and *Scleroderma areolatum* and grown for 90 days in a commercial nursery

Fungus	Commercial[1]	Calcium alginate beads with mycelium per mini-cutting				Mean
		0	5	10	15	
		Root dry mass (mg per plant)				
A. muscaria		410*	401*	368*	448*	407 AB
E. anthracinus		435*	375*	439*	437*	422 AB
P. microcarpus		381*	377*	342*	373*	368 B
S. areolatum		486*	522*	403*	445*	464 A
Mean	747	428	419*	388	426	415
		Dickson Quality Index				
A. muscaria		0.03*	0.04*	0.03*	0.05*	0.04
E. anthracinus		0.04*	0.04*	0.04*	0.05*	0.04
P. microcarpus		0.04*	0.04*	0.03*	0.03*	0.04
S. areolatum		0.04*	0.05*	0.03*	0.05*	0.04
Mean	0.11	0.04	0.04*	0.03	0.04	0.04

[1] Commercial: non-inoculated rooted cuttings without substrate fertilization reduction. Means followed by * differ from commercial rooted cuttings at 5 % by the Tukey test.

rate (Table 1), no regression equation could be fitted (Figure 1c). Plants inoculated with 15 beads had higher SDM (862 mg per plant), 1.2 times higher than non-inoculated plants (733 mg per plant). Colonized root length percentage increased together with application rates for all fungi in a similar manner, showing quadratic behavior; and the largest colonization (16 %) was found at the highest rate (Figure 1d). The non-inoculated plants grown in substrate with 50 % fertilization reduction had an average of 1% colonization.

Leaf P concentration and content were influenced by inoculum application rates and fungal species interaction (Table 1, Figures 2a, 2b). Among the EMF evaluated, only *P. microcarpus* did not increase plant P concentration and content at increasing inoculum application rates. For the others, leaf P concentration and content increased up to 10 beads per mini-cutting. However, regression equations were fitted solely for leaf P concentration and contents in plants inoculated with *A. muscaria* and contents in plants inoculated with *S. aerolatum* (Figures 2a, 2b). In such cases, there is quadratic behavior with mean values double those of non-inoculated plants. Although no regression equation was fitted, the highest P concentrations were observed for *S. aerolatum* inoculated plants (2.2 times higher than non-inoculated ones) and

