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Influence of arbuscular mycorrhizal fungi inoculum produced on-farm and phosphorus on growth and nutrition of native woody plant species from Brazil

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ABSTRACT

Mycorrhizal fungus inoculum produced on-farm can be used during production of woody plant seedlings to reduce costs associated with purchase of commercial inoculant and fertilization. This study aimed to test the efficiency of a mycorrhizal inoculant produced on-farm to promote growth and nutrition of woody species in combination with different levels of phosphorus. Plants were submitted to different treatments of phosphorus (0, 40 and 80 mg P/dm^3) and mycorrhizal inoculation (uninoculated, and inoculation with *Rhizophagus clarus* [Rc] or *Claroideoglomus etunicatum* [Ce]). Species included were *Luehea divaricata, Centrolobium robustum, Schinus terebinthifolius, Garcinia gardneriana, Cedrella fissilis,* and *Lafoensia pacari.* The inoculum was produced using the on-farm methodology. Mycorrhizal colonization of plants inoculated with Rc and Ce ranged from 44.8 to 74.8%, except for *Garcinia gardneriana*. Inoculation treatment increased plant height and stem diameter of *Luehea divaricata, Centrolobium robustum* and *Cedrella fissilis* while phosphorus, inoculation and the interaction affected these parameters for *G. gardneriana* and *Lafoensia pacari*. Shoot biomass increased significantly with inoculation treatment in four species. For most species, mycorrhizal fungus inoculation and the addition of phosphorus increased the shoot phosphorus content. Mycorrhizal fungus inoculum produced on-farm successfully colonized tree seedlings and improved growth and/or nutrition under nursery conditions, producing seedlings useful for revegetation of degraded lands.

Keywords: biofertilizers, inoculation, mycorrhizal efficiency, nursery, seedlings growth, tropical species

Introduction

Forest woody species have great commercial potential for their use as timber, charcoal, urban landscaping, and as a source of therapeutic compounds. Moreover, some woody native species have the capacity to adapt to degraded soil turning them important components in projects aiming the recuperation of degraded areas (Kageyama & Gandara 2003). In Brazil, commercial nurseries usually grow seedlings of woody species for the purpose of reclamation of degraded areas (ABRAF 2012). Survival and adequate growth under field conditions depend on several factors including the nutritional status of seedlings before transplanting (Noland *et al.* 2001). However, commercial substrates used for seedling production are usually inert and free of plant growth promoting microorganisms.

Arbuscular mycorrhizal fungi (AMF - phylum Glomeromycota) are among soil microorganisms that greatly impact plant nutrition and growth under nursery and field conditions as they establish the arbuscular mycorrhizal association with their hosts. These fungi colonize the plant root cortex and spread their hyphae into the surrounding soil where they scavenge for low mobility nutrients like phosphorus, which they translocate back to the plant host; in turn they receive from the

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plants carbon compounds to grow and complete their life cycle (Smith & Read 2008). They also confer to the plants resistance against pathogen (Wehner *et al.* 2011), and improvement in water relations (Auge 2004), as well as impacting soil structure (Leifheit *et al.* 2014). Considering all these benefits of the mycorrhizal association, inoculation of woody species' seedlings under nursery conditions is a strategy to reduce costs of chemical fertilizers and to produce seedlings with good vigor which would translate into high survival and growth at the field (Zangaro *et al.* 2003; Vandressen *et al.* 2007; Carneiro *et al.* 1996).

Successfulness of mycorrhizal inoculation depends partially on the mycorrhizal dependency of each woody species (Siqueira & Saggin Junior 2001) which varies accordingly to the successional stage that a species belongs; pioneer and early successional species being more dependent compared to late successional and climax species (Carneiro *et al.* 1996; Siqueira & Saggin-Júnior 2001; Zangaro *et al.* 2007; Pasqualini *et al.* 2007).

