


Division - Soil Processes and Properties | Commission - Soil Biology

Effect of Inoculation of Pineapple Plantlets with Arbuscular Mycorrhizal Fungi Obtained from Different Inoculum Sources Multiplied by the On-Farm Method

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ABSTRACT: The production of inoculum from arbuscular mycorrhizal fungi (AMF) at a large scale and low cost is essential for establishing methods to assist in producing pineapple plantlets with high nutritional and phytosanitary quality. However, this objective is difficult to accomplish because of the biotrophic nature of these fungi. The on-farm multiplication method for AMF inoculum presents a good alternative to supply the demand for the production of glomerospores. This study aimed to multiply and evaluate AMF inoculum originating from isolated species (including *Rhizophagus clarus*, *Claroideoglomus etunicatum*) versus native AMF from pineapple and coffee plantations multiplied by the on-farm method on the colonization in pineapple plantlets. Initially, inocula of *R. clarus*, *C. etunicatum*, and native AMF (pineapple and coffee) were multiplied by the on-farm method in *Sorghum bicolor*. After four months, the number of AMF spores and the percentage of viable spores at the layers of 0.00-0.05 and 0.05-0.10 m were evaluated. There were no differences in spore numbers in relation to the source of the inoculum (*R. clarus*, *C. etunicatum*, pineapple, and coffee) and evaluated layers, with an average number of 605 spores per 100 cm³ of soil. The percentage of viable spores was greater at the layer of 0.00-0.05 m (76.32 %) compared to the layer of 0.05-0.10 m (72.05 %), regardless of the inoculum source. The viability of the inoculum obtained from *C. etunicatum* was higher than that from the coffee crop (77.93 and 68.06 %, respectively). Subsequently, the spores were inoculated in pineapple plantlets to assess the rate of colonization. Pineapple plantlets inoculated with AMF had an average of colonization

of 18 and 67.73 % after 50 and 180 days cultivation, respectively, with no significant difference being detected between treatments. Therefore, on-farm inoculum production was effective at multiplying the AMF of both isolates of *R. clarus* and *C. etunicatum*, as well as for commercial crops (pineapple and coffee), with spores having high viability. Arbuscular mycorrhizal fungi colonized pineapple plantlets independently of the inoculum utilized and favored its growth.

Keywords: mycorrhizal inoculant, spore viability, AMF, *Rhizophagus clarus*, *Claroideoglossum etunicatum*.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are important components of soil that can be exploited to enhance the development of crops and contribute to the establishment of more sustainable agriculture. This approach would reduce, or even eliminate, the need for chemical fertilizers and pesticides in organic farming (Douds Jr et al., 2008, 2010). This is possible because plants colonized by AMF exploit higher volumes of soil (Smith and Read, 1997) and have higher nutrient uptake (Smith et al., 2011), as well as increased tolerance to drought, saline stress (Augé, 2001), and heavy metals (Rozpadek et al., 2014). Thus, they provide many additional benefits to acting as plant growth promoting agents (Azcón-Aguilar et al., 1997) and as a biological control against phytopathogens (Pozo et al., 2002; Moreira et al., 2016).

Arbuscular mycorrhizal fungi are obligate biotrophic (Douds Jr et al., 2006, 2008), and complete their life cycles solely associated with the roots of living plants. Consequently, they cannot be multiplied separately in a defined culture medium (Douds Jr et al., 2006). Because of this, it is very difficult to develop low-cost methods for high-quality inocula production at large scales (Ijdo et al., 2011). Thus, these fungi are usually multiplied in host plants in culture pots, aeroponic cultivation, hydroponics, or *in vitro* culture with genetically transformed roots (Ijdo et al., 2011). These processes are carried out under controlled or semi-controlled conditions, such as greenhouses or growth chambers (Ijdo et al., 2011).

To stimulate inocula production, studies have been developed to test the multiplication of spores under field conditions, called the on-farm method. These studies explore mycorrhizal colonization with fungal isolates that are environmentally adapted to local conditions, potentially representing a low-cost alternative for farmers (Douds Jr et al., 2006, 2008, 2010; Schlemper and Stürmer, 2014). This method allows farmers and nursery workers to access inoculums with the most effective strains for their culture and their soil and climate conditions; furthermore, they can produce seedlings already mycorrhizal, with this benefit enhancing the establishment of seedlings in the field (Douds Jr et al., 2008). Schlemper and Stürmer (2014) showed that multiplication on-farm with *Rhizophagus clarus* and *Claroideoglossum etunicatum* grown in agro-industrial residues, such as sugarcane bagasse, is a good strategy for the multiplication of AMF, leading to excellent inoculum potential and large numbers of spores.

The production of pineapple plantlets [*Ananas comosus* (L.) Merrill] inoculated with AMF at the acclimatization stage improves the growth and nutrient uptake by the plants, especially under conditions with low P levels (Moreira et al., 2015). This approach also enhances the activity of various antioxidant enzymes responsible for ensuring greater resistance to pathogens (Moreira et al., 2016). The inoculum production in large-scale, easy to apply and low cost would then be the next step to establishing a methodology that would help in the production of pineapple plantlets with nutrition and phytosanitary

quality, and environmentally friendly. Thus, on-farm technology represents a potentially viable alternative to achieve these goals.