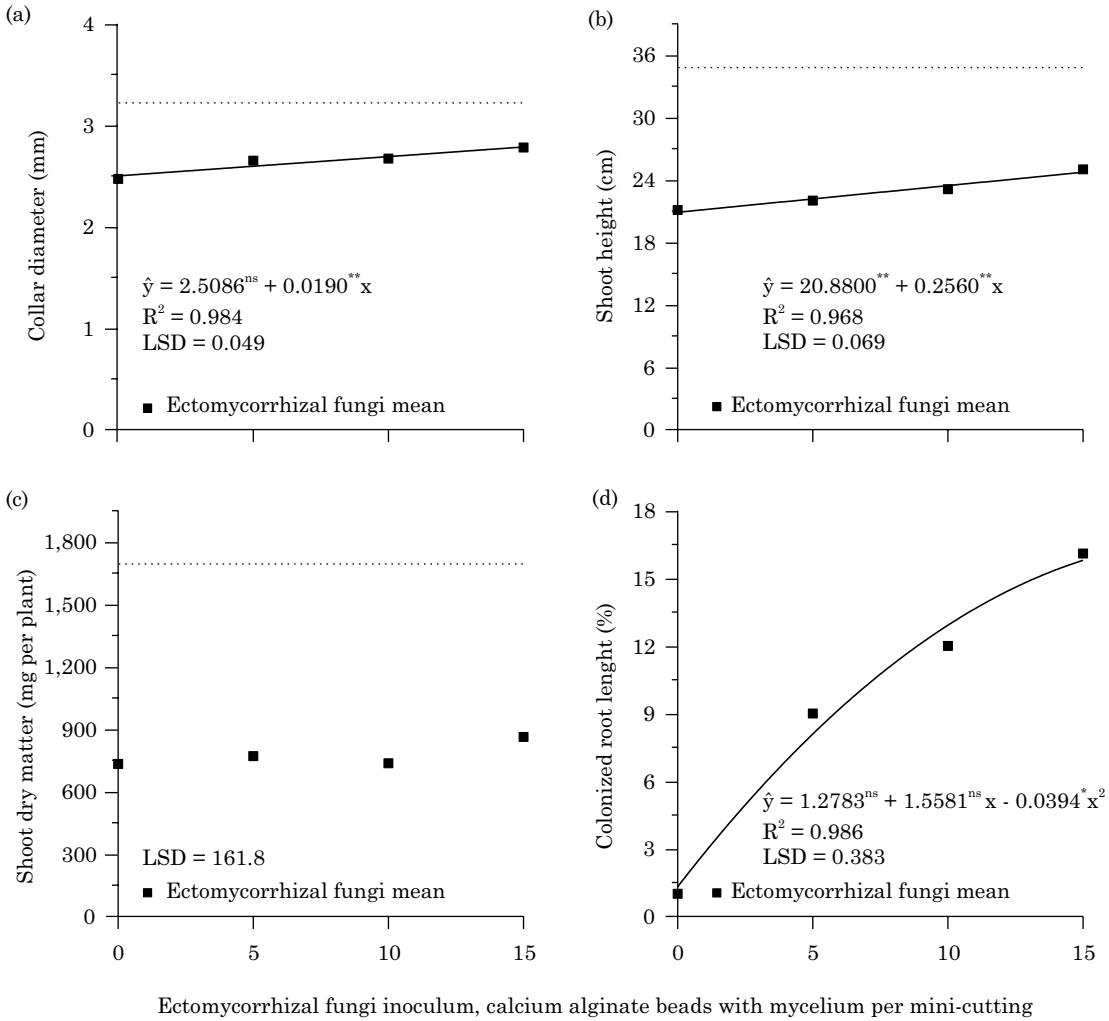

Figure 1. Collar diameter (a), shoot height (b), shoot dry matter (c) and colonized root length (d) of *Eucalyptus urophylla* rooted cuttings that received increasing inoculum application rates of *Amanita muscaria*, *Elaphomyces anthracinus*, *Pisolithus microcarpus*, and *Scleroderma areolatum* and grown for 90 days in a commercial nursery. Dash-dot line shows results for non-inoculated rooted cuttings without substrate fertilization reduction (commercial). Since the commercial sapling was not colonized, the dotted line is absent in (d). * and **: significant at, respectively, 5 and 1 % by the "t" test; ns: not significant. LSD: least significant difference.

the highest contents were observed for plants inoculated with *E. anthracinus* (2.0 times higher than non-inoculated ones), both cases at the 10 bead per mini-cutting application rate.

Both leaf concentration and N and K content were influenced by inoculum application rates and not by the fungal species (Table 1, Figures 2c, 2d, 2e, 2f). Leaf N and K concentration and content increased quadratically with inoculum application rates (Figures 2c, 2d, 2e, 2f). The highest N concentrations were observed in plants receiving the rate of 14 beads with inoculum (18.9 g kg^{-1}), 1.6 times higher than non-inoculated plants (12.0 g kg^{-1}) (Figure 2c). Leaf N content was higher in plants supplied with 13 beads (13.7 mg per plant), which was 2.4 times higher than in non-inoculated plants (5.39 mg per plant) (Figure 2d). Regarding K, larger concentrations (27.1 g kg^{-1}) were observed in plants treated with 11 beads, which were on average 1.6 times higher than concentrations in non-inoculated plants (16.9 g kg^{-1}) (Figure 2e). In addition, the highest leaf K contents were found for saplings with 15 beads (9.9 mg per plant), 30 % times higher than contents for non-inoculated ones (7.6 mg per plant) (Figure 2f).

Colonization did not correlate with the CD, SH, SDM and RDM, but was positively correlated with the contents of all nutrients evaluated (Table 3). However, only K contents correlated with SH and SDM.