Large scale inoculation in nurseries depends on the availability of a AMF inoculant that can be either purchased commercially or produced by the nurseryman. Many studies in Brazil have shown that nutrition and growth of native woody species are improved by the mycorrhizal association and that many species are moderate to very highly dependent on the association (Siqueira & Saggin Júnior 2001; Zangaro *et al.* 2007). Despite this, mycorrhizal inoculation of seedlings under nursery conditions is not a common practice and no inoculant is available commercially. Production of AMF inoculant using the on-farm methodology is an avenue to overcome this problem. Using the on-farm methodology, an inoculant can be developed by the nursery owner using several available substrates and at a low cost (Douds *et al.* 2005). Methods for the on-farm production of AMF inoculant utilize large plastic bags (Douds *et al.* 2010) or raised beds (Sieverding 1991), with soil-based or composted substrates mixed with vermiculite, perlite or peat (Gaur *et al.* 2000; Douds *et al.* 2010) and grasses usually as host plants to multiply the fungi (Douds *et al.* 2010; Sieverding 1991). Application of on-farm mycorrhizal fungus inoculant improved fruit production of pepper (Douds *et al.* 2012), potatoes (Douds *et al.* 2007) and phosphorus content in cassava (Sieverding 1991).

Despite the effectiveness of the on-farm mycorrhizal fungus inoculum to improve growth of some crop plants and the simple technology and low cost with which this inoculum can be produced, there is no report on the effect of this inoculant and phosphorus fertilization on initial growth and nutrition of tropical woody species. In this context, the aim of this paper was to test the efficiency of a mycorrhizal inoculum produced by the on-farm methodology to promote plant growth and phosphorus nutrition of woody species in the presence of different levels of soil phosphorus.

Materials and methods

On-farm AMF inoculum

The method to produce the on-farm mycorrhizal fungus inoculum used herein is described in Schlemper & Stürmer (2014). AMF isolates used were *Rhizophagus clarus* (Nicolson & Schenck) Walker & Schussler RJN102A (Rc) or *Claroideoglomus etunicatum* (Becker & Gerdemann) Walker & Schussler RJN101A (Ce) and they were obtained from the International Culture Collection of Glomeromycota (CICG at FURB, Blumenau, SC, Brazil - www.furb.br/cicg). Inoculum was stored at 4° C for 6 months before using it to set up the plant growth experiment. Mycorrhizal propagules measured by the most probable number method were 350 and 283 cm^{-3} for *R. clarus* and *C. etunicatum*, respectively.

Native woody species

Seedlings of woody species were obtained from a nursery where they were grown in 100 cm^3 plastic cones in a peat-based commercial substrate for three months. The following species were used: *Luehea divaricata,* Mart (Malvaceae), *Centrolobium robustum* (Vell.) Mart ex Benth. (Fabaceae), *Schinus terebinthifolius* Raddi (Anacardiaceae), *Garcinia gardneriana* (Planch etTriana) Zappi (Clusiaceae), *Cedrela fissilis* Vell (Meliaceae), and *Lafoensia pacari* A. St. Hill (Lythraceae). Mycorrhizal colonization and plant height were not verified at this time as previous analysis (data not shown) indicated that the peat-based substrate was free of AMF.

Species were selected based on their potential to be used for revegetation programs in degraded areas or riparian sites. Wood of all host species is used in building construction and to make furnitures and tools (machetes, hammers). *L. pacari, L. divaricata, S. terebinthifolius,* and *C. fissilis* have been used for landscaping and in reforestation processes. *Garcinia gardneriana* fruits are edible (bacupari) and seeds of *S. terebinthifolius* used as a condiment (Carvalho 1994). Response of *G. gardneriana* and *C. robustum* to inoculation with AMF is not reported in the literature and all other species are highly responsive to mycorrhizal fungi (Siqueira & Saggin Júnior 2001; Zangaro *et al.* 2003).

Plant growth experiment

The substrate used in the plant growth experiment is that used by the Forestry Nursery Station of the Universidade Regional de Blumenau (FURB) to produce seedlings of native woody species and consisted of a non-sterilized mixture (1:1, v/v) of a silty loam soil with carbonized rice shell. The silty loam soil had the following chemical properties: pH 4.8, 5% clay, P 27 mg dm^{-3}, organic matter

0.6%, Al 0.10 cmolc dm^{-3}, CEC 20.46 cmolc dm^{-3}. Plastic cones (270 mL) were filled with this mixture, inoculated with mycorrhizal fungus inoculum (10% by volume) prior to receiving one seedling of each plant species.

