Mycorrhiza (2009) 19:149–158
DOI 10.1007/s00572-008-0222-1

ORIGINAL PAPER

Mycorrhizas on nursery and field seedlings of *Quercus garryana*

Darlene Southworth · Elizabeth M. Carrington · Jonathan L. Frank · Peter Gould · Connie A. Harrington · Warren D. Devine

Received: 4 August 2008 / Accepted: 22 December 2008 / Published online: 13 January 2009

Abstract Oak woodland regeneration and restoration requires that seedlings develop mycorrhizas, yet the need for this mutualistic association is often overlooked. In this study, we asked whether *Quercus garryana* seedlings in nursery beds acquire mycorrhizas without artificial inoculation or access to a mycorrhizal network of other ectomycorrhizal hosts. We also assessed the relationship between mycorrhizal infection and seedling growth in a nursery. Further, we compared the mycorrhizal assemblage of oak nursery seedlings to that of conifer seedlings in the nursery and to that of oak seedlings in nearby oak woodlands. Seedlings were excavated and the roots washed and examined microscopically. Mycorrhizas were identified by DNA sequences of the internal transcribed spacer region and by morphotype. On oak nursery seedlings, predominant mycorrhizas were species of *Laccaria* and *Tuber* with single occurrences of *Entoloma* and *Peziza*. In adjacent beds, seedlings of *Pseudotsuga menziesii* were mycorrhizal with *Hysterangium* and a different species of *Laccaria*; seedlings of *Pinus monticola* were mycorrhizal with *Geneabea*, *Tarzetta*, and *Thelephora*. Height of *Q. garryana* seedlings correlated with root biomass and mycorrhizal abundance. Total mycorrhizal abundance and abundance of *Laccaria* mycorrhizas significantly predicted seedling height in the nursery. Native oak seedlings from nearby *Q. garryana* woodlands were mycorrhizal with 13 fungal symbionts, none of which occurred on the nursery seedlings. These results demonstrate the value of mycorrhizas to the growth of oak seedlings. Although seedlings in nursery beds developed mycorrhizas without intentional inoculation, their mycorrhizas differed from and were less species rich than those on native seedlings.

Keywords Ectomycorrhizas · Garry oak · *Laccaria* · Oregon white oak · *Tuber*

D. Southworth (✉) · E. M. Carrington · J. L. Frank
Department of Biology, Southern Oregon University,
Ashland, OR 97520-5071, USA
e-mail: southworth@sou.edu

P. Gould · C. A. Harrington · W. D. Devine
USDA Forest Service Pacific Northwest Research Station,
Olympia, WA 98512, USA

Introduction

Oak regeneration and oak woodland restoration is of interest to private land owners and to managers of public lands, but survival of oak seedlings is often poor both in natural regeneration and in managed restoration projects (Fuchs et al. 2000; McCreary 2004; Hosten et al. 2006; Quero et al. 2006; Tyler et al. 2006; Devine et al. 2007). One factor often overlooked in studies of oak regeneration is the community of mycorrhizal fungi associated with oak seedlings. Seedlings acquired from a nursery may not form any mycorrhizas or the types of ectomycorrhizas that would maximize their chance of establishment after planting (Garbaye et al. 1986). This is not a problem if the planting site includes mycorrhizal species normally associated with seedlings. However, in restoration projects, seedlings are often planted on sites where vegetative and mycorrhizal communities have been substantially altered over time (Berman and Bledsoe 1998; Marx et al. 2002). In these cases, it is important that seedlings have already developed the mycorrhizas that will benefit their postplanting growth and survival. In this study, we ask whether *Quercus garryana* seedlings in nursery beds acquire mycorrhizas without artificial inoculation or access to a mycorrhizal network. We also assess the relationship between mycor-

rhizal infection and seedling growth in the nursery and compare those to mycorrhizas on seedlings in local *Q. garryana* woodlands.

The mycorrhizal nature of oaks is well documented beginning with the initial descriptions of mycorrhizas by A. B. Frank in 1885 (Cairney and Chambers 1999; translation, Frank 2005). In southern Oregon, virtually all root tips of *Q. garryana* are ectomycorrhizal with an average diversity of four fungal morphotypes per tree and about 40 fungal species per stand (Valentine et al. 2004; Moser et al. 2005, 2008; Frank et al. 2008).

The effects of mycorrhizal fungi on seedling growth, either as single fungal species or in assemblages, are difficult to assess in oaks, such as *Q. garryana*, that are adapted to Mediterranean climates with seasonal drought (Stein 1990). Because acorns store abundant carbon and nutrients, any effect of mycorrhizas, positive or negative, would be difficult to detect in first year seedlings. Acorn germination proceeds with growth of a long taproot that is nonmycorrhizal; subsequently, lateral roots form near the soil surface (Devine and Harrington 2005). These are the first roots to become colonized by mycorrhizal fungi; thus, the time of mycorrhizal inoculation is separate from the time of seed dispersal and germination (Southworth, unpublished data).

Greenhouse studies on container-grown oak seedlings have shown benefits due to mycorrhizas. Inoculation of *Quercus alba*, *Quercus robur*, and *Quercus velutina* with *Pisolithus tinctorius*, *Suillus* spp., *Thelephora terrestris*, and *Cenococcum geophilum* resulted in taller shoots and greater root mass after 20 weeks in greenhouse conditions (Dixon et al. 1984; Mitchell et al. 1984). Addition of soils from under mature *Q. garryana* led to formation of mycorrhizas with *C. geophilum* and improved growth in container-grown *Q. garryana* seedlings (Devine et al. 2009). Addition of soils from under an oak canopy increased mycorrhizal colonization, leaf mass, and foliar N in *Quercus ellipsoidalis* (Dickie et al. 2007b). In *Q. robur*, inoculation with *Paxillus involutus* did not increase seedling growth in the first year but did improve seedling survival in the second year (Von Herrmann et al. 1992). However, greenhouse studies on the effects of single fungal species on plant growth offer limited insights about natural systems because in situ communities contain more mycorrhizal fungal species, and other ecosystem factors (e.g., competing fungi, hyphal predators, and seasonal drought) may alter the interaction between hosts and mycorrhizas (Kennedy et al. 2007).

