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Ectomycorrhizal fungi of *Pinus pinaster*

Abstract A study was undertaken to determine the ability to form ectomycorrhizae with *Pinus pinaster* Ait. in pure culture syntheses of 98 isolates of putative mycorrhizal fungi, mainly collected in northern Spain. A total of 35 species in 16 genera – *Amanita*, *Cenococcum*, *Collybia*, *Cortinarius*, *Hebeloma*, *Laccaria*, *Lactarius*, *Lyophyllum*, *Melanogaster*, *Paxillus*, *Pisolithus*, *Rhizopogon*, *Scleroderma*, *Suillus*, *Thelephora* and *Xerocomus* – formed ectomycorrhizae. Many of these fungal species were not previously reported as symbiotic with *Pinus pinaster*. Results obtained increase the range of potential fungal candidates for inoculation of nursery seedlings.

Key words Maritime pine · Mycorrhizae · Pure culture synthesis · Reforestation · Host specificity

Introduction

Maritime pine (*Pinus pinaster* Ait.) is a major conifer species in Spain, covering 27% of the total surface of 5.5 million ha occupied by conifers. Maritime pine was the forest species most frequently planted in the Spanish reforestation programmes of the period 1945–1980 (Anonymous 1989). At present there are 800000 ha of man-made forests and approximately 600000 ha of natural masses distributed mostly in the northern half of the country (Gil et al. 1990).

Maritime pine is an undemanding, hardy species which will grow where no other forest species of economic importance will grow. Therefore, it has been used widely in the reforestation of infertile, sandy and slightly acid soils (Scott 1962; Nicolas and Gandullo 1967).

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The beneficial effects that selected ectomycorrhizal fungi can exert on the performance of outplanted conifer seedlings are well known (Trofymow 1990; Villeneuve et al. 1991; Marx 1991). The criteria applied in the selection process of ectomycorrhizal fungi for inoculation of nursery seedlings used in reforestation have been reviewed by Trappe (1977). To select appropiate fungi knowledge is needed on the fungal symbionts of the plant species. Past literature contains little information on the mycorrhizal fungi of maritime pine. The information available has been primarily obtained from field observation of association of sporocarps and the host. Few of these associations have been confirmed as symbiotic by mycorrhiza synthesis under controlled conditions. The objective of this work was to broaden knowledge of the ectomycorrhizal fungi of maritime pine in order help assess their potential use in the inoculation of seedlings for reforestation.

Materials and methods

The fungal isolates used in this study were obtained from sporocarps collected in natural forests and in forest plantations of northern Spain, covering a wide geographical area and a range of habitats (Table 1). Herbarium specimens were deposited in the Instituto Antonio Jose Cavanilles, Real Jardín Botánico, Madrid, Spain. Isolations were made by explants from sporocarp tissue on modified Melin-Norkrans agar (MMN) (Marx 1969) and maintained by transfer on that medium every 3 months (Molina and Palmer 1982). *Cenococcum geophilum* was isolated from surface-sterilized sclerotia by the method of Trappe (1969). Additional fungi of potential practical importance were obtained from colleagues at other institutions and included in the study (Table 2).

Seeds of *Pinus pinaster*, obtained from plantations in north-eastern Spain, were rinsed overnight in running tap water, shaken 60 min in tap water with Tween 20 (2 drops/10 ml), rinsed in running tap water, shaken 60 min in 30% H_2O_2 and washed in 2 l of sterile distilied water. Disinfected seeds were seeded on malt extract agar (1% malt extract, 1% agar-agar) (Difco) in screwcap vials and stratified 3–4 weeks at 4° C. Vials were then removed from the refrigerator for seed germination at room temperature (20±2° C) and after 2 weeks incubation, vials showing contamination were discarded. When the radicle was 1–2 cm long, aseptically germinated seedlings were transferred to synthesis tubes con-

Table 1 Fungal isolates obtained from sporocarps collected in northern Spain and tested in pure culture syntheses for ectomycorrhiza formation with *Pinus pinaster*. (*A gra Abies grandis* (Dougl.) Lind., *C ave Corylus avellana* L., *C sat Castanea sativa* Mill., *F syl Fagus sylvatica* L., *P abi Picea abies* (L.) Karst., *P men Pseudotsuga menziesii* (Mirb.) Franco, *P pin Pinus pinaster* Ait., *P rad Pinus radiata* D.Don, *P syl Pinus sylvestris* L., *Q ile Quercus ilex* L., *Q pet Quercus petraea* (Matts.) Liebl., *Q rob Quercus robur* L., *Q sub Quercus suber* L.)