This study aimed to multiply and evaluate the AMF inoculum of *R. clarus*, *C. etunicatum* species, and native AMF from pineapple and coffee plantations, using spores multiplied by the on-farm method. In addition, this study aimed to evaluate the effects of these inoculants on the colonization and growth of pineapple plantlets.

MATERIALS AND METHODS

In vitro culture

Pineapple plantlets of the cultivar Imperial were subcultivated in liquid MS (Murashige and Skoog, 1962). The medium was supplemented with 30 g L⁻¹ sucrose, 1.8 mg L⁻¹ α-naphthaleneacetic acid, 2 mg L⁻¹ indole-3-butyric acid, and 2.1 mg L⁻¹ kinetin at pH 5.5. The cultures were grown in 250-mL glass jars containing 15 mL culture medium and were sealed with rigid polypropylene covers. Cultures were kept in a growth room at 26±2 °C under a photoperiod of 16 h light/8 h dark and under an irradiance of 36 μmol m⁻² s⁻¹, provided by white fluorescent lamps. Subcultures were performed every 40 days.

Fungal inoculants

Four mycorrhizal inocula were used in this study: a) *Claroideoglossum etunicatum* RJN101A; b) *Rhizophagus clarus* RJN102A; c) mycorrhizal community from pineapple plantation; and d) mycorrhizal community from an organic coffee plantation. The isolates of AMF *Claroideoglossum etunicatum* RJN101A (= *Glomus etunicatum*) and *Rhizophagus clarus* RJN102A (= *Glomus clarum*) were obtained from the International Culture Collection of Glomeromycota (CICG, www.furb.br/cicg) at the Universidade Regional de Blumenau, Santa Catarina, Brazil. Pure cultures obtained from the CICG were established following a certain procedure. In brief, spores were extracted from trap cultures, separated by morphotypes, and inoculated on the roots of 15-day-old *Sorghum bicolor* seedlings that had been grown on sterilized substrate. Sorghum seedlings were then transplanted to cones (270 cm³) in a mix of sterilized sand:expanded clay:soil (2:2:1 v:v:v), and were grown for four months under greenhouse conditions. Subsequently, the cones were checked for sporulation. Plants were allowed to dry in situ, and the contents of the cones were stored in zip lock plastic bags at 4 °C for 6 months. The native AMF inocula were obtained from: (1) a pineapple plantation with four years of successive crops located in Maria Nunes district, Diamantina, Minas Gerais, Brazil and (2) an organic coffee experimental crop of Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil for representing a different AMF community those already adapted to pineapple plantations and its effect on pineapple plantlets. Ten random soil samples (layer of 0.00-0.20 m) were collected from pineapple and coffee plantation and pooled to form a composite sample, that were used in the experiment to multiply the AMF spores by the on-farm method.

Experimental design

Experiment 1

This experiment was conducted in a completely randomized design, with four treatments consisting of inoculum containing spores, hyphae, and parts of roots colonized with *C. etunicatum*, *R. clarus*, or inoculum obtained from pineapple and organic coffee crops, with four replicates.

For inoculum multiplication by the on-farm method, a substrate containing soil:vermiculite:sugarcane bagasse (1:1:1 v:v:v) was prepared and placed in black polyethylene bags with 20 L capacity (50 m high × 40 m wide) containing 35 drainage

holes (JB Embalagens Ltda). The soil used was an Oxisol (*Latossolo Vermelho-Amarelo Distrófico*) with low natural fertility. Each polyethylene bag was prepared with 18 L of substrate and was positioned at a distance of 0.5 m from adjacent bags. Polyethylene canvas was used to exert a physical barrier to weeds and to avoid direct contact with the soil.

Arbuscular mycorrhizal fungi were added separately by directly inoculating 300 cm³ soil containing the inoculum for testing on the prepared substrate. Then, 20 seeds of *Sorghum bicolor* were sown and covered with a 1-3 cm layer of the initial substrate. After germination, one thinning was performed to standardize the number of plants per bag, leaving 10 remaining plants. The plants were grown outdoors from 120 days onwards and were irrigated as needed. After this period, irrigation was suspended for 90 days to facilitate the production of spores.

The number of spores was quantified in the initial inoculum (used for multiplication) and after multiplication at the end of the growing period, at 0.00-0.05 and 0.05-0.10 m layers in the polyethylene bags. For each depth, three replicates were obtained to have repeated samples for each treatment. Spores were extracted from 100 cm³ soil of each composite sample.

Experiment 2

This experiment was conducted in a greenhouse, in a completely randomized design, with five treatments consisting of inoculum previously multiplied by the on-farm method (*C. etunicatum*, *R. clarus*, and obtained from pineapple crops and organic coffee) and using a control treatment (without AMF), with eight replicates.