In field settings, the proximity of oak seedlings to mature oak trees positively affects the rate of mycorrhizal infection and seedling survival, as seedlings obtain ectomycorrhizal inocula from roots of established trees (Dickie et al. 2002; Cline et al. 2005; Dickie and Reich 2005; Luoma et al. 2006). Seedlings of *Quercus macrocarpa* and *Q. ellipsoidalis* had lower rates of mycorrhizal infection at distances of 35 m from mature trees, and survival was lower, but after 3 years, heights of surviving seedlings were only slightly less than those with higher mycorrhizal infections closer to mature trees (Dickie et al. 2007a). In nurseries, conifer seedlings may acquire mycorrhizas even if they are not specifically inoculated. Bare-root seedlings of *Pinus sylvestris* and *Picea abies* located in nursery beds distant from mature trees were colonized by about 30 fungi including *Laccaria*, *Rhizopogon*, *Suillus*, and *Thelephora*, species also found on tree roots in the field (Menkis et al. 2005).

Here, we asked the following questions regarding *Q. garryana* seedlings in nursery beds: (1) Which species of mycorrhizal fungal do they acquire? (2) Are these the same species as those on seedlings in local natural stands? (3) Do conifer seedlings in the same nursery beds acquire the same species of fungi? (4) Do more vigorous oak seedlings have more mycorrhizas? (5) Are more vigorous oak seedlings associated with particular species of mycorrhizal fungi? We predicted that if seedling size were determined by microenvironment or seedling genetics, then size would not correlate with mycorrhizal abundance or fungal species. If seedling sizes were a function of mycorrhizal capacity, then larger seedlings would have greater species richness or greater total mycorrhizal abundance of one or several fungal species.

Materials and methods

Field methods

Acorns of *Q. garryana* Dougl. ex Hook. were collected in October 2004 from two trees in Shelton, WA, USA and sown in beds of Tumwater sandy loam at the Washington Department of Natural Resources Webster Nursery, Olympia, WA, USA (46°58′ N, 122°54.1′ W). Seedlings were transplanted after the first growing season and were lifted in January 2006 and held in bare-root condition in cool storage until April 2006 when they were transplanted into two nursery beds. These second year beds had been planted with Western red cedar (*Thuja plicata* D. Don) in spring 2004, those seedlings lifted in spring 2005, and the field fumigated with methyl bromide in July 2005. Oak seedlings were irrigated in summer. A total of 100 kg ha^{-1} elemental N was applied as calcium nitrate between April and October 2006, with 91% applied by mid-July. Potassium was applied as potassium sulfate in October at a rate of 42 kg K_2O ha^{-1}. Douglas-fir (*Pseudotsuga menziesii* [Mirbel] Franco) and western white pine (*Pinus monticola* Dougl. ex D. Don) seedlings were planted into adjacent

beds in July 2005. In August 2006, the oak seedlings were root wrenched by cutting the taproots at a depth of 15 cm to encourage development of lateral roots.

In January 2007, when seedlings were in winter condition without leaves, we lifted ten oak seedlings from two blocks in each of two nursery beds (N=40) plus two seedlings each of Douglas-fir and western white pine from adjacent nursery beds. Blocks 1 and 2 in nursery bed 1 were approximately 150 m apart and blocks 3 and 4 in nursery bed 2 were approximately 20 m apart. The two

Table 1 Molecular identification of fungal symbionts based on DNA sequences of the ITS region

Concensus taxon GenBank #	Host	Site	Length (bp)	Nearest BLAST match to vouchered specimen	Query coverage (%)	*E* value	Maximum identification (%)
Cenococcum FJ348362	QUGA	G	528	*Cenococcum geophilum* AY394919	99	0	98
Cenococcum FJ348363	QUGA	G	389	*Cenococcum geophilum* EU427331	96	7e−168	96
Cenococcum FJ348364	QUGA	S	524	*Cenococcum geophilum* AY394919	93	0	96
Cenococcum FJ348365	QUGA	W	520	*Cenococcum geophilum* AY394919	99	0	99
Cenococcum FJ348366	QUGA	W	462	*Cenococcum geophilum* AY394919	99	0	99
Clavulinaceae FJ348367	QUGA	G	390	*Clavulina cinerea* AY456339	93	2e−110	86
Clavulinaceae FJ348368	QUGA	W	496	*Clavulina cinerea* AY456339	99	3e−130	81
Cortinarius sp.1 FJ348369	QUGA	S	184	*Cortinarius pulchripes* FJ039537	100	2e−83	98
Cortinarius sp. 1 FJ348371	QUGA	W	178	*Cortinarius pulchripes* FJ039537	99	5e−83	99
Cortinarius sp. 2 FJ348370	QUGA	W	442	*Cortinarius scandens* FJ039555	100	0	97
Entoloma FJ348372	QUGA	N	488	*Entoloma* sp. DQ974695	100	0	95
Genabea FJ348373	PIMO	N	371	*Genabea cerebriformis* DQ206866	99	0	99
Genea FJ348374	QUGA	S	612	*Genea harknessii* DQ218288	85	0	95
Hebeloma FJ348375	QUGA	S	358	*Hebeloma cavipes* AY948193	100	3e−159	95
Humaria FJ348376	QUGA	S	272	*Humaria hemisphaerica* DQ200832	98	1e−59	85
Humaria FJ348377	QUGA	W	427	*Humaria hemisphaerica* DQ200832	100	0	94
Hysterangium FJ348378	PSME	N	229	*Hysterangium* sp. DQ974736	41	4e−22	87
Inocybe FJ348379	QUGA	G	164	*Inocybe leiocephala* AM882793	84	5e−32	84
Laccaria sp. 1 FJ348380	PSME	N	513	*Laccaria proxima* AY750156	100	0	99
Laccaria sp. 2 FJ348381	QUGA	N	607	*Laccaria nobilis* DQ149863	97	0	99
Laccaria sp. 2 FJ348382	QUGA	N	396	*Laccaria nobilis* DQ149863	100	3e−178	98
Laccaria sp. 2 FJ348383	QUGA	N	441	*Laccaria nobilis* DQ149863	99	0	96
Otidea FJ348384	QUGA	G	567	*Otidea smithii* EU834207	100	1e−172	82
Peziza FJ348385	QUGA	N	547	*Peziza ostracoderma* EU819461	98	0	96
Russula FJ348386	QUGA	S	503	*Russula pectinatoides* AY880930	99	0	99
Russula FJ348387	QUGA	W	442	*Russula pectinatoides* AY880930	99	0	100
Tarzetta FJ348388	PIMO	N	562	*Tarzetta* cf. *cupularis* FJ235145	100	8e−157	83
Thelephora FJ348389	PIMO	N	550	*Thelephora terrestris* U83486	100	0	98
Tomentella FJ348390	QUGA	G	600	*Tomentella* sp. U83482	98	0	96
Tomentella FJ348391	QUGA	S	585	*Tomentella* sp. U83482	100	0	94
Tomentella FJ348392	QUGA	W	378	*Tomentella* sp. U83482	100	3e−179	97
Tuber sp. 1 FJ348393	QUGA	N	548	*Tuber* sp. AM900418	99	0	98
Tuber sp. 1 FJ348395	QUGA	N	364	*Tuber* sp. AM900418	98	1e−177	99
Tuber sp. 1 FJ348396	QUGA	N	592	*Tuber* sp. EU379679	92	0	99
Tuber sp. 1 FJ348397	QUGA	N	634	*Tuber* sp. AM900418	94	0	100
Tuber sp. 1 FJ348398	QUGA	N	771	*Tuber* sp. AM900418	74	0	96
Tuber sp. 1 FJ348399	QUGA	N	627	*Tuber* sp. AM900418	91	0	99
Tuber sp. 1 FJ348400	QUGA	N	625	*Tuber* sp. AM900418	95	0	100
Tuber sp. 1 FJ348401	QUGA	N	564	*Tuber* sp. AM900418	99	0	99
Tuber sp. 1 FJ348402	QUGA	N	549	*Tuber* sp. AM900418	91	0	98
Tuber sp. 1 FJ348403	QUGA	N	737	*Tuber* sp. AM900418	77	0	95
Tuber sp. 2 FJ348404	QUGA	S	572	*Tuber candidum* AY830856	99	0	96
Xerocomus FJ348405	QUGA	G	624	*Xerocomus chrysenteron* DQ822793	98	0	97
Xerocomus FJ348406	QUGA	S	327	*Xerocomus chrysenteron* EU350581	99	9e−52	89

