

## COMPARISON OF ECTOMYCORRHIZAS OF *QUERCUS GARRYANA* (FAGACEAE) ON SERPENTINE AND NON-SERPENTINE SOILS IN SOUTHWESTERN OREGON<sup>1</sup>

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The diversity of ectomycorrhizal communities associated with *Quercus garryana* on and off serpentine soils was compared and related to landscape-level diversity. Serpentine soils are high in magnesium, iron, and heavy metals and low in fertility. In plant communities on serpentine soils, a high proportion of flowering plant species are endemic. At three sites with paired serpentine and nonserpentine soils in southwestern Oregon, we sampled *Q. garryana* roots and categorized ectomycorrhizas by morphotyping and by restriction fragment length patterns. Ectomycorrhizas were abundant at all sites; no single fungal species dominated in the ectomycorrhizas. Of 74 fungal species characterized by morphotype and pattern of restriction fragment length polymorphisms, 46 occurred on serpentine soils, and 32 were unique to serpentine soil. These species are potentially endemic to serpentine soil. Similarities in species composition between paired serpentine and nonserpentine soils were not significantly lower than among three serpentine sites or among three nonserpentine sites. We conclude that mycorrhizal communities associated with oaks on serpentine soil do not differ in species richness or species evenness from those on neighboring nonserpentine soil.

**Key words:** ectomycorrhizas; heavy metals; morphotyping; mycorrhizas; Oregon white oak; *Quercus garryana*; serpentine soil; ultramafic.

Serpentine soils are associated with a unique vegetation that is sparse and often includes a high proportion of endemics (Reeves et al., 1983; Kruckeberg, 1984, 1992). Serpentine floras provide an opportunity to examine causes of endemism and rarity, as well as molecular mechanisms that have evolved to withstand heavy metals. Among serpentine-tolerant plants are species that accumulate heavy metals (Reeves et al., 1983; Brooks, 1987). The distinctive features of serpentine vegetation above ground may be mirrored in the ectomycorrhizal community below ground.

Contact between plants and soil is mediated by mycorrhizas, modified roots formed of fungi in close association with root tissues. Ectomycorrhizas, found on conifers and oaks, for example, have a fungal mantle that encases the root tip, a network of hyphae that grow between root cortical cells, and emanating hyphae that extend into the soil, thus expanding the surface area of the root (Smith and Read, 1997). The fungal partner of the mycorrhizal symbiosis is in direct contact with soil solutions.

Several factors make it difficult to predict how mycorrhizas on serpentine soil will differ from those on soils derived from metamorphic rocks of different composition (Leyval et al., 1997). Serpentine minerals may be toxic to some fungal species and thus select for a suite of resistant or tolerant fungal taxa different from those on nonserpentine soils. Mycorrhizas on serpentine soils may adsorb minerals by virtue of extensive

hyphae or hyphal walls with adsorbant properties and thus protect trees from toxic concentrations of elements (Gadd, 1990; Meharg and Cairney, 2000). Alternatively, mycorrhizas may not contribute to metal tolerance, but may help overcome low soil fertility. Reduced soil fertility might promote mycorrhizal formation. In soils of low fertility, trees are more dependent on ectomycorrhizas for mineral nutrition (Smith and Read, 1997). Some notable serpentine endemics and nickel hyperaccumulators, e.g., *Thlaspi montanum* L. var. *siskiyouense* P. Holmgren, are members of the Brassicaceae (Kruckeberg, 1984; Brooks, 1987; Heath et al., 1997) and lack mycorrhizas.

As compared to adjacent metavolcanic and metasedimentary rocks, ultramafic serpentine soils contain higher magnesium, iron, nickel, chromium, and cobalt and have a higher magnesium-to-calcium ratio, but contain less silicon, calcium, potassium, sodium, aluminum, and phosphorus (Coleman, 1977; Garcia, 1979; Alexander, 1988; O'Hanley, 1996; Harper, 2003). Thus serpentine or ultramafic soils are lower in fertility and may contain relatively high amounts of potentially phytotoxic elements.

Previous studies of mycorrhizas associated with conifers on serpentine soils found a lower diversity of ectomycorrhizal fungi. The number of fungal species as measured by macrofungal fruiting bodies collected on serpentine soils was about half that on nonserpentine soils in Washington (Maas and Stuntz, 1969). Diversity of basidiocarps, mycorrhizas, and mycorrhizal inocula was lower at a serpentine outcrop than on nearby nonserpentine soils in Virginia (Sheets et al., 2000).

*Cenococcum geophilum* Fr., a widespread, multihost ectomycorrhizal fungus, has been isolated from roots of *Pinus virginiana* P. Mill. on both serpentine and nonserpentine soils (Panaccione et al., 2001). Molecular analyses indicate that *C. geophilum* on serpentine soils differs from that on nonserpentine soils, yet the serpentine isolates also differ among themselves, as do the nonserpentine isolates.

<sup>1</sup> Received for publication 15 April 2004; revision accepted 30 September 2004.

The authors thank Robert Zasoski, William Horwath, and Caroline Bledsoe at the University of California–Davis and Robert G. Coleman at Stanford University for helpful comments. This research was supported by National Science Foundation Grants DEB-9981337 through the Biocomplexity Program and Research at Undergraduate Institutions and DBI-0115892 to the Biotechnology Center at Southern Oregon University.