Pineapple plantlets of 4.4 cm height on average with 10 to 12 leaves were transplanted to plastic pots with a 1 kg capacity containing a commercial substrate (Tropstrato HA Hortaliças). For each treatment obtained from experiment 1 (*R. clarus*, *C. etunicatum*, mycorrhizal communities from pineapple, and coffee), a sample containing 200 cm³ soil was prepared for each replicate from which the first 0.10 m soil was used as inoculum in experiment 2. The moment that pineapple plantlets were transferred to the substrate, they were inoculated near the root system with about 605 spores in 100 cm³ of soil obtained from the composite samples containing the AMF inoculum. The controls consisted of a pure commercial substrate. The seedlings were monitored for moisture in the substrate and irrigation was performed whenever necessary. After 50 days of cultivation in a greenhouse, five plants were selected at random to determine the percentage of colonization of pineapple plantlets, as well as their growth parameters. The remaining three plants were kept in the greenhouse for 180 days to quantify the percentage of colonization after this period.

Number and viability of spores and mycorrhizal colonization

Arbuscular mycorrhizal fungi spores were extracted using 100 cm³ soil using the wet sieving technique (Gerdeman and Nicholson, 1963), followed by centrifugation in water and then in 50 % sucrose solution. Approximately 0.5 g of the root system from each plant was diaphanized in 10 % KOH (w:v) for 12 h, followed by successive washing in tap water. After washing, the root system was kept in HCl 2 % (w:v) for 5 min and then it was stained with 0.05 % trypan blue in lactoglycerol (w:v) at 70 °C for 30 to 40 min. The sample was then stored in lactoglycerol (Phillips and Hayman, 1970). Root colonization was quantified by using the gridline intersect method (Giovannetti and Mosse, 1980). Both spore counts and mycorrhizal colonization were performed under a stereoscopic microscope.

Spore viability was assessed by immersion in 100 µL iodinitrotetrazolium chloride (INT) solution at 1 mg mL⁻¹ for 72 h at room temperature (Walley and Germida, 1995; Carvalho et al., 2001). Viable spores were considered those that reacted with INT

and showed a characteristic reddish color. Viability was expressed as a percentage of viable spores.

Evaluation of growth parameters

After the growing period in the greenhouse, the height of the plants (H), number of leaves (LN), shoot fresh matter (SFM), and shoot dry matter (SDM) were determined. The SDM was determined after drying to a constant weight at 70 °C in an oven with forced ventilation.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) at an α level of 10 %. The means were compared using Tukey's test ($p \leq 0.10$). The spore count data were previously normalized via the $\log(x + 1)$. The mycorrhizal colonization and percentage of viable spores were transformed by $\arcsen(x/100)^{1/2}$ to perform the ANOVA.

RESULTS

The initial inoculum containing *C. etunicatum* stood had more AMF spores than the other inoculums, averaging 1,512 spores in 100 cm³ of soil (Figure 1). There was no difference in the number of spores in the inoculum obtained from the coffee and pineapple plantations and *R. clarus*, averaging around 577 spores in 100 cm³ of soil (Figure 1).

After the period of multiplication on-farm of the inoculum, the spores were counted again. There was no difference between soil layers (0.00-0.05 and 0.05-0.10 m) for the different treatments or the same treatments (Figure 2).

There was no interaction between inoculum obtained from different backgrounds and soil layers (0.00-0.05 and 0.05-0.10 m) in relation to the percentage of viable spores in on-farm inocula. However, each factor alone was significant ($p \leq 0.10$). Regardless treatment type, the percentage of viable spores in the 0.00-0.05 m layer was higher than that in the 0.05-0.10 m layer, with an average of 76.32 and 72.05 %, respectively. By comparing each inoculum, regardless of layer in the soil profile, the inoculum obtained

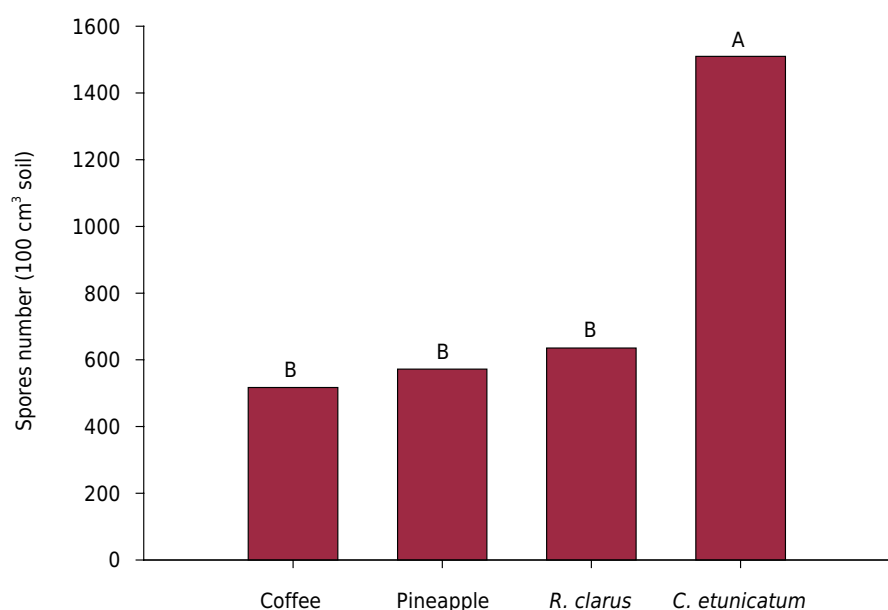


Figure 1. Number of arbuscular mycorrhizal fungi spores in 100 cm³ of soil from coffee and pineapple plantations, and isolates of *Rhizophagus clarus* and *Claroideoglossum etunicatum* used for multiplication by the on-farm method. Means followed by the same letter did not differ when using the Tukey test ($p \leq 0.05$).

from the coffee plantation had the lowest percentage of viable spores (68.06 %) compared to the inoculum containing *C. etunicatum* (77.93 %) (Figure 3).

