SEÇÃO III - BIOLOGIA DO SOLO

COMPATIBILITY AND ECTOMYCORRHIZA FORMATION AMONG *Pisolithus* ISOLATES AND *Eucalyptus* SPP[(1)]

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SUMMARY

Twenty-nine isolates of the ectomycorrhiza fungus *Pisolithus* sp. from different geographical and host origins were tested for their ability to form ectomycorrhizae on *Eucalyptus grandis* and *E. urophylla* seedlings under greenhouse conditions. The ectomycorrhiza-forming capacity of isolates varied greatly from one eucalypt species to the other. All isolates from *Eucalyptus,* nine from *Pinus* spp. and two isolates from unknown hosts formed mycorrhizae with *E. grandis* and *E. urophylla*. Root colonization rates varied from 0 to 5.2 % for all *Pinus* isolates and those from unknown hosts. Colonization rates for these isolates were lower than those observed for *Eucalyptus* isolates (0.8 to 89.4 %). Three isolates from unknown hosts formed mycorrhizae with neither *Eucalyptus* species. The main characteristic for distinguishing *Pinus* from *Eucalyptus* isolates was mantle color. These data corroborate previous results obtained in our laboratory indicating that the isolates tested represent at least two distinct different species within the genus *Pisolithus*.

Index terms: ectomycorrhiza colonization, *Eucalyptus grandis, Eucalyptus urophylla,* morphological characterization, sclerotia.

RESUMO: *COMPATIBILIDADE E FORMAÇÃO DE ECTOMICORRIZAS ENTRE ISOLADOS DE Pisolithus E Eucalyptus SPP*

Vinte e nove isolados do fungo ectomicorrízico Pisolithus sp., de diferentes regiões geográficas e hospedeiros, foram testados quanto à capacidade de formar ectomicorrizas

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em plântulas de Eucalyptus grandis *e* E. urophylla *sob condições de casa de vegetação. Os isolados apresentaram grande variação na capacidade de formar ectomicorrizas com ambas as espécies de eucalipto. Todos os isolados originalmente obtidos de Eucalyptus, 9 originalmente obtidos de Pinus spp. e dois isolados de hospedeiros desconhecidos formaram micorrizas com* E. grandis *e* E. urophylla. *A taxa de colonização radicular dos isolados originalmente obtidos de Pinus e dos isolados de hospedeiros desconhecidos variou de 0 a 5,2 %. A taxa de colonização para esses isolados foi menor do que as obtidas para os isolados originalmente obtidos de* Eucalyptus *(0,8 a 89,4 %). Três isolados obtidos de hospedeiros desconhecidos não formaram micorrizas com nenhuma das espécies de* Eucalyptus. *A característica mais marcante para distinção das ectomicorrizas formadas pelos isolados de Pinus e pelo de Eucalyptus foi a cor do manto fúngico. Esses dados corroboram resultados prévios obtidos em laboratório, indicando que os isolados estudados devem representar ao menos duas espécies distintas dentro do gênero Pisolithus.*

Termos de indexação: colonização ectomicorrízica, Eucalyptus grandis, Eucalyptus urophylla, *caracterização morfológica, esclerócio.*

INTRODUCTION

The ectomycorrhizal genus *Pisolithus* is widespread worldwide and associates with several tree genera. It is one of the most commonly used fungi for inoculation in controlled mycorrhization programs (Marx et al., 1982; Burgess et al., 1995). This fungus has been used successfully to improve the growth of eucalypt (Burguess et al., 1994; Garbaye et al., 1988; Brundrett, 1996) and pine plantations (Marx et al., 1977; Delwaulle et al., 1982). It is also known to have a broad host range (Molina et al., 1992), although literature reports a large variation among isolates regarding mycorrhizal colonization and plant growth stimulation rate (Burgess et al., 1994; Oliveira et al., 1994; Rosado et al., 1994; Burgess et al., 1995; Kropp, 1997; Costa, 2002).

Early studies by Malajczuk et al. (1982) showed few differences in the ectomycorrhiza-forming ability of several eucalypt species from geographically distinct areas with several fungi, including *Pisolithus*, thus indicating no evidence for *Eucalyptus* fungus-specificity. However, this observation was not confirmed since *Eucalyptus* species form little or no association with *P. tinctorius* strains originally isolated from pine (Burgess et al., 1994; Malajczuk et al., 1990; Oliveira et al., 1994).

