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

Luis Claudio Goetten¹, Geraldo Moretto² and Sidney Luiz Stürmer²*

Received: July 7, 2015
Accepted: September 28, 2015

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/dm³) 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...
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 Junior 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, perlitie 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 RJJ102A (Rc) or *Claroideoglomus etunicatum* (Becker & Gerdemann) Walker & Schussler RJJ101A (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 cm³ 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 Bentl. (Fabaceae), *Schinus terebinthifolius* Raddi (Anacardiaceae), *Garcinia gardneriana* (Planch et Triana) Zappi (Clusiaceae), *Cedrela fissilis* Vell (Meliaceae), and *Lafaoensia 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 K$_2$HPO$_4$ (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 caliper. 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* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinthifolius* (1989). Pigmented roots of *S. terebinth...
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).

| Species                  | 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 |
| Cedrela 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 = probability 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)).

| Species                  | Mycorrhizal | Phosphorus (mg/kg) |          |        |
|--------------------------|-------------|--------------------|----------|--------|
|                          |             | 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           | 70.8 A a | 66.2 A b a|
| Garcinia 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 a|
| Cedrela 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|

*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.*
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-
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 around 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

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)).

| Phytophthora (mg/kg) | Mycorrhizal 0 | 40 | 80 |
|---------------------|--------------|----|----|
| 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 |
|                      | 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 |
|                      | 0.73 ± 1.41 A b | 0.80 ± 1.41 A a | 0.63 ± 1.41 A c |
| Rc                  | 0.67 ± 1.41 A c | 1.30 ± 1.41 A 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 |
|                      | 0.90 ± 1.79 A a | 0.47 ± 1.79 A b | 0.83 ± 1.79 A b |
| Ce                  | 0.33 ± 1.79 A a | 1.23 ± 1.79 A ab | 1.70 ± 1.79 A a |
|                      | 0.90 ± 1.79 A a | 1.53 ± 1.79 A a | 1.57 ± 1.79 A a |
| 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.
and Ce are within the range reported by Zangaro et al. (2003) and Carneiro et al. (1996), while colonization of L. pacari (average 50%) and C. 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 & Sagggin-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 & Sagggin-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.

**Acknowledgements**

This study was supported by grants from the Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (grant FAPESC 5286/2011-8) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (grant CNPq 562.651/2010-1) (Edital REPENSA 22/2010). LCG thanks CAPES, Brazil for a Master’s assistantship, and SLS thanks CNPq for a Research Assistantship (Process 302343/2012-1). We thank Dr. Paulo Emilio Lovato and two anonymous reviewers for suggestions on the manuscript. Program Bunge Natureza of Bunge Alimentos S/A is acknowledged for donation of seedlings.

**References**

ABRAF - Associação Brasileira de Produtores de Florestas Plantadas. Brasília, Anuário Estatístico de 2012.

Auge RM. 2004. Arbuscular mycorrhizae and soil/plant water relations. Canadian Journal of Soil Sciences 84: 373-381.

Carneiro MAC, Siqueira JO, Davide AC. 2004. Fósforo e inoculação com fungos micorrízicos arbusculares no estabelecimento de mudas de embáuca (*Cecropia pachystachya* Trec). Pesquisa Agropecuária Tropical 34: 119-125.

Carneiro MAC, Siqueira JO, Davide AC, Gomes LJ, Curi N, Vale FR. 1996. Fungos micorrízicos e superfosfato no crescimento de espécies arbóreas tropicais. Scientia Forestalis 50: 20-36.

Carvalho, PER. 1994. Espécies florestais brasileiras: recomendações silviculturais, potencialidades e uso da madeira. Colombo, Embrapa-CNPF.

Douds DD, Lee J, Rogers L, Lohman ME, Pinzon N, Ganser S. 2012. Utilization of inoculum of AM fungi produced on-farm for the production of *Capsicum annuum*: A summary of seven years of field trials on a conventional vegetable farm. Biological Agriculture and Horticulture 28: 129-145.

Douds DD, Nagahashi G, Hepperly PR. 2010. Production of inoculum of indigenous AM fungi and options for diluents of compost for on-farm production of AM fungi. Bioresource Technology 101: 2326-2330.
Douds DD, Nagahashi G, Pfeffe PE, Kayser WH, Reider C. 2005. On-farm production and utilization of mycorrhizal fungus inoculum. Canadian Journal of Plant Sciences 85:15-21.

Douds DD, Nagahashi G, Reider C, Hepperly PR. 2007. Inoculation with arbuscular mycorrhizal fungi increases the yield of potatoes in a high P soil. Biological Agriculture and Horticulture 25: 67-78.

