Plant Soil (2009) 315:185–194
DOI 10.1007/s11104-008-9743-9

REGULAR ARTICLE

Ectomycorrhizal communities of *Quercus garryana* are similar on serpentine and nonserpentine soils

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Received: 17 March 2008 / Accepted: 1 August 2008 / Published online: 16 August 2008

Abstract Serpentine soils, rich in iron, magnesium, and heavy metals, select for unique plant communities and for endemic species. Because mycorrhizal fungi mediate the interaction between plants and soil, we hypothesized that distinct ectomycorrhizal fungi would colonize *Quercus garryana* roots on serpentine and nonserpentine soils. We sampled roots of *Q. garryana* on serpentine soils at two locations in the Klamath-Siskiyou Mountains of southwestern Oregon and identified ectomycorrhizas by morphological and molecular methods. The same six most abundant and most frequent mycorrhizal species, *Cenococcum geophilum*, *Tuber candidum*, *Genea harknessii*, *Tomentella* sp., *Sebacina* sp., and *Inocybe* sp., were found on serpentine and nonserpentine soils. Based on similarities calculated using the Sørensen index in Non-metric Multidimensional Scaling, mycorrhizal communities on serpentine and nonserpentine soils were not significantly different. This study showed that ectomycorrhizal species associated with *Q. garryana* exhibit edaphic tolerance and were neither reduced nor excluded by serpentinite or peridotite parent materials.

Keywords Ectomycorrhiza · Heavy metal tolerance · Josephine ophiolite · Non-metric multidimensional scaling · Oaks · *Quercus garryana* · Serpentine

Electronic supplementary material The online version of this article (doi:10.1007/s11104-008-9743-9) contains supplementary material, which is available to authorized users.

Responsible Editor: Erik A. Hobbie.

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Introduction

The ectomycorrhizal community of *Quercus garryana* Dougl. ex Hook. exhibits rich biodiversity (Valentine et al. 2004). On alluvial soils in Southern Oregon, roots formed mycorrhizas with 40 fungal species; the most common were *Cenococcum geophilum* and *Tuber candidum*. Mycorrhizas mediate the contact between plant and soil through the interface of the ectomycorrhizal mantle, a sheath of fungal tissue that surrounds root tips. If soil composition influences ectomycorrhizal fungi, then serpentine soils may select for different fungi. In order to evaluate the role of soil composition on mycorrhizal communities, we sought a soil type that differed significantly from the metamorphic and alluvial soils that make up much of *Q. garryana* habitat.

Serpentine soils, rich in iron, magnesium, and heavy metals such as chromium and nickel, have a suite of soil properties that differ from nonserpentine soils derived from metasedimentary rocks (Brooks

1987; Alexander 1988; O'Hanley 1996; Lee et al. 2001; Oze et al. 2004). The effect of these soil differences is a change in vegetation density and in woody plant species (Kruckeberg 2006). Although edaphic factors are widely recognized for their importance in the ecology and evolution of serpentine plants, the effects of ultramafic soils on ectomycorrhizas and on the role of mycorrhizas in serpentine tolerance and sensitivity is often overlooked (Kruckeberg 1984, 1986, 1992; Brooks 1987; Brady et al. 2005; Grace et al. 2007). For example, a study looking at the physiological adaptation of Ponderosa pines to serpentine soils over 20 years did not consider the development of a mycorrhizal community (Wright 2007). However, Alexander et al. (2007) recognized the need to include a mycorrhizal component in studies of the adaptation of plants to serpentine soils.

Moser et al. (2005) compared the diversity of ectomycorrhizas associated with *Q. garryana* at three sites with paired serpentine and nonserpentine soils. Ectomycorrhizas were abundant at all sites; communities on serpentine soils were similar in morphotype richness to those on neighboring nonserpentine soils with no single fungal morphotype dominating on either soil type. These results were unexpected because fungi take up heavy metals and because some fungi have ecotypes or strains with tolerance to heavy metals (Gadd and deRome 1988; Gadd 1993; Galli et al. 1994; Jentschke and Godbold 2000; Meharg and Cairney 2000; Panaccione et al. 2001; Colpaert et al. 2000). Possible explanations for the finding of similar ectomycorrhizal communities on serpentine and nonserpentine soils by Moser et al. (2005) include too great a distance between sites, the weakly serpentinic composition of two of the sites, and lack of molecular confirmation of morphotype identity. Ectomycorrhizas on paired serpentine and nonserpentine sites (1 km apart) were more similar to each other than to those on distantly separated serpentine sites (up to 50 km apart). Only one serpentine site had levels of heavy metals greater than 1000 μg/g and a magnesium-to-calcium ratio greater than two (Moser et al. 2005).

Here we studied the ectomycorrhizas of *Q. garryana* on serpentine in ways that represent several advances. First, we sampled mycorrhizas at strongly serpentinic sites with high concentrations of chromium and nickel within 26 km of each other (Garcia 1979; Ramp and Peterson 1979; Harper 2003; Alexander et al. 2007). Second, we added molecular methods to identify ectomycorrhizal fungi. Third, we used ordination methods rather than pairwise similarity indices to compare communities. We test the hypotheses that mycorrhizal fungi would differ on serpentine soils, that they would be sparser in the drier soils, have less diversity, and be characterized by a unique set of serpentine-specific fungi.

