Mycorrhiza (2000) 9:279–285

ORIGINAL PAPER

J.L. Dahlstrom · J.E. Smith · N.S. Weber

Mycorrhiza-like interaction by *Morchella* with species of the Pinaceae in pure culture synthesis

Accepted: 23 October 1999

Abstract Isolates from two species of *Morchella* were tested for ability to form mycorrhizae in pure culture synthesis with *Arbutus menziesii*, *Larix occidentalis, Pinus contorta*, *Pinus ponderosa,* and*Pseudotsuga menziesii*. Ectomycorrhizal structures (mantle and Hartig net) formed with the four species of the Pinaceae but not with *A. menziesii*. Results are compared to previous studies on morel mycorrhizae and discussed in an ecological context.

Key words *Morchella · Pinus contorta · Pinus ponderosa · Pseudotsuga menziesii · Larix occidentalis*

Introduction

The trophic status of morels (*Morchella* spp.) has been a topic of interest for many years. Mycologists of the mid-to-late 19th century believed that morels were parasitic on the roots of higher plants (Weber 1995; Wipf 1997). For example, mycelia of *Morchella rotunda* (Pers.) Boud. were seen adhering to roots of *Cornaceae* and *Oleaceae* species (Robert 1865), and mycelia of *M. esculenta* (L.: Fr.) Pers. were seen connecting with tubers of Jerusalem artichoke (*Helianthus tuberosus* L.) (Roze 1882, 1883). The possibility that morels are facultatively mycorrhizal has been debated for decades (Ulbrich 1936; Buscot and Roux 1987; Buscot 1989, 1992a,b,c; Buscot and Kottke 1990; Wipf et al. 1997; Harbin and Volk 1999).

Morels are commonly considered to be saprobes, obtaining nutrition from dead organic matter. Their mycelia grow rapidly and abundantly on various media (Brock 1951; Robbins and Hervey 1965; Kaul 1977), and some produce ascocarps (Ower 1982; Ower et al. 1986, 1988). In nature, morels commonly fruit in areas disturbed by herbicide application, fire, or tree disease (Turnau 1984, 1987; Carpenter et al. 1987; Weber 1995; Parks and Schmitt 1997). Such disturbances significantly increase dead organic matter in the rhizosphere and subsequent formation of abundant ascocarps of saprobic fungi.

A possible ectomycorrhizal association between Norway spruce (*Picea abies* (L.) Karst.) and two species of morel, *M. rotunda* and *M. esculenta*, *in vivo* has been reported (Buscot and Kottke 1990; Buscot 1992a,c). Several reports suggest that in stable forest ecosystems the life cycle of *Morchella* includes both saprotrophic and mycorrhizal phases, oscillating in a biannual cycle (Buscot and Roux 1987; Buscot 1989; Buscot and Bernillon 1991). The saprotrophic phase, characterized by nutrient accumulation and formation of sclerotia, occurs in summer and autumn and is followed by the mycorrhizal phase, in which mycelia growing from the sclerotia colonize fine roots in spring. Ower (1982) showed that mycelia from morel sclerotia may produce ascocarps. Miller et al. (1994) reported the presence of *Morchella* sclerotia in soils in both adjacent unburned and burned forests and noted a correlation between location of *Morchella* sclerotia with abundant *Morchella* ascocarps. Several researchers have hypothesized about the role of sclerotia in morel ascocarp production and detail probable morel life cycles (Volk and Leonard

Mention of trade or firm names does not constitute an endorsement by the US Department of Agriculture

J.L. Dahlstrom
Marlboro College,
Marlboro, VT 05344, USA

J.E. Smith (✉)
US Department of Agriculture, Forest Service,
Pacific Northwest Research Station,
Forestry Sciences Laboratory,
3200 SW Jefferson Way,
Corvallis, OR 97331, USA
e-mail: smithj@fsl.orst.edu
Fax: +1-541-750-7329

N.S. Weber
Department of Forest Science, Oregon State University,
Corvallis, OR 97331, USA

1989a,b, 1990; Volk 1991; Buscot 1992b; Weber et al. 1996).

