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AND TECHNOLOGY SERIES

**HANDBOOK ON AGROFORESTRY:
MANAGEMENT PRACTICES AND
ENVIRONMENTAL IMPACT**

LAWRENCE R. KELLIMORE
EDITOR

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Chapter 12

AGROFORESTRY IN DRY FOREST, BRAZIL: MYCORRHIZAL FUNGI POTENTIAL

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ABSTRACT

Afforestation of mixed plantation provides wood supply for local communities thus minimizing exploratory actions in biological reserves. Within the Endomycorrhizas, arbuscular mycorrhizas (AM) are the most frequent fungi supported by legumes trees, enhancing the uptake of phosphorus, nitrogen and other nutrients, consequently being of high interest for agroforestry. This symbiosis links the biotic and geochemical components of the ecosystem and their nonnutritional effects, such as reducing plant diseases and modifying water relationships or stabilizing soil structure, are also very important. Ectomycorrhizas (EM) improve water balance of host plants, reduce impacts on trees from root pathogens, and mobilize essential plant nutrients directly from the soil. This chapter discusses agroforestry and mycorrhizal fungi potential drawing on results of research on native agroforestry trees, mostly noduliferous legumes, intercropped with *Eucalyptus* spp. in Brazil. The studies found that all plant species show mycotrophy. Also, native agroforestry trees show arbuscular mycorrhizal mycotrophy and increased growth when inoculated with rhizobia and AM. On the other hand, *Eucalyptus camaldulensis* presents about 50% colonization by AM in the dry period, and *Eucalyptus grandis* is more dependent on EM. The benefits of AM fungi inoculation as well as the importance of AM and EM are discussed. Root colonization in native and exotic plant species used for agroforestry as well as AM spore diversity found in their rhizospheres are illustrated, and relevant findings are emphasized, such as the presence of significant amounts of nutrients, in *Eucalyptus* spp., in wood localized in superior parts of trunk and bark, revealing the importance of leaving vegetal waste on the site in order to reduce the loss of tree productivity in this semi-arid region.

Keywords: Agroforestry - Native trees - *Eucalyptus* - Mycorrhizal symbioses - Dry forest – Brazil.

INTRODUCTION

The basic objective of agroforestry is to produce systems that exhibit an ecological structure more similar to that of natural forests. Woody perennials (trees, shrubs, palms, bamboos, etc.) are deliberately used on the same land management unit as agricultural crops and/or animals, or as tree cover with a multipurpose utilization, resulting in both ecological and economic interactions between the different components (Sinclair 1999).

Therefore, agroforestry systems (AS) are advantageous over conventional agricultural and forest production methods due to increased productivity, economic benefits, social outcomes and the ecological goods and services provided.

Biodiversity in AS is typically higher than in conventional agricultural systems, because it incorporates several plant species into a given land area and creates a more complex habitat that can support a wider variety of birds, insects, and other animals. Agroforestry also has the potential to help reduce climate change since trees take up and store carbon at a faster rate than crop plants (Verchot et al. 2007). AS are of higher interest in the tropics to maintain soil fertility (Cardoso and Kuyper 2006).

Interplanting legume trees usually facilitate the growth of non legumes, since Leguminosae can support rhizobia and both ectomycorrhizae and arbuscular mycorrhizae; the latter being the most frequent (Frioni et al. 1999). In legumes, AMF may conceivably enhance plant performance by promoting plant vigor and hence biomass production and nitrogen (N) uptake. The response of legume species to AMF inoculation can be markedly different depending on the species of AMF inoculated, differences in root architecture and AMF dependency. AMF can also directly take up inorganic nitrogen from the soil and transfer it to the host plant (Govindarajulu et al. 2005).

Eucalyptus species, due to their fast growth, good adaptation to different soils and climatic conditions, and high timber value, are increasingly used for reforestation (DeBell et al. 1985, Khanna 1997, May and Attiwill 2003), and in tropical regions their use in reforestation programs is increasing.

In Brazil the area forested with *Eucalyptus* is approximately 3.55 million ha, mostly in zones with low fertility soil that are associated with problems of water deficit which make the production of profitable wood difficult (Marques Júnior et al. 1996). One of the impacts of this reforestation is a decrease in bioavailable nutrients (Carrenho et al. 2008). However, *Eucalyptus* spp. have the capacity to form two types of mycorrhizas, arbuscular (AM) and ectomycorrhiza (EM) (Zambolim and Barros 1982) and the potential uses of microbiological inoculants may be more fully studied.

Eucalyptus plantations are very important in Brazil as an energy source and to produce pulp/paper and charcoal; however, large-scale plantations cause environmental impacts. *Eucalyptus* monoculture may reduce the fungal species found in the soil after several years of continuous culture as well as arbuscular mycorrhizal (AM) colonization percentage, number of spores and arbuscular mycorrhizal fungi (AMF) infectivity (reviewed by Carrenho et al. 2008). The decrease in productivity of *Eucalyptus* can be due to infertile soils as well as the lack of indigenous symbiotic mycorrhizal fungi. Some *Eucalyptus* species form two different types of mycorrhizas, i.e., AM and ectomycorrhiza (EM) (Malajczuk et al. 1981, Zambolim

and Barros 1982, Wang and Qiu 2006). AM colonization has been reported in young individuals of *Eucalyptus* species that usually form EM (Chilvers et al. 1987). Ectomycorrhizal fungi (EMF) 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 (Landeweert et al. 2001).

Eucalypts associate with numerous species of EMF (Molina et al. 1992). The genera *Scleroderma*, *Pisolithus*, *Hymenogaster*, *Hydnangium* and *Laccaria* are of interest as possible candidates for incorporation into nursery inoculation programmes. *Pisolithus tinctorius* (Pers) Cocker & Couch is the most important EM fungus for forestation and has been used to increase the growth of a plantation of eucalypts (Garbaye et al. 1988). Mason et al. (2000) reported that *P. tinctorius* colonized the roots of the largest seedlings at low phosphorus (P) fertilization. Moreover, some EMF (rhizomorph formers) form extensive mycelia connected by different hyphal strands called rhizomorphs which transport water and nutrients over long distances (Read 1984).

