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Efficiency of the On-Farm Mycorrhizal Inoculant and Phonolite Rock on Growth and Nutrition of *Schinus terebinthifolius* and *Eucalyptus saligna*

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ABSTRACT: The production of tree seedlings with high survival rate and growth is influenced by the substrate, which may be composed of biological and non-biological materials that help plant growth. The objective of this study was to evaluate the efficiency of a mycorrhizal inoculant on farm and the use of a potassic rock (phonolite) on growth and nutrition of *Eucalyptus saligna* Sm. and *Schinus terebinthifolius* Raddi under greenhouse conditions. Both species were assigned to three mycorrhizal treatments [no inoculated control (SP), no inoculation + pulp sludge and carbonized rice husk (LC), and mycorrhizal inoculant (IN)] and four phonolite rock treatments [no phonolite (F_0) and addition of phonolite in particle sizes of 0.037 mm (F_1), 0.074 mm (F_2), and 0.149 mm (F_3)]. The IN treatment consisted of inoculum of *Claroideoglomus etunicatum* (Becker & Gerd.) C. Walker & A. Schussler (isolate MGR288A) and *Dentiscutata heterogama* (Nicol. & Gerd.) Sieverd. F.A. Souza & Oehl (isolate PNB102A) produced on farm with pulp sludge and carbonized rice husks. After 120 days, plants were evaluated for height, stem diameter, shoot and root dry weight, shoot K and P, and mycorrhizal colonization. Growth parameters were used to calculate the Dickson Quality Index (DQI) for seedlings. Growth parameters of *S. terebinthifolius* demonstrated synergistic and positive effects when different particle sizes of phonolite and on-farm mycorrhizal inoculum were used together. For *E. saligna*, phonolite and on-farm mycorrhizal inoculum had little effect on growth parameters, although the mycorrhizal inoculum increased K and P content in the F_0 and F_2 treatments. The DQI of *S. terebinthifolius* was higher with IN compared to SP and LC with phonolite, whereas this index was not influenced by most treatment combinations for *E. saligna*. Overall, the percentage of mycorrhizal root colonization for both species was significantly higher when phonolite was present in the substrate. The interaction of mycorrhizal inoculum produced on farm and phonolite rock has the potential to increase growth and nutrition of *S. terebinthifolius* and *E. saligna*.

Keywords: woody species, mycorrhiza, phonolite, biofertilizers, mycorrhizal colonization, potassium.

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INTRODUCTION

Reforestation includes planting of native and exotic woody species through human intervention and represented 7 % of the total area covered by forests in 2012 (FAO, 2012). This activity contributes to forest (e.g., wood) and non-forest products, such as carbon sequestration, rehabilitation of degraded environments, and protection of watershed and agricultural areas (Evans, 2009). To support this activity, it is necessary to produce high quality seedlings, especially those with high survival and growth rates (Ritchie, 1984). Among factors influencing seedling growth and quality are the plant genetic material, water and nutrition regime, containers, and type of substrate (Silva et al., 2012).

Inoculation with microorganisms that help plant growth and nutrition, such as nitrogen-fixing bacteria and arbuscular mycorrhizal fungi (AMF), represents a management practice that improves substrate quality for growing seedlings of woody species (Abiala et al., 2013; Pellegrino and Bedini, 2014). Furthermore, addition of powdered rocks containing nutrients important for plant growth provides a better substrate for raising seedlings (Ramos et al., 2014).

Seedling inoculation with AMF significantly increases growth, nutrition, and survival rate under field conditions of many native woody species (Carneiro et al., 1998; Siqueira et al., 1998). Arbuscular mycorrhizal fungi colonize the plant root cortex without causing any morphological change in root external morphology and once mycorrhizal symbiosis is established, the plant host provides energetic substrate to the fungus to complete its life cycle while the fungus takes up nutrients from the soil solution and translocates them to the plant (Smith and Read, 2008). Mycorrhizal association is able to provide up to 80 % of the P and 10 % of the K required for plant development (Marschner and Dell, 1994).

The benefits to plants provided by AMF make these microorganisms suitable for use as components of biofertilizers. However, production of AMF-based biofertilizers must overcome some challenges, such as the selection of efficient strains and an appropriate carrier (Malusa et al., 2012; Herrmann and Lesueur, 2013). The on-farm method for producing mycorrhizal inoculant is very promising (Douds Jr et al., 2005; Siddiqui and Kataoka, 2011), considering the low cost associated with its production and the possibility of utilizing native AMF strains adapted to local soil conditions (Douds Jr et al., 2005; Schlemper and Stürmer, 2014).

Addition of powdered rock improves the nutrient availability of the soil and substrate used for seedling production (Theodoro et al., 2013), aiming to achieve the same seedling growth rate as that achieved using soluble fertilizers (Stamford et al., 2011). Using powdered rock as a fertilizer also decreases the use of imported synthetic fertilizers (Cola and Simão, 2012) and helps to give an appropriate destination to some waste material from mining activities (Carvalho, 2012). The main rocks used as fertilizers are igneous rocks such as basalt, andesite, phonolite, anorthosite, and sienite, and metamorphic rocks such as serpentinite (Leonardos et al., 2000; Bernardi et al., 2002). Phonolite is used as a soil amendment and source of Ca and K (van Straaten, 2006). Phonolite represents an alternative source of K for plants, considering the importance of this mineral for plant nutrition (Epstein and Bloom, 2006) and the widespread occurrence of this rock in the world (Toledo et al., 2011). von Wilpert and Lukes (2003) and Teixeira et al. (2012) have demonstrated the potential of using phonolite in crop production to stabilize soil pH, to correct soil for K deficiency, and to influence soil base saturation.

