

Oaks Belowground: Mycorrhizas, Truffles, and Small Mammals¹

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Abstract

Oaks depend on hidden diversity belowground. Oregon white oaks (*Quercus garryana*) form ectomycorrhizas with more than 40 species of fungi at a 25-ha site. Several of the most common oak mycorrhizal fungi form hypogeous fruiting bodies or truffles in the upper layer of mineral soil. We collected 18 species of truffles associated with Oregon white oak. Truffles do not release spores directly into the air, but remain closed belowground. In conifer ecosystems, animals eat truffles and disperse the spores, providing mycorrhizal inoculum for new roots. We did a survey to determine the extent that small mammals eat hypogeous fungi and defecate the spores, thus dispersing mycorrhizal inoculum. We trapped small mammals near Oregon white oaks and examined fecal pellets for hypogeous fungal spores. Three species of rodents, California voles (*Microtus californicus*), deer mice (*Peromyscus maniculatus*), and harvest mice (*Reithrodontomys megalotis*), had 12 species of fungal spores in their fecal pellets. The most common spores in fecal pellets were those of *Tuber candidum*/*T. quercicola*, *Hydnootryopsis setchellii*, and *Cazia flexiascus*, all Ascomycota. Seedlings growing in the root zone of mature oaks have access to the mycorrhizal network of parent trees, but seedlings germinating outside the root zone may lack mycorrhizal sources. If the mycorrhizal community on saplings located away from mature oaks includes hypogeous fungi, then small mammals may be dispersing fungal spores into shrublands where saplings are located. We examined roots of oak saplings at distances up to 72 m from mature oaks and found mycorrhizas of *Tuber candidum* and *Peziza infossa*, both hypogeous species, suggesting that small mammals disperse spores for mycorrhizal inoculum. We propose a model that identifies the major players in oak ecosystems and hypothesize that regeneration of oak woodlands depends on the dispersal of mycorrhizal fungal spores by small mammals.

Keywords: Hypogeous fungi, mycophagy saplings, mycorrhizal inoculum, Oregon white oak, *Quercus garryana*.

Introduction

Roots of oaks form mycorrhizas with fungi (Cairney and Chambers 1999). These are beneficial fungi, not related to sudden oak death. A mycorrhiza, literally a fungus root, is an oak root tip growing closely with a particular fungus (Agerer 1991, Smith and Read 1997). In ectomycorrhizas, fungal cells form a sheath or mantle around the root. Fungal hyphae penetrate between the outer cortical cells to form the Hartig net, a network of hyphae that exchange nutrients with the root cells. Also, hyphae emanate from the mantle outward into the soil where they take up water and nutrients, particularly nitrogen and phosphorus, and transfer these to the root. The tree provides carbon compounds to the fungi.

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In the upper layers of soil, virtually every root tip of Oregon white oak (*Quercus garryana*) is covered with the mantle of a mycorrhizal fungus (Valentine and others 2004, Moser and others 2005). So this is not a casual occurrence, but a regular feature of oak root structure. Ectomycorrhizas are found on all oak species and other members of the beech family (beech, tanoak), as well as in a few other families, for example, the pine family and the birch family.

Oregon white oak forms ectomycorrhizas with more than 40 species of fungi at a 25-ha site (Valentine and others 2004). The identity of ectomycorrhizal fungi associated with roots is hinted at by collecting the fungal fruiting bodies under the oaks, but confirmation of the identity is determined by comparing DNA of mycorrhizas with that of fruiting bodies or with fungal DNA sequences in a public database, for example, GenBank (Gardes and Bruns 1993).

Several of the most common oak mycorrhizal fungi form hypogeous fruiting bodies or truffles (both Ascomycota and Basidiomycota) in the upper 10 to 20 cm of mineral soil. Truffles do not release spores directly into the air, but remain closed belowground (Castellano and others 2004). Spores are dispersed by mycophagists, animals that eat the fruiting body and disperse the spores by defecation. In pine and Douglas fir forests of California and the Pacific Northwest, hypogeous fungi are eaten by small mammals, for example, flying squirrels, red-backed voles, and chipmunks (Hayes and others 1986, Izzo and others 2005, Maser and others 1978, Meyer and others 2005, North and others 1997), that disperse spores in fecal pellets.