Materials and methods

Study sites

We selected two sites in the Klamath-Siskiyou Mountains (Josephine County, OR, USA), each with *Q. garryana* Dougl. ex Hook. (Oregon white oak) growing on serpentine and nonserpentine soils in relatively close proximity (Ramp and Peterson 1979; Alexander et al. 2007): Waldo Mountain, south of Cave Junction, OR, USA on the Rattlesnake Creek Terrane (serpentine—42°03′ N, 123°39′ W, elevation 640 m; nonserpentine—42°02′ N 123°39′ W, elevation 720 m, 1.4 km apart) and Eight Dollar Mountain, west of Selma, OR, USA on the Josephine ophiolite (serpentine—42° 17′ N 123° 41′ W, elevation 460 m; nonserpentine—42° 17′ N 123° 42′ W, elevation 420 m, 2.1 km apart). The Waldo Mountain and Eight Dollar Mountain sites were 26 km apart. On serpentine soil at Eight Dollar Mountain, the oak was *Q. garryana* var. *breweri* (Engelm.) Jepson, the shrubby Brewer's oak with many stems about 2 m tall. At all other sites, the Oregon white oak was the tree form *Q. garryana* var. *garryana*, with single stems up to 7 m tall, although there was some overlap of form between the two varieties. At both sites, identity of serpentine and nonserpentine soils was confirmed by visual appraisal of rock characteristics and by soil analyses.

Sampling

Root samples for mycorrhizas were taken in February 2004. Each sample unit consisted of a total volume of 200 to 600 mL pooled from four soil cores from one tree taken along radii in the four cardinal directions at the canopy drip line. Soil samples were extracted with a soil corer (2.5 cm diameter×25 cm long) from the upper 15–20 cm of mineral soil. Six trees, at least

10 m apart, were sampled on each soil type at both sites. Soil samples were washed and sieved. And the ectomycorrhizal roots picked out under a dissecting microscope at ×10.

For analyses of soil composition, an additional four soil cores per tree were collected from each soil type at both sites. Composite samples were dried, ground, and analyzed at DANR Analytical Laboratory, University of California, Davis (http://danranlab.ucanr.org). Methods of elemental analyses followed those in Moser et al. (2005).

Morphotyping

All mycorrhizas were sorted by morphotype, a suite of characters including color, branching pattern, emanating hyphae, and mantle peels (Agerer 1991; Valentine et al. 2004; Moser et al. 2005). To quantify mycorrhiza abundance, we counted individual mycorrhizal root tips. We selected the most abundant mycorrhizal morphotypes from both soil types at each site for molecular analysis.

Molecular methods

Molecular data were obtained by sequencing of the internal transcribed spacer (ITS) region, including ITS1, the 5.8S ribosomal DNA gene and ITS2. DNA was extracted from ectomycorrhizas in cetyl trimethylammonium bromide with chloroform and amplified in polymerase chain reactions (PCR) with fungal specific primers ITS1F and ITS4 (White et al. 1990, Gardes and Bruns 1993, Bruns et al. 1998). Selected PCR products were cleaned in Montage PCR Centrifugal Filter Devices (Millipore Corporation), prepared for sequencing with BigDye Terminator Ready Reaction Mix and sequenced by an ABI 310 Genetic Analyzer (Applied Biosystems). Sequences were edited with Chromas 1.45 (McCarthy 1998) and compared to other fungal DNA sequences in GenBank with Basic Local Alignment Search Tool (BLAST; Altschul et al. 1990). Identifications were based on strength of match, similarity over the entire fragment length, consistency of matches, the pattern of top matches to vouchered specimens, and dissimilarity to other genera. Sequences were aligned and compared to each other in ClustalX to determine whether multiple species were present (Thompson et al. 1997)

Statistics

Soil properties were compared by one-way analysis of variance (ANOVA) with Minitab Release 15; the numbers of tips per sample, number of tips per soil volume, or fraction of the total number of tips for that sample were compared by two-way ANOVA. Coefficients of dispersion were calculated for tip abundance of the six most common species (Sokal and Rohlf 1996).