AM are the most frequent fungi supported by legumes trees, enhancing the uptake of phosphorus, nitrogen and others nutrients, consequently being of high interest for agroforestry. This symbiosis links the biotic and geochemical components of the ecosystem and their nonnutritional effects, such as reducing plant diseases and modifying water relationships or stabilizing soil structure, are also very important. In fact, AMF form a uniformly distributed mycelium in soil, hyphal proliferation occurs in response to several types of organic material and near potential host roots (Olsson et al 2003), improving physical soil quality (reviewed by Cardoso and Kuyper 2006).

The use of the AM symbioses provides an alternative to high inputs of fertilizers and pesticides in sustainable plant production systems (Gianinazzi and Schüepp 1994). However, AM inoculation technology is limited by the lack of production of commercial inocula, because AMF cannot be multiplied on artificial growth media without a host (Sieverding 1991).

Nowadays there is need to further investigate the composition and distribution patterns of AMF species in different AS. Such a study will facilitate the utilization and management of AMF to improve agroforestry tree productivity, as well as to obtain other benefits.

The environmental benefits of agroforestry in the semi-arid region of Brazil have been scarcely studied (Maia et al. 2006). In their evaluation of the impact of agroforestry and conventional systems on the physical and chemical soil characteristics, in the semi-arid region of Ceará, Brazil, Maia et al. (2006) found that silvopasture systems maintained the soil quality and food production for that region.

In the State of Minas Gerais (Brazil) there is a need for minimizing the impacts of eucalypt and coffee monocultures. Cardoso et al. (2001) stressed the complexity of participatory development of AS and the need for their continual development. Some reports have shown intercropping *E. camaldulensis* with rice and beans (Cecon 2005) as well as AS including coffee (Cardoso et al. 2003) in this State.

The semiarid region (Caatinga biome), in NE Brazil, (2°54' to 17°21' S), comprises a strip in Minas Gerais State, following the São Francisco river. This dry forest presents the highest solar radiation and annual average temperature, and the lowest precipitations and nebulosity (Prado 2003). This region still presents fragments of natural vegetal cover, being important for conservation of the patterns of climate and biodiversity of the planet (Tabarelli and Silva 2003). Plants from Caatinga enter a dormant phase during the major part of the year

(dry season), while leaves, flowers, and the herbaceous vegetation grow in the short and sporadic rainy season (Rizzini 1997).

After forest clearing or in burned areas, early-successional trees (attaining up to 5 m height) and microfanerophytic shrub species, a vegetation called "Carrasco", covers the area and significantly retards woody natural succession (Pagano et al. 2008). The Jaíba Project is the most important irrigation program in the Semi-arid of Minas Gerais State and its goal is to increment the agricultural production, economic and social growth of the region. A project of wood provision for the local populations was established using AS of mixed native species and *Eucalyptus* in disturbed sites at the Jaíba region (Pagano et al. 2009). The successful growth of native plants for AS demands an understanding of the dependency of these plants to mycorrhizal fungi (Pagano 2007).

This chapter presents an overview of field studies conducted in the Jaíba region, in the north of Minas Gerais, Brazil. Data showing mycorrhizal root colonization, AMF occurrence and effects of inoculation on plant species from AS of native legume and non-legume trees, intercropped with *Eucalyptus camaldulensis* and *E. grandis* are here compiled.

Our goal was to answer the following questions: (1) Do plant species (Table 1) form mycorrhizae and what types of mycorrhizae are present?; (2) Do AMF affect plant growth on native low nutrient soils; under monoculture and mixed plantations with *Eucalyptus* sp.?; and (3) What levels of additional phosphorus (P) are required to improve seedling growth? Our experimental results provide land managers with information on the AMF occurrence and productivity of AS.

AGROFORESTRY SYSTEMS IN THE NORTH OF MINAS GERAIS, BRAZIL

AS in this region include, in general, one native legume species (N-fixing tree) chosen to increase the input of N, interplanting with a second non legume species, and with a species of *Eucalyptus* that tolerates dry conditions, which can be a source of wood for local community use. The native species used at Jaíba, Minas Gerais State, were caducifolious trees considered late secondary species such as *Tabebuia heptaphylla* (Vell.) Tol. (Bignoniaceae), *Schinopsis brasiliensis* Engl. (Anacardiaceae), or pioneer species: *A. peregrina* (L.) Spegazzini (Mimosoideae), *P. reticulata* Benth (Leguminosae) and *E. contortisiliquum* (Vell.) Morong. Some of them (*S. brasiliensis* Engl., *M. urundeuva*) are classified within the threatened or near threatened category of the official Brazilian endangered species list.

Attention is being increasingly focused on the agroforestry potential of *E. camaldulensis*, a fast growing tree species which can tolerate extended dry seasons (Marcar et al. 2002). In Jaíba, we used two timber *Eucalyptus* (Myrtaceae) species (*E. camaldulensis* Dehnh and *E. grandis* Hill ex Maiden), considered of good adaptation for the region. *E. camaldulensis* Dehnh presents high resistance to drought and a taproot.

Enterolobium contortisiliquum (Vell. Conc.) Morong (Mimosaceae), *Plathymenia reticulata* Benth (Leguminosae), *Schinopsis brasiliensis* Engler (Anacardiaceae) and *Tabebuia heptaphylla* (Vell.)Tol. (Bignoniaceae), were studied in mixture with *Eucalyptus camaldulensis* Dehnh or *Eucalyptus grandis* W. Hill (Mirtaceae), and in monocultures at experimental sites (1.5 ha) (Table 2). Details of the original experimental design and

sampling are provided by (Pagano et al. 2008, 2009); we present here an overview necessary to place in perspective the findings from the present chapter.