Our study was conducted to expand upon previous findings of morel mycorrhizae. Under aseptic conditions and with the addition of mycorrhization helper bacteria, Buscot (1992a) revealed an ectomycorrhizal relation between *M. esculenta* and *Picea abies*. It was unclear, however, why helper bacteria effected mycorrhiza formation, and we omitted helper bacteria from our study. We used pure culture synthesis (Molina 1979) to evaluate the potential of two *Morchella* species to form mycorrhizae. Because prior pure culture research (Buscot 1992c) revealed an ectomycorrhizal relation only between *M. esculenta* and *Picea abies*, our experiment was broadened to include two species of *Morchella* with five tree species: Pacific madrone (*Arbutus menziesii* Pursh), western larch (*Larix occidentalis* Nutt.), lodgepole pine (*Pinus contorta* Loudon), ponderosa pine (*Pinus ponderosa* Laws.), and Douglas-fir *(Pseudotsuga menziesii* (Mirb.) Franco). Morels have been observed to fruit in the vicinity of these tree species.

The main objective of our study was to test the ability of *Morchella* to form mycorrhizae with various tree species. Our second objective was to examine effects of dextrose levels on mycorrhizal development.

Materials and methods

Identity and establishment of cultures

Because debate currently exists about the delimitation of species and their names within *Morchella*, we have chosen to refer to the two isolates in our study by common group rather than scientific names. Isolate NSW 7753 superficially resembles members of the group of taxa around *M. esculenta sensu lato.* Ascocarps have a gray hymenium when young that becomes tan to ochraceous in age, with ivory ribs that may be stained amber; however, main ribs are strongly vertically oriented to nearly parallel, producing elongated pits. We refer to this as the "mountain blond morel". Isolate NSW 7749b corresponds to *M. elata* Fr.:Fr. *sensu lato*. Ascocarps have a dusky gray to light brown hymenium at all ages and ribs that darken to black or dark blackish-brown as ascospores mature. We refer to this as the "natural black morel". In Oregon, the appellation "natural" is often used by commercial mushroom pickers to denote morels found in forests not recently burned or otherwise disturbed.

Ascocarps of the mountain blond morel and the natural black morel were collected in May 1996 from Malheur National Forest in Grant County and Wallowa-Whitman National Forest in Union County, Oregon, respectively. Voucher specimens will be deposited in the Oregon State University Herbarium (OSC). Both collections of morels were growing in forests with minimal insect infestation and not recently disturbed by fire. Spore isolates from the mountain blond morel were obtained by suspending 0.5–1 cm^3 sections of an ascocarp, hymenium side down, over sterile petri dishes containing malt yeast extract agar (MYA) (Stamets 1993) for 3–5 min. Spore isolates from the natural black morel were obtained by wrapping together an ascoma and glass slide in wax paper for 3–5 h, after which time the slide was removed, labeled, and stored in a slide container at room temperature. After 1 week, spores were scraped from the slide, suspended in sterile, deionized water and spread onto sterile petri dishes containing MYA. Isolates were incubated at ambient room temperature (19–21 C) for 3 days, after which noncontaminated sections (about 1 cm^2 surface area) were transferred singly onto fresh plates containing MYA. Stock cultures are stored in the Forest Mycology Team culture collection of ectomycorrhizal fungi at the Pacific Northwest Research Station, Corvallis Forestry Sciences Laboratory.

Preparation of fungal inoculum

Vegetatively colonized discs (9 mm diameter) of both isolates were cut from 7-day-old plates. Five discs from each isolate were floated in separate sterile 1000-ml flasks containing 300 ml of liquid malt yeast extract (MY) media (Stamets 1993). Liquid inoculum was selected because we had had better colonization success with it than with agar inoculum. Flasks were agitated for about 5 s daily to increase gas exchange. After 5 weeks, an unquantified amount of hyphae was aseptically transferred from the exhausted media into flasks containing fresh MY. Eight to 12 weeks after the initial transfer into liquid media, mycelia from each isolate were aseptically removed from the flasks and filtered through Buchner funnels containing grade 615 Eaten-Dikeman filter paper to remove nutrients before inoculation. The mycelia were then resuspended through submersion in a beaker containing 400 ml sterilized, deionized H_2O. The mycelia-water mixtures were blended for about 5 s in a sterile Waring blender.