Similarities among samples were investigated on species abundance measured as tips/sample, tips/L, and on the proportion of each species as a fraction of the total mycorrhizal root tips from each sample using nonmetric multidimensional scaling (NMS) in PC-ORD 5 (McCune and Mefford 1999; McCune and Grace 2002). Distance measures were Jaccard (presence/absence) and Sørensen (abundance values). To minimize stress and reduce noise, datasets were tested as raw data; modified with sequential elimination of species present in one to four samples and with elimination of *Cenococcum*, the only outlier species; and with proportional abundance data transformed to arcsine squareroots and tip count data to logarithms

Table 1 Soil analyses for serpentine (Serp) and nonserpentine (Non) soils (four samples each) at Waldo Mountain (WM) at and Eight Dollar Mountain (EDM) in southwestern Oregon, USA

Soil	Site	pH	N %	C %	P ppm	X-Ca meq/100 g	X-Mg meq/100 g	Mg:Ca	Fe ppm	Cr ppm	Ni ppm
Serp	WM	5.7	0.23	4.4	11.3	11.5	24.0	2.1	15.45	1162.3	1581
Serp	EDM	6.08	0.19	3.42	2.22	4.2	11.0	2.6	7.2	1878.5	3036
Mean	(SD)	5.9 (0.3)[a]	0.2 (0.1)	3.8 (1.0)	5.9 (6.5)	7.1 (4.8)	16.2 (8.6)[a]	2.3 (0.4)[a]	10.5 (5.4)	1592 (489)[a]	2454 (802)[a]
Non	WM	5.35	0.3	5.06	2.7	13.2	8.6	0.7	5.5	227	246
Non	EDM	5.1	0.14	2.75	6.25	9.8	4.8	0.5	5.65	112	77
Mean	(SD)	5.2 (0.1)[a]	0.2 (0.1)	4.5 (2.1)	4.5 (2.1)	11.5 (2.3)	6.7 (2.4)[a]	0.6 (0.1)[a]	5.6 (0.1)	169 (67)[a]	161 (99)[a]

[a] Soil characteristics within a column differed significantly between soil types using one-way ANOVA ($P>0.05$).

Table 2 Abundance of ectomycorrhizas (EMs) as measured by number of ectomycorrhizal root tips per liter of soil under *Quercus garryana* in serpentine and nonserpentine soils at Waldo Mountain (WM) and Eight Dollar Mountain (EDM), OR, USA

Site	Nonserpentine			Serpentine		
	Mean (se) tips/L	Range	Total EMs	Mean (se) tips/L	Range	Total Ems
WM	653 (196)	113–1488	971	409 (150)	83–1091	1088
EDM	360 (76)	74–686	851	630 (257)	140–1716	926
Both	507 (110)		1822	520 (498)		2014

For each site and soil type, $n=6$. Differences in abundance of ectomycorrhizas between soil types were not significant using two-way ANOVA ($P>0.05$).

(McCune and Grace 2002). We analyzed a matrix of 18 mycorrhiza species from 24 plots (Supplement 1). NMS was performed with 50 runs of real data along with 100 runs with randomized data for a Monte Carlo test of significance. Groups were compared using multi-response permutation procedures (MRPP) in PC-ORD 5 to determine the significance of differences (McCune and Mefford 1999; McCune and Grace 2002).

Results

Soil characteristics

At both the Eight Dollar Mountain and Waldo Mountain sites, the pH, the concentrations of Mg, Cr, and Ni, and the Mg:Ca ratio were significantly higher on serpentine than on nonserpentine soils (Table 1). Iron was significantly higher on serpentine

Table 3 Identification of ectomycorrhizas from *Quercus garryana* in southwestern Oregon, based on BLAST matches from GenBank

Concensus taxon	GenBank accession	Soil	Length (bp)	Closest vouchered BLAST match	Max score	Query coverage (%)	E value	Max ident
Boletus sp.	EU018562	N	623	*Boletus pseudoregius* AY680996	838	96	0.0	91%
Cortinarius sp.	EU018564	S	409	*Cortinarius parvannulatus* AY669664	419	91	4e-114	87%
Genea sp.	EU018565	N	368	*Genea* sp. AY920529	444	98	9e-122	89%
Genea sp.	EU018566	S	674	*Genea harknessii* DQ218292	755	99	0.0	84%
Gilkeya sp.	EU018567	S	533	*Gilkeya compacta* DQ206862	937	100	0.0	98%
Gilkeya sp.	EU018568	S	403	*Gilkeya compacta* DQ206862	652	97	0.0	97%
Inocybe sp.	EU018569	S	330	*Inocybe* sp. DQ974804	329	100	4e-87	83%
Inocybe sp.	EU018570	S	597	*Inocybe inodora* AM882901	641	89	1e-180	88%
Inocybe sp.	EU018572	N	444	*Inocybe subnudipes* AM882809	511	95	9e-142	87%
Lactarius sp.	EU018573	N	555	*Lactarius substriatus* DQ974746	872	98	0.0	95%
Otidea sp.	EU018574	N	552	*Otidea umbrina* DQ974738	906	97	0.0	97%
Russula sp.	EU018575	S	130	*Russula delica* AY061671	219	99	2e-54	97%
Sebacina sp.	EU018576	S	684	*Sebacina* sp. DQ974768	706	72	0.0	94%
Sebacina sp.	EU018577	S	575	*Sebacina* sp. DQ974768	870	99	0.0	94%
Sebacina sp.	EU018578	S	460	*Sebacina* sp. DQ974770	500	96	2e-138	87%
Sebacina sp.	EU018579	S	416	*Sebacina* sp. DQ974770	434	97	2e-118	86%
Sebacina sp.	EU018580	S	454	*Sebacina* sp. DQ974770	605	97	5e-170	90%
Sebacina sp.	EU018581	N	568	*Sebacina* sp. DQ974768	765	99	0.0	90%
Sebacina sp.	EU018582	N	679	*Sebacina* sp. DQ974768	729	84	0.0	89%
Tomentella sp.	EU018583	N	609	*Tomentella* sp. U83482	899	99	0.0	93%
Tomentella sp.	EU018585	S	442	*Tomentella* sp. U92537	630	100	1e-177	93%
Tomentella sp.	EU018586	N	431	*Tomentella* sp. AJ534913	551	99	1e-153	90%
Tomentella sp.	EU018587	N	263	*Tomentella stuposa* AY010277	372	97	3e-100	92%
Tuber candidum	EU018589	N	637	*Tuber candidum* AY830856	987	99	0.0	95%
Tuber candidum	EU018590	S	685	*Tuber candidum* AY830856	881	94	0.0	91%
Tuber whetstonense	EU018592	S	587	*Tuber whetstonense* AY830855	829	100	0.0	97%

