SHORT COMMUNICATION

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# Arbuscular and ectomycorrhizal colonization of two *Eucalyptus* species in semiarid Brazil

**Abstract** Our goal was to evaluate the mycorrhizal colonization, as well as the density of arbuscular mycorrhizal (AM) fungal spores, in *Eucalyptus camaldulensis* and *E. grandis* monocultures at 2 years in a semiarid part of Brazil. Soil and root samples were collected in 2 consecutive years. *Eucalyptus camaldulensis* showed varied AM colonization level according to season of sampling, and *Glomus* was dominant in spore numbers. *Eucalyptus grandis* showed dominant ectomycorrhizal (ECM) colonization and lower AM fungal spore density. Overall results suggest that *E. camaldulensis* has both AM and ECM dependencies, whereas *E. grandis* is solely ECM dependent in the monocultures.

**Key words** Arbuscular mycorrhizae · Ectomycorrhizae · *Eucalyptus camaldulensis* · *Eucalyptus grandis* · Semiarid region

Arbuscular mycorrhizas (AM) are ecologically obligate symbionts of a wide range of plants, and both fungi and plants establish long-term compatible interactions. Mycorrhizal symbioses improve plant nutrient acquisition in infertile soils. Moreover, their nonnutritional effects such as reducing plant diseases and modifying water relationships (Smith and Read 1997), or stabilizing soil structure (Rillig 2004) are also very important. Smith and Read (1997) stressed that mycorrhizal studies must elucidate the quantitative aspects of different mycorrhizal types and their functional contributions to the ecosystem, because mycorrhizas have ameliorative effects on plant nutrition and are used in afforestation programs to enhance forest productivity.

AM have ecological and agronomic importance, and their development may be influenced by environmental factors such as climatic conditions, soil chemical and physical properties, and the variety and age of host plant species (Smith and Read 1997). As arbuscular mycorrhizal fungal (AMF) species composition seems to be affected by plant species composition, management of local plant diversity is important to sustain the AMF community. In fact, monocultures appear to change AMF species composition and reduce their population diversity (Hendrix et al. 1995; Pagano 2007). Even in the tropics AM are significant for soil fertility and constitute an important biological resource, enhancing soil sustainability (Cardoso and Kuyper 2006).

Some *Eucalyptus* species form two different types of mycorrhizas, i.e., AM and ectomycorrhiza (ECM) (Malajczuk et al. 1981; Zambolim and Barros 1982). AM colonization has been reported in young individuals of *Eucalyptus* species that usually form ECM (Chilvers et al. 1987; Wang and Qiu 2006). ECM improve water balance of host plants, reduce impacts on trees from root pathogens (Smith and Read 1997), and mobilize essential plant nutrients directly from soil through excretion of organic acids (Landeweert et al. 2001). *Pisolithus tinctorius* (Pers) Cocker & Couch is the most important ECM fungus for forestation and has been used to increase the growth of plantation eucalypts (Garbaye et al. 1988).

For reasons of maintaining biological diversity and recognizing the magnitude of fungal diversity, accurate mycological data in man-made, native, or disturbed forests are required (Giachini et al. 2004). In Brazil, establishment of the AM association in *Eucalyptus* plantations has been known for more than 20 years, and the benefits of symbiosis are commercially relevant (Zambolim and Barros 1982; Coelho et al. 1997; Gomes and Trufem 1998; Grazziotti et al. 1998; Santos et al. 2001). The most recent studies on eucalypt forestation were carried out at São Paulo State, the coastal region (Diaz-Balteiro and Rodriguez 2006; Bouillet et al. 2008). In Minas Gerais State (southeastern Brazil), *Eucalyptus* plantations for agroforestry appear to be an adequate alternative to integrate timber and food production, and *E. camaldulensis* has been increasingly studied in the Cerrado savanna region (Ceccon 2005; Pereira and Oliveira 2005) and more arid regions (Lima et al. 2006).