Schinus terebenthifolius Raddi and *Eucalyptus saligna* Sm. are important woody species considering their environmental and economic uses. *S. terebinthifolius* is commonly used during revegetation of degraded areas due to its fast growth rate under adverse environmental conditions, interaction with fauna, and high rates of survival under field conditions (Sacramento et al., 2012; Santana et al., 2012). *E. saligna* is one the most widely cultivated species of *Eucalyptus* for commercial use in the South of Brazil (Lorenzi et al.,

2003) due to fast growth rates (Iwakiri et al., 2012). Studies with *S. terebinthifolius* demonstrated that this species has high mycorrhizal dependency (Carneiro et al., 1996; Pasqualini et al., 2007), while no information is found in the literature on the interaction of *E. saligna* with mycorrhizal fungi and the effects of phonolite application on plant growth. Therefore, we tested the hypothesis that application of a mycorrhizal inoculant produced on farm and of phonolite has a synergistic effect on the growth parameters of both species.

Considering the potential of AMF inoculation for increasing plant growth and nutrition and the use of powdered rock as a source of some plant nutrients, application of a mycorrhizal inoculant with powdered rock can promote the growth and nutrition of *S. terebinthifolius* and *E. saligna*. The goal of this study was to evaluate the effect of the interaction between arbuscular mycorrhizal fungi and phonolite on the growth of *Eucalyptus saligna* and *Schinus terebinthifolius* under controlled conditions.

MATERIALS AND METHODS

Biological material

Seeds of *Eucalyptus saligna* and *Schinus terebinthifolius* were purchased from commercial companies, immersed in 70 % alcohol for 30 s for surface disinfestation, washed with sterilized water, and seeded in trays containing sterilized vermiculite. After germination, seedlings were watered daily and received a Hoagland nutrient solution (10 mL per seedling) after 30 days of germination. The nutrient solution contained the following nutrient concentration (in 10 mL): 1.36 mg of KH_2PO_4, 0.01546 mg of H_3BO_3, 0.003 mg of $MnCl_2 + H_2O$, 0.002 mg of $ZnCl_2$, 0.00085 mg of $CuCl_2$, 0.00081 mg of H_2MoO_4, 0.1084 mg of FeEDTA, 4.92 mg of $MgSO_4$, 5.05 mg of KNO_3, and 11.8 mg of $Ca(NO_3)_2$ (Hoagland and Arnon, 1950).

Fungal isolates used in the plant growth experiment were *Claroideoglomus etunicatum* (Becker & Gerd.) C. Walker & Schuessler (isolate MGR288A) and *Dentiscutata heterogama* (Nicolson & Gerd.) Sieverd., F.A. Souza & Oehl (isolate PNB102A), obtained from the International Culture Collection of Glomeromycota (http://www.furb.br/cicg - Universidade Regional de Blumenau - FURB). On-farm mycorrhizal inoculant of both isolates were produced according to Czerniak and Stürmer (2014) over a 3-month period in a substrate composed of a mixture of pulp sludge, carbonized rice husks, and soil (1:1:1). The inoculum potential of the inoculant was evaluated using the most probable number method and numbered 23 to 28 infective propagules of AMF per cm^3 of substrate (Czerniak and Stürmer, 2014).

Phonolite

Phonolite used in plant growth experiments consisted of potassic rock originating from the Chapada dos Índios, Distrito Alcalino of Lages, SC, Brazil, and has the following chemical composition as determined by flame atomic absorption spectrometry: SiO_2 56.6 %; Al_2O_3 22 %; Fe_2O_3 2.3 %; TiO_2 0.2 %; CaO 0.9 %; MgO 0.1 %; K_2O 6 %; Na_2O 9.8 %, and MnO 0.2 % (Aumond and Scheibe, 1996). Phonolite rock was ground in a ball mill and sieved over 100 and 200 mesh sieves to obtain particle sizes of 0.149 and 0.074 mm, respectively. To obtain material with particle size of 0.037 mm, phonolite rock was ground in a commercial laboratory (T-cota Engenharia e Minerais Industriais, Tijucas, SC) in a ball mill with a 1,000 mL capacity.