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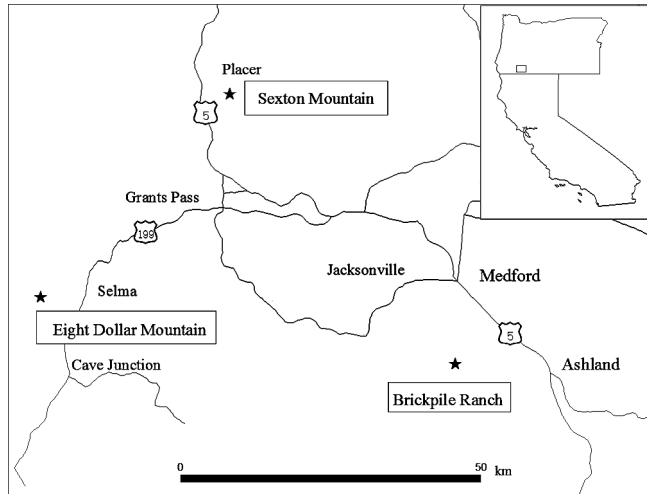


Fig. 1. Sites with paired serpentine and nonserpentine soils in southwestern Oregon. Inset shows Oregon and California and location of map.

Vesicular-arbuscular mycorrhizas also occur in plant roots on serpentine soils. Roots of grasses and forbs on serpentine soils in California were abundantly colonized by vesicular-arbuscular mycorrhizas (Hopkins, 1987). Four species of nickel hyperaccumulators in the Asteraceae were colonized by arbuscular mycorrhizal fungi on South African serpentine soils (Turnau and Mesjasz-Przybyłowicz, 2003).

Oaks also form ectomycorrhizas. Over 40 ectomycorrhizal fungal species, characterized by morphotype, were found in association with roots of *Quercus garryana* Hook. in southern Oregon (Valentine et al., 2002, 2004). In addition, *Q. garryana* roots also develop vesicular mycorrhizas as a small component of the total mycorrhizal infection (Valentine et al., 2002).

Many *Quercus* species occur on serpentine soils worldwide (Hull and Wood, 1984; Brooks, 1987). Although *Q. garryana* is not common on serpentine soils in southern Oregon, it does occur on low-elevation serpentine soils, both as the tree form, *Q. garryana* Dougl. Ex Hook. var. *garryana*, and as the shrub form, *Q. garryana* var. *breweri* (Engelm.) Jepson. In the Klamath-Siskiyou bioregion of southern Oregon, serpentine rocks and soils occur as isolated outcrops and as extensive ophiolite zones (Wells, 1956; Ramp and Peterson, 1979; Alexander, 1988).

The purpose of this study was to compare ectomycorrhizal diversity of *Q. garryana* growing on and off serpentine soils at three sites. We examined ectomycorrhizas directly and did not depend on collection of fungal fruiting bodies to estimate mycorrhizal diversity. In addition, two factors were held constant: (1) The ectomycorrhizas were on a single host species, *Q. garryana*, both on and off serpentine soil. (2) The differing soil types, serpentine and nonserpentine, were close to each other, minimizing microclimate differences. We hypothesized that roots on serpentine soil would have lower mycorrhizal diversity and a unique set of serpentine-specific fungi.

## MATERIALS AND METHODS

**Sites**—In southwest Oregon three sites were chosen; each included both serpentine and nonserpentine soils in close proximity, with *Q. garryana* growing on both soil types. (Fig. 1). All sites had the tree form, *Q. garryana* var. *garryana*, with the exception of Eight Dollar Mountain serpentine soil that

had the shrub form, *Q. garryana* var. *breweri*. The sites were: (1) Brickpile Ranch (BR), south of Jacksonville, Jackson County, serpentine soil (42°08' N, 122°51' W) and nonserpentine soil (42°08' N, 122°52' W) 1.8 km apart; (2) Sexton Mountain (SM), south of Placer, Josephine County, serpentine soil (42°38' N, 123°18' W) and nonserpentine soil (42°37' N, 123°19' W), 0.6 km apart; and (3) Eight Dollar Mountain (8\$), west of Selma, Josephine County, serpentine soil (42°17' N, 123°41' W) and nonserpentine soil (42°17' N, 123°42' W), 2.1 km apart.

Selection of sampling sites was facilitated by use of geologic maps—Wells (1956) for the Brickpile Ranch site, and Ramp and Peterson (1979) for the Sexton Mountain and Eight Dollar Mountain sites. We verified by field inspection of sites that serpentine soils were derived from ultramafic or serpentine sources and that nonserpentine soils were derived from plutonic or nonserpentine metamorphic sources.

All serpentine sites had components of serpentine soils with some variability. The Eight Dollar Mountain serpentine soil had no alluvial component. At Sexton Mountain, loose rocks were consistently serpentine, but some alluvial material had washed down from plutonic material above the serpentine bench. Brickpile Ranch was located in a drainage surrounded by nonserpentine metamorphic rocks and had greater soil development resulting from retention of alluvium and organic matter.

**Soil and root sampling**—For mycorrhizas, four soil samples per tree for each of four trees were collected along radii in the four cardinal directions at the canopy drip line. From February to June 2003, soil samples were extracted with a soil corer (2.5 cm diameter × 25 cm long) from the upper 15–20 cm of soil. Roots were washed and stored in water at 4°C before analysis.

