Biodiversity of Mycorrhizas on Garry Oak (*Quercus garryana*) in a Southern Oregon Savanna[1]

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Abstract

Garry oak or Oregon white oak (*Quercus garryana*) is the dominant vegetation on the Whetstone Savanna in Jackson County, Oregon. The site is located on the western edge of the Agate Desert, an alluvial fan capped with shallow clay loam over a cemented hardpan. The landform exhibits patterned ground with mounds and vernal pools. The oaks are associated with buck brush (*Ceanothus cuneatus*) and with native and exotic grasses. In preparation for a study of the biocomplexity of common mycorrhizal networks among oaks and grasses, we examined the mycorrhizal morphotypes on Garry oak. We sampled soil cores at distances half way to the canopy edge, at the canopy edge, and outside the canopy and have identified over 40 ectomycorrhizal morphotypes including *Cenococcum geophilum*. Infection rates on oak roots were lowest on trees growing in or near vernal pools and highest on oaks growing in groves with closed canopies. Using the fungal specific primers ITS1-F and ITS4 and the restriction enzymes *HinfI* and *TaqI*, we amplified DNA via polymerase chain reaction (PCR) to compare ectomycorrhizal morphotypes. Small differences in mycorrhizal morphology correlated with differences in restriction fragment-length polymorphism (RFLP) patterns, suggesting that there were many different species. We also observed the unusual occurrence of endomycorrhizas with intraradical hyphae and vesicles in oak roots.

Introduction

Although the association of oaks with fungal fruiting bodies or mushrooms (Arora 1979) and mycorrhizal fungi are well known (Trappe 1962), the below-ground interactions of oaks and fungi receive less scientific attention. Another major inference is that the fleshy fungal fruiting bodies associated with oaks correlate with the fungi that form ectomycorrhizas on roots (Arora 1979). However, although both ectomycorrhizal fungi and fungal fruiting bodies associate with Garry oak (*Quercus garryana*), the two fungal forms correlate poorly. That is, the ectomycorrhizas appear to be different species from the mushrooms.

Some studies on inoculations of oak roots clearly identify the fungi involved in mycorrhizal formation. For example *Pisolithus tinctorius*, *Suillus granulatus*, *S. luteus*, *Thelephora terrestris*, and *Cenococcum geophilum* form ectomycorrhizas with *Q. robur*, black oak (*Q. velutina*), and eastern white oak (*Q. alba*) in culture (Dixon

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and others 1984). In culture, *P. tinctorius* and *Scleroderma auranteum* form ectomycorrhizas with red oak (Beckjord and others 1985). Neither of these studies described the morphotypes. At that time, methods for correlating DNA of ectomycorrhizas with that of inoculum were not available. Furthermore, there is little information whether the fungi used as inocula in culture form the major part of field inocula or whether they are common or rare morphotypes.

Many studies of ectomycorrhizas on oaks have not distinguished among morphotypes and hence did not enhance our understanding of systematic and functional biodiversity. Wasserman and others (1987) identified heavy metals in mycorrhizas of eastern white oak and red oak without regard for the species of oak or the ectomycorrhizal morphotypes. Brundrett and others (1990) examined the structure of ectomycorrhizas on three species of oaks, but did not document morphotypes. Berman and Bledsoe (1998) provided descriptions for nine morphotypes and photographs of six morphotypes, but no DNA information or taxonomic identification.

New methods to identify ectomycorrhizas have led to more precise descriptions, at first as morphotypes without certainty about taxonomic identification, more recently with comparative DNA analyses, and with DNA sequencing to identify fungi at various taxonomic levels (Bruns and others 1998). Standardized descriptions of ectomycorrhizal characteristics have facilitated comparisons of ectomycorrhizal morphotypes even without knowing the taxonomic identity of the fungus (Agerer 1987-1998, Agerer and others 1996, Goodman and others 1996). Amplification of mycorrhizal DNA by fungal-specific primers, followed by restriction digests, has added a central quantitative character to morphological descriptions so that one can ask whether two similar morphotypes are the same fungal species. Comparison of mycorrhizal restriction fragment-length polymorphisms (RFLPs) with fungal fruiting body RFLPs helps identify morphotypes. This allows us to correlate ectomycorrhizal fungi with fungal species.

