Mycorrhiza (1996) 6:51–55

ORIGINAL PAPER

J. Parladé · I.F. Álvarez · J. Pera

Ability of native ectomycorrhizal fungi from northern Spain to colonize Douglas-fir and other introduced conifers

Accepted: 28 August 1995

Abstract Thirty-six isolates from 27 species of native ectomycorrhizal fungi collected in northern Spain were tested for ectomycorrhiza formation with *Pseudotsuga menziesii* seedlings in pure culture syntheses. Thirteen of those species were also tested for ectomycorrhiza formation with six other species of conifers (two native and four introduced) to compare their colonization potential. Twenty-three fungal isolates from 18 species formed ectomycorrhizas with *Pseudotsuga menziesii*. The colonization level of the root system varied markedly among the different fungal species. Eight fungi colonized over 50% of the short roots. Nine fungi did not form ectomycorrhizas even though some of them were collected in pure stands of *Pseudotsuga menziesii*. *Laccaria laccata*, *Lyophyllum decastes*, *Pisolithus tinctorius*, and *Scleroderma citrinum* formed abundant ectomycorrhizas on all the conifers tested. *Lactarius deliciosus*, *Rhizopogon* spp., and *Suillus luteus* showed the greatest host specificity. The success in the introduction of some exotic conifers for reforestation in northern Spain is discussed in relation to their compatibility with native ectomycorrhizal fungi.

Key words Douglas-fir · Ectomycorrhizas · Host specificity · Pure culture syntheses · Reforestation

Introduction

Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] was introduced to Europe in 1827. It was extensively planted after 1945 in many countries, and has become the most important North American tree for timber production in Europe, especially in France and Germany (Matthews 1983). Experimental tests to select adequate provenances of Douglas-fir for Spain have been carried out since 1963 (Toval et al. 1993). The ecological conditions in many areas of northern Spain are appropriate for the growth of Douglas-fir. Also, economic analysis of the revenues obtained per hectare from plantations of different conifer or hardwood species in this region indicate that Douglas-fir plantations are the most profitable (Anonymous 1992). Despite this, Douglas-fir has not been planted extensively in this region.

The strategy for the introduction of an exotic tree species should consider the occurrence of populations of compatible mycorrhizal fungi in the outplanting area (Trappe 1977; Marx 1980; Alvarez et al. 1993). Although Douglas-fir-associated ectomycorrhizal fungi may exceed 2000 species in its natural range (Trappe 1962a, 1977), the diversity of fungi associated with nursery seedlings is low, and fungal species present in nurseries may be replaced by others after outplanting (Le Tacon et al. 1984). Also, native populations of mycorrhizal fungi may be low in both afforested and disturbed areas (Le Tacon et al. 1984; Perry et al. 1987) or incompatible with the introduced species. In such cases, nursery inoculations with selected ectomycorrhizal fungi adapted to the ecological conditions of the planting area may be necessary (Mikola 1973; Trappe 1977; Marx 1980). Field trials of seedlings inoculated with selected ectomycorrhizal fungi often show increased seedling survival and growth in relation to uninoculated plants, especially in areas where compatible ectomycorrhizal fungi are lacking (Castellano and Trappe 1985; Stenström et al. 1985; Marx and Cordell 1988; Villeneuve et al. 1991; Le Tacon et al. 1992).

In this study, we conducted pure culture synthesis experiments between isolates from native ectomycorrhizal fungi collected in northern Spain and seven different species of conifers as part of a screening program to select ectomycorrhizal fungi for reforestation purposes. Our objectives were: (1) to test the symbiotic compatibility of the native fungi with Douglas-fir seedlings, and (2) to compare the colonization potential of different

J. Parladé (✉) · I.F. Álvarez · J. Pera
Departament de Patologia Vegetal, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Ctra. de Cabrils s/n, E-08348 Cabrils (Barcelona), Spain
Fax: +34–3–75 33 954

Table 1 Collections of the fungal isolates tested in pure culture synthesis with Douglas-fir. Fungal isolates preceded by an *asterisk* were also tested with Norway spruce, lodgepole pine, Monterey pine, ponderosa pine, maritime pine and black pine. (A.gra *Abies grandis* (Dougl.) Lindl., 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.rob *Quercus robur* L., Q.sub *Quercus suber* L.)