Fungus	Isolate	Associated host	Year isolated	Geographical location	Elevation (m)	Soil pH
Amanita aspera (Fr.) Hooker	A-48	Q rob	1986	Asturias	320	3.7
A. citrina (Schff.) S.F. Gray	A-145	*Picea* sp.	1990	Girona	1100	5.6
	A-157	Q ile	1990	Girona	200	6.2
A. muscaria (L. ex Fr.) Pers. ex Hooker	A-17	C sat	1985	Pontevedra	40	5.2
	A-124	A gra	1989	Girona	1100	5.5
	A-151	*Abies* sp.	1990	Girona	1100	5.5
A. pantherina (D.C. ex Fr.) Kummer	A-67	Q rob	1986	Asturias	40	5.2
	A-149	P men	1990	Girona	1100	5.6
A. rubescens (Pers. ex Fr.) S.F. Gray	A-73	C sat	1986	Asturias	340	—
	A-148	F syl, *Quercus* sp.	1990	Girona	1100	5.6
A. spissa (Fr.) Kummer	A-150	*Abies* sp.	1990	Girona	1100	5.6
Boletus edulis Bull. ex Fr.	A-79	C sat	1986	Asturias	340	—
	A-39	P syl	1986	Asturias	460	3.7
B. erythropus (Fr. ex Fr.) Pers.	A-45	F syl	1986	Asturias	530	4.1
	A-154	Q ile	1990	Girona	1000	5.6
B. pulverulentus Opat.	A-47	C sat	1986	Asturias	320	3.7
	A-146	P men	1990	Girona	1000	5.6
Cenococcum geophilum Fr.	A-144	F syl	1990	Girona	1200	—
Clitopilus prunulus (Scop. ex Fr.) Kummer	A-58	Q rob	1986	Asturias	300	3.9
Collybia dryophyla (Bull. ex Fr.) Kummer	A-63	Q pet, C ave	1986	Asturias	730	4.0
C. maculata (Alb. & Schff. ex Fr) Kummer	A-65	C ave	1986	Asturias	730	4.0
C. succinea (Fr.) Quél.	A-49	P syl	1986	Asturias	600	5.1
Cortinarius caerulascens Schff. ex Secr.	A-88	C sat	1986	Pontevedra	—	—
C. glaucopus (Schff. ex Fr.) Fr.	A-125	A gra	1989	Girona	1100	5.5
C. purpurascens Fr. ex Fr.	A-14	C sat	1985	Pontevedra	10	—
Elaphomyces granulatus Fr.	A-136	P pin	1987	Pontavedra	—	4.7
Hebeloma sinapizans (Paul. ex Fr.) Gill.	A-126	P abi	1989	Barcelona	20	7.5
Hysterangium clathroides Vitt.	A-11	P rad	1986	Asturias	240	4.2
Inocybe maculipes Favre	A-44	F syl	1986	Asturias	550	4.1
Laccaria amethystina (Bolt. ex Hooker) Murr.	A-52	Q pet	1986	Asturias	740	4.0
L. farinacea (Huds. ex Gray) Sing.	A-32	P rad	1986	Asturias	500	3.7
L. laccata (Scop. ex Fr.) Berk. & Br.	A-54	F syl	1986	Asturias	780	—
	A-127	Q ile	1989	Girona	1000	4.9
L. proxima (Boud.) Orton ss. Favre, Sing.	A-40	F syl	1986	Asturias	610	3.4
Lactarius acerrimus Britz	A-46	Q rob	1986	Asturias	320	3.7
L. chrysorrheus Fr.	A-156	Q ile	1990	Girona	200	6.2
L. deliciosus L. ex Fr.	A-81	P pin	1987	Asturias	80	—
	A-120	P pin	1988	Girona	200	5.6
	A-159	P pin	1990	Girona	200	5.6
L. rufus (Scop. ex Fr.) Fr.	A-34	P syl	1986	Asturias	610	5.8
Lepista nuda (Bull. ex Fr.) Cke.	A-76	P pin	1986	Pontevedra	—	—
Lycoperdon perlatum Pers. ex Pers.	A-72	P pin	1985	Asturias	80	4.6
	A-77	P pin	1986	Pontevadra	—	—
L. pyriforme Pers. ex Schff.	A-83	F syl	1986	Cantabria	550	—
Lyophyllum decastes (Fr.) Sing.	A-71	P rad	1986	Asturias	240	4.2
Melanogaster ambiguus (Vitt.) Tul. & Tul.	A-132	P men	1989	Girona	1100	5.6
Paxillus involutus (Batsch. ex Fr.) Fr.	A-41	P syl	1986	Asturias	600	5.0
	A-87	C sat	1986	Pontevedra	—	4.7
Pisolithus arhizus (Scop:Pers.) Rausch.	A-93	Q sub	1986	Girona	50	—
	A-141	Q sub	1990	Girona	200	6.2
Rhizopogon luteolus Fr. & Nordh.	A-5	P pin	1985	Pontevedra	—	—
	A-106	P pin	1987	Pontevedra	—	4.7
R. roseolus (Corda ex Sturm) Fr.	A-7	P rad	1985	Asturias	350	—
	A-96	P syl	1987	Tarragona	1000	7.2
	A-160	P pin	1991	Girona	100	5.6
R. subareolatus Smith	A-104	P men	1987	Girona	1100	4.7
	A-116	P men	1987	Girona	1100	5.5
R. ventricisporus Smith	A-97	P syl	1987	Tarragona	1000	7.2
R. vulgaris (Vitt.) M. Lange	A-56	P rad	1986	Asturias	100	5.1
	A-98	P pin	1987	Pontevedra	—	4.7
	A-101	P pin	1987	Pontevedra	—	4.7

Table 1 (continued)

