Current Research in Environmental & Applied Mycology (Journal of Fungal Biology)
8(4): 455–467 (2018) ISSN 2229-2225

www.creamjournal.org

Article

Doi 10.5943/cream/8/4/5

Ectomycorrhizal fungi associated with the roots of planted *Eucalyptus grandis* in northeastern Brazil

Coelho IL[1]*, Nelsen DJ[2], Ben Hassine Ben Ali M[1] and Stephenson SL[1]

[1] Department of Biological Sciences, University of Arkansas, Fayetteville, Arkansas, 72701, USA.
[2] Kansas Biological Survey, University of Kansas, Lawrence, Kansas 66047, USA.

Coelho IL, Nelsen DJ, Ben Hassine Ben Ali M, Stephenson SL 2018 – Ectomycorrhizal fungi associated with the roots of planted *Eucalyptus grandis* in northeastern Brazil. Current Research in Environmental & Applied Mycology (Journal of Fungal Biology) 8(4), 455–467, Doi 10.5943/cream/8/4/5

Abstract

Eucalyptus, like other members of the family Myrtaceae, form symbiotic associations with various ectomycorrhizal fungi. The purpose of this study was to assess the ectomycorrhizal taxa associated with *Eucalyptus* plantations in the municipalities of Gloria do Goita and Moreno, Pernambuco, Brazil. Root-tip samples were collected from *Eucalyptus grandis* trees to identify the ectomycorrhizal and other root-associated fungi present. Twelve taxa of fungi were determined from the root-tips collected in these two plantations. Four taxa were identified to species, and five were identified as putative ectomycorrhizae. *Scleroderma albidum* was the only species found both fruiting and in the root-tip study. Overall, species of *Tomentella* were the dominant taxa present in the two study areas.

Key words – ecology – ITS ribosomal DNA region – mycorrhiza – *Tomentella* – tree plantations

Introduction

Ectomycorrhizal fungi, first described by Frank (1885), have the ability to colonize and provide benefits to species of vascular plants. For example, ectomycorrhizal associations increase the area of root absorption, allowing the vascular plant to obtain more water and nutrients such as phosphorus (P), nitrogen (N) and potassium (K) from the soil (Glowa et al. 2003, Sawyer et al. 2003, Zhao et al. 2015). The fungi that form these associations also increase host-plant resistance to water stress, higher temperatures, soil acidity, and their presence results in improved tolerance to toxic soil substances and root pathogens (Marx & Cordell 1989, Smith & Read 1997, Hall 2002). Ectomycorrhizal fungi also are important to the establishment and growth of plants, particularly in nutrient-poor or degraded soils (Marx & Ruehle 1988, Marx & Cordell 1989, Ortiz et al. 2015), examples of which occur in white-sand forests, in Brazil and French Guiana, composed of clay soils known to have low water-retaining capacity, and low levels organic matter (Roy et al. 2016).

The first attempts to establish ectomycorrhizal trees outside of their native habitats showed that these plants struggled in the absence of their symbiotic fungi (Vozzo & Hacskaylo 1971, Mikola 1973). Turjaman et al. (2011), Alberton et al. (2014) discussed the difficulty in establishing non-native trees outside of their native ranges and the importance of inoculation of compatible fungi to the planting substrate to ensure the adaptation of the introduced species. Further investigation highlighted the importance of ectomycorrhizal fungi-tree interactions during early

Submitted 13 April 2018, Accepted 6 August 2018, Published 29 August 2018
Corresponding Author: Isadora L. Coelho – e-mail – icoelho@uark.edu

seedling growth, plant growth promotion, and nutrient acquisition (Holste et al. 2017). These findings emphasize the importance of ectomycorrhizal associations and demonstrate the value of understanding the belowground interactions between ectomycorrhizal fungi in forest ecosystems.

The ectomycorrhizal status of *Eucalyptus* has been known for almost a century (Samuel 1926), and the benefits of the symbiosis have been commercially exploited in many countries (e.g., St. John 1980, Zambolim & Barros 1982, Coelho et al. 1997).

Fossil records indicate that the genus *Eucalyptus* originated in the central portion of the Australian continent (Pryor 1976). The more than 600 described species are endemic to Australia, New Guinea, and surrounding islands (Pryor 1976). However, *Eucalyptus* plantations have been established around the world to provide pulp and the various pharmaceutical and hygiene products derived from this tree (Christie 2008, Rocha et al. 2015). Many species of *Eucalyptus* are known for their rapid growth, high pulp yield, and resistance to adverse environmental conditions and diseases, thus allowing their use on a commercial scale (Santos 2001, Vera et al. 2017). For these characteristics, their introduction in the tropics has been highly favourable.

In 1825 seedlings of *Eucalyptus robusta* Sm. and *E. tereticornis* Sm. were the first species introduced to Brazil, and these were planted in the southeastern region of the country in response to deforestation (Moura et al. 1980). Currently, *Eucalyptus* is the most cultivated forest genus in Brazil, the second largest area planted in the world, surpassing 4.7 million hectares distributed throughout several regions of the country (de Souza et al. 2017). The largest *Eucalyptus* plantations are in areas with low soil fertility and substantial drought problems since it is one of the best adapted non-native timber species for such conditions (Marques Júnior et al. 1996, Lima et al. 2013).

Due to the economic and ecological value of *Eucalyptus*, and considering the importance of ectomycorrhizal associations for the establishment of these trees, there have been considerable efforts directed towards characterizing the fungi associated with these hosts in Brazil (Singer & Araujo 1979, Singer et al. 1983, Yokomizo 1986, Giachini et al. 2000, 2004). However, the majority of these investigations are limited to mushroom-forming fungi and *in situ* observation of fruiting bodies (Oliveira et al. 1997, Giachini et al. 2004). Such an approach neglects an important portion of the ectomycorrhizal community, which is likely to be observed only with the implementation of molecular techniques. Moreover, despite the increasing interest in this specific group of ectomycorrhizal fungi, most studies are concentrated in the southern portion of the country. Therefore, knowledge regarding ectomycorrhizal communities associated with commercial *Eucalyptus* plantations in northeastern Brazil remain limited.

The objectives of this study were first to evaluate the presence of root-associated fungi and then to determine the overall biodiversity of ectomycorrhizal fungi in two *Eucalyptus grandis* W. Hill ex Maiden plantations in Brazil (Fig. 1), located in the municipalities of Gloria do Goita and Moreno, Pernambuco, Brazil. This study provides a better understanding of the ecology of these plantation forests, and increases our knowledge relating to the ecological relationships which exist between fungi and plants.