Experimental design

Plant growth experiments were conducted under greenhouse conditions using plastic cones (270 cm^3) and began in December 2013 for *E. saligna* and January 2014 for *S. terebinthifolius*. The substrate consisted of a silty loam soil:sand:expanded clay mixture (1:2:2) and was sterilized twice in an autoclave (121 °C for 60 min with a 24 h interval). Soil chemical properties were the following: pH in water 4.13, P 4.10 mg dm^{-3}, K 17.33 mg dm^{-3}, organic matter 0.47 %, Al^{3+} 5.3 $cmol_c$ dm^{-3}, Ca^{2+} 0.20 $cmol_c$ dm^{-3}, Mg^{2+} 0.03 $cmol_c$ dm^{-3}, H+Al 12.31 $cmol_c$ dm^{-3}, and CEC 12.59 $cmol_c$ dm^{-3}. The pH level was measured in water (1:1) and P and K were extracted with

HCl and H_2SO_4. Exchangeable Ca, Mg, and Al were extracted with 1 mol L^{-1} KCl and H+Al was extracted with 1.0 mol L^{-1} calcium acetate. Organic matter was analyzed using the Walkley-Black method. All analyses were according to the methods described in Tedesco et al. (1995).

The experiment followed a completely randomized design using a 3 × 4 factorial arrangement, consisting of three inoculation treatments and four phonolite treatments, with 10 replicates. Inoculation treatments were: 1) non-mycorrhizal control (SP), 2) no inoculation + pulp sludge and carbonized rice husk (LC), and 3) on-farm mycorrhizal inoculant produced using pulped sludge and carbonized rice husk (IN). Phonolite treatments consisted of no phonolite addition (F_0) and addition of phonolite with particle sizes of 0.037 mm (F_1), 0.074 mm (F_2), and 0.149 mm (F_3). Treatments F_1, F_2, and F_3 received 3.7 g of phonolite. Considering that the on-farm mycorrhizal inoculant was produced in a substrate containing pulp sludge, which is used as a soil conditioner and therefore influences plant nutrition (Vaz and Gonçalves, 2002), we opted to add a second non-mycorrhizal treatment (LC) that received 5 mL of a sterilized mixture of pulp sludge and carbonized rice husk. The IN treatment received 5 mL of the on-farm mycorrhizal inoculant.

Seedlings of *E. saligna* and *S. terebinthifolius* were pre-germinated in sterilized vermiculite and selected based on height before transplanting to plastic cones. Plants were maintained under greenhouse conditions and watered daily. After 60 days, plants received 50 mL of Hoagland nutrient solution with the following nutrient concentrations (in 50 mL): 6.8 mg of KH_2PO_4, 0.077 mg of H_3BO_3, 0.019 mg of $MnCl_2 + H_2O$, 0.013 mg of $ZnCl_2$, 0.0042 mg of $CuCl_2$, 0.004 mg of H_2MoO_4, 0.54 mg of Fe EDTA, 24.64 mg of $MgSO_4$, 25.27 mg of KNO_3, and 59.04 mg of $Ca(NO_3)_2$.

Data sampling

Plants were harvested 120 days after transplanting and stem diameter was measured at 5 cm from the substrate using a digital caliper (King Tools model 502.150 BL). Height was measured from the substrate level up to the apical meristem using a metric tape measure.

Shoots were separated from the roots and dried at 65 °C for 72 h to obtain shoot dry biomass (SDB). Roots were washed under tap water to free soil particles and blot dried. A 0.1 g subsample of fresh root biomass was obtained to measure mycorrhizal root colonization and the remainder dried at 65 °C for 72 h to obtain root dry biomass (RDB).

The sum of SDB and RDB resulted in total dry biomass (TDB), which was used with height (H) and stem diameter (SD) readings to evaluate seedling quality using the Dickson Quality Index (DQI) (Dickson et al., 1960) according to equation (1).

$$DQI\,(\%) = \frac{TDB\ (g)}{\frac{H\ (cm)}{SD\ (mm)} + \frac{SDB\ (g)}{RDB\ (g)}} \qquad \text{Eq. 1}$$

Shoot K and P concentrations were determined in a commercial laboratory following the methods described in Tedesco et al. (1985). Shoots were placed in an oven at 60 °C for three days and ground using a Willey mill. Samples were solubilized in digestion tubes with a 6:1 solution of nitric acid (65 %) and percloric acid (70 %) and digested at 190 °C in a digestion block system. Phosphorus was determined colorimetrically by the molybdate method, and K was determined by atomic absorption spectroscopy.

To evaluate mycorrhizal root colonization, root samples were stained according to the method of Koske and Gemma (1989) and colonization measured using the gridline intersect method of Giovannetti and Mosse (1980).

Statistical analyses

Detection of outliers was performed with the Mahalanobis test using JMP® software (SAS, 2002). Data were evaluated using two-way analysis of variance (Anova) and means

separated using the Tukey post hoc test at 5 % probability by the Statistica® 7.0 statistical software. *S. terebinthifolius* plants assigned to SP and LC treatments did not produce enough biomass to allow measurement of shoot P and K, and these parameters were evaluated using one-way Anova followed by the Tukey test. Mycorrhizal root colonization data were transformed to arcsine square root values before analyses.

RESULTS

For *S. terebinthifolius*, plant height and SDM were significantly affected by phonolite, mycorrhizal inoculation, and the interaction factor, while stem diameter and RDM were significantly affected only by mycorrhizal inoculation and the interaction factor (Table 1). For *E. saligna*, shoot dry matter and K concentration were significantly affected by mycorrhizal inoculation and the interaction. Other parameters for *E. saligna* were significantly affected by the interaction factor (stem diameter and RDM) or mycorrhizal inoculation (shoot dry matter and P).

