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Mycorrhizal fungi in *Pinus ponderosa* introduced in Central Patagonia (Argentina)

by

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With 1 figure and 3 tables

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Abstract: A study to survey the ectomycorrhizal fungi found in ponderosa pine plantations and bare-root nurseries in Central Patagonia (Argentina) was conducted. Fourteen plantations and eight nurseries were sampled for hypogeous and epigeous fungi for three years. *Rhizopogon ellenae, Tricholoma muricatum, Cortinarius* sp. *subg. Telamonia* and *Radiigera* sp. were found exclusively in plantations. *Laccaria tortilis, Hebeloma hiemale, Scleroderma areolatum, Inocybe kauffmanii, Scleroderma fuscum, Thelephora terrestris, Rhizopogon subolivascens, Amanita* sp. and *Tuber* sp. were found exclusively in nurseries. *Hebeloma mesophaeum, Rhizopogon roseolus, Suillus luteus, Tomentella atramentaria* and *Amphinema byssoides* were found in nurseries and plantations. Overall, eighteen ectomycorrhizal species were found in association with ponderosa pine plantations and nurseries. The most widely distributed species were *Amphinema byssoides, Hebeloma mesophaeum, Suillus luteus* and *Rhizopogon roseolus* in plantations, and *R. roseolus* and *H. mesophaeum* in nurseries. Because of their wide distribution and abundance in plantations and their relative abundant spore concentration, *Rhizopogon roseolus, Suillus luteus* and *Hebeloma mesophaeum* are potential candidates that could be used in mycorrhizal inoculation programs for ponderosa pine in forest nurseries.

Key words: ectomycorrhizal fungi, exotic plantations, nurseries, ponderosa pine

Introduction

Planting of exotic forest trees to increase timber production is a common practice in many parts of the world (Le Maitre 1998). In Argentina, along the piedmont of the Patagonian Andes, there are vast grasslands located within the ecotone between native

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forests on the west and steppe on the east, which are suitable for afforestation with fast growing non-native conifers. The main objectives of this practice are to produce high quality timber in order to foster social and economic development of the region, and also to ameliorate or even reverse erosion in areas that have been heavily overgrazed.

Ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) is the most widely planted species in Patagonia (Gonda 2001). The environmental conditions along the piedmont of the eastern slope of the Andes are very similar to those found on the eastern side of the Cascades of north western North America, where ponderosa pine grows naturally (Oliver & Ryker 1991). Both regions have a mediterranean precipitation regime with a well defined dry summer season, volcanic soils, and western winds that bring the moisture from the Pacific across the mountains. Plantations were initiated around 50 years ago. Approximately 50,000 ha are currently forested with ponderosa pine, yet 2,225,000 ha of suitable lands are still not forested (Andenmatten et al. 2002).

Mycorrhizae are extremely important in many terrestrial ecosystems (Malloch et al. 1980, Smith & Read 1997), and their importance as a fundamental requirement for the normal growth and survival of conifers is widely documented (Meyer 1973, Smith & Read 1997). The effect of ectomycorrhizas (EM) varies according to environmental conditions and with the specific association between host and fungi (Trappe 1977, Bledsoe 1992). Different species of fungi vary in their ability to reduce the effects of drought on a given host (Dixon et al. 1983, Parke et al. 1983). Knowledge of the distribution and ecology of EM fungi is important for monitoring and retention of diversity and selection of species for use in forest nurseries (Trappe 1977, Castellano & Molina 1989).

Many species of EM fungi have been reported for *P. ponderosa* in its natural distribution area (Trappe 1962, States 1983, 1984, Melichar et al. 1985, States & Gaud 1997, Stendell et al. 1999), but little is known about the existing fungal symbionts in Patagonia. Ponderosa pine plantations in Patagonia occur in grasslands with a typical vesicular-arbuscular flora lacking EM fungi compatible with pines (Godoy et al. 1994, Fontenla et al. 1998) so it is likely that the EM fungal diversity will be low. Reports on these taxa are scanty; Singer (1969) and Schroeder et al.[1] recorded *Suillus luteus*, *Rhizopogon* sp., and *Hebeloma* sp. from several plantations. Peredo et al. (1989, 1992) performed an inoculation trial with *Laccaria laccata*, *Hebeloma crustuliniforme*, *Thelephora terrestris* and *Pisolithus tinctorius* in one nursery in Neuquén province (North Patagonia) but, to date, there is no confirmation that those taxa were effectively incorporated to the nursery or any plantation. Barroetaveña & Rajchenberg (2003) found a poor mycorrhization degree of ponderosa pine seedlings produced by forest nurseries from Central Patagonia, and reported 11 EM taxa, pointing out the need to select appropriate EM fungal species and to initiate regular inoculation programs.