Composites of eight subsamples per soil type at each site were dried and sieved. Soils were analyzed at DANR Analytical Laboratory, University of California, Davis, California, USA. Methods of analyses are described at <http://danranlab.ucanr.org>. Standard operating procedures used were pH from a saturated soil paste; soil nitrate and extractable ammonium by flow injection analyzer method; carbon by combustion gas analyzer method; extractable phosphorus by the Olsen method; exchangeable potassium, calcium, and magnesium by ammonium acetate extraction; and extractable micronutrients using diethylenetriaminepentaacetic acid (DTPA) for iron and total elements via inductively coupled plasma atomic emission spectroscopy for chromium and nickel.

**Morphotyping**—Ectomycorrhizal assessment and morphotype descriptions followed Agerer (1991), Goodman et al. (2002), and Valentine et al. (2002, 2004). Color, shape, branching pattern, surface texture, hyphal structure and abundance, and mantle pattern were recorded. Mycorrhizal tips of each morphotype were counted. Micrographs of each morphotype and its mantle peel were taken with a Spot RT digital camera (Diagnostic Instruments, Inc., Sterling Heights, Michigan, USA) using Leica DMLB compound and MZ75 dissecting microscopes. Detailed descriptions of ectomycorrhizal morphotypes are listed in the Supplemental Data accompanying the online version of this article. Numbers of morphotypes at each site and soil category were compared by two-way ANOVA.

Morphotypes were categorized as dark or light and as fuzzy or smooth, with or without abundant emanating hyphae. Data were analyzed using a chi-square 2 × 2 contingency table (Brower et al., 1998).

For individual sites we calculated Simpson's diversity index,  $D_s = 1 - [\sum n_i(n_i - 1) / (N(N - 1))]$  where  $n_i$  is the number of individuals in the  $i$ th species, and  $N$  is the total number of morphotypes, and Shannon's diversity index,  $H' = -\sum p_i \log p_i$  where  $p_i$  is the proportion of the total number of individuals that belong to species  $i$ . In addition, maximum values of  $H'$  and  $D_s$  were calculated based on even distributions of the actual numbers of morphotypes from each site. To compare pairs of soils or sites, we calculated Sørensen's index of similarity,  $CC_s = 2c / (s_1 + s_2)$  where  $c$  is the number of species in common and  $s_1$  and  $s_2$  are the total number of species on two sites (Brower et al., 1998). Two-tailed  $t$  tests were used to evaluate significance of differences between means.

TABLE 1. Soil analyses for paired serpentine and nonserpentine soils at three sites, Sexton Mountain (SM), Brickpile Ranch (BR), and Eight Dollar Mountain (8\$), in southwestern Oregon, USA.

Soil type	Site	pH	%C	µg/g			meq/100g <sup>a</sup>		ratio	µg/g		
				Fe	Cr	Ni	Mg	Ca	Mg:Ca	K	P	NO <sub>3</sub> + NH <sub>4</sub>
Nonserpentine	SM	6.4	2.5	35	349	359	7.6	9.4	0.81	70	1.1	9.8
	BR	6.0	1.9	48	75	58	1.2	11.5	0.10	268	33.9	3.0
	8\$	6.0	2.5	43	204	68	4.6	12.2	0.38	182	11.8	4.4
Serpentine	SM	6.5	3.1	53	652	775	14.1	12.3	1.15	113	3.6	5.9
	BR	5.9	7.1	135	299	302	4.8	18.1	0.27	282	18.7	13.6
	8\$	6.5	3.6	61	1771	2885	13.0	5.4	2.41	96	1.3	7.5

<sup>a</sup> milliequivalent.

**Molecular methods**—DNA was extracted from ectomycorrhizal tips using CTAB and amplified via polymerase chain reaction (PCR) using the fungal-specific primers ITS1-F and ITS4 (Gardes and Bruns, 1993; Valentine et al., 2004). PCR products were cut with restriction enzymes *Hinf*I and *Taq*I, and the restriction fragments were separated on a 4% acrylamide gel. Gels were stained with ethidium bromide and photographed using a UV transilluminator and camera with Fotodyne imaging software. Gels were analyzed with ONE-Dscan software. Patterns of restriction fragment length polymorphisms (RFLPs) were used as characters to distinguish among similar morphotypes (see Data Supplement accompanying the online version of this article).

## RESULTS

**Soil composition**—All serpentine soils were higher in iron than all nonserpentine soils (Table 1). Among pairs of soils at all three sites, the serpentine soil was higher in magnesium, chromium, nickel, and magnesium-to-calcium ratio than the nonserpentine soil (Table 1).

Soils varied in composition of nutrients (Table 1). None of the serpentine soils was particularly low in nitrogen. At Brickpile Ranch and Eight Dollar Mountain, nitrogen (ammonium plus nitrate) was higher on the serpentine soil, but at Sexton Mountain, nitrogen on serpentine soil was lower than on nonserpentine soil. Phosphorus was lower on the serpentine soil at Eight Dollar Mountain and Brickpile Ranch, but higher on serpentine soils at Sexton Mountain. The Brickpile Ranch serpentine site was highest in carbon. Neither soil type was particularly acidic: pH ranged from 5.9 to 6.4. At Brickpile Ranch, the magnesium-to-calcium ratio was less than 1.0 for both serpentine and nonserpentine soils, a value below that for nonserpentine soils at the other sites. At Eight Dollar Mountain, chromium and nickel concentrations and magnesium-to-calcium ratios were highest, but iron was comparable to concentrations at Brickpile Ranch and Sexton Mountain.

