MYCORRHIZAL FUNGI BIODIVERSITY AND APPLICATIONS OF INOCULATION TECHNOLOGY

菌根生物多样性及其应用研究

——中澳合作广州国际菌根研讨会论文集

弓明钦．徐大平．仲崇录．陈应龙
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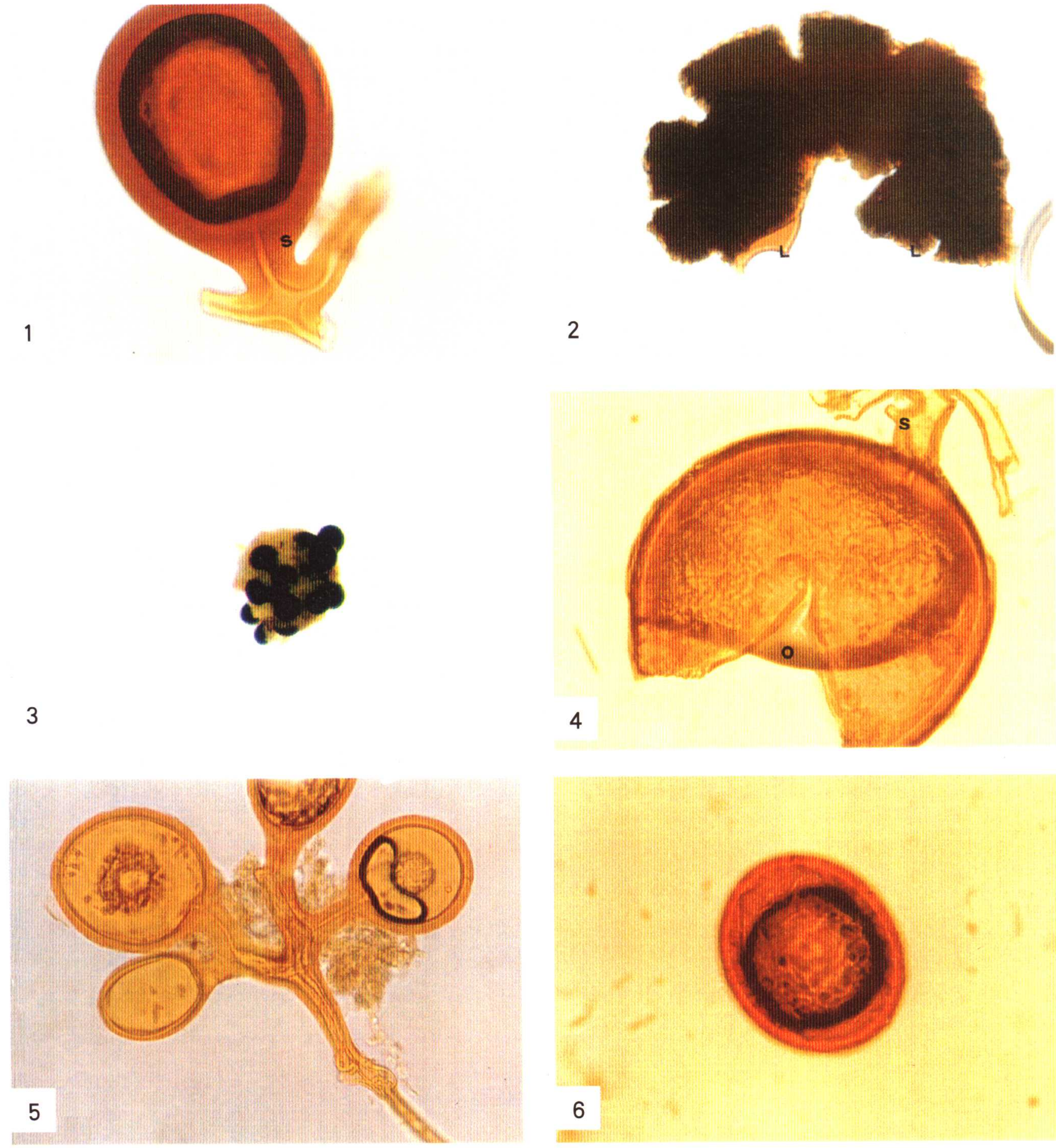