Seed germination and seedling growth conditions

Seeds were surface sterilized in 30% H_2O_2 (*A. menziesii,* 20 min; *L. occidentalis,* 40 min; *Pinus contorta,* 45 min; *Pinus ponderosa,* 55 min; and *Pseudotsuga menziesii,* 50 min), rinsed in sterile, deionized H_2O, aseptically sown in petri dishes containing 25 ml of PDA (Difco Laboratories Detroit, Mich) and left at ambient room temperature (19–21 °C) for 7 days, during which time contaminated seeds and media were cut from the plates. Seeds were then cold stratified (4–5 °C) to stimulate and synchronize germination (Young and Young 1992) for 3 weeks and left at room temperature on a lab bench under fluorescent lights to germinate. Germinants of each species were planted individually into 35 × 300-mm glass culture tubes following techniques for pure culture synthesis described by Molina (1979). Each culture tube contained an autoclaved mixture of 10 cm^3 peatmoss, 110 cm^3 vermiculite, and 70 ml of either modified Melin Norkrans medium (MMN) (Marx 1969) unaltered (50 mgl^{-1} $CaCl_2$, 25 mgl^{-1} NaCl, 500 mgl^{-1} KH_2PO_4, 250 mgl^{-1} $(NH_4)_2$ HPO_4, 150 mgl^{-1} $MgSO_4 \cdot 7H_2O$, 20 mgl^{-1} sequestrene, 0.1 mgl^{-1} thiamine HCl, 3 gl^{-1} malt extract, 10 gl^{-1} dextrose, 839 ml H_2O/l), MMN with 1/4 strength dextrose, or MMN with no dextrose. Synthesis tubes were covered with either 50-ml glass beakers or translucent plastic test tube lids, and placed in racks with the bottom third of the tubes submerged in a water bath (16–20 °C). Seedlings were grown under fluorescent-incandescent light of an average of 157.6 $\mu mol \cdot m^{-2} s^{-1}$ with a 16-h photoperiod. At both 6 and 8 weeks after germination, seedlings were inoculated with 10 ml of either the mountain blond morel or the natural black morel isolate from the blended mycelia-water mixtures previously described. Seedlings were harvested 6–9 months after germination.

The experimental design comprised five tree species, three dextrose treatments (MMN with full strength dextrose, MMN with 1/4 strength dextrose, and MMN with no dextrose), and two morel treatments (mountain blond morel or natural black morel). Six replicates were planted for each treatment combination. Damping off reduced seedling survival to 92%, resulting in three to six replicates per treatment combination.

Colonization assessment

Seedlings were removed intact from the tubes and plates, with any visible contamination noted, and washed with deionized H_2O

to remove substrate. Roots were examined by stereomicroscopy and representative putative colonized roots removed and either sectioned by hand or prepared for microtome-sectioning. Free-hand sections were stained in cotton blue in aqueous solution and mounted in lactoglycerol. Roots for microtome sectioning were fixed and postfixed by procedures described by Massicotte et al. (1985, 1986), rinsed and dehydrated in a graded ethanol series, and embedded in LR White resin (London Resin Company Ltd.). Sections (1.0–1.5 μm) were cut with glass knives and stained for light microscopy with 0.05% toluidine blue O in 1% sodium borate. All sections were examined for mantle, Hartig net, and intercellular and intracellular hyphal development.

Quantification and statistical analysis of colonization

Colonization was often difficult to detect without sectioning and microscopic examination of individual root tips. No attempt was made to quantify percentage of colonized root tips per seedling, but we did quantify the number of colonized seedlings for each tree species by morel isolate and MMN dextrose treatment (Table 1). In *Pinus* and *Pseudotsuga* species, root tips lacking root hairs (hereafter termed "hairless roots") were closely examined because hairless roots can indicate ectomycorrhizal colonization (Brown and Sinclair 1981; Massicotte et al. 1987, 1990; Thomson et al. 1989). Because bifurcation can be a sign of mycorrhiza formation in *Pinus* (Agerer 1991), such root tips were also closely examined for colonization.

Chi-square tests were used to compare differences in numbers of pooled tree seedlings colonized among MMN dextrose treatments and between morel isolates. Fisher's exact test was used to compare differences in numbers of seedlings colonized among the tree species for each of the two isolates.

Formation of sclerotia and confirmation of isolates

Formation of sclerotia, their abundance, and size were observed in the morel treatments. We used polymerase chain reaction (PCR) methods for added confirmation of the identity of the fungal symbionts. DNA extraction, PCR amplifications, and restriction fragment length polymorphism (RFLP) protocols follow Gardes and Bruns (1993). We identified sclerotia formed in pure culture synthesis by comparing RFLP patterns to those from the voucher specimens of ascocarps. The internal transcribed spacer (ITS) region of the nuclear ribosomal repeat was amplified from sclerotia and ascocarps by using the fungal specific primer pair ITS1f and ITS4 (Gardes et al. 1991). Fungal ITS-RFLP patterns were produced using restriction enzymes *AluI*, *HinfI*, and *DpnII*.