**Species richness**—Ectomycorrhizal morphotypes were abundant and diverse on serpentine and nonserpentine soils (Figs. 2–29, Table 2, Supplemental Data). Mantle peels (Figs. 10–13) and RFLP patterns (Supplemental Data) were used to distinguish among morphotypes that varied in form, pigmentation, and extent of emanating hyphae. No single form dominated. Some were single unbranched tips, others clustered (Figs. 9, 18, 28) or crowded with multiple morphotypes. All root tips were mycorrhizal. Pigmentation ranged from pale (Figs. 2, 22, 27) to very dark (Figs. 5, 15, 25, 26). Emanating hyphae varied from none, to short cystidial, to longer ones along the length of the mycorrhiza (Figs. 5, 7, 20). Others formed dense hyphal fans or mycelial strands (Figs. 4, 19, 23). Emanating hyphae were either the same color as the mantle (Figs. 5, 20) or white (Figs. 4, 19).

**Properties of serpentine morphotypes**—We examined characteristics of serpentine mycorrhizal morphotypes to determine whether particular characters such as pigmentation and extensive emanating hyphae prevailed on serpentine soils. Dark morphotypes were slightly more abundant on serpentine than on nonserpentine soils, although the differences were not statistically significant (Table 3). All pairs of sites had slightly more dark morphotypes on serpentine soils. Abundance of emanating hyphae (“fuzziness”) among morphotypes on serpentine soils was not consistently or significantly higher than among those on nonserpentine soils (Table 3). Serpentine-specific morphotypes exhibited the same patterns as did the serpentine morphotypes, including those that were not soil specific.

**Specificity of morphotypes with respect to soil type**—Of 74 morphotypes from the six sites, 46 (62%) occurred on serpentine sites, and 32 (43%) were unique to serpentine sites (Table 4). Fewer morphotypes (42) were found on nonserpentine sites, and 28 of those were restricted to nonserpentine sites. The majority (90%) of soil-specific morphotypes were unique to a single site. Only one serpentine-specific morphotype (SS02, Fig. 5) was found on all three serpentine soils. Two serpentine-specific morphotypes (SS12 and SS21, Figs. 3, 8) were found on two sites.

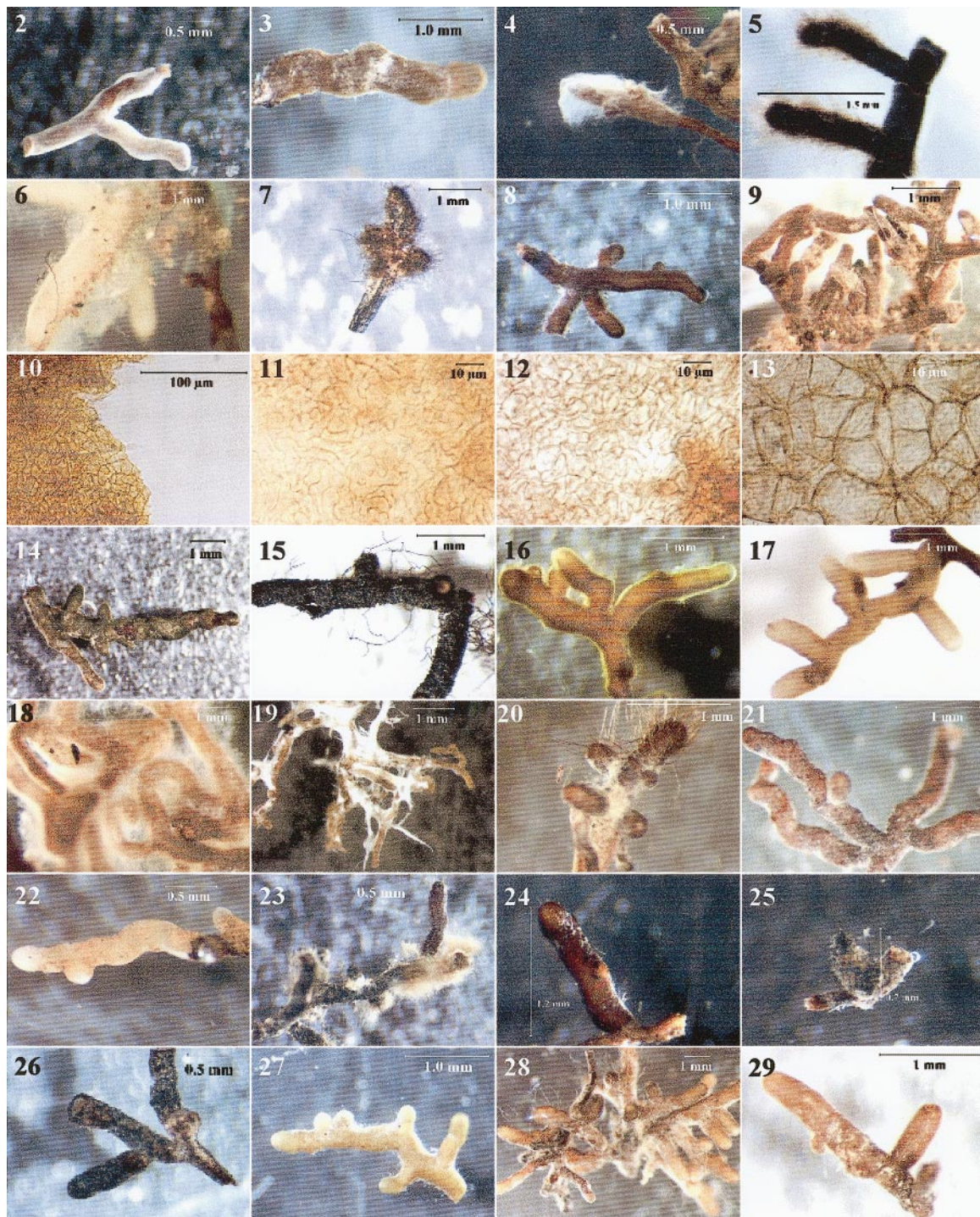
No morphotype that was found only on nonserpentine soils occurred at all three sites (Table 4). Of the 14 nonspecific morphotypes, half were found at one site on both soil types. Only *C. geophilum* (SS03, Fig. 25), the most abundant morphotype, was found at all six sites in both serpentine and nonserpentine soils.