Soil type: N, nonserpentine; S, serpentine.

soils at Waldo Mountain, but not at Eight Dollar Mountain. Exchangeable Ca was slightly higher on nonserpentine soils, but the difference from nonserpentine soils was not significant. The significantly higher exchangeable Mg on serpentine soils contributed to the higher Mg/Ca ratio. Serpentine and nonserpentine soils did not differ significantly in C, N, or P.

Ectomycorrhiza abundance

In single soil samples, total numbers of ectomycorrhizas ranged from 140 to 1716 ectomycorrhizal root tips per liter of soil (Table 2). Mean differences in abundance of ectomycorrhizas on serpentine and nonserpentine soils were not significant.

Classification of ectomycorrhizas

DNA extractions from 32 morphotype collections yielded sequence data of usable quality with sequence matches ranging in strength from 83% to 98%. Based on BLAST results, 13 species of ectomycorrhizal fungi were identified (Table 3). For example, a 623 bp signal with no base pair ambiguities closely matched 17 sequences in the genus *Boletus*, without a consistent match to any single species. The shortest sequence, a 130 bp fragment matching only *Russula delica*, was entirely in the highly variable ITS1 region with a maximum identity match of 97%. Alignments with ClustalX separated *Tuber* into two phyletic groups. The genus *Tuber* includes two distinct clades, distinguishable by morphology of their ectomycor-

Table 4 Morphotype descriptions of ectomycorrhizas of *Quercus garryana* from Southwestern Oregon

Concensus taxon	Soil	Morphotype
Boletus sp. Fig. 8	N	Tan; monopodial pyramidal, bent and tortuous; smooth; few rhizomorphs; few white hyphae; i: nets, o: netp
Cortinarius sp. Fig. 19	N, S	White; long white thick hyphal fans; monopodial pyramidal, bent; smooth; i: nets, o: netp
Genea sp. Figs. 5, 6, 7	N, S	Dark red-brown, lighter red-brown tips; smooth; monopodial pyramidal; straight brown hyphae; i: nets, o: reg
Gilkeya compacta Figs. 11, 12	S	Tan; short dichotomous, pale tips; smooth, coralloid and straight; sparse white hyphae; i: nets, o: non
Inocybe sp. Fig. 18	N, S	White; tan base; smooth; monopodial pinnate and pyramidal, club-shaped tips, coralloid branching; short white hyphae; isolated or cottony; i: netp, o: nets
Lactarius sp. Figs. 14, 15	N, S	Yellow-tan; smooth to grainy; monopodial pyramidal, irregular branching, tortuous; white hyphae; i: int, o: netp or nets.
Otidea sp. Fig. 13	N	Yellow-tan; smooth; monopodial pyramidal, straight; white cottony hyphae; o: netp
Russula sp. Fig. 16	N, S	Brown to light brown; reddish layer under mantle; cystidia flask-shaped; i:int, o: nets.
Sebacina sp., Fig. 17	N, S	Yellow-white over gray-tan base; monopodial pinnate or pyramidal, tortuous; tufts of cottony white hyphae; i: int, o: nets.
Tomentella sp. Figs. 3, 4	N, S	Black, dark brown; often with brown tips; monopodial pinnate, tortuous; bent; grainy; visible mantle; pale hyphae; i: nets netp; o: non to reg
Tuber candidum Figs. 9, 10	N, S	Orange-tan to brassy tan, often with pale tips; monopodial pinnate or pyramidal, tortuous, bent; smooth; few short white hyphae; i: nets, o: int to non.
Tuber whetstonense	N, S	Orange-tan to brassy, often with pale tips; monopodial pinnate or pyramidal, tortuous, bent; smooth; some with cystidia; i: nets, o: int to non.
Cenococcum geophilum Figs. 1, 2	N, S	Black; grainy; most unbranched; straight and tortuous; long black hyphae; star syn
Unknown 1	N	Black with jade green patches; nets
Unknown 2	S	Brown; bifurcate; cystidial; i: netp to nets, o: reg
Unknown 3	S	Brown; with extensive hyphae; i: netp, o: nets
Unknown 4	S	Brown; surface rough; i: nets, o: reg
Unknown 5, Fig. 20	S	White; thick bifurcated tips; white rhizomorphs; i: nets, o: netp

Soil types: *S* Serpentine, *N* nonserpentine; mantle types: *i* inner mantle, *o* outer mantle, *netp* net prosenchyma, *nets* net synenchyma, *int* interlocking irregular synenchyma, *non* non-interlocking irregular synenchyma, *reg* regular synenchyma, *syn* synenchyma (Agerer 1991).

rhizas and by large differences in their ITS sequences (Frank et al. 2006a). These were separated by morphotype and identified to species, *T. candidum* and *T. whetstonense*.