Variations in sexual compatibility, diversity in basidiospore spine morphology, heterogeneity of phenotypic traits, such as polypeptide expression among isolates, culture characteristics, and the morphology of basidiospores and basidiomes suggest the occurrence of biological species within the *Pisolithus* complex (Kope and Fortin, 1990; Burgess et al., 1995; Carvalho et al., 1997; Sales, 2001). Genetic variability at the molecular level was also found in *Pisolithus* isolates from different geographic locations and associated hosts by molecular tools such as the RAPD-PCR analysis (Junghans et al., 1998), rDNA PCR-RFLP (Gomes et al., 1999), and alignment of rDNA ITS sequences (Gomes et al., 2000). All these studies suggested and demonstrated that the taxonomy of this genus was unclear and ought to be revised.

The area in Brazil currently aforested with *Eucalyptus* and *Pinus* species cover approximately 4.8 million ha (SBS, 2002). This represents an enormous potential for the development of large scale inoculation procedures in commercial nurseries. The adoption of routine seedling mycorrhization programs requires the previous selection of fungal isolates able to promote the formation of ectomycorrhizae with the host species (Oliveira et al., 1994; Brundrett et al., 1996). The extent of mycorrhizal colonization can be used as an indicator of the isolate's aggressiveness and, consequently, of its potential to promote tree growth, since it correlates positively to growth stimulation under greenhouse conditions (Burgess et al., 1994).

The objectives of this research were to study the compatibility between two eucalypts species and *Pisolithus* sp. isolates and to characterize the resulting ectomycorrhizae.

MATERIAL AND METHODS

Eucalyptus species and *Pisolithus* sp. isolates

Twenty-nine isolates of the fungus *Pisolithus* sp. from the ectomycorrhizal fungal collection of the Departamento de Microbiologia, Universidade Federal de Viçosa, Viçosa, State of Minas Gerais, Brazil, maintained on Melin-Norkrans medium (MNM) (Marx, 1969) at 5 °C were tested in this experiment (Table 1). The isolates had been obtained from different geographical regions throughout the world. The Brazilian isolates were

originally collected from areas in the southern, southeastern, and western regions of Brazil. *Eucalyptus grandis* and *E. urophylla* are widely used in reforestation programs in Brazil and have been selected for this study due to their commercial importance in the production of coal, timber, and pulp for the paper industry.

Seedling production

Eucalyptus seeds were superficially disinfected in a laminar flux hood by immersion in 20 % H_2O_2 for 6 min followed by 5 rinses with sterile distilled water. The disinfected seeds were germinated at room temperature in sterile acid-washed sand in the laboratory. After one week they were transferred to a greenhouse, where they received 15 mL diluted Clark's solution at ¼ strength and 0.2 mg L^{-1} P twice a week. The temperatures varied from 25 to 30 °C. Uniformly sized and undamaged seeds from a single seed lot were selected to minimize variability.

Inoculum production

Three agar discs containing mycelium were inoculated onto Petri dishes containing 25 mL modified MNM (Marx, 1969). The cultures were incubated for 30 days at 28 °C. From the resulting colonies 9 mm agar discs were cut off from the edge of actively growing colonies. The agar discs were tested for their viability by incubating at 28 °C on 25 mL MMN. As soon as mycelial growth was clearly distinguishable (4 days) the discs were used to inoculate the seedlings.

Table 1. Number, code, host, geographical origin and root colonization of *Pisolithus* sp. isolates inoculated onto *E. grandis* and *E. urophylla*