The objective of this study was to survey the mycorrhizal fungi from *Pinus ponderosa* in Central Patagonia, Argentina.

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[1]SCHROEDER, J., P. CWIELONG & M. RAJCHENBERG (unpublished). Zum Ectomykorizastatus von *Pseudotsuga menziesii* und *Pinus ponderosa* in Patagonien.

Material and Methods

PLANTATION AND NURSERY LOCATION AND CHARACTERISTICS. – Eight bare-root nurseries located between 71° 35' to 71° 21'W and 41° 08' to 42° 55'S, and fourteen pure ponderosa pine plantations were surveyed, all located in the Andean region of Río Negro and Chubut provinces (i.e. Central Patagonia, Argentina) (Fig. 1). The region has winter concentrated precipitation, with annual rates between 500 to 1000 mm. Nurseries' characteristics are as in Barroetaveña & Rajchenberg (2003), except for Huinganco nursery (cfr. Table 3 as Nursery 9, not included in the former study) from north Neuquén province, from where some collections were also studied. Plantations GIS localization, age and soil type are described in Table 1.

SAMPLING AND CURATORIAL PROCEDURES. – Fungal fruiting bodies were collected in spring

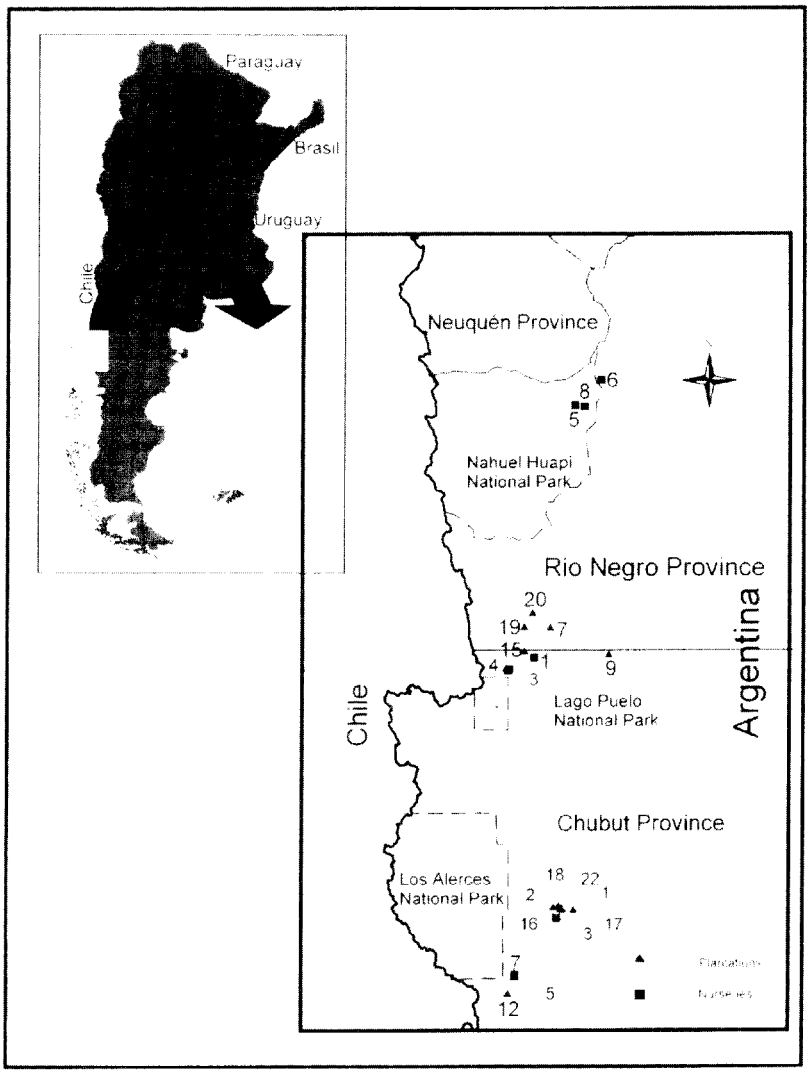

Fig. 1. Location of surveyed ponderosa pine nurseries and plantations. For number explanation cfr. tables 1 and 3.

Table 1. Plantations location and characteristics.

Plantation	GPS location	Age (year 2002)	Soil type
1- Laguna la Z, Esquel-Chubut	41°54'01"S 71°20'07.2"W	22	sandy loam
2- Camino al Percey, Esquel-Chubut	42°53'34.2"S 71°22'49.4"W	12-13	sandy
5- INTA Trevelin- Chubut	43°08'08.2"S 71°33'42.4"W	40	loamy sands
7- Camino a Maitén, ruta 29-Chubut	41°54'50,2"S 71°24'26"W	31	loamy sands, compacted
9- El Maitén- Chubut	42°00'19"S 71°08'01.6"W	41	silty
12- Piscicultura, Ruta 259 a Río Grande- Chubut	43°11'34.5"S 71°35'27.4"W	16	sandy loam (clay loam from 60 cm)
15- INTA Golondrinas Chubut	41°59'44"S 71°31'40.32"W	47	silty clay with stones
16- Laguna la Z, Esquel-Chubut	42°53'51.9"S 71°20'32.4"W	13	sandy with stones
