

Mycorrhizas on nursery and field seedlings of *Quercus garryana*

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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*, *Tarsetta*, 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*

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-

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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 K₂O 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

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

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

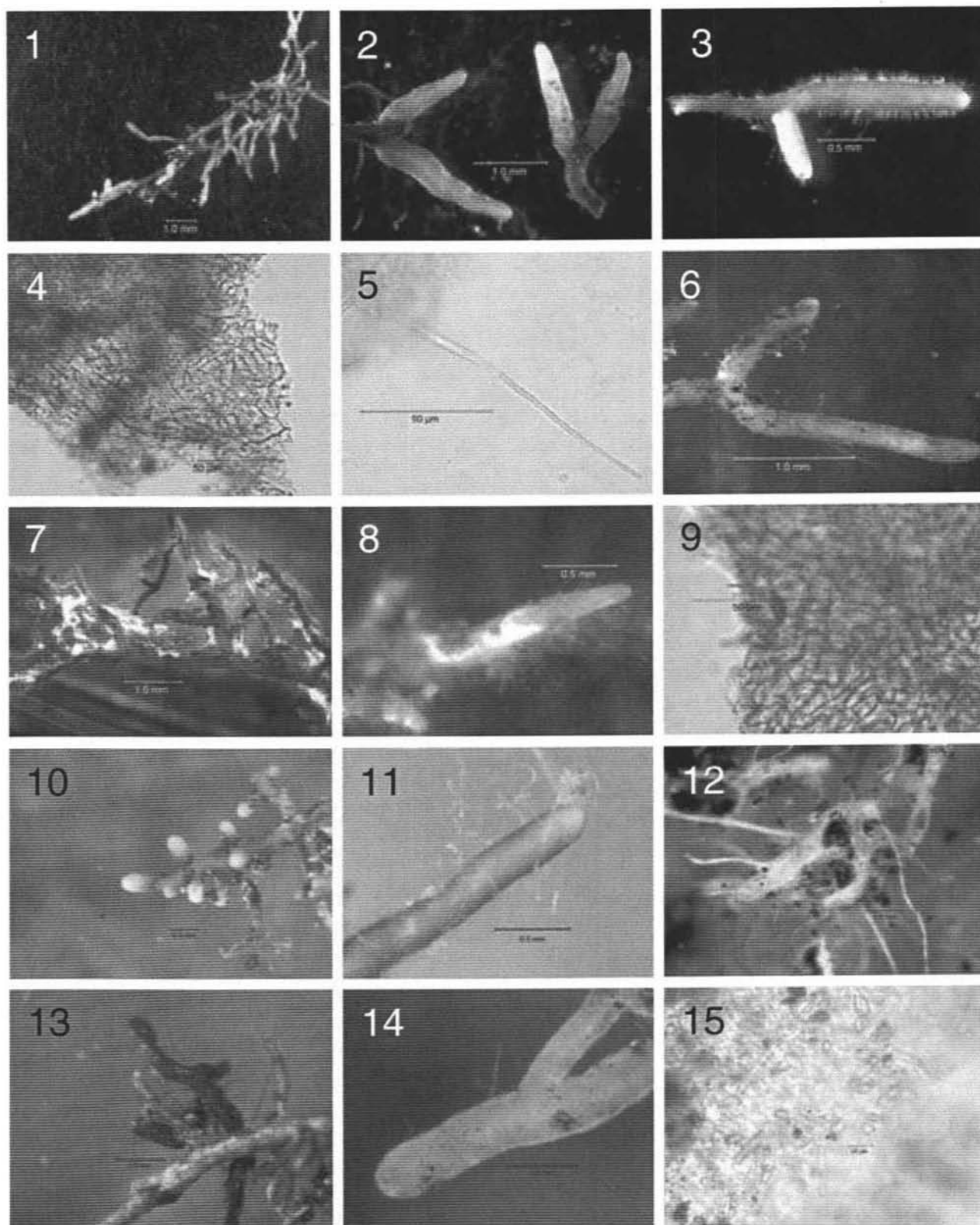
primer ITS1F (5'-ggcatttagaggaagtaa-3') and universal eukaryote primer TW13 (5'-ggtcggtgtttcaagacg-3'; White et al. 1990; Gardes and Bruns 1993, 1996). PCR reactions (20 µl) were performed using 0.6 units GoTaq (Promega, Madison, WI, USA) and 4 µL 5× colorless buffer, 200 µM each dNTP, 0.3 µM each primer, 2.5 mM MgCl₂, 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'-tccgtaggtgacacgtgcgg-3') and reverse primer ITS4 (5'-tctccgcttattgatgc-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



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 $\alpha=0.05$.