Isolate number	Isolate code	Host	Origin(1)	*E. grandis*	*E. urophylla*
				— root colonization, % —	
1	PT França	unknown	France	0.6 Af	0.6 Ae
2	PF	unknown	France	0.0 Af	0.0 Ae
3	PT 571	unknown	-	0.0 Af	0.0 Ae
4	PT 1 USA	unknown	USA	0.0 Af	0.0 Ae
5	PT 5 USA	unknown	USA	0.4 Af	0.8 Ae
6	PT 301	*Pinus* sp.	DF - Brazil	4.3 Af	0.6 Ae
7	PT 306	*Pinus* sp.	USA	3.0 Af	0.2 Ae
8	PT 306 (94R)	*Pinus* sp.	USA	3.0 Af	2.4 Ae
9	PT 303	*P. taeda*	USA	nd**	nd
10	PT taeda	*P. taeda*	-	1.8 Af	5.2 Ae
11	PT taeda R	*P. taeda*	-	0.6 Af	0.6 Ae
12	PT 185	*P. taeda*	USA	2.8 Af	2.2 Ae
13	PT 185 R	*P. taeda*	USA	2.5 Af	3.0 Ae
14	PT 185 (6,0R)	*P. caribaea*	USA	1.2 Af	0.6 Ae
15	PT 185 (6,0R)R	*P. caribaea*	USA	4.0 Af	1.6 Ae
16	PT 185 (6,5R)	*P. caribaea*	USA	nd	nd
17	PT 26	*Eucalyptus* sp.	SC-Brazil	13.4 Ae	3.4 Ae
18	PT SILV 1	*Eucalyptus* sp.	MG-Brazil	24.6 Ad	19.8 Ad
19	PT 145	*Eucalyptus* sp.	SC-Brazil	22.8 Bd	80.0 Aa
20	PT 90A	*Eucalyptus* sp.	MG-Brazil	7.8 Af	11.8 Ad
21	PT 90B	*Eucalyptus* sp.	MG-Brazil	1.2 Af	0.8 Ae
22	PT 1 MG	*Eucalyptus* sp.	MG-Brazil	32.2 Bd	70.4 Ab
23	IS 83	*Eucalyptus* sp.	MG-Brazil	63.2 Ab	38.6 Bc
24	RV 82	*Eucalyptus* sp.	MG-Brazil	78.0 Aa	70.6 Ab
25	RS 20	*Eucalyptus* sp.	RS-Brazil	58.0 Ab	66.2 Ab
26	RS 23	*Eucalyptus* sp.	RS-Brazil	48.5 Bc	83.8 Aa
27	RS 24	*Eucalyptus* sp.	RS-Brazil	89.4 Aa	26.4 Bd
28	RS 26	*Eucalyptus* sp.	RS-Brazil	64.6 Ab	66.4 Ab
29	RS 27	*Eucalyptus* sp.	RS-Brazil	77.6 Aa	76.0 Aa
Control	-	-	-	0.0 Af	0.0 Ae

The means followed by the same capital letter in a row are not significantly different by Tukey's test at 5 % probability.
The means followed by the same lower case letter in a column are not significantly different by the Scott-Knott test at 5 % probability.
(1) SC = Santa Catarina State; MG = Minas Gerais State and RS = Rio Grande do Sul State.
** nd = not determined, despite the presence of a low amount of ectomycorrhizae.

Seedling inoculation

Forty five-day old seedlings at a uniform stage of development were selected and transplanted to 170 mL containers filled with a 1:1 (v:v) mixture of sand and vermiculite. This mixture had been previously autoclaved twice at 121 °C and 1 atm for 20 min. At the moment of transplanting, the root system of each seedling was inoculated with three agar discs obtained as described above.

Seedling growth and evaluation of ectomycorrhiza colonization

Seedlings were maintained in a greenhouse and were irrigated weekly with 15 mL of a ¼ diluted Clark solution (Clark, 1975) with 0.2 mg L^{-1} P per container. Seventy days after transplanting the inoculation was reinforced by the application of 10 mL $plant^{-1}$ of a suspension containing fragmented mycelium. The suspension was prepared in two erlenmeyer flasks filled with 100 mL MMN solution inoculated with three agar discs containing mycelium. After a 25-day incubation at 28 °C the mycelium was collected, washed and fragmented in a blender with 150 mL autoclaved distilled water, applying 5 pulses of 1 second each.

The experiment was installed in a completely randomized design with five replicates. Forty days after the reinforcement inoculation, the root system with the intact substrate was removed from the containers for a visual evaluation of the presence of ectomycorrhizae and fungal mycelium. The root system was washed and samples were taken for evaluation of mycorrhizal colonization as described by Brundrett et al. (1996).