Fungus	Isolate	Associated host	Year isolated	Geographical location	Elevation (m)	Soil pH
Scleroderma cepa Pers.	A-18	C sat	1985	Pontevedra	—	—
S. citrinum Pers.	A-37	C sat	1986	Asturias	—	—
S. polyrhizum Gmel. ex Pers.	A-61	P syl	1986	Asturias	610	5.0
	A-99	P pin	1987	Pontevedra	—	—
Suillus bovinus (L. ex Fr.) Kuntze	A-21	P rad	1985	Asturias	350	—
	A-22	P pin	1985	Asturias	100	3.9
	A-75	C sat	1986	Pontevedra	—	—
S. granulatus (L. ex Fr.) Kuntze	A-82	P pin	1986	Asturias	80	—
	A-140	P syl	1990	Barcolona	1400	—
S. luteus (L. ex Fr.) S.F. Gray	A-33	P rad	1986	Asturias	100	—
	A-57	P rad	1986	Asturias	240	4.2
	A-155	P syl	1990	Barcelona	1400	—
S. variegatus (Swartz ex Fr.) Kuntze	A-142	P syl	1990	Barcelona	1400	—
Tricholoma columbetta (Fr.) Kummer	A-74	Q rob	1986	Asturias	300	—
T. portentosum (Fr.) Quél.	A-92	P pin	1986	Pontevedra	—	—
T. saponaceum (Fr. ex Fr.) Kummer	A-121	P men	1988	Girona	1100	5.5
T. sulphureum (Bull. ex Fr.) Kummer	A-80	Q rob	1986	Asturias	300	—
Xerocomus chrysenteron (Bull. ex St. Amans) Quél.	A-119	P men	1988	Girona	1100	5.5
	A-147	P men	1990	Girona	1100	5.5

taining sterilized peat-vermiculite with nutrient solution (Molina 1979). The synthesis tubes were inoculated with 12 ml of a mycelial culture of each fungal strain grown in MMN liquid medium. A total of five replicates were prepared for each fungal isolate tested. Inoculated seedlings were grown for 3–4 months in a water bath (20–25° C) under fluorescent lights (135 μmol s^{-1} m^{-2}, 16 h/day). At the end of the growing period, seedlings were removed intact from the synthesis tubes and root systems washed and examined for ectomycorrhizae. Infected rootlets from each seedling were sampled for microscopic examination of hand-made transverse sections to detect Hartig net formation. Doubtful infections and unsuccessful syntheses were repeated to confirm negative results.

Results

Only 19 of the nearly 70 fungal species collected in the field in association with maritime pine were previously known to be ectomycorrhizal (Table 3). Out of 98 fungal isolates tested for ectomycorrhiza formation, 57 representing 35 species formed ectomycorrhiza; 20 of these species were previously unknown to form ectomycorrhizae with *Pinus pinaster* (Table 4). The fungi that did not form mycorrhizae with maritime pine under the experimental conditions grew sparsely on the root surfaces and did not penetrate the cortex except *Rhizopogon hawkeri* S-274, *R. subareolatus* A-104 and A-116, and *R. ventricisporus* A-97. These fungi formed what appeared to be a weak mantle on only a small proportion of the short roots (3–10%). Microscopic examination of these rootlets showed the presence of superficial surface mycelium but the absence of a well-developed Hartig net. None of the fungi tested were observed to stimulate seedling growth significantly in pure culture synthesis conditions.

Our results and other reports in the literature (see Table 3) increase to 40 the fungal species proven to be ectomycorrhizal with *Pinus pinaster*.

Discussion

Formation of ectomycorrhizae is the essential proof that an ectomycorrhizal relationship can exist between a fungus and a tree. Pure culture syntheses are done under artificial conditions that may not be representative of those found in nature. Members of *Boletus* and *Tricholoma* have been cited as ectomycorrhizal with pine and other conifers (Kropp and Trappe 1982; Malajczuk et al. 1982) but none of the isolates tested in our experiments formed ectomycorrhizae with *Pinus pinaster*. The difficulties in obtaining ectomycorrhiza formation by species of *Boletus* and *Tricholoma* in pure culture conditions have been cited before (Kropp 1982; Kropp and Trappe 1982; Godbout and Fortin 1983; Danielson et al. 1984). Both genera are considered to be mainly associated with adult trees (Deacon et at. 1983; Mason et al. 1983; Dighton and Mason 1985; Fleming et al. 1986). Hence, the pure culture synthesis conditions may not be conducive to the expression of their symbiotic capabilities. Negative results in pure culture syntheses indicate that the experimental conditions were inappropriate for ectomycorrhiza formation. This information is useful in order to omit such fungi as candidates for nursery inoculations by present technology, but it does not definitively preclude mycorrhizal association between that fungus and host in nature.

Positive results presented in this work demonstrate the capability of a fungal isolate to form mycorrhizae with juvenile *Pinus pinaster*. *Rhizopogon ellenae*, *R. luteolus*, *R. roseolus* and *R. volgaris*, members of the subgenus *Rhizopogon*, formed abundant ectomycorrhizae with maritime pine. *Rhizopogon colossus*, *R. hawkeri*, *R. mutabilis* and *R. subareolatus*, belonging to the subgenus *Villosuli*, and *R. vinicolor*, belonging to the sub-

Table 2 Fungal isolates obtained from other institutions and tested in pure syntheses for ectomycorrhiza formation with *Pinus pinaster*