Feldmann F, Grotkass C. 2002. Direct inoculum production - shall we be able to design populations of arbuscular mycorrhizal fungi to achieve predictable symbiotic effectiveness? In: Gianinazzi S, Schuepp H, Barea JM, Haselwandter K (eds.) Mycorrhizal Technology in Agriculture. Birkhäuser, Basel. p. 261-279.

Gaur A, Adholeya A, Mukerji KG. 2000. On-farm production of VAM inoculum and vegetable crops in marginal soil amended with organic matter. Tropical Agriculture 77: 21-26.

Giovannetti M, Mosse B. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytologist 84: 489-500.

Hart MM, Reader RJ. 2002. Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. New Phytologist 153: 335-344.

Jannes D. 1980. Vesicular-arbuscular mycorrhizae affect lowland tropical rain forest plant growth. Ecology 61: 151-162.

Kageyama YP, Gandara BF. 2003. Resultado do programa de restauração com espécies arbóreas no convênio ESALQ/USP e CESP. In: Galvão APM, Porfírio-da-Silva V. (eds.) Restauração florestal - fundamentos e estudos de caso. Colombo, Embrapa Florestas. p. 59-86.

Koske RE, Gemma JN. 1989. A modified procedure for staining roots to detect VA mycorrhizas. Mycological Research 9: 486-488.

Leifheit EF, Veresoglou SD, Lehmann A, Morris EK, Rillig MC. 2014. Multiple factors influence the role of arbuscular mycorrhizal fungi in soil aggregation – a meta-analysis. Plant and Soil 374: 523-537.

Newsham KK, Fitter AH, Watkinson AR. 1995. Multi-functionality and biodiversity in arbuscular mycorrhizas. Trends in Ecology and Evolution 10: 407-411.

Noland TL, Mohammed GH, Wagner RG. 2001. Morphological characteristics associated with tolerance to competition from herbaceous vegetation for seedlings of jack pine, black spruce and white pine. New Forests 2: 199-215.

Pasqualini D, Uhmann A, Stürmer SL. 2007. Arbuscular mycorrhizal fungal communities influence growth and phosphorus concentration of woody plants species from the Atlantic rain forest in South Brazil. Forest Ecology and Management 245: 148-145.

Pouyu-Rojas E, Siqueira JO, Santos JGD. 2006. Compatibilidade simbiótica de fungos micorrízicos arbusculares com espécies arbóreas tropicais. Revista Brasileira de Ciência do Solo 30: 413-424.

Schleper TR, Stürmer SL. 2014. On farm production of arbuscular mycorrhizal fungi inoculum using lignocellulosic agrowastes. Mycorrhiza 24: 571-580.

Shi GX, Liu YJ, Johnson NC, et al. 2014. Interactive influence of light intensity and soil fertility on root-associated arbuscular mycorrhizal fungi. Plant and Soil 378: 173-188.

Sieverding E. 1991. Vesicular Arbuscular Mycorrhiza Management in Tropical Agroecosystems. Eschborn, Gesellschaft fur Technische Zusammenarbeit.

Siqueira JO, Saggin-Júnior OJ. 2001. Dependency of arbuscular mycorrhizal fungi and responsiveness of some Brazilian native woody species. Mycorrhiza 11: 245-255.

Smith SE, Read DJ. 2008. Mycorrhizal Symbiosis. London, Academic Press.

Sreenivassa MN. 1992. Selection of an efficient vesicular-arbuscular mycorrhizal fungi for Chili (Capsicum annum L.). Scientia Horticulturae 50: 53-58.

Vandressen J, Nishidatea FR, Torezan JMD, Zangaro W. 2007. Inoculação de fungos micorrízicos arbusculares e adubação na formação e pós-transplante de mudas de cinco espécies arbóreas nativas do sul do Brasil. Acta Botanica Brasilica 21: 753-765.

Wehner J, Antunes PM, Powell JR, Caruso T, Rillig MC. 2011. Indigenous arbuscular mycorrhizal fungal assemblages protect grassland host plants from pathogens. Plos One 6(11):e27381. DOI:10.1371/journal.pone.002738.

Zangaro W, Nishidate FR, Camargo FRS, Romagnoli GG, Vandressen J. 2005. Relationships among arbuscular mycorrhizas, root morphology and seedling growth of tropical native woody species in southern Brazil. Journal of Tropical Ecology 21: 529-540.

Zangaro W, Niszaki SMA, Domingos JCB, Nakano EM. 2003. Mycorrhizal response and successional status in 80 woody species from South Brazil. Journal of Tropical Ecology 19: 315-324.

Zangaro W, Nishidate FR, Vandressen J, Andrade G, Nogueira MA. 2007. Root mycorrhizal colonization and plant responsiveness are related to root plasticity, soil fertility and successional status of native woody. Journal of Tropical Ecology 23: 53-61.