Results

Mycorrhiza-like formation

Microscopic examination often confirmed mantle or Hartig net development, or both, in hairless root tips in *Pinus* and *Pseudotsuga* and in bifurcated root tips of *Pinus* seedlings. The structure and appearance of the fungal-root association formed with *Larix occidentalis, Pinus contorta, Pinus ponderosa*, and *Pseudotsuga menziesii* were similar to descriptions of ectomycorrhizae formed by other members of Pezizales in that they were thin, fairly straight and infrequently branched (Ingleby et al. 1990). Roots of*Arbutus menziesii* were not colonized. The fungal-root associations were bifurcate or simple root tips for *Pinus* or simple root tips in the other genera (Fig. 1a,b). The fungal-root association typically formed a shiny, cream-colored mantle surface and slightly enlarged root tips. Often the basal region of the feeder root was colonized while the tip remained noncolonized (Fig. 1b). Mantle formation was either continuous (Fig. 1d) or patchy (Fig. 1e). Mantle formation on seedlings inoculated with the mountain blond morel isolate was frequently a well-formed net prosenchyma to net synenchyma. Outer mantles were loosely organized with elongated interhyphal spaces, with wider and more frequently branched hyphal cells in comparison to cells emanating from the mantle, and with compact inner mantles without obvious interhyphal spaces (Ingleby et al. 1990). Mantles on seedlings inoculated with the natural black morel isolate were usually felt prosenchyma (loose, unorganized mantles with abundant interhyphal spaces) (Ingleby et al. 1990), varying occasionally to net prosenchyma-net synenchyma. Sloughing off of cortical cells was observed with *Pseudotsuga menziesii* and *L. occidentalis* root tips, and these were often infused with collapsed layers of cortical cells. Free-hand sections showed scattered intercellular hyphae and incomplete Hartig net formation (Fig. 1d–f). Hyphae penetrating intercellularly were most frequently observed between the first and second cortical cell layers, occasionally extending between the second and third cortical cell layers. Hartig net formation was occasionally complete around two to three cells in the first and second cortical cell layers. Mycorrhiza-like features

Table 1 Number of colonized seedlings for each tree species by modified Melin Norkrans (MMN) dextrose treatment and morel isolate (*Bld* mountain blond morel isolate, *Blk* natural black morel isolate)

Tree species	MMN dextrose treatment					
	no dextrose		1/4 dextrose		full dextrose	
	Bld	Blk	Bld	Blk	Bld	Blk
Arbutus menziesii	0/6	0/6	0/6	0/6	0/6	0/6
Larix occidentalis	6/6	4/6	4/6	4/5	5/6	4/6
Pinus contorta	4/5	4/5	4/6	5/5	5/6	1/6
Pinus ponderosa	6/6	5/5	6/6	6/6	6/6	6/6
Pseudotsuga menziesii	3/6	4/5	3/3	1/3	5/5	2/5