Fungus	Isolate	Associated host	Year isolated	Research institution
Heboloma crustuliniforme (Bull. ex St. Amans) Quél.	S-66	—	—	University of Surrey, Guildford, UK
Hebeloma cylindrosporum Romagn.	S-20	—	—	University of Surrey, Guildford, UK
Hebeloma subsaponaceum Karst.	S-68	—	—	University of Surrey, Guildford, UK
Laccaria bicolor (R.Mre.) Orton	S-238	*Larix occidentalis*	—	Forestry Service, Corvallis, Ore., USA
Laccaria proxima (Boud.) Orton ss. Favre, Sing.	S-64	—	—	University of Surrey, Guildford, UK
Rhizopogon colossus Smith	S-148	*Pseudotsuga menziesii*	1965	Forestry Service, Corvallis, Ore., USA
Rhizopogon ellenae Smith	8974	*Pinus ponderosa*	1986	Forestry Service, Corvallis, Ore., USA
Rhizopogon hawkeri Smith	S-274	*Pseudotsuga menziesii*	1976	Forestry Service, Corvallis, Ore., USA
Rhizopogon luteolus Fr. & Nordh	G-823	*Pinus pinaster*	—	Centro Forestal de Lourizan, Galicia, Spain
Rhizopogon mutabilis Smith	S-483	—	—	Forestry Service, Corvallis, Ore., USA
Rhizopogon occidentalis Zeller & Dodge	7544	*Pinus ponderosa*	1983	Forestry Service, Corvallis, Ore., USA
Rhizopogon roseolus (Corda ex Sturm) Fr.	M-1	*Pinus halepensis*	1989	University of Murcia, Spain
	M-2	*Pinus pinaster*	1989	University of Murcia, Spain
Rhizopogon vinicolor Smith	S-47	—	—	University of Surrey, Guildford, UK
Suillus collinitus (Fr.) O. Kuntze	I-1	*Pinus nigra*	1989	Instituto Sperimentale per la Patologia Vegetale, Rome, Italy
Thelephora terrestris (Ehrh.) Fr.	R-34	—	—	University of Surrey, Guildford, UK
	R-38	—	—	University of Surrey, Guildford, UK
	S-63	—	—	University of Surrey, Guildford, UK

Table 3 Putative ectomycorrhizal fungi of *Pinus pinaster*. Species and references in bold type report ectomycorrhizal syntheses using various procedures, others are field observations

Amanita citrina (Schff.) Gray – Fernández de Ana et al. 1989
A. gemmata (Fr.) Gill. – Fernández de Ana et al. 1989
A. muscaria (L. ex Fr.) Pers. ex Hook. – **Azevedo** (in Marx 1980); Fernández de Ana et al. 1989
A. phalloides (Vaill. ex Fr.) Secr. – Fernández de Ana et al. 1989
A. rubescens (Pers. ex Fr.) Gray – Fernández de Ana et al. 1989
A. spissa (Fr.) Kummer – Fernández de Ana et al. 1989
Boletus edulis Bull. ex Fr. – Fernández de Ana et al. 1989
B. erythropus (Fr.) Krombh. non Pers. – Fernández de Ana et al. 1989
B. fragrans Vitt. – **Ferreira dos Santos 1941**; Trappe 1962
Cantharellus cibarius Fr. – Fernández de Ana et al. 1989
C. tubaeformis Bull. es Fr. – Fernández de Ana et al. 1989
Cenococcum geophilum Fr. – **Trappe** 1962, **1994**
Chroogomphus rutilus (Schaeff. ex Fr.) Miller – Guinberteau et al. 1990
Clavulina rugosa (Fr.) Schrost. – Guinberteau et al. 1990
Elaphomyces granulatus Fries. – Alvaraz et al. 1993
Geopora arenicola (Lév.) Kers – Alvarez et al. 1993
Gomphidius viscidus L. ex Fr. – Guinberteau et al. 1990
Hebeloma crustuliniforme (Bull. ex Fr.) Quél. – Guinberteau et al. 1990
H. cylindrosporum Romagn. – Mousain et al. 1977; **Mousain and Lamond 1978; Mousain et al. 1979; Rancillac 1982; David et al. 1983; Dabaud and Gay 1987; Plassard et al. 1988; Scheromm et al. 1990**
H. sacchariolens Quél. – Guinberteau et al. 1990
H. sinapizans (Paul. ex Fr.) Gill. – **Branzanti and Zambonelli 1987**
Hydnum repandum L. ex Fr. – Fernández de Ana et al. 1989
Hygrophorus hypothejus (Fr. ex Fr.) Fr. – Guinberteau et al. 1991
Hymenogaster arenarius L. & Ch. Tul. – Vidal 1991
H. hessei Soehner – Vidal 1991
H. rehsteineri Bucholtz – Vidal 1991
H. spictensis Pat. – Vidal 1991
H. thwaitesii Berk. & Br. – Vidal 1991
Inocybe decipientoides Peck. – Guinberteau et al. 1990
Laccaria amethystina (Bolt. ex Hooker) Murr. – Fernández de Ana et al. 1989
L. laccata (Scop. ex Fr.) Berk. & Br. – **Branzanti and Zambonelli 1987**; Fernández de Ana et al. 1989; Guinberteau et al. 1990
Lactarius deliciosus (L. ex Fr.) S.F. Gray – Barsali 1922; Trappe 1962; **Mousain et al. 1979; Poitou et al. 1982**; Fernández de Ana et al. 1989
L. hepaticus Plowr. ex Boud. (ss. Neuh.) – Guinberteau et al. 1990
L. piperatus (L. ex Fr.) Gray – Fernández de Ana et al. 1989
L. vellereus (Fr.) Fr. – Fernández de Ana et al. 1989
L. volemus (Fr.) Fr. – Barsali 1922; Trappe 1962
Paxillus involutus (Batsch. ex Fr.) Fr. – **Branzanti and Zambonelli 1987**
Pisolithus tinctorius (Pers.) Cok. & Couch (=*P. arhizus* (Scop:Pors.) Rausch.) – **Marx and Bryan 1970**; Marx 1977; **Mousain et al. 1977, 1979; Ruehle et al. 1981; Azevedo 1982; Rancillac 1982; David et al. 1983; Plessard et al. 1983; Dexheimer et al. 1986; Doumas et al. 1986**
Rhizopogon luteolus Fries & Nordh. – **Azevedo 1982**; Guinberteau et al. 1990; Vidal 1991; Alvarez et al. 1993
R. roseolus (Corda ex Sturm) Fries (=*R. aestivus* (Tul. & Tul.) Tul. & Tul., *R. rubescens* Tul. & Tul.) – Vidal 1991; Alvarez et al. 1993
R. vulgaris (Vitt.) M. Lange (=*R. provincialis* Tul. & Tul.) Heim et al. 1934; Alvarez et al. 1993