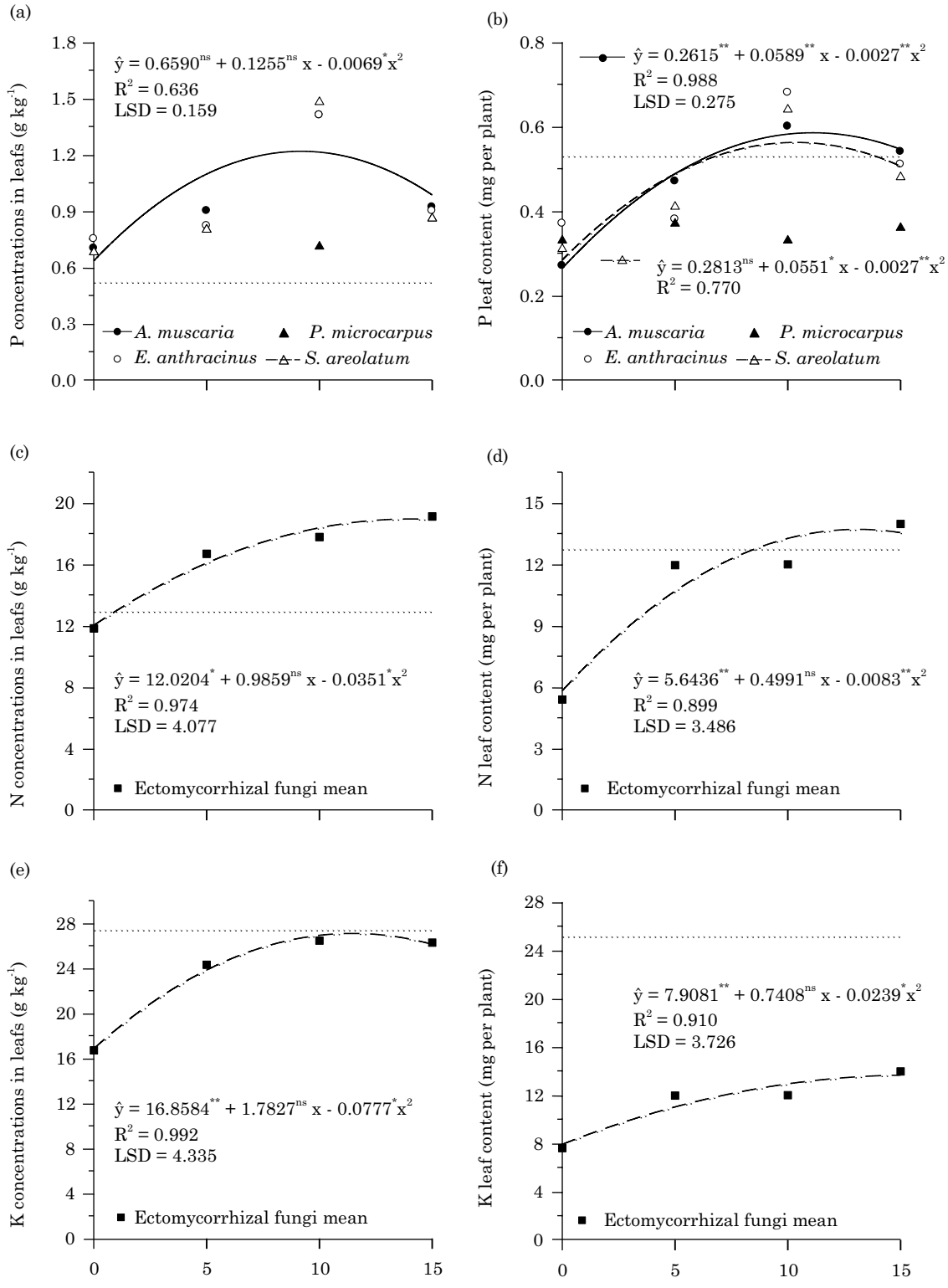

Figure 2. Leaf concentrations and contents of P (a, b), N (c, d), and K (e, f) for *Eucalyptus urophylla* rooted cuttings that received increasing inoculum application rates of *Amanita muscaria*, *Elaphomyces anthracinus*, *Pisolithus microcarpus*, and *Scleroderma areolatum* and grown for 90 days in a commercial nursery. Dash-dot line shows results for non-inoculated rooted cuttings without substrate fertilization reduction (commercial). * and **: significant at, respectively, 5 and 1 % by the "t" test; ns: not significant. LSD: least significant difference.