Hosts were seedlings of *Quercus garryana*, *Pseudotsuga menziesii*, and *Pinus monticola* from nursery beds or from local field sites, Glacial Heritage Preserve, Scatter Creek Wildlife Area, and Upper Weir Prairie in Washington

QUGA Quercus garryana, *PSME Pseudotsuga menziesii*, *PIMO Pinus monticola*, *N* nursery beds, *G* Glacial Heritage Preserve, *S* Scatter Creek Wildlife Area, *W* Upper Weir Prairie

nursery beds were 500 m apart. Our sampling of oak seedlings was designed to select seedlings representing the full range of size and biomass present in the nursery beds. To this end, we used a stratified random sampling approach: Samples were randomly selected from the tallest 25% of seedlings and the shortest 25% of seedlings in each block. Seedlings were carefully excavated, bagged individually with surrounding soil, and transported on ice to Southern Oregon University.

Seedling stem diameter at groundline and total stem height were measured for all seedlings after transplanting in April 2006 and again after the growing season in October 2006. After seedlings were excavated, shoot length (from top of root collar to shoot apex) and root length (from top of root collar to maximum root extent) were measured; shoots were dried at 65°C for 3 days and weighed. All roots were examined for mycorrhizas (see below).

To determine whether the mycorrhizal fungi on nursery seedlings were typical of those on oak seedlings in natural habitats in the Puget Trough, we collected three seedlings in April 2007 from each of three *Q. garryana* woodland sites: Glacial Heritage Preserve (46°54.1′ N, 123°01.0′ W), near Littlerock, WA, USA, Scatter Creek Wildlife Area (46° 50.2′ N, 123°00.24′ W), Thurston County, WA, USA, and Upper Weir Prairie (47°06.8′ N, 122°32.8′ W), Ft. Lewis, WA, USA located at distances of 16, 25, and 26 km from the nursery. Relative to the nursery, these were among the nearest oak-dominated stands.

Mycorrhizas

Roots were rinsed with gentle agitation, the fine roots clipped into 7-cm segments, and the entire root system of each seedling examined in water with a Leica MZ75 dissecting microscope. Mycorrhizal morphotypes were described by a suite of characters including color, branching pattern, emanating hyphae, and mantle peels (Agerer 1991; Goodman et al. 1996; Valentine et al. 2004; Moser et al. 2005, 2008). Voucher images were captured with a SPOT RT camera (Diagnostic Instruments, Sterling Heights, MI, USA). To quantify mycorrhizal abundance, we counted all the individual mycorrhizal root tips on each seedling. Representative mycorrhizas of each morphotype were stored in buffer for DNA identification. Root and shoot segments were dried and weighed.

Molecular methods

Ectomycorrhizas were stored in buffer (0.1 M Tris, 0.3 M NaCl, 0.04 M ethylenediaminetetraacetic acid) at 4°C. DNA was extracted from ectomycorrhizas in 2% cetyltrimethyl ammonium bromide with chloroform. DNA was amplified in polymerase chain reactions (PCR) with fungal primer ITS1F (5′-ggtcatttagaggaagtaa-3′) and universal eukaryote primer TW13 (5′-ggtccgtgtttcaagacg-3′; White et al. 1990; Gardes and Bruns 1993, 1996). PCR reactions (20 μl) were performed using 0.6 units GoTaq (Promega, Madison, WI, USA) and 4 μL 5× colorless buffer, 200 μM each dNTP, 0.3 μM each primer, 2.5 mM $MgCl_2$, and 2 μL undiluted DNA template. An initial 3 min at 93°C was followed by 30 cycles of 30 s at 95°C, 2 min at 56°C, and 3 min at 72°C, with a final cycle for 10 min at 72°C.