Fig.1 Red brown spherical spore showing subtending hypha (S) without septum, 400×

Fig.2 Dark coloure spore without subtending hypha showing different layers (L), 400×

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I. 菌根生物多样性及生态学

Ecological, Physiological and Molecular Genetic Studies on *Eucalyptus* and *Pinus* Mycorrhizas in Minas Gerais, Brazil*

Maria Catarina Megumi Kasuya[1], Arnaldo Chaer Borges[1], Elza Fernandes de Araújo[1], Marcos Rogério Tótola[1], Nairam Félix de Barros[2], Roberto Ferreira de Novais[2] and Júlio César Lima Neves[2]

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ABSTRACT

In Brazil, extensive areas of tropical soils are being reforested with exotic forest species, specially *Eucalyptus* (57%) and *Pinus* (42%) in a total of 1.4 million hectares. Mycorrhizal associations assume an important role in those areas where the soils are of low nutrient status or are degraded.

In eucalypt plantations, ectomycorrhizas are associated with sporocarps of *Pisolithus, Clavaria, Scleroderma bovista, S. uruguayensis and Telephora terrestris. A*rbuscular mycorrhizal fungi, *Acaulospora longula, A. denticulata, A. mellea, A. scrobiculata, Glomus microcarpum, G. clarum, G. fasciculatum, G. invermayanum, G. macrocarpum, Scutelospora pellucida* and *S. heterogama* have also been observed associated with *Eucalyptus*. Furthermore, superior plant growth was achieved by inoculation of *Eucalyptus* with ectomycorrhizal fungi. With tree age, ectomycorrhizas become dominant to the detriment of arbuscular mycorrhizas. However, presently, there is no inoculation program for eucalypts in commercial nurseries in Brazil

In *Pinus* plantations, that have been initiated with seedlings previously inoculated by soil/litter, the occurrence of basidiocarps of *Inocybe lanuginella, Scleroderma fuscum, Suillus granulatus, Suillus* sp., *Rhizopogon nigrescens, R. reaii* and *R.roseolus* have been recorded.

In vitro studies of ectomycorrizal fungi have shown differences among genera, species, and even among isolates from the same species, in relations to the parameters of cultural growth conditions, such as pH, temperature and the tolerance to aluminum. The fungi were characterized in relation to mycorrhizal formation in *Eucalyptus* and *Pinus* seedlings, competitivity and enzyme production, such as phosphatases and nitrogen assimilation enzymes.

Using sample of a latosol, soil highly leached and acidic and with low concentration of exchangeable K, Ca, and Mg, mycorrhizal formation by *Pisolithus* spp. in *E. grandis* was favored by addition of 8.6 $mg.kg^{-1}$ of P in soil. In *Pinus caribaea* the best *Pisolithus* sp. colonization was obtained by addition of 161.47 $mg.kg^{-1}$ of P, while *Rhizopogon nigrescens* required 108.43 $mg.kg^{-1}$ of P.

Production and regeneration of mycorrhizal fungi protoplasts of *Pisolithus* sp. and *Suillus granulatus* were established. Studies using RAPD, PCR-RFLP of rDNA and RFLP of mtDNA have shown differences among ectomycorrhizal fungi isolates and the need to review *Pisolithus* taxonomy. Currently, the use of molecular markers is under investigation.

1 INTRODUCTION

Eucalyptus and *Pinus* are genera of commercial forest trees with many desirable characteristics such as rapid growth, high cellulose production and resistance to diseases and adverse environmental conditions. Thus, their use in reforestation programs in the tropics has been increasing steadily. In Brazil, according to Associação Nacional dos Fabricantes de Papel e Celulose - ANFPC (1996), the section of paper and cellulose contribute with 1% of Gross National Product - GNP, corresponding to US$ 7.75 billion. The reforested area for that was, in 1996, 1.4 million hectares, 879000 ha with eucalypt, 449000 hm^2 with pine and 31,000 hm^2 with other species.

The mutualistic association of ectomycorrhizal fungi with plant roots is essential for plant health and nutrition. Artificial fungus inoculation can guarantee uniformity and production of healthy seedlings, even if natural inoculum is present.