Growth parameters of *S. terebinthifolius* were significantly higher in the IN treatment compared to the SP and LC treatments, regardless of the phonolite treatment (Table 2). No significant differences were detected between SP and LC with the phonolite treatments

Table 1. Summary of two-way analysis of variance of height, stem diameter, shoot (SDB) and root (RDB) dry biomass, shoot phosphorus (P), shoot potassium (K), and the Dickson Quality Index (DQI) for seedlings of *Schinus terebinthifolius* and *Eucalyptus saligna* with phonolite (PH) and mycorrhizal inoculum (MYC) as independent factors

Parameter	*S. terebinthifolius*		*E. saligna*	
	F value	p	F value	p
Height (cm)				
PH	3.29	0.02	2.53	0.059
MYC	166.62	<0.001	10.66	<0.001
PH × MYC	2.41	0.03	5.58	<0.001
Stem diameter (mm)				
PH	2.40	0.07	0.47	0.69
MYC	158.61	<0.001	1.09	0.33
PH × MYC	2.65	0.019	6.55	<0.001
SDB (g)				
PH	4.91	0.03	0.58	0.62
MYC	285.29	<0.001	6.66	0.001
PH × MYC	4.26	<0.001	3.86	0.001
RDB (g)				
PH	0.99	0.39	0.73	0.53
MYC	140.26	<0.001	1.70	0.18
PH × MYC	5.04	<0.001	3.94	0.001
P (g kg^{-1})				
PH	nd	nd	0.37	0.77
MYC	nd	nd	6.92	0.001
PH × MYC	nd	nd	2.04	0.06
K (g kg^{-1})				
PH	nd	nd	1.83	0.14
MYC	nd	nd	15.25	<0.001
PH × MYC	nd	nd	2.72	0.01
DQI				
PH	3.39	<0.02	0.081	0.97
MYC	267.7	<0.001	2.43	0.09
PH × MYC	9.11	<0.001	3.94	0.0013

nd: not determined because plants did not produce enough biomass in non-mycorrhizal SP and LC treatments.
p: probability associated with the F value.

Table 2. Height, stem diameter, shoot (SDB) and root (RDB) dry biomass, shoot phosphorus (P), and shoot potassium (K) of *Schinus terebinthifolius* and *Eucalyptus saligna* at different particle sizes of phonolite [no phonolite (F_0) and addition of phonolite in particle sizes of 0.037 mm (F_1), 0.074 mm (F_2), and 0.149 mm (F_3)] and mycorrhizal inoculation