Morphological characterization

Macroscopic characteristics and microscopic details of the ectomycorrhiza samples were observed using stereo and light microscopes according to the procedures described by Ingleby et al. (1990) and Agerer (1992). Ectomycorrhizae was hand-sectioned to confirm the presence of the Hartig net. The ectomycorrhizae formed were evaluated for overall morphology and color, hyphal dimensions (length and diameter), presence of septa and clamp connections, emanating hyphae, rhizomorphs, sclerotia, and mantle structures. The colors were recorded under bright tungsten illumination and designated according to Munsell (1990).

RESULTS

Ectomycorrhiza formation

Twenty-six out of 29 isolates tested were able to form ectomycorrhizae with both *Eucalyptus* species. Three isolates did not form any ectomycorrhizae with meither species (Table 1). The formation of ectomycorrhizae with both *Eucalyptus* species was also observed for the isolates PT 303 and PT 185 (6.5R). However, the low amount of ectomycorrhizae formed was not detected under the stereomicroscope during colonization evaluation (Table 1).

The root colonization rates of all isolates from *Pinus* and unknown hosts varied from 0 to 5.2 %. No significant differences in root colonization were observed between *Pinus* isolates and those from unknown hosts in association with the eucalypt species tested. The colonization rates for these isolates were lower than those obtained for the *Eucalyptus* isolates. For the latter, colonization rates varied from 0.8 to 89.4 % (Table 1).

The *Eucalyptus* isolates presented significantly different colonization rates when associated with either *E. grandis* or *E. urophylla*. For the former species, the highest values were obtained with isolate RS 24, followed by isolates RV 82, RS 27, RS 26, IS 83, RS 20, RS 23, PT 1 MG, PT SILV 1, PT 145, PT 26, PT 90A, and PT 90B. For *E. urophylla*, the highest colonization rates were obtained with the isolates RS 23, followed by isolates PT 145, RS 27, RV 82, PT 1 MG, RS 26, RS 20, IS 83, RS 24, PT SILV 1, PT 90A, PT 26, and PT 90B.

Most isolates presented colonization rates that were roughly the same for both *E. grandis* and *E. urophylla* (Table 1). However, colonization rates for the isolates PT 145, PT 1 MG, and RS 23 were higher in *E. urophylla* than in *E. grandis*. An inverse colonization pattern was observed for the isolates IS 83 and RS 24.

Morphological characterization

Some of the morphological characteristics varied according to the host species from which the isolate was originally obtained rather than in relation to its geographical origin. *Eucalyptus grandis* ectomycorrhizae are very similar to those of *E. urophylla* regarding the length of the main axis and the basal and apical diameter (Tables 2 and 3). All ectomycorrhizae presented a monopodial pyramidal branching pattern. Ectomycorrhizae formed by the *Pinus* isolates were predominantly light-brown, HUE 10 YR 6/3, and those formed by the *Eucalyptus* isolates were yellow, HUE 5Y 8/8 (Munsell Color Charts, 1990). All ectomycorrhizae formed by eucalypt isolates had pletenchymatous mantles and verrucose hyphae with thickened cell walls, while the ectomycorrhizae mantle formed by *Pinus* isolates had a loosely arranged surface. Rhizomorphs were observed in all host and fungal isolate combinations in which the formation of ectomycorrhizae was assessed. Rhizomorphs presented smooth margins, with a constriction at the point of connection to the mantle. Numerous clamp connections were observed in the hyphae constituting the mantle as well as in

Table 2. Macroscopic and microscopic characteristics of ectomycorrhizae formed on *Eucalyptus grandis* by *Pisolithus* sp. isolates, 110d after cultivation under greenhouse conditions