Our group has tried to demonstrate the importance of mycorrhizal associations for eucalypt and pine under Brazilian field conditions, the use of se-

* All data are from the Laboratory of Mycorrhiza, Departament of Microbiology, Federal University of Viçosa, Brazil. Financial support: the Crawford Foundation (Australia), FINEP, CNPq and FAPEMIG (Brazil).

lected fungal isolates that can improve the performance of seedlings transplanted to field conditions, as well as to study the persistence of inoculated fungi over time. Here, some results we have obtained are presented.

2 ECOLOGICAL STUDIES

2.1 Occurrence and identification of mycorrhizal fungi

Some studies have been conducted to evaluate the occurrence of fruitbodies, arbuscular mycorrhizal spores and/or mycorrhizal formation under *Eucalyptus* and *Pinus* plantations. In pine forests, in Minas Gerais (Table 1), the dominance of some species, mainly Suillus, *Scleroderma* and *Rhizopogon* has been observed.

Table 1 Main species of ectomycorrhizal fungi found under Pine plantations in Minas Gerais

Pine species	Place	Ectomycorrhizal fungi species (identified by fruitbodies)
P. caribaea var. *hondurensis*	Viçosa (MG)	*Suillus granulatus* *Scleroderma fuscum* *Rhizopogon nigrescens* *R. reaii*
P. elliottii	Viçosa (MG)	*S. fuscum* *Rhizopogon nigrescens* *R. reaii* *Rhizopogon* sp.
P. glabra *P. patula*	Itabira (MG)	*Inocybe lanuginella* *R. roseolus*
P. elliottii	Itabira (MG)	*Rhizopogon* sp. *Scleroderma* sp.

Root samples taken over four season plantations of three hybrids of *Eucalyptus grandis* × *E. urophyla*, in three areas of Aracruz and São Mateus, in Espírito Santo State, showed that colonization presented high variation among areas and period of samples (Table 2). Ectomycorrhizas were greater in the summer, when the temperatures are higher. The colonization by arbuscular mycorrhizal fungi was highly variable with sites and sample seasons.

Fumigated soils samples, brought from the field of the above areas, were put in pots and used to grow hybrid eucalypt seedlings, in green house. The seedlings were inoculated as follow; *Pisolithus* sp. alone, *Glomus fasciculatum* alone, *Pisolithus* sp. and *G. fasciculatum*, and non-inoculated control. Clone 3918 showed greatest colonization by *Pisolithus* sp. and AM, but in only one of the studied sites (Table 3). The existence of the difference on the fungi compatibility and/or susceptibility by eucalypt hybrids, *E. grandis* x *E. urophylla*, to colonization by *Pisolithus* sp. and *G. fasciculatum*, is consistent with the hypothesis that soil and clone can conditioner the intensity of colonization and reflect the effect on the growth and plant nutrition.

Table 2 Percentage of fine root colonization by ecto and arbuscular mycorrhizal fungi in 3 *Eucalyptus* clones, at four sampling times, in three areas of Espírito Santo State.