**Variation among sites**—Although sites differed in total morphotypes and in the proportion of serpentine-specific morphotypes, neither total numbers of morphotypes nor numbers of soil-specific morphotypes differed significantly among sites ( $P = 0.95$  and  $P = 0.82$ , respectively) or soils ( $P = 0.87$  and  $P = 0.78$ , respectively; Table 2).

Dominant morphotypes varied among sites (see Data Supplement accompanying the online version of this article). At Sexton Mountain, the predominant morphotypes were SS07 (Fig. 26), SS24 (Fig. 23), and SS40 (Fig. 14). At Brickpile Ranch, SS36 (Fig. 22), SS52 (Fig. 15), SS59 (Fig. 18), and SS60 (Fig. 28) were abundant. At Eight Dollar Mountain, SS36 (Fig. 22) was abundant, as were SS78 (Fig. 19) and SS89 (not shown).

Diversity at all sites was similar, with no single morphotype dominating (Table 5). Neither Simpson’s diversity index nor





Figs. 2–13. Serpentine-specific morphotypes. **2.** SS25, white transparent, bent tip. **3.** SS12, tan, semitransparent, tortuous. **4.** SS28, white, wooly emanating hyphae. **5.** SS02, brown-black with dark emanating hyphae. **6.** SS62, light yellow, cottony hyphae with orange soil debris. **7.** SS37, black, matte, pyramidal. **8.** SS21, dark brown, felty, monopodial. **9.** SS67, milky brown, matte, irregular branching. **10.** SS62, inner mantle regular synenchyma. **11.** SS01, inner mantle interlocking irregular synenchyma. **12.** SS67, inner mantle interlocking irregular synenchyma. **13.** SS65, inner mantle regular synenchyma.

Figs. 14–21. Nonserpentine specific morphotypes. **14.** SS40, dark green, monopodial. **15.** SS52, black, warty, shiny, with long black emanating hyphae. **16.** SS46, orange-tan with transparent coating. **17.** SS56, milky brown, straight tips. **18.** SS59, brown, clustered. **19.** SS78, tan-yellow with white hyphal fans. **20.** SS77, brown, matte, with long hyphae. **21.** SS61, brown, grainy, tortuous tips.

Figs. 22–29. Nonspecific morphotypes. **22.** SS36, white to yellow tips with orange soil debris. **23.** SS24, dark brown with tufts of tan hyphae. **24.** SS01, red-brown, reflective. **25.** SS03, black, *Cenococcum geophilum*. **26.** SS07, dark brown to black. **27.** SS15, yellow, felty. **28.** SS60, brown with lighter tips, clustered monopodial. **29.** SS73, orange, smooth, straight tips.



TABLE 2. Soil specificity of ectomycorrhizal fungal morphotypes on *Quercus garryana* at three sites: Brickpile Ranch (BR), Sexton Mountain (SM), and Eight Dollar Mountain (8\$). Serpentine soils did not differ significantly from nonserpentine soils in number of morphotypes or in number of soil-specific morphotypes by two-way ANOVA.

Site	Serpentine soils			Nonserpentine soils		
	Serpentine-specific	Non-specific	Total morphotypes	Non-serpentine-specific	Nonspecific	Total morphotypes
BR	16	7	23	5	9	14
SM	14	7	21	13	5	18
8\$	6	7	13	13	9	22

Shannon's diversity index differed significantly ( $P > 0.1$ ) between serpentine and nonserpentine soils.

Based on Sørensen's index of similarity, mycorrhizal communities on serpentine soils did not differ significantly from those on the nearest nonserpentine soil (mean  $S_s = 0.27$ ) at the same site. Mycorrhizal communities on serpentine soils were also similar to each other (mean  $S_s = 0.24$ ), as were those on nonserpentine soils (mean  $S_s = 0.28$ ; Table 6).