Ectomycorrhizas of oaks also form with epigeous fungi—mushrooms, puffballs, jelly fungi, and crust fungi (Valentine and others 2004, Avis and others 2003, Walker and others 2005). Epigeous fungi emerge above the leaf litter and release their spores into the air where they are dispersed by air currents.

Oregon white oak seedlings initially obtain mineral nutrients and carbon from the cotyledons of the acorn for development of the initial root, which consists largely of a long tap root. By spring of the first year, the leaves emerge providing photosynthetic tissue for a source of carbon; the roots form lateral branches. Roots of seedlings germinating from acorns that fall under parent trees would grow in the fungal network of the parent tree. Thus the network of mycorrhizal mycelia emanating from parent tree mycorrhizas could serve as a source of mycorrhizal inoculum for the tips of lateral roots.

However, not all acorns germinate under the parent tree. Some are carried away by animals, such as ground squirrels and scrub jays, that move acorns and cache or bury them. Some of the cached or buried acorns also germinate, but the roots of these seedlings may not reach the mycorrhizal network of mature trees in areas where there are no ectomycorrhizal plants.

There is the common perception that fungi are “everywhere,” and indeed some fungi are widespread. However, mycorrhizal fungi are not ubiquitous because they are obligate heterotrophs on green plant hosts—they must be attached to roots for food. Mycorrhizal fungi live where they can interact with tree roots, and not just any tree roots but those of appropriate host trees. In conifers, transfers of soil or of truffle spores enhanced mycorrhizal formation of seedlings (Amaranthus and Perry 1987; Castellano and Trappe 1985).

Our questions relate to dispersal of inoculum for oak seedlings. How do seedlings germinating outside the root zone of mature trees encounter fungi that can

form mycorrhizas? If seedlings are successful, they grow into saplings. Do saplings, derived from successful seedlings, have mycorrhizas? Is the ectomycorrhizal community of saplings composed predominantly of epigeous fungi with airborne spores or does it include hypogeous fungi that would be dispersed by animals? Because so few seedlings survive beyond a few years, we chose to investigate the mycorrhizal communities of saplings, which are those seedlings that clearly have survived. This gives a longer-term view of mycorrhizal communities associated with survival.

The answers to these questions will influence our understanding of the elements required for natural regeneration in an oak woodland ecosystem. In addition, we could define important components of restoration practices where acorns or oak seedlings are planted to restore oak woodlands.

Methods

We collected truffles, trapped small mammals, and sampled sapling roots of Oregon white oak (Garry oak, *Quercus garryana*) at Whetstone Savanna Preserve (42°25'N, 122°54'W) north of Medford, Oregon, at an elevation of 400 m. Shrublands of buck brush (*Ceanothus cuneatus*) surround the oaks (Valentine and others 2004). No other ectomycorrhizal shrubs or trees were present.

We found truffles by raking under and around Oregon white oaks and examining the leaf litter and loose soil. Fungi were identified by use of keys and descriptions (Gilkey 1916, 1954; Trappe 1979, 1989; Arora 1986; Castellano and others 1989; Trappe and Castellano 1991; Hansen 2001; Fogel and States 2002, 2003; Frank and others 2006a, 2006b, 2006c). Preliminary fieldwork showed that spring (April to June) was the primary fruiting season for truffles under Oregon white oaks in this climatic region (Frank and others 2006).

For small mammal trapping, 60 Sherman live traps were set in a 10-m grid around Oregon white oaks and in the surrounding buck brush in spring 2003 and 2004 (Frank 2005, Frank and others 2006). In spring 2006, 80 traps were set at 10-m intervals up to 50 m from the trunks of Oregon white oaks. Trapped animals were identified and released; fecal pellets were stored at 4° C.

For spore identification, slides of fresh fecal pellets were stained with Melzer's reagent and viewed with a compound microscope. Spores were identified, based on comparisons to sporocarps collected at the research site and to descriptions in Castellano and others (1989) and in consultation with J. M. Trappe at Oregon State University.