Endomycorrhizas, a second major class of mycorrhizas found on herbs, forbs, and some tree species, occasionally have been reported on oaks: *Q. rubra* (Grand 1969), *Q. imbricaria* (Rothwell and others 1983), and *Q. agrifolia* (Egerton-Warburton and Allen 2001). These observations extend our understanding of the complex ecto- and endomycorrhizal support system for oaks.

No studies have addressed the diversity or distribution of ectomycorrhizal fungi on Garry oak (*Quercus garryana* Hook). In spite of interest in habitat, systematics, tree health, natural regeneration, and restoration of Garry oaks, no studies have gone below ground to see which fungal associates are present. For example, we do not know how much variability there is among oaks at different sites, nor do we know the effects of grazing, fire, or non-native grasses on mycorrhizas. In other oak species, ectomycorrhizas are influenced by light intensity and by soil factors including aeration, moisture, organic matter, pH, temperature, and fertility (Garrett and others 1979). Diversity data from ectomycorrhizas on a Garry oak savanna can provide an initial base for comparing other stand conditions. The purpose of our study was to describe the diversity of ectomycorrhizas on Garry oak roots.

Methods and Materials

Whetstone Savanna, a 58-ha Preserve owned by The Nature Conservancy, is located on the western edge of the Agate Desert in Jackson County, Oregon (42 degrees 25

minutes North latitude 122 degrees 54 minutes West longitude T365 RSW sec 23, 26). The Agate Desert, an alluvial fan capped with a shallow layer of clay loam over cemented hardpan, is characterized by patterned ground with mounds and vernal pools. This landform is not true desert as it receives 48 cm of precipitation annually. The Whetstone Savanna site supports a mix of scattered lone trees and denser groves of Garry oaks. Buck brush (*Ceanothus cuneatus*) grows adjacent to Garry oaks, but not under the canopy. The grassland understory of Garry oaks supports perennial native bunch grasses including Lemmon's needlegrass (*Achnatherum lemmonii*), California oatgrass (*Danthonia californica*), and many introduced annual grasses including medusa head (*Taeniatherum caput-medusae*) and several *Bromus* species. Whetstone Savanna has a recent history of cattle grazing and logging of Ponderosa pines. (Borgias 1994)

Soil samples were extracted with a soil corer (2.5-cm diameter by 15 cm) from under Garry oaks at edges of oak stands where tree canopies overlapped grasses, buckbrush or other Garry oaks. Soil samples were collected along 4 or 5 radii at three distances from the trunk: 0.5 x canopy, 1.0 x canopy, and 1.5 x canopy. Samples (8-20 per tree) were immersed in water over a 1.0-mm sieve. Ectomycorrhizal morphotypes were distinguished by macroscopic and microscopic characteristics (Goodman and others 1996).

For endomycorrhizas, roots were cleared in KOH, washed in NH_4OH and H_2O_2 to remove phenolic compounds, stained in Chlorazol Black E, and mounted in PVLG (Brundrett and others 1996). Digital pictures of macroscopic and microscopic root tip characters were taken with Spot RT color digital camera.

DNA was extracted from ectomycorrhizal tips following a CTAB protocol, and amplified via polymerase chain reaction (PCR) using the fungal-specific primers ITS1-F and ITS4 (Bruns and others 1998). The PCR product was cut with the restriction enzymes *HinfI* and *TaqI* (Bruns and Gardes 1993, Gardes and Bruns 1996). The PCR product and the restriction fragments were run on a 4 percent acrylamide gel. Gels were stained with ethidium bromide and photographed using a UV transilluminator and camera with Fotodyne imaging software. Gel analysis was done using ONE-Dscan software (ONE-Dscan 1998).