Site	Clone	Sampling 5/1992	8/1992	11/1993	2/1993	Mean
		Ectomycorrhiza (%)				
077-05	3918	15.0ABb	11.3 Ab	30.3 Aa	20.0 Aab	19.2 A
	4172	7.0 Bc	8.7 Abc	27.0 Aa	19.0 Aab	15.4 A
	10400	22.3 Aa	15.3 Aa	19.0 Aa	16.7 Aa	18.3 A
	Mean	14.8 b	11.8 b	25.4 a	18.6 ab	17.6
061-12	3918	17.7 Aab	11.3Aab	10.3 Bb	22.7 Aa	15.5 A
	4172	11.3 A b	19.8Aab	24.7 Aa	18.0 Aab	18.5 A
	10400	18.8 Aa	14.3 Aa	13.0 ABa	20.3 Aa	16.6 A
	Mean	15.9 a	15.2 a	16.0 a	20.3 a	16.9
023-03	3918	3.0 Ab	1.3 Ab	18.7 Aa	12.7 Aa	8.9 A
	4172	6.0 Ab	3.0 Ab	1.7 Bb	24.1 Aa	8.7 A
	10400	3.0 Ab	2.7 Aa	4.3 Ba	7.7 Ba	4.4 A
	Mean	4.0 bc	2.3 c	8.2 b	14.8 a	7.3
		Arbuscular Mycorrhiza (%)				
077-05	3918	7.7 Aab	4.0 Aab	1.7 Ab	9.5 Aa	5.7 A
	4172	17.7 Aa	4.7 Ab	1.0 Ab	6.3 Ab	7.4 A
	10400	11.7 Aa	3.0 Aab	1.3 Ab	7.0 Aab	5.8 A
	Mean	12.3 a	3.9 b	1.3 c	7.6 ab	6.3
061-12	3918	9.5 Ab	10.3 Ab	22.7 Aa	7.0 Ab	12.4 A
	4172	4.7 Aa	8.8 Aa	7.7 Ba	6.9 Aa	7.0 A
	10400	12.0 Aa	8.3 Aa	4.0 Ba	5.7 Aa	7.5 A
	Mean	8.7 a	9.2 Aa	11.4 a	6.5 a	9.0
023-03	3918	6.7 Aab	2.0 Bb	9.3 Aa	7.7 Aab	6.4 A
	4172	6.3 Ab	9.3 Aab	3.3 Ab	20.3 Aa	9.8 A
	10400	9.3 Aa	16.3 Aa	2.7 Ab	14.4 Aa	10.7 A
	Mean	7.4 ab	9.2 ab	5.1 b	14.1 a	9.0

1. Data transformed by arcsine $(x/100)^{1/2}$ * 180, where x=percentage of colonized roots. 2. Mean of Clones, within each area and sampling date of evaluation, followed by the same small letters, do not differ at 5%, by Newman Kels test. 3. Mean of collection time, within area and clone, followed by the same capital letters, do not differ at 5%, by Newman Kels test.

In *E. camaldulensis* plantations, in Paraopeba, Bocaiúva and João Pinheiro, in Minas Gerais State, the occurrence of ecto and arbuscular mycorrhizal fungi were also evaluated. The age of eucalypt plantations varied from 1 to 8 years old. *Pisolithus* was observed in all three places and *Scleroderma* sp. in Bocaiúva and João Pinheiro. The occurrence of *Pisolithus* was more frequent in João Pinheiro (Pat), which soil is a Dark Red Latosol, argyle texture, while in Bocaiúva (Boc) and Paraopeba (Ita), where soils are Yellowish Red Latosol, media texture, the *Scleroderma* was more frequent. This observation suggests that there is an ecological adaptation of ectomycorrhizal fungi to the different soils. Basidiocarps of *Telephora terrestris* were also observed in some areas of Bocaiúva, but no connection between fungus hyphae and roots were observed. So, it was not possible to confirm mycorrhizal association between eucalypt and *T. terrestris* at that time.

Arbuscular mycorrhizal fungi were identified by spores characteristics (Table 4). Colonization was greater in dry season and number of spores was greater in wet season.

Table 3 Percentage of fine root length colonized by *Pisolithus* sp. (ECM) or *Glomus fasciculatum* (AM) and number of AM fungi spores (ESP), according to the soil, clone and fungal inoculation. Plants were grown for 120 days in green house. Inoculum was provided as mycelia. NI = non-inoculated control.