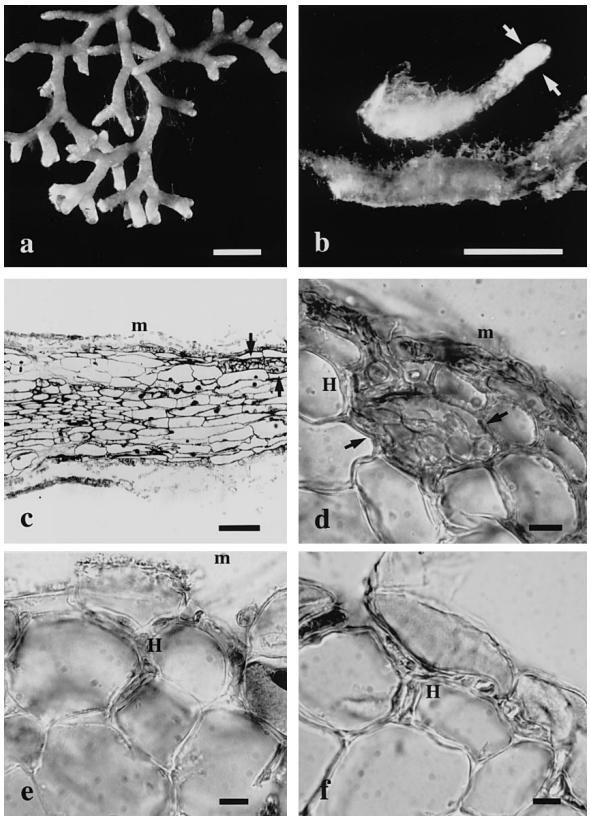

Fig. 1a–f Mycorrhiza-like interactions with *Morchella* spp. + *Pinus* spp. (*m* mantle, *H* Hartig net). **a** Macroscopic view of fungal-root association of natural black morel + *Pinus contorta* showing numerous bifurcate root tips; *bar* 1 mm. **b** Macroscopic view of mycorrhiza-like interaction between mountain blond morel + *Pinus contorta* showing enlarged basal region and uncolonized root tip (*arrows*); *bar* 1 mm. **c** Longitudinal section of mountain blond morel + *Pinus ponderosa* showing hyphal mantle and intracellular penetration (*arrows*); *bar* 100 μm. **d** Cross section of mountain blond morel + *Pinus ponderosa* showing intracellular penetration (*arrows*), mantle, and Hartig net; *bar* 10 μm. **e** Cross section of mountain blond morel + *Pinus ponderosa* showing intercellular penetration, mantle, and Hartig net; *bar* 10 μm. **f** Cross section of mountain blond morel + *Pinus ponderosa* showing intercellular penetration, and Hartig net; *bar* 10 μm

did not visibly differ in structure or appearance between the MMN dextrose treatments.

Intracellular penetration occurred in all tested members of the Pinaceae but was most frequent in *Pinus ponderosa* (Table 2). When present, intracellular penetration was most frequently observed within cells of the first or second cortical layer, occasionally entering cells of the third cortical layer (Fig. 1c,d). MMN dextrose treatments had no visible effect upon intracellular penetration.

Quantification and statistical analysis of colonization

Eighty percent of the Pinaceae host seedlings were colonized by *Morchella* (Tables 1, 2). *Pinus ponderosa* had the highest percentage of colonized seedlings (100%) and *Pseudotsuga menziesii* the lowest (67%); *L. occidentalis* and *Pinus contorta* had 77% and 70%, respectively (Table 2). Colonization of the pooled Pinaceae host seedlings did not differ among MMN dextrose treatments ($X_2^2=1.38, P=0.50$) or between morel isolates ($X_1^2=2.55$, $P=0.11$) (Table 1). When data were not pooled, colonization did not differ among the Pinaceae species for the mountain blond morel isolate ($P=0.14$) but did differ for the natural black morel isolate ($P=0.007$). However, when *Pinus ponderosa* was excluded from the analyses, because it was colonized 100% of the time, there were no differences in colonization among *L. occidentalis, Pinus contorta,* and *Pseudotsuga menziesii* for either the natural black morel ($P=0.69$) or the mountain blond morel ($P=0.91$) isolates (Table 1).

Formation of sclerotia and confirmation of isolates

Formation of sclerotia throughout the media was abundant in treatments with both morel isolates and was observed 1–2 weeks after the first inoculations. For both isolates, sclerotia developed more frequently (visual estimation) in treatments containing MMN with full amounts of dextrose than in MMN treatments with 1/4 strength or no dextrose. Sclerotia formed by the mountain blond morel were larger in size than those formed by the natural black morel isolate. RFLP patterns of the sclerotia matched those from the voucher specimens.

Discussion

The root morphology observed in the experiment reveals a mycorrhiza-like relation between the roots of several species of the Pinaceae and *Morchella.* Features of both ectomycorrhizae and ectendomycorrhizal colonization, particularly with *Pinus ponderosa*, were observed in all genera of the Pinaceae.

The often poor or incomplete development of Hartig net and mantle structures in our study concurred with Buscot's (1992a,c) findings. In contrast to Buscot's (1992a,c) results, however, the morel isolates in our study formed similar structures in an aseptic system without the addition of helper bacteria (Garbaye 1994) or primary colonizing fungi.

We found that enlarged root tips, along with possessing well-developed mantles, exhibited multiple hyphal penetrations. Buscot (1992c) suggested that the bulbous appearance of mycorrhizal root tips is a result of supplementary mitosis behind the root meristem. Cross sections of our roots showed the enlargement likely was due to the large diameter of morel hyphae (4–6 μm between cortical cells) invading the roots (Fig. 1d–f).