Each plant species was exposed to phosphorus and mycorrhizal treatments in a 3 x 3 full factorial design. Mycorrhizal treatments were non-inoculated (Ni), and inoculated with on-farm mycorrhizal fungus inoculum of *Rhizophagus clarus* (Rc) or *Claroideoglomus etunicatum* (Ce). The Phosphorus treatment had three levels: none (P0), 40 mg kg^{-1} (P40), and 80 mg kg^{-1} (P80). Phosphorus was added as a solution of KH_2PO_4 (10 mL/cone). A supplemented solution of KCl was added in the P0 and P40 treatment to provide the same amount of K received by the P80 treatment. Sterilized on-farm mycorrhizal fungus inoculum was added to the Ni treatment. Pots were arranged following a completely randomized design with 10 replicates per host plant x AMF x P level treatment combination.

Plants were grown for 120 days under nursery conditions from December 2012 to March 2013. Monthly temperature and rainfall during this period averaged 19° C and 1,230 mm, respectively. At harvest, plant height was measured with a scaler and stem diameter was measured with a digital calipter. Plant tops were cut at the soil line and oven dried at 65 °C to obtain shoot dry biomass.

Plants did not produce enough shoot biomass to measure shoot P content for each replicate. Therefore, shoots from three to four replicates per treatment were randomly pooled to form one composited sample of a minimum of 0.70 g of plant shoots for P analysis, yielding only three replicates per treatment. Shoot phosphorus analyses were performed at a commercial laboratory (EPAGRI, Caçador, SC).

Root systems were gently washed under tap water to remove substrate debris and stained with trypan blue (0.05%) according to the method of Koske & Gemma (1989). Pigmented roots of *S. terebinthifolius*, *L. pacari* and *C. robustum* required an extra step for clearing by immersing roots in a freshly prepared solution of alkaline H_2O_2 (3 mL of 20% NH_4OH in 30 mL 3% H_2O_2). After staining, root systems were observed under a dissecting microscope and root colonization measured using the grid line intersect method (Giovannetti & Mosse 1980).

Statistical Analyses

Plant height, stem diameter and shoot dry biomass of each plant species were screened for outliers using the Mahalanobis distance and then for the homogeneity of variance according to Levene's test. The effect of each single factor (mycorrhizal and phosphorus) and the interactions between factors were analyzed by a two-way analysis of variance followed by a Tukey's HSD test to separate means. Statistical analyses were performed using the software Statistica and JMP 5.1.

Results

Mycorrhizal colonization of roots was influenced by mycorrhizal treatment, phosphorus addition and the interaction (Tab. 1). Mycorrhizal colonization was very low in the Ni treatment ranging from 0.4 to 12.0 % of root length. Colonization in plants inoculated with Rc or Ce varied from 44.8 to 74.8 % of root lenght, except for *Garcinia gardneriana* in which colonization varied from 10.3 to 34.2 % (Tab. 2). Vesicles, arbuscules, and mycelium colonizing the root cortex were observed in all plant species.

Mycorrhizal treatment significantly influenced plant height and stem diameter of *L. divaricata, C. robustum,* and *C. fissilis* (Tab. 1) while phosphorus affected both parameters in *L. divaricata* and height in *S. terebinthifolius*. No significant effect of mycorrhizal, P and the interaction factor on height and stem diameter was observed for *G. gardneriana* and *L. pacari*.