Table 3 (continued)

Russula amoenolens Romagn. – Fernández de Ana et al. 1989
R. cessans Pearson – Guinberteau et al. 1990
R. cyanoxantha Schff. ex Fr. – **Azevedo** (in Marx 1980); Fernández de Ana et al. 1989
R. emetica (Schaeff. ex Fr.) Pers. ex Fr. – Barsali 1922; Trappe 1962
R. foetens Fr. – Fernández de Ana et al. 1989
R. nigricans (Bull.) Fr. – Fernández de Ana et al. 1989
R. palumbina Quél. (= *R. grisea* Romagn.) – Barsali 1922; Trappe 1962
R. queletii Fr. in Quél. – Fernández de Ana et al. 1989
R. sardonia Fr. ex Rom. – Fernández de Ana et al. 1989; Guinberteau et al. 1990
R. torulosa Bres. – Guinberteau et al. 1990
R. turci Bres. ss. Maire – Fernández de Ana et al. 1989
Scleroderma citrinum Pers. (= *S. aurantium* (Vaill.) Pers., *S. vulgare* Fr.) – **Takacs 1961**; Trappe 1962; **Azevedo 1982**; Fernández de Ana et al. 1989
S. bovinus (L. ex Fr.) O. Kuntze – Barsali 1922; Rayner and Levisohn 1941; Trappe 1962; **Mousain 1971**; Plassard et al. 1988; Fernández de Ana et al. 1989; Guinberteau et al. 1990
S. granulatus (L. ex Fr.) O. Kuntze (= *Boletus granulatus* Fr. ex L.) – Barsali 1922; Costantini 1923; Singer 1945; Heim 1957; **Takacs 1961**; Trappe 1962; **Azevedo** (in Marx 1980); **Poitou et al. 1982; Branzanti and Zambonelli 1987**
S. luteus (L. ex Fr.) S.F. Gray (= *Boletus luteus* Fr. ex L.) – **Poitou et al. 1982**
Thelephora terrestris (Ehrh.) Fr. – **Marx and Bryan 1970; Ruehle et al. 1981**; Guinberteau et al. 1990
Tricholoma colossus (Fr.) Quél. – Fernández de Ana et al. 1989
T. flavovirens (Pers. ex Fr.) Lund. – **Poitou et al. 1982**; Fernández de Ana et al. 1989
T. malluvium (Batt. ex Fr.) Sacc. – Fernández de Ana et al. 1989
T. pessundatum (Fr.) Quél. – Guinberteau et al. 1990
T. portentosum (Fr.) Quél. – Fernández de Ana et al. 1989
T. saponaceum (Fr.) Kummer – Fernández de Ana et al. 1989
T. terreum (Schff. ex Fr.) Kummer – **Azevedo** (in Marx 1980)
Tuber aestivum Vitt. – Vidal 1991
T. borchii Vitt. – Ceruti 1965; Vidal 1991
Xerocomus badius (Fr. ex Fr.) Gilbert – Fernández de Ana et al. 1989

genus *Fulviglebae*, failed to form ectomycorrhizae with maritime pine. The subgenus *Rhizopogon* has been cited as usually associated with *Pinus* spp. (Trappe 1962), whereas members of the subgenus *Villosuli* have been considered specific for Douglas fir (Molina and Trappe 1982).

Differences in root colonization capacity were detectable among fungal species and isolates of a same species (Table 4). These differences were not related to the age of the isolate or the associated host. Formation of only a few ectomycorrhizae suggests that under these experimental conditions the fungus is a less vigorous ectomycorrhiza former than others. Such information could be useful in selecting aggresive fungal isolates for nursery inoculations with vegetative inocula. However, the value of such fungi to the host under nursery or field conditions cannot be inferred.

The list of mycorrhizal associates given in this work does not represent the total potential mycorrhizal fungi associated with *Pinus pinaster*, but demonstrates that maritime pine is receptive to a broad range of fungi. Some of the ectomycorrhizal fungi reported as aggressive colonizers of *Pinus pinaster* are either not host specific (*Amanita* spp., *Hebeloma* spp., *Laccaria* spp., *Pisolithus tinctorius*, *Scleroderma citrinum*) or conifer specific (*Rhizopogon* spp., *Suillus* spp.). The success of *Pinus pinaster* as invader of eroded disturbed soils may be attributed in part to its compatibility with many different ectomycorrhizal fungi with a broad host range. A natural succession of mycorrhizal fungi occurs as stands mature (Mason et al. 1982, 1983), and this succession tends over time from broad-host-range fungi towards dominance by host-specific fungi. This possibility and its implications in the selection of fungi for nursery inoculations needs to be further explored.

The results obtained in our work increase knowledge of fungi with potential for use in nursery inoculations. Little effort was made to select the best fungi for *Pinus pinaster* plantings and further research is needed to optimize fungus-host combinations for different sites if maximum gains are to be achieved in future forestation programmes.