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 seed-

lings, 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 Pyrenomataceae, distinct phylotypes were assigned to genera (*Genea*, *Geneabea*, *Humaria*, *Otidea*, and *Tarzetia*) 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 *Geneabea*, *Tarzetia*, 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			
<i>Cenococcum</i> (Fig. 1 13)	Black, 0.5–1.5 mm long; stiff, angular black hyphae; mantle: regular synenchyma	0	7
<i>Genea</i>	Brown, dark red-brown; mantle: regular synenchyma	0	1
<i>Humaria</i> (Fig. 1 11)	Reddish tan to red-brown; mantle: regular synenchyma, emanating hyphae	0	3
<i>Peziza</i> (Fig. 1 6)	White to pale tan, smooth	1	0
<i>Otidea</i>	White-tan, long, reflective	0	1
<i>Tuber</i> sp. 1 (Fig. 1 1–5)	Pale tan to rusty tan; mantle: net synenchyma, cystidia	29	0
<i>Tuber</i> sp. 2 (Fig. 1 10)	Tan with pale tips; pinnate	0	1
Basidiomycota			
Clavulinaceae	White or gray-tan, white surface	0	1
<i>Cortinarius</i> ^a (Fig. 1 12)	White tortuous, reflective; om: felt prosenchyma, emanating hyphae, rhizomorphs	0	5
<i>Entoloma</i>	Tan	1	0
<i>Hebeloma</i>			
<i>Inocybe</i>	Tan cottony	0	1
<i>Laccaria</i> sp. 2 (Fig. 1 7–9)	White, wefty, clamp connections	33	0
<i>Russula</i> (Fig. 1 14, 15)	Pale tan shiny; om: net prosenchyma, im: net synenchyma, flask-shaped cystidia	0	2
<i>Tomentella</i>	Black, warty, large (1.5–5 mm); clamp connections; om: noninterlocking irregular synenchyma, short light brown hyphae, some with white tips	0	2
<i>Xerocomus</i>	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 $\pm$ standard deviation) by nursery bed plot

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

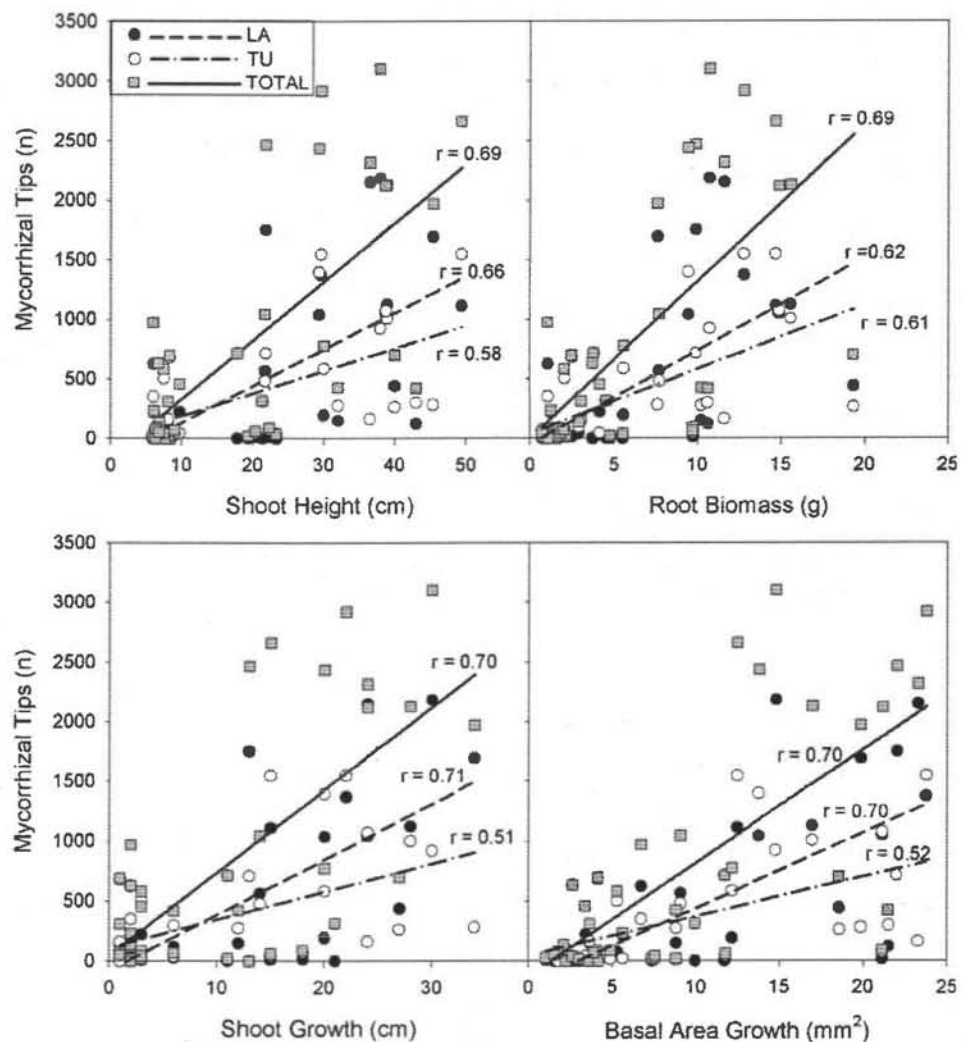
Potential variables were total mycorrhizal tips per seedling, number of *Laccaria* tips, number of *Tuber* tips, and initial basal area. Only variables significant at the 0.05 level were retained
BA basal area

Laccaria 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).

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