Fungal species	Isolate	Associated tree	Province	Altitude (m)	Soil pH	Isolation year
**Amanita aspera* (Fr.) Hooker	A-48	Q.rob	Oviedo	320	3.7	1986
A. citrina (Schff.) S. F. Gray	A-145	A.gra	Girona	1100	5.6	1990
**A. muscaria* (L. ex Fr.) Hooker	A-17	C.sat	Pontevedra	40	5.2	1985
A. muscaria (L. ex Fr.) Hooker	A-124	A.gra	Girona	1100	5.5	1989
A. rubescens (Pers. ex Fr.) Gray	A-148	F.syl	Girona	1100	5.6	1990
Boletus edulis Bull ex Fr.	A-39	P.syl	Oviedo	460	3.7	1986
B. erythropus Fr. ex Pers.	A-45	F.syl	Oviedo	530	4.1	1986
B. pulverulentus Opat.	A-146	P.men	Girona	1000	5.6	1990
Cenoccocum geophilum Fr.	A-144	F.syl	Girona	1200	5.5	1990
Cortinarius purpurascens Fr.	A-14	C.sat	Pontevedra	10	—	1985
Hebeloma sinapizans (Paulet ex Fr.) Gill.	A-126	P.abi	Barcelona	20	7.5	1990
**Laccaria laccata* (Scop. ex Fr.) Bk. & Br.	A-127	Q.ile	Girona	1000	4.9	1989
Lactarius deliciosus Fr.	A-81	P.pin	Oviedo	80	—	1986
**L. deliciosus* Fr.	A-120	P.pin	Girona	200	5.6	1988
L. deliciosus Fr.	A-159	P.pin	Barcelona	200	5.6	1990
L. rufus (Scop.) Fr.	A-34	P.syl	Oviedo	610	5.8	1986
**Lyophyllum decastes* (Fr.) Sing	A-71	P.rad	Oviedo	240	4.2	1986
Melanogaster ambiguus (Vitt.) Tul. & Tul.	A-132	P.men	Girona	1100	5.6	1989
**Paxillus involutus* (Batsch) Fr.	A-87	C.sat	Pontevedra	—	4.7	1986
**Pisolithus tinctorius* (Pers.) Coker & Couch	A-93	Q.sub	Girona	50	—	1986
**Rhizopogon luteolus* Fr. Nord.	A-5	P.pin	Pontevedra	5	—	1985
R. luteolus Fr. Nord.	A-106	P.pin	Pontevedra	400	4.7	1987
R. roseolus (Corda ex Sturm) Th. Fr.	A-7	P.rad	Oviedo	350	—	1985
**R. roseolus* (Corda ex Sturm) Th. Fr.	A-96	P.syl	Tarragona	1000	7.2	1987
R. subareolatus Smith	A-116	P.men	Girona	1100	5.5	1987
R. ventricisporus Smith	A-97	P.syl	Tarragona	1000	7.2	1987
R. vulgaris (Vitt.) M. Lange	A-56	P.rad	Oviedo	100	5.1	1986
**R. vulgaris* (Vitt.) M. Lange	A-101	P.pin	Pontevedra	—	4.7	1987
R. vulgaris (Vitt.) M. Lange	A-98	P.pin	Pontevedra	—	4.7	1987
**Scleroderma citrinum* Pers.	A-37	C.sat	Oviedo	—	—	1986
**Suillus bovinus* (L. ex Fr.) O. Kuntze	A-75	C.sat	Pontevedra	—	4.7	1986
S. bovinus (L. ex Fr.) O. Kuntze	A-21	P.rad	Oviedo	350	—	1985
**S. luteus* (L. ex Fr.) S. F. Gray	A-33	P.rad	Oviedo	100	—	1986
Tricholoma saponaceum (Fr.) Kummer	A-121	P.men	Girona	1100	5.5	1988
Xerocomus chrysenteron (Bull. ex St. Am.) Quél.	A-119	P.men	Girona	1100	5.5	1988
X. chrysenteron (Bull. ex St. Am.) Quél.	A-147	P.men	Girona	1100	5.5	1990

species of fungi on introduced and native conifer species.

Materials and methods

Seeds of seven species of conifers, five introduced into Spain [Douglas-fir *Pseudotsuga menziesii* (Mirb.) Franco, Norway spruce *Picea abies* (L.) Karst., lodgepole pine *Pinus contorta* Dougl. ex Loud., Monterey pine *Pinus radiata* D. Don. and ponderosa pine *Pinus ponderosa* Dougl. ex Laws.] and two native (maritime pine *Pinus pinaster* Ait. and black pine *Pinus nigra* Arnold) were surface sterilized with 30% hydrogen peroxide for 1 h, rinsed with sterile distilled water and germinated in glass vials containing 2% malt agar (Difco). When the radicle was 2 cm long, seedlings were transferred into glass synthesis tubes prepared according to the technique described by Molina (1979), modified with double the amount of peat.