Shoot biomass of *L. divaricata, C. robustum, G. gardneriana* and *C. fissilis* was significantly influenced by mycorrhizal treatment while phosphorus affected shoot biomass of *S. terebinthifolius* and *C. fissilis* (Tab. 1). The interaction factor significantly affected shoot biomass of *C. fissilis* only; biomass for this species increased at 80P for Ni and Rc, but it decreased for Ce. Mean shoot biomass of *L. divaricata* was 0.66 and 0.67 g when inoculated with Rc and Ce, respectively, and it was significantly greater than Ni plants (mean = 0.36 g) (Fig. 1). Similarly, shoot biomass of *C. robustum* was 0.70 and 0.66 g for plants inoculated with Rc and Ce, respectively, and these values were significantly higher than Ni plants (mean = 0.35 g). Biomass of *S. terebinthifolius* was significantly higher when plants were grown at P80 (1.03 g) compared to P40 and P0 (0.82 and 0.73 g, respectively). Shoot biomass of *G. gardneriana* was significantly higher in Ni plants compared to Ce plants. Biomass of G. gardneriana plants inoculated with Rc did not differ from Ni and Ce plants in all phosphorus levels (Fig. 1). For *C. fissilis*, no differences between fungal treatments were observed for biomass at P0, while at P40 and P80 Ni and Ce plants were significantly different from Rc plants.

Shoot P concentration was influenced by each factor and the interaction factor in *C. robustum*, *S. terebinthifolius,* and *G. gardneriana,* while this parameter was affected only by phosphorus treatment in *L. divaricata* (Tab. 1). Shoot P in *L. divaricata* was significantly higher at P80 compared to P40 and P0. Shoot P of *C. robustum* and *S. terebinthifolius* was significantly higher at P80 compared to P40 and P0 only when inoculated with Rc. For both plant species, inoculation with Ce at P0 significantly increased shoot P compared to Ni e Rc. For *G. gardneriana*, shoot P was influenced by phosphorus levels added to the soil only when inoculated with Rc. Shoot P of *L. pacari* was not affected by phosphorus treatment when inoculated with Rc and

Table 1. Summary for the two way analysis of variance of height, stem diameter, shoot biomass, shoot phosphorus (P) and root colonization of woody species following mycorrhizal inoculation (Myc) and phosphorus addition (P).

	Height		Stem diameter		Shoot biomass		Shoot P		Root colonization	
	F value	*P*	F value	*P*	F value	*P*	F value	*P*	F value	*P*
Luehea divaricata										
Myc	13.67	< 0.001	9.20	< 0.001	19.20	< 0.001	0.64	ns	4.07	< 0.001
P	3.67	0.03	3.18	0.04	1.10	ns	8.98	0.002	10.9	< 0.001
Myc x P	0.36	ns	0.52	ns	0.29	ns	2.12	ns	1.36	ns
Centrolobium robustum										
Myc	14.07	< 0.001	13.4	< 0.001	14.14	< 0.001	17.42	< 0.001	410	< 0.001
P	1.70	ns	2.22	ns	0.41	ns	24.68	< 0.001	3.41	< 0.038
Myc x P	1.86	ns	0.23	ns	0.42	ns	10.00	< 0.001	4.10	0.004
Schinus terebinthifolius										
Myc	1.889	ns	1.51	ns	0.17	ns	21.46	< 0.001	251	< 0.001
P	9.31	< 0.001	1.08	ns	6.47	0.003	17.57	< 0.001	14.1	< 0.001
Myc x P	1.90	ns	0.61	ns	0.95	ns	21.82	< 0.001	3.53	< 0.01
Garcinia gardneriana										
Myc	0.90	ns	1.54	ns	3.94	0.02	8.47	0.003	196.5	< 0.001
P	0.60	ns	0.70	ns	0.09	ns	10.05	0.01	0.93	ns
Myc x P	1/34	ns	1.14	ns	1.38	ns	5.50	0.004	7.96	< 0.001
Cedrella fissilis										
Myc	4.99	0.009	2.98	0.05	5.61	0.005	ND	ND	240	< 0.001
P	2.93	ns	1.84	ns	3.29	0.04	ND	ND	4.02	< 0.02
Myc x P	0.87	ns	3.01	0.02	5.42	< 0.001	ND	ND	2.42	< 0.05
Lafoensia pacari										
Myc	1.86	ns	2.97	ns	1.12	ns	6.08	ns	327	< 0.001
P	0.39	ns	0.21	ns	0.81	ns	3.64	ns	0.09	ns
Myc x P	1.42	ns	2.44	ns	1.83	ns	4.74	ns	5.81	< 0.001

ns = not significant, ND = not determined
P = probabilty associated with the F value.