All tips were sorted into morphotypes based on microscopic characters (Table 4).

Black morphotypes C. geophilum was identified by the black mantle with star-patterned synenchyma and stiff, black, often angular emanating hyphae (Figs. 1, 2). *Tomentella* sp. varied from black to dark brown with flexible, pale brown emanating hyphae (Figs. 3, 4). One black morphotype, Unknown black-green (U-1) from one sample did not fit either pattern.

Brown morphotypes Genea sp. mycorrhizas were dark red-brown with slightly lighter red-brown tips and pale brown hyphae (Table 4, Fig. 5). An outer mantle of regular synenchyma was often visible under the dissecting microscope (Figs. 6, 7). Three brown morphotypes lacked the red-brown tips or outer mantle that defined *Genea*. The most frequent of these was unknown brown rough (U-4); others were unknown brown hyphal (U-3) and unknown brown cystidial (U-2).

Tan morphotypes Boletus sp. lacked pale tips and appeared slightly more yellow than *Tuber* morphotypes (Fig. 8). *T. candidum* (Fig. 9) and *T. whetstonense* were rusty or orange-tan color with paler tips; older *Tuber* mycorrhizas lacked pale tips. The inner mantle was net synenchyma; the outer mantle graded from interlocking to non-interlocking synenchyma (Fig. 10). Cystidia were present on some tan mycorrhizas indicating *T. whetstonense*. *Gilkeya compacta* had bi- and trifurcate tips and an outer mantle pattern of interlocking synenchyma that was visible under the dissecting microscope (Figs. 11, 12). *Otidia* sp. was yellow-tan with pale emanating hyphae (Fig. 13). *Lactarius* sp. (Figs. 14, 15) and *Russula* sp. (Fig. 16) were cystidial.

Pale tan to white morphotypes Sebacina sp. varied from tan-white to gray-tan (Fig. 17). *Inocybe* sp. varied from pale tan to white with thicker tips (Fig. 18). *Cortinarius* was similarly tan, but with white rhizomorphs (Fig. 19). Unknown white bifurcate (U-5, Fig. 20) resembled the color and thickness of *Inocybe*, but none of the sequenced *Inocybe* mycorrhizas demonstrated this bifurcate pattern.

Fig. 1 Ectomycorrhizas of *Q. garryana* on serpentine and nonserpentine soils. *C. geophilum* with stiff black emanating hyphae

Fig. 2 Ectomycorrhizas of *Q. garryana* on serpentine and nonserpentine soils. *C. geophilum* mantle, star synenchyma

Fig. 3 Ectomycorrhizas of *Q. garryana* on serpentine and nonserpentine soils. *Tomentella* sp., pale soft emanating hyphae

Fig. 4 Ectomycorrhizas of *Q. garryana* on serpentine and nonserpentine soils. *Tomentella* sp. lacking hyphae

Fig. 5 Ectomycorrhizas of *Q. garryana* on serpentine and nonserpentine soils. *Genea* sp., red-brown, pale hyphae

Fig. 6 Ectomycorrhizas of *Q. garryana* on serpentine and nonserpentine soils. *Ge.* sp. inner mantle, net synenchyma

Fig. 7 Ectomycorrhizas of *Q. garryana* on serpentine and nonserpentine soils. *Ge.* sp. outer mantle, regular synenchyma

Fig. 8 Ectomycorrhizas of *Q. garryana* on serpentine and nonserpentine soils. *Boletus* sp., tan

Fig. 9 Ectomycorrhizas of *Q. garryana* on serpentine and nonserpentine soils. *T. candidum*, orange-tan, pale tips

Fig. 10 Ectomycorrhizas of *Q. garryana* on serpentine and nonserpentine soils. *T. candidum* outer mantle, interlocking and non-interlocking regular synenchyma

Fig. 11 Ectomycorrhizas of *Q. garryana* on serpentine and nonserpentine soils. *G. compacta*, bifurcate golden tan tips

Fig. 12 Ectomycorrhizas of *Q. garryana* on serpentine and nonserpentine soils. *G. compacta* outer mantle, thin-walled regular synenchyma

Fig. 13 Ectomycorrhizas of *Q. garryana* on serpentine and nonserpentine soils. *Otidea* sp. yellow-tan, emanating hyphae