Sporocarps of putative ectomycorrhizal fungi were collected in northern Spain in association with a variety of host plants and ecological habitats (Table 1). Fungal cultures were isolated from fragments of sporocarp tissue grown on modified MMN (Marx 1969) or PDA (Difco) as described by Molina and Palmer (1982). *Cenococcum geophilum* was isolated from surface-sterilized sclerotia (Trappe 1969) collected by sieving forest soil.

One month after sowing, seedlings were inoculated with 12 ml of mycelial slurries. Slurries were prepared by homogenizing 25-day-old fungal colonies in a Waring blender in sterile distilled water. A total of 36 fungal isolates, belonging to 27 species, was tested in pure culture with Douglas-fir seedlings. From them, a subset of 13 fungal species previously reported as mycorrhizal with *Pinus pinaster* (Pera and Alvarez 1995) was also tested on the remaining six species of conifers (Table 1). A total of five replicates was prepared for each host-fungus combination. The synthesis tubes were maintained for 3 months in a pure culture synthesis apparatus as described by Parladé and Alvarez (1993).

At the end of the growing period, seedlings were carefully removed from the tubes and the roots gently washed in tap water to clean off the substrate. Root systems were examined by stereomicroscopy to evaluate the percentage of ectomycorrhizal short roots. Four colonization levels were established from the median of five replicates: 1 = 1–25% of the short roots colonized, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100%. The median was chosen over the mean because the former is unaffected by erratic extreme values (Snedecor and Cochran 1980). Doubtful ectomycorrhizas were checked microscopically for Hartig net formation. Reisolation of the fungus from the substrate was carried out at the end of the growing period to detect possible contaminants.

Table 2 Fungal isolates that formed ectomycorrhizas with Douglas-fir seedlings in pure culture synthesis. Root colonization levels are assigned from the median of the percentage of ectomycorrhizas from five replicates: 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–100%

Fungal species	Isolate	Colonization level	First reference to association
Amantia aspera (Fr.) Hooker	A-48	2	—
A. muscaria (L. ex Fr.) Hooker	A-17	1	Chu-Chou and Grace (1983)
A. muscaria (L. ex Fr.) Hooker	A-124	1	Chu-Chou and Grace (1983)
A. rubescens (Pers. ex Fr.) Gray	A-148	3	—
Cenococcum geophilum Fr.	A-144	1	Trappe (1962b)
Hebeloma sinapizans (Paulet ex Fr.) Gill.	A-126	3	—
Laccaria laccata (Scop. ex Fr.) Bk & Br.	A-127	4	Trappe and Strand (1969)
Lactarius deliciosus Fr.	A-159	1	Molina and Trappe (1982)
L. rufus (Scop.) Fr.	A-34	1	—
Lyophyllum decastes (Fr.) Sing	A-71	4	—
Melanogaster ambiguus (Vitt.) Tul. & Tul.	A-132	2	—
Paxillus involutus (Batsch) Fr.	A-87	3	Laiho (1970)
Pisolithus tinctorius (Pers.) Coker & Couch	A-93	3	Marx (1977)
Rhizopogon roseolus (Corda ex Sturm) Th. Fr.	A-7	2	Parladé and Alvarez (1993)
R. roseolus (Corda ex Sturm) Th. Fr.	A-96	1	Parladé and Alvarez (1993)
R. subareolatus Smith	A-116	4	Parladé and Alvarez (1993)
R. vulgaris (Vitt.) M. Lange	A-56	2	—
R. vulgaris (Vitt.) M. Lange	A-98	1	—
R. vulgaris (Vitt.) M. Lange	A-101	1	—
Scleroderma citrinum Pers.	A-37	3	Jansen and De Nie (1988)
Suillus bovinus (L. ex Fr.) O. Kuntze	A-75	2	—
S. bovinus (L. ex Fr.) O. Kuntze	A-21	1	—
S. luteus (L. ex Fr.) S. F. Gray	A-33	1	—

Results

Douglas-fir seedlings were on average 9 cm tall after the growing period and formed well-developed root systems in all fungal treatments. All the fungi totally or partially colonized the substrate of the synthesis tubes by the end of the experiment. Twenty-three isolates in 18 species formed ectomycorrhizas (Table 2). Of these, nine species are confirmed as ectomycorrhizal with Douglas-fir for the first time.