Table 2. Mycorrhizal root colonization (%) in woody species at three levels of phosphorus added in the soil (0, 40 and 80 mg/kg) and three mycorrhizal treatment (inoculated with *Rhizophagus clarus* (Rc), *Claroideoglomus etunicatum* (Ce) or non inoculated (Ni)).

		Phosphorus (mg/kg)		
	Mycorrhizal	0	40	80
Luehea divaricata	Ni	1.6 B b	4.8 A b	9.0 A b
	Rc	53.9 A a	68.6 A a	65.7 A a
	Ce	55.3 A a	61.4 A a	61.7 A a
Centrolobium robustum	Ni	0.9 B b	9.1 A b	1.9 B b
	Rc	71.3 A a	63.1 A a	66.9 A a
	Ce	70.1 A a	74.8 A a	65.6 A a
Schinus terebinthifolius	Ni	8.1 A b	12.0 A b	9.8 A b
	Rc	49.9 B a	59.0 AB a	73.7 A a
	Ce	51.7 B a	70.6 A a	66.2 AB a
Garcinea gardneriana	Ni	0.4 A b	0.8 A c	1.0 A c
	Rc	19.1 B a	33.5 A a	34.2 A a
	Ce	20.6 A a	13.9 AB b	10.3 B b
Cedrella fissilis	Ni	2.7 B b	8.2 A b	10.3 A b
	Rc	54.6 A a	52.9 A a	49.7 A a
	Ce	53.7 A a	62.3 A a	62.2 A a
Lafoensia pacari	Ni	0.5 B b	2.6 AB b	7.7 A b
	Rc	58.4 A a	52.3 A a	44.8 A a
	Ce	53.0 A a	51.3 A a	50.3 A a

[a)] Means followed by the same letter are not different by Tukey's HSD test at 0.05 level. Small letters compare Mycorrhizal treatments and capital letters compare Phosphorus treatments.