## DISCUSSION

**Soil composition**—All of the sites had parts of the set of elements that define serpentine soils (Brooks, 1987). The Eight Dollar Mountain site on the Josephine ophiolite in the Klamath-Siskiyou bioregion of southwestern Oregon supports a classic serpentine flora on soils with levels of chromium and nickel sufficient for mining (Brooks, 1987; Kruckeberg, 1992). The relatively lower magnesium-to-calcium ratio in the well developed Brickpile Ranch serpentine soils may result from weathering (Brooks, 1987). The Brickpile Ranch serpentine outcrop is located in a drainage surrounded by metamorphic rocks, but its high iron and significant chromium and nickel demonstrate a serpentine influence. At Sexton Mountain, the levels of chromium and nickel and a magnesium-to-calcium ratio greater than 1.0 support its inclusion as a serpentine site.

On the serpentine soil with the greatest concentration of serpentine elements (Eight Dollar Mountain), the oak is *Q. garryana* var. *breweri*, a shrub form of *Q. garryana*. The other two sites with apparently greater alluvial influence supported *Q. garryana* var. *garryana*, the tree form. It is possible that *Q. garryana* grows on serpentine soil in areas where there are intrusions of nonserpentine materials, alluvial influences, greater weathering or greater soil development.

**Species richness**—Oak mycorrhizas on serpentine soils are abundant and diverse. Oak ectomycorrhizal diversity on serpentine soils is greater than that on alluvial soils derived from

TABLE 4. Specificity of ectomycorrhizal morphotypes on *Quercus garryana* with respect to soil type and frequency of occurrence on serpentine and nonserpentine soils at Brickpile Ranch, Sexton Mountain and Eight Dollar Mountain.

Number of sites with a morphotype	Numbers of morphotypes per number of sites			Total
	Morphotype specificity			
	Serpentine	Nonserpentine	Both soil types	
1	29	25	N/A	54
2	2	3	7	12
3	1	0	2	3
4	N/A	N/A	2	2
5	N/A	N/A	2	2
6	N/A	N/A	1	1
Total	32	28	14	74

volcanic rocks in southern Oregon (Valentine et al., 2004). This contrasts with studies on conifers, based on fruiting bodies and mycorrhizas, in which diversity of mycorrhizal fungi was lower on serpentine soils (Maas and Stuntz, 1969; Sheets et al., 2000). The relatively higher diversity of ectomycorrhizas associated with oaks on serpentine soils may result from climatic differences. Our sites differed from the conifer sites cited above in that they were in a region with a Mediterranean climate and extreme seasonal drying of soils.

Soils polluted with heavy metals from industrial wastes are extreme examples of low-fertility soils. On such sites, fungal responses vary. Some asexual forms of Ascomycota increased while others decreased (Gadd, 1990). Soils high in heavy metals may select tolerant species or genotypes. In greenhouse-grown plants, nickel (100  $\mu\text{g/g}$ ) reduced ectomycorrhizal colonization of *Quercus rubra* L. by *Suillus luteus* (Fries) S. F. Gray (Dixon, 1988). Mycorrhizas of Scots pine with zinc-tolerant genotypes of *Suillus* continued to take up phosphorus at control levels even when zinc levels were high (Adriaensen et al., 2003). This protection is in the form of a mycorrhiza that maintains normal nutrient uptake in the presence of heavy metal contaminants.

**Properties of serpentine mycorrhizal morphotypes**—Dark morphotypes were not significantly more abundant on serpentine than on nonserpentine soils. Although melanin in cell walls can adsorb heavy metals (Gadd and de Rome, 1988), these pigments appear to play a minor role in selection of serpentine mycorrhizal morphotypes. The percentage of morphotypes with abundant hyphae was not consistently higher on serpentine than on nonserpentine soils. Abundant hyphae are not correlated with development of serpentine morphotypes. Neither dark morphotypes nor abundance of hyphae indicates the serpentine level of a soil. It appears that elements such as

TABLE 3. Morphotype pigmentation and emanating hyphae (fuzzy) on serpentine and nonserpentine soils at three sites: Brickpile Ranch (BR), Sexton Mountain (SM), and Eight Dollar Mountain (8\$). No significant differences were found with either dark or fuzzy morphotypes between soil types.

Site	Serpentine morphotypes (%)		Nonserpentine morphotypes (%)		Serpentine-specific morphotypes (%)		Nonserpentine-specific morphotypes (%)	
	Dark	Fuzzy	Dark	Fuzzy	Dark	Fuzzy	Dark	Fuzzy
BR	69.6	34.8	64.3	50.0	56.0	25.0	40.0	40.0
SM	42.9	61.9	38.9	44.4	28.6	57.1	30.8	38.5
8\$	69.2	46.1	50.0	31.8	66.6	50.0	46.2	30.8
Mean (SE)	60.6 (8.8)	47.6 (7.9)	51.1 (7.4)	42.1 (5.4)	50.3 (11.3)	44.0 (9.7)	39.0 (4.5)	36.4 (2.8)

TABLE 5. Diversity of ectomycorrhizal morphotypes for each site and soil type as measured by Simpson's diversity index ( $D_s$ ) and Shannon diversity index ( $H'$ ). Maximum values of each diversity index were calculated based on even distribution of the actual number of morphotypes. Ratios of observed to maximum diversity did not differ significantly.