17- Campo Sr.Vargas, Esquel-Chubut	42°54'04.5"S 71°17'05.3"W	13	sandy
18- Laguna la Z oeste- Chubut	42°53'14.5"S 71°21'11"W	15	clay loam
19- Mallin Ahogado-Río Negro	41°54'46.9"S 71°31'40.8"W	19	sandy
20- Guadal- Río Negro	41°51'43.9"S 71°29'24.2"W	21	clay loam
21- Estancia Leleque-Chubut	42°17'44.6"S 71°19'34.8"W	12	sandy
22- Laguna la Z Este, Esquel-Chubut	42°53'45.8"S 71°20'32.3"W	14	sandy

(September-October) and autumn (April-May) from 1998 to 2002 from plantations (except of plantations 15 and 21, which were visited only once). All nurseries were visited during autumn 1999 and 2000, and some of them also in autumn 2001 and 2002 (excepting nursery 9, from which we received collections but was not visited personally). Both nursery beds and plantations were walked to collect epigeous fungi and then raked in several points to search for hypogeous fruitbodies.

Fresh specimens were described for macroscopic characteristics, some were photographed, and dried at 35-40°C. Sporocarp tissue reactions were tested with KOH 15%, FeSO4 10% and Melzer's reagent (Moser 1983). Microscopic features used for identification were recorded by hand-cut sections using phloxine 1%, KOH 5% and Melzer's reagent. Species identification was completed using the criteria of Smith & Thiers (1964), Smith & Zeller (1966), Guzmán (1970), Eriksson & Ryvarden (1973), Smith & Smith (1973), Moser (1983), Smith et al. (1983), Arora (1986), Singer (1986), Mueller (1992), Domínguez de Toledo & Castellano (1996), Kõljalg (1996), Shanks (1996) and Martín (1996). Voucher specimens were deposited in the Herbarium, Centro Forestal CIEFAP, Esquel, Chubut, Argentina.

Results

In all, five hundred and fourteen collections were made from 1998 through 2002. Eight orders, eleven families and eighteen taxa were represented, many of them being first records for the region and some for the country (Table 2).

Table 2. EM fungal taxa associated with *Pinus ponderosa* plantations and nurseries in Central Patagonia, Argentina.