Table 3. Correlation matrix between collar diameter, shoot height, shoot dry matter, root dry matter, Dickson Quality Index, colonized root length, and leaf concentrations and contents of P, N, and K for *Eucalyptus urophylla* rooted cuttings that received increasing inoculum application rates of *Amanita muscaria*, *Elaphomyces anthracinus*, *Pisolithus microcarpus*, and *Scleroderma areolatum* and grown for 90 days in a commercial nursery

Variable	Collar diameter	Shoot height	Shoot dry matter	Root dry matter	Dickson Quality Index	Colonized root length	P leaf concentration	P leaf content	N leaf concentration	N leaf content	K leaf concentration
Shoot height	0.638**										
Shoot dry matter	0.608**	0.794**									
Root dry matter	0.286*	0.563**	0.681**								
Dickson Quality Index	0.489**	0.659**	0.963**	0.771**							
Colonized root length	0.139^{ns}	0.070^{ns}	-0.104^{ns}	-0.235^{ns}	-0.214^{ns}						
P leaf concentration	-0.009^{ns}	-0.182^{ns}	-0.296^{ns}	-0.266^{ns}	-0.329^{ns}	0.401**					
P leaf content	0.336**	0.165^{ns}	0.390**	0.101^{ns}	0.352**	0.361**	0.616**				
N leaf concentration	0.098^{ns}	0.025^{ns}	-0.063^{ns}	-0.250^{ns}	-0.132^{ns}	0.570**	0.282*	0.286*			
N leaf content	0.393**	0.406**	0.611**	0.253*	0.566**	0.328**	-0.013^{ns}	0.591**	0.660**		
K leaf concentration	0.238^{ns}	0.347**	0.142**	0.030^{ns}	0.035^{ns}	0.441**	0.382**	0.375**	0.453**	0.343**	
K leaf content	0.516**	0.656**	0.808**	0.485**	0.742**	0.163^{ns}	-0.034^{ns}	0.615**	0.233^{ns}	0.768**	0.599**

* and **: significant at, respectively, 5 and 1 % by the "t" test; ^{ns}: not significant.

Effect of substrate fertilization reduction

Plants fertilized at a level of 50 % and inoculated with EMF showed a smaller CD, SH, SDM, RDM, and DQI than the commercial treatment (non-inoculated and 100 % fertilization) (Table 2, Figure 1). Commercial plants had no ectomycorrhizal colonization (Figure 1d). Those non-inoculated, but grown with 50 % reduction in fertilizers showed 1 % of their root length colonized by EMF.

Root dry matter was 1.5 times and DQI was 2.7 times higher for commercial plants than those grown under 50 % fertilization reduction (Table 2). For plants under fertilization reduction, the DQI index classified the non-inoculated plants as equal to those that had the highest rate of inoculum (Table 2). Despite reducing fertilization by half, leaf P, N, and K concentrations and contents for inoculated saplings with the highest inoculum rates were greater than or equal to the commercial ones (Figures 2a, 2b, 2c, 2d, 2e), except for leaf K content (Figure 2f). Plants inoculated with 10 beads of *S. areolatum* inoculum had a leaf P concentration (1.48 g kg^{-1}) 2.8 times higher than commercial plants (0.53 g kg^{-1}) (Figure 2a). Rooted cuttings inoculated with 15 beads had an N concentration (19.1 g kg^{-1}) 1.5 times greater than the commercial cuttings (12.9 g kg^{-1}) (Figure 2c).

DISCUSSION

Inoculation effect

Inoculation with the highest EMF application rate in eucalypt mini-cuttings in a commercial nursery promoted growth, ectomycorrhizal symbiosis, and improved nutrient uptake. Ectomycorrhizal fungi species differed in their capacity to increase leaf P concentration and content (Figures 2a, 2b); however, they did not differ in terms of growth promotion (Figures 1a, 1b, 1c) and root colonization ability (Figure 1d), and they also raised leaf K and N concentration and content (Figures 2c, 2d, 2e, 2f). This similar behavior among EMF species observed in growth promotion, root colonization, and plant nutrition for some nutrients differs from most studies found in the literature (Alves et al., 2001; Silva et al., 2003; Souza et al., 2004, 2008; Chen et al., 2006c).