Site	Simpson's diversity index $D_s$				Shannon diversity index $H'$			
	Serpentine		Nonserpentine		Serpentine		Nonserpentine	
	$D_s$	$D_s/D_{smax}$	$D_s$	$D_s/D_{smax}$	$H'$	$H'/H'_{max}$	$H'$	$H'/H'_{max}$
Brickpile Ranch	0.89	0.91	0.94	0.99	2.42	0.77	2.22	0.84
Sexton Mountain	0.81	0.83	0.92	0.95	2.13	0.70	2.46	0.85
Eight Dollar Mountain	0.9	0.95	0.97	0.99	2.28	0.89	2.76	0.89
Mean (SE)		0.89 (0.04)		0.98 (0.01)		0.79 (0.05)		0.86 (0.02)

iron, nickel, and chromium do not select for mycorrhizas with these physical characteristics.

**Specificity to soil type**—Many morphotypes were unique to serpentine soils. These are possible serpentine endemics and may tolerate or resist heavy metals. Another set of ectomycorrhizal morphotypes occurs on nonserpentine soils only, indicating species that avoid serpentine soil. A third group of species found on both serpentine and nonserpentine soils have the ability to survive and the potential to infect oaks in diverse soil types.

The proportion of serpentine-specific ectomycorrhizas with *Q. garryana* (43%) is greater than that reported by Maas and Stuntz (1969), who found that 19% of 212 fungal fruiting bodies occurred only on serpentine soils, 63% only on nonserpentine soils, and 18% on both soil types. However, different tree species occupied their serpentine and nonserpentine sites, so the proportion of potentially similar morphotypes would be lower.

Most morphotypes were found at only one site. One morphotype was found in all three serpentine soils. *Cenococcum geophilum*, an asexual ascomycete, was found at all six sites (serpentine and nonserpentine), as it is in most ectomycorrhizal communities world wide.

**Variation among sites**—Mycorrhizal species richness on serpentine soils is similar to that on nonserpentine soils, indicating that serpentine soils do not inhibit mycorrhizal formation. This may be the result of microclimate similarities such as temperature, precipitation, wind, soil influences, and available inocula. Beta diversity, the change in species composition with distance, remained relatively constant across the

TABLE 6. Sørensen's index of similarity ( $S_s$ ) measured at three sites, Sexton Mountain (SM), Brickpile Ranch (BR), and Eight Dollar Mountain (8\$), among paired soils: serpentine (s) and nonserpentine (ns) soils pairwise at each site, serpentine soils pairwise among sites, and nonserpentine soils pairwise among sites.  $S_s$  values did not differ significantly.

Comparison (site 1–site 2)	Total morphotypes		Common morphotypes	Sørensen's index $S_s$	Mean $S_s$ (SE)
	Site 1	Site 2			
SMs–SMns	21	18	4	0.21	
BRs–BRns	23	14	6	0.32	0.27 (0.03)
8\$s–8\$ns	13	22	5	0.29	
SMs–BRs	21	23	7	0.32	
SMs–8\$s	21	13	4	0.24	0.24 (0.04)
BRs–8\$s	23	13	3	0.17	
SMns–BRns	18	14	4	0.25	
SMns–8\$ns	18	22	5	0.25	0.28 (0.03)
BRns–8\$ns	14	22	6	0.33	

scale of this project. The value of Sørensen's index of similarity for adjacent sites was comparable to that among nonserpentine sites.

Our findings of rich ectomycorrhizal diversity on serpentine soils contrast with those of Maas and Stuntz (1969) and Sheets et al. (2000). Several factors in our study contributed to the contrast between our results and those cited. We examined ectomycorrhizas directly and did not use collections of fungal fruiting bodies to estimate mycorrhizal diversity. This allowed us a more direct estimation of mycorrhizal species richness and diversity without depending on fruiting body production. We found no fungal fruiting bodies on most sites, so for our sites, fungal fruiting body production would have been a poor predictor of ectomycorrhizal diversity.

We conclude that oaks on serpentine soils have abundant mycorrhizal diversity with a significant fraction of fungal species found only on serpentine soils. The serpentine properties of low nutrient status combined with the seasonal dryness of Mediterranean climates may promote or support mycorrhizal diversity and abundance and balance the potentially toxic effects of heavy metals.

LITERATURE CITED

ADRIAENSEN, K., D. VAN DER LELIE, A. VAN LAERE, J. VANGRONSVELD, AND J. V. COLPAERT. 2003. A zinc-adapted fungus protects pines from zinc stress. *New Phytologist* 161: 549–555.

AGERER, R. 1991. Characterization of ectomycorrhizas. *Methods in Microbiology* 23: 25–73.

ALEXANDER, E. B. 1988. Morphology, fertility, and classification of productive soils on serpentinized peridotite, California, USA. *Geoderma* 41: 337–351.

BROOKS, R. R. 1987. Serpentine and its vegetation. Dioscorides Press, Portland, Oregon, USA.

BROWER, J. E., J. H. ZAR, AND C. N. VON ENDE. 1998. Field and laboratory methods for general ecology. McGraw-Hill, Boston, Massachusetts, USA.

COLEMAN, R. 1977. Ophiolites: ancient oceanic lithosphere? Springer-Verlag, New York, New York, USA.

DIXON, R. K. 1988. Response of ectomycorrhizal *Quercus rubra* to soil cadmium, nickel and lead. *Soil Biology and Biochemistry* 20: 555–559.

GADD, G. M. 1990. Metal tolerance. In C. Edwards [ed.], *Microbiology of extreme environments*, 178–210. McGraw-Hill, New York, New York, USA.

