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Ectomycorrhizal fungi of *Pinus pinea* L. in northeastern Spain

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Abstract Although *Pinus pinea* L. is an important forest species in the Mediterranean region, few reports exist on its ectomycorrhizal associates. Sixty isolates, obtained from fungal sporocarps collected in mixed forests of *P. pinea* in Catalonia (northeastern Spain), were tested for ectomycorrhiza formation on containerized *P. pinea* seedlings when applied as mycelial inoculum produced in peat-vermiculite. A total of 17 isolates, in 8 genera (*Amanita*, *Hebeloma*, *Laccaria*, *Lactarius*, *Pisolithus*, *Rhizopogon*, *Scleroderma* and *Suillus*), formed ectomycorrhizas and the percentages of mycorrhizal short roots varied among isolates and species from 13% to 89%. Some of these fungi are cited for the first time in association with *P. pinea*. The results indicate further fungal candidates for controlled inoculation of *P. pinea* seedlings in the nursery.

Key words Stone pine · Synthesis of mycorrhizas · Containerized seedlings · Mycelial inoculum produced in peat-vermiculite

Introduction

Pinus pinea L. is one of the most representative conifer species of the Mediterranean region. It extends from Portugal to the Syrian and Lebanese coastal areas, although 75% of its world-wide distribution is located in the Iberian peninsula (Agrimi and Ciancio 1994). It tolerates the hot and dry conditions associated with the Mediterranean climate and occurs in a wide range of soils and sites, but is usually found in infertile, sandy soils unfavourable for other forest species (Montoya 1989). *Pinus pinea* is mainly valued for its edible nuts and it plays an important ecological role in arid and semi-arid zones by preventing erosion (Montero et al. 1997). This species is thus useful in standard reforestation and the afforestation of marginally economic agricultural lands.

Different species of *Pinus* have been used frequently in reforestation programs around the world (Castellano 1994, 1996). The association of plant roots with ectomycorrhizal (EM) fungi is an important factor in the performance of outplanted conifers (Marx 1991; Castellano 1996) and it has been demostrated that *Pinus* spp. are dependent on the symbiosis to reach optimal development under natural conditions (Marx 1980; Molina et al. 1992). The application of controlled inoculation techniques to nursery-grown seedlings can be a useful tool for enhancing field performance of outplanted seedlings (Cordell et al. 1987). However, not all fungi produce the same effects on a particular tree species and various criteria must be taken into account in the selection of the most effective EM fungi in a nursery inoculation program (Trappe 1977; Marx 1980; Molina and Trappe 1984).

For selection of appropiate fungi, the fungal symbionts of the plant species must first be defined. Various methods have been developed for determining the ability of a fungus to form ectomycorrhizas with a particular tree species (Melin 1922; Hacskaylo 1953; Marx and Zak 1965; Fortin et al. 1980; Molina and Palmer 1982; Duddridge 1986; Stenström and Unestam 1987; Wong and Fortin 1989). Most require axenic conditions but non-axenic methods have also been used (Marx 1976; Danielson et al. 1984; Duñabeitia et al. 1996).

Cenococcum geophilum Fr. (Trappe 1962), *Hebeloma crustuliniforme* (Bull. ex St. Am.) Quél. (Branzanti et al. 1985), *Hebeloma sinapizans* (Paul. ex Fr.) Gillet (Branzanti and Zambonelli 1988), *Laccaria laccata* (Scop. ex Fr.) Bk. & Br. (Branzanti et al. 1985; Branzanti and Zambonelli 1988), *Lactarius deliciosus* (L. ex Fr.) S. F. Gray (Trappe 1962), *Lactarius hygrophoroides* Berk & Curt. (Barsali 1922), *Paxillus involutus*

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(Batsch. ex Fr.) Fr. (Branzanti and Zambonelli 1988), *Pisolithus tinctorius* (Pers.) Cok. & Couch (Mousain et al. 1987, 1994), *Rhizopogon roseolus* (Corda ex Sturm) Fries (Mousain et al. 1987, 1994), *Suillus collinitus* (Fr.) O. Kuntze (Mousain et al. 1987; 1994), *Suillus granulatus* (L. ex Fr.) O. Kuntze (Takacs 1961; Branzanti and Zambonelli 1988), *Suillus luteus* (L. ex Fr.) S. F. Gray (Trappe 1962), *Tuber albidum* Pico (Branzanti and Zambonelli 1984; Pascualini et al. 1992) and *Tuber borchii* Vitt. (Barsali 1922; Ceruti 1965) have been cited in association with *P. pinea*.