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However, there is no report concerning AM colonizations on *E. camaldulensis* and *E. grandis* in the semiarid regions of the State.

The density of AMF spores in soil and their species diversity are vary greatly, depending on environmental conditions (Smith and Read 1997). Different species and isolates of AMF and ectomycorrhizal fungi (ECMF) affect the nutrient uptake and growth of their host plants differently (Sanders et al. 1977). *Eucalyptus camaldulensis* colonized by AMF decreased soil compaction and increased phosphorus uptake (Santos 1995). Working with five *Eucalyptus* species, Santos et al. (2001) observed a successional colonization of mycorrhizal types, initially dominated by AM and then replaced by ECM. Almost all studies, except for that by Adjoud-Sadadou and Halli-Hargas (2000), report such temporal replacement. Thus, there is some controversy concerning *Eucalyptus* growth responses to inoculation with AMF. There is to date scarce knowledge concerning AM symbiosis on *Eucalyptus* in the plantations in Brazil. Within the scope of a broad project dealing with *Eucalyptus* monocultures and mixed cultures with the aim of providing wood supply and minimizing exploratory actions in biological reserves at Jaíba, in Minas Gerais State, Brazil, we focused on *Eucalyptus* monocultures. The objective of the present study is to document AM and ECM colonization and the diversity of AMF spores in *E. camaldulensis* and *E. grandis* plantations.

The present study was carried out at a Legal Reserve in Jaíba (15°09′03″ S, 43°49′26″ W) in the north of Minas Gerais State, which has been degraded after the woody Caatinga (dry forest) was cut and has become covered with invasive vegetation. The study area is a semiarid region and is characterized by annual mean precipitation of 800 mm, concentrated in the spring–summer season from November to March; the rest of the year is a dry period (Jaíba Irrigation Project). According to Köppen, the climate type of the region is BSh. Predominant soil type is acid Quartzarenic Neosoil, with high infiltration rate and small amounts of soil organic matter.

One experimental stand (0.8 ha) in each *E. camaldulensis* and *E. grandis* monoculture was established and surveyed. Study plots were established in November to March 2002, using a randomized block design with 48 plants and three blocks. Each block was composed of three plots of 432 m$^2$ (24 × 18 m), where each line was cultivated with a 3 × 3 m spacing. Details of the study plots have been described previously (Pagano 2007). Both stands were fertilized with triple superphosphate (500 kg/ha), KCl (382 kg/ha), $MgSO_4 \cdot 7H_2O$ (50 kg/ha), $ZnSO_4 \cdot 7H_2O$ (46.8 kg/ha), $Mo_7O_2 \cdot 4H_2O$ (1.76 kg/ha), and urea (222 kg/ha) following Somasegaran and Hoben (1994), at the beginning of the plantation (November 2002).

Roots of *Eucalyptus* were collected in August 2003 and in March and October 2004, by excavating from the trunk to the lateral root system of each tree, and were fixed in FAA (formalin:acetic acid:70% ethanol = 5:5:90) solution until samples were processed. Three samples were collected from each plot. Fixed roots were rinsed with tap water, stained with trypan blue for AM colonization according to Phillips and Hayman (1970), and assessed. Roots were cut into 1-cm segments, and 31 root segments were examined in each sample for their AM status under a compound microscope (100×). If at least 1 root segment was found to be colonized by hyphae, arbuscules, or vesicles, the sample was considered as AM colonization. Mycorrhizal colonization level was estimated according to McGonigle et al. (1990), and obtained data were expressed as percentage of colonized segments. Root samples were also checked for ECM colonization (presence of Hartig net). Roots colonized by ECMF were included when calculating the percentage of total mycorrhizal colonization. When dual mycorrhization was observed, AM and ECM colonizations were recorded individually for the colonization level. These data were arcsin $(x/100)^{1/2}$ transformed. The data were subjected to one-way analysis of variance (ANOVA) (MINITAB, version 13.2), and means were compared by the Tukey test ($P < 0.05$). Photographs of mycorrhizal structures and AMF spores were taken by a camera attached to the compound microscope (Olympus BH-2).