Species	Family	Order	New to
Amanita sp.	Amanitaceae	Agaricales	?
Amphinema byssoides (Fr.) J. Erikss.	Atheliaceae	Stereales	Argentina
Cortinarius sp. subg. *Telamonia*	Cortinariaceae	Cortinariales	Argentina
Hebeloma hiemale Bres.	Cortinariaceae	Cortinariales	Argentina
Hebeloma mesophaeum (Pers.: Fr.) Quél.	Cortinariaceae	Cortinariales	*
Inocybe kauffmanii A.H. Sm.	Cortinariaceae	Cortinariales	Argentina
Laccaria tortilis (Bolton) Cooke	Tricholomataceae	Agaricales	Patagonia
Radiigera sp.	Geastraceae	Geastrales	?
Rhizopogon ellenae A.H. Sm.	Rhizopogonaceae	Boletales	Argentina
Rhizopogon roseolus (Corda) Th. Fr.	Rhizopogonaceae	Boletales	Patagonia
Rhizopogon subolivascens A.H. Sm.	Rhizopogonaceae	Boletales	South America
Scleroderma areolatum Ehrenb.	Sclerodermataceae	Sclerodermatales	Patagonia
Scleroderma fuscum (Corda) Fischer	Sclerodermataceae	Sclerodermatales	Patagonia
Suillus luteus (Fr.) S.F. Gray	Boletaceae	Boletales	*
Thelephora terrestris Fr.: Fr.	Thelephoraceae	Thelephorales	*
Tomentella atramentaria Rostr.	Thelephoraceae	Thelephorales	South America
Tricholoma muricatum Shanks	Tricholomataceae	Agaricales	South America
Tuber sp.	Tuberaceae	Pezizales	?

*Already known from the area. ? not determined (generic determination).

Species distribution and occurrence was different in nurseries and plantations. *Hebeloma mesophaeum* and *Rhizopogon roseolus* were the most widely distributed in nurseries, while *Amphinema byssoides* was found in all plantations, followed by *Hebeloma mesophaeum*, *Suillus luteus* and *R. roseolus* (Table 3). The species *Hebeloma hiemale*, *Inocybe kauffmanii*, *Laccaria tortilis*, *Amanita* sp., *Scleroderma fuscum*, *Scleroderma areolatum*, *Thelephora terrestris*, *Rhizopogon subolivascens* and *Tuber* sp. were found only in nurseries, while *Cortinarius* sp. subg. *Telamonia*, *Tricholoma muricatum*, *Radiigera* sp. and *Rhizopogon ellenae* were found fruiting only in plantations. The other taxa, *Amphinema byssoides, Hebeloma mesophaeum*, *Suillus luteus, Rhizopogon roseolus* and *Tomentella atramentaria* were found in both nurseries and plantations.

Considering fruiting seasons, the species present in plantations showed that *Rhizopogon roseolus*, *Suillus luteus*, *Hebeloma mesophaeum*, *Amphinema byssoides* and *Tomentella atramentaria* can be found fruiting both in autumn and spring, while *Rhizopogon ellenae*, *Cortinarius* sp. subg. *Telamonia* and *Tricholoma muricatum* were found only in autumn and *Radiigera* sp. only in spring. Nurseries were visited only in autumn.

Table 3. Distribution of mycorrhizal fungal species in *Pinus ponderosa* plantations (Pl) and nurseries (N); rates indicate number of visits each species was recorded out of total visits of a site.