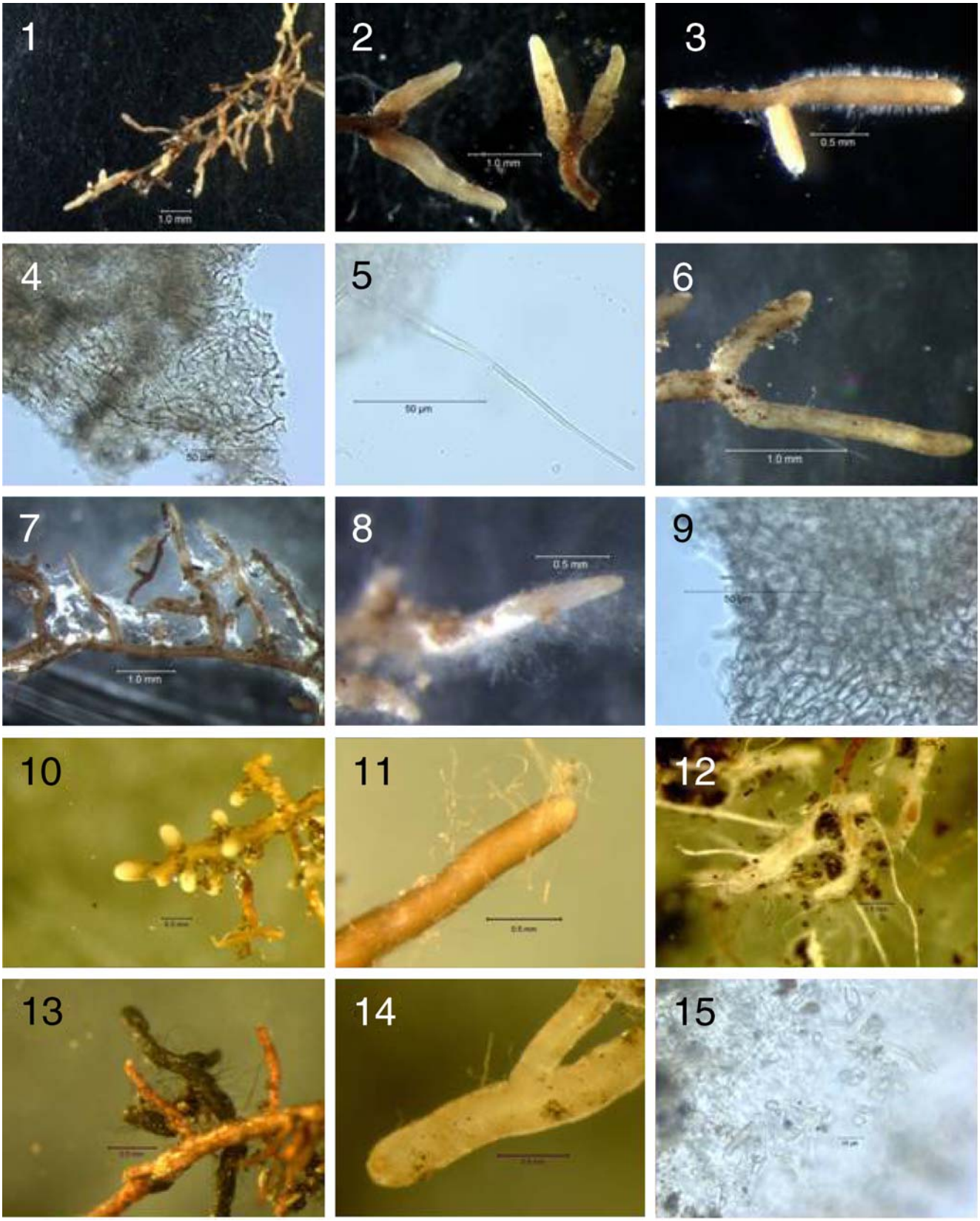
PCR products were purified with QIAquick PCR Purification kits (Qiagen, Valencia, CA, USA) and then prepared with BigDye Terminator Ready Reaction Mix v3.1 prior to sequencing in an ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) in the Biotechnology Center at Southern Oregon University. Molecular data were obtained by sequencing the internal transcribed spacer (ITS) region, including ITS1, the 5.8S ribosomal DNA gene and ITS2, with forward primers ITS1F and ITS1 (5′-tccgtaggtgaacctgcgg-3′) and reverse primer ITS4 (5′-tcctccgcttattgatatgc-3′).

Sequences were edited with Chromas 1.45 (McCarthy 1998) and Sequencher v4.7 (Gene Codes Corp. Ann Arbor, MI, USA) and compared to other fungal ITS DNA sequences in GenBank with BLAST (Altschul et al. 1990). Sequences with >96% similarity to vouchered specimens were assigned to a genus. Phylotypes were considered the same species when sequences were >97% similar. We examined the top 50 BLAST matches for sequences with <96% similarity to a vouchered specimen and assigned them to a genus or family based on a combination of similarity and phylogeny (Parrent et al. 2006).

Statistical analysis

We applied regression analysis using PROC Mixed (SAS Institute, Cary, NC, USA) to determine if mycorrhizal abundance, quantified as total number of mycorrhizal root

Fig. 1 Mycorrhizas of *Q. garryana* seedlings. *1–5 Tuber* sp. 1 from nursery bed. *1* Abundant mycorrhizas on tall nursery seedlings. *2* Typical pattern of two mycorrhizas at the end of a fine root. *3* Pale mycorrhizas with cystidia; older darker tips coated with cystidia. *4* Mantle of net synenchyma. *5* Cystidium. *6 Peziza*, nursery bed, with two mycorrhizas at the end of a fine root. *7–9 Laccaria* sp. 2 from *Q. garryana* seedlings in nursery bed. *7* Wefts of hyphae around mycorrhizas. *8* White emanating hyphae and reflective surface. *9* Mantle cells with straight or curved walls—regular synenchyma or noninterlocking irregular synenchyma. *10–15* Mycorrhizas of native seedlings from field sites. *10 Tuber* sp. 2, Scatter Creek, mycorrhizas rusty tan with pale tips. *11 Humaria*, Upper Weir Prairie, straight rusty tan with slightly pale tips; visible mantle of regular synenchyma. *12 Cortinarius* sp. 1, Scatter Creek, white felty surface over tan mantle, rhizomorphs. *13 Cenococcum* Upper Weir Prairie, black with stiff black hyphae. *14 Russula*, Scatter Creek, white to pale tan. *15 Russula*, Scatter Creek, flask-shaped cystidia


1
1.0 mm
2
1.0 mm
3
0.5 mm
4
50 µm
5
50 µm
6
1.0 mm
7
1.0 mm
8
0.5 mm
9
10
11
12
13
14
15

tips per seedling, was related to seedling height and basal area growth increment during the 2006 growing season. Stem basal area growth was calculated from groundline diameter measurements before and after the growing season. To account for initial variation in seedling size and as a surrogate for initial variation in root mass, seedling basal area prior to the 2006 growing season was included in the models. Block was included as a random effect. Seedling growth variables and number of mycorrhizal root tips for the most prevalent types of mycorrhizas were compared by correlation analysis (PROC CORR). Statistical significance was determined at α=0.05.