The increase in eucalypt CD, SH, and SDM promoted by inoculation with increasing application rates of different EMF species (Figures 1a, 1b, 1c) was also observed by other authors in eucalypt seedlings (Alves et al., 2001; Souza et al., 2004, 2008). *E. dunnii* seedlings had greater CD (1.4 times), SH (1.3 times), and SDM (1.7 times) levels when inoculated with *Pisolithus* sp. (UFSC-Pt24)

isolate compared to non-inoculated plants (Alves et al., 2001); and they had greater SDM when inoculated with *Scleroderma* sp. (UFSC-Sc68) isolate (3.3 times) (Souza et al., 2008), with *P. microcarpus* (UFSC-Pt116) isolate (1.2 times), and with *C. angustisporus* (UFSC-Ch163) isolate (1.3 times) (Souza et al., 2004). These results are greater than or equal to the CD and SDM observed in this study, which were 1.1 and 1.2 times higher respectively, always at the highest application rate without reaching a maximum (Figures 1a, 1b, 1c). In contrast, the growth of rooted cuttings did not correlate with colonization (Table 2).

Major growth observed at the highest application rate (Figures 1a, 1b, 1c) differs from results of other studies. In *E. dunnii* seedlings inoculated with *Pisolithus* sp. (UFSC-pT24) and cultivated for 100 days, the largest CD (2.5 mm) and SH (26.2 cm) were observed in seedlings that received 3 % inoculum produced in a mixture of a vermiculite-peat culture medium (Alves et al., 2001). This difference may be due to different types of inocula used in this study. Another explanation may be that in this study a possible effect of the association with arbuscular mycorrhiza fungi that could be present together with ECM fungi in the initial growth of eucalyputs saplings was not considered.

The ideal inoculant application rate depends on the goals of the study. If the expected benefit of ectomycorrhizal fungi is after transplanting, by increasing the survival and growth of plants in the field, the rate that would provide greatest root colonization should be used, even if it did not result in increased growth under nursery conditions. This should occur without preventing the saplings from reaching the standard considered ideal for transplanting in the same growing period. However, if the expected benefit is greater rooting of promising clones, the most extensive colonization could undermine the rooting process rather than promote it.

Low colonization in the control (non-inoculated with 50 % fertilization) (Figure 1d) may have been because the substrate for rooted cuttings was composed of vermiculite, carbonized rice husk, and coconut fiber, that is, only inert materials. Ectomycorrhizae presence in the control may be due to spores brought by irrigation water or wind-borne spores, since the nursery is located near a eucalypt plantation. Notably, in this study, the substrate for rooted cuttings was not sterilized and the irrigation water was not distilled, unlike other studies with EMF (Alves et al., 2001; Souza et al., 2004). This shows that by using suitable rates of P for colonization, sapling production conditions used in the forestry sector may allow the introduction of selected fungal isolates.

The increase in colonization rate observed in inoculated rooted cuttings from increasing application rates (Figure 1d) was also observed in *E. dunnii* seedlings that received *Pisolithus* sp. inoculum produced in a vermiculite-peat-culture medium (Alves et al., 2001). Root length colonization of 16 %, observed at the highest application rate of inoculum (15 beads), was higher than the highest percentage observed in *E. dunnii* inoculated by 10 species and, or, EMF isolates (Souza et al., 2008). However, it was lower than that reported in eucalypt by other authors (Alves et al., 2001; Chen et al., 2006c). Nevertheless, as mentioned earlier, all these studies had lower P fertilization application rates.

The similar behavior among fungal species in promoting plant growth and root colonization and the lower intensity of inoculation benefits on growth characteristics (Figure 1) compared to the literature may be due to the different type of inocula and different experimental conditions. Furthermore, the interaction between symbionts may have been poor because the fungi tested were obtained from various species of eucalypt and other genera, also from different regions. This demonstrates the need for selection of specific isolates. The aforementioned studies differ from the current study because they did not use clonal saplings and were developed under greenhouse conditions with sterilized substrates, low P fertilization, higher plant growth time (100 and 120 days), and inoculum production in a vermiculite-peat-culture medium mixture. In these studies, the amount of P for seedlings ranged from 0.25 mg using $Ca(H_2PO_4)_2.H_2O$ (Souza et al., 2004) to 8.7 mg using 14N-8P-8K slow-release fertilizer (Nutricote®) (Alves et al., 2001), that is, up to two times lower than the 18 mg P for rooted cuttings provided in the MAP source (60.8 % P_2O_5) plus slow-release fertilizer 19N-6P-10K (Osmocote®) used in this study, without considering the weekly fertirrigation with P.