Materials & Methods

Study areas

The study areas were two *Eucalyptus grandis* plantations located in the municipalities of Gloria do Goita (8º00'0.05"S and 35 º17'2.67"W) and Moreno (8º06'7.32"S and 35º12'5.00"W), both in the state of Pernambuco (Fig. 1a, b). Previously used as farmland for sugarcane monoculture, both plots received dolomitic lime (calcium magnesium carbonate) treatments to correct soil acidity before *Eucalyptus* was planted. *Eucalyptus* cultivation at both sites began in 2002, with seedlings placed in 30 centimeters deep holes, spaced two or three meters apart and supplied with chemical fertilizers. The collection sites were in humid tropical areas at between 80 m and 130 m elevation.

This region of Brazil is often called the "sea of hills," the landform consists of rocks from the Pre-Cambrian Age (between 650 million and one billion years ago), carved out by chemical decomposition processes and precipitation runoff (CPRH 1999a, 1999b). As a result of various disruptions that occurred during different geological ages, these rocks have a large number of faults and fractures (de Andrade & Lins 1984). The soils usually have a reddish and sandy clay composition, which varies from sandy-loamy to sandy (dos Santos et al. 2014), and are susceptible to erosion and vary in depth depending on decomposed rock type, sometimes reaching a depth of more than 20 meters (CPRH 1999a, 1999b, dos Santos et al. 2014).

Fig. 1 – *Eucalyptus grandis* plantations in the municipalities of Gloria do Goita. a at approximately 8 years of age and Moreno. b at approximately 9 years of age in the state of Pernambuco, Brazil.

The climate is hot and humid, being classified as type As' (pseudotropical), according to the Köppen classification system. Autumn-winter rains characterize the rainforest zone, with an annual rainfall of approximately 2500 L/m^2, which is relatively well distributed throughout the year. The driest period extends from October to December (Alvares et al. 2013). The average annual temperature is 23ºC, with a maximum average high of 29ºC and a minimum average low of 19ºC (Le Sann 1983, CPRH 2013).

Root-tip collection and DNA extraction

In June 2015 (rainy season), root-tips were collected from the rhizosphere at depths of 5 to 20 cm of five individuals of *E. grandis*, located 10 m from the border of the plot and 10 m apart from each other in each of the two study areas. The root-tips selected occurred within a distance of 90 cm from the trunk of the tree and collecting was carried out in all directions.

Before microscopic examination and subsequent DNA extraction, the root-tip samples were carefully washed with deionized water to remove soil residues. The cleaned roots were transferred to a polystyrene Petri dish. Digital images of ECM morphologies were obtained with a Leica DFC495 binocular microscope, using black background illumination at various magnifications (Fig. 2). Individual ECM root-tips were then transferred to clean sterile 1.5 ml microfuge tubes. Samples were homogenized using a Geno/Grinder 2010 with 3 mm glass beads (10 min, 1620 rpm). DNA extraction of homogenized tissue was carried out using the NucleoSpin Plant II kit (Macherey-Nagel, Bethlehem, Pennsylvania). Protocol steps were modified from the

manufacturer's original protocol to achieve optimal DNA extraction. Modifications included dividing the volumes of PL_1 Buffer solution, Rnase A and PC Buffer solution PC by half, and performing a single wash of extracted DNA with 350 ml PW1 Buffer solution. DNA samples were eluted in 25 µl of PE Buffer solution.

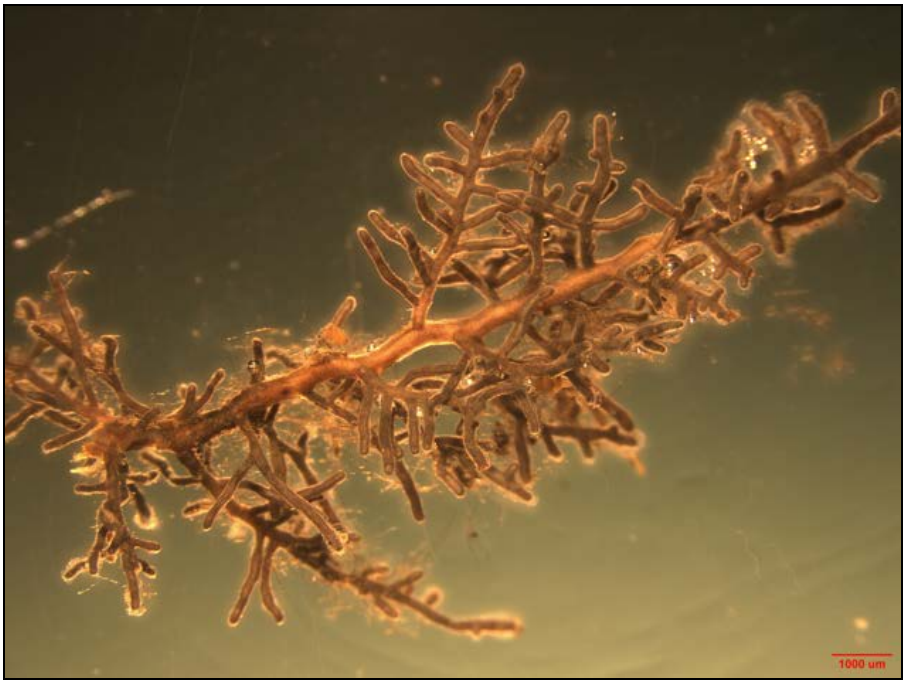

Fig. 2 – Hyphae of ectomycorrhizal fungi (*Tomentella* sp.) associated with the roots of planted *Eucalyptus grandis* from Gloria do Goita, Pernambuco, Brazil.

It is also important to mention that even though the collecting efforts were directed primarily to root-tips, epigeous sporocarps of *Scleroderma albidum* Pat. & Trab were observed and collected in the field, and sequences obtained from these were compared to sequences obtained from root-tips. Macroscopic and microscopic characters (e.g., yellowish color of the sporocarp, spores 11-16 µ in diameterwith blunt spines) corroborated by DNA-based identification methods were used to identify the fruiting bodies of *S. albidum* (Kirk 2015). When compared, the sequences of *S. albidum* obtained from root-tips and sporocarp tissue were identical.