Rhizosphere soil samples were collected at a depth of 10–30 cm in each plot of *E. camaldulensis* and *E. grandis*. AMF spores were extracted from each 100 g soil sample and were recovered by wet sieving (Gerdemann and Nicolson 1963) and decanting by sucrose centrifugation (Walker et al. 1982) procedures. Healthy spores were counted by the number, which was expressed as spore density/100 g soil dry weight. Each spore type was mounted sequentially in polyvinyl alcohol lactic acid glycerol (PVLG) and a mixture of PVLG and Melzer's reagent (Morton 1988) to obtain a permanent voucher specimen, and was observed under a light microscope at 100×. Identification was based on spore color, size, surface ornamentation, and wall structure, with reference to the descriptions [Schenk and Pérez 1988; International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM: West Virginia, USA; http://invam.caf.wvu.edu)]. Spore numbers were square root transformed and statistically analyzed. The frequency of occurrence of each AMF species was calculated with the formula $x_i/x_0 \times 100$, where $x_i$ = the spore density for an individual species and $x_0$ = the total spores; the Shannon–Weaver biodiversity index ($H'$), species richness ($S$), and evenness ($E'$) were obtained according to Magurran (1988).

Some basic properties of the soil are presented in Table 1. Base saturation was medium, phosphorus content was low, and acidity was moderate. The soil from the *E. camaldulensis* plot showed greater K, Mg, and Fe content, whereas the soil from the *E. grandis* plot presented higher Zn, Cu, and Mn content. Base saturation was similar to a 20-year-old *E. grandis* plantation in Ethiopia (Lemma et al. 2006), although soil bulk density was higher in this study site.

Although typical AM structures such as vesicles and extra- and intraradical hyphae were observed on the *E. camaldulensis* root samples, arbuscules were not observed on any of the samples (Fig. 1a–e). Spherical to oblong vesicles (42–48 × 28–42 μm) were often observed within root segments. Hyphae varied in diameter (4.5–9.0 μm), and

often grew for some distance in parallel to each other, connected by an "h"-shape anastomosis pattern. Intraradical hyphae ranged from 3.4 to 6.0 μm in diameter. This Glomineae-type colonization was in line with the presence of *Glomus* spores in the rhizosphere soil (Fig. 1f). We observed ECM (Fig. 1g,h) and a dominance of Hartig net on the root samples of *E. grandis*, but no AM colonization was observed on any of the root samples. The percentage of mycorrhizal colonizations varied in both eucalypt species (Fig. 2, Table 2). The extent of mycorrhizal colonization on *E. camaldu-lensis* ranged from 55% to 23% depending on the sampling season, and the higher trend of AM colonization values was suggested in the dry season. *Eucalyptus grandis* did not show AM colonization in any sampling season, and only ECM colonization was observed (Table 2). Abundant *Pisolithus*-like basidiocarps in *E. grandis*, but fewer in *E. camaldulensis* stands, were also observed. As mycorrhizal colonization on *E. grandis* was investigated only in the dry period, sampling in the rainy period is required to conclude that *E. grandis* in monoculture lacks AM.