Despite the fact of greater ectomycorrhizal colonization (16 times) and nutrient uptake in inoculated plants, they and the control plants grown under fertilization reduction had the same DQI values (Table 2), which may be due to the fact that this index does not consider those characteristics.

The increase in inoculated plant P content for three out of four EMFs used, according to inoculum application rates and their differential ability in promoting P uptake (Figures 2a, 2b) corroborates with some results reported in the literature (Alves et al., 2001; Chen et al., 2006b; Souza et al., 2008). *E. dunnii* seedlings inoculated with *P. microcarpus* (UFSC-Pt188) accumulated nine times more P (528 µg per plant) than non-inoculated seedlings (60 µg per plant) (Souza et al., 2008). For *E. urophylla* seedlings inoculated with six species or isolates of *Scleroderma*, the increase was up to 1.8 times (Chen et al., 2006b). Evaluating seven application rates of P for maximum efficiency

of mycorrhizal association between seedlings of *Eucalyptus grandis* and *Pisolithus tinctorius* (Pt 854) grown in 2.0 dm^3 of soil, Vieira and Peres (1988) observed increased P content only up to 23 mg kg^{-1} P (third rate), in which inoculated plants stocked 5.1 times more P than non-inoculated ones. *E. grandis* seedlings inoculated with three isolates of *Pisolithus* sp. increased P content from 1.3 to 2.0 times compared to non-inoculated ones (Andreazza et al., 2004). Thus, it can be noted that the current study, despite the high P fertilization rate, the two-fold increase of P content in inoculated seedlings over the control was similar to results from two out of four outcomes listed above. Moreover, in the present study, EMF inoculation also increased leaf P concentration (2.2 times), indicating that the increased production of SDM in inoculated plants was not sufficient to lead to a dilution effect on P and showing that three out of four EMF were able to promote better P nutritional status and "luxury" uptake. These observations are confirmed by the correlation between colonization and P concentrations, and also the absence of correlation between P concentration and growth (Table 3). These increased P concentrations can enhance plant growth after transplanting in the field compared to non-inoculated plants (Chen et al., 2006a). Usually, in the literature, for data on EMF inoculation in eucalypt, nutrient levels are usually not shown, probably due to the absence of an inoculation effect on this variable.

Just as for P concentration, there are few studies on the effect of EMF inoculation on N and K concentration and content in forest species. This may also be due to lack of observation of inoculation effects on these variables, because it is known that the greatest EMF benefit is on the uptake of low-mobility elements in the soil, such as P. Studies on eucalypt showing results from these variables are even rarer. In *E. grandis* saplings inoculated with the Pt 854 isolate of *P. tinctorius*, which received growing application rates of P, increases of 3.3 times for N content and 2.6 times for K were observed when compared to non-inoculated plants at the rate of 23 mg kg^{-1} P (Vieira and Peres, 1988). In *E. grandis* seedlings inoculated with eight fungal treatments (four *Pisolithus* sp. isolates or mixtures thereof), increases in N content were observed for seven treatments and increases in K content for three, with the largest increases in N of 2.3 times and K of 1.9 times compared to the control (non-inoculated) (Silva et al., 2003). In *E. urophylla* seedlings inoculated with spores of six species or isolates of *Scleroderma*, increases of up to 2.2 times in N content and up to 1.7 times in K content was observed for seedling shoots (Chen et al., 2006b). In general, the rises in N and K content shown above are the best results of the species and/or isolates evaluated. In some cases they were higher and in others lower than the increase in N (1.6 times) and K (1.8 times) content observed in this study (Figures 2d, 2f). In addition, unlike the present study, the effect of inoculation on these nutrients was influenced by the species and isolate. Once again, it is important to remember that fertilization rates in the present study, although reduced by 50 %, were much larger than those reported in the literature, and this may have influenced both the effective response and lack of variation of the results among species. The studies mentioned above do not show any results on N, P, and K concentrations, which suggests that the higher nutrient contents came from increased plant growth from the EMF effect on other factors, such as water uptake, which go against the results of this study, which affirms the influence of nutrient uptake. Therefore, in addition to the increase in K content and concentration promoted by EMF inoculation (Figure 2), the positive correlation between K concentration and colonization, and the lack of correlation between all the growth characteristics and colonization (Table 3), indicates that the observed increase in growth (Figure 1), although not very expressive, was due to better K nutrition.