After 50- and 180-days cultivation of pineapple plantlets inoculated with the AMF in a greenhouse, the root colonization of inoculated seedlings was 18 and 67.73 %, respectively, with no difference in the origin of the inoculum used for the same period of evaluation (Figure 4).

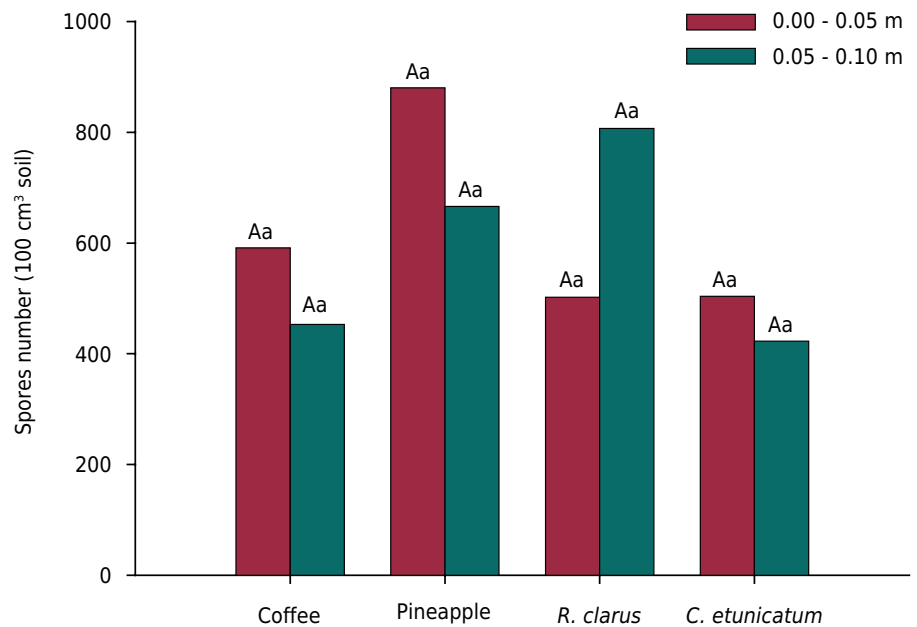


Figure 2. Number of arbuscular mycorrhizal fungi spores in 100 cm³ of soil inoculum that was obtained after multiplication by the on-farm method for four months. Samples were collected at soil layers of 0.00-0.05 and 0.05-0.10 m from coffee and pineapple plantations and isolates of *Rhizophagus clarus* and *Claroideoglossum etunicatum*. Means followed by the same capital letter did not differ among treatments or at the same layer when using the Tukey test ($p \leq 0.10$). Averages followed by the same lowercase letter did not differ between layers within the same treatment when using the Tukey test ($p \leq 0.10$).