The percentage of ectomycorrhizas formed on Douglas-fir seedlings varied markedly among species. *Amanita rubescens*, *Hebeloma sinapizans*, *Laccaria laccata*, *Lyophyllum decastes*, *Paxillus involutus*, *Pisolithus tinctorius*, *Rhizopogon subareolatus* and *Scleroderma citrinum* colonized over 50% of the short roots. *Amanita citrina*, *Boletus edulis*, *Boletus erythropus*, *Boletus pulverulentus*, *Cortinarius purpurascens*, *Rhizopogon luteolus*, *Rhizopogon ventricisporus*, *Tricholoma saponaceum*, and *Xerocomus chrysenteron* did not form ectomycorrhizas. Less variation in the level of colonization was observed within isolates of the same species in *Amanita muscaria*, *Rhizopogon roseolus*, *Rhizopogon vulgaris*, and *Suillus bovinus*. In almost all cases, variability among replicates in each host-fungus combination was not so high as to change the colonization levels, irrespective of whether we calculate them from the mean or from the median.

Of the 13 species of fungi tested on native and introduced conifers, *Laccaria laccata*, *Lyophyllum decastes*, *Pisolithus tinctorius*, and *Scleroderma citrinum* formed a high percentage of mycorrhizas with all host plants (Table 3). *Amanita aspera*, *Amanita muscaria*, *Paxillus involutus*, and *Suillus bovinus* were compatible with all the host plants but showed variable colonization levels. *Lactarius deliciosus*, *Rhizopogon* spp., and *Suillus luteus* showed higher specificity than the other fungi tested and reached maximal root colonization levels on *Pinus* spp., especially on *P. pinaster* and *P. radiata*.

Discussion

Many of the fungi associated with either conifers or hardwoods in northern Spain are able to form mycorrhizas with Douglas-fir under aseptic conditions. Among them are known broad-host-range, worldwide fungi such as *Laccaria laccata*, *Paxillus involutus*, and *Pisolithus tinctorius* (Trappe 1962a; Molina and Trappe 1982). The successful adaptation of Douglas-fir to many areas in Europe may be related to its compatibility with broad-host-range fungi, as Malajczuk et al. (1982) found when comparing the ectomycorrhizal fungi of *Pinus radiata* to those of *Eucalyptus* spp. In addition, Le Tacon et al. (1984) pointed out that Douglas-fir shares fungal symbionts with *Betula*, *Fagus*, *Picea* and *Quercus* species in *Calluna* heathlands in central France.

Of the fungal species tested in pure culture with Douglas-fir, eight colonized over 50% of the short roots. Positive field responses to inoculation with certain fungi may require a high colonization level of the root system (Marx and Cordell 1988; Marx et al. 1991). Therefore, those fungi showing a high colonization po-

Table 3 Level of mycorrhizal colonization obtained by pure culture synthesis on seven species of conifers (two native and five exotic) inoculated with 13 species of native ectomycorrhizal fungi. Colonization levels as in Table 2. Species abbreviations as in Table 1 plus P.con *Pinus contorta* Dougl. ex Loud., P.nig *Pinus nigra* Arnold, P.pon *Pinus ponderosa* Dougl. ex Laws (— no mycorrhizas, *nd* not determined)

Fungal species	Isolate	Colonization level						
		P.men	P.nig	P.pin	P.abi	P.con	P.pon	P.rad
Amanita aspera (Fr.) Hooker	A-48	2	2	3	2	1	2	3
A. muscaria (L. ex Fr.) Hooker	A-17	1	nd	2	4	3	nd	3
Laccaria laccata (Scop. ex Fr.) Bk. & Br.	A-127	4	4	4	nd	4	4	3
Lactarius deliciosus Fr.	A-120	—	1	4	2	3	—	3
Lyophyllum decastes (Fr.) Sing	A-71	4	2	3	nd	3	3	3
Paxillus involutus (Batsch) Fr.	A-87	3	nd	3	2	2	3	2
Pisolithus tinctorius (Pers.) Coker & Couch	A-93	3	3	4	4	3	1	3
Rhizopogon luteolus Fr. Nord.	A-5	—	—	3	3	—	3	2
R. roseolus (Corda ex Sturm) Th. Fr.	A-96	1	1	3	—	nd	—	1
R. vulgaris (Vitt.) M. Lange	A-101	1	2	3	2	—	1	4
Scleroderma citrinum Pers.	A-37	3	4	3	3	3	2	3
Suillus bovinus (L. ex Fr.) O. Kuntze	A-75	2	1	3	nd	nd	2	1
S. luteus (L. ex Fr.) S. F. Gray	A-33	1	2	3	—	—	1	1

tential would be preferred as potential candidates for further research in the selection process for nursery inoculations.