PCR, sequencing, and analysis

The DNA extracted from each root-tip was amplified by the polymerase chain reaction (PCR), using the fungal-specific primers ITS1F and ITS4 (Bruns et al. 1998). PCR amplifications were carried out in a Bio-Rad T100^{TM} thermal cycler. The PCR program consisted of an initial denaturation at 95 °C for 5 min, followed by 37 cycles of denaturation at 95 °C for 20 s, annealing at 56 °C for 30 s, amplification at 72 °C for 1.30 min, and a final extension at 72 °C for 7 min. PCR products were verified via electrophoresis in a 1.5% agarose gel in 0.5× TAE buffer, stained by SYBR safe. MassRuler Express Forward DNA ladder Mix (Thermo Scientific) was used as a size standard. DNA was sent for single-pass Sanger sequencing to Beckman-Coulter Genomics (Danvers, Massachusetts).

Sequences were edited using the software SeqMan-program version 7.1.0 (44.1) and manually corrected before alignment to obtain a consensus sequence from forward and reverse reads. For a DNA-based identification, all sequences were in-silico compared with the results of a nucleotide search using the Basic Local Alignment Search Tool (BLAST) available at the National Center for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov).

Sequences were clustered using a 97% identity threshold in the algorithm Cluster Database at High Identity with Tolerance (CD-HIT) for nucleotide sequences (CD-HIT-EST) using the CD-

HIT online suite (http://weizhongli-lab.org/cdhit_suite/cgi-bin/index.cgi) (Huang et al. 2010). The resulting identified fungal taxa were assigned putative ecological function based on taxonomy using the dataset of Tedersoo et al. (2014) as a reference and community composition was assessed.

Results

From the total of 100 tips collected from both study areas, 68 were selected for DNA extraction based on hyphal colonization observed on root surfaces. Of the 68, 40 produced ITS DNA fragments that could be sequenced and identified, mostly to the taxonomic level of genus (Table 1). The fungus identified as present on more than half (25) of the colonized root-tips was a member of the ectomycorrhizal genus *Tomentella* (Thelephoraceae), with ITS sequences most closely resembling those deposited in GenBank from collections made in New Caledonia. Only four of the fungi associated with the root-tips could be identified to the level of species. These were *Aureobasidium pullulans* (de Bary & Lowenthal) G. Arnaud (Aureobasidiaceae), *Subulicystidium longisporum* (Pat.) Parmasto (Hydnodontaceae), *Gymnopus gibbosus* (Corner) A.W. Wilson, Desjardin & E. Horak (Omphalotaceae), and *S. albidum*. (Sclerodermataceae). Two sporocarps of *S. albidum* were collected in the field beneath *Eucalyptus* in the same study areas from which root-tips were obtained (Fig. 3a, b).

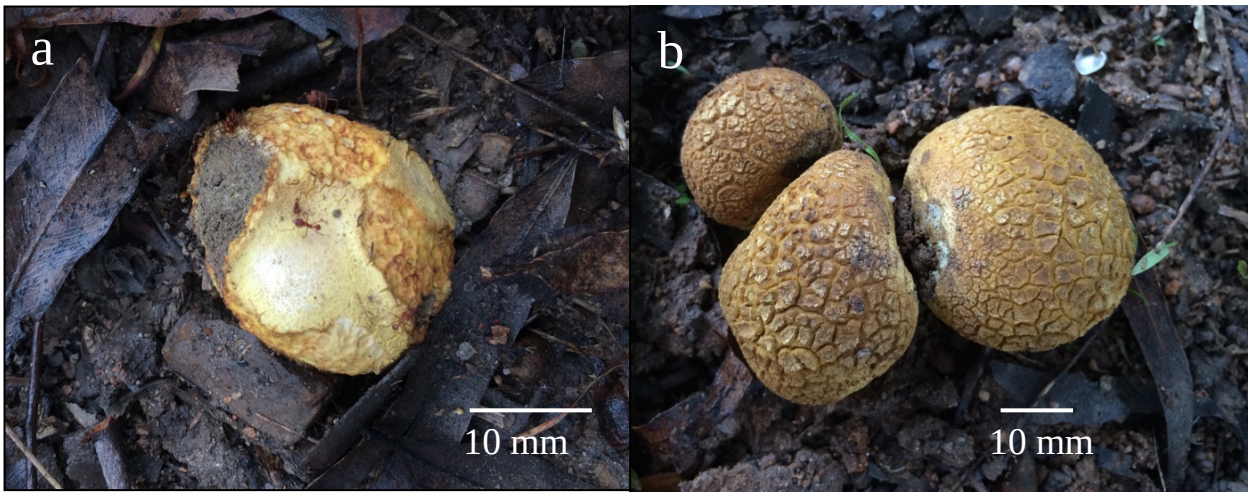

Fig. 3 – Epigeous sporocarps of *Scleroderma albidum* (a–b).

The ITS sequences were clustered to determine conspecific sequence identity and also to assess the diversity of fungi on the roots of *Eucalyptus* in the Brazilian plantations being surveyed (Table 2). A total of 12 fungal taxa were identified, based on clustering at a 97% identity for homologous sequences out of the original 40 sequences. Among the most common taxa resolved for the entire set of data were two different species of *Tomentella*, with one of these more than four times more common than the other.

Discussion

In the present study, the assemblage of fungi associated with root-tips of *Eucalyptus* was assessed to examine the relative proportions of (1) the different ecological functional groups and (2) the different fungal taxa present (Fig. 4). Out of the 40 sampled root-tips, 72% were colonized by ectomycorrhizal fungi representing five taxa. The largest proportion of the ectomycorrhizal fungi present (76%) was represented by members of the genus *Tomentella*, which is known for its cosmopolitan distribution (Rachid et al. 2015). The smallest proportion of the root-associated community was made up by saprotrophic fungi (3%), with *Subulicystidium longisporum* (at 84% sequence similarity, possibly an undescribed species) and *Gymnopus gibbosus* the two species present.

Table 1 Taxonomic identity assigned to fungi present on the roots of *Eucalyptus grandis* in northern Brazil based on ITS rDNA identification. Of the 40 root-associated fungi identified, 25 were members of the genus *Tomentella*, a group of ectomycorrhizal corticioid fungi.