Table 2 Occurrence of *Morchella* hyphal structures in tree seedlings grown in pure culture synthesis, combining morel isolates and MMN dextrose treatments. Data are expressed as percentage of seedlings within the indicated colonization categories

Tree species	*n*	*Morchella* colonization categories			Total colonized
		Intercellular hyphae only	Intracellular hyphae only	Intercellular and intracellular hyphae	
Arbutus menziesii	36	0	0	0	0
Larix occidentalis	35	60	6	11	77
Pinus contorta	33	53	7	10	70
Pinus ponderosa	35	40	3	57	100
Pseudotsuga menziesii	27	59	4	4	67

We observed at least one occurrence of hyphae within root cells with all tested hosts in the Pinaceae (Table 2). Some fungi routinely form ectendomycorrhizae (Smith and Read 1997), and many ectomycorrhizal plants can form ectendomycorrhizae (Molina et al. 1992). Some postfire ascomycetes can form ectendomycorrhizal associations with members of the Pinaceae (Danielson 1984; Egger and Paden 1986). Wipf (1997) also observed intracellular penetration with *M. esculenta* and *Fraxinus excelsior* L., suggesting an ectendomycorrhizal association. Buscot and Roux (1987), Buscot and Kottke (1990), and Buscot (1992c) make no mention of intracellular penetration.

In our study, hairless or bifurcate root tips frequently possessed either or both mantles or hyphal penetration between the cortical cells. Bifurcation, however, can occur in *Pinus* roots in the absence of mycorrhizal fungi (Slankis 1973; Smith and Read 1997).

Soon after inoculation with pure cultures of the mountain blond morel or the natural black morel, fungal and/or bacterial contamination, or both, appeared within nearly all the synthesis tubes containing *Pinus contorta*. Surface sterilization times for the seeds may have been insufficient. Contaminants were examined by light microscopy but were not identified. Most of the contaminants were restricted to the top surface of the media, and careful examination revealed little association between the contaminant fungus and the roots. Because potential effects of the unidentified contaminants could not be surmised, the possibility exists that they affected the formation of mycorrhizae between the morel isolates and seedlings. No differences were observed in the structure or appearance of the fungal-root associations formed by contaminant-free and the contaminated *Pinus contorta* seedlings.

The ability of *Morchella* to form a mycorrhiza-like interaction in pure culture synthesis on all members of the Pinaceae tested but not on *Arbutus* was in contrast to the findings of numerous researchers working with other fungi. Many fungi that form EM with members of the Pinaceae also form mycorrhizae on *Arbutus* hosts, as demonstrated in pure culture (Zak 1976a,b; Molina and Trappe 1982a, 1994), in greenhouse seedling bioassays in pasteurized soil (Molina et al. 1997), as well as in forest soils (Molina et al. 1992). Some fungi that form mycorrhizae in pure culture fail to form mycorrhizae in greenhouse or natural soil conditions (Molina and Trappe 1982b; Duddridge 1986a,b; Massicotte et al. 1994; Molina et al. 1997). Duddridge (1986a,b) suggests that the artificial nature of pure-culture syntheses, specifically the addition of sugar to the media, yields abnormal ectomycorrhizal anatomy and creates problems in interpreting host-fungus compatibility. In our experiment, MMN treatments with dextrose produced similar mycorrhiza-like structures to those with no dextrose.

Our results in pure culture synthesis emphasize the need to evaluate the ability of *Morchella* to form mycorrhizae under natural soil conditions. Ecologically, the ability of some species of *Morchella* to form mycorrhiza-like interactions is significant. Even if weakly mycorrhizal, morels would be able to obtain a constant supply of fixed carbon. Perhaps morels form facultative mycorrhizae not only to acquire nutrients from living trees but also to position themselves to decompose fine roots as they senesce or in the event of tree death. On the other hand, we may find morel colonization provides no mycorrhizal benefit or may even act as a parasite. These possibilities also have ecological significance in understanding carbon dynamics of the rhizosphere microbial community.

Acknowledgments Our sincere thanks to François Buscot for valuable suggestions, Donaraye McKay and George Mosley for technical assistance, and Annette Simonson for advice about culturing techniques. Special thanks to Lewis Melville at the University of Guelph for sectioning and photography. We thank the USDA Forest Service Nursery in Bend and the Seven Oaks Nursery in Corvallis, Ore., for providing seeds. In addition, we are grateful to Manuela Huso, Randy Molina, David Pilz, James Trappe, Yana Valachovic, and Tom Volk for critical review of the manuscript. Jennifer Dahlstrom thanks Jennifer Ramsetter and Kimberly Woodbury of Marlboro College for their guidance and support. Participation of J.L.D. was funded in part by a grant from the Howard Hughes Medical Institute to Marlboro College. Participation of N.S.W. was made possible by support from the National Science Foundation through grants DEB 9007186 and DEB 9400545. Supplies and equipment for PCR and RFLP were funded in part by NRI Agricultural Systems Grant 9603859.