Results

Identification of mycorrhizas

A total of 22 mycorrhizal phylotypes were identified: four on oak seedlings in nursery beds, five on conifer seedlings in nursery beds, and 13 on oak seedlings in local woodlands (Table 1; Fig. 1 1–15). ITS sequence data placed *Tuber* sp. 1, from nursery seedlings, near the reticulate-spored *Tuber menseri* nom. prov. (JT1161; Bonito, unpublished phylogeny) and placed *Tuber* sp. 2, from a field seedling, near *T. candidum*, a species known to be ectomycorrhizal with *Q. garryana* (Frank et al. 2006b). Sequence data placed *Laccaria* sp. 1, from conifer seedlings, close to *L. proxima* and *Laccaria* sp. 2, from oak seedlings, near *Laccaria nobilis*. Two species of *Cortinarius* were identified from *Q. garryana* field seedlings, both distinct from *Inocybe* with a relatively short sequence that included most of the informative ITS2. Within the Pyronemataceae, distinct phylotypes were assigned to genera (*Genea*, *Geneabea*, *Humaria*, *Otidea*, and *Tarzetta*) although the family is not well represented in GenBank. Data from all other genera were interpreted as one species each.

Four species of fungi were mycorrhizal symbionts of *Q. garryana* seedlings in the nursery bed (Table 1). *Tuber* sp. 1 and *Laccaria* sp. 2 were the dominant taxa, occurring on 73% and 83% of seedlings, respectively. *Entoloma* and *Peziza* were found on one seedling each. *Tuber* phylotypes were identified from 16 samples taken from 11 seedlings; all were the same species. *Laccaria* phylotypes were identified from six samples taken from three seedlings of *Q. garryana*; all on *Q. garryana* seedlings were the same species. *Tuber* mycorrhizas were identified from 18 additional seedlings and *Laccaria* mycorrhizas from 30 additional seedlings based on morphological comparison to sequenced specimens (Table 2; Fig. 1 1–15).

Conifer seedlings in adjacent nursery beds were also mycorrhizal (Tables 1 and 2). One seedling of *P. menziesii* was mycorrhizal with *Hysterangium* and *Laccaria* sp. 1; *P. monticola* seedlings were mycorrhizal with *Genabea*, *Tarzetta*, and *Thelephora*. None of these species were shared by *Q. garryana* seedlings.

Table 2 Morphology and frequency of mycorrhizas on *Q. garryana* seedlings collected from nursery beds (n=40) or from field sites (n=9) at Glacial Heritage Preserve, Scatter Creek Wildlife Area, and Upper Weir Prairie in Washington

Fungal symbiont	Morphotype	Nursery	Field
Ascomycota			
Cenococcum (Fig. 1 13)	Black, 0.5–1.5 mm long; stiff, angular black hyphae; mantle: regular synenchyma	0	7
Genea	Brown, dark red-brown; mantle: regular synenchyma	0	1
Humaria (Fig. 1 11)	Reddish tan to red-brown; mantle: regular synenchyma, emanating hyphae	0	3
Peziza (Fig. 1 6)	White to pale tan, smooth	1	0
Otidea	White-tan, long, reflective	0	1
Tuber sp. 1 (Fig. 1 1–5)	Pale tan to rusty tan; mantle: net synenchyma, cystidia	29	0
Tuber sp. 2 (Fig. 1 10)	Tan with pale tips; pinnate	0	1
Basidiomycota			0
Clavulinaceae	White or gray-tan, white surface	0	1
Cortinarius[a] (Fig. 1 12)	White tortuous, reflective; om: felt prosenchyma, emanating hyphae, rhizomorphs	0	5
Entoloma	Tan	1	0
Hebeloma			
Inocybe	Tan cottony	0	1
Laccaria sp. 2 (Fig. 1 7–9)	White, wefty, clamp connections	33	0
Russula (Fig. 1 14, 15)	Pale tan shiny; om: net prosenchyma, im: net synenchyma, flask-shaped cystidia	0	2
Tomentella	Black, warty, large (1.5–5 mm); clamp connections; om: noninterlocking irregular synenchyma, short light brown hyphae, some with white tips	0	2
Xerocomus	Pale yellow-tan	0	2

om outer mantle, *im* inner mantle

[a] *Cortinarius* species cannot be distinguished by morphotype

Oak seedlings from local field sites had a richer diversity of fungal symbionts; 13 species were identified by phylotype and morphotype (Tables 1 and 2). Mycorrhizal symbionts of field seedlings did not overlap those on nursery seedlings. One seedling at Scatter Creek was mycorrhizal with a different *Tuber* species (Fig. 1 10) most similar to *T. candidum*, which places it in the spiny-spored clade of *Tuber*, clearly distinct from *Tuber* sp. 1 on the nursery seedlings (Table 1; Frank et al. 2006b). The most frequent mycorrhizal species was *Cenococcum* (Fig. 1 13) found on seven seedlings at all three sites, followed by *Tomentella* (Fig. 1 12) on five seedlings at three sites.

Seedling growth and mycorrhizal abundance

Prior to the 2006 growing season, seedlings were similar in height and diameter among the four blocks (Table 3). At the end of their second growing season (2006), nursery oak seedlings ranged in height from 5.9 to 49.4 cm and in diameter from 1.9 to 9.2 mm. Dry shoot weight ranged from 0.2 to 7.6 g and dry root weight from 0.7 to 19.3 g. Growth during 2006 was variable within blocks, although there was a trend toward greater growth in block 3. This trend was evident among seedlings selected from the largest quartile per block which had a height growth increment of 24.2±7.3 cm on block 3 compared to an average of 15.1± 6.9 cm for the other blocks.

The number of mycorrhizal root tips per seedling varied within blocks, and the magnitude of the mean and standard deviation varied among blocks (Table 3). Values were greatest in block 3, particularly for *Laccaria*. When abundance of mycorrhizal tips was expressed per gram dry root weight, mean values were again greatest for block 3.