Taxon	**Sample No.**	**%ID**	**%Coverage**	**Accession No.**	**Accession location**
Ascomycota (unidentified sp.)	BR51	94%	96%	FJ999654.1	Yunnan, China
Aureobasidium pullulans	BR41	100%	100%	AY225166.1	Thailand
Aureobasidium pullulans	BR42	99%	100%	KP131645.1	Sydney, Australia
Aureobasidium pullulans	BR38	100%	100%	KX184282.1	China
Subulicystidium longisporum	BR33	84%	99%	JX998773.1	Costa Rica
Dothideomycetes sp.	BR53	97%	100%	KR818855.1	Brazil
Dothideomycetes sp.	BR24	96%	100%	KR818855.1	Brazil
Dothideomycetes sp.	BR18	97%	100%	KR818855.1	Brazil
Dothideomycetes sp.	BR17	96%	100%	KR818855.1	Brazil
Fungus (unidentified sp.)	BR21	100%	100%	KJ690092.1	Senegal and Reunion Island
Gymnopus gibbosus	BR3	100%	100%	KU194327.1	Hong Kong
Pezizomycotina sp.	BR40	93%	99%	EF027382.1	California
Scleroderma albidum	BR67	100%	100%	KJ676532.1	Southern Brazil
Scleroderma albidum	BR64	100%	100%	KJ676532.1	Southern Brazil
Scleroderma albidum	BR11	100%	100%	KJ676532.1	Southern Brazil
Tomentella sp.	BR54	95%	98%	AB777489.1	NE Thailand
Tomentella sp.	BR76	99%	99%	LC122063.1	New Caledonia
Tomentella sp.	BR55	99%	99%	LC122063.1	New Caledonia
Tomentella sp.	BR34	99%	99%	LC122063.1	New Caledonia
Tomentella sp.	BR32	99%	100%	LC122063.1	New Caledonia
Tomentella sp.	BR7	98%	100%	LC122078.1	New Caledonia
Tomentella sp.	BR4	98%	100%	LC122078.1	New Caledonia
Tomentella sp.	BR2	97%	98%	LC122078.1	New Caledonia
Tomentella sp.	BR89	98%	97%	LC122078.1	New Caledonia
Tomentella sp.	BR65	98%	100%	LC122078.1	New Caledonia
Tomentella sp.	BR58	98%	100%	LC122078.1	New Caledonia
Tomentella sp.	BR57	98%	99%	LC122078.1	New Caledonia
Tomentella sp.	BR56	99%	99%	LC122078.1	New Caledonia
Tomentella sp.	BR28	99%	96%	LC122078.1	New Caledonia
Tomentella sp.	BR27	98%	97%	LC122078.1	New Caledonia
Tomentella sp.	BR26	98%	98%	LC122078.1	New Caledonia
Tomentella sp.	BR19	98%	91%	LC122078.1	New Caledonia
Tomentella sp.	BR13	98%	98%	LC122078.1	New Caledonia
Tomentella sp.	BR12	99%	99%	LC122078.1	New Caledonia
Tomentella sp.	BR9	98%	99%	LC122165.1	New Caledonia
Tomentella sp.	BR78	98%	96%	LC122165.1	New Caledonia
Tomentella sp.	BR75	98%	99%	LC122166.1	New Caledonia

Table 1 Continued.

Taxon	Sample No.	%ID	%Coverage	Accession No.	Accession location
Tomentella sp.	BR14	98%	100%	LC122260.1	New Caledonia
Tomentella sp.	BR36	99%	96%	LC122260.1	New Caledonia
Tomentella sp.	BR35	99%	99%	LC122260.1	New Caledonia

Table 2 Clustering of ITS sequences using CD-HIT-EST algorithm resulted in 12 taxa based on 97% identity across the length of the amplicon.

Sample Number	Taxonomic identification	Ecological group	Number of sequences clustered
BR54	*Tomentella* sp. 1	Ectomycorrhizal	18 sequences
BR32	*Tomentella* sp. 2	Ectomycorrhizal	4 sequences
BR78	*Thelephora* sp. 3	Ectomycorrhizal	3 sequences
BR64	*Scleroderma albidum*	Ectomycorrhizal	3 sequences
BR42	*Aureobasidium pullulans*	Endophyte	3 sequences
BR21	*Ruhlandiella* sp. 1	Ectomycorrhizal	1 sequence
BR53	Dothideomycetes sp. 1	Unresolved	2 sequences
BR17	Dothideomycetes sp. 2	Unresolved	2 sequences
BR33	*Subulicystidium longisporum*	Saprotroph	1 sequence
BR51	Sordariomycetes sp. 1	Unresolved	1 sequence
BR3	*Gymnopus gibbosus*	Saprotroph	1 sequence
BR40	*Diatrypella* sp. 1	Plant pathogen	1 sequence

The diversity of ectomycorrhizal fungi present on the roots of *E. grandis* was relatively low (only five taxa) when compared to previous studies. According to Chilvers (2000), more than 140 identified species of ectomycorrhizal fungi are known to be associated with *Eucalyptus* trees around the world. Among these, 104 have been reported from Australia, where native *Eucalyptus* forests occur or plantations have been established, and 37 have been recorded outside Australia. In Brazil, Giachini et al. (2000) reported a higher diversity of ectomycorrhizal fungi, including some sequestrate species, in plantations of *Eucalyptus* and *Pinus* than previously reported. Later, Giachini et al. (2004) listed 23 ectomycorrhizal fungi associated with *E. dunnii* Maiden plantations in southern Brazil. More recently, studies focused on specific groups have expanded this list (Gurgel et al. 2008, Pagano & Scotti 2008, Abreu et al. 2013, Paz et al. 2015).

Factors that may have contributed to the differences in ectomycorrhizal diversity reported in the various studies include climatic and topological characteristics, soil properties, plantation age, tree species and land history (Chen et al. 2007). Giachini et al. (2004) observed that occurrence and diversity of ectomycorrhizal species were influenced by season, mentioning a decline in fungal

populations during the rainy season and an increase during dry periods throughout the year. The fact that high temperatures and occasional precipitation during summer appear to cause an increase of ectomycorrhizal colonization rates, as reported by Grazziotti et al. (1998), Abreu et al. (2013), could have influenced the number of species found in the present study. Walker et al. (2008), Corcobado et al. (2015), who examined the seasonal dynamics of ectomycorrhizal fungi, reported that changes in the composition of the assemblage of species present occur throughout the year. Consequently, our data represent a "window" into just one period during the year. Sampling at a different period of time might well have yielded other taxa. Furthermore, soil constitution, compaction, and aeration may influence mycorrhizal communities. Mycelial growth and mycorrhizal formation are inhibited in clayey soils due to its low aeration and oxygen concentration. Thus, the clayey nature of the soil in this region would be a further disadvantage to ectomycorrhizal associations.