Isolate code	Macroscopic characteristics				Microscopic characteristics									
	Ectomycorrhiza				Emanating hyphae				Rhiz	SC	Mantle			
	Color	Main axis		Shape	Freq	Len	Diam	CC			Edge	IPV	Hyphae	
		Len	Diam										Diam	Freq
		cm	µm			—µm—							µm	
1	Pb	0.15	200	MP	++	Lo	2.9	+	+	-	L	NP	3.0	++
5	Ye	0.31	248	MP	++	Lo	3.3	+	+	-	L	NP	3.1	++
6	Pb	0.18	208	MP	++	Lo	3.4	+	+	+	L	NP	3.2	++
7	Pb	0.12	208	MP	++	Lo	3.0	+	+	-	L	NP	3.0	++
8	Pb	0.17	217	MP	++	Lo	3.1	+	+	+	L	NP	3.0	++
10	Pb	0.13	223	MP	++	Lo	3.6	+	+	+	L	NP	3.4	++
11	Pb	0.20	200	MP	++	Lo	3.1	+	+	-	L	NP	3.0	++
12	Pb	0.18	207	MP	++	Lo	2.6	+	+	-	L	NP	2.5	++
13	Pb	0.23	205	MP	++	Lo	3.0	+	+	+	L	NP	2.9	++
14	Pb	0.25	193	MP	++	Lo	3.1	+	+	-	L	NP	3.3	++
15	Pb	0.21	214	MP	++	Lo	3.0	+	+	-	L	NP	3.0	++
16	Pb	0.30	227	MP	++	Lo	3.1	+	+	-	L	NP	3.0	++
17	Ye	0.11	161	MP	++	Lo	3.1	+	+	-	C	NP	3.0	++
18	Ye	0.20	431	MP	++	Lo	3.1	+	+	-	C	NP	3.0	++
19	Ye	0.27	230	MP	++	Lo	4.0	+	+	-	C	NP	3.2	++
20	Ye	0.21	240	MP	++	Lo	3.2	+	+	-	C	NP	3.0	++
21	Ye	0.10	188	MP	++	Lo	3.2	+	+	-	C	NP	3.1	++
22	Ye	0.10	214	MP	++	Lo	3.2	+	+	-	C	NP	3.3	++
23	Ye	0.25	210	MP	++	Lo	3.3	+	+	-	C	NP	3.4	++
24	Ye	0.23	250	MP	++	Lo	2.6	+	+	-	C	NP	3.1	++
25	Ye	0.28	213	MP	++	Lo	3.1	+	+	-	C	NP	3.0	++
26	Ye	0.25	185	MP	++	Lo	2.8	+	+	-	C	NP	3.1	++
27	Ye	0.19	279	MP	++	Lo	2.9	+	+	-	C	NP	3.0	++
28	Ye	0.20	218	MP	++	Lo	3.1	+	+	-	C	NP	3.0	++
29	Ye	0.21	236	MP	++	Lo	3.1	+	+	-	C	NP	3.2	++

Pb = pale brown HUE 10YR 6/3; Ye = Yellow HUE 5Y 8/8; Rhiz = rhizomorph; CC = clamp connection; SC = sclerotia; + presence and – absence; Len = length; Diam = diameter; C = compact; L = loosely formed; IPV = inner plan view: NP = net prosenchyma; Freq = frequence; MP = monopodial pyramidal; ++ = frequent but not abundant; Lo= long: majority of emanating hyphae are 100 mm. Terminology of mantle edge and inner plan view is based on Ingleby et al. (1990). Terminology of color is based on Munsell (1990).

those emanating from the surface of the ectomycorrhizae. The isolates PT taeda, PT 185R, and PT 306 (94R) in association with *E. grandis*, formed sclerotia in the host's rhizosphere. The surface of the sclerotia consisted of thick-walled cells, forming an irregular synenchyma. The other isolates did not form such structures.

DISCUSSION

We have observed a large variation among *Pisolithus* isolates from different geographical locations and natural hosts in the ability to form mycorrhizae with *E. grandis* and *E. urophylla* seedlings under controlled conditions. The *Pisolithus* isolates were either incompatible with the host plants or compatible forming typical ectomycorrhizae. The incompatible isolates established only a superficial, loose association of the mycelia with the host roots. Such variation has been frequently reported in literature and involves several fungal species and host plants (Burgess et al., 1994; Oliveira et al., 1994; Rosado et al., 1994; Burgess et al., 1995; Kropp, 1997; Costa, 2002).

Isolate aggressiveness can be described as the rate of mycorrhizal development (Burgess et al., 1994). In this study, we could divide the isolates into two groups: group I, containing the *Eucalyptus* isolates, and group II, containing the *Pinus* isolates. Isolates from group II were poor colonizers of

Table 3. Macroscopic and microscopic characteristics of ectomycorrhizae formed on *Eucalyptus urophylla* by *Pisolithus* sp. isolates, 110d after cultivation under greenhouse conditions