Positive results in pure culture syntheses should be interpreted in the light of the artificial experimental conditions. They may not reflect the ectomycorrhizal associations occurring in the field (Harley and Smith 1983). *Rhizopogon roseolus* and *Rhizopogon vulgaris* belong to the section *Rhizopogon* within the genus (Smith and Zeller 1966) and are considered pine specific (Molina and Trappe 1994). In our experiments, they formed a relatively low percentage of mycorrhizas with Douglas-fir and, although they developed a Hartig net, they lacked some of the typical morphological features of *Rhizopogon* mycorrhizas, such as the presence of abundant rhizomorphs. All the isolates of *Rhizopogon subareolatus* of Spanish origin proved to be good colonizers of Douglas-fir roots in pure culture synthesis.

The negative results obtained indicate that the experimental conditions were not adequate for the establishment of the symbiosis, but it cannot be inferred that the association is not possible in nature. Thus, the lack of effectivity of *Boletus pulverulentus*, *Tricholoma saponaceum*, and *Xerocomus chrysenteron*, collected in pure stands of Douglas-fir, may be due to the inappropriate experimental conditions. These species also grew slowly in the synthesis tubes.

Of the sporocarps collected regularly from northern Spain, only *Rhizopogon subareolatus* has been considered host specific to Douglas-fir (Molina and Trappe 1982, 1994; Ho and Trappe 1987). The origin of this fungal species and its mode of entry into Spain is still unknown (Alvarez et al. 1993). Malajczuk et al. (1982) suggested that successful long-term development of exotic plantations is generally accompanied by the appearance of host-specific fungi. Searches for the presence of specific mycorrhizal fungi in adult plantations of Douglas-fir in Spain and other European countries have not been extensive. Further mycological investigations are needed in European exotic plantations of Douglas-fir.

Cross-tests between several native fungi and both native and introduced conifers allowed us to demonstrate similar patterns of specificity to those described by Molina and Trappe (1982) for native conifers from the northwestern United States. Broad-host-range fungi colonized all host species, irrespective of their origin (native or introduced) and formed a relatively high percentage of mycorrhizas. A second group of fungi was able to colonize all the species of conifers but showed host preferences with marked variation in colonization level. Some of the mycorrhizas formed by fungi from this group lacked typical morphological characteristics of the species, or the colonization level was very low. Finally, a third group showed a narrow range of host compatibility. Species in this group were not able to form mycorrhizas with several host plants and showed a marked affinity for the genus *Pinus*, especially in the genera *Lactarius*, *Suillus* and pine-specific *Rhizopogon*.

The information obtained from pure culture synthesis experiments is useful to complement field observations of sporocarp-host associations. The presence of a fungal species forming mycorrhizas with several hosts does not necessarily mean that the fungus will produce sporocarps (Trappe 1962a). Also, the number of potential ectomycorrhizal hosts may be more than expected from observed associations in the field (Molina and Trappe 1982). In spite of the limitations of the methodology, Massicotte et al. (1994) found clear similarities in patterns of specificity when comparing pure culture (mycelium inoculations) and greenhouse (spore inoculations) experiments for the genus *Rhizopogon*.

This study provides experimental evidence of the degree of potential compatibility between some native ectomycorrhizal fungi from Spain and introduced conif-

ers. These data will be used in studies aimed to elucidate the need for inoculation of conifer seedlings with appropriate ectomycorrhizal fungi and to improve reforestation practices in northern Spain.

Acknowledgements Financial support for this study was provided in part by the Comisión Interministerial de Ciencia y Tecnología (CICYT AGF92–0979) and by the European Union (EEC MA1B1–0345). We wish to thank Dr. Randy Molina for reviewing the manuscript.

References

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

Anonymous (1992) Analisis y diagnóstico de los sistemas forestales de la Comunidad Autónoma del País Vasco. Servicio Central de Publicaciones del Gobierno Vasco. Depto. Agric. y Pesca, Vitoria-Gasteiz, Spain

Castellano MA, Trappe JM (1985) Ectomycorrhizal formation and plantation performance of a Douglas-fir nursery stock inoculated with *Rhizopogon* spores. Can J For Res 15:613–617

Chu-Chou M, Grace LJ (1983) Characterization and identification of mycorrhizas of Douglas-fir in New Zealand. Eur J For Pathol 13:251–260

Harley JL, Smith SE (1983) Mycorrhizal symbiosis. Academic Press, London

Ho I, Trappe JM (1987) Enzymes and growth substances of *Rhizopogon* species in relation to mycorrhizal hosts and infrageneric taxonomy. Mycologia 79:553–558

Jansen AE, De Nie HW (1988) Relations between mycorrhizas and fruitbodies of mycorrhizal fungi in Douglas-fir plantations in The Netherlands. Acta Bot Neerl 37:243–249

Laiho O (1970) *Paxillus involutus* as a mycorrhizal symbiont of forest trees. Acta For Fenn 106:1–72

Le Tacon F, Lamoure D, Guimberteau J, Fiket C (1984) Les symbiotes mycorhiziens de l'épicéa commun et du Douglas dans le Limousin. Rev For Fr 36:325–338

Le Tacon F, Alvarez IF, Bouchard D, Henrion B, Jackson RM, Luff S, Parladé J, Pera J, Stenström E, Villeneuve N, Walker C (1992) Variations in field response of forest trees to nursery ectomycorrhizal inoculation in Europe. In: Read DJ, Lewis DH, Fitter AH, Alexander IJ (eds) Mycorrhizas in ecosystems. CAB, Wallingford, pp 119–134

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 23:217–233

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

Marx DH, Cordell CE (1988) Specific ectomycorrhizae improve reforestation and reclamation in the eastern United States. In: Lalonde M, Piché Y (eds) Proceedings of the Canadian Workshop on Mycorrhizae in Forestry, 1–4 May, Centre de Recherche en Biologie Forestière. Faculté de Foresterie et de Géodésie, Université Laval, Sainte-Foy, Québec

Marx DH, Ruehle JL, Cordell CE (1991) Methods for studying nursery and field response of trees to specific ectomycorrhiza. In: Norris JR, Read DJ, Varma AK (eds) Methods in microbiology, vol 23. Academic Press, London, pp 384–411

Massicotte HB, Molina R, Luoma DL, Smith JE (1994) Biology of the ectomycorrhizal genus *Rhizopogon*. II. Patterns of host-fungus specificity following spore inoculation of diverse hosts grown in monoculture and dual culture. New Phytol 126:677–690

Matthews JD (1983) The role of north-west American trees in western Europe. MacMillan HR, Lectureship in Forestry. University of British Columbia, Vancouver

Mikola P (1973) Application of mycorrhizal symbiosis in forestry practice. In: Marks GC, Kozlowski TT (eds) Ectomycorrhizae: their ecology and physiology. Academic Press, New York, pp 383–411

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 28:423–458

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

Parladé J, Alvarez IF (1993) Co-inoculation of aseptically-grown Douglas-fir with pairs of ectomycorrhizal fungi. Mycorrhiza 3:93–96

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

Perry DA, Molina R, Amaranthus MP (1987) Mycorrhizae, mycorrhizospheres and reforestation: current knowledge and research needs. Can J For Res 17:929–940

Smith AH, Zeller SM (1966) A preliminary account of the North American species of *Rhizopogon*. Mem N Y Bot Gard 14:1–178

Snedecor GW, Cochran WG (1980) Statistical methods. Iowa State University Press, Ames, Iowa

Stenström E, Ek M, Unestam T (1985) Prolonged effects of initially introduced mycorrhizae of pine plants after outplanting. In: Gianinazzi-Pearson V, Gianinazzi S (eds) Physiological and genetical aspects of mycorrhizae. Proceedings of the First European Symposium on Mycorrhizae, Dijon 1–5 July 1985. INRA, Paris, pp 503–506

Toval Hernández G, Vega Alonso G, Puerto Arribas G, Jenkinson JL (1993) Screening Douglas-fir for rapid early growth in common-garden tests in Spain. General Technical Report PSW-GTR-146, Pacific Southwest Research Station, Forest Service, USDA, Albany, Calif

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

Trappe JM (1962b) *Cenococcum graniforme* – its distribution, ecology, mycorrhiza formation, and inherent variation. PhD thesis, University of Washington, Seattle

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

Trappe JM, Strand RF (1969) Mycorrhizal deficiency in a Douglas-fir region nursery. For Sci 15:381–389

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