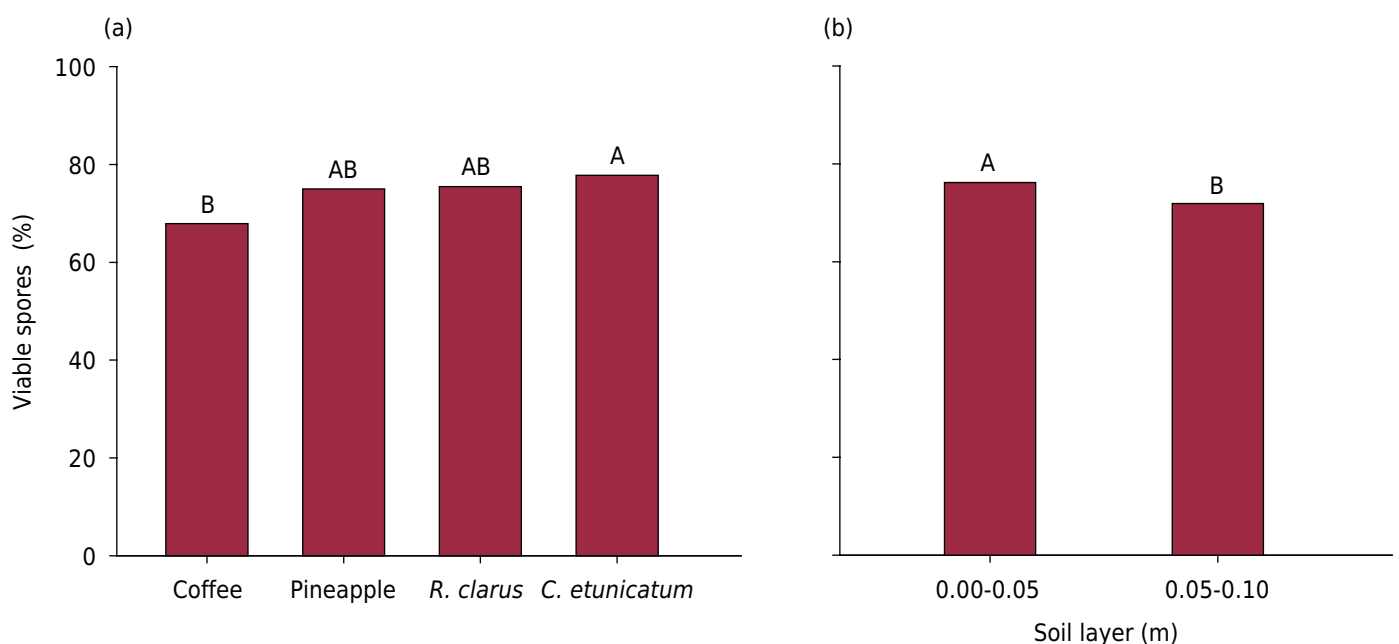


Figure 3. Percentage of viable spores in the inoculum obtained from coffee and pineapple plantations, and in isolates of *Rhizophagus clarus* and *Claroideoglossum etunicatum* after multiplication by the on-farm method (a). Viable spores of AMF at different soil layers independent of source of inoculum (b). Means followed by the same letter did not differ when using the Tukey test ($p \leq 0.10$).

After 50 days cultivation, no difference in the growth parameters of pineapple plantlets was observed ($p \leq 0.10$) among AMF inoculated seedlings, regardless of the source of inoculum or plants without mycorrhizal fungi inoculation (control) (Table 1).

DISCUSSION

The multiplication of AMF inoculum by the on-farm method has been tested over the years in various countries, including Colombia (Sieverding, 1991), India (Gaur et al., 2000), USA (Douds Jr et al., 2006, 2008, 2010), and, more recently, in Brazil (Czerniak and Stürmer, 2014; Schlemper and Stürmer, 2014; Goetten et al., 2016). Promising results have been obtained regarding the use of these fungi by the producers themselves. However, the effects of AMF inoculum on the viability of pineapple seedling production has not been previously tested. In particular, this approach represents an alternative to using agro-residues, such as cane sugar bagasse and royal palm tree sheath (Schlemper and Stürmer, 2014).

Pineapple seedlings inoculated with AMF have higher growth and better nutritional status than seedlings not colonized by these fungi (Rodríguez-Romero et al., 2011; Moreira et al., 2015). These benefits were observed in pineapple plantlets that grow larger with greater nutrient absorption, even when plants were grown with high doses of P (Moreira et al.,

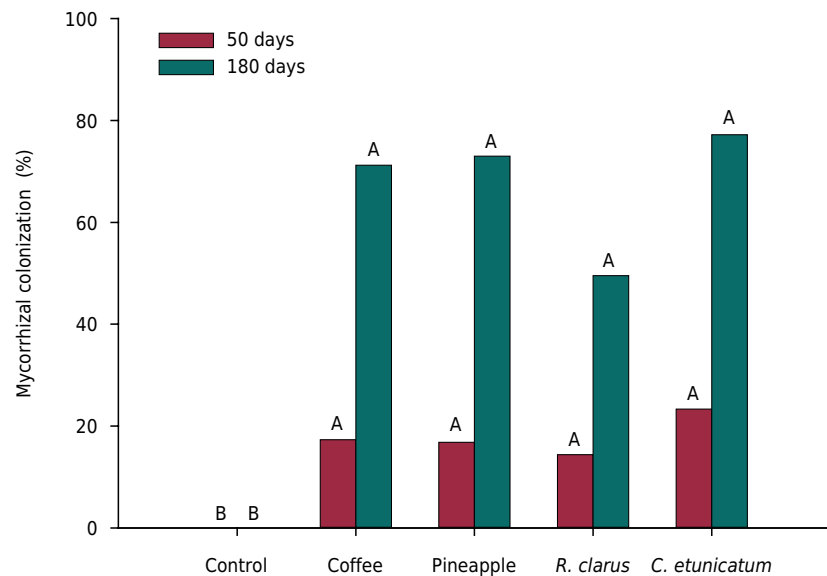


Figure 4. Mycorrhizal colonization of pineapple plantlets after 50 and 180 days of cultivation in a greenhouse. The plantlets were inoculated with spores obtained from coffee plantations, pineapple, and isolates of *Rhizophagus clarus* and *Claroideoglossum etunicatum* multiplied by the on-farm method. Means followed by the same letter did not differ when compared to the Tukey test ($p \leq 0.10$).

Table 1. Growth parameters in pineapple plantlets after 50 days of cultivation in a greenhouse, inoculated with spores obtained from coffee and pineapple plantations and isolated *Rhizophagus clarus* and *Claroideoglossum etunicatum* multiplied by the on-farm method

Treatments	Leaves number	Height	Shoot	
			fresh matter	dry matter
		cm	g	
Control	18.50 A	16.30 A	11.79 A	1.21 A
Pineapple	19.75 A	15.65 A	11.20 A	1.19 A
Coffee	19.25 A	13.33 A	8.46 A	0.97 A
<i>R. clarus</i>	17.50 A	14.18 A	8.26 A	1.08 A
<i>C. etunicatum</i>	20.50 A	15.95 A	13.76 A	1.47 A

Means followed by the same letter do not differ by Tukey test ($p \leq 0.10$).