Regression models indicated that the number of mycorrhizal root tips per seedling was a significant predictor of 2006 height growth (model R^2=0.69; P<0.0001) and basal area growth (model R^2=0.32; P=0.0001; Table 4). In each model, number of mycorrhizal root tips was a stronger predictor of growth than initial seedling basal area. Correlation analyses showed positive relationships between numbers of *Laccaria*, *Tuber*, and total mycorrhizal tips per seedling and final seedling height, root biomass, height growth, and basal area growth (Fig. 2). While all correlations with 2006 height and basal area growth were statistically significant (P<0.001), correlations involving *Laccaria* or total number of mycorrhizal tips (r=0.70 to 0.71) were stronger than correlations involving *Tuber* (r= 0.51 to 0.52).

Discussion

In nursery beds distant from any ectomycorrhizal hosts, most seedlings of *Q. garryana* were mycorrhizal, indicating that inocula—spores or hyphae—had reached them. The predominant fungal symbionts on nursery seedlings were species of *Tuber* and *Laccaria*. Based on the DNA sequence of the ITS region, the *Tuber* was an undescribed species, *T. menseri* nom. prov. (Bonito, personal communication). *Tuber* species are common mycorrhizal symbionts of *Q. garryana* but have not been reported as nursery fungi (Frank et al. 2006a, b; Valentine et al. 2004).

The DNA sequence of *Laccaria* sp. 2 most closely matched that of *L. nobilis*. *Laccaria* is a common nursery fungus but has not been found as ectomycorrhizas on *Q. garryana* (Molina and Trappe 1984; Valentine et al. 2004; Moser et al. 2005, 2008). A widespread genus often occurring as a pioneer species or in disturbed sites, *Laccaria*, forms mycorrhizas primarily with conifers, but a few species form mycorrhizas with oaks (Cairney and Chambers 1999). Seedling responses to infection with

Table 3 Initial seedling size (prior to the 2006 growth season), 2006 growth, final root weight, and number of mycorrhizal tips per seedling (mean ± standard deviation) by nursery bed plot

Parameter	Plot			
	1	2	3	4
Initial seedling height (cm)	6.2±2.6	6.7±3.5	7.0±4.9	6.4±3.9
Initial seedling diameter (mm)	4.7±1.8	4.0±1.7	3.8±1.4	3.4±1.6
2006 height growth (cm)	7.4±6.6	10.3±11.1	15.4±12.6	6.5±6.5
2006 diameter growth (mm)	0.6±0.6	0.7±0.7	1.1±1.1	0.8±0.6
Final root weight (g)	4.4±3.1	6.6±6.4	7.8±5.9	4.9±4.1
Laccaria mycorrhizal tips (no.)	25±45	218±348	1,028±836	387±581
Tuber mycorrhizal tips (no.)	180±277	261±320	596±620	408±443
Total mycorrhizal tips (no.)	205±278	498±637	1,624±1,227	795±928
Laccaria mycorrhizal tips per gram root wt.	9±16	22±25	151±172	68±68
Tuber mycorrhizal tips per gram root wt.	58±95	35±33	85±95	76±84
Total mycorrhizal tips per gram root wt.	67±96	62±51	236±256	145±101

Table 4 Regression models resulting from stepwise variable selection

Dependent variable	Model R^2	Parameter	Estimate	Standard Error	Pr>F	Partial R^2
2006 height growth	0.69	Intercept	0.5920	1.4711		
		Laccaria tips	0.0100	0.0014	<0.0001	0.52
		Initial BA	0.3581	0.0773	<0.0001	0.18
2006 BA growth	0.32	Intercept	1.3224	1.4861		
		Total tips	0.0051	0.0012	0.0001	0.32

Potential variables were total mycorrhizal tips per seedling, number of *Laccaria* tips, number of *Tuber* tips, and initial basal area. Only variables significant at the 0.05 level were retained
BA basal area

Lacarria spp. range from positive through neutral to negative and vary with host species, *Laccaria* species, soil type, and culture methods.

The fungi forming mycorrhizas on nursery seedlings differed from those on native oak seedlings in the Puget Trough. Field grown seedlings of *Q. garryana* had a more diverse ectomycorrhizal assemblage with 13 fungal species, none of which were found on nursery seedlings. Thus, nursery seedlings were not acquiring a subset of fungi native to oak communities. It is unclear how this difference in mycorrhizal fungi will affect seedlings after they are outplanted and subsequently infected by other species. One or more fungal species best suited to the site and host may become dominant, although it is possible that seedling growth could be negatively affected in the interim (Chilvers et al. 1987; Egerton-Warburton and Allen 2001).