References

Agerer R (1991) Characterisation of ectomycorrhiza. In: Norris JR, Read DJ, Varma AK (eds) Techniques for the study of mycorrhiza (Methods in microbiology, vol 23) Academic, London, pp 25–73

Brock TD (1951) Studies on the nutrition of *Morchella esculenta* Fries. Mycologia 43:403–422

Brown AC, Sinclair WA (1981) Colonization and infection of primary roots of Douglas-fir seedlings by the ectomycorrhizal fungus *Laccaria laccata*. For Sci 27:111–124

Buscot F (1989) Field observations on growth and development of *Morchella rotunda* and *Mitrophora semilibera* in relation to forest soil temperature. Can J Bot 67:589–593

Buscot F (1992a) Mycorrhizal succession and morel biology. In: Read DJ, Lewis DH, Fitter AH, Alexander IJ (eds) Mycorrhizas in ecosystems. CAB International, Wallingford, pp 220–224

Buscot F (1992b) Stratégies écologiques et biologiques des morilles. Crypt Mycol 13:171–179

Buscot F (1992c) Synthesis of two types of association between *Morchella esculenta* and *Picea abies* under controlled culture conditions. J Plant Physiol 141:12–17

Buscot F, Bernillon J (1991) Mycosporins and related compounds in field and cultured mycelial structures of *Morchella esculenta*. Mycol Res 95:752–754

Buscot F, Kottke I (1990) The association between living roots and ascocarps of*Morchella rotunda* Boudier with roots of *Picea abies* (L.) Karst. New Phytol 116:425–430

Buscot F, Roux J (1987) Association between living roots and ascocarps of *Morchella rotunda*. Trans Br Mycol Soc 89:249–252

Carpenter SE, Trappe JM, Ammirati J, JR (1987) Observations of fungal succession in the Mount St. Helens devastation zone, 1980–1983. Can J Bot 65:716–728

Danielson RM (1984) Ectomycorrhiza formation by the operculate discomycete *Sphaerosporella brunnea* (Pezizales). Mycologia 76:454–461

Duddridge JA (1986a) 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 carbohydrate. New Phytol 103:457–464

Duddridge JA (1986b) The development and utlrastructure of ectomycorrhizas. IV. Compatible and incompatible interactions between *Suillus grevillei* (Klotzsch) Sing. and a number of ectomycorrhizal hosts *in vitro* in the presence of exogenous carbohydrate. New Phytol 103:465–471

Egger KN, Paden JW (1986) Biotrophic associations between lodgepole pine seedlings and postfire ascomycetes (Pezizales) in monoaxenic culture. Can J Bot 64:2719–2725

Garbaye J (1994) Tansley Review No. 76, Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytol 128:197–210

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes–application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118

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

Harbin M, Volk TJ (1999) The relationship of *Morchella* with plant roots. Abstracts XVI International Botantical Congress, St. Louis, Miss, USA, p 559

Ingleby K, Mason PA, Last FT, Fleming LV (1990) Identification of ectomycorrhizas. Pub. No. 5. Institute of Terrestrial Ecology Research, London

Kaul TN (1977) Physiological studies on *Morchella* species; 2-nitrogen utilization. Mushrooms J 58:328–332

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

Massicotte HB, Ackerley CA, Peterson RL (1985) An improved fixation protocol for ultrastructural studies of ectomycorrhizae, Proc Microsc Soc Can 12:68–69

Massicotte HB, Peterson RL, Ackerley CA, Piche Y (1986) Structure and ontogeny of *Alnus crispa – Alpova diplophloeus* ectomycorrhizae. Can J Bot 64:177–192

Massicotte HB, Melville LH, Peterson RL (1987) Scanning electron microscopy of ectomycorrhizae: potential and limitations. Scan Microsc 1:1439–1454

Massicotte HB, Peterson RL, Ackerley CA, Melville LH (1990) Structure and ontogeny of *Betula alleghaniensis – Pisolithus tinctorius* ectomycorrhizae. Can J Bot 68:579–593

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

Miller SL, Pilar T, McClean TM (1994) Persistence of basidiospores and sclerotia of ectomycorrhizal fungi and *Morchella* in soil. Mycologia 86:89–95

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

Molina R, Trappe JM (1982a) Lack of mycorrhizal specificity by the ericaceous hosts *Arbutus menziesii* and *Arctostaphylos uva-ursi*. New Phytol 90:495–509

Molina R, Trappe JM (1982b) 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 synthesis. New Phytol 125:653–675