Ten AS were inoculated with AMF and rhizobia (for legumes), and another ten were not inoculated, totaling 20 experimental AS, planted to evaluate the effect of inoculation on plant growth and to study plant consorts. The noduliferous leguminous were inoculated with rhizobia and AMF, because dual inoculation improves legume production.

Rhizobia are bacteria classified as *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, etc., which can survive and reproduce in the soil, and fix atmospheric N inside the nodules that develop on legume plants roots (reviewed by Denison and Kiers 2004).

Efficient rhizobial strains were pre-screened for high fixed nitrogen under greenhouse conditions. The bacterial inoculum (which is specific for each legume) 1 mL (10^7 cfu mL/L) of the rhizobial culture grown in liquid YEM (Yeast Extract Mannitol) medium in a density corresponding to the mid-log phase of growth, according to Somasegaran and Hoben (1985), was applied in the seedling root at planting.

Table 1. Trees used in agroforestry systems in Jaíba region, Minas Gerais, Brazil (listed per family names and species with local names in brackets)

Family	Species (local name)
Anacardiaceae	<i>Myracrodouon urundeuva</i> (Aroeira)
Bignoniaceae	<i>Tabebuia heptaphylla</i> (Ipê)
Mimosaceae	<i>Anadenanthera peregrina</i> (Angico vermelho)
	<i>Enterolobium contortisiliquum</i> (Tamboril, Orelha-de-macaco)
Leguminosae	<i>Plathymenia reticulata</i> (Guanambira)
Anacardiaceae	<i>Schinopsis brasiliensis</i> (Braúna)
Mirtaceae	<i>Eucalyptus camaldulensis</i> (Eucalipto)
	<i>Eucalyptus grandis</i> (Eucalipto)

Table 2. Some of the experimental AS in Jaíba region, Minas Gerais, Brazil

	No interplantings
A1	<i>A. peregrina</i> † monoculture
P1	<i>P. reticulata</i> † monoculture
S1	<i>S. brasiliensis</i> monoculture
E1	<i>E. contortisiliquum</i> † monoculture
EC1	<i>E. camaldulensis</i> monoculture
EG1	<i>E. grandis</i> monoculture
	Legume tree interplanted
A2	<i>A. peregrina</i> † monoculture interplanted with <i>M. urundeuva</i> and <i>E. camaldulensis</i>
P2	<i>P. reticulata</i> † interplanted with <i>T. heptaphylla</i> (T) and <i>E. camaldulensis</i> (EC2)
S2	<i>S. brasiliensis</i> interplanted with <i>E. camaldulensis</i>
E2	<i>E. contortisiliquum</i> † interplanted with <i>E. grandis</i> (EG2)

†Legume tree. The different AS were inoculated or not with AMF and rhizobia (legumes).

It has become customary to use AM spores as inoculum (Read 2003), and using three representative Genus of AMF (mixed inocula) is a common inoculation strategy.

In AS at Jaíba, AM inoculation was done at transplantation using 1 ml of inoculum containing about 150 spores of three species: *Gigaspora margarita* Becker and Hall, *Scutellospora heterogama* (Nicol. and Gerdemann) Walker and Sanders, and *Glomus brohultii* Sieverding and Herrera, which were isolated from pot cultures with *Brachiaria decumbens* Stapf. Inoculum was applied in a 5 cm furrow and mixed with soil at planting. The AMF strains used were from the Biological Science Institute, Belo Horizonte (BHCB) culture collection. The plant species *T. heptaphylla* was not inoculated and *E. camaldulensis* was inoculated with AMF.

SOIL CONDITIONS IN AS

The predominant soil type in the studied site at Jaíba is Quartzarenic Neosol with high infiltration rates. Furthermore, it is moderately acid and has small amounts of soil organic matter. In our study, we observed little variation in soil characteristics between the legume or non-legume monocultures and the mixed plantations. Soil pH was almost higher under *P. reticulata* monoculture in comparison to that under mixed plantation, whereas under *S. brasiliensis* or *E. contortisiliquum*, the monocultures presented lower pH. Soil organic matter was low both in the monocultures and mixed plantations. Soil texture was sandy with lower levels of clay and silt found. P content was very low (Pagano et al. 2008, 2009).

EFFECT OF MYCORRHIZA INOCULATION ON PLANT GROWTH IN AS

The effect of mycorrhization on plant growth is well documented on AS (Haselwandter and Bowen 1996, Cardoso and Kuyper 2006). However, some tropical trees benefit from AM inoculation, while others do not (Bâ et al. 2000) because plant response to inoculation with AMF differs (Siqueira et al. 1998, Siqueira and Saggin Júnior 2001).

Root colonization, relative mycorrhizal dependency, hyphal P transport, and P concentrations in shoots indicate the magnitude of benefits from improved management of AMF.

In our study, the inoculated native species (*Plathymania reticulata* and *Anadenanthera peregrina*) showed higher height and diameter growth when mixed with *E. camaldulensis* from AS in Minas Gerais.

Under field conditions, uninoculated *A. peregrina* plants intercropped with *E. camaldulensis* that had been inoculated with AMF presented higher height and diameter growth (Pagano et al. 2008). The double inoculation of *A. peregrina* also improved dry matter production and nutrient content, especially of N and P, confirming that the productivity of inoculated AS was greater than that of uninoculated AS, especially regarding above-ground biomass and basal area. Most of the nutrients were concentrated in the leaves, stem, and in the bark especially in inoculated plants.

AS using *Plathymania reticulata* Benth, *E. camaldulensis* Dehnh and *T. heptaphylla* (Vell.) Tol. (Bignoniaceae) also showed that the legume inoculated with rhizobia and AMF

presented higher height growth than non-inoculated plants and an increase especially in diameter after 2 years of cultivation.