In relationship to AMF spore density, the *E. grandis* stand was significantly lower than that in *E. camaldulensis* stand at the same season (Table 3). Total spore number was significantly higher in the rainy season for both eucalypt stands, and showed codominance of *Glomus* sp. 1. Although the *E. camaldulensis* stand presented higher AMF species richness than the *E. grandis* stand regardless of sampling season (Table 3), the season had an impact on AMF species richness, diversity, and evenness (Table 4). The stand of *E. camaldulensis* presented a significantly higher spore density as compared to that in the stand of *E. grandis* in spite of there being no significant difference in species richness. The AMF species diversity presented here is low when compared to the data obtained in mixed plantations of *Eucalyptus* species with native leguminous species at the same experimental area (Pagano 2007), but species richness

**Table 1.** Soil chemical properties sampled from *Eucalyptus camaldulensis* and *E. grandis* plantations

| Soil property | Stand | |
|---|---|---|
| | *E. camaldulensis* | *E. grandis* |
| pH ($H_2O$) 1:1 | 6.0 | 5.7 |
| Organic matter (mg/g) | 0.9 | 0.9 |
| Available P (mg/l) | 4.09 | 3.76 |
| Available K (mg/l) | 94 | 72 |
| Exchangeable $Al^{3+}$ (mmol/kg) | 0 | 0.1 |
| Exchangeable $Ca^{2+}$ (mmol/kg) | 2.4 | 2.5 |
| Exchangeable $Mg^{2+}$ (mmol/kg) | 0.7 | 0.5 |
| CEC (mmol/kg) | 5.5 | 5.6 |
| Base saturation (%) | 61.4 | 57.8 |
| Total porosity | 47 | 46.3 |
| Macroporosity | 31.2 | 29.6 |
| Microporosity | 14.4 | 13.4 |
| Density | 1.3 | 1.2 |
| Zn | 0.6 | 1 |
| Cu | 0.4 | 0.8 |
| Mn | 318 | 431 |
| Fe | 207 | 117 |
| Texture (%)[a] | | |
| Coarse sand | 50 | 50 |
| Fine sand | 34 | 31 |
| Silt | 0 | 4 |
| Clay | 16 | 15 |

CEC, cation-exchange capacity
Particle size: coarse sand, 2–0.2 mm; fine sand, 0.2–0.02 mm; silt, 0.02–0.002 mm; clay, < 0.002 mm
[a] Mean of two measures from one composite sample

**Table 2.** Mycorrhizal colonization (%) on *Eucalyptus camaldulensis* and *E. grandis* in dry season (October 2004)

| Host plant species | AM | | ECM Colonization |
|---|---|---|---|
| | Colonization[†] | Vesicles[‡] | |
| *E. camaldulensis* | 43.33 a | 23.75 a | 23.33 b |
| *E. grandis* | 0 b | 0 b | 50 a |

AM, arbuscular mycorrhizae; ECM, ectomycorrhizae
†, % AM hyphae in roots; ‡, % AM vesicles in roots
Values followed by a different letter are significantly different (*P* < 0.05)

**Table 3.** Arbuscular mycorrhizal fungus (AMF) spore density in the *Eucalyptus camaldulensis* and *E. grandis* plantations in the rainy (March 2004) and dry (October 2004) seasons

| AMF species | Number of spores (per 100 g soil) in the following stand/season | | | |
|---|---|---|---|---|
| | *E. camaldulensis* | | *E. grandis* | |
| | Rainy | Dry | Rainy | Dry |
| Gigasporaceae | | | | |
| *Gigaspora margarita* Becker & Hall | 0 | 2.5 NS | 0 | 1 NS |
| *Gigaspora* sp. 1 | 0 | 0 | 0 | 1 NS |
| *Scutellospora heterogama* (Nicol. & Gerd.) Walker & Sanders | 0 | 0 | 0 | 1 NS |
| Acaulosporaceae | | | | |
| *Acaulospora mellea* Spain & Schenck | 2.5[†] | 9.1 NS | 0 | 0 |
| *A. scrobiculata* Trappe | 4.7 b | 27.9 a | 8 | 2 NS |
| *Acaulospora* sp. 1 | 5.1 NS | 0.8 | 0 | 0 |
| *Acaulospora* sp. 2 | 0 b | 1.6 a | 0 | 0 |
| Glomeraceae | | | | |
| *Glomus* sp. 1 | 203.4 a | 123 b | 70 a | 13 b |
| Total | 211.1 a | 157.4 b | 78 a | 17 b |