GADD, G. M., AND L. DE ROME. 1988. Biosorption of copper by fungal melanin. *Applied Microbiology and Biotechnology* 29: 610–617.

GARCIA, M. 1979. Petrology of the Rogue and Galice Formations, Klamath Mountains, Oregon: identification of a Jurassic Island arc sequence. *Journal of Geology* 86: 29–41.

GARDES, M., AND T. D. BRUNS. 1993. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizas and rusts. *Molecular Ecology* 2: 113–118.

GOODMAN, D. M., D. M. DURALL, J. A. TROFYMOW, AND S. M. BERCH [EDS.]. 2002. Concise descriptions of North American ectomycorrhizas. Mycologue Publications and Canada–BC Forest Resource Development

- Agreement, Canadian Forest Service. Victoria, British Columbia, Canada. Also available at: <http://www.pfc.forestry.ca/biodiversity/bcern/manual>, accessed 20 September 2004.
- HARPER, G. 2003. Fe-Ti basalts and propagating-rift tectonics in the Josephine Ophiolite. *Geological Society of America Bulletin* 115: 771–789.
- HEATH, S. M., D. SOUTHWORTH, AND J. A. D'ALLURA. 1997. Localization of nickel in epidermal subsidiary cells of leaves of *Thlaspi montanum* var. *siskiyouense* (Brassicaceae) using energy dispersive X-ray microanalysis. *International Journal of Plant Sciences* 158: 184–188.
- HOPKINS, N. A. 1987. Mycorrhiza in a California serpentine grassland community. *Canadian Journal of Botany* 65: 484–487.
- HULL, J. C., AND S. G. WOOD. 1984. Water relations of oak species on and adjacent to a Maryland serpentine soil. *American Midland Naturalist* 112: 224–234.
- KRUCKEBERG, A. R. 1984. California serpentines: flora, vegetation, geology, soils, and management problems. University of California Press, Berkeley, California, USA.
- KRUCKEBERG, A. R. 1992. Plant life of western North American ultramafics. In B. A. Roberts and J. Proctor [eds.], The ecology of areas with serpentinized rocks: a world view, 31–73. Kluwer Academic Publishers, Dordrecht, Netherlands.
- LEYVAL, C., K. TURNAU, AND K. HASELWANDTER. 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. *Mycorrhiza* 7: 139–153.
- MAAS, J. L., AND D. E. STUNTZ. 1969. Mycoecology on serpentine soil. *Mycologia* 61: 1106–1116.
- MEHARG, A. A., AND J. W. G. CAIRNEY. 2000. Co-evolution of mycorrhizal symbionts and their hosts to metal-contaminated environments. *Advances in Ecological Research* 30: 69–112.
- O'HANLEY, D. S. 1996. Serpentinities: records of tectonic and petrologic history. Oxford University Press, London, UK.
- PANACCIONE, D. G., N. L. SHEETS, S. P. MILLER, AND J. R. CUMMING. 2001. Diversity of *Cenococcum geophilum* isolates from serpentine and non-serpentine soils. *Mycologia* 93: 645–652.
- RAMP, L., AND N. V. PETERSON. 1979. Geologic map of Josephine County, Oregon, 1979. Bulletin 100, Geology and mineral resources of Josephine County, Oregon. Department of Geology and Mineral Industries, State of Oregon, Salem, Oregon, USA.
- REEVES, R. D., R. M. MACFARLANE, AND R. R. BROOKS. 1983. Accumulation of nickel and zinc by western North American genera containing serpentine-tolerant species. *American Journal of Botany* 70: 1297–1303.
- SHEETS, N. L., J. R. CUMMING, S. P. MILLER, AND D. G. PANACCIONE. 2000. Diversity of ectomycorrhizal fungal communities and *Cenococcum geophilum* populations from serpentine and nonserpentine soils. *Phytotaxonomy* 90: S71 (Abstract).
- SMITH, S. E., AND D. M. READ. 1997. Mycorrhizal symbiosis. Academic Press, London, UK.
- TURNAU, K., AND J. MESJASZ-PRZYBYLOWICZ. 2003. Arbuscular mycorrhiza of *Berkheya coddii* and other Ni-hyperaccumulating members of Asteraceae from ultramafic soils in South Africa. *Mycorrhiza* 13: 185–190.
- VALENTINE, L. L., T. L. FIEDLER, S. R. HANEY, H. K. BERNINGHAUSEN, AND D. SOUTHWORTH. 2002. Biodiversity of mycorrhizas on Garry oak (*Quercus garryana*) in a southern Oregon savanna. In R. B. Standiford, D. McCreary, and K. L. Purcell [eds.], Proceedings of the fifth symposium on oak woodlands: oaks in California's changing landscape. USDA Forest Service General Technical Report PSW-GTR-184, 151–157. Also available at <http://danr.ucop.edu/ihrmp/proceed/valentine.pdf>, accessed 20 September 2004.
- VALENTINE, L. L., T. L. FIEDLER, A. N. HART, C. A. PETERSEN, H. K. BERNINGHAUSEN, AND D. SOUTHWORTH. 2004. Biodiversity of ectomycorrhizal fungi associated with *Quercus garryana*. *Canadian Journal of Botany* 82: 123–135.
- WELLS, F. G. 1956. Geologic map of the Medford Quadrangle, Oregon-California. Map GQ-89. U.S. Geological Survey, Washington, DC, USA.