

Mammal Mycophagy and Dispersal of Mycorrhizal Inoculum in Oregon White Oak Woodlands

Abstract

Oregon white oak (*Quercus garryana*) forms ectomycorrhizas with many fungi that are hypogeous, forming sporocarps that mature underground. Because oak seeds are heavy, most germinate under parent trees where they compete for light and water. Animals disperse acorns to more distant sites, often in shrublands, contributing to the expansion of oak woodlands. While dispersed seeds escape from competition, they may lack access to mycorrhizal inoculum or to the common mycorrhizal network of oak woodlands. The purpose of this study was to determine whether small mammals eat hypogeous sporocarps and defecate fungal spores thus dispersing mycorrhizal inoculum in oak woodlands. Mycorrhizal fungi, particularly truffles, are not “everywhere” because they are obligately mycorrhizal on trees. In addition, hypogeous fungi remain below ground and do not release spores into the air, further inhibiting dispersal of inoculum. We collected 21 species of hypogeous fungi near Oregon white oaks and confirmed the mycorrhizal status of six species. We set live traps and captured three species of rodents, *Microtus californicus*, *Peromyscus maniculatus*, and *Reithrodontomys megalotis*. Spores of twelve species of hypogeous fungi were in their fecal pellets. The diets of these small mammals differed in fungal composition indicating unique foraging strategies. We found that mycorrhizal communities on Oregon white oak saplings located in buck brush shrublands at distances away from mature trees included hypogeous species suggesting that small mammals disperse spores for mycorrhizal inoculum. Dispersal of mycorrhizal inoculum is an important consideration in restoration of oak habitats, in natural regeneration of oak communities, and in their possibilities for range expansion with global warming.

Introduction

Oaks are considered foundation species creating habitat for other organisms and providing food for wildlife (McShea and Healy 2002). Yet, oaks, too, depend on other organisms for basic functions: they are mycorrhizal, utilizing fungi to take up water and nutrients, and their seeds are dispersed by animals (Montecchi and Sarasini 2000, Que-rejeta et al. 2003, Frank 2005).

Because oak seeds are heavy, most germinate under parent trees where they compete for light and water. Birds and small mammals disperse acorns to more distant sites, often in shrublands, contributing to the expansion of oak woodlands. While dispersed seeds escape from competition, they lack access to mycorrhizal inoculum or to the common mycorrhizal network of oak woodlands.

In reforestation projects with Oregon white oak (*Quercus garryana*), belowground factors such as mycorrhizas are often overlooked (McCreary 2004, Vesely and Tucker 2004). Mycorrhizas are essential for successful maintenance and regeneration of oak woodland ecosystems. Mycorrhizal inoculum may be derived from a variety of potential

sources. Acorns germinating in the root-hyphal zone of mature trees develop mycorrhizas via contact with hyphae or spores of the fungi that form mycorrhizas on the mature trees (Valentine et al. 2004). At distances outside the root-hyphal zone of ectomycorrhizal trees, seedlings may obtain inoculum as spores distributed by air or by animals including small mammals, deer, and invertebrates (Johnson 1996, Lilleskov and Bruns 2005, Ashkannejhad and Horton 2006).

Many ectomycorrhizal species with Oregon white oak are hypogeous fungi (truffles) that form sporocarps below the surface of the ground in mineral soil. Valentine et al. (2004) characterized 39 ectomycorrhizas associated with Oregon white oak roots. DNA restriction fragment patterns of three ectomycorrhizas matched those of hypogeous sporocarps of *Tuber* spp. and *Peziza infossa* collected under Oregon white oaks in southern Oregon (Valentine et al. 2004, Frank 2005).