Research is currently under way, mainly in France and Spain, to select EM fungi to improve field performance of outplanted *P. pinea* seedlings. This present work is an initial step to increase current knowledge of EM fungi associated to *P. pinea* in order to develope a selection process for their use in nursery inoculation programs.

Materials and methods

Sporocarps of putative EM fungi were collected throughout the year in mixed forest of *P. pinea* in diverse locations of Catalonia (northeastern Spain). Site characteristics were recarded. Isolation and taxonomic identification were carried out on the different sporocarps collected. To obtain pure culture isolates, the sporocarps were externally brushed and pieces of inner tissue were taken and placed in Petri dishes filled with modified Melin-Norkrans agar medium (MMN) (Marx 1969). Cultures were incubated for 7–30 days at 25 °C, depending on the species. The isolates obtained were transferred to MMN-filled tubes and maintained by transfer on MMN every 3 months (Molina and Palmer 1982; Smith and Onions 1994).

Pinus pinea seeds were obtained from natural forests in the Montnegre and Montseny Sierras in Catalonia. Seeds were rinsed overnight in running tap water, surface disinfected by shaking for 60 min in 30% H_2O_2, and washed in two changes of sterile distilled water. Disinfected seeds were sown on trays (10 seeds per tray) filled with wet, sterilized perlite (30 min, 120 °C), and germinated in a greenhouse under controlled temperature (18–25 °C) and humidity conditions (>40%).

The synthesis of ectomycorrhizas was carried out on containerized seedlings under greenhouse conditions, using vegetative mycelial inocula of 60 different fungal isolates (Table 1). To obtain the vegetative inoculum, each fungal isolate was grown in MMN liquid medium (25 °C, 7–15 days) and then 40–50 ml of the mycelial suspension was transferred to 1-l bottles containing an autoclaved (20 min, 120 °C) mixture of peat-vermiculite (50–550 ml) moistened with 350 ml of MMN liquid medium (glucose reduced to 2.5 g/l). Inoculated bottles were incubated at 25 °C in the dark for 1 month. The peat-vermiculite inoculum was mixed with sterilized substrate (peat-vermiculite, 1:1, v:v; pH 5.5) in the proportion 1:4 (inoculum:substrate by volume). Emergent *P. pinea* seedlings were transferred from germination trays to 175-ml containers (Sherwood, Rootrainers, Spencer-Lemaire) filled with the inoculated substrate. A total of 14 plants were inoculated with each fungal isolate tested. The inoculated plants were grown in a shaded greenhouse with a controlled enviroment (20–25 °C and >40% humidity) for 4 months. At the end of this period, plants were removed from the containers and their root systems washed prior to examination for the presence of ectomycorrhizas. To assess the percentage of colonized short roots, at least 200 randomly selected short roots of each plant were counted under the stereomicroscope. Samples of infected roots from each seedling were sectioned transversely for microscopic examination of Hartig net formation.