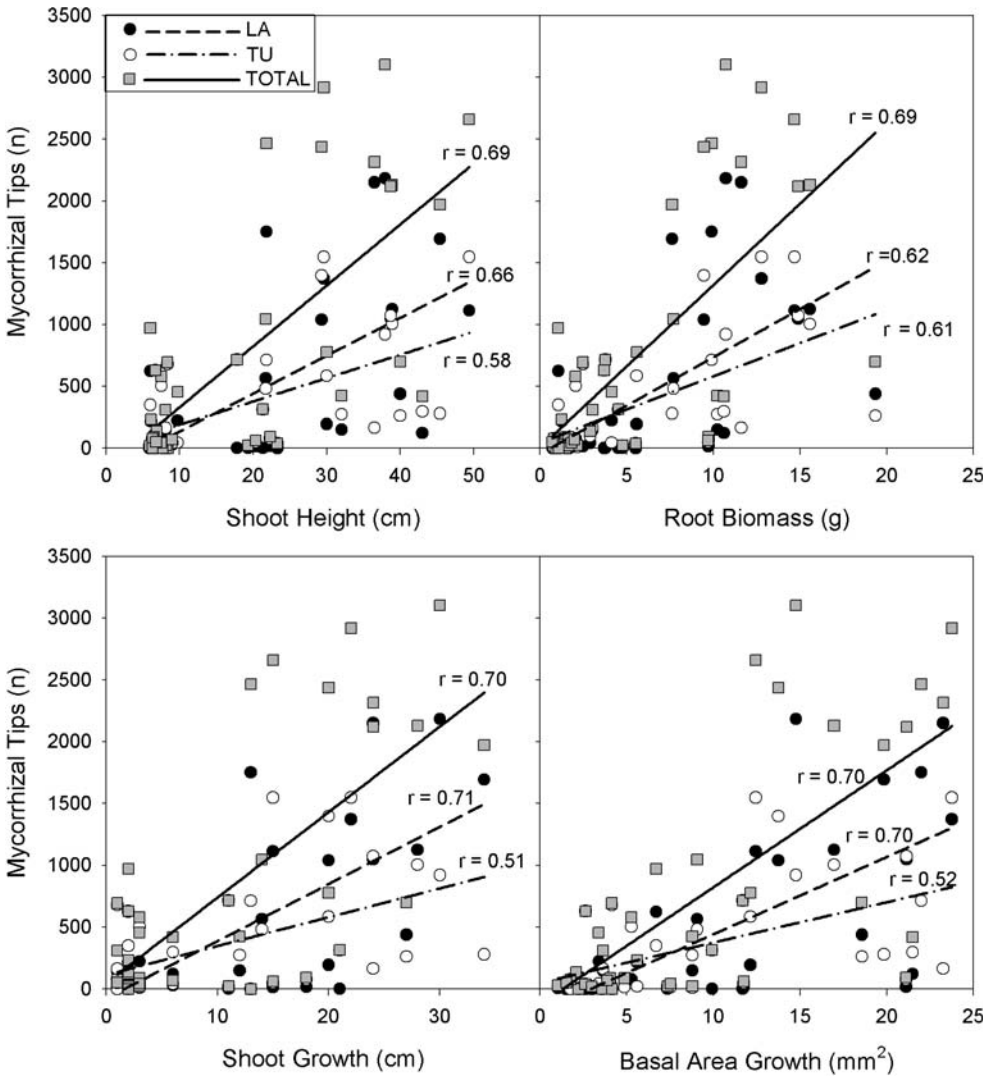


Fig. 2 Correlations between the number of root tips infected by *Laccaria* sp. 2 (*LA*), *Tuber* sp. 1 (*TU*), or the total number of infected root tips (*TOTAL*) and shoot height (*top left*), root biomass (*top right*), shoot growth (*bottom left*), and basal area growth (*bottom right*)

Conifer seedlings in adjacent nursery beds also were mycorrhizal with *Laccaria* sp. 1, close to *L.proxima*, and with four other fungal species but not with *Tuber*. The difference in mycorrhizal assemblages suggests differences in host specificity of fungal species. Oak and conifer seedlings did not acquire mycorrhizal inocula from each other or from a common source.

Abundance of *Laccaria* and *Tuber* and total mycorrhizas per seedling correlated positively with height, root biomass, height growth, and basal area growth of *Q. garryana* seedlings. The correlation of growth, height, and root biomass was stronger with *Laccaria* than with *Tuber*.

Variation in mycorrhizal infection among blocks demonstrates the heterogeneity of available inocula. Although seedlings in all beds formed mycorrhizas with *Tuber* and with *Laccaria*, the average number of infected root tips varied among beds. Because seedlings were mixed before planting, any spatial patterns in infection can be attributed to heterogeneity of inoculum in the soil into which they were planted. This finding and the positive relationship between infection and growth suggest that if the oak seedlings in the nursery beds were consistently infected with the same mycorrhizas, uniformity of seedling size and mean seedling size would increase.

The finding that mycorrhizal abundance was a stronger predictor of individual seedling growth than initial seedling size supports the conclusion of a causal relationship between mycorrhizal infection and nursery seedling growth. Mycorrhizal infection is clearly one component of optimized nursery performance of *Q. garryana*. If oak seedlings in nursery beds acquire mycorrhizal fungi from natural sources, it may not be necessary to artificially inoculate, but monitoring for the presence of mycorrhizas on nursery stock would be valuable and may help explain variation in seedling performance. *Q. garryana* apparently benefits from a variety of mycorrhizal species (Valentine et al. 2004; Moser et al. 2005, 2008). Although nursery mycorrhizas differed from those on seedlings in native woodlands, the resultant increase in seedling size will likely benefit postplanting seedling performance (Devine et al. 2007).

Acknowledgments This study was supported by National Science Foundation Grant DEB-0516229 to D. S. and DBI-0115892 to the Biotechnology Center at Southern Oregon University. We thank David Peter for collecting oak seedlings from the three field sites.

References

Agerer R (1991) Characterization of ectomycorrhizae. Methods Microbiol 23:25–73. doi:10.1016/S0580-9517(08)70172-7

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Berman JT, Bledsoe CS (1998) Soil transfers from valley oak (Quercus lobata Nee) stands increase ectomycorrhizal diversity and alter root and shoot growth on valley oak seedlings. Mycorrhiza 7:223–235. doi:10.1007/s005720050185

Cairney JWG, Chambers SM (eds) (1999) Ectomycorrhizal fungi: key genera in profile. Springer, New York, p 370

Chilvers GA, Lapeyrie FF, Horan DP (1987) Ectomycorrhizal versus endomycorrhizal fungi within the same root system. New Phytol 107:441–448. doi:10.1111/j.1469-8137.1987.tb00195.x

Cline ET, Ammirati JF, Edmonds RL (2005) Does proximity to mature trees influence extomycorrhizal fungus communities of Douglas-fir seedlings. New Phytol 166:993–1009. doi:10.1111/j.1469-8137.2005.01387.x

Devine WD, Harrington CA (2005) The root system morphology of Oregon white oak on a glacial outwash soil. Northwest Sci 79:179–188

Devine WD, Harrington CA, Leonard LP (2007) Post-planting treatments increase growth of Oregon white oak (*Quercus garryana* Dougl. ex Hook.) seedlings. Restor Ecol 15:212–222. doi:10.1111/j.1526-100X.2007.00205.x

Devine WD, Harrington CA, Southworth D (2009) Improving root growth of containerized Oregon white oak seedlings. Tree Plant Notes 54 (in press)

Dickie IA, Reich PB (2005) Ectomycorrhizal fungal communities at forest edges. J Ecol 93:244–255. doi:10.1111/j.1365-2745.2005.00977.x

Dickie IA, Koide RT, Steiner KC (2002) Influences of established trees on mycorrhizas, nutrition, and growth of *Quercus rubra* seedlings. Ecol Monogr 72:505–521

Dickie IA, Schnitzer SA, Reich PB, Hobbie SE (2007a) Is oak establishment in old-fields and savanna openings context dependent? J Ecol 95:309–320. doi:10.1111/j.1365-2745.2006.01202.x

Dickie IA, Montgomery RA, Reich PB, Schnitzer SA (2007b) Physiological and phenological responses of oak seedlings to oak forest soil in the absence of trees. Tree Physiol 27:133–140