Fig. 14 Ectomycorrhizas of *Q. garryana* on serpentine and nonserpentine soils. *Lactarius* sp., brown-tan, pale tips

Fig. 15 Ectomycorrhizas of *Q. garryana* on serpentine and nonserpentine soils. *Lactarius* sp., cystidia

Fig. 16 Ectomycorrhizas of *Q. garryana* on serpentine and nonserpentine soils. *Russula* sp., brown-tan

Fig. 17 Ectomycorrhizas of *Q. garryana* on serpentine and nonserpentine soils. *Sebacina* sp., gray-tan cluster

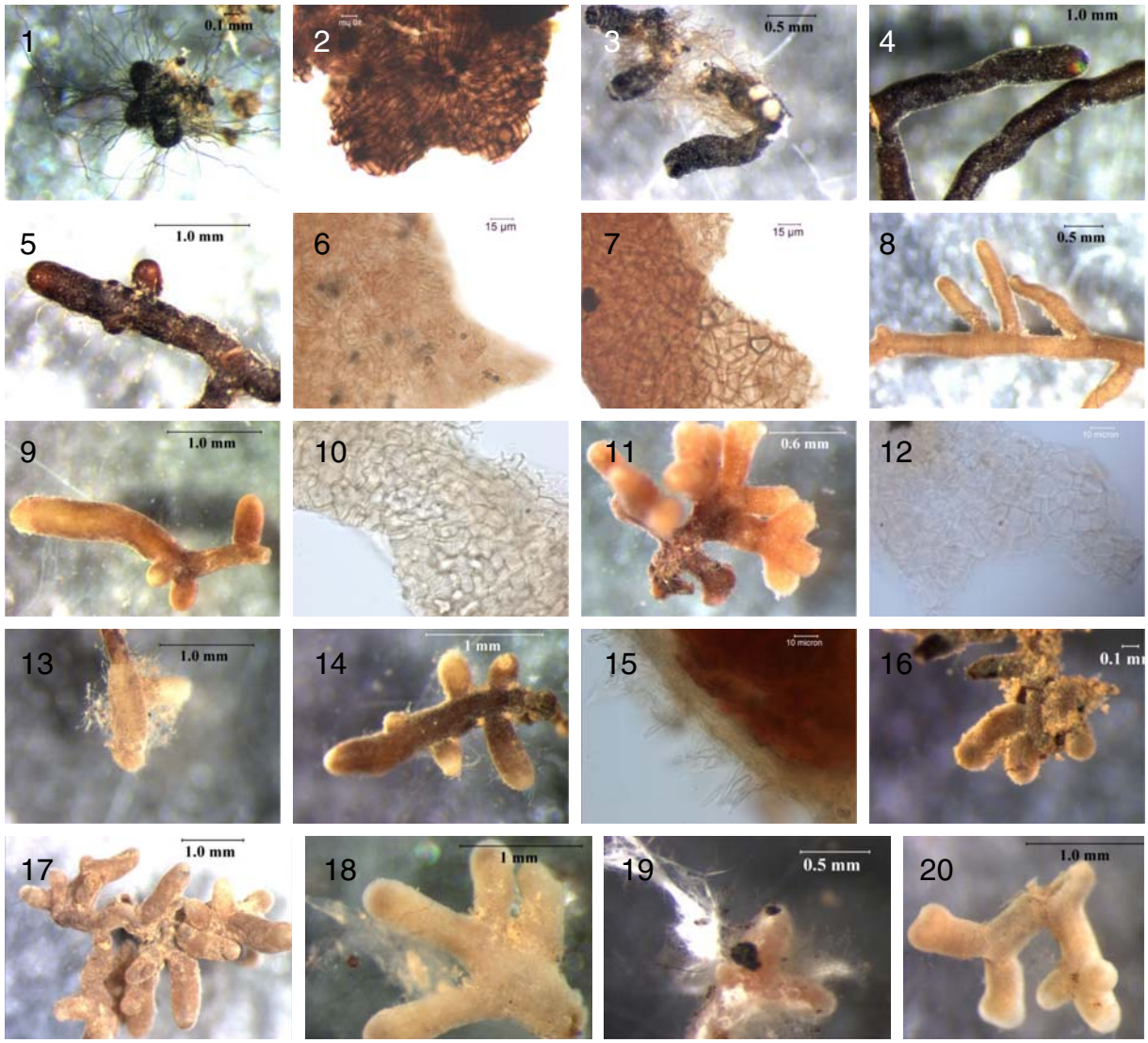
Fig. 18 Ectomycorrhizas of *Q. garryana* on serpentine and nonserpentine soils. *Inocybe* sp., white to light tan

Fig. 19 Ectomycorrhizas of *Q. garryana* on serpentine and nonserpentine soils. *Cortinarius* sp., tan with white rhizomorphs

Fig. 20 Ectomycorrhizas of *Q. garryana* on serpentine and nonserpentine soils. Unknown-5, white bifurcate

Abundance and frequency of ectomycorrhizal species

Of the 18 species only six, all identified by DNA sequences, occurred in more than five samples and in both soil types (Fig. 21, Table 5, Supplement 1). Nearly 80% of all mycorrhizal tips fit into these categories. The proportions of each species did not differ significantly among soil types or sites (ANOVA, $P>0.05$) when calculated as number of tips per sample, number of tips per soil volume or as fraction of the total number of tips for that sample. The largest fraction was of *T. candidum*, followed by



C. geophilum, *Tomentella* sp., *Sebacina* sp., *Genea* sp., and *Inocybe* sp. (Fig. 21). Coefficients of dispersion were greater than three for all six of the most common species indicating a clumped, nonrandom distribution (Sokal and Rohlf 1996).