Soil[2]	Clone	Inoculation treatment	Colonization[1] ECM	AM	spores/ g of soil
A	3918	ECT	11.6	0.8	29.4
		AM	4.2	0.4	28.1
		E + AM	9.4	6.4	24.2
		NI	2.4	2.7	25.6
		Avarage[3]	6.9A	4.8A	26.8A
	4172	ECT	3.9	0.4	19.7
		AM	1.3	5.3	25.4
		E + AM	2.6	2.9	24.0
		NI	4.1	1.6	28.8
		Avarage[3]	3.0B	2.6B	24.5B
	10400	ECT	6.4	2.5	28.2
		AM	2.1	6.3	28.6
		E + AM	6.3	2.4	25.5
		NI	7.3	2.4	27.0
		Avarage[3]	5.5B	3.4B	27.3A
B	3918	ECT	3.4	2.8	23.2
		AM	0.8	2.8	25.2
		E + AM	1.6	2.0	25.0
		NI	1.1	0.6	20.4
		Avarage[3]	1.7A	2.0A	24.3A
	4172	ECT	1.5	0.3	26.6
		AM	0.8	2.4	22.8
		E + AM	2.9	1.9	23.6
		NI	3.0	1.9	25.4
		Avarage[3]	2.0A	1.6A	24.6A
	10400	ECT	1.2	0.1	25.5
		AM	1.5	2.5	26.5
		E + AM	1.1	2.4	21.0
		NI	0.3	0.2	24.3
		Avarage[3]	1.0A	1.3A	24.3A

1. The data were transformed to Arcsine $(x/100)^{1/2} * 180$, where x = percentage of colonized roots or number of AM spores per gram of soil. 2. The soils A and B were from areas 077-05 and 061-1, respectively; 3/ media of clones, within each soil, followed by the same letter, did not differ at 5%, by the de Newman Kels test.

Table 4 Arbuscular mycorrhizal fungal spores found under *Eucalyptus camaldulensis* plantations, in Paraopeba, Bocaiúva and João Pinheiro, in Minas Gerais States

Site	Species of Arbuscular Mycorrhizal Fungi
Ita 1/86-35	*Acaulospora longula, Glomus macrocarpum, Scutellospora pellucida*
Ita 1/87-38	*Acaulospora longula*
Ita 2/88-37	*Acaulospora longula, Glomus macrocarpum, Glomus microcarpum, Scutellospora pellucida, Acaulospora* sp., *Acaulospora trappei*
Pat 5/83-30	*Acaulospora longula, Glomus* sp.
Pat 3/84-05	*Acaulospora longula, Scutellospora pellucida and Acaulospora* sp.
Pat 4/85-06	*Acaulospora longula, Glomus* sp., *Glomus microcarpum*
Pat 6/87-01	*Acaulospora longula, Glomus* sp., *Acaulospora* sp.
Pat 6/88-31	*Glomus invermayanum*
Boc 1/84-08	*Acaulospora mellea, Acaulospora* sp., *Glomus invermayanun, Scutellospora heterogama*
Boc 1/85-20	*Acaulospora* sp., *Acaulospora denticulata, Glomus invermayanun*
Boc 1/86-25	*Acaulospora longula, Acaulospora* sp.
Boc 3/87-77	*Acaulospora denticulata, Glomus invermayanum, Glomus microcarpum*
Boc 3/88-78	*Glomus invermayanum, Scutellospora pellucida.*

Percentage of root colonization by AM fungi increased in all of the three regions, during the dry season, while the number of spores increased during the wet season. The ectomycorrhizal association did not present a defined variation during sampled period.

2.2 Succession of ecto-arbuscular mycorrhizas

The occurrence of arbuscular mycorrhizas (AM) and ectomycorrhizas (Ecto) in the same root system was observed when *Eucalyptus urophylla*, *E. citriodora*, *E. grandis*, *E. cloeziana* or *E. camaldulensis* were simultaneously inoculated with *Glomus etunicatum* and *Pisolithus* sp. The succession between the two fungi was observed. In general, the increase in ectomycorrhizal colonization was followed by a decrease in AM (Figure 1). *Pisolithus* sp. was favored by simultaneous inoculation with *G. etunicatum*, and the positive effect occurred up to 60 days after inoculation. After 120 days, root colonization by *G. etunicatum* decreased in the presence of *Pisolithus* sp. The proportions of AM and *Pisolithus* varied with time. The total percentage of roots colonized approached the maximum and then became constant by 60 days. This suggests that there is a limit to the amount of fungal biomass that a *Eucalyptus* root system will support. It is likely that there is a competition for infection sites and for carbohydrates between the two types of mycorrhizal fungi. However, the maximum percentage of mycorrhizal colonization varied with *Eucalyptus* species, and this was a consequence of the different root dry mass, root system growth rates, and symbiont compatibility. The