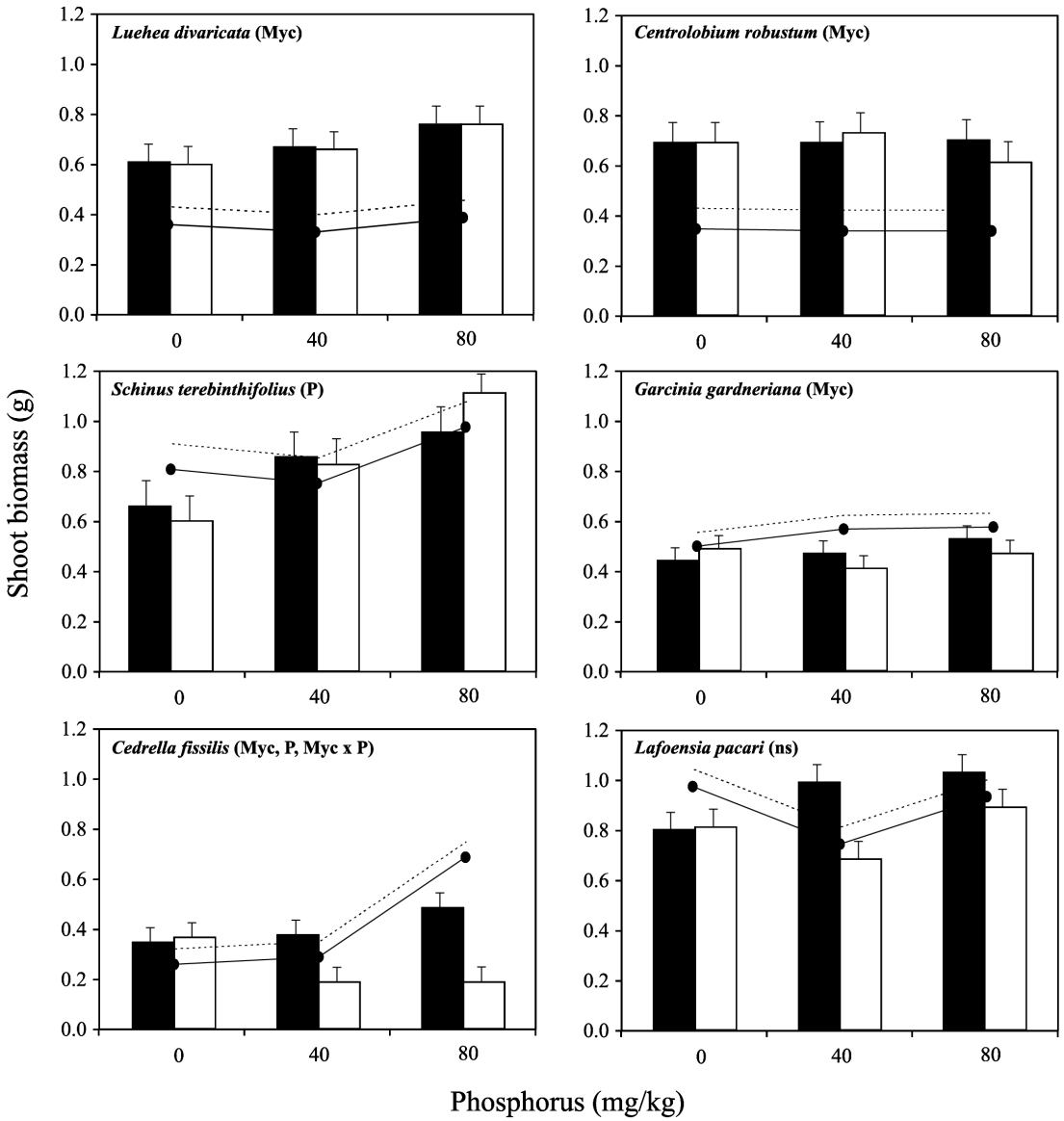

Figure 1. Shoot biomass (g) produced by woody species at different levels of phosphorus added in the soil (0, 40 and 80 mg kg^{-1}) and associated with *Rhizophagus clarus* (black bars) and *Claroideoglomus etunicatum* (white bars). Horizontal line represent shoot biomass produced by non-inoculated (Ni) plants. Treatment effects are indicated in parenthesis after species name (Myc = mycorrhizal, P = phosphorus, Myc x P = interaction, and ns = non significant). Means of 10 ± standard error of the mean. The dashed line (for the Ni) represents standard errors.

Ce and no differences between fungal treatments were detected at P40 and P80 for P shoots (Tab. 3).

Discussion

To the best of authors' knowledge, this study represents the first attempt to test the efficiency of a mycorrhizal fungus inoculum produced by the on-farm method to increase growth and phosphorus nutrition of tropical woody species under nursery conditions. Inocula of AMF produced on farm had been shown to have positive yield effects on vegetable plants such as potatoes and peppers (Douds *et al.* 2007; Douds *et al.* 2012), cassava (Sieverding 1991), and coriander, fenugreek and carrot (Gaur *et al.* 2000) under field conditions. AMF inoculum used in this study was produced in a sugarcane bagasse, carbonized rice shell and sand mixture with pre colonized grain sorghum plants to yield 283 to 350 AMF propagules cm^{-3} (Schlemper & Stürmer 2014). Efficiency of this inoculum is attested by the high levels of root colonization achieved by hosts and by the increase of biomass production and shoot phosphorus for some hosts tested.

The on-farm mycorrhizal fungus inoculum used in this study was produced outdoors under the same climatic con-

Table 3. Treatment effects on shoot phosphorus (g/kg) in woody species at different levels of phosphorus added in the soil (0, 40 and 80 mg/kg) and mycorrhizal treatment (inoculated with *Rhizophagus clarus* (Rc), *Claroideoglomus etunicatum* (Ce) or non inoculated (Ni)).