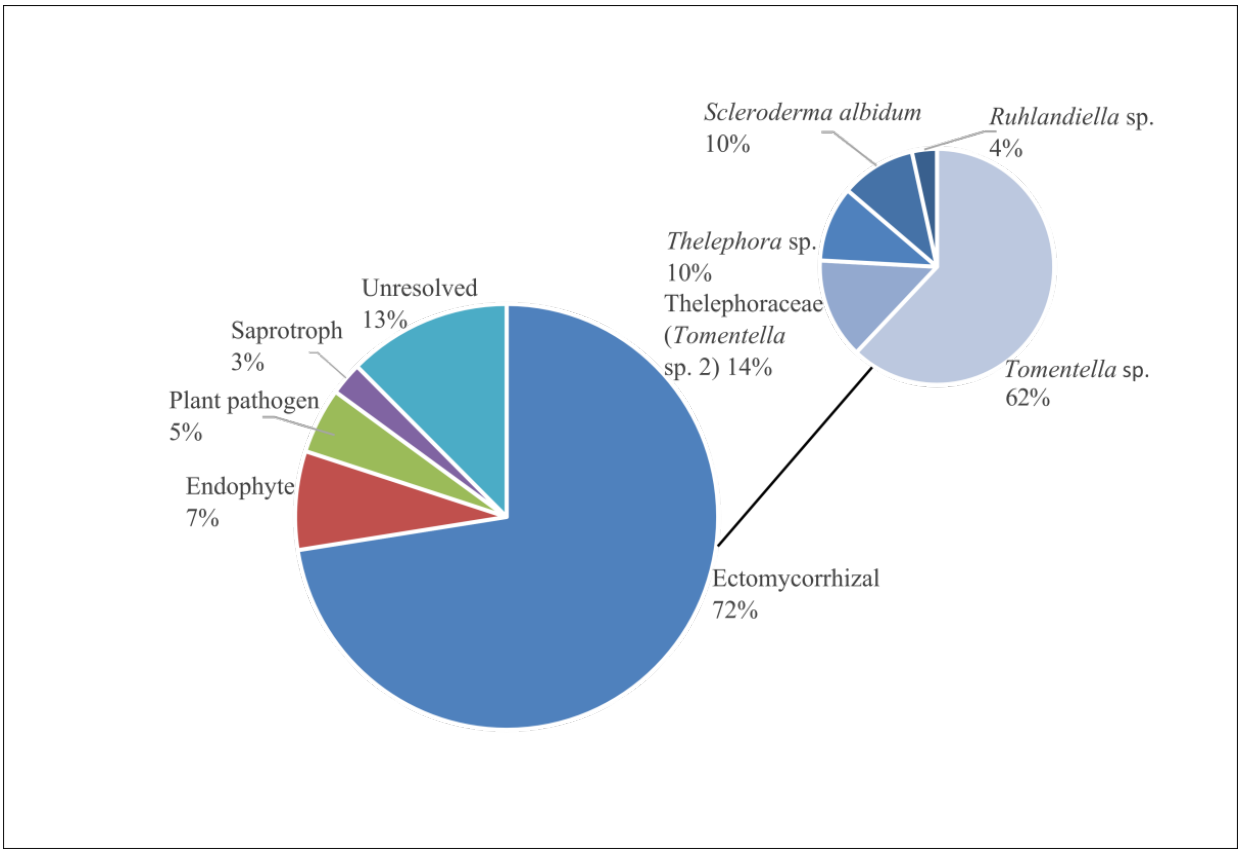

Fig. 4 – Relative proportions of the different ecological functional groups (left) and the taxa of the ectomycorrhizal fungi (right) sampled from root-tips collected from *Eucalyptus* in Brazil. The largest portion of root-associated fungi is made up of taxa considered to be ectomycorrhizal, but saprotrophic and pathogenic taxa also were identified.

An additional consideration would be sampling strategy and molecular methodologies used to assess root-associated fungal communities. A larger number of samples from a higher number of individual trees might have resulted in an increase in the number of taxa recorded. It also should be noted that some of the root-associated fungi recovered in the present study are putative pathogens, saprotrophs, and endophytes. Examination of the extent to which these root-associated fungi are functionally active in those roles would be another area of future research.

However, the proportion of root-associated fungi that were ectomycorrhizal was relatively high even though species diversity was relatively low. Ducousso et al. (2012) observed similar results in their study of ectomycorrhizal fungi associated with *Eucalyptus* roots in Africa. This observation can be corroborated by the premise that many ectomycorrhizal associations are species-

specific (Prescott & Grayston 2013), and most native ectomycorrhizal fungi are unable to form associations with exotic trees that are not closely related to local host trees (Buyck 1994, Härkönen et al. 1995). The spread of *Amanita muscaria* from introduced species of oak to *Nothofagus* in New Zealand represents a noteworthy exception (Bagley & Orlovich 2004). As such, the present study supports previous research that *Eucalyptus* root-associated fungi are limited to a few fungal species that are cosmopolitan or introduced with other exotic taxa (Ducousso et al. 2012).

A comparable study, using sporocarps collected in the field to assess fungal diversity on *Eucalyptus* in tree plantations in Ethiopia reported a range of 5-10 different species of fungi (Dejene et al. 2017). An anatomotyping integrated with sequencing survey in Seychelles revealed seven species of ECM fungi on root-tips of introduced *Eucalyptus robusta* Sm. (Tedersoo et al. 2007). Evaluating two *Eucalyptus* species in semiarid Brazil, Pagano & Scotti (2008) observed lower ectomycorrhizal diversity on *Eucalyptus* plantations when compared to native leguminous species in the same experimental area. Another study, which used DNA-based identification of root-associated fungi on *Eucalyptus* in tree plantations in Kenya, found the diversity of ectomycorrhizal fungi to be relatively low, with only eight taxa identified (Kluthe et al. 2016). The low diversity reported herein for *Eucalyptus* in Brazil thus makes sense, since the trees and a low diversity of affiliated fungi were imported from Australia. Some early introductions of *Eucalypus* certainly involved seedlings growing in pots with soil, which increases the chances of native ectomycorrhizal fungi also being introduced. More stringent quarantine measures which require *Eucalyptus* to be introduced as seeds would not allow this to take place.

The majority of ectomycorrhizal fungi found on the roots of Brazilian *Eucalyptus* in the present study were a species of *Tomentella* that matched with 97% or greater sequence identity a specimen deposited in Genbank from New Caledonia, an island not far from mainland Australia– the native habitat of species of *Eucalyptus* and on which a few members of this genus also occur. This at least suggests that root-associated fungi, specifically ectomycorrhizal fungi, present on plantation-grown *Eucalyptus* in Brazil could have been transported from native habitats along with their host trees. *Scleroderma albidum*, previously described to form ectomycorrhizal associations with *Eucalyptus* trees (Guzmán 1970, Malajczuk et al. 1982, Gurgel et al. 2008, Sulzbacher et al. 2013) was found on the sampled roots. *Scleroderma albidum* also has been found to commonly occur on exotic forest plantations of pine in Brazil (Nouhra et al. 2012), and may have been transplanted from non-native habitats.