The six most common species occurred on both serpentine and nonserpentine soils at both sites. Among the less frequent species, *Russula* sp. and *Lactarius* sp. occurred on both soil types; *Boletus* sp., *Otidea* sp. and unknown-1 on nonserpentine only; and *G. compacta* and unknown-4 on serpentine only and at both sites. These were abundant in one or few samples.

Based on similarities calculated using the Sørensen index in NMS, no distinct patterns of mycorrhizal communities were detected among the sites (Waldo Mountain and Eight Dollar Mountain) and soil types (serpentine and nonserpentine; Fig. 22). Mycorrhizal assemblages overlapped in ordination space so that no pattern of distinct groups by site or soil type emerged in any of the ordinations. The same patterns of randomness were found when species were measured by number of tips per sample, by number of tips per soil volume, and by percent composition. The random intermixing of samples occurred whether the ordination included entire dataset, the dataset modified to eliminate species found in fewer than four plots, or the log transform of entire dataset. The only species outlier was *Cenococcum*, which was present in the

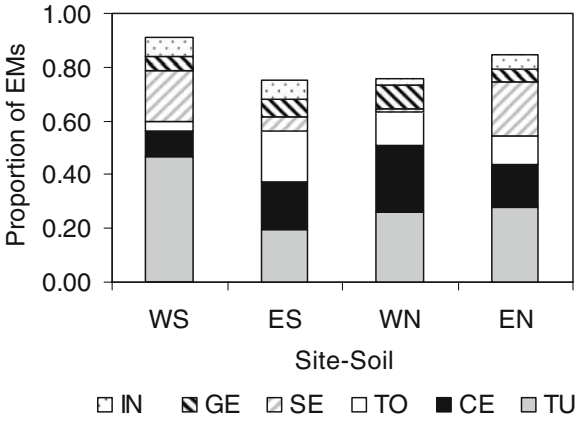


Fig. 21 Relative abundance of the most common ectomycorrhizas (EMs) as a proportion of total EMs on serpentine (S) and nonserpentine (N) soils at Waldo Mountain (W) and Eight Dollar Mountain (E) in Southwestern Oregon. *TU*, *T. candidum*, *CE*, *C. geophilum*, *TO*, *Tomentella* sp., *SE*, *Sebacina* sp., *GE*, *Genea* sp., and *IN*, *Inocybe* sp

most samples. Removing *Cenococcum* from the dataset also did not change the overlap of communities. One sample (on serpentine at Waldo Mountain) had only one species, *T. candidum* and that was not plotted by the ordination process (Fig. 22). The one pattern that emerged was that assemblages on serpentine soils were less similar to each other (average distance 0.82)

Table 5 Frequency of occurrence of mycorrhizal species on serpentine (S) and nonserpentine (N) soils at Waldo Mountain (W) and Eight Dollar Mountain (E), six trees per soil type at each site

Ectomycorrhiza	ES	WS	EN	WN	All
Tuber candidum	5	6	6	6	23
Cenococcum geophilum	6	3	6	5	20
Tomentella sp.	3	1	5	5	14
Genea sp.	2	2	4	5	13
Inocybe sp.	3	2	2	4	11
Sebacina sp.	3	2	5	1	11
Cortinarius	2	0	1	2	5
Tuber whetstonense	0	1	0	3	4
Gilkeya compacta	2	1	0	0	3
Lactarius sp.	1	0	1	1	3
Russula sp.	1	1	0	1	3
Unknown 4	1	2	0	0	3
Otidea sp.	0	0	0	1	1
Unknown 1	0	0	1	0	1
Unknown 2	1	0	0	0	1
Unknown 3	1	0	0	0	1
Unknown 5	0	1	0	0	1
Boletus sp.	0	0	0	1	1