Results

A total of 289 sporocarps (80 species in 18 genera) of putative EM fungi were collected and 109 isolates obtained in pure culture. Fungi belonging to the genera *Boletus*, *Lactarius*, *Pisolithus*, *Rhizopogon* and *Suillus* were easily isolated in pure culture (in more than 50% of the cases), in contrast to species of *Laccaria* and *Russula*, which were difficult to isolate from sporocarps (10% of the cases). From a total of 60 fungal isolates tested (Table 1), 17 (nine species) formed ectomycorrhizas with containerized *P. pinea* seedlings (Tables 2, 3). The morphological characteristics of the ectomycorrhizas formed are summarized in Table 3. The percentages of ectomycorrhizas formed by *P. pinea* seedlings varied among species and among isolates of the same species (Table 2). *Rhizopogon roseolus* isolates were the most infective, producing percentages of mycorrhizal short roots of 63–89%. *Lactarius chrysorrheus* and *Pisolithus tinctorius* isolates showed a variable infectivity, with percentages of mycorrhizal short roots of 39–76% and 22–71%, respectively. This variability among isolates of the same species was also observed in *Hebeloma crustuliniforme* and *Laccaria laccata* (Table 2). Other species in the genera *Boletus*, *Cortinarius*, *Hygrophorus*, *Leccinum*, *Lepista*, *Tricholoma* and *Xerocomus* failed to form ectomycorrhizas under the experimental conditions used in this study.

Discussion

As with other synthesis methods, the non-axenic synthesis used here was done under relatively artificial conditions and the results must be interpreted cautiously when drawing conclusions about their natural occurrence. Formation of ectomycorrhizas under synthesis conditions indicates that such a fungus-plant association is possible, but negative results are not conclusive and at best suggest that such relationships seem improbable (Molina and Palmer 1982; Brundrett et al. 1996).

Fungal species belonging to the genera *Amanita*, *Boletus*, *Cortinarius*, *Tricholoma* and *Xerocomus* failed to form ectomycorrhizas with containerized *P. pinea* seedlings, although members of these fungal genera have been cited as ectomycorrhizal with other tree species (Trappe 1962). Similar results with*Boletus*, *Tricholoma* and *Xerocomus* species have been reported for other tree species (Kropp 1982; Kropp and Trappe 1982; Godboud and Fortin 1983; Danielson et al. 1984; Pera and Alvarez 1995; Parlade et al. 1996). These fungi may be particularly prone to losing infectivity when grown in artificial media (Marx 1981) or the type of inoculum used in these experiments may not have been optimal for these fungi. All of these fungal genera belong to the late-stage group of fungi that form symbioses with mature trees instead of seedlings (Deacon and Fleming

Table 1 Fungal isolates tested in synthesis of ectomycorrhizas with containerized *Pinus pinea* seedlings (Aalb *Abies alba*, Fsyl *Fagus sylvatica*, Phal *Pinus halepensis*, Pmen *Pseudotsuga menziesii*, Pnig *Pinus nigra*, Ppin *Pinus pinea*, Ppter *Pinus pinaster*, Prad *Pinus radiata*, Psyl *Pinus sylvestris*, Qile *Quercus ilex*, Qpub *Quercus pubescens*, Qsub *Quercus suber*)

Fungus[a]	Isolate number	Potential associated host	Year isolated	Location
Amanita sp.	185	Ppter, Prad	1994	Breda[c]
Amanita citrina (Schaeff. ex) S. F. Gray	181	Ppin, Qile	1994	Cabrera de Mar[b]
Amanita pantherina (D.C. ex Fr.) Kummer	207	Qsub, Ppin	1994	Forallac (Fitor)[c]
	234	Psyl	1995	St. Hilari Sacalm[c]
Amanita rubescens (Pers. ex Fr.) S. F. Gray	208	Qpub	1994	Forallac (Fitor)[c]
Boletus sp.	203	Ppin, Qile	1994	Breda[c]
	241	Qsub	1995	Llinars del Vallès[b]
	260	Qsub, Qile	1995	Forallac (Fitor)[c]
	267	Ppter	1995	Forallac (Fitor)[c]
	268	Ppter	1995	Forallac (Fitor)[c]
Boletus erythropus (Fr. ex Fr.) Pers.	292	Aalb	1995	Fogars de Montclús[b]
Cortinarius sp.	258	Fsyl	1995	Fogars de Montclús[b]
Hebeloma crustuliniforme (Bull. ex St. Amans)	265	Abies sp.	1995	Arbúcies[c]
	290	Abies sp.	1995	Arbúcies[c]
Hygrophorus russula (Schaeff. ex Fr.) Quél.	225	Ppter	1994	Fogars de Montclús[b]
Laccaria laccata (Scop. ex Fr.) Berk. & Br.	127	Qile	1989	Arbúcies[c]
	226	Qsub, Ppin	1994	Forallac (Fitor)[c]
Lactarius chrysorrheus Fr.	231	Ppin	1994	St Celoni[b]
	273	Ppin	1995	Forallac (Fitor)[c]
	275	Qsub	1995	Arbúcies[c]
	201	Ppin, Qile	1994	Breda[c]
	262	Pnig	1995	Arbúcies[c]
Lactarius deliciosus L. ex Fr.	178	Ppin	1994	Cabrera de Mar[b]
	216	Ppin	1994	Forallac (Fitor)[c]
	274	Ppin	1995	Forallac (Fitor)[c]
Lactarius sanguifluus Paulet ex Fr.	261	Prad	1995	Arbúcies[c]
	263	Ppter	1995	Forallac (Fitor)[c]
Leccinum sp.	278	Qsub, Qile	1995	Forallac (Fitor)[c]
Lepista nuda (Bull. ex Fr.) Cooke	209	Qsub	1994	Forallac (Fitor)[c]
Pisolithus tinctorius (Pers.) Coker & Couch.	93	Qsub	1986	Tossa de Mar[c]
	193	Qsub, Ppin	1994	Sta Coloma de Farners[c]
	202	Qsub	1994	Forallac (Fitor)[c]
	233	Qsub	1995	St Hilari Sacalm[c]
	249	Qile, Qsub	1995	Fogars de Montclús[b]
Rhizopogon luteolus Fr. & Nordh.	252	Pnig	1995	Fogars de Montclús[b]
Rhizopogon roseolus (Corda ex Sturm) Fr.	173	Ppin	1994	Orrius[b]
	196	Ppter, Prad	1994	Breda[c]
	183	Ppin	1994	Breda[c]
	179	Ppter	1994	Mozoncillo (Segovia)
	294	Prad	1995	Breda[c]
Russula sp.	200	Ppin, Qub	1994	Palautordera[c]
Russula foetens Pers. ex Fr.	269	Qsub, Qile	1995	Forallac (Fitor)[c]
Scleroderma sp.	281	Abies sp.	1995	Arbúcies[c]
Scleroderma verrucosum Bull. ex Pers. ss. Grév.	266	Abies sp.	1995	Arbúcies[c]
Suillus sp.	164	Phal	1994	Breda[c]
Suillus bellinii (Inz.) Watling	174	Ppin	1994	Cabrils[b]
	240	Qsub	1995	Llinars del Vallès[b]
	255	Ppin	1995	Forallac (Fitor)[c]
Suillus granulatus (L. ex Fr.) O. Kuntze	232	Psyl	1995	St Hilari Sacalm[c]
	277	Ppter	1995	Forallac (Fitor)[c]
Suillus luteus (L. ex Fr.) S. F. Gray	251	Fsyl	1995	Forallac (Fitor)[c]
Tricholoma spp.	175	Ppin, Qile	1994	Vallgorguina[b]
Xerocomus badius (Fr.) Kühner ex Gilbert	167	Qsub	1994	Sta Coloma de Farners[c]
	271	Qsub, Qile	1995	Forallac (Fitor)[c]
Xerocomus ferrugineus Schaeffer	168	Qsub	1994	Sta Coloma de Farners[c]
Xerocomus subtomentosus (L. ex Fr.) Quél.	176	Ppin, Qsub	1994	Vilalba Saserra[b]
	177	Ppin, Qsub	1994	Palautordera[c]
	247	Qile, Qsub	1995	Fogars de Montclús[b]
	250	Qsub	1995	St Feliu de Buixalleu[c]
	279	Qsub	1995	Forallac (Fitor)[c]

[a] Voucher specimens of those fungal isolates that formed ectomycorrhizas with containerized *P. pinea* seedlings were deposited in the Real Jardín Botánico de Madrid Herbarium (Spain), others are being kept in the herbarium of the Department de Patologia Vegetal (IRTA)