Mycorrhizal treatment	Phonolite treatment			
	F_0	F_1	F_2	F_3
		Schinus terebinthifolius		
Height (cm)				
SP	4.77 ± 1.21 a B	4.37 ± 0.46 a B	3.96 ± 0.90 a B	5.82 ± 4.14 a B
LC	3.95 ± 1.11 a B	3.8 ± 0.44 a B	4.19 ± 0.53 a B	4.63 ± 1.70 a B
IN	9.48 ± 4.77 b A	13.38 ± 1.08 a A	12.35 ± 1.88 ab A	12.75 ± 1.29 a A
Stem diameter (mm)				
SP	0.57 ± 0.12 a B	0.55 ± 0.16 a B	0.55 ± 0.09 a B	0.65 ± 0.20 a B
LC	0.56 ± 0.09 a B	0.57 ± 0.11 a B	0.56 ± 0.15 a B	0.55 ± 0.16 a B
IN	0.98 ± 0.39 b A	1.31 ± 0.11 a A	1.26 ± 0.15 a A	1.21 ± 0.14 ab A
SDB (g)				
SP	0.05 ± 0.02 a B	0.02 ± 0.01 a B	0.03 ± 0.02 a B	0.07 ± 0.10 a B
LC	0.03 ± 0.02 a B	0.03 ± 0.01 a B	0.05 ± 0.02 a B	0.06 ± 0.04 a B
IN	0.29 ± 0.21 b A	0.50 ± 0.07 a A	0.44 ± 0.06 a A	0.47 ± 0.11 a A
RDB (g)				
SP	0.05 ± 0.02 a B	0.02 ± 0.01 a B	0.02 ± 0.01 a B	0.05 ± 0.08 a B
LC	0.05 ± 0.04 a B	0.03 ± 0.05 a B	0.03 ± 0.01 a B	0.03 ± 0.03 a B
IN	0.13 ± 0.10 b A	0.23 ± 0.04 a A	0.23 ± 0.04 a A	0.20 ± 0.05 ab A
P (g kg^{-1})				
SP	nd	nd	nd	nd
LC	nd	nd	nd	nd
IN	1.91 ± 0.39 a	1.78 ± 0.23 a	1.63 ± 0.33 a	1.79 ± 0.26 a
K (g kg^{-1})				
SP	nd	nd	nd	nd
LC	nd	nd	nd	nd
IN	23.32 ± 10.11 a	22.64 ± 6.97 a	23.44 ± 8.82 a	27.82 ± 8.39 a
		Eucalyptus saligna		
Height (cm)				
SP	15.04 ± 3.61 b B	17.46 ± 2.73 ab B	18.86 ± 3.93 ab AB	21.84 ± 1.4 a A
LC	20.2 ± 4.09 ab AB	23.68 ± 2.02 a A	15.76 ± 3.52 b B	20.07 ± 5.8 ab A
IN	22.61 ± 3.6 a A	21.02 ± 3.3 a AB	22.73 ± 2.73 a A	21.99 ± 4.15 a A
Stem diameter (mm)				
SP	0.91 ± 0.21 a A	0.99 ± 0.19 a AB	1.11 ± 0.19 a A	1.1 ± 0.1 a A
LC	0.91 ± 0.16 a A	1.17 ± 0.09 b A	0.87 ± 0.11 a A	1.00 ± 0.19 ab A
IN	1.14 ± 0.18 a A	0.91 ± 0.16 a B	1.10 ± 0.11 a A	1.00 ± 0.16 a A
SDB (g)				
SP	0.19 ± 0.11 a B	0.21 ± 0.08 a A	0.27 ± 0.11 a AB	0.29 ± 0.06 a A
LC	0.26 ± 0.09 ab AB	0.30 ± 0.09 a A	0.15 ± 0.07 b B	0.25 ± 0.11 ab A
IN	0.35 ± 0.08 a A	0.27 ± 0.11 a A	0.31 ± 0.05 a A	0.29 ± 0.12 a A
RDB (g)				
SP	0.05 ± 0.04 a A	0.06 ± 0.05 a A	0.09 ± 0.05 a A	0.08 ± 0.05 a A
LC	0.07 ± 0.04 ab A	0.09 ± 0.03 a A	0.03 ± 0.02 b B	0.06 ± 0.03 ab A
IN	0.08 ± 0.03 a A	0.08 ± 0.05 a A	0.07 ± 0.02 a AB	0.07 ± 0.03 a A
P (g kg^{-1})				
SP	1.32 ± 0.96 a A	2.19 ± 0.93 a A	2.13 ± 1.24 a AB	1.11 ± 0.55 a A
LC	1.93 ± 1.47 a A	1.13 ± 0.72 a A	1.01 ± 0.64 a B	1.51 ± 1.28 a A
IN	1.86 ± 1.42 a A	2.22 ± 1.33 a A	2.82 ± 0.93 a A	2.53 ± 1.44 a A
K (g kg^{-1})				
SP	1.16 ± 1.13 a B	1.16 ± 0.51 a A	1.81 ± 1.21 a AB	1.72 ± 0.94 a A
LC	1.93 ± 1.11 a AB	1.21 ± 0.72 a A	0.52 ± 0.49 a B	1.74 ± 1.41 a A
IN	3.38 ± 1.25 a A	2.26 ± 1.42 a A	2.59 ± 0.30 a A	2.06 ± 1.49 a A

Values are mean ± standard deviation. Means followed by the same letter are not different by the Tukey HSD test at the 0.05 level. Lowercase letters compare phonolite treatments and uppercase letters compare mycorrhizal treatments. nd: not determined.

for growth parameters. In the IN treatment, growth parameters were significantly higher in phonolite treatments F_1, F_2, and F_3 compared to F_0, and phonolite treatments have no influence on shoot P and K, with values averaging 1.77 and 24.3 g kg^{-1}, respectively.

The DQI of *S. terebinthifolius* was significantly higher for plants in the IN treatment compared to the SP and LC treatments, regardless of the phonolite treatment (Figure 1a). Within the IN treatment, the DQI ranged from 0.053 to 0.06 for phonolite treatments F_1, F_2, and F_3, while it averaged 0.031 without the addition of phonolite (F_0).

Growth parameters of *E. saligna* were not significantly different among SP, LC, and IN within the F_3 phonolite treatment (Table 2). In F_0, plants in IN showed a significant increase in height and SDM compared to plants in SP. Plants in IN were significantly taller, produced more SDM, and had a higher concentration of P and K compared to plants in LC when grown in F_2.

The DQI of *E. saligna* was not significantly different among mycorrhizal inoculation treatments for seedlings growing in the F_0, F_1, and F_2 treatments, and DQI values in the SP and IN treatments were significantly higher than in the LC treatment for seedlings in the F_3 treatment (Figure 1b). Among phonolite treatments, no significant differences were detected in DQI values for seedlings growing in SP and IN treatments.

Mycorrhizal root colonization in *S. terebinthifolius* was significantly higher in phonolite treatments F_1, F_2, and F_3 compared to no addition of phonolite treatment (F_0) (Figure 2). For *E. saligna*, the percentage of mycorrhizal colonization was significantly higher in F_1 compared to F_0, but no differences were detected in Eucalyptus mycorrhizal colonization between F_0, F_2, and F_3.