For some of these plants the benefic effect of arbuscular mycorrhizal associations enhancing uptake of mineral nutrients, namely phosphorus was corroborated. In greenhouse tests, phosphorus fertilization improved growth of *A. peregrina*, *E. contortisiliquum* and *P. reticulata*. Phosphorus increased to enhance the positive effects of AMF in the three studied species. Tissue nutrient concentrations were influenced by both AMF inoculation and phosphorus addition. Plants inoculated and fertilized with higher doses of phosphorus (but lower than the commercial fertilizer doses) showed more vigorous seedlings than uninoculated ones (Pagano et al. 2007). These results suggested that in low fertility soils *A. peregrina*, *E. contortisiliquum* and *P. reticulata* seedlings should be inoculated with AMF to enhance plant growth.

As regards nutritional demand plants species differ due to their ecophysiological characteristics, such as successional group and their capacity to form symbioses with soil microorganisms, especially with mycorrhizal fungi. The need to know the nutrient requirements of the plant species and their mycorrhizal dependency is thus crucial.

Besides, some plant species respond to fertilizer application and show a higher occurrence of spores of some AMF species and low occurrence of others. The need to develop sustainable AS in natural undisturbed ecosystems, with reduced external inputs of pesticides and manufactured fertilizers has encouraged the survey of other microorganisms that can likewise benefit plant production.

Our results showed that nutrient content of *E. grandis* presented little variation compared with that of *E. camaldulensis*. Wood localized in superior parts of trunk presented a higher concentration of P and bark contained significant amounts of nutrients, especially in *E. grandis*. The highest nutrient concentrations were found in the leaves and the lowest concentrations of N, P, K, Ca and Mg were found in the stem wood, those of S being in the stem wood and bark; wood localized in superior parts of trunk presented higher concentration of P and bark contained significant amounts of nutrients, especially in *E. grandis*. This showed that leaving vegetal waste on the site is important to reduce the loss of tree productivity in this semiarid region.

ROOT COLONIZATION IN AS

Variations in occurrence of fungal structures provide information about the fungi in relation to nutrient transfer and plant growth (Jakobsen et al. 2003).

Root colonization of *Enterolobium contortisiliquum* (Vell.) Morong, *Plathymania reticulata* Benth, *Schinopsis brasiliensis* Engl. and *Tabebuia heptaphylla* (Vell.) Tol varied according to the species and the sites (monoculture or mixed plantation). In general, the native trees showed the highest arbuscular mycorrhizal (AM) colonization when mixed compared to their respective monoculture. Only *E. contortisiliquum* presented lower levels of colonization. Values for colonization were higher in the wet period than in the dry period. All the native species presented *Arum*-type colonization in their roots, and significant AM morphological structures were documented (Figure 3 b-e).

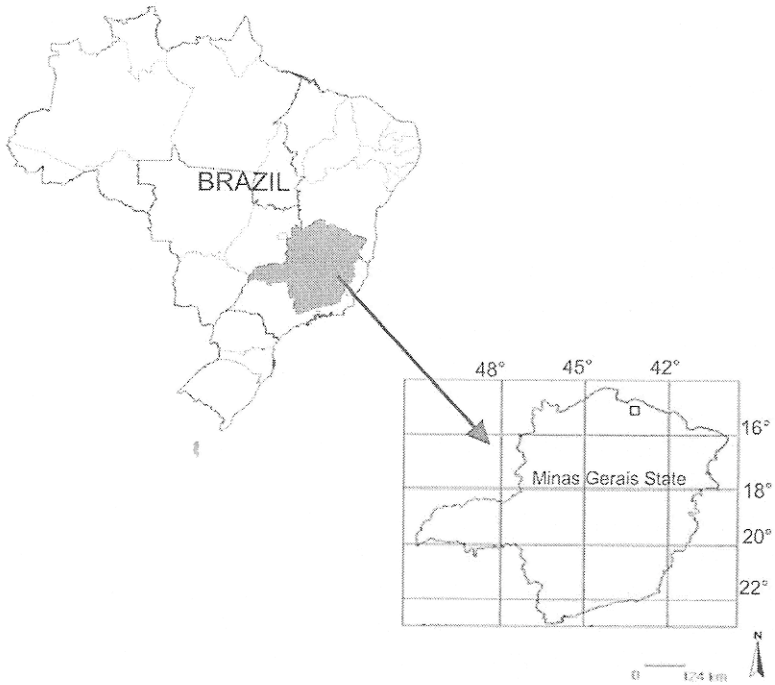


Figure 1. The location of the experimental site at Jaíba in the state of Minas Gerais, Brazil.

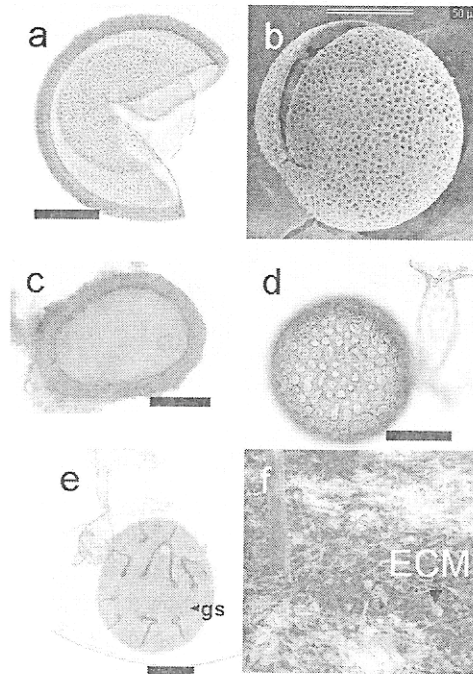


Figure 2. Spores of species of AMF found in AS at Jaíba, Minas Gerais, Brazil: (a-b) *Acaulospora scrobiculata*, (b) ovoid concave depressions on the surface, scanning electron micrograph (SEM). (c) *Glomus brohultii* (a dominant AM species), (d) *Acaulospora birreticulata*, (e) *Scutellospora cerradensis* spore with germination shield (arrow head); (f) basidiocarp of EMF (arrow) in eucalypt monoculture. Bars: 50 µm.