†Values represent the mean of three samples taken from the pooled soil samples in each plot
Values followed by a different letter differ at *P* < 0.05 for each eucalypt species; NS, not significantly different
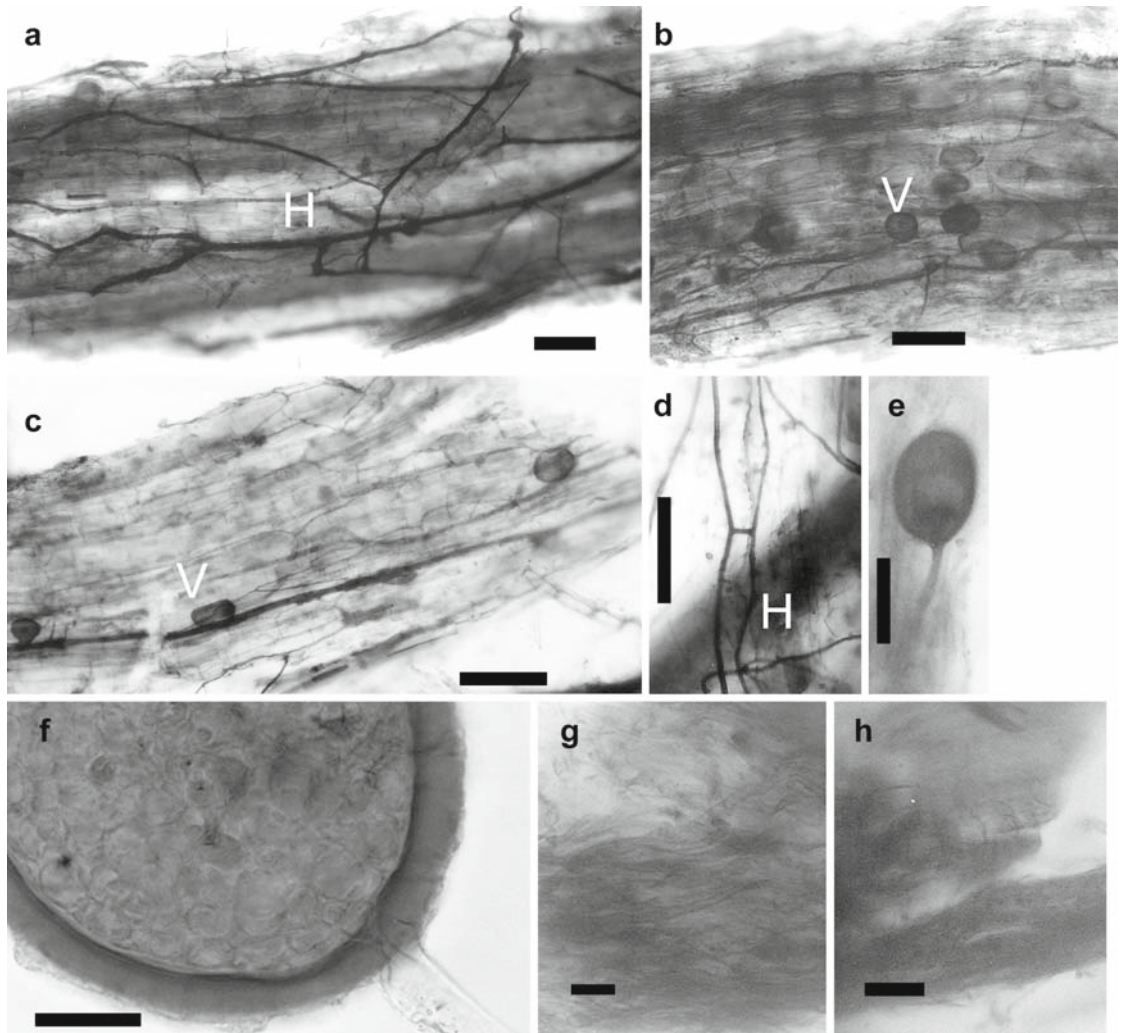
**Fig. 1.** Arbuscular mycorrhizal (AM) structures observed in the roots of *Eucalyptus* species. **a–d** Intraradical hyphae on *E. camaldulensis* roots differing in diameter (**a**; indicated with a letter *H*), bearing vesicles (**b**, **c**; indicated with a letter *V*), and often growing in parallel to each other, connected by a distinct "h" branching pattern (**d**; indicated with a letter *H*). **e** Terminal vesicle on an *E. camaldulensis* root. **f** A spore of *Glomus* sp. 1 showing a subtending hypha at the spore base. **g** Ectomycorrhizal (ECM) fungal mantle of *E. grandis*. **h** ECM fungal mantle and rhizomorph of *E. camaldulensis*. *Bars* **a–d** 100 μm; **e**, **g**, **h** 50 μm; **f** 10 μm