DISCUSSION

The efficiency of using an on-farm mycorrhizal inoculum associated with ground rock as a source of K to promote growth of two woody species was verified in this study. Overall, phonolite used as a potassic rock had little influence on growth parameters, although application of the mycorrhizal inoculum and its interaction with phonolite influenced plant growth. Bhardwaj et al. (2014) point out that to ensure sustainable primary production

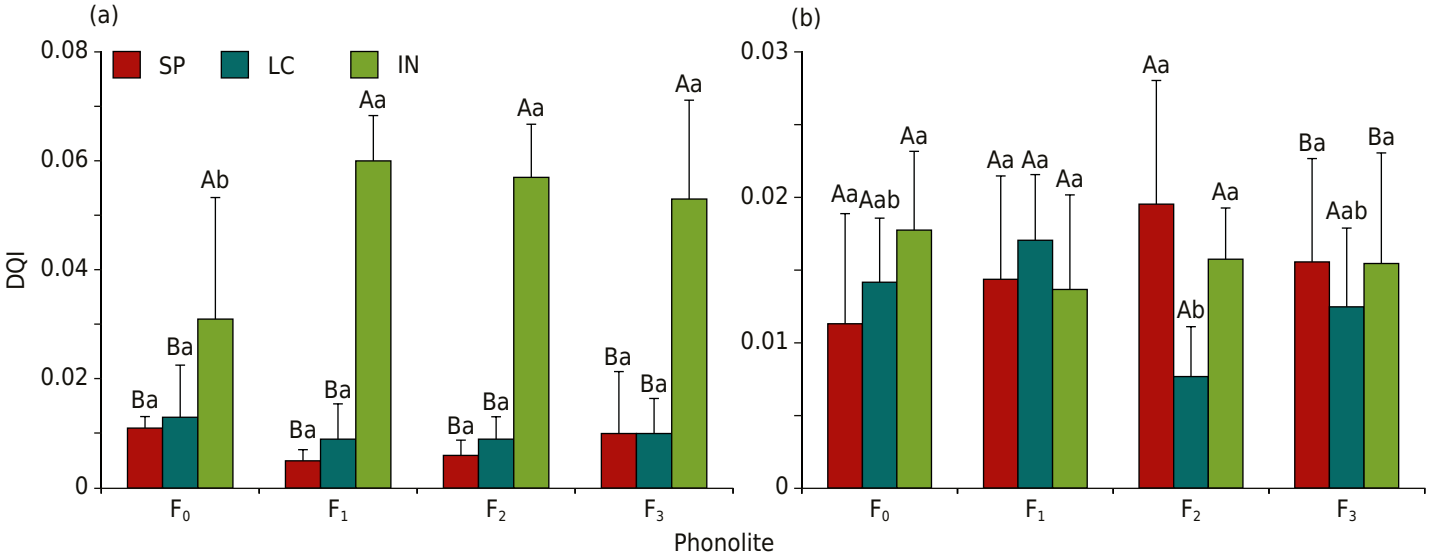

Figure 1. Dickson Quality Index (DQI) for seedlings of (a) *Schinus terebinthifolius* and (b) *Eucalyptus saligna* in mycorrhizal (SP, LC, and IN) and phonolite (F_0, F_1, F_2, and F_3) treatments. F_0: no phonolite, and addition of phonolite in particle sizes of 0.037 mm (F_1), 0.074 mm (F_2), and 0.149 mm (F_3). Uppercase letters compare means between mycorrhizal treatments, and lowercase letters compare means between phonolite treatments. Within each treatment, bars followed by the same letter are not statistically different according to the Tukey *post hoc* test ($p<0.05$).

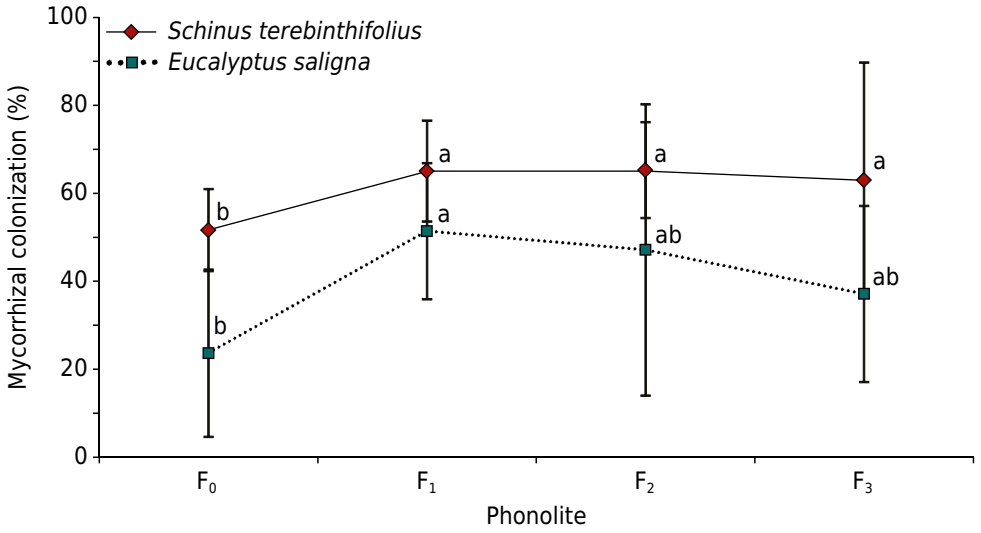

Figure 2. Mycorrhizal colonization of *Schinus terebinthifolius* and *Eucalyptus saligna* in mycorrhizal (SP, LC, and IN) and phonolite (F_0, F_1, F_2, and F_3) treatments. F_0: no phonolite, and addition of phonolite in particle sizes of 0.037 mm (F_1), 0.074 mm (F_2), and 0.149 mm (F_3). For each species, means followed by the same letter are not statistically different according to the Tukey post hoc test ($p<0.05$).

systems, it is necessary to enhance the benefits provided by soil microorganisms and to meet K demands in plants in order to reduce the use of chemical fertilizers. Results obtained herein partially corroborate our hypothesis and indicate that using AMF-based biofertilizers and potassic rock represents a sustainable strategy for promoting plant growth of *S. terebinthifolius* and *E. saligna*.