Isolate code	Macroscopic characteristics				Microscopic characteristics									
	Ectomycorrhiza				Emanating hyphae				Rhiz	SC	Mantle			
	Color	Main axis		Shape	Freq	Len	Diam	CC			Edge	IPV	Hyphae	
		Len	Diam										Diam	Freq
		cm	µm			— µm —							µm	
1	Pb	0.14	215	MP	++	Lo	2.9	+	+	-	L	NP	3.0	++
5	Ye	0.16	240	MP	++	Lo	3.0	+	+	-	L	NP	3.1	++
6	Pb	0.21	227	MP	++	Lo	2.4	+	+	-	L	NP	2.9	++
7	Pb	0.12	113	MP	++	Lo	3.0	+	+	-	L	NP	3.0	++
8	Pb	0.18	200	MP	++	Lo	3.1	+	+	-	L	NP	3.0	++
10	Pb	0.21	176	MP	++	Lo	3.4	+	+	-	L	NP	3.2	++
11	Pb	0.20	210	MP	++	Lo	3.0	+	+	-	L	NP	2.9	++
12	Pb	0.23	225	MP	++	Lo	3.1	+	+	-	L	NP	3.1	++
13	Pb	0.19	253	MP	++	Lo	3.1	+	+	-	L	NP	3.0	++
14	Pb	0.11	187	MP	++	Lo	3.3	+	+	-	L	NP	2.4	++
15	Pb	0.21	235	MP	++	Lo	3.6	+	+	-	L	NP	3.0	++
16	Pb	0.09	147	MP	++	Lo	3.5	+	+	-	L	NP	3.0	++
17	Ye	0.15	181	MP	++	Lo	3.1	+	+	-	C	NP	3.0	++
18	Ye	0.14	237	MP	++	Lo	3.2	+	+	-	C	NP	3.2	++
19	Ye	0.20	195	MP	++	Lo	3.0	+	+	-	C	NP	3.1	++
20	Ye	0.15	213	MP	++	Lo	3.0	+	+	-	C	NP	3.0	++
21	Ye	0.12	218	MP	++	Lo	3.2	+	+	-	C	NP	3.1	++
22	Ye	0.24	212	MP	++	Lo	3.2	+	+	-	C	NP	3.1	++
23	Ye	0.17	235	MP	++	Lo	3.3	+	+	-	C	NP	3.2	++
24	Ye	0.15	227	MP	++	Lo	3.4	+	+	-	C	NP	3.0	++
25	Ye	0.16	213	MP	++	Lo	3.2	+	+	-	C	NP	3.0	++
26	Ye	0.15	167	MP	++	Lo	3.2	+	+	-	C	NP	3.1	++
27	Ye	0.17	235	MP	++	Lo	3.1	+	+	-	C	NP	3.8	++
28	Ye	0.15	218	MP	++	Lo	3.6	+	+	-	C	NP	3.9	++
29	Ye	0.18	222	MP	++	Lo	3.0	+	+	-	C	NP	3.0	++

Pb = pale brown HUE 10YR 6/3; Ye = Yellow HUE 5Y 8/8; Rhiz = rhizomorph; CC = clamp connection; SC = sclerotia; + presence and – absence; Len = length; Diam = diameter; C = compact; L = loosely formed; IPV = inner plan view: NP = net prosenchyma; Freq = frequence; MP = monopodial pyramidal; ++ = frequent but not abundant; Lo = long: majority of emanating hyphae are 100 mm. Terminology of mantle edge and inner plan view is based on Ingleby et al. (1990). Terminology of color is based on Munsell (1990).

eucalypt or did not form any ectomycorrhizae at all. The few ectomycorrhizae formed presented a loose mantle with incomplete development. These two groups coincide with the geographical origin and host plant of the isolates. Southern hemisphere isolates constitute the isolates from group I and Northern hemisphere isolates constitute the isolates from group II. This grouping agrees with previous studies based on molecular analysis using RAPD-PCR (Junghans et al., 1998), rDNA PCR-RFLP analyses (Gomes et al., 1999), and nucleotide sequence of ITS from rDNA genes (Gomes et al., 2000). This strongly suggests that the tested isolates represent at least two distinct species within *Pisolithus*. The isolates PT 145, PT 1 MG, RS 23, IS 83, and RS 24 from group I displayed significant differences in the capacity to form ectomycorrhizas with both eucalypt species, suggesting a degree of preference to a given host species (Cairney, 1999) which may be a reflection of interspecific variation amongst isolates. However, whether isolates in group I represent more than one species is yet to be determined since no classical taxonomic study with these isolates and their corresponding basidiomes have been concluded so far. Alternatively, the isolates may belong to the same *Pisolithus* species and possess varied ability to colonize *Eucalyptus* spp. or even different host genotypes of the same *Eucalyptus* species (Cairney, 1999). Both possibilities indicate the importance of a previous selection of isolates for inoculum production taking into account the host plant and fungal genotypes.