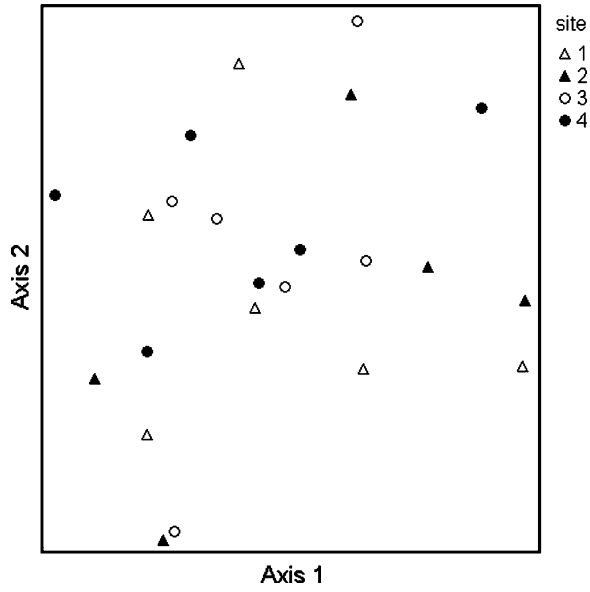


Fig. 22 NMS ordination, using Sørensen distance, of ectomycorrhizas on serpentine (*solid symbols*) and nonserpentine (*open symbols*) soils at Waldo Mountain (*triangles*) and Eight Dollar Mountain (circles) in Southwestern Oregon. The data were counts of EMs per sample. The samples intermix with no particular pattern related to soil type or site. Serpentine samples were less similar and more widely spread in ordination space than nonserpentine samples

and spread over a larger area of ordination space than nonserpentine assemblages (average distance 0.71). Based on MRPP using the same index of similarity as for NMS, the distances between groups (serpentine and nonserpentine) were not significantly different from random (A=0.00075, P=0.41).

Discussion

The serpentine sites at both Eight Dollar Mountain and Waldo Mountain were strongly serpentinic with higher levels of Mg, Cr, and Ni. These sites provide extreme differences between serpentine and nonserpentine soils (Brooks 1987; Alexander 1988; Alexander et al. 2007).

The most abundant and most frequent species of ectomycorrhizas associated with oaks on serpentine soils were the very common fungal species associated with oaks on all soils world wide: *C. geophilum*, an asexual Ascomycota with sclerotia; hypogeous Ascomycota (*Tuber*, *Genea*, *Gilkeya*, and other Pezizales); crust-forming resupinate Basidiomycota (*Sebacina*

and *Tomentella*); and fleshy Basidiomycota (*Russula*, *Boletus*, *Inocybe*, and *Cortinarius*; Cairney and Chambers 1999; Avis et al. 2003; Valentine et al. 2004; Moser et al. 2005; Richard et al. 2005; Walker et al. 2005; Frank et al. 2006b).

Serpentine soils supported extensive mycorrhizal communities. Serpentine mycorrhizal communities were not dominated by a single species nor were they distinguishable as groups of similar species distinct from nonserpentine mycorrhizal communities. Variability of ectomycorrhizal communities was as great between sites (Eight Dollar Mountain versus Waldo Mountain) as between soil types (serpentine versus nonserpentine) at each site.

Maas and Stuntz (1969) compared epigeous fungi under conifers on serpentine and nonserpentine soils in Washington. Several genera in the Basidiomycota, including *Inocybe* and *Suillus*, were found on both soil types. Only two hypogeous genera were reported (*Rhizopogon* and *Thaxterogaster*). Of 13 Ascomycota, only three were found on serpentine soils; these did not include *Tuber* or *Genea*. They did not examine ectomycorrhizas directly. Fruiting bodies and mycorrhizas are overlapping sets of species. The correlation between fruiting body abundance and mycorrhizal abundance is generally poor, especially where sampling is done only once and where fruiting is erratic due to dry soils (Horton and Bruns 2001; Taylor 2002).

The common ectomycorrhizal species with *Q. garryana* were similar on serpentine and nonserpentine soils. The abundances and proportions of epigeous, hypogeous, and resupinate fungal species, along with *Cenococcum*, were similar in these diverse soil types. Occasionally, individual epigeous species and hypogeous species occurred on one or the other soil types, but not in a widely repeated pattern across the landscape.

There may be uncommon species or ecotypes that prefer or avoid serpentine. Isolates of *Suillus luteus* from mineral-rich soils showed greater tolerance to zinc and cadmium, but not to copper or nickel (Colpaert et al. 2000). *C. geophilum* isolates from serpentine and nonserpentine soils differed in restriction and amplified fragment length polymorphism patterns (Panaccione et al. 2001). *Cenococcum* from other sites also harbors significant phylogenetic divergence, unexpected in an organism not known to have sexual reproduction (Douhan and Rizzo 2005). This study was designed to distinguish species, but not ecotypes.

Although some fungal species were found only on serpentine soils, the heterogeneity of soils and the patchiness of fungal dispersion, as evidence by the high coefficients of dispersion, prevented recognition of serpentine-specific species. The common mycorrhizal species are widespread and tolerant of serpentine conditions, thus they do not create distinct soil-specific groups. This study showed that the most frequent ectomycorrhizal species associated with *Q. garryana* were not excluded by serpentinite or peridotite parent materials and that no new species with frequent occurrences appeared solely on serpentine soils. The implication of this finding is that new root tips of either seedlings or established plants may obtain mycorrhizal inoculum from fungi on adjacent non-serpentine sites. Ectomycorrhizal plants on serpentine soils are not restricted by dispersal of spores among serpentine sites.

Acknowledgements This research was supported by National Science Foundation Grant DEB-0516229 to D. Southworth through Research at Undergraduate Institutions.

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