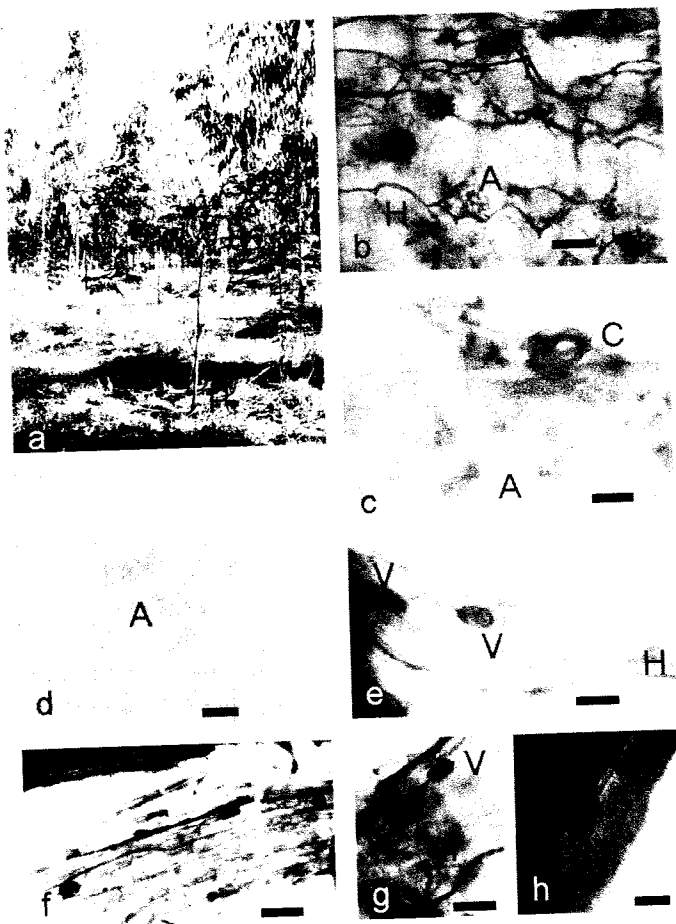


Figure 3. (a) AS at Jaíba, Minas Gerais, Brazil; P= *Plathymeria reticulata*; (b) AM colonization in *S. brasiliensis* fine roots. Arbuscules in root cells of *S. brasiliensis*; (c-d) *P. reticulata* root bearing arbuscules, and coils (C) and detail of an arbuscule (d); (e) *T. heptaphylla* intra radical hyphae bearing vesicles; (f) *E. camaldulensis* showing "h" and "y" branching pattern and terminal vesicles (V); (g) EM colonization in roots of eucalypt. Bars for figures b, f and g = 100 μm ; c, e, and h = 50 μm , and d = 10 μm .

Plathymeria reticulata AS maintained high AMF colonization levels compared with monocultures, proving an efficient system for productivity and sustainability. Plants in monocultures presented lower hyphae, vesicles and arbuscular colonization than in mixed plots (Pagano et al. 2009). Moreover, the roots of *T. heptaphylla* (which was not inoculated with AMF) showed significantly higher hyphal colonization levels when intercropped with the inoculated plants than when intercropped with uninoculated ones.

The monocultures of *S. brasiliensis*, and *E. contortisiliquum* also exhibited lower colonization (Table 3) than in the mixed plantations.

Significant AM morphological structures, such as extraradical hyphae, intraradical hyphae, hyphal coils, arbuscules and vesicles were documented (Figure 3 b-g). It has been shown that the external hyphae produced by a mycorrhizal fungus can indicate its relative ability to uptake phosphorus (Jones et al. 1990).

Table 3. AM root colonization intensity of some of the plants used for AS in Jaíba region, Minas Gerais, Brazil

Vegetal species	AS [†]	Rainy season [#]	Dry season
		Root colonization intensity (%) [‡]	Root colonization intensity (%)
<i>E. contortisiliquum</i>	E2	ND	0.25
<i>E. camaldulensis</i>	EC1	3.42 aA	0.68 bB
	EC2	2.88 aA	5.27 aA
<i>P. reticulata</i>	P1	1.55 bA	1.29 bA
	P2	19.44 aA	7.13 aB
<i>S. brasiliensis</i>	S1	ND	1 b
	S2	ND	4.3 a
<i>T. heptaphylla</i>	T	17.68 A	5.48 B

[†] Agroforestry systems (see Table 2); [‡] Hyphae %; [#] Period; Values followed by the same lowercase letter (compare means in column within the same plant species) or same capital letter (compare means in row of different seasons), did not differ by Tukey's test at ($P \leq 0.05$). ND= not determined.

Table 4. Arbuscular mycorrhizal fungal status in roots and spore numbers in rooting zone soils of the studied plant species

Host	AS	Length	Coils	A	V	Type	PC [‡]	SN	S
<i>E. camaldulensis</i>	EC1	45	-	+	25.4	<i>Arum</i>	h, eh, ov	340	2
	EC2	32.05	-	+	7.05	<i>Arum</i>	h, eh, ov	503.7	2
<i>E. grandis</i>	EG1	-	-	-	-	-	-	20	2
	EG2	-	-	-	-	-	-	53.5	4
<i>E. contortisiliquum</i>	E1	35	-	-	18	<i>Arum</i>	h, ar, iv	142	4
	E2	30.1	-	-	20	<i>Arum</i>	h, ar, iv	59	2
<i>P. reticulata</i>	P1	60	+	++	41.2	<i>Arum</i>	h, ar, eh, ov	154.5	5
	P2	65	+	+	31.2	<i>Arum</i>	h, ar, eh, ov	180.5	6
<i>S. brasiliensis</i>	S1	45	-	++	22.5	<i>Arum</i>	h, ar, eh, ov	158.5	5
	S2	60	-	++	55	<i>Arum</i>	h, ar, iv, ov	88	6
<i>T. heptaphylla</i>	T	58.7	-	+	20	<i>Arum</i>	h, ar, ov	163.8	5

Length AM root length %; Coils hyphal coils; A arbuscules; V vesicles; Type *Arum*, *Paris* or intermediate; Relative development of structures shown as: ++ always present in significant numbers, + always present; - not detected [‡]PC: Patterns of AM colonization; *h*: intra- or intercellular aseptate hyphae, *ar*: arbuscules, *ov*: oval vesicles, *iv*: irregular vesicles; SN spore numbers; S species richness.