2015). In addition, the mycorrhizal pineapple plantlets tend to have higher antioxidant enzyme activity, enhancing plant resistance to attack by pathogens (Moreira et al., 2016). Thus, this study is the first to report the inoculation of pineapple plantlets with AMF multiplied on-farm as an easy way of obtaining inoculum by the producer that also facilitates the efficient colonization of seedlings.

On average, there were 605 spores per 100 cm³ of soil obtained in the layers of 0.00-0.05 and 0.05-0.10 m in all treatments, supporting previous studies on AMF multiplication using the on-farm method (Douds Jr et al., 2006, 2010; Pozzan and Stürmer, 2014; Schlemper and Stürmer, 2014). Counting the number of spores in the top layer has been prioritized because the number of spores noticeably drops in deeper layers, as demonstrated by Pozzan and Stürmer (2014). These authors found that the average spore reduced from 384 to 72 and later 5 at the layers of 0.00-0.10, 0.10-0.20, and 0.20-0.30 m in the soil. This phenomenon might be related to a decline in the viability of spores in the deeper layers, as noted in the current study. Furthermore, the higher root concentration in the first layers of the substrate might contribute to the higher number of spores found in this region.

The high number of spores obtained by the current study demonstrates that the on-farm method produces a viable source of inoculum for AMF. Of note, Douds Jr et al. (2010) found that spores are not the only propagating material of these fungi, which also use roots colonized with vesicles and/or infective hyphal fragments.

The percentage of viable spores ranged 68.06 and 77.93 %, demonstrating the effectiveness of the on-farm method in producing good quality inoculum. Carvalho et al. (2001) recorded averages of 67-71 % viable spores, supporting the best results obtained by Druille et al. (2013a,b) in experiments with zero doses to glyphosate. Our results were higher than those obtained by Bharadwaj et al. (2007) in monoculture crop samples.

In many situations, plants might be colonized by different taxa of AMF simultaneously (Smith et al., 2011). Previous experiments on pineapple plantlets colonized with one AMF species versus multiple AMF species simultaneously showed that seedlings receiving mixed inoculum were superior for almost all parameters (including growth, nutrient absorption efficiency, photosynthetic production, and antioxidant enzymes), regardless of P level in the soil or cultivating analyzed (Moreira et al., 2015, 2016). Thus, the production of inoculum on-farm could obtain similar results because non-sterile soil is used, resulting in highly taxonomically diverse inocula (Douds Jr et al., 2006; Schlemper and Stürmer, 2014). Second, native AMF that are more suited to local conditions can be multiplied and could be used to help restore the native community of these fungi, particularly in degraded regions (Douds Jr et al., 2005, 2006, 2010).

Fungi adapted to local conditions could maximize the absorption of limiting nutrients (Johnson et al., 2010). Furthermore, because fungi carry different amounts of nutrients for plants, they may affect the growth of plants differently (Shukla et al., 2012). This phenomenon could provide more benefits compared to colonization with a single species. Furthermore, the mixed inoculation of AMF might have the characteristic of complementarity, exploiting the best of each species that colonizes the plant (Smith et al., 2011).

Therefore, it was expected that, in the current experiment, the obtained mixed inoculum of a pineapple plantation and multiplication on-farm would produce better results for colonization and seedling growth compared to the other treatments, as found in previous studies (Moreira et al., 2015, 2016). However, after 180 days of cultivation, there was high mycorrhizal colonization, supporting data from previous experiments, where plants were grown for 210 (Moreira et al., 2015) and 230 days (Moreira et al., 2016) in the greenhouse.

The incubation time might explain the low percentage of mycorrhizal colonization, which is subsequently reflected in growth parameters (LN, H, SFM, and SDM). Growth

parameters did not differ to those of non-inoculated plants, contrasting with previous studies (Moreira et al., 2015, 2016). However, while low colonization was documented at 50 days in this experiment, Rodríguez-Romero et al. (2011) obtained similar results seven months after inoculation with *Funneliformis mosseae*, but in this work, the inoculated pineapple plantlets were higher than controls in relation to SFM, SDM, and the absorption of N, P, and K. However, when producing pineapple plantlets, the average length of stay of the seedlings in the greenhouse is six months (Farahani, 2013), which is enough to gain benefits from mycorrhizal colonization, as previously demonstrated for this crop (Moreira et al., 2015, 2016). In addition, mycorrhiza might colonize a variety of agricultural crops (Cozzolino et al., 2013; Rodríguez-Romero et al., 2011), being detectable about 5-days after inoculation (Song et al., 2015).