**Fig. 2.** Mycorrhizal colonization of *Eucalyptus camaldulensis* sampled in different (dry or rainy) seasons. ▤ AM; □, ECM. Values followed by a different letter differ at $P < 0.05$ between the sampling seasons
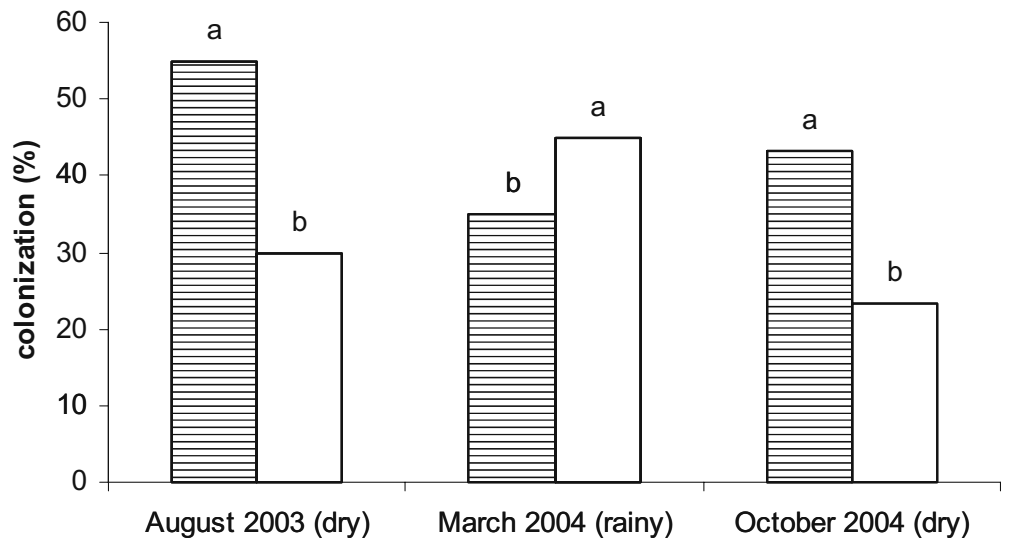
**Table 4.** AMF spore diversity in the *Eucalyptus camaldulensis* and *E. grandis* plantations in rainy (March 2004) and dry (October 2004) seasons

| Diversity index | Stand/season | | | |
|---|---|---|---|---|
| | *E. camaldulensis* | | *E. grandis* | |
| | Rainy | Dry | Rainy | Dry |
| *S* | 4 NS | 6 NS | 2 NS | 5 NS |
| *H'* | 0.34 NS | 0.98 NS | 0.33 NS | 0.78 NS |
| *E'* | 0.17 NS | 0.37 NS | 0.33 NS | 0.33 NS |

Values do not differ at *P* < 0.05

was almost similar to that found in *E. grandis* monocultures (Mello et al. 2006).