Dixon RK, Garrett HE, Cox GS, Marx DH, Sander IL (1984) Inoculation of three *Quercus* species with eleven isolates of ectomycorrhizal fungi. I. Inoculation success and seedling growth relationships. For Sci 30:364–372

Egerton-Warburton L, Allen MF (2001) Endo- and ectomycorrhizas in *Quercus agrifolia* Nee. (Fagaceae): patterns of root colonization and effects on seedling growth. Mycorrhiza 11:283–290. doi:10.1007/s005720100134

Frank B (2005) On the nutritional dependence of certain trees on root symbiosis with belowground fungi (an English translation of A. B. Frank's classic paper of 1885). Mycorrhiza 15:267–275. doi:10.1007/s00572-004-0329-y

Frank JL, Barry S, Southworth D (2006a) Mammal mycophagy and dispersal of mycorrhizal inoculum in Oregon white oak woodlands. Northwest Sci 80:264–273

Frank JL, Southworth D, Trappe JM (2006b) NATS truffle and truffle-like fungi 13: *Tuber quercicola* and *Tuber whetstonense*, new species from Oregon, and *Tuber candidum* redescribed. Mycotaxon 95:229–240

Frank JL, Barry S, Madden J, Southworth D (2008) Oaks belowground: mycorrhizas, truffles and small mammals. In: A Merenlender, D. McCreary, KL Purcell (tech coords) Proceedings of the sixth California oak symposium: today's challenges, tomorrow's opportunities. Gen Tech Rep PSW-GTR-217, US Department of Agriculture, Forest Service, Pacific Southwest Research Station, Albany, CA, pp 131–138

Fuchs MA, Krannitz PG, Harestad AS (2000) Factors affecting emergence and first-year survival of seedlings of Garry oaks (*Quercus garryana*) in British Columbia, Canada. For Ecol Manag 137:209–219

Garbaye J, Menez J, Wilhelm ME (1986) Mycorrhizae of young oaks in nurseries and natural regenerations in north-eastern France. Acta Oecol 7:87–96

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizas and rusts. Mol Ecol 2:113–118. doi:10.1111/j.1365-294X.1993.tb00005.x

Gardes M, Bruns TD (1996) ITS–RFLP matching for identification of fungi. In: Clapp JP (ed) Methods in molecular biology, species diagnostics protocols: PCR and other nucleic acid methods, vol. 50. Humana, New Jersey, pp 177–186

Goodman DM, Durall DM, Trofymow JA, Berch SM (1996) A manual of concise descriptions of North American ectomycorrhizas. Mycologue, Sidney, Australia

Hosten PE, Hickman OE, Lake FK, Lang FA, Vesely D (2006) Oak woodlands and savannas. In: Apostol D, Sinclair M (eds) Restoring the Pacific Northwest. Island, Washington, DC, pp 63–96

Kennedy PG, Hortal S, Bergemann SE, Bruns TD (2007) Competitive interactions among three ectomycorrhizal fungi and their relation to host plant performance. J Ecol 95:1338–1345. doi:10.1111/j.1365-2745.2007.01306.x

Luoma DL, Stockdale CA, Molina R, Eberhart JL (2006) The spatial influence of *Pseudotsuga menziesii* retention trees on ectomycorrhizal diversity. Can J Res 36:1–13. doi:10.1139/X06-143

Marx DH, Marrs LF, Cordell CE (2002) Practical use of the mycorrhizal fungal technology in forestry, reclamation, arboriculture, agriculture, and horticulture. Dendrobiology 47:27–40

McCarthy C (1998) Chromas 1.45. School of Health Science, Griffith University, Southport, Queensland, Australia

McCreary D (2004) Managing and restoring California's oak woodlands. Nat Areas J 24:269–275

Menkis A, Vasiliauskas R, Taylor AFS, Stenlid J, Finlay R (2005) Fungal communities in mycorrhizal roots of conifer seedlings in forest nurseries under different cultivation systems, assessed by morphotyping, direct sequencing and mycelial isolation. Mycorrhiza 16:33–41. doi:10.1007/s00572-005-0011-z

Mitchell RJ, Cox GS, Dixon RK, Garrett HE, Sander IL (1984) Inoculation of three *Quercus* species with eleven isolates of ectomycorrhizal fungi. II. Foliar nutrient content and isolate effectiveness. For Sci 30:563–572

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

Moser AM, Petersen CA, D'Allura JA, Southworth D (2005) Comparison of ectomycorrhizas of *Quercus garryana* (Fagaceae) on serpentine and nonserpentine soils in southwestern Oregon. Am J Bot 92:224–230. doi:10.3732/ajb.92.2.224

Moser AM, Frank JL, D'Allura JA, Southworth D (2008) Ectomycorrhizal communities of *Quercus garryana* are similar on serpentine and nonserpentine soils. Plant Soil.. doi:10.1007/s11104-008-9743-9

Parrent JL, Morris WF, Vilgalys R (2006) CO_2-enrichment and nutrient availability alter ectomycorrhizal fungal communities. Ecology 87:2278–2287. doi:10.1890/0012-9658(2006)87[2278:CANAAE]2.0.CO;2

Quero JL, Villar R, Marañón T, Zamora R (2006) Interactions of drought and shade effects on seedlings of four *Quercus* species: physiological and structural leaf responses. New Phytol 170:819–834. doi:10.1111/j.1469-8137.2006.01713.x

Stein WI (1990) Oregon white oak. In: Burns RM, Honkala BH (tech coords) Silvics of North America, vol 2. Hardwoods. USDA Agric. Handbook, No. 654, pp 650–660 online http://www.na.fs.fed.us/spfo/pubs/silvics_manual/volume_2/quercus/garryana.htm

Tyler CM, Kuhn B, Davis FW (2006) Demography and recruitment limitations of three oak species in California. Q Rev Biol 81:127–152. doi:10.1086/506025

Valentine LL, Fiedler TL, Hart AN, Peterson CA, Berninghausen HK, Southworth D (2004) Diversity of ectomycorrhizas associated with *Quercus garryana* in southern Oregon. Can J Bot 82:123–135. doi:10.1139/b03-117

Von Herrmann S, Ritter T, Kottke I, Oberwinkler F (1992) Steigerung der leistungsfahigkeit von forstpflanzen (*Fagus silvatica* L. und *Quercus robur* L.) durch kontrollierte Mycorrhizierung. Allge Forst Jagdzeit 163:72–78

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, San Diego, pp 315–322