Molina R, Massicotte H, Trappe JM (1992) Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. In: Allen MF (ed) Mycorrhizal functioning: an integrative plant-fungal process. Chapman and Hall, New York, pp 357–423

Molina R, Smith JE, McKay D, Melville LH (1997) Biology of the ectomycorrhizal genus, *Rhizopogon*. III. Influence of co-cultured conifer species on mycorrhizal specificity with the arbutoid hosts *Arctostaphylos uva-ursi* and *Arbutus menziesii*. New Phytol 137:519–528

Ower R (1982) Notes on the development of the morel ascocarp: *Morchella esculenta*. Mycologia 74:142–168

Ower R, Mills G, Malachowski J (1986) Cultivation of *Morchella*. US Patent No. 4 594 809

Ower R, Mills G, Malachowski J (1988) Cultivation of *Morchella*. US Patent No. 4 757 640

Parks CG, Schmitt CL (1997) Wild edible mushrooms in the Blue Mountains: resource and issues. USDA, Portland, Ore, PNW-GTR-393

Robbins WJ, Hervey A (1965) Manganese, calcium and filtrate factor for *Morchella crassipes*. Mycologia 57:262–274

Robert E (1865) Relation entre la famille des oleïnées et les morilles. Bull Soc Bot Fr 12:244–246

Roze ME (1882) Adherence de la base d'appareils ascospores de *Morchella* sur *Helianthus tuberosus*. Bull Soc Bot Fr 19:166–167

Roze ME (1883) Le parasitisme du *Morchella esculenta* Pers. sur *l'Helianthus tuberosus* L. Bull Soc Bot Fr 30:139–143

Slankis V (1973) Hormonal relationships in mycorrhizal development. In: Marks GC, Kozlowski TT (eds) Ectomycorrhizae: their ecology and physiology. Academic, New York, pp 213–298

Smith SE, Read DJ (1997) Mycorrhizal symbiosis. Academic, New York

Stamets P (1993) Growing gourmet and medicinal mushrooms. Ten Speed, Berkeley, Calif

Thomson J, Melville LH, Peterson RL (1989) Interaction between the ectomycorrhizal fungus *Pisolithus tinctorius* and root hairs of *Picea mariana* (Pinaceae). Am J Bot 76:632–636

Turnau K (1984) Post-fire cup-fungi of Turbacz and Stare Wiechy mountains in the Gorge Range (Polish Western Carpathians). Prace Bot 12:145–170

Turnau K (1987) An emergence of *Morchella semilibera* D.C. Fr. after application of Gesaprim. 50. Prace Bot 15:153–157

Ulbrich E (1936) Ergebnisse neuerer Forschungen über die Mykorrhiza. Sitzungsber Ges Naturforsch Freunde Berlin 1936(4–7):260–270

Volk TJ (1991) Understanding the morel life cycle: key to cultivation. McIlvainea 10:76–81

Volk TJ, Leonard TJ (1989a) Experimental studies on the morel. I. Heterokaryon formation between monoascosporous isolates of *Morchella*. Mycologia 81:523–531

Volk TJ, Leonard TJ (1989b) Physiological and environmental studies of sclerotium formation and maturation in *Morchella*. Appl Env Microbiol 55:3095–3100

Volk TJ, Leonard TJ (1990) Cytology of the life-cycle of *Morchella*. Mycol Res 94:399–406

Weber NS (1995) A morel hunter's companion. Thunderbay, Holt, Mich

Weber NS, Pilz D, Carter C (1996) Study 7:Morel life histories – beginning to address the unknowns with a case study in the Fremont National Forest near Lakeview, Ore. In: Pilz D, Molina R (eds) Managing forest ecosystems to conserve fungus diversity and sustain wild mushroom harvests. USDA, Portland, Ore, PNW-GTR-371, pp 62–68

Wipf D (1997) Polymorphismes protéique et génomique au sein des Morchellaceae – Mise au point d'un outil moléculaire adapté à l'étude de l'écologie du genre *Morchella* en milieu forestier. PhD thesis, Laboratoire de Biologie Forestière, Nancy, France

Wipf D, Koschinsky S, Clowez P, Munch JC, Botton B, Buscot F (1997) Recent advances in ecology and systematics of morels. Crypt Mycol 19:95–109

Young JA, Young CG (1992) Seeds of woody plants in North America. Dioscorides, Portland, Ore

Zak B (1976a) Pure culture synthesis of bearberry mycorrhizae. Can J Bot 54:1297–1305

Zak B (1976b) Pure culture synthesis of Pacific madrone ectendomycorrhizae. Mycologia 68:362–369