Mycorrhizal oak roots were obtained from soil samples collected under saplings of Oregon white oak. Saplings, 0.7 to 4.5 m in height at distances of 3.4 to 72.0 m from mature trees, were sampled April to June 2004. Soil samples were rinsed through sieves with 0.59 and 0.15 mm openings (Moser and others 2005). Mycorrhizas were grouped by morphotype (Agerer 1991, Goodman and others 1996, Valentine and others 2004).

Results

Eighteen species of truffles were collected over two years (*table 1*). Most were Ascomycota; five (*Gymnomyces* sp., *Hymenogaster boozeri*, *Melanogaster eurypermus*, *Scleroderma cepa*, and *Zelleromyces gardneri*) were Basidiomycota.

Spores from 12 taxa were found in fecal pellets of small mammals. California voles ate the greatest diversity of species, followed by deer mice and harvest mice (*table 1*).

The ectomycorrhizal community on sapling roots included *Tuber* species, *Peziza infossa*, and *Scleroderma* sp. (*table 1*). These were the most abundant hypogeous fruiting bodies under mature Oregon white oaks at this site. Epigeous fungi were also present on sapling roots. All sapling root tips were mycorrhizal.

Table 1—Truffle fruiting bodies collected at Whetstone Savanna Preserve in southern Oregon; truffle spores in fecal pellets of 10 California voles, *Microtus californicus* (MI); 10 deer mice, *Peromyscus maniculatus* (PE); and 8 harvest mice, *Reithrodontomys megalotis* (RE); and truffle fungi as mycorrhizas on roots of Oregon white oak saplings. Fruiting bodies were collected and rodents trapped near Oregon white oaks at Whetstone Savanna Preserve in southern Oregon, USA, in 2003 and 2004.

Fungus	Small mammal			Sapling
	MI	PE	RE	
<i>Balsamia alba</i>	0	0	0	0
<i>Cazia flexiascus</i>	+	+	+	0
<i>Genabea cerebriformis</i>	+	+	0	0
<i>Genea gardneri</i>	0	0	0	0
<i>Genea harknessii</i>	+	0	0	0
<i>Gymnomyces</i> sp	+	+	+	0
<i>Hydnotryopsis setchellii</i>	+	+	0	0
<i>Hymenogaster boozeri</i>	+	0	0	0
<i>Melanogaster euryspermus</i>	0	0	0	0
<i>Pachyphloeus citrinus</i>	+	+	0	0
<i>Pachyphloeus austro-oregonensis</i>	+	+	0	0
<i>Peziza ellipsospora</i>	+	+	0	0
<i>Peziza infossa</i>	+	+	0	+
<i>Scleroderma cepa</i>	0	0	0	+
<i>Tuber candidum</i> / <i>T. quercicola</i>	+	0	+	+
<i>Tuber whetstonense</i>	0	+	0	+
<i>Zelleromyces gardneri</i>	0	0	0	0

Rodents did not remain particularly close to trees. In 2006, rodents were trapped at 40 m from trees with the greatest number trapped at 30 m from trees (*table 2*). Saplings also occurred in this range with 87 percent of saplings within 40 m of mature trees.

Table 2—Rodents trapped at distances from mature Oregon white oaks at Whetstone Savanna Preserve in southern Oregon in 2006. MI, *Microtus californicus*; PE, *Peromyscus maniculatus* RE, *Reithrodontomys megalotis*.

Distance (m)	MI	PE	RE	Total
10	1	1	0	2
20	1	6	1	8
30	14	8	2	24
40	11	6	0	17

Discussion

Our data show that the ectomycorrhizal communities of saplings of Oregon white oak, growing outside the root zone of mature trees, include both epigeous and hypogeous fungal species. The presence of hypogeous fungi on sapling roots suggests that animal vectors disperse truffle spores from mature trees, where the fruiting bodies occur, out into shrublands, where saplings occur. Truffle spores were found in the fecal pellets of small mammals, and these animals were trapped in shrublands where saplings grow. The correlation of presence of spores in rodent fecal pellets with the general forming ectomycorrhizas on saplings distant from parent trees suggests a causal link in the dispersal of mycorrhizal inoculum by small mammals.

Truffles with hypogeous fruiting bodies have evolved under selection pressure for survival in warm dry climates (Castellano and others 2004). In such Mediterranean climates with seasonal drought, hypogeous fungi may be important survival elements for oak ecosystems. They provide food for small mammals in the spring when seeds are scarce. In addition, in the spring, oak seedlings that germinated in winter and formed a tap root, develop fine roots that become mycorrhizal.