[b] Barcelona

[c] Girona

Table 2 Fungal isolates that formed ectomycorrhizas with containerized *P. pinea* seedlings (new syntheses with numbers) and ectomycorrhizal fungi previously cited in the literature for this conifer (– not given)

Fungus	Isolate number or literature reference	Mycorrhizae colonization (% ± SD)
Amanita sp.	185	56 ± 20.2
Cenococcum geophilum Fr.	Trappe (1962)	–
Hebeloma crustuliniforme (Bull. ex St. Am.) Quél.	290	45 ± 5.6
	265	13 ± 6.8
	Branzanti et al. (1985)	87
Hebeloma sinapizans (Paul. ex Fr.) Gillet	Branzanti and Zambonelli (1988)	90
Laccaria laccata (Scop. ex Fr.) Bk. & Br.	127	24 ± 13.2
	Branzanti et al. (1985)	93
	Branzanti and Zambonelli (1988)	72
Lactarius chrysorrheus Fr.	275	71 ± 8.1
	262	22 ± 18.8
Lactarius deliciosus (L. ex Fr.) S. F. Gray	274	50 ± 15.2
	Trappe (1962)	–
Lactarius hygrophoroides Berk & Curt.	Barsali (1922)	–
Paxillus involutus (Batsch. ex Fr.) Fr.	Branzanti and Zambonelli (1988)	64
Pisolithus tinctorius (Pers.) Cok, & Couch (=*P. arhizus* (Scop:Pors.) Rausch.)	249	58 ± 5.6
	193	39 ± 14.8
	93	76 ± 13.0
	Mousain et al. (1987; 1994)	–
Rhizopogon roseolus (Corda ex Sturm) Fries	179	89 ± 9.9
	183	88 ± 5.8
	196	63 ± 24.9
	Mousain et al. (1987; 1994)	–
Scleroderma sp.	281	82 ± 15.7
Suillus collinitus (Fr.) O. Kuntze	Mousain et al. (1987; 1994)	–
Suillus bellinii (Inz.) Waltling	164	48 ± 16.2
	174	48 ± 15.9
	240	41 ± 11.6
Suillus granulatus (L. ex Fr.) O. Kuntze	Takacs (1961)	–
	Branzanti and Zambonelli (1988)	58
Suillus luteus (L. ex Fr.) S. F. Gray	Trappe (1962)	–
Tuber albidum Pico	Branzanti and Zambonelli (1984)	–
	Pascualini et al. (1992)	–
Tuber borchii Vitt.	Barsali (1922)	–
	Ceruti (1965)	–

Table 3 General morphological characteristics of ectomycorrhizas formed on *P. pinea*

Fungus	Isolate	Color of mantle	Hartig net[a]	Mycelial growth[b]	Rhizomorphs/ mycelial strands[c]
Amanita sp.[d]	185	White	+ +	+	+ +
Hebeloma crustuliniforme	290	White	+ +	+ + +	−
	265	White	+ +	+ + +	−
Laccaria laccata	127	Beige-violet	+ +	+	−
Lactarius chrysorrheus[d]	275	Beige-pale orange	+ +	+	−
	262	Beige-pale orange	+ +	+	−
Lactarius deliciosus	274	Orange-green	+ +	+	−
Pisolithus tinctorius	249	Yellow	+ + +	+ + +	+ + +
	193	Yellow	+ + +	+ + +	+ + +
	93	Yellow	+ + +	+ + +	+ + +
Rhizopogon roseolus	179	White-pink	+ + +	+ + +	+ + +
	183	White-pink	+ + +	+ + +	+ + +
	196	White-pink	+ + +	+ + +	+ + +
Scleroderma sp.[d]	281	White	+ +	+ + +	+ +
Suillus bellinii[d]	164	White-pink	+ +	+ + +	+ +
	174	White-pink	+ +	+ + +	+ +
	240	White-pink	+ +	+ + +	+ +

[a] Hartig net development: + outer layer of cortex cells, + + first and second layers of cortex, + + + first to third layers of cortex
[b] Mycelial growth extended from mantle: − undetectable, + sparse, + + abundant, + + + very abundant
[c] Rhizomorphs or mycelial strands: − absent, + scarce, + + abundant, + + + very abundant
[d] First report of this species together with *P. pinea*

1992). Other fungal isolates belonging to the genera *Hygrophorus*, *Leccinum* and *Lepista* did not form ectomycorrhizas with *P. pinea*. Some species of these genera are known to be saprophytic, while others have been reported more frequently in association with angiosperms than with gymnosperms (Trappe 1962). The negative results do not exclude the possibility that *P. pinea* could form ectomycorrhizas with these fungi under natural conditions but it precludes future evaluations of these fungi for seedling inoculation.

Most of the isolates that formed ectomycorrhizas with containerized *P. pinea* seedlings belong to species commonly observed in association with stone pine in the field: *Lactarius* spp., *Pisolithus tinctorius*, *Rhizopogon roseolus* and *Suillus* spp. Of the 17 isolates that formed ectomycorrhizas, nine infected over 50% of *P. pinea* short roots. Such information may be useful in selecting aggressive fungal isolates for nursery inoculations with vegetative inocula. The colonization ability of a fungus is a selection criterium for candidates in nursery inoculation programs. It has been suggested that the colonization level of the root system is related to the fungal effect on outplanted seedlings (Marx 1980; Ruehle et al. 1981; Trofymow 1990), although the minimal colonization level needed to ensure expression of a potential beneficial effect is unknown for large numbers of EM fungi. Among the 17 isolates which formed ectomycorrhizas with *P. pinea* in our experiments, *Laccaria laccata* and *Pisolithus tinctorius* are known to be broad-host-range, worldwide fungi (Molina 1982; Marx 1991). These, in addition to *Hebeloma crustuliniforme*, *Rhizopogon roseolus* and *Suillus* spp. have been cited previously for *P. pinea* (Table 2). Our results extend the number of EM fungi of *P. pinea* to *Amanita* sp., *Lactarius chrysorrheus*, *Scleroderma* sp. and *Suillus bellinii*.

Rhizopogon, *Scleroderma* and *Suillus* spp. produce large quantities of rhizomorphs, structures related to the enhancement of water uptake by the plant (Duddridge et al. 1980; Cairney 1992). This characteristic could be of interest in the selection of ectomycorrhizal fungi for *P. pinea*, which is usually grown under the dry conditions associated with a Mediterranean climate.

The difficulty in obtaining EM seedlings inoculated with mycelial inoculum of *Rhizopogon* spp. has been reported previously (Molina 1980; Molina and Trappe 1994). The results obtained in this present study indicate the possibility of inoculating *P. pinea* seedlings with *Rhizopogon* mycelia at high inoculum rates, although, for practical application in nursery inoculations, more research is needed to determine the success of this type of inoculum when applied at lower rates.

The results obtained are the beginning of an ectomycorrhizal selection process to obtain *P. pinea* seedlings inoculated with ectomycorrhizal fungi and suitable to withstand Mediterranean field conditions. Field experiments are currently in progress to determine the performance of *P. pinea* seedlings inoculated with most of the above-mentioned EM fungi (Parladé et al. 1997, 1998). At the same time, currently available molecular techniques (Armstrong et al. 1989; Gardes et al. 1991) could become extraordinarily powerful tools for identifying fungal species or fungal strains associated with their hosts in field situations, even on a single mycorrhiza. This would help in assays of distribution, persistence and competitive ability of introduced fungi. Data obtained on plant growth and survival, and on fungal persistence, will enable the selection of the most efficient EM strains.