species	sites																			
	Pl.1	**Pl.2**	**Pl.5**	**Pl.7**	**Pl.9**	**Pl.12**	**Pl.15**	**Pl.16**	**Pl.17**	**Pl.18**	**Pl.19**	**Pl.20**	**Pl.21**	**Pl.22**	**N.1**	**N.2**	**N.3**	**N.4**	**N.7**	**N.8**	**N.9***
Amanita sp.																				1/2	
Amphinema byssoides	2/6	4/9	7/10	1/3	5/7	4/6	1/1	2/3	4/4	4/4	4/4	4/4	1/1	3/4	2/5		1/5				
Cortinarius sp. subg. *Telamonia*			4/10																		
Hebeloma mesophaeum	4/6	3/9	7/10		1/7	6/6		1/3	2/4	3/4	3/4	3/4	1/1	2/4	4/5	1/2	1/5	2/3	3/5	2/2	
Hebeloma hiemale															1/5		2/5	1/3			
Inocybe kauffmanii															2/5						
Laccaria tortilis															2/5	1/2	3/5				
Radiigera sp.										1/4											
Rhizopogon ellenae						3/6					1/4	1/4									1
Rhizopogon roseolus	3/6			1/3		6/6			1/4	2/4	4/4	3/4			4/5		1/5	2/3	3/5	1/2	1
Rhizopogon subolivascens															1/5						
Scleroderma areolatum															1/5						
Scleroderma fuscum																					2
Suillus luteus	1/6	4/9	4/10			4/6		3/3	2/4	2/4	2/4	2/4		1/4					3/5		
Thelephora terrestris															1/5	1/2	1/5				
Tomentella atramentaria		1/9	2/10					1/3		1/4	1/4			1/4							
Tricholoma muricatum						3/6															
Tuber sp.																	1/5				

* collections sent by the nursery manager.

Discussion

All taxa are new records for the region, with the exception of *Suillus luteus*, *Hebeloma mesophaeum* and *Thelephora terrestris*, and belong to ectomycorrhizal genera commonly associated with *Pinus* spp. elsewhere. *Hebeloma mesophaeum* was recorded by Singer (1969) in mixed native *Nothofagus* forests from Argentina and Chile, *S. luteus* was recorded growing with Pinaceae and *T. terrestris* was recorded growing with *Nothofagus antarctica* and with planted trees like *Cedrus* by the same author. Guzmán (1970) cited *Scleroderma areolatum* and *S. fuscum* from central Argentina. *Radiigera* sp. corresponds to a single, immature specimen whose features did not match appropriately to any of the species in the genus (Domínguez de Toledo & Castellano 1996). *Hebeloma hiemale, Tomentella atramentaria, Inocybe kauffmanii, Rhizopogon ellenae, Rhizopogon subolivascens and Laccaria tortilis* are new records for Argentina. Neighboring regions like Southern Chile has reports of *Rhizopogon roseolus*, *Tricholoma pessundatum* (a species similar to *T. muricatum*), *Suillus luteus* and two undetermined species of *Inocybe* (Garrido 1986) and *Hebeloma mesophaeum* and *S. luteus* (Valenzuela 1993) from Pinaceae plantations; surveys carried out in *Pinus* spp. plantations in Northern Argentina reported on some of the same genera as found in this study but with different species (Takacs 1961, Salusso & Moraña 1995, Nourha 1997, Wright & Albertó 2002).

All the registered genera are known to be mycorrhizal (Cairney & Chambers 1999); the genus *Tomentella*, usually not considered as mycorrhizal, has recently been demonstrated to be so, in particular the species *T. atramentaria* (Kõljalg et al. 2000). All the registered genera except for *Tomentella* were cited by Trappe (1962) or were registered in collection data bases associated with ponderosa pine [i.e., Data Base, Department of Forest Science Herbarium, Oregon State University, Corvallis, Oregon, USA (OSC)]. Regarding the natural distribution of species, *Rhhizopogon ellenae* and *R. subolivascens* are probably restricted to USA as there is only one record of each species from Europe (Martín 1996). *Suillus luteus* and *Rhizopogon roseolus* are rather abundant and widely distributed in pine plantations around the world (Dunstan et al. 1998). *Tricholoma muricatum* pertains to the *T. pessundatum* complex of species and might have been recorded as such from Chile (Garrido 1986), New Zealand (Hall et al. 1998) and southern Argentina (Singer 1969). For *Rhizopogon roseolus* followed Martín (1996). *Laccaria tortilis* is a cosmopolitan but uncommon taxon, apparently associated with both Pinaceae and Fagaceae (Mueller 1992) that was previously recorded from Central Argentina (i.e. Buenos Aires province) by Spegazzini (fide Mueller 1992). *Scleroderma areolatum, S. fuscum, Hebeloma mesophaeum, H. hiemale* and *Amphinema byssoides* have a boreal distribution (Kauffman 1918, Guzmán 1970, Moser 1983, Erland & Taylor 1999, Gilbertson 1974). Both *Tomentella atramentaria* and *Thelephora terrestris* present a worldwide distribution (Kõljalg 1996).