The roots of non-legume *S. brasiliensis* presented hyphae in parallel to each other and to the root axis and numerous arbuscules stained darkly in trypan blue (Figure 3b). Their colonization level was higher, especially when mixed (Table 3).

Plathymenia plants showed hyphae, vesicles and arbuscules production (Table 3, Figure 3 c-d) in the finest roots. Arbuscules formed from a trunk hypha that was generally 9–10 μm wide (Figure 3d).

Table 5. Arbuscular mycorrhizal genera found in the plants used for agroforestry system in Brazil

AMF genus	A1	A2	E1	E2	EC1	EC2	EG1	EG2	P1	P2	S1	S2	T [#]
Gigasporaceae													
<i>Gigaspora</i>	X	X	X	-	-	X	-	X	X	X	X	X	X
<i>Scutellospora</i>	X	X	-	-	-	-	-	-	-	-	X	X	-
Acaulosporaceae													
<i>Acaulospora</i>	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Entrophospora</i>	-	-	-	X	-	-	-	-	-	-	-	-	-
Glomeraceae													
<i>Glomus</i>	X	X	X	X	X	X	X	X	X	X	X	X	X

[†]AS; [#]*T. heptaphylla* in mixed plot (P2).

Tabebuia heptaphylla roots presented a higher colonization rate in the rainy season; whereas in the dry season the levels were lower (Table 4). Their roots presented arbuscules, vesicles and higher hyphae colonization frequency (Table 3). Hyphae (4 µm in diameter) and oval vesicles were recorded (Figure 3e).

Enterolobium contortisiliquum roots contained mostly intraradical hyphae and sporadic spherical to oblong vesicles (18-27 x 45-80 µm) (Table 3). Hyphae connected by "h"-shape anastomosis pattern were often observed within root segments. This Glomineae-type colonization is in line with the presence of *Glomus* spores in their rhizosphere (Table 5). We were not able to evaluate the roots of *E. contortisiliquum* and *S. brasiliensis* for mycorrhizal colonization in the rainy season, because roots had not been collected.

In general, aseptate intra and intercellular hyphae, vesicles, were the most frequent AM structures present in the studied tree species. Arbuscules or hyphal coils were less frequent (Table 3), the latter being observed only in *P. reticulata* (Figure 3c). All plant species presented *Arum*-type colonization.

Mycorrhizal colonization values of plant species presented here in tables 3 and 4, are in accordance with those obtained by Patreze and Cordeiro (2005) for *E. contortisiliquum*, and with those reported by Soares et al. (2003), who found a similar percent of root colonization (55%) in *T. heptaphylla* inoculated with *G. margarita*, found in high numbers in the rhizosphere of the same plant (this study). *Plathymenia reticulata* exhibited the highest values for root colonization. Carrenho et al. (2002) also found higher AM colonization in legumes than in non-legumes.

In our study the *Arum*-type was seen to be dominant in the woody trees. The patterns of colonization were in accordance with Dickson et al.'s review (2007) reporting the *Arum*-type colonization in Fabaceae and Anacardiaceae. It is well known that the *Arum*-type is formed in most plants that usually grow in sunlight, and that the spreading rate of colonization is faster in this type of colonization (Brundrett and Kendrick 1990). However, other environmental factors such as temperature and soil moisture content may influence AM morphology in roots (Cavagnaro et al. 2001). In AS at Jaiba, vesicles and hyphae were the most common AM structures in the plant roots. It has been shown that storage vesicles appear at later stages of colonization, being important contributors to carbon drain from the plant (Jakobsen et al. 2003), as well as propagules (Smith and Read 1997).

It is difficult to generalize about plant colonization by AM fungi is difficult because different methodologies are used to estimate them, as Koide and Schreiner (1992) pointed out. Some authors used the degree of colonization (class) (Wubet et al. 2003, Becerra et al. 2007); however, in this study the intensity or rate of colonization was calculated according to Trouvelot et al. (1996).

The intensity of AM colonization is the proportion or rate of cortical cells colonized by the fungi. According to Trouvelot et al. (1986) method, the intensity was estimated on the same 3 lots of 30 root segments under the light microscope. AM colonization > 50%, 50–20% and <20% were considered as high, medium and low respectively, according to other authors.

The highest AM colonization rate of *P. reticulata* occurred in the mixed plantation, whereas the lowest colonization was observed in the monoculture (< 2%) during the two periods (Table 4). Compared to their respective monoculture, the intensity of *S. brasiliensis* root colonization (Table 4) was significantly higher when this species was mixed with *E. camaldulensis*.

In general, *P. reticulata* showed AM root colonization rate medium to low, compared to other native species of Brazil (Carneiro et al., 1998). Jesus et al. (2005) observed a lower rate (16%) in another legume, *Piptadenia paniculata*, inoculated with AM and fertilized with N, and higher rates when this species was inoculated with rhizobia. The cost of the symbiosis (hyphae formation, nutrient uptake and interchange) does not seem to be much greater due to the medium colonization rates presented. It is also known that legumes are generally more mycotrophic than other plants (Plenchette et al. 2005), and they can increase the concentration of AMF spores in the soil (Colozzi and Cardoso 2000). It is known that the morphology of AM in roots is the result of the interplay between both the plant and the fungal species (Kubota et al. 2005).

Plants control the AM colonization, and root anatomy may also play a role in determining the rate at which internal infections develop (Koide and Schreiner 1992). The medium colonization rate presented in *T. heptaphylla* roots in the rainy season could be due to the presence of a legume tree intercropped, which demands more P for nitrogen fixation (Hokka et al. 2004). Moreover, fixed nitrogen can be transferred to non-nitrogen fixing plants via AM fungal hyphae without entering the soil solution (Martensson et al. 1998). Other reports have showed *Tabebuia roseo-alba*, native of Brazil, being colonized by AMF in nursery conditions (Carneiro et al. 1998).