Effect of substrate fertilization reduction

Plants grown in substrate with reduced fertilization and colonized with fungi were healthy; they did not show any symptoms of nutritional deficiency and pathogen damages; clods were well-shaped; plants had more than four pairs of well-formed leaves; and SH was greater than 20 cm, as proposed by Alfenas et al. (2004). Nevertheless, these plants were smaller than those of the commercial treatment (Table 2, Figure 1).

Ectomycorrhizal absence in commercial saplings (Figure 1d), different from that observed in control plants, might be due to the larger amount of fertilizer used. It is known that high rates of fertilizers, especially phosphates, inhibit ectomycorrhizal colonization (Soares et al., 1990; Souza et al., 2004). This result demonstrates the importance of better defining the best P application rate that provides colonization able to promote benefits to the plants without the need for increasing sapling production time.

As the calculation of DQI considers CD, SH, and SDM, and these characteristics were higher for commercial plants (Figure 1), this index classified commercial plants as being more suitable for transplanting (Table 3). Nevertheless, it is reported in the literature that colonized and well-nourished plants have adaptive advantages after transplanting to the field, such as increased survival and growth (Chen et al., 2006a). In addition, as discussed above, the plants grown in the substrate with reduced fertilization were suitable for transplanting. Thus, the DQI is not adequate for evaluating the quality of EMF colonized rooted cuttings.

The higher P, N, and K concentrations, and content of N and P in plants receiving the highest application rates of inoculum compared to commercial plants showed that EMF inoculation was effective in promoting enhanced nutrient supply. In addition, providing plants with mycorrhizae at the time of transplanting can help with nutritional balance under commercial nursery conditions. Inoculated plants also showed higher N and K concentrations (Figures 1c, 1e) than those considered suitable for rooted cuttings of *E. grandis* (N: 13-15 g kg^{-1}; K: 15-20 g kg^{-1}) at an age of 80 to 100 days (Alfenas et al., 2004). Regarding P concentration, although it was lower in the inoculated plants than the level adequate for rooted cuttings of eucalypt (P 1.5-2.0 g kg^{-1}) (Alfenas et al., 2004), the concentration observed in plants inoculated with 10 beads was 2.4 times higher than the concentration in commercial plants.

Enhanced nutrient uptake by rooted cuttings promoted by EMF compared to non-inoculated plants is unprecedented in the literature and displays the possibility of eucalypt forest establishment using previously colonized plants, within Brazilian conditions. This reinforces the need for studies to evaluate the influence of ectomycorrhizal symbiosis and the need for sapling quality indexes to consider the root ectomycorrhizal colonization rate at the time of transplanting. It is recommend that the colonized root length percentage be one of the key parameters in assessing seedling quality since it is related to plant vigor and fitness for growth and development under field conditions, increasing the chances of plant survival (Garbaye, 1990).

CONCLUSIONS

EMF inoculation promoted similar increases in growth, colonization, and N and K uptake for rooted cuttings of eucalypt grown in commercial nurseries.

The highest inoculum application rate provided increased shoot height, collar diameter, dry matter production, and fungal colonization.

Application rates near 10 beads per mini-cutting provided higher concentrations and contents of P, N, and K.

Inoculation for eucalypt rooted cuttings by *Amanita muscaria* (UFSC-Am161), *Elaphomyces anthracinus* (Amance), and *Scleroderma areolatum* (UFSC-Sc129) increased concentrations and contents of P in a different manner.

The rooted cuttings colonized by ectomycorrhizal fungi have sufficient quality for transplanting at 90 days.

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