Results

Above ground we collected fruiting bodies of 39 fungal species located under or near oaks. Ten species were Ascomycetes of which five were hypogeous (fruiting below ground). The other 29 species were Basidiomycetes of which 24 were fleshy fungi likely to be mycorrhizal.

In two collecting seasons (1999, 2000), we found over 40 ectomycorrhizal morphotypes at one site on Garry oak roots in the upper 15 cm of mineral soil (Southworth and others 2001). Most morphotypes were found only once, but five *(figs. 1.1-1.5)* were found on 5 percent or more of trees. The most common extomycorrhizal morphotype was *Cenococcum geophilum* (SOU 3; *fig. 1.1*) that forms sclerotia, but not fruiting bodies. SOU9, SO12, SOU13, and SOU30, the other four morphotypes *(figs. 1.2-1.5)* are unidentified at present.

In addition to morphotyping, we used molecular methods to identify fungal species on roots by comparing RFLP patterns from mycorrhizal roots with RFLP patterns from fruiting bodies. We produced RFLPs from 28 ectomycorrhizal

morphotypes and from 31 fungal fruiting bodies. The RFLP bands, derived from *HinfI* and *TaqI*, showed that only one ectomycorrhizal morphotype matched a fungal fruiting body: a "truffle-like" *Peziza* (Arora 1979).

In addition to ectomycorrhizas, oak roots formed endomycorrhizas *(fig. 1.6)* characterized by non-septate hyphae and vesicles. Arbuscules were not seen. We also observed dark septate hyphae associated with oak roots *(fig. 1.6)*. Dark septate endophytes were not organized as ectomycorrhizas into a mantle and Hartig net, but grew as individual hyphae over the root surface or between root cells.