The possibility that the composition of the assemblages of ectomycorrhizal fungi associated with native trees in Brazil is changing as a result of the introduction of non-native root-associated fungi is a cause for concern. Examining changes in macrofungal communities in tree plantations established in southern Brazil, Paz et al. (2015) mentioned that native forests might be vulnerable to invasion by exotic ectomycorrhizal species together with the introduction of their exotic hosts (e.g., *Pinus* spp. and *Eucalyptus* spp). They noticed that most of the ectomycorrhizal species associated with native forests are not found in exotic tree plantations, indicating that conservation of native species and functional group diversification depends on the preservation of native forests. It highlights the importance of safeguarding native species in their natural habitats. It is difficult to assess the status of invasive ectomycorrhizal fungi, but there are indications that it is happening in Brazil (e.g., the occurrence of *Russula emetica* associated with *Araucaria angustifolia* forests in Brazil (Paz et al. 2015)) and other parts of the world. The introduction of *Amanita phalloides* across North America (Pringle & Vellinga 2006) and the introduction of *A. muscaria* to the southern beech forests of New Zealand (Nuñez & Dickie 2014) already noted provide other examples of this same phenomenon.

Additional studies of root-associated fungi for non-native trees in Brazilian plantations is essential to develop a more complete understanding of the belowground ecology and potential for displacement of native fungi. Future research should include efforts to sample neighboring native forest tree roots and soils to assess the diversity of native root-associated fungal assemblages and also to determine whether or not plantation-based root-associated fungi are expanding into neighboring habitats. This research sets the stage for future more comprehensive studies.

Acknowledgments

Appreciation is extended to Rafael Aroxa, Eduardo Jorge, Andrea Carla, and Pedro Coelho for providing valuable technical assistance. We also wish to thank Dr. Fred Spiegel for providing the photographical equipment and technical support. The authors acknowledge the Southern Regional Education Board (SREB) for the financial support. Isadora L. Coelho is a SREB fellow.

References

Abreu VP, Martins GSL, Campos ANR. 2013 – Basidiocarps of ectomycorrhizal fungi in *Eucalyptus urograndis* plantation at the zona da mata de minas gerais: Main genera and seasonal distribution. Boletim do Observatório Ambiental Alberto Ribeiro Lamego 6(2), 23–36. DOI: https://doi.org/10.5935/2177-4560.20120013

Alvares CA, Stape JL, Sentelhas PC, de Moraes GJL, Sparovek G. 2013 – Köppen's climate classification map for Brazil. Meteorologische Zeitschrift 22(6), 711–728. DOI: https://doi.org/10.1127/0941-2948/2013/0507

Alberton O, Aguiar D, Gimenes RMT, Carrenho R. 2014 – Meta-analysis for responses of eucalyptus and pine inoculated with ectomycorrhizal fungi in Brazil. Journal of Food, Agriculture & Environment, 12, 1159–1163.

de Andrade GO, Lins RC. 1984 – Pirapama um estudo geográfico e histórico. Recife, Editora Massangana.

Bagley SJ, Orlovich DA. 2004 – Genet size and distribution of *Amanita muscaria* in a suburban park, Dunedin, New Zealand. New Zealand Journal of Botany 42, 939–947.

Bruns TD, Szaro TM, Gardes M, Cullings KW et al. 1998 – A sequence database for the identification of ectomycorrhizal basidiomycetes by phylogenetic analysis. Molecular Ecology 7, 257–272 DOI: https://doi.org/10.1046/j.1365-294X.1998.00337.x

Buyck B. 1994 – Ubwoba: les champignons comestibles de l'ouest du Burundi. Burundi, Administration générale de la coopération au dévelopment.

Chen YL, Liu S, Dell B. 2007 – Mycorrhizal status of eucalyptus plantations in south china and implications for management. Mycorrhiza 17(6), 527–35. DOI: http://0-dx.doi.org.library.uark.edu/10.1007/s00572-007-0125-6

Chilvers GA. 2000 – Mycorrhizas of eucalypts. In: Keane PJ, Kile GA, Podger FD, Brown BN (eds) Diseases and pathogens of eucalypts. CSIRO, Melbourne.

Christie S. 2008 – Energy, chemicals and carbon: future options for the *Eucalyptus* value chain. Southern Forests: A Journal of Forest Science 70, 175–182. DOI: https://doi.org/10.2989/SOUTH.FOR.2008.70.2.13.541

Coelho FB, Borges AC, Neves JCL, Barros NF, Muchovej RM. 1997 – Caracterização e incidência de fungos micorrízicos em povoamentos de *Eucalyptus grandis* e *Eucalyptus saligna*, nos municípios de Botucatu, São José dos Campos e São Miguel Arcanjo, São Paulo. Revista Árvore 21(4), 563–573.

Corcobado T, Moreno G, Azul AM, Solla A. 2015 – Seasonal variations of ectomycorrhizal communities in declining *Quercus ilex* forests: interactions with topography, tree health status and *Phytophthora cinnamomi* infections. Forestry: An International Journal of Forest Research 88, 257–266.

CPRH/DFID. 1999a – Diagnóstico ambiental integrado da bacia do rio Pirapama. Recife, Companhia Pernambucana do Meio Ambiente/Department for International Development (Publicações Projeto Pirapama).

CPRH/DFID. 1999b – Diagnóstico ambiental e zoneamento ecológico-econômico costeiro do litoral sul de Pernambuco. Recife, Companhia Pernambucana do Meio Ambiente/Department for International Development (Publicações Projeto Pirapama).

CPRH/DFID. 2013 – Relatório Anual de Atividades – 2013. Recife, Companhia Pernambucana do Meio Ambiente/Department for International Development.

de Souza EL, Antoniolli ZI, Machado RG, Pazzini DE et al. 2017 – Fungos Ectomicorrízicos na produção de mudas de *Eucalyptus grandis* W. Hill ex. Maiden em neossolo quartzarênico. Ciência Florestal 27(2), 471–484.

Dejene T, Oria-de-Rueda JA, Martín-Pinto P. 2017 – Fungal diversity and succession under *Eucalyptus grandis* plantations in Ethiopia. Forest Ecology and Management 405, 179–187. DOI: https://doi.org/10.1016/j.foreco.2017.08.050

dos Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA et al. 2014 – Sistema brasileiro de classificação de solos. Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) Solos, Ministério da Agricultura, Pecuária e Abastecimento Sistema Brasileiro de Classificação de Solos. Brasília, Embrapa (4th ed.).

