Eucalypt as trap plant to capture associative fungi in soil samples from great depth

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***Abstract*—** *There are only very few papers reporting on mycorrhizal and endophytic fungi along a whole soil profile in great depth. The objective of our study was to find propagules of arbuscular mycorrhizal (AMF), ectomycorrhizal (ECM) and dark septate endophytic fungi (DSE) using Eucalyptus grandis as trap plant. We sampled soil of the surface to eight m down in a four-years-old eucalypt plantation. The sampled soil was used as seeding substrate in 300 ml-pots in the greenhouse at a temperature of 28ºC. Plants were grown for 100 days before harvest and evaluation of fungal presence in the plantlets. In most of the treatments (soil layers of increasing depth) we found root colonization of plantlets by different fungi. In some of the treatments, even in some originating from very deep soil layers, we detected the synthesis of ectomycorrhizal fruiting bodies. Some of the fungi in the plants could be isolated and identified. Therefore, we conclude that in every one of the different soil layers there was the presence of one or more potentially associative fungi with this tree.*

***Keywords*— Arbuscular Mycorrhiza, Dark septate Endophytes, Deep soil layers, Ectomycorrhiza.**

I. INTRODUCTION

Most plants can associate with symbiotic mycorrhizal fungi, a feature that promotes increased nutrient and water absorption, producing vigorous plants resistant to environmental stresses (Smith & Read 2008). Generally, every plant presents only one type of mycorrhizal interaction, however, in a few kinds, as Eucalyptus, one can find a simultaneous colonization with AMF and ECM (Campos et al. 2011).

DSE may also coexist on the same host plant with either kind of mycorrhizal fungi (Wagg et al. 2008; Newsham, 2011; Bonfim et al. 2016). Surveys on the structure of mycorrhizal fungi in plants are normally restricted to the uppermost soil layer (zero to 20 cm) where we find the greatest root biomass (Brundrett 1991). Only a few reports include a survey of more profound soil layers (Oehl et al. 2005; Becerra et al. 2014). Nevertheless, there is a direct relation between the height of a tree and the deepness of its root in the soil. For example, forest plants can have very deep root systems, down to 8 m or more (Pereira 2014). Therefore, several questions may be asked, as: are there associative fungi with Eucalyptus roots in deep soil layers?

To respond to this question our research group followed down the growth and vertical distribution of four year-old Eucalyptus roots and evidenced the presence of a rich and varied microbiota in all soil layers, including ecto and endomycorrhizal fungi, besides other endophytes, even at very deep sites (PEREIRA, 2014).

However, during this survey we could not find out if these fungal communities had the potential to infect Eucalyptus roots. The fact of the fungi being detected at those locations does not necessarily indicate their activity. Therefore, our hypothesis was that there are fungal propagules able to associate with these tree roots down to the greatest soil depth (to 8 m deep). Therefore, we set up a greenhouse experiment in which the sampled soil was transferred into plastic pots to serve as seeding substrate for Eucalyptus grandis seeds to obtain trap plants for these propagules.

This is a pioneer research because it shows the activity of fungi present in soil layers down to 8 m deep. It is of surmount importance to understand the life cycle of the fungal community and the functionality of the fine roots which are essential in furnishing water and nutrients to the trees, especially during scarceness of water in tropical soils in Brazil.

II. MATERIAL AND METHOD

2.1 Experimental Area and Seeding Substrate

The soil samples were collected in a four years-old Eucalypt stand, at the Forestry Experimental Station of Itatinga, located in the municipality of Itatinga, São Paulo – Brazil (23º02' S 48º38' W). For sampling of soil we manually built some deep

trenches beside a tree, measuring 1.80 x 0.6 m x 8 m deep. Six soil layers (0-1; 1-2; 2-3; 3-4; 4-5 and 5-8 m) were sampled from three trenches. For each layer four repetitions were obtained, resulting in 24 samples. About 25 cm of the external soil from each trench was discarded to avoid cross contamination among soil layers. The sampled soil was immediately stored in sterile plastic bags, that were maintained completely closed, without any external contact, at 4ºC, up to the moment of the installation of the experiment.

The soil samples were mixed with washed and sterilized river sand, in the proportion of 1:1. The greenhouse experiment had a completely aleatory design, with six treatments related to increasing depth and four repetitions. The Eucalypt seedlings were cultivated for 100 days at an average temperature of 28ºC. The experimental unit consisted of a 300 ml plastic pot with one plant. We used pre-sterilized E. grandis seeds. During the first 30 days of growth the seedlings were weak and showed little growth, especially those growing in deep sub-soil layers. At this time we started weekly applications of Hoagland's solution (Hoagland & Arnon 1950) containing ¼ of the preconized P strength (Sarruge 1970). Forty days after sowing each pot received an 8 g application of a sterilized commercial potting mix (Tropstrato Florestal Tubete Plus). The sand and substrate were sterilized in an autoclave at 120oC, for one hour.