We propose a model for natural regeneration of oaks (figure 1). In autumn, some acorns are dispersed by animals away from the parent trees. Some are cached in shrublands and not all are eaten. Acorns of Oregon white oak germinate in winter, producing a long tap root. In spring, lateral roots develop. At this time, truffles produce fruiting bodies in the upper soil layers around mature oaks. Small mammals eat them and move into the shrublands. Spores in fecal pellets, as well as airborne spores of epigeous fungi, form mycorrhizas with the young lateral root tips. These mycorrhiza are likely critical for their survival. The regeneration of oaks out from under mature trees may require two animal vectors—one for acorns in autumn and one for truffle spores in spring. We recognize that this model is largely hypothetical and needs further research for verification.

Currently, we are testing the hypotheses that mycorrhizal inoculum declines as a function of distance from mature oaks and that the mycorrhizal community on first-year seedlings outside the root zone of mature trees is composed of the fungi found as spores in rodent fecal pellets.

If our model is correct, natural regeneration of oaks would require fungi and small mammals as part of a healthy ecosystem. Restoration practices for oaks, in which people serve as the vectors for acorn dispersal, would also require inoculum dispersal—either as fungal spores or as mycorrhizal fungal hyphae in soil from under mature trees.

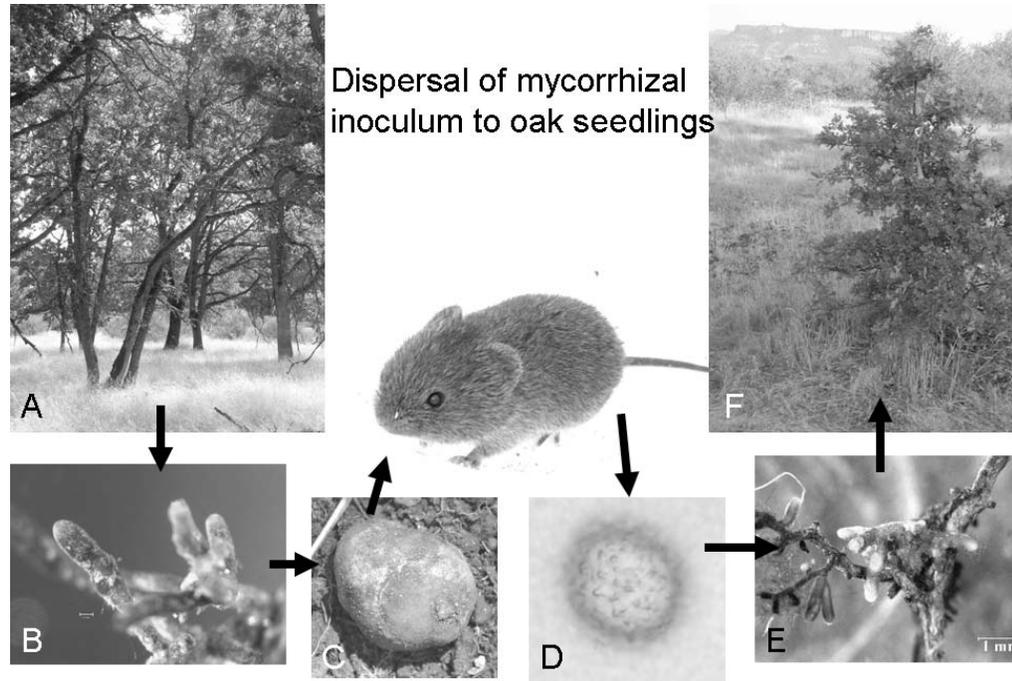


Figure 1—Model of dispersal of mycorrhizal inoculum to oak seedlings. Oregon white oaks (A) form mycorrhizas on virtually every root tip (B). Mycorrhizal fungi produce fruiting bodies seasonally (C). Hypogeous ones (truffles) (C) are eaten by rodents such as the California vole (D) that disperse truffle spores via fecal pellets (D). Truffles form mycorrhizas with seedling roots (E) that develop into saplings (G).

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