Mycorrhizal colonization values of *Eucalyptus* species presented here are in accordance with those obtained by Santos et al. (2001) and by Malajczuk et al. (1981). We observed a dominant ECM colonization in *E. camaldulensis* roots in the rainy period, which decreased in the dry periods. Consequently, the AM/ECM ratio values decreased in the rainy period. Studies dealing with such dual symbiosis of *Eucalyptus* seedlings in pot culture or young plantations reported a successional pattern of colonization, i.e., ECM taking over from AM in older seedlings (Lapeyrie and Chilvers 1985; Chilvers et al. 1987; Gardner and Malajczuk 1988; Bellei et al. 1992). In contrast, Adjoud-Sadadou and Halli-Hargas (2000) showed AM colonization on different *Eucalyptus* species at different ages, up to over 50 years, suggesting that replacement of AM by ECM during plant growth is not a general pattern in *Eucalyptus* under exotic conditions.

ECM colonization on *E. grandis* roots may be related to the host preference. Reis and Krügner (1990) also found low AM colonization level on this eucalypt species. Silva et al. (2003) found that *E. grandis* seedlings respond to ECMF inoculation when using low levels of phosphorus in soil and showed 54% of ECM colonization. The percent of ECM colonization in *E. grandis* roots in the dry season suggests the presence of ECM that can survive during drought periods, as mentioned by Pietro et al. (2007). Evidence of ECMF basidiocarps in the rainy season (data not shown) reinforces this idea.

*Eucalyptus camaldulensis* presented the highest percentages of AM root colonization, as was also found by Santos et al. (2001). The absence of arbuscules could suggest a nonfunctional association, but the presence of vesicles is distinct evidence of AM. Santos et al. (2001) and Araújo et al. (2004) also found few arbuscules in *E. cloeziana*, possibly because of the presence of tannin that interfered with arbuscule observation. The small and round vesicles found in root segments suggest colonization by Glomeraceae.

Three AMF species, i.e., *A. scrobiculata*, *G. margarita*, and *S. heterogama*, and several *Glomus* species were found from soil of the *E. grandis* plantation, which corroborated the results of Mello et al. (2006) in the same eucalypt species. However, the number of *Glomus* species was higher in this study than in their results (8 spores/100 g) for a 3-year-old plantation in Rio Grande do Sul State, Brazil. In contrast, Reis et al. (1985) found dominance of *Acaulospora* sp. in *Eucalyptus* spp. rhizospheres in greenhouse conditions in Brazil. In the present study, the spore composition of AMF in a single plantation was found to differ seasonally. The difference of species composition between our plots suggests that vegetation structure is an important determinant of the AMF community structure.

Our data suggest that *E. camaldulensis* is both AM- and ECM dependent, whereas *E. grandis* is not dependent on AM, as was found by Reis and Krügner (1990). Root colonization by a given AMF varied greatly depending on the eucalypt species (Reis and Krügner 1990; Adjoud et al. 1996; Adjoud-Sadadou and Halli-Hargas 2000; Santos et al. 2001; Araújo et al. 2004). Mycorrhizal characterization in *Eucalyptus* plantations is prerequisite to decide which species will be useful for the mycorrhizal inoculation programs. According to the previously reported results in exotic *Eucalyptus* plantations (Reis and Krügner 1990; Adjoud et al. 1996; Adjoud-Sadadou and Halli-Hargas 2000; Chen et al. 2000; Santos et al. 2001; Araújo et al. 2004), dominance of AM in *E. camaldulensis* in the dry periods in the present study suggests that AM may play an important role in the plantation soils. *Glomus* sp. seems to be a potential AMF inoculum for *E. camaldulensis* plantations in Minas Gerais, Brazil. The presence of *Pisolithus* basidiocarps in the *E. grandis* plot implies the ECMF as a potential inoculum at the plantation site. Our study contributes to evaluating the mycorrhizal fungal community in the managed reforestation programs in Brazil.

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