Application of mycorrhizal inoculum contributed to higher production of *S. terebinthifolius* biomass compared to both non-mycorrhizal treatments (LC and SP). A significant response in growth parameters of *S. terebinthifolius* to IN may be due to the fact that this plant is a pioneer during secondary succession, and pioneer plants have been shown to be highly mycorrhizal (Siqueira et al., 1998). Santos et al. (2008) also observed an increase in growth response for *S. terebinthifolius* due to inoculation with *Paraglomus occultum* (C. Walker) J.B. Morton & D. Redecker. Mycorrhizal colonization of S. teribinthifolius in our study ranged from 52 to 62 %, while in other studies, these values ranged from 1 to 20 % (Carneiro et al., 1998; Siqueira et al., 1998). These differences can possibly be explained by compatibility between AMF isolates and host plants (Pouyu-Rojas et al., 2006). Considering the large growth response of *S. terebinthifolius* to AMF inoculation as observed in this study and others (Pasqualini et al., 2007), this species can be recommended for use in revegetation programs (Scabora et al., 2011).

The potential of using a mycorrhizal inoculum produced on farm to produce *S. terebinthifolius* seedlings is shown by our results. Czerniak (2014) tested the mycorrhizal inoculum used in this study on growth of *Centrolobium tomentosum* Guillem. ex Benth., *Myracrodruon urundeuva* Allemão, *Ficus insipida* Willdenow, and *Ilex paraguariensis* A. St.-Hil. and observed that growth parameters were influenced by mycorrhizal inoculation in only the last two species. The use of a mycorrhizal inoculum produced on farm has been effective in increasing growth and yield of different crops when other substrates were used as carriers. Douds Jr et al. (2014) and Schlemper and Stürmer (2014) observed the effectiveness of the on-farm methodology in producing mycorrhizal inoculum using pellets of biochar and lignocellulotic residues as carriers (sugarcane bagasse, leaf sheaths of king palm and barley hulls), respectively.

Mycorrhizal inoculation did not have significant effects on the growth and nutrition parameters of *E. saligna*, although other studies have shown a significant response to AMF inoculation for other species of Eucalyptus. Adjoud et al. (1996) inoculated 11

species of eucalypts with AMF and observed significant differences in height and shoot biomass between inoculated and non-inoculated plants of E. macarthurii H. Deane & Maiden, *E. dives Schauer*, *E. delegatensis* R.T. Baker, and *E. viminalis* Labill. *Eucalyptus globulus* Labill. responded to mycorrhizal inoculation with a significant increase in growth and P uptake (Arriagadaa et al., 2014). Karthikeyan and Krishnakumar (2012) also observed an increase in growth and uptake of N, P, and K for *E. tereticornis* Sm. inoculated with AMF and grown in a substrate containing residues from bauxite mining. Nevertheless, mycorrhizal inoculation contributed to K nutrition in *E. saligna* in our study in some phonolite treatments, as compared to SP and LC. This result demonstrates that AMF can contribute to plant nutrition without necessarily being reflected in an increase in biomass production.

Growth and nutrition parameters of both plant species were also influenced by the synergistic effect of phonolite and mycorrhizal inoculum application. One possible explanation for this synergistic interaction is the presence in high amounts of silicone oxide (SiO_2 = 56.6 %) in the phonolite rock, a characteristic of this type of rock (Toledo et al., 2011). Silicon-based fertilizers can benefit plants by mitigating effects due to abiotic stresses and stimulate reaction and defense mechanisms, resulting in increased plant resistance to diseases (Ma and Yamaji, 2006; Liang et al., 2007). In this study, silicon oxide may have reduced soil acidity (Ramos et al., 2006) (pH = 4.13), providing favorable conditions for growth of the AMF species present in the inoculum. Indeed, mycorrhizal colonization for both hosts was generally significantly higher in treatments with phonolite compared to the treatment without the addition of phonolite.

The low growth response for both plant species when only phonolite was applied may be due to two factors: 1) low solubilization of K, and 2) sodium (Na) release. Experiments in our study were conducted for 120 days, a period that may have been too short to analyze the effectiveness of phonolite application. Phonolite, characterized as an alkaline rock (Sichel et al., 2012), releases nutrients to plants in the medium to long term, even when this application occurs with small size particles (Prates et al., 2012). Furthermore, the phonolite used here had 9 % Na, compared to 6 % K. The presence of Na in the substrate represents an abiotic stress to plants, affecting uptake, transport, and use of some nutrients essential to plant growth (Cruz et al., 2006). von Wilpert and Lukes (2003), evaluating how the application of a phonolite rock with 4.2 % K influenced *Picea* sp. growth and soil chemical conditions, observed benefits only after 4-5 years under field conditions. They also attributed the absence of plant response to phonolite application to Na release, a common process that occurs with phonolites with K levels lower than 12-13 %.