Notably, in our study the colonization rate increased in the rainy period, which may be related to the improvement of soluble nutrients, and in the case of legumes, to P supply by AMF to host plant and nodules. Silva Junior and Cardoso (2006) also reported higher AM colonization in the rainy season in *Theobroma grandiflorum* and Allen et al. (1998) for Mexican tropic trees. This was related to a higher root activity in that season.

Studies of AM colonization are important for seedling production and preparation of technologies for successful afforestation, because of the fact that vegetal species exhibit different AM dependency (Siqueira and Saggin-Junior 2001). Climax species can show low AM percent of colonization, and the literature shows that different plant species often harbor quite distinct AM fungal communities (Eom et al. 2000).

Our results were consistent with the reports of many authors (Zangaro et al. 2002, Dhar et al. 2006, Zhao et al. 2006, Becerra et al. 2007), showing that plant species have a narrow to broad range of colonization. We believe that more studies including molecular identification directly in host plant roots are important to select efficient AM isolates.

In Brazil, the occurrence of AMF in agroforestry trees is not yet well documented. Our studies provides the first detailed report ever published on the mycorrhizal status of some of the species examined, which belong to the Anacardiaceae, Bignoniaceae, Leguminosae and Mimosaceae families. It also confirms the mycotrophic nature of the tree species at Brazilian Dry forest.

MYCORRHIZAE IN EUCALYPTUS

EMF fungi are particularly dominant in ecosystems where plants are limited by N (Read 1991), but elevated levels of N from fertilizers inhibit growth of external EM and fruit bodies (Olsson et al. 2003).

For the purpose 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, the establishment of AM associations in *Eucalyptus* plantations has been known for over 20 years, and the benefits of symbiosis are known to be commercially relevant (Zambolim and Barros 1982, Coelho et al. 1997, Gomes and Trufem 1998, Graziotti et al. 1998, Santos et al. 2001). However, a better understanding of symbioses is necessary.

Colonization percentages by AMF in *Eucalyptus* are very varied, and both AMF and EMF can be present in roots (Chilvers et al. 1987). Differences in growth response to EMF and AMF seem to be related to the higher increase of P by AMF (reviewed by Carrenho et al. 2008); nonetheless, increases in eucalypt growth have been shown to be positively correlated with the extent of EMF colonization (Thomson et al. 1994).

In Jaíba, the two eucalypt species showed mycotrophy. *Eucalyptus camaldulensis* presented both types of root colonization, by AMF and EMF (Figure 3 f-h).

Eucalyptus camaldulensis showed varied AM colonization level according to season of sampling. During the rainy season the AMF colonization decreased in monocultures as well as mixed plantations inoculated or not, while the native EMF colonization levels improved. In contrast, in the dry seasons EMF was reduced and AMF colonization increased. Although typical AM structures such as vesicles and extra- and intraradical hyphae were observed on the *E. camaldulensis* root samples, arbuscules were observed only in some of the samples. Spherical to oblong vesicles were often observed within root segments. Hyphae often grew for some distance in parallel to each other, connected by an "h"-shape anastomosis pattern (Figure 3f), indicating a Glomineae-type colonization that was in line with the presence of *Glomus* spores in the rhizospheric soils (Pagano and Scotti 2008), suggesting that this AM genus could be a potential inoculum for this plant species in this region.

According to the previously reported results in exotic *Eucalyptus* plantations (Reis and Krüger 1990, Adjoud et al. 1996; Adjoud-Sadadou and Halli-Hargas 2000, Chen et al. 2000, Santos et al. 2001), dominance of AM in *E. camaldulensis* in the dry periods in our 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.

On the other hand, *E. grandis* showed dominant ectomycorrhizal colonization, suggesting that *E. camaldulensis* has both AM and EMF dependencies, whereas *E. grandis* is solely

EMF dependent in the monocultures (Pagano and Scotti 2008). As mycorrhizal colonization on *E. grandis* was investigated only in the dry period, sampling in the rainy period would be required to conclude that *E. grandis* in monoculture lacks AM.

Overall results suggested that *E. camaldulensis* has both AM and EMF dependencies, whereas *E. grandis* is solely EMF dependent in the monocultures.

Mycorrhizal characterization in *Eucalyptus* plantations is a prerequisite to decide which species will be useful for the mycorrhizal inoculation programs. We observed EMF and a dominance of Hartig net on the root samples of *E. grandis*. Moreover, abundant *Pisolithus*-like basidiocarps in *E. grandis*, but fewer in *E. camaldulensis* stands, were also observed. The presence of *Pisolithus* basidiocarps in the *E. grandis* plot implies the EMF as a potential inoculum at this site. In this sense, our study contributes to evaluating the mycorrhizal fungal community in the managed reforestation programs in Brazil.

AM SPORE DISTRIBUTION AND DIVERSITY IN AS

Mycorrhizal fungi are ubiquitous in tropical savannas, grasslands and tropical forests (Newman et al. 1986). Agroforestry systems are usually more productive and allow a larger diversity and/or abundance of AM than monocultures (Cardoso et al. 2003, Cardoso and Kuyper 2006, Muleta et al. 2008, Pagano et al. 2009). Jefwa et al. (2006) have showed that the occurrence and persistence of AMF species are influenced by agroforestry combinations, and that the spores of most AM species are tolerant to dry conditions. Management practices have also great implication in the persistence of spore propagules of AM species.

In general, fertilizers and nutrient supply imbalance especially higher levels of N and P can decrease the AMF occurrence and sporulation (Johnson and Gehring 2007), which could be reduced in these conditions (Guillemin et al. 1992, Olsson et al. 2003). However, a decrease in AMF species by inorganic fertilizers depends on the differential response in spore production of AMF species to different types of fertilizer (Jefwa et al. 2006). Therefore, different AMF species shows different responses to fertilization. Bhadalung et al. (2005) have showed that some spores of *Glomus* (an important AMF genus in agriculture) were classified as slightly sensitive to fertilization and others species of *Glomus*, as highly sensitive.