		Phosphorus (mg/kg)		
	Mycorrhizal	0	40	80
Luehea divaricata	Ni	1.20 ± 0.39 B ns [a)]	1.60 ± 0.39 B ns	2.60 ± 0.39 A ns
	Rc	1.17 ± 0.38 B ns	1.37 ± 0.38 B ns	3.33 ± 0.38 A ns
	Ce	2.13 ± 0.38 B ns	2.07 ± 0.38 B ns	2.27 ± 0.38 A ns
Centrolobium robustum	Ni	1.63 ± 0.30 A b	1.97 ± 0.30 A b	2.53 ± 0.30 A b
	Rc	1.57 ± 0.30 B b	2.40 ± 0.30 B ab	5.30 ± 0.30 A a
	Ce	3.47 ± 0.30 A a	3.17 ± 0.30 A a	3.73 ± 0.30 A ab
Schinus terebinthifolius	Ni	0.73 ± 1.41 A b	0.80 ± 1.41 A a	0.63 ± 1.41 A c
	Rc	0.67 ± 1.41 C c	1.30 ± 1.41 B a	2.27 ± 1.41 A a
	Ce	1.50 ± 1.41 A a	1.47 ± 1.41 A a	1.43 ± 1.41 A b
Garcinea gardneriana	Ni	0.90 ± 1.79 A a	0.47 ± 1.79 A b	0.83 ± 1.79 A b
	Rc	0.33 ± 1.79 B a	1.23 ± 1.79 A ab	1.70 ± 1.79 A a
	Ce	0.90 ± 1.79 A a	1.53 ± 1.79 A a	1.57 ± 1.79 A a
Lafoensia pacari	Ni	0.53 ± 0.21 A a	0.17 ± 0.21 B a	0.13 ± 0.21 B a
	Rc	0.10 ± 0.21 A b	1.17 ± 0.21 A a	1.13 ± 0.25 A a
	Ce	0.37 ± 0.21 A a	0.90 ± 0.21 A a	0.63 ± 0.18 A a

[a)] Values are mean ± standard error. Means followed by the same letter are not different by Tukey's HSD test at 0.05 level. Small letters compare Mycorrhizal treatments and capital letters compare Phosphorus treatments. ns = not significant

ditions of the nursery where the plant growth experiment was conducted. Our results demonstrate the potential and feasibility of the incorporation of mycorrhizal fungus inoculum in media for seedling production with the goal of producing vigorous seedlings and saving fertilizer application. For nursery owners, producing a on-farm mycorrhizal fungus inoculum is an attractive alternative relative to commercially available inoculum as it saves costs associated with processing and shipping (Douds *et al.* 2005) and allows the multiplication of locally adapted fungal isolates (Sreenivasa 1992). Another benefit for nursery owners is the production of the inoculum all year round in tropical climates, allowing mycorrhizal fungus inoculation to be a continuous practice during seedlings production. Considering that seedlings are produced in relatively inert substrates that are usually free of symbiotic microorganisms, addition of on-farm mycorrhizal fungus inoculant into the growth media can results in "mycorrhizal seedlings", a product that can be highly marketed by nursery owners. Mycorrhizal seedlings of woody species have been demonstrated to have higher survival and growth rates under field conditions (Carneiro *et al.* 2004).

Addition of on-farm mycorrhizal fungus inoculum composed by *Rhizophagus clarus* (Rc) or *Claroideoglomus etunicatum* (Ce) increased dramatically mycorrhizal fungus colonization of hosts compared to non-inoculated treatment. Presence of mycorrhizal fungus colonization in plants under the Ni treatment was expected as the experiment used non-sterile soil to mimic conditions routinely used by nurseries. The moderate to high values of mycorrhizal fungus colonization for species when inoculated with the on-farm inoculum may be explained by three factors. First, life traits characteristics of Rc and Ce that allows them to rapidly initiate root colonization and spread inside root cortex (Hart & Reader 2002). Second, plant-fungal compatibility that is important for the symbiotic efficiency for the host plant (Pouyu-Rojas *et al.* 2006). Indeed isolates of *R. clarus* and *C. etunicatum* screened by Pouyu-Rojas *et al.* (2006) were among the most efficient fungi from 11 isolates tested in promoting growth of woody species, suggesting that geographically distinct isolates of both species are effective in promoting plant growth under distinct conditions and should be components of a mycorrhizal fungus inoculum. Finally, the high inoculum potential of the mycorrhizal inoculant, whose levels can be characterized as mass production according to Feldmann & Grotkass (2002), and relative high proportion of inoculum in the potting mixture (10%) could have contributed to the high levels of mycorrhizal fungus colonization measured. This is particularly important in nurseries where field soil is a component of the potting media to produce seedlings, as AMF present in the on-farm inoculum can outcompete indigenous AMF species.