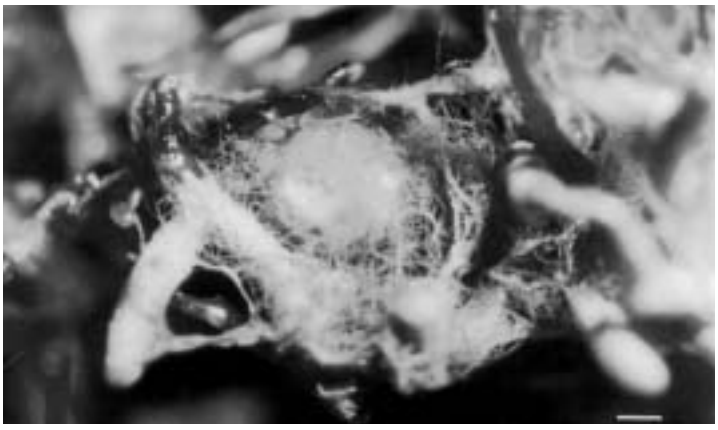

Figure 1. Scletrotium formed in the rhizosphere of *Eucalyptus grandis* seedling inoculated with the isolate PT 306 (94R) of *Pisolithus* sp. Scale bar = 200 µm.

The main contrasting morphological traits in the ectomycorrhizae formed by isolates from group I and group II were mantle color, according to the Munsell Color charts, and mantle structure. The latter character is indicative of isolate compatibility (Burgess et al., 1994; Malajczuck et al., 1984). In general, compatible isolates induce the formation of thick, compact mantles around the host rootlets.

Other signs of isolate-host incompatibility such as the deposition of tannins in the root tissues, lysis of hyphae and of epidermal and cortical cells (Malajczuck et al., 1984) were not evaluated in our study. However, roots in contact with the *Pinus* isolates frequently had a brown color, indicating an increased synthesis of phenolics due to isolate incompatibility (Baptista et al., 1999).

The *Pinus* isolates Pt taeda, Pt 185 R and Pt 306 (94R) in association with *E. grandis* formed sclerotia in the host's rhizosphere (Table 3). Such structures are generally produced under stress conditions and may remain dormant for long periods and then germinate when favorable conditions return. Sclerotium formation by *Pisolithus tinctorius* has already been reported in association with pine seedlings (Grenville et al., 1985a,b) but to our knowledge this is the first report of sclerotium formation by *Pisolithus* sp. in association with *Eucalyptus* species. Thus, sclerotia production may be entirely dependent on the isolate and not the host species. Additionally, the sclerotia production by some *Pisolithus* sp. isolates from *Pinus* in association with a non-original host (*Eucalyptus*) may indicate the existence of unfavorable conditions in the rhizosphere leading to the production of such structures and probably represent one more sign of fungus - plant incompatibility.

Further studies employing classical and molecular taxonomic tools will be necessary to indicate the extent and degree of *Pisolithus* diversity in Brazilian eucalypt plantations and the compatibility phenomena between isolates and host plants.

CONCLUSIONS

1. No evidence for specificity at the species level was observed in this study. However, the compatibility range observed among isolates reinforces the need to previously select the most compatible isolate within a fungal species to promote ectomycorrhiza formation with a given host tree species.

2. Despite the wide range in host compatibility observed for *Pinus* and *Eucalyptus* isolates, the morphological traits of ectomycorrhizae did not differ significantly among the *Pisolithus* isolates under study. Since the color of ectomycorrhizae reflects that of the mycelia, the mantle structure was the main morphological indicator of compatibility between the isolates and the host plants.

3. The establishment of two distinct compatibility groups among all tested strains, based on the fungal capacity to form ectomycorrhizae with *E. grandis* and *E. urophylla* corroborates the previous studies with molecular markers suggesting that *Pisolithus* isolates may belong to at least two distinct species.

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