Due to the difficulty in making specific fertilization recommendations, requirements of native plants needs to be more studied. Nowadays, the study of plant species from Brazil is increasing, because of their importance for reforestation and restoration purposes, and a complete understanding of plant life histories should include traits related to AM formation.

Our study in the semiarid of Minas Gerais State, the AS presented Glomeraceae as the dominant family, with the genus *Glomus*. *Glomus* was the dominant genus in most of the plant rhizospheres studied, followed by *Acaulospora* spp., *Gigaspora* sp. and *Scutellospora* sp.

AS including leguminous trees maintained AMF spore numbers in soils (Table 4). Notably, in the AS denominated P2 (Table 2), *P. reticulata* and *T. heptaphylla* presented higher *Gigaspora margarita* (See *Gigaspora*, Table 5) spore numbers in their rhizospheres, suggesting that this AM species can be a potential inoculum for these trees.

The AMF spore number was high in the *P. reticulata* inoculated plants in monoculture as well as in mixed plantations. In *S. brasiliensis* and *E. contortisiliquum* monocultures higher

spore number was observed than in mixed plots. This can be due to the fact that legumes are more mycotrophic than other plants (Plenchette et al. 2005, Colozzi and Cardoso 2000, Muleta et al. 2008).

AMF species richness was in general higher in mixed plots (EC2, EG2, P2, S2), than in the monocultures of these species. In the rhizospheres of native species spore populations were found to belong to five genera: *Acaulospora*, *Entrophospora*, *Glomus*, *Gigaspora* and *Scutellospora*. In general, the number of AMF spores in soil increased with time in the AS after transplantation.

Regarding the rhizosphere of the exotic species used, *E. camaldulensis* showed greater AM fungal spore numbers (mean data from the dry and rainy periods) (Table 4). In the rhizospheres of *Eucalyptus*, the AM sporulation increased in the rainy season compared to the dry period (Pagano et al. 2009).

In *E. camaldulensis* monocultures mean spore number was significantly higher in the rainy season compared with the dry season (Pagano et al. 2009). In *Eucalyptus* rhizospheres *Glomus* was also dominant in spore numbers.

On the other hand, *E. grandis* showed lower AM fungal spore numbers. In *Eucalyptus grandis* monocultures, higher spore numbers were recovered in the rainy period (about 64 spores/ g soil) than in the dry period (19.5 spores/ g soil) and *Glomus* was the dominating genus (data not showed).

Among AMF species in the studied areas a total of 14 taxa were found. Of these, one belonged to the genus *Glomus*, eight to *Acaulospora*, three to *Gigaspora* and two to *Scutellospora* (Table 5). *Acaulospora scrobiculata*, a common AMF species, was also present in higher numbers.

Glomus brohultii (Figure 2c) was found during all samplings in all treatments. During the dry season the spore numbers of this species did not differ; whereas, during the rainy season, the highest spore number was found in mixed plantations inoculated with AMF and/ or *Rhizobium*.

Species richness ranged from three to eight. The highest species richness was found in the mixed plantations. The lowest species richness was found in *Eucalyptus* monocultures, presenting only three to six species. The rooting-soil of *P. reticulata* showed more AMF species richness than *Eucalyptus* and AMF diversity was higher in mixed plantations (Table 2). We thus verified that *Glomus* was the dominant genus in both, native trees and eucalypt rhizospheres.

CONCLUSION

In the introduction to this chapter, we briefly described that mycorrhizal fungi provide a wide range of significant benefits to their plant hosts, enhancing mineral nutrition, tolerance to drought stress, and soil fertility, and that many efforts have been made in recent years to accrue benefits from mycorrhizae for forestry and agroforestry.

Throughout the chapter, we have showed that AS at Jaíba, Minas Gerais State, Brazil, maintain higher root colonization and spore numbers than monocultures, confirming the benefic role of AMF, and highlighting the importance of mycorrhizae as an essential

component for establishment and sustainability of plant communities. Nonetheless, further studies are required to achieve maximum benefits from these microorganisms and their associations. All these results show that *P. reticulata*, *S. brasiliensis* and *T. heptaphylla*, AM-dependent species, are indicated for mixture with *E. camaldulensis* when there is a need for minimizing the impacts of eucalypt monoculture. Additionally, AS are alternatives to preserve the biome of Dry Forest by using native species of this biome; thus restoration programs should take mycorrhizae into account.

Since this chapter is primarily concerned with AMF occurrence and benefits, we have refrained from discussing soil fertility which most studies have often focused on, disregarding soil biological properties such as mycorrhizal occurrence and interactions between plants, fungi, and the environment. The evidence presented here suggests that the introduction of AMF fungi appear to offer much advantage to enhanced nutrition and plant growth. Our results emphasize the need to consider the symbiotic fungi in agroforestry management practices, which show great implication in the persistence of AMF species. The choice of agroforestry tree species would therefore have great implication in the manipulation and conservation of AMF species. The ability of native AMF to colonize plants in natural conditions and the loss of these fungi with disturbance require more studies. Highly dependent tree hosts should be selected over mycorrhizal-independent hosts in AS for regions like the semiarid of Minas Gerais.

Finally, this chapter argues that *Eucalyptus grandis* and *E. camaldulensis* in a semiarid region did not reduce growth and aboveground biomass production, showing nutrient concentrations similar to those reported in the literature. The highest nutrient concentrations were found in the leaves and the lowest in the stem wood; wood localized in superior parts of trunk presented higher concentration of P and bark contained significant amounts of nutrients, especially in *E. grandis*. We have attempted to highlight the importance of leaving vegetal slash (mostly crown) on the site in order to decrease the loss of tree productivity. Consequently, further research is necessary on *Eucalyptus* in the Southern Brazilian region, especially regarding litter accumulation, below-ground biomass, and nutrient dynamics.

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