Mycorrhizal colonization was above 50% in plants inoculated with Rc and Ce compared to 0.4-12% for non-inoculated plants, except for *Garcinia gardneriana*. Values of mycorrhizal colonization found in this study for *Luehea divaricata* and *Schinus terebinthifolius* inoculated with Rc

and Ce are within the range reported by Zangaro *et al.* (2003) and Carneiro *et al.* (1996), while colonization of *Lafoensia pacari* (average 50%) and *Cedrella fissilis* (average 56%) was higher than values reported by both authors. To our knowledge, this is the first report of mycorrhizal root colonization for *Garcinia gardneriana* and *Centrolobium robustum*. Mycorrhizal colonization within a host plant is determined by several factors including soil environment (Shi *et al.* 2014), AMF identity (Hart & Reader 2002) and host properties (Zangaro *et al.* 2007). Seed mass is one attribute shown to be negatively correlated with mycorrhizal colonization (Zangaro *et al.* 2005). Among the six species studied, seed mass of *Garcinia gardneriana,* which also had the lowest AMF colonization, averaged 35 g compared to seed mass of other species that ranged from 0.02 to 9.0 g. Janos (1980) suggested that seed reserves are important for seedling growth before they become colonized by AMF.

Application of on-farm mycorrhizal fungus inoculum positively influenced at least one parameter of host growth or nutrition on five of six host species while applied soil phosphorus influenced growth parameters in three species and shoot P in five species. Growth response and nutrition of woody species have been shown to be influenced by AMF inoculation, P addition and the interaction of both parameters (Carneiro *et al.* 1996; Janos 1980; Siqueira & Saggin-Júnior 2001) and results with the application of the on-farm mycorrhizal inoculum corroborate these findings.

Pioneer and early secondary species are usually more responsive to mycorrhizae than late secondary and climax species (Zangaro *et al.* 2003; Siqueira & Saggin-Júnior 2001). Our results support this observation for climax species *Garcinia gardneriana* and *Cedrella fissilis,* both not responding regarding biomass accumulation when inoculated with Rc and Ce relative to Ni. However, for *S. terebinthifolius* (pioneer) and *L. pacari* (early secondary), association with Rc and Ce did not influence biomass accumulation although other studies have reported both species as highly responsive to mycorrhizal inoculation (Zangaro *et al.* 2003; Pasqualini *et al.* 2007). Compatibility between plant species and fungal isolates does not explain this result as mycorrhizal fungus colonization was high for both species and the ability of a mycorrhizal fungus to colonize well not necessarily means a better chance for a growth response. We speculate that both species may not have a high demand for phosphorus for growth and that phosphorus levels in the soil were enough to promote adequate growth of these species. However, it is interesting that shoot P of *G. gardneriana* was significantly higher when associated with Rc and Ce at doses of 40 and 80 mg kg^{-1} P. Therefore, at early stages of seedling development for climax species that are characterized by slow growth, mycorrhizal association may influence plant mineral nutrition more than biomass accumulation. Our results emphasize the multifunctionality of arbuscular mycorrhizal fungi as some combinations of plant-fungus improve growth parameters while others increase plant mineral nutrition (Newsham *et al.* 1995).

Our goal was to test the efficiency of mycorrhizal inoculum produced on-farm to increase growth and phosphorus nutrition of tropical woody species under nursery conditions. Results achieved were positive as AMF present in the inoculum colonized all hosts in greater levels compared to non-inoculated plants and increased at least one parameter of growth or nutrition. Lack of response for growth parameters might be a result of intrinsic characteristics of the host species that reduce or eliminate dependency on mycorrhizas. Mycorrhizal fungus inoculum produced on-farm has been used to promote growth and yield of some crops (Sieverding 1991; Douds *et al.* 2012) and results obtained in this study extend its use for nursery conditions for production of seedlings of woody species. Application of mycorrhizal fungus inoculum produced on-farm can decrease the use of chemical fertilizers and produce a "mycorrhizal seedlings" that has a better chance of survival after transplanting.

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