There is strong evidence that the majority of the EM fungal species found are introduced; none of them, with the exception of *Thelephora terrestris* and *Hebeloma mesophaeum* have been registered from native Patagonian forest in spite of intensive surveys (Singer 1969, Horak 1979, Garrido 1988, Valenzuela 1993). On the contrary,

all of them have been reported associated with *Pinus* spp. around the world (Smith & Thiers 1964, Smith & Zeller 1966, Guzmán 1970, Bruchet 1973, Eriksson & Ryvarden 1973, Moser 1983, Arora 1986, Nishida 1989, Mueller 1992, Shanks 1996, Colpaert 1999, Kőljalg et al. 2000). How these EM fungal taxa have been introduced to Patagonia is not documented. Apparently, the original seedlings used for the first ponderosa pine plantations in this region came from Estación Forestal Puerto Anchorena, in Isla Victoria, Río Negro, Argentina, not extant since the end of the sixties. The Isla Victoria nursery started production in 1925 and imported seeds were used initially. The first ponderosa pine plantations in Isla Victoria, though, were installed in 1920, and the origin of seeds or seedling is unknown. The nursery did not exist at that time, and the activities described in that period were cattle and wood extraction, so the seedlings may have come from somewhere else. The first plantations installed after the nursery was created were in 1926 and 1927, with 3-5 year old seedlings, so it is hard to tell whether the plants were produced there before the nursery was formally created, or if they were brought from any other nursery (Koutché 1942). In the early '50 two public forest experimental stations at Las Golondrinas and Trevelin (Chubut province) were created. Both began afforestation activities with seedlings brought from Isla Victoria nursery but fairly soon also with seedlings obtained in their own nurseries; the latter employed either imported seeds from the USA or from stands at Isla Victoria. Seedlings from Isla Victoria nursery were also taken to Parque Nacional Los Alerces nursery (Chubut province) around 1954. Other nurseries in Patagonia have also imported seeds from USA, so the possibility exists of the introduction of mycorrhizal spores with them (Dunstan et al. 1998).

Cairney & Chambers (1999), Dahlberg & Finlay (1999) and Erland & Taylor (1999) have reviewed the ecology of several species of *Rhizopogon*, *Laccaria*, *Hebeloma*, *Scleroderma*, *Amphinema* and *Suillus* in the Northern Hemisphere and afforested areas around the world. Nevertheless, their behavior needs critical examination under the conditions of Patagonia. *Amphinema byssoides*, for example, was found to have a wide distribution in ponderosa pine plantations of different ages and precipitation conditions in Patagonia (data not shown), suggesting that it is a successful competitor of other EM fungi under Patagonian conditions, as is the case of *Thelephora terrestris* with Pines in Western Australia (Dunstan et al. 1998).

This study did not intend to quantify species abundance, but some field observations are remarkable for future research lines. The abundance of *Rhizopogon roseolus*, *Suillus luteus* and *Hebeloma mesophaeum* was very high in plantations. Because of this, together with their wide distribution and fruitbody characteristics like size and spore concentration and previous experience using them as inoculum, they appear as good candidates to be tested for inoculation programs.

Many EM species do not produce sporocarps in certain conditions, or they fruit occasionally, so their occurrence in a certain area may represent only a limited fraction of the ectomycorrhizal community (Dahlberg 2001). Ectomycorrhizas can be morphologically characterized as morphotypes, and their diversity is a complementary information to determine the real mycorrhizal diversity (Jansen & De Nie 1988, Gardes & Bruns 1996). Fifteen morphotypes found in ponderosa pine nurseries were described by Barroetaveña & Rajchenberg (2003), but studies including detailed

morphotype descriptions and PCR identification of mycorrhizal tips are needed to better asses the real diversity present in ponderosa pine plantations in Patagonia. This study is a first approach to the knowledge of EM fungi of ponderosa pine plantations and nurseries in Patagonia, Argentina.

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