Ducousso M, Duponnois R, Thoen D, Prin Y. 2012 – Diversity of ectomycorrhizal fungi associated with *Eucalyptus* in Africa and Madagascar. International Journal of Forestry Research. DOI: https://doi.org/10.1155/2012/450715.

Frank B. 1885 – Ueber die auf Wurzelsymbiose beruhende Ernahrung gewiser Baume durchunterirdische Pilze. Berichte der Deutschen Botanischen Gesellschaft 3, 128–145.

Giachini AJ, Oliveira VL, Castellano MA, Trappe JM. 2000 – Ectomycorrhizal fungi in *Eucalyptus* and *Pinus* plantations in southern Brazil. Mycologia, 1166–1177.

Giachini AJ, Souza LAB, Oliveira VL. 2004 – Species richness and seasonal abundance of ectomycorrhizal fungi in plantations of *Eucalyptus dunnii* and *Pinus taeda* in southern Brazil. Mycorrhiza 14(6), 375–381. DOI: https://10.1007/s00572-004-0297-2

Glowa KR, Arocena JM, Massicotte HB. 2003 – Extraction of potassium and/or magnesium from selected soil minerals by *Piloderma*. Geomicrobiology Journal 20, 99–111. DOI: https://doi.org/10.1080/01490450303881

Grazziotti PH, Barros NF, Borges AC, Neves JCL, Fonseca S 1998 – Variação sazonal da colonização de raízes de clones de híbridos de eucalipto por fungos micorrízicos no estado do Espírito Santo. Revista Brasileira de Ciência do Solo 22, 613–619. DOI: http://dx.doi.org/10.1590/S0100-06831998000400006

Gurgel FE, Silva BDB, Baseia IG. 2008 – New records of *Scleroderma* from Northeastern Brazil. Mycotaxon 105, 399–405. DOI: https://doi.org/10.5248/129.169

Guzmán G. 1970 – Monografia del género *Scleroderma* Pers. emend. Fr. (Fungi-Basidiomycetes). Darwiniana 16, 233–407.

Hall JL. 2002 – Cellular mechanisms for heavy metal detoxification and tolerance. Journal of Experimental Botany 53(366), 1–11. DOI: https://doi.org/10.1093/jexbot/53.366.1

Härkönen M, Saarimäki T, Mwasumbi L. 1995 – Edible mushrooms of Tanzania. Yliopistopaino, Helsinki.

Holste EK, Kobe RK, Gehring CA. 2017 – Plant species differ in early seedling growth and tissue nutrient responses to arbuscular and ectomycorrhizal fungi. Mycorrhiza 27(3), 211–223. DOI: https://10.1007/s00572-016-0744-x

Huang Y, Niu B, Gao Y, Fu L, Li W. 2010 – CD-HIT Suite: a web server for clustering and comparing biological sequences. Bioinformatics 26(5), 680–682. DOI: https://doi.org/10.1093/bioinformatics/btq003

Kluthe GB, Ben Ali BHM, Nelsen DJ, Stephenson SL. 2016 – A preliminary study of the ectomycorrhizal fungi associated with introduced *Eucalyptus* in Kenya. Mycosphere *7*(1), 81–86. DOI: https://doi.org/10.5943/mycosphere/7/1/8

Le Sann JG. 1983 – Documento cartográfico: considerações gerais. Revista Geografia e Ensino, Belo Horizonte 1(3), 3–17.

Lima FdS, Soares ACF, Sousa CdS. 2013 – Ocorrência e atividade de fungos micorrízicos arbusculares em plantios de eucalipto (eucalyptus sp.) no litoral norte da bahia, brasil. Revista Árvore 37(2), 245–255. DOI: https://10.1590/S0100-67622013000200006

Kirk PM. 2015 – Species Fungorum (version Feb 2014). In: Roskov Y, Abucay L, Orrell T, Nicolson D, Kunze T, Flann C, Bailly N, Kirk P, Bourgoin T, DeWalt RE, Decock W, De Wever A (Eds) Species 2000 & ITIS Catalogue of Life. Species 2000: Naturalis, Leiden.

Malajczuk N, Molina R, Trappe JM. 1982 – Ectomycorrhiza formation in *Eucalyptus*. New Phytologist, 91(3), 467–482.

Marques Júnior OG, Andrade HB, Ramalho MAP. 1996 – Avaliação de procedências de *Eucalyptus cloeziana* F. Muell e estimação de parâmetros genéticos e fenótipos na região noroeste do estado de Minas Gerais. Cerne 2(1), 12–19.

Marx DH, Cordell CE. 1989 – The use of specific ectomycorrhizas to improve artificial forestation practices. In: Whipps JM, Lumsden RD (eds.). Biotechnology of fungi for improving plant growth. New York, Cambridge, University Press. pp. 1–25.

Marx DH, Ruehle JL. 1988 – Ectomycorrhizae as biological tools in reclamation and revegetation of waste lands. In: Mahadevan A, Raman N, Natarajan K (eds.). Mycorrhizae for green Asia. Madras, Centre for Advanced Studies in Botany, University of Madras. pp. 336–344.

Mikola P. 1973 – Application of mycorrhizal symbiosis in forestry practice. In: Marks GC, Kozlowski TT. (eds.). Ectomycorrhizae. London, Academic Press. pp. 383–411.

Moura VPG, Caser RL, Albino JC, Guimaraes DP et al. 1980 – Avaliação de espécies e procedências de Eucalyptus em Minas Gerais e Espirito Santo: resultados parciais. Planaltina, EMBRAPA-CPAC.