Because many benefits are obtained by mycorrhizal colonization, exploitation of this symbiosis is desired, especially when aiming to minimize or eliminate the use of chemical fertilizers and synthetic pesticides (Douds Jr et al., 2008). One way to best take advantage of these benefits is to perform the inoculation with AMF still in the seedling production phase with isolated more adapted for later transplanting in the field (Douds Jr et al., 2008). Thus, the multiplication of inoculum on-farm represents a viable option for farmers because it saves the costs associated with processing and transporting inoculum, which would be included in the prices of commercial inoculants (Douds Jr et al., 2005). Besides being an economically viable strategy, it is also environmentally friendly as waste residues typically produced on the farm can be used as substrate for producing high quality inoculum (Schlemper and Stürmer, 2014).

CONCLUSIONS

The production of inoculum on-farm is effective for multiplying AMF from isolates of *R. clarus* and *C. etunicatum*, as well as from commercial crops (pineapple and coffee). In particular, highly viable spores were produced. Regardless of the source of inoculum, mycorrhizal colonization is established over time, reaching high levels of colonization of culture.

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REFERENCES

- Augé RM. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza*. 2001;11:3-42. <https://doi.org/10.1007/s005720100097>
- Azcón-Aguilar C, Cantos M, Troncoso A, Barea JM. Beneficial effect arbuscular mycorrhizas on acclimatization of micropropagated cassava plantlets. *Sci Hortic*. 1997;72:63-71. [https://doi.org/10.1016/S0304-4238\(97\)00120-9](https://doi.org/10.1016/S0304-4238(97)00120-9)
- Bharadwaj DP, Lundquist P-O, Alström S. Impact of plant species grown as monocultures on sporulation and root colonization by native arbuscular mycorrhizal fungi in potato. *Appl Soil Ecol*. 2007;35:213-25. <https://doi.org/10.1016/j.apsoil.2006.04.003>
- Carvalho LM, Caçador I, Martins-Loução MA. Temporal and spatial variation of arbuscular mycorrhizas in salt marsh plants of the Tagus estuary (Portugal). *Mycorrhiza*. 2001;11:303-9. <https://doi.org/10.1007/s00572-001-0137-6>
- Cozzolino V, Di Meo V, Piccolo A. Impact of arbuscular mycorrhizal fungi applications on maize production and soil phosphorus availability. *J Geochem Explor*. 2013;129:40-4. <https://doi.org/10.1016/j.gexplo.2013.02.006>
- Czerniak MJ, Stürmer SL. Produção de inoculante micorrízico *on farm* utilizando resíduos da indústria florestal. *Rev Bras Cienc Solo*. 2014;38:1712-21. <https://doi.org/10.1590/S0100-06832014000600006>
- Douds Jr DD, Nagahashi G, Pfeiffer PE, Kayser WM, Reider C. On-farm production and utilization of arbuscular mycorrhizal fungus inoculum. *Can J Plant Sci*. 2005;85:15-21. <https://doi.org/10.4141/P03-168>
- Douds Jr DD, Nagahashi G, Pfeiffer PE, Reider C, Kayser WM. On-farm production of AM fungus inoculum in mixtures of compost and vermiculite. *Bioresource Technol*. 2006;97:809-18. <https://doi.org/10.1016/j.biortech.2005.04.015>
- Douds Jr DD, Nagahashi G, Reider C, Hepperly PR. Choosing a mixture ratio for the on-farm production of AM fungus inoculum in mixtures of compost and vermiculite. *Compost Sci Util*. 2008;16:52-60. <https://doi.org/10.1080/1065657X.2008.10702355>
- Douds Jr DD, Nagahashi G, Hepperly PR. On-farm production of inoculum of indigenous arbuscular mycorrhizal fungi and assessment of diluents of compost for inoculum production. *Bioresource Technol*. 2010;101:2326-30. <https://doi.org/10.1016/j.biortech.2009.11.071>
- Druille M, Omacini M, Golluscio RA, Cabello MN. Arbuscular mycorrhizal fungi are directly and indirectly affected by glyphosate application. *Appl Soil Ecol*. 2013a;72:143-9. <https://doi.org/10.1016/j.apsoil.2013.06.011>
- Druille M, Cabello MN, Omacini M, Golluscio RA. Glyphosate reduces spore viability and root colonization of arbuscular mycorrhizal fungi. *Appl Soil Ecol*. 2013b;64:99-103. <https://doi.org/10.1016/j.apsoil.2012.10.007>

- Farahani F. Growth, flowering and fruiting in vitro pineapple (*Ananas comosus* L.) in greenhouse conditions. *Afr J Biotechnol.* 2013;12:1774-81. <https://doi.org/10.5897/AJB12.1421>
- Gaur A, Adholeya A, Mukerji KG. On-farm production of VAM inoculum and vegetable crops in marginal soil amended with organic matter. *Trop Agr.* 2000;77:21-6.
- Gerdeman JW, Nicholson TH. Spores of mycorrhizal endogene extracted from soil by wet sieving and decanting. *T Brit Mycol Soc.* 1963;46:235-44. [https://doi.org/10.1016/S0007-1536\(63\)80079-0](https://doi.org/10.1016/S0007-1536(63)80079-0)
- Giovannetti M, Mosse B. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytol.* 1980;84:489-500. <https://doi.org/10.1111/j.1469-8137.1980.tb04556.x>
- Goetten LC, Moretto G, Stürmer SL. Influence of arbuscular mycorrhizal fungi inoculum produced on-farm and phosphorus on growth and nutrition of native woody plant species from Brazil. *Acta Bot Bras.* 2016;30:9-16. <https://doi.org/10.1590/0102-33062015abb0175>
- Ijdo M, Cranenbrouck S, Declerck S. Methods for large-scale production of AM fungi: past, present, and future. *Mycorrhiza.* 2011;21:1-16. <https://doi.org/10.1007/s00572-010-0337-z>
- Johnson NC, Wilson GWT, Bowker M, Wilson J, Miller RM. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. *PNAS.* 2010;107:2093-8. <https://doi.org/10.1073/pnas.0906710107>
- Moreira BC, Mendes FC, Mendes IR, Paula TA, Prates Junior P, Salomão LCC, Stürmer SL, Otoni WC, Guarçoni MA, Kasuya MCM. The interaction between arbuscular mycorrhizal fungi and *Piriformospora indica* improves the growth and nutrient uptake in micropropagation-derived pineapple plantlets. *Sci Hortic.* 2015;197:183-92. <https://doi.org/10.1016/j.scienta.2015.09.032>
- Moreira BC, Prates Junior P, Jordão TC, Silva MCS, Stürmer SL, Salomão LCC, Otoni WC, Kasuya MCM. Effect of inoculation of symbiotic fungi on the growth and antioxidant enzymes' activities in the presence of *Fusarium subglutinans* f. sp. *ananas* in pineapple plantlets. *Acta Physiol Plant.* 2016;38:235. <https://doi.org/10.1007/s11738-016-2247-y>
- Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plantarum.* 1962;15:473-97. <https://doi.org/10.1111/j.1399-3054.1962.tb08052.x>
- Phillips JM, Hayman DS. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *T Brit Mycol Soc.* 1970;55:158-61. [https://doi.org/10.1016/S0007-1536\(70\)80110-3](https://doi.org/10.1016/S0007-1536(70)80110-3)
- Pozo MJ, Cordier C, Dumas-Gaudot E, Gianinazzi S, Barea JM, Azcón-Aguilar C. Localized versus systemic effect of arbuscular mycorrhizal fungi on defense responses *Phytophthora* infections in tomato plants. *J Exp Bot.* 2002;53:525-34. <https://doi.org/10.1093/jexbot/53.368.525>
- Pozzan AM, Stürmer SL. Produção de inóculo micorrízico mono específico e multiespecífico utilizando o método on farm. In: *FertBio 2014: Fertilidade e biologia do solo: integração e tecnologia para todos; setembro 2014; Araxá. Viçosa, MG: Sociedade Brasileira de Ciência do Solo; 2014.*
- Rodríguez-Romero AS, Azcón R, Jaizme-Vega MC. Early mycorrhization of two tropical crops, papaya (*Carica papaya* L.) and pineapple [*Ananas comosus* (L.) Merr.], reduces the necessity of P fertilization during the nursery stage. *Fruits.* 2011;66:3-10. <https://doi.org/10.1051/fruits/2010036>
- Rozpadek P, Wezowicz K, Stojakowska A, Malarz J, Surówka E, Sobczyk Ł, Anielska T, Wazny R, Miszalski Z, Turnau K. Mycorrhizal fungi modulate phytochemical production and antioxidante activity of *Cichorium intybus* L. (Asteraceae) under metal toxicity. *Chemosphere.* 2014;112:217-24. <https://doi.org/10.1016/j.chemosphere.2014.04.023>
- Schlemper TR, Stürmer SL. On farm production of arbuscular mycorrhizal fungi inoculum using lignocellulosic agrowastes. *Mycorrhiza.* 2014;24:571-80. <https://doi.org/10.1007/s00572-014-0576-5>
- Sieverding E. Vesicular-arbuscular mycorrhiza management in tropical agrosystems. Technical cooperation, Eschborn, Federal Republic of Germany; 1991.

Shukla A, Kumar AJA, Ajit DVK, Rao N. Phosphorus threshold for arbuscular mycorrhizal colonization of crops and tree seedlings. *Biol Fert Soils*. 2012;48:109-16. <https://doi.org/10.1007/s00374-011-0576-y>

Smith SE, Read DJ. *Mycorrhizal symbiosis*. 2nd ed. Cambridge: Academic Press; 1997.

Smith SE, Jakobsen I, Gronlund M, Smith FA. Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. *Plant Physiol*. 2011;156:1050-7. <https://doi.org/10.1104/pp.111.174581>

Song F, Qi D, Liu X, Kong X, Gao Y, Zhou Z, Wu Qi. Proteomic analysis of symbiotic proteins of *Glomus mosseae* and *Amorpha fruticosa*. *Sci Rep*. 2015;5:18031. <https://doi.org/10.1038/srep18031>

Walley FL, Germida JJ. Estimating the viability of vesicular-arbuscular mycorrhizae fungal spores using tertrazolium salts as vital stains. *Mycologia*. 1995;87:273-9. <https://doi.org/10.1080/00275514.1995.12026530>