2.2 Evaluation of seedling colonization by fungi

Plants were harvested 100 days after germination. To evaluate AMF and DSE colonization the roots were rinsed with water and heated at 60°C in 10% KOH for 10 minutes, dyed with a 5% solution of blue ink (Parker) and 5% acetic acid in a water bath at 90°C for 3 min (Vierheilig et al. 1998). Afterwards the roots were preserved in lacto-glycerol (Koske & Gemma 1989). To determine the presence of ECM the roots were observed under the stereomicroscope without any pre-treatment. AMF spores were extracted from 50 g of soil by wet sieving (Gerdemann & Nicolson 1963), followed by a centrifugation in 60% sucrose for 3 min at 3,500 rpm. Only viable spores were caught, identified by being resistant to the touch of a dissecting needle, fixed on glass slides, in PVLG (polyvynilic alcohol, lactic acid and glycerol) + Melzer's reagent (Morton et al. 1993; INVAM 2000; Bonfim et al. 2015). ECM fruiting bodies grew out of the soil close to the seedlings.

III. RESULTS AND DISCUSSION

We found AMF root colonization in all analyzed plantlets (Table 1, Figure1) and all samples of rhizospheric soil contained viable AMF spores.

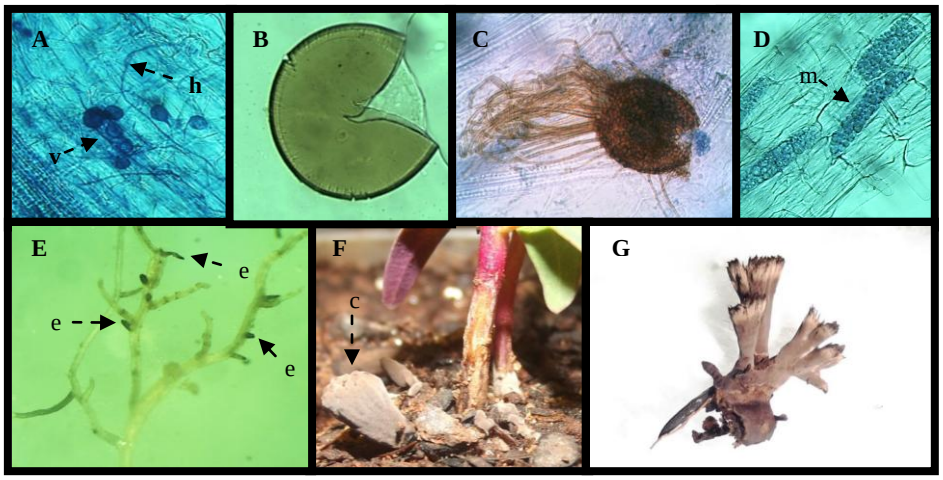

FIGURE 1. A) AM MYCORRHIZAL STRUCTURES: (H) = HYPHA AND (V) = VESICLES. B) AMF SPORE EXTRACTED FROM SOIL. C) REPRODUCTIVE STRUCTURE OF DSE IN ROOTS. D) MICROSCLEROTIA OF DSE IN ROOTS. E) ECM STRUCTURE IN ROOTS (E). F, G) BASIDIOMYCETE FRUITING BODY CLOSE TO THE PLANTLET (C)

Taxonomic identification (Caproni et al. 2005) was possible only for a few of the spores. The genera Acaulospora and Gigaspora were predominant. DSE were also found in the root cortex of plantlets growing in four of the six tested depths (Barrow & Aaltonen 2001) and some presented microsclerotia (Barrow 2003). We also found cleistothecia or picnidia of DSE in the root of one plantlet (Carris et al. 2012). We verified the presence of typical ECM structures (Agerer 2001) at the edges of roots in all treatments (Table 1, Figure 1).

TABLE: 1
AMF, DSE AND EMC ROOT COLONIZATION IN THE SIX DIFFERENT SOIL DEPTH LAYERS, PRESENCE OF AMF IN SOIL AND ECM FRUITING BODIES EMERGING FROM SOIL

DEPTH (m)	AMF Col.	AMF Spores	DSE Col.	ECM Col.	EMC FB
0-1	+	+	+	+	+
1-2	+	+	-	+	-
2-3	+	+	+	+	+
3-4	+	+	+	+	-
4-5	+	+	-	+	+
5-8	+	+	+	+	-

*** + and - = presence or absence. AMF = Arbuscular mycorrhizal fungus, DSE = Dark septate endophyte, ECM = Ectomycorrhiza, FB = fruiting body, Col. = Colonization.**

Although unexpected, starting at 90 days after emergence, we could follow the outgrowth of basidiomycetal fruiting bodies (FB) (Table 1, Figure 1) in some of the pots with the treatments 0-1, 2-3 and 4-5m, which we classified tentatively as belonging to the Thelephoraceae (Agerer & Weiss 1989).

V. CONCLUSION

The trap plant method worked quite well to detect the type of fungal propagules present in increasing soil depth layers down to 8 m. Since we took great care not to contaminate our soil samples between layers, we can affirm that all the fungal structures that were detected originated from propagules found in each of the soil layers. However, it is not possible to guarantee that all of them would have colonized plant roots in the soil profile in the field, since the physical and chemical soil attributes in great depth are quite different from those of the more superficial soil. There are variations in texture, aeration, pH, mineral nutrients, etc. In our greenhouse experiment we modified those attributes to turn them more similar to those of the soil surface, just to allow for plant growth in the sampled soil.

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