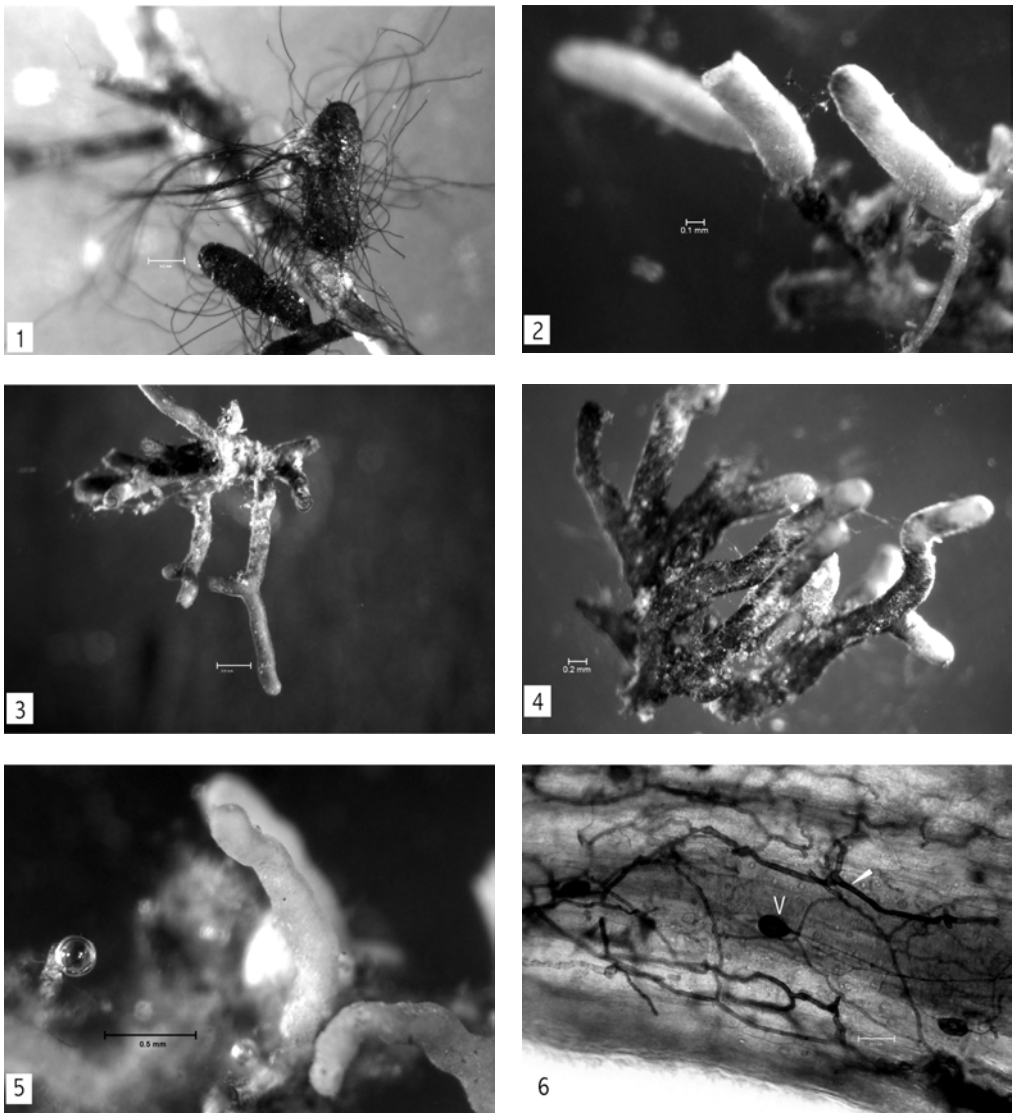

Figure 1—Ectomycorrhizal morphotypes on *Q. garryana* roots collected at Whetstone Savanna. **1.1)** *Cenococcum geophilum* (SOU3). Bar=0.2 mm. **1.2)** SOU9. Bar=0.1 mm. **1.3)** SOU12. Bar=0.5 mm. **1.4)** SOU13. Bar=0.2 mm. **1.5)** SOU30. Bar=0.5 mm. **1.6)** *Q. garryana* root with endomycorrhizal vesicles (V) attached to thin, non-septate hyphae. Branched dark-septate hyphae (arrowhead) are also present. Bar=25 µm.

Discussion

A large number of fungi form ectomycorrhizas with Garry oaks. It is likely that these differ in their physiological abilities such as nutrient uptake capacity. The result is an unrecognized complex of belowground components in Garry oak savannas.

The most abundant ectomycorrhizal morphotypes are leading candidates for participants in common mycorrhizal networks. *Cenococcum geophilum*, the most abundant ECM on roots of Garry oak, is ubiquitous and has low host specificity. These features may allow *C. geophilum* to play a key role in common mycorrhizal networks between plants. Endomycorrhizas are also potential participants in common mycorrhizal networks and may link oak networks to grass networks.

The disagreement in identity between ectomycorrhizal morphotypes and fruiting bodies may be explained in several ways. Mycorrhizal fungi may not produce fruiting bodies or may do so infrequently. Our sampling scheme may be too limited. We may need to take more mycorrhizal samples across the site and to sample more frequently and more exhaustively for fruiting bodies. Alternatively, not all fleshy fungi associated with oaks may form mycorrhizas; some fungi, "suspected" of being mycorrhizal, may in fact not be so.

Careful descriptions of ectomycorrhizal morphotypes can extend our ability to understand the mycorrhizal status of oak roots without knowing the identity of the actual fungus. If identification is important, we can apply PCR and RFLP methods that support classification of ectomycorrhizas and enable cross comparison of morphotypes as well as correlation with fungal fruiting bodies.

Conclusions

- The diversity of ectomycorrhizal morphotypes in a stand with one predominant tree species is remarkable.
- Species of fleshy fungi associated with oaks do not completely correspond to species of ectomycorrhizal fungi.
- Oaks form both ecto- and endomycorrhizas.
- Standardized descriptions of ectomycorrhizas provide a source of information about plant responses to environmental conditions.

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