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References

Alvarez IF, Parladé J, Trappe JM, Castellano MA (1993) Hypogeous mycorrhizal fungi of Spain. Mycotaxon 47:201–217

Anonymous (1989) Anuario de estadística agraria. Ministerio de Agricultura Pesca y Alimentación Madrid

Azevedo N (1982) Ectomicorrizas del *Pinus pinaster* Sol. ex Ait. Bol Estación Cental Ecol (ICONA) 11:37–42

Barsali E (1922) Contribuzione allo studio del rapporti delle micorize ectotrofiche di alcune essenze arboree. Atti Soc Toscana Sci Nat Proc Verb 31:16–20

Branzanti B, Zambonelli A (1987) Effetti della micorrizazione sullo sviluppo di semenzali di *Pinus pinaster*. Microbiol Ital 2:53–57

Ceruti A (1965) La tartuficoltura in Italia. Ann Accad Agr Torino 107:1–12

Costantini J (1923) La vie mystérieuse des champignons. Mise au point du problème des mycorhizes de conifers. Rev Sci 61:733–737

Danielson RM, Visser S, Parkinson D (1984) Production of ectomycorrhizae on container-grown jack pine seedlings. Can J For Res 14:33–36

David A, Faye M, Rancillac M (1983) Influence of auxin and mycorrhizal fungi on the in vitro formation and growth of *Pinus pinaster* roots. Plant Soil 71:501–505

Deacon JW, Donaldson SJ, Last FT (1983) Sequences and interactions of mycorrhizal fungi on birch. Plant Soil 71:257–262

Debaud JC, Gay G (1987) In vitro fruiting under controlled conditions of the ectomycorrhizal fungus *Hebeloma cylindrosporum* associated with *Pinus pinaster*. New Phytol 105:429–435

Dexheimer J, Aubert-Dufresne M-P, Gèrard J, Le Tacon F, Mousain D (1986) Étude de la localisation ultrastructurale des activités phosphatasiques acides dans deux types d'ectomycorhizes: *Pinus nigra nigricans/Hebeloma crustuliniforme* et *Pinus pinaster/Pisolithus tinctorius*. Bull Soc Bot Fr 133:343–352

Table 4 Percentage infection and general morphological characteristics of ectomycorrhizae formed on *Pinus pinaster*. Fungal species in bold type indicate new syntheses

Fungus	Isolate	Mycorrhizae			Mycelial growth[c]	Rhizomorphs/ mycelial strands[b]
		%[a]	Color of mantle	Hartig net[b]		
Amanita aspera	A-48	71	White–pale beige	+ +	–	–
A. citrina	A-157	76	White	+	+ +	+ + +
A. muscaria	A-17	46	White	+ +	+	+ + +
	A-124	38	White	+ +	+	+ + +
	A-151	58	White	+ +	+	+ + +
A. pantherina	A-67	27	White	+ +	–	+ + +
	A-149	62	White	+ +	–	+ + +
A. rubescens	A-73	45	White–beige	+ +	–	+ + +
	A-148	75	White–beige	+ +	–	+ + +
A. spissa	A-150	13	White	+ +	–	+
Cenococcum geophilum	A-144	9	Black	+	+	–
Collybia maculata	A-65	42	White	+ +	+ + +	+
Cortinarius glaucopus	A-125	40	White	+ + +	+ + +	+ +
Hebeloma crustuliniforme	S-66	25	White	+ + +	+ + +	–
H. cylindrosporum	S-20	86	White	+ + +	+ + +	–
H. sinapizans	A-126	56	White	+ +	+ + +	–
H. subsaponaceum	S-68	69	White	+ + +	+ + +	–
Laccaria bicolor	S-238	89	Beige–white, violet	+ + +	+ +	–
L. laccata	A-127	79	Beige–white, violet	+ +	+	–
L. proxima	S-64	32	Beige	+	+ +	–
Lactarius chrysorrheus	A-156	60	Pale orange	+	+	–
L. deliciosus	A-81	29	Orange–green	+ +	+	–
	A-120	75	Orange–green	+ +	+	–
	A-159	46	Orange–green	+ +	+	–
L. rufus	A-34	40	Orange–red	+ +	+	–
Lyophyllum decastes	A-71	64	White	+ +	+ +	–
Melanogaster ambiguus	A-132	38	Brown-gold	+	+ + +	+ + +
Paxillus involutus	A-41	8	White–beige	+	+ + +	–
	A-87	52	White–beige	+	+ + +	–
Pisolithus arhizus	A-93	81	Yellow	+ + +	+ + +	+ + +
	A-141	35	Yellow	+ + +	+ + +	+ + +
Rhizopogon ellenae	8974	81	White	+ + +	+ + +	+ + +
R. luteolus	A-5	66	White–beige	+ + +	+ + +	+
	A-106	15	White–beige	+ + +	+ + +	+
	G-823	95	White–beige	+ + +	+ + +	+
R. roseolus	A-7	55	White–pinkish	+ + +	+ + +	+ + +
	A-96	53	White–pinkish	+ + +	+ + +	+ + +
	A-160	17	White–pinkish	+ + +	+ + +	+ + +
	M-1	53	White–pinkish	+ + +	+ + +	+ + +
	M-2	60	White–pinkish	+ + +	+ + +	+ + +
R. vulgaris	A-56	70	White–pinkish	+ +	+ + +	+ + +
	A-98	44	White–pinkish	+ +	+ + +	+ + +
	A-101	52	White–pinkish	+ +	+ + +	+ + +
Scleroderma citrinum	A-37	75	White	+ + +	+ + +	+ + +
Suillus bovinus	A-21	35	White	+	+ +	–
	A-22	19	White	+	+ +	–
	A-75	58	White	+	+ +	–
S. collinitus	I-1	80	White	+ +	+ + +	–
S. granulatus	A-140	65	White–beige	+ + +	+ + +	+ + +
S. luteus	A-33	52	White	+	+ + +	+ + +
	A-57	33	White	+	+ + +	+ + +
	A-155	77	White	+	+ + +	+ + +
S. variegatus	A-142	74	White	+ +	+ + +	+ + +
Thelephora terrestris	R-34	32	Pale–beige	+	+	+
	R-38	8	Pale–beige	+	+	+
	S-63	30	Pale–beige	+	+	+
Xerocomus chrysenteron	A-147	17	White–brown	+ +	+ + +	+ + +