Nouhra ER, Caffot MLH, Pastor N, Crespo EM. 2012 – The species of *Scleroderma* from Argentina, including a new species from the *Nothofagus* forest. Mycologia 104(2), 488–495. DOI: https://doi.org/10.3852/11-082

Nuñez MA, Dickie IA. 2014 – Invasive belowground mutualists of woody plants. Biological Invasions 16, 645–661. DOI: https://doi.org/10.1007/s10530-013-0612-y

Oliveira VL, Schmidt VDB, Bellei MM. 1997 – Patterns of arbuscular-and ecto-mycorrhizal colonization of *Eucalyptus dunnii* in southern Brazil. In Annales des sciences forestières 54(5), 473–481. DOI: https://doi.org/10.1051/forest:19970505

Ortiz N, Armada E, Duque E, Roldán A, Azcón R. 2015 – Contribution of arbuscular mycorrhizal fungi and/or bacteria to enhancing plant drought tolerance under natural soil conditions: effectiveness of autochthonous or allochthonous strains. Journal of Plant Physiology 174, 87–96. DOI: https://doi.org/10.1016/j.jplph.2014.08.019

Pagano MC, Scotti MR. 2008 – Arbuscular and ectomycorrhizal colonization of two *Eucalyptus* species in semiarid Brazil. Mycoscience 49, 379–384. DOI: https://doi.org/10.1007/s10267-008-0435-3

Paz CP, Gallon M, Putzke J, Ganade G. 2015 – Changes in macrofungal communities following forest conversion into tree plantations in southern brazil. Biotropica 47(5), 616–625. DOI: https://doi.org/10.1111/btp.12240

Prescott CE, Grayston SJ. 2013 – Tree species influence on microbial communities in litter and soil: Current knowledge and research needs. Ecology Manage 309, 19–27.

Pringle A, Vellinga EC. 2006 – Last chance to know? Using literature to explore the biogeography and invasion biology of the death cap mushroom *Amanita phalloides* (Vaill. ex Fr.: Fr.) Link. Biological Invasions 8(5), 1131–1144. DOI: https://doi.org/10.1007/s10530-005-3804-2

Pryor L. 1976 – Biology of *Eucalyptus*. London, Edward Arnold.

Rachid CT, Balieiro, FC, Fonseca ES, Peixoto RS et al. 2015 – Intercropped silviculture systems, a key to achieving soil fungal community management in *Eucalyptus* plantations. PloS one 10(2), e0118515.

Rocha EPA, Gomes FJB, Sermyagina E, Cardoso M, Colodette JL. 2015 – Analysis of Brazilian biomass focusing on thermochemical conversion for energy production. Energy & Fuels 29(12), 7975–7984. DOI: https://doi.org/10.1021/acs.energyfuels.5b01945

Samuel G. 1926 – Note on the distribution of mycorrhiza. Transactiom of the Royal Society of South Australia 50, 245–246.

Santos IS. 2001 – Fungos micorrízicos arbusculares em ambiente de mata atlântica e de Eucaliptos na região de Entre Rios, Bahia. Salvador. MSc Thesis, Universidade Federal da Bahia, Salvador.

Sawyer NA, Chambers SM, Cairney JWG. 2003 – Utilization of inorganic and organic phosphorus sources by isolates of *Amanita muscaria* and *Amanita* species native to temperate eastern Australia. Australian Journal of Botany 51, 151–158. DOI: https://doi.org/10.1071/BT02073

Singer R, Araffljo IJS. 1979 – Litter decomposition and ectomycor- rhiza in Amazonian forests. 1. A comparison of litter decom- posing and ectomycorrhizal basidiomycetes in latosol-terra-firme rain forest and white podzol campinarana. Acta Amazonica 9, 25–41

Singer R, Araujo I, Ivory MH. 1983 – The ectotrophically mycorrhizal fungi of the neotropical lowlands, especially central Amazonia. Beih Nova Hedwigia 77, 1–352

Roy M, Schimann H, Braga-Neto R, Da Silva RA et al. 2016 – Diversity and distribution of ectomycorrhizal fungi from amazonian lowland White sand forests in Brazil and French Guiana. Biotropica 48(1), 90–100. DOI: https://10.1111/btp.12297

Smith SE, Read DJ. 1997 – Mycorrhizal Symbiosis. London, Academic Press.

St. John TV. 1980 – Uma lista de espécies de plantas tropicais brasileiras naturalmente infectadas com micorriza vesicular-arbuscular. Acta Amazonica 10(1), 229–234. DOI: http://dx.doi.org/10.1590/1809-43921980101229

Sulzbacher MA, Giachini AJ, Grebenc T, Silva BD et al. 2013 – Survey of an ectotrophic sand dune forest in the northeast Brazil. Mycosphere 6, 1106–1115. DOI: http://dx.doi.org/10.5943/mycosphere/4/6/8

Tedersoo L, Suvi T, Beaver K, Kõljalg U. 2007 – Ectomycorrhizal fungi of the Seychelles: Diversity patterns and host shifts from the native *Vateriopsis seychellarum* (Dipterocarpaceae) and *Intsia bijuga* (Caesalpiniaceae) to the introduced *Eucalyptus robusta* (Myrtaceae), but not *Pinus caribaea* (Pinaceae). The New Phytologist 175(2), 321–333. DOI: https://10.1111/j.1469-8137.2007.02104.x

Tedersoo L, Bahram M, Põlme S, Kõljalg U et al. 2014 – Global diversity and geography of soil fungi. Science 346(6213), 1256688. DOI: https://doi.org/10.1126/science.1256688

Turjaman M, Santoso E, Susanto A, Gaman S et al. 2011 – Ectomycorrhizal fungi promote growth of *Shorea balangeran* in degraded peat swamp forests. Wetlands Ecology and Management 19(4), 331–339. DOI: https://10.1007/s11273-011-9219-1

Vera DS, Agüero G, Villalba G, Silveira DS. 2017 – Economic profitability of charcoal production from reforested woods with Eucalyptus sp. Custos e agronegócio (Special Edition) 13, 132–154.

Vozzo JA, Hacskaylo E. 1971 – Inoculation of *Pinus caribaea* with ectomycorrhizal fungi in Puerto Rico. Forest Science 17, 239–241. DOI: https://doi.org/10.1093/forestscience/17.2.239

Walker JF. Miller OK Jr, Horton JL. 2008 – Seasonal dynamics of ectomycorrhizal fungus assemblages on oak seedlings in the southeastern Appalachian Mountains. Mycorrhiza 18, 123–132.

Yokomizo NKS. 1986 – Micorrizas em essÞncias florestais. Anais da I Reuni1⁄4o Brasileira sobre Micorrizas. UFLA, Lavras, Brazil, pp 212.

Zambolim L, Barros NF. 1982 – Constatação de micorriza vesicular-aruscular em *Eucalyptus* spp. na região de Viçosa, MG. Revista Árvore 6(1), 95–97.

Zhao R, Guo W, Bi N, Guo J et al. 2015 – Arbuscular mycorrhizal fungi affect the growth, nutrient uptake and water status of maize (Zea mays L.) grown in two types of coal mine spoils under drought stress. Applied Soil Ecology, 88, 41–49. DOI: https://doi.org/10.1016/j.apsoil.2014.11.016