In treatments with mycorrhizal inoculation (IN), the adverse effects of Na on plants may have been ameliorated by AMF, which are known to influence plant tolerance to abiotic factors (Porcel et al., 2011). In corn inoculated with *Funneliformis mosseae* (Nicolson & Gerd.) C. Walker & Schuessler, Feng et al. (2001) observed that under high levels of soil Na, plants with mycorrhizal fungi produced increased shoot biomass, soluble sugars, and root electrolyte concentrations compared to plants without mycorrhizal fungi. Authors suggested that higher concentrations of soluble sugars and root electrolytes in plants with mycorrhizal fungi provided for higher osmotic regulation in these plants, which, in turn, increased resistance to high levels of Na. Giri and Mukerji (2003) also observed that inoculation of *Sesbania aegyptiaca* (L.) Merr. and *S. grandiflora* (L.) Pers. with *Glomus macrocarpum* Tul. & C. Tul. reduced Na uptake from soil while increasing P, N, and Mg uptake.

Although the application of phonolite rock had no significant effects on growth and nutrition for either plant species, the stonemeal technique has been effective using other rocks like phosphate rock (Gafsa) and potassic rock (biotite) (Silva et al., 2014). In addition to the type of rock, the effectiveness of the stonemeal technique depends on time of contact

between the rock and the soil and on particle size (Barbosa Filho et al., 2006). We tested three different particle sizes of phonolite (0.149, 0.074, and 0.037 mm), but no significant differences were observed among them on improving plant growth and nutrition. One explanation is that the particle sizes used in our experiments were too coarse to release K in the short time that the experiments were conducted. When the stonemeal technique is applied, Theodoro et al. (2006) recommended the use of a particle size <0.002 mm to make nutrients readily available to plants. This requirement, however, could make the stonemeal technique unfeasible, due to high energy costs in obtaining fine particle size and difficulties in large-scale production (Bolland and Baker, 2000; Pádua, 2012).

Mycorrhizal root colonization was usually significantly higher in treatments with phonolite compared to the treatment with no phonolite added. This suggests that the release of some nutrients from the phonolite, possibly K, favors the process of fungal growth in the root cortex. Furlan and Bernier-Cardou (1989) observed that the addition of fertilizer containing K tended to increase mycorrhizal colonization in Allium cepa, and they attributed this increase to adequate levels of K, which decreases plant root exudation and favors the build-up of carbohydrates in the root cortex, which are readily available to AMF. Silva et al. (2008) observed an increase in AMF spore numbers and mycorrhizal colonization of *Astronium fraxinifolium* Schott when the stonemeal technique was used to apply basalt powder. Carvalho (2012) used gneiss powder on the bean crop and observed that mycorrhizal colonization was not affected by the powdered rock, although mycorrhizal colonization was higher than 60 %. Similarly, Bildusas et al. (1986) observed no significant influence of K fertilization on mycorrhizal colonization of *Bromus inermis* Leyss when associated with *Rhizophagus fasciculatum* (Thaxt.) C. Walker & Schuessler.

The Dickson Quality Index (DQI) for seedlings can vary according to the plant species, management practices during seedling production (e.g., type and fertility of the substrate, container volume), and time of evaluation after seeding (Carneiro, 1995). For *S. terebinthifolius*, DQI values were lower than those registered in the literature (José et al., 2005), although a synergistic effect on this parameter was observed when phonolite and the mycorrhizal inoculum were applied together, indicating that both factors should be considered to produce seedlings with high quality. Values for the eucalyptus species were low compared to those previously reported for other species in the genus (Kratz and Wendling, 2013).

The role of soil microorganisms in providing ecosystem services and influencing the productivity and sustainability of forest and agriculture systems is well established (Gianinazzi et al., 2010). The use of an AMF inoculum for crops and forest species still face some challenges, such as the application of effective fungal strains and the choice of an adequate carrier for inoculum production (Herrmann and Lesueur, 2013; Verbruggen et al., 2013). From a management perspective, the on-farm mycorrhizal inoculum tested here was effective enough to be incorporated in the seedling production system under nursery conditions. Although the stonemeal technique has potential for use in forestry, there are still some challenges to be overcome to ensure its effectiveness in improving plant growth compared to the use of conventional fertilizers (Manning, 2010). Our results suggest that under field conditions, application of the on-farm mycorrhizal inoculum associated with phonolite might increase root colonization in seedlings of woody species, which may represent an advantage for growth and nutrient uptake after transplanting.

CONCLUSIONS

The application of on-farm mycorrhizal inoculum and different particle sizes of phonolite rock synergistically influences the growth and nutrition parameters of *Schinus terebinthifolius* and *Eucalyptus saligna*.

The application of phonolite in the substrate increases mycorrhizal root colonization of *S. terebinthifolius* and *E. saligna*.

The simultaneous use of on-farm mycorrhizal inoculum and phonolite represents a strategy for producing high quality seedlings of *Schinus terebenthifolius*.

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