[a] Percentage of ectomycorrhizal short roots

[b] Hartig net development: + outer layer of cortex, + + first and second layers of cortex, + + + reaching third layer of cortex

[c] Mycelial growth extending from mantle around infected rootlets: – undetectable, + sparse, + + abundant, + + + very abundant

[d] Rhizomorphs or mycelial strands: – absent, + scarce, + + abundant, + + + very abundant

Dighton J, Mason PA (1985) Mycorrhizal dynamics during forest tree development. In: Moore D, Casselton LA, Woods DA, Frankland JC (eds) Development biology of higher fungi. (British Mycology Society Symposium 10) Cambridge University Press, Cambridge, pp 117–139

Doumas P, Berjaud C, Calléja M, Coupé M, Espiau C, d'Auzac J (1986) Phosphatases exatracellulaires et nutrition phosphatée chez les champignons ectomycorhiziens et les plantes hôtes. Physiol Veg 24:173–184

Fernandez de Ana FJ, Rodríguez A, Rodríguez-Fernández RJ (1989) A influencia dos tratamentos silvicolas na micetacion dos macromicetos. III Congreso Luso-Galaico de Macromicoloxía, Vila Real, Portugal, pp 1–20

Ferreira dos Santos N (1941) Elementos para o estudio das micorrizas ectendotróficas do *Pinus pinaster* Sol. Publ Serv Florestais Agric Portugal 8:65–95

Fleming LV, Deacon JW, Last FT (1986) Ectomycorrhizal succession in a Scottish birch wood. In: Gianinazzi-Pearson V, Gianinazzi S (eds) Mycorrhizae: physiology and genetics. INRA, Paris, pp 259–264

Gil L, Gordo J, Alia R, Catalán G, Pardos JA (1990) *Pinus pinaster* Aiton en el paisaje vegetal de la Peninsula Ibérica. Ecología 1:469–495

Godbout C, Fortin JA (1983) Morphological features of synthesized ectomycorrhizae of *Alnus crispa* and *A. rugosa*. New Phytol 94:249–262

Guinberteau J, Ducamp M, Poipou N, Mamoun M, Olivier JM (1990) Ecology of various competitors from an experimental plot of *Pinus pinaster* inoculated with *Suillus granulatus* and *Lactarius deliciosus*. Agric Ecosyst Environ 28:161–165

Heim R (1957) Les champignons d'Europe, vol I. Boubée, Paris

Heim R, Font Quer P, Codina J (1934) Fungi Iberici. Observations sur la Flore Mycologique Catalane. Treb Mus Cienc Nat Barcelona, Ser Bot 15:1–146

Kropp BR (1982) Fungi from decayed wood as mycorrhizal symbionts of western hemlock. Can J For Res 12:36–39

Kropp BR, Trappe JM (1982) Ectomycorrhizal fungi of *Tsuga heterophylla*. Mycologia 74:479–488

Malajczuk N, Molina R, Trappe JM (1982) Ectomycorrhiza formation in *Eucalyptus. I. Pure culture synthesis, host specificity and mycorrhizal compatibility with Pinus radiata*. New Phytol 91:467–482

Marx DH (1969) The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. Phytopathology 59:153–163

Marx DH (1977) Tree host range and world distribution of the ectomycorrhizal fungus *Pisolithus tinctorius*. Can J Microbiol 23:217–223

Marx DH (1980) Ectomycorrhizal fungus inoculations: a tool for improving forestation practices. In: Mikola P (ed) Tropical mycorrhiza research. Oxford University Press, New York, pp 13–71

Marx DH (1991) The practical significance of ectomycorrhizae in forest establishment. In: Ecophysiology of ectomycorrhizae of forest trees. Proceedings of the Marcus Wallenberg Foundation, no 7, Stockholm, Sweden, pp 54–90

Marx DH, Bryan WC (1970) Pure culture synthesis of ectomycorrhizae by *Thelephora terrestris* and *Pisolithus tinctorius* on different conifer hosts. Can J Bot 48:639–643

Mason PA, Last FT, Pelham J, Ingleby K (1982) Ecology of some fungi associated with an ageing stand of birches (*Betula pendula* and *B. pubescens*). For Ecol Manag 4:19–39

Mason PA, Wilson J, Last FT, Walker C (1983) The concept of succession in relation with the spread of sheathing mycorrhizal fungi on inoculated tree seedlings growing in unsterile soil. Plant Soil 71:247–256

Molina R (1979) Pure culture synthesis and host specificity of red alder mycorrhizae. Can J Bot 57:1223–1228

Molina R, Palmer JG (1982) Isolation, maintenance, and pure culture manipulation of ectomycorrhizal fungi. In: Schenck NC (ed) Methods and principles of mycorrhizal research. American Phytopathological Society, St Paul, Minn, pp 115–129

Molina R, Trappe JM (1982) Patterns of ectomycorrhizal host specificity and potential among Pacific Northwest conifers and fungi. For Sci 28:423–457

Mousain D (1971) Essai d'analyse de la symbiose ectomycorhizienne chez le Pin maritime. PhD thesis, University of Science and Technology, Montpellier

Mousain D, Lamond M (1978) Influence de la concentration en phosphate ($H_2PO_4^-$) de la solution nutritive sur la mycorhization du *Pinus pinaster* par le *Pisolithus tinctorius* et l'*Hebeloma cylindrosporum* (élevage sur caisson à aèrosol nutritif). In: Riedacker A, Gagnaire-Michard J (eds) IUFRO C R Symposium: Physiologie des Racines et Symbioses, Nancy, pp 473–475

Mousain D, Delmas J, Poitou N (1977) Influences of the ectomycorrhizae of *Hebeloma cylindrosporum* and *Pisolithus tinctorius* on the growth of young cluster pine. Proceedings of the 3rd North American Conference on Mycorrhizae, Athens, Georgia, p 94

Mousain D, Couteaudier Y, Pierson J (1979) Synthèses de mycorhizes du *Lactarius deliciosus* chez le *Pinus pinaster*. Ann Phytopathol 11:130

Mousain D, Poitou N, Delmas J (1979) La symbiose mycorhizienne: resultats obtenus avec l'*Hebeloma cylindrosporum* et le *Pisolithus tinctorius*, et perspectives d'application agronomique. Mushroom Sci 10:949–956

Nicolas A, Gandullo JM (1967) Ecología de los pinares españoles. I. *Pinus pinaster* Ait. IFIE, Madrid

Plassard C, Mousain D, Salsac L (1983) Dosage de la chitine sur des ectomycorhizes de pin maritime (*Pinus pinaster*) à *Pisolithus tinctorius*: évaluation de la masse mycèlienne et de la mycorhization. Can J Bot 22 61:692–699

Plassard C, Coll A, Mousain D, Salsac L (1988) Estimation quantitative de l'infection ectomycorhizienne du Pin maritime (*Pinus pinaster* Aiton) en pépinière. Acta Oecol Plant 9:381–391

Poitou N, Mamoun M, Delmas J (1982) Quelques résultats obtenus concernant la mycorhization de plantes-hôtes par les champignons mycorhiziens comestibles. In: Gianinazzi S, Gianinazzi-Pearson V, Trouvelot A (eds) Les mycorhizes: biologie et utilization. (Les Colloques de l'INRA, no 13) INRA, Paris, pp 295–301

Rancillac M (1982) Multiplication vegetative *in vitro* et synthese mycorhizienne: pin maritime – hebelome, pisolithe. In: Gianinazzi S, Gianinazzi-Pearson V, Trouvelot A (eds) Les micorhizes: biologie et utilization. (Les Colloques de l'INRA, no 13) INRA, Paris, pp 351–357

Rayner MC, Levisohn I (1941) The mycorrhizal habit in relation to forestry. IV. Studies on mycorrhizal response in *Pinus* and other conifers. Forestry 15:1–36

Ruehle JL, Marx DH, Aborouh M (1981) Development of *Pisolithus tinctorius* and *Thelephora terrestris* ectomycorrhizae on seedlings of coniferous trees important to Marocco. Ann Rech For Maroc 21:283–296

Scheromm P, Plassard C, Salsac L (1990) Nitrate nutrition of maritime pine (*Pinus pinaster* Soland in Ait.) ectomycorrhizal with *Hebeloma cylindrosporum* Romagn. New Phytol 114:93–98

Scott CW (1962) A summary of information of *Pinus pinaster* Ait. For Abstr 23

Singer R (1945) Notes on Farlow's Agaricales from Chocorua. Farlowia 2:39–51

Takacs EA (1961) Algunas especies de hongos formadores de micorrizas en árboles forestales en la Argentina. Rev For Argentina 5:80–82

Trappe JM (1962) Fungus associates of ectotrophic mycorrhizae. Bot Rev 28:538–606

Trappe JM (1964) Mycorrhizal hosts and distribution of *Cenococcum graniforme*. Lloydia 27:100–106

Trappe JM (1969) Studies on *Cenococcum graniforme*. I. An efficient method for isolation from sclerotia. Can J Bot 47:1389–1390

Trappe JM (1977) Selection of fungi for ectomycorrhizal inoculation in nurseries. Annu Rev Phytopathol 15:203–222

Trofymow JA (1990) A review of outplanting trials with ectomycorrhizal conifer seedlings. Proceedings of the 8th North American Conference on Mycorrhizae, Jackson, Wyoming, p 289

Vidal JM (1991) Contribución al conocimiento de la flora micológica del Baix Emporda y zonas limítrofes (Catalunya). IV. Hongos hipogeos (Zygomicotina, Ascomycotina y Basidiomycotina). Bull Soc Catalana Micol 14–15:143–194

Villeneuve N, Le Tacon F, Bouchard D (1991) Survival of inoculated *Laccaria bicolor* in competition with native ectomycorrhizal fungi and effects on the growth of outplanted Douglas fir seedlings. Plant Soil 135:95–107