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References

Agrimi M, Ciancio O (1994) Le pin pignon (*Pinus pinea* L.). 16ème Session du Comité CFFSA/CEF/CFPO des Questions Forestières Méditerranées. FAO. Silva Mediterranea. Larnaca, Chypre

Armstrong JL, Fowles NL, Rygiewicz PT (1989) Restriction fragment length polymorphisms distinguish ectomycorrhizal fungi. Plant Soil 116:1–7

Barsali E (1922) Contribuzione allo studio dei rapporti delle micorize ectotrofiche de alcune essenze arboree. Atti Soc Toscana Sci Nat Pisa 31:16–20

Branzanti B, Zambonelli A (1984) Sintesi micorrizica del *Tuber albidum* con *Pinus pinea*. Microbiol Ital 2:51–53

Branzanti B, Zambonelli A (1988) Influenza della micorrizazione su semenzali di *Pinus pinea* allevati in contenitore. Monti Boschi 4:53–56

Branzanti B, Zambonelli A, Govi G (1985) Micorrizazione di *Pinus pinea* con *Laccaria laccata* et *Hebeloma crustuliniforme*. Microbiol Ital 3:3–10

Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N (1996) Working with mycorrhizas in forestry and agriculture. Monograph 32. ACIAR. Canberra, Australia

Cairney JWG (1992) Translocation of solutes in ectomycorrhizal and saprotrophic rhizomorphs. Mycol Res 96:135–141

Castellano MA (1994) Current status of outplanting studies using ectomycorrhiza-inoculated forest trees. In: Pfleger FL, Linderman RG (eds) Mycorrhizae and plant health. AP, St. Paul, Minn, pp 261–281

Castellano MA (1996) Outplanting performance of mycorrhizal inoculated seedlings. In: Mukerji KG (ed) Concepts in mycorrhizal research. (Handbook of vegetation science 19/2) Kluwer, Dordrecht, The Netherlands, pp 223–301

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

Cordell CE, Owen JH, Marx DH (1987) Mycorrhizae nursery management for improved seedling quality and field performance. Meeting the challenge of the nineties: Proceedings of the Intermountain Forest Nursery Association. Oklahoma City, Okla. General Technical Report RM-151, pp 105–115

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

Deacon JW, Fleming LV (1992) Interactions of ectomycorrhizal fungi. In: Allen MJ (ed) Mycorrhizal functioning. An integrative plant-fungal process. Chapman & Hall, New York, pp 249–300

Duddridge JA (1986) The development and ultrastructure of ectomycorrhizas. III. Compatible and incompatible interactions between *Suillus grevillei* (Klotzsch) Sing. and 11 species of ectomycorrhizal hosts in vitro in the absence of exogenous carbohydrates. New Phytol 103:457–464

Duddridge JA, Malibari A, Read DJ (1980) Structure and function of ectomycorrhizal rhizomorphs with special reference to their role in water transport. Nature 287:834–836

Duñabeitia MK, Hormilla S, Salcedo I, Peña JI (1996) Ectomycorrhizae synthesized between *Pinus radiata* and eight fungi associated with *Pinus* spp. Mycologia 88:897–908

Fortin JA, Piché Y, Lalonde M (1980) Technique for observation of early morphological changes during ectomycorrhiza formation. Can J Bot 58:361–365

Gardes M, White TJ, Fortin JA, Bruns TD, Taylor JW (1991) Identification of indigenous and introduced symbiotic fungi in ectomycorrhizae by amplification of nuclear and mitochondrial ribosomal DNA. Can J Bot 69:180–190

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

Hacskaylo E (1953) Pure culture synthesis of pine mycorrhizae in Terra-Lite. Mycologia 45:971–975

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

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 (1976) Synthesis of ectomycorrhizae on Loblolly pine seedlings with basidiospores of *Pisolithus tinctorius*. For Sci 22:13–20

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

Marx DH (1981) Variability in ectomycorrhizal development and growth among isolates of *Pisolithus tinctorius* as affected by source, age and reisolation. Can J For Sci 11:168–174

Marx DH (1991) The practical significance of ectomycorrhizae in forest establishment. In: Ecophysiology of ectomycorrhizae of forest trees. Proceedings of the Marcus Wallenberg Foundation Symposium 7:54–90

Marx DH, Zak B (1965) Effect of pH on mycorrhizal formation of slash pine in aseptic conditions. For Sci 11:66–75

Melin E (1922) Untersuchungen über die *Larix*-Mykorhiza. I. Synthese der Mykorhiza in Reinkultur. Sven Bot Tidskr 16:161–196

Molina R (1980) Ectomycorrhizal inoculations of containerized western conifer seedlings. USDA For Serv Res Note PNW-357

Molina R (1982) Use of the ectomycorrhizal fungus *Laccaria laccata* in forestry. I. Consistency between isolates in effective colonization of containerized conifer seedlings. Can J For Res 12:469–473

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 Phytopathology Society, St. Paul, Minn, pp 115–119

Molina R, Trappe JM (1984) Mycorrhiza management in bareroot nurseries. In: Duryea M, Landis TD (eds) Forest nursery manual. production of bareroot seedlings. Nijhoff/Junk, The Hague, pp 211–223

Molina R, Trappe JM (1994) Biology of the ectomycorrhizal genus *Rhizopogon*. I. Host associations, host-specificity and pure culture synthesis. New Phytol 126:653–675

Molina R, Massicotte H, Trappe JM (1992) Specificity phenomena in mycorrhizal symbiosis: community-ecological consequences and practical implications. In: Allen MJ (ed) Mycorrhizal functioning. Chapman & Hall, New York, pp 357–423

Montero G, Candela JA, Gutiérrez M, Pavón J, Ortega C, García CG, Cañellas I (1997) Manual de claras para repoblaciones de *Pinus pinea* L. EGMASA / Junta de Andalucía, Huelva

Montoya JM (1989) El pino piñonero. Agroguias Mundi-Prensa, Madrid

Mousain D, Falconnet J, Gruez J, Chevalier G, Tillard P, Bousquet N, Plassard C, Cleyet-Marel JC (1987) Controlled ectomycorrhizal development of mediterranean forest seedlings in the nursery. First results and prospects. In: Sylvia DM, Hung L-LL, Graham JH (eds) Proceedings of the 7th North American Conference on Mycorrhizae. University of Florida, Florida

Mousain D, Plassard C, Argillier C, Sardin T, Leprince F, El Karkouri K, Arvieu J-C, Cleyet-Marel JC (1994) Stratégie d'amélioration de la qualité des plants forestiers et des reboisements méditerranéens par utilisation de la mycorhization contrôlée en pépinière. Acta Bot Gallica 141:571–580

Parladé J, Alvarez IF, Pera J (1996) Ability of native ectomycorrhizal fungi from northern Spain to colonize Douglas-fir and other introduced conifers. Mycorrhiza 6:51–55

Parladé J, Pera J, Alvarez IF (1997) La mycorhization contrôlée du Douglas dans le nord de l'Espagne: premiers résultats en plantation. Rev For Fr 49:163–173

Parladé J, Alvarez IF, Rincón A, Pera J (1998) Updated results from experimental plantations with ectomycorrhizal trees in northern Spain. Ahonen-Jonnarth U, Danell E, Fransson P, Karén O, Lindahl B, Rangel I, Finlay R (eds) Abstracts of the Second International Conference on Mycorrhiza, July, 1998. SLU, Uppsala, Sweden, p 133

Pascualini S, Panara F, Antonielli M (1992) Acid phosphatase activity in *Pinus pinea-Tuber albidum* ectomycorrhizal association. Can J Bot 70:1377–1383

Pera J, Alvarez IF (1995) Ectomycorrhizal fungi of *Pinus pinaster*. Mycorrhiza 5:193–200

Ruehle JL, Marx DH, Bunett JP, Pawuk WH (1981) Survival and growth of container-grown and bare-root shortleaf pine seedlings with *Pisolithus tinctorius* and *Thelephora terrestris* ectomycorrhizas. South J Appl For 5:20–24

Smith D, Onions AHS (1994) The preservation and maintenance of living fungi. IMI technical handbooks, no. 2, International Mycological Institute. CAB, Wallingford, UK

Stenström E, Unestam T (1987) Ecological variation in pine mycorrhizae. Sylvia DM, Hung L-LL, Graham JH (eds) Proceedings of the 7th North American Conference on Mycorrhizae. University of Florida, Gainesville, Fla

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

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

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, Wyo

Wong KKY, Fortin JA (1989) A Petri dish technique for the aseptic synthesis of ectomycorrhizae. Can J Bot 67:1713–1716