In addition to the well-known association of hypogeous fungi, particularly *Tuber* spp., with oaks in Mediterranean Europe (Agerer 1991, Montecchi and Sarasini 2000), hypogeous fungi associate with oaks in the western United States. *Geopora cooperi*, *Hysterangium separabile*, *Tuber dryophilum*, *T. rufum* and *Balsamia magnata* occur with oaks in the American Southwest (States

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1990). *Genea* spp., *Genabea cerebriformis*, *Pachyphloeus citrinus* and *Tuber* spp. associate with oaks, primarily coast live oak in California (Arora 1986). *Hydnotryopsis setchellii*, *Hymenogaster mcmurphyi*, and *Hysterangium separabile* are found with oaks in the Pacific Northwest (Molina et al. 1992). New truffle species, indicative of an understudied ecosystem, have been described recently: *Peziza infossa* with Gambel's oak in Utah (Fogel and States 2002, 2003), *Cazia flexiascus* from a single specimen with black oak in southern Oregon (Trappe 1989), and *Tuber whetstonense*, *T. quercicola*, and *Pachyphloeus austro-oregonensis* with Oregon white oak in southern Oregon (Frank et al. 2006, 2007).

Hypogeous fungi are by nature sequestered: the sporocarps do not open to shed spores (Trappe 1979, Bruns et al. 1989, Fogel 1992, Castellano et al. 2004). Hypogeous fungal spores are often dispersed by mycophagists, animals that eat the sporocarps and defecate the spores. The location of sporocarps in shallow layers of soil makes them accessible to ground-feeding animals that are attracted by the aromas of mature sporocarps (Pyare and Longland 2001). In conifer ecosystems, small mammals such as the western red-backed vole (*Clethrionomys californicus*), the northern flying squirrel (*Glaucomys sabrinus*), Townsend's chipmunk (*Tamias townsendii*), and the lodgepole chipmunk (*Neotamias speciosus*) consume significant quantities of hypogeous sporocarps, spreading their spores (Fogel and Trappe 1978, Maser et al. 1978, Hayes et al. 1986, North et al. 1997, Colgan et al. 1997, Meyer et al. 2005). Small mammal mycophagy of hypogeous fungi also occurs in Australian eucalypt forests (Malajczuk et al. 1987, Claridge and May 1994).

Mammal mycophagy has not been reported from Oregon white oak savannas and woodlands or from other oak species in Mediterranean climates of western North America. Yet the components of a mutualism among mycophagists, fungi and oaks are present: hypogeous fungi form ectomycorrhizas with oaks, and their sporocarps occur near oaks.

We asked the following questions. Do small mammals eat hypogeous sporocarps and disperse fungal spores in oak woodlands? Does the ectomycorrhizal community of oak saplings, located out of the root zone of mature trees, include hypogeous taxa? The implications of a linkage among oaks, hypogeous fungi, and small mammals are considerable. Natural regeneration

in oaks, particularly Oregon white oak, is poor (McCreary 2004). Because oaks depend on ectomycorrhizas, seedlings require fungal inoculum to establish mycorrhizal associations as nitrogen in the cotyledons is depleted. Mycorrhizas assist with N uptake (Chalot et al. 2002). The problem of oak regeneration is two-fold: acorns must be carried away from parent trees to reduce competition for sunlight and water, and mycorrhizal inoculum must reach seedling roots. Birds and small mammals disperse acorns. We hypothesize that small mammals disperse fungal inoculum in Oregon white oak woodlands.

Methods

Study Sites

We surveyed three sites for hypogeous sporocarps with Oregon white oak or Garry oak, *Quercus garryana* Dougl. ex Hook. The primary site was Whetstone Savanna Preserve (42°25'N, 122°54'W) north of Medford, Oregon, elevation 400 m, with shrublands of buck brush, *Ceanothus cuneatus* (Hook.) Nutt. surrounding the oaks (Valentine et al. 2004). Secondary sites were Emigrant Lake (42°13'N, 122°40'W) east of Ashland, Oregon, elevation 700 m and Brickpile Ranch (42°8'N, 122°51'W) south of Jacksonville, Oregon, elevation 1100 m (Moser et al. 2005). Rainfall in this region is 40-50 cm per year, chiefly between October and May.

Collection of Hypogeous Sporocarps and Mycorrhizas

We collected sporocarps using a modified adaptive sampling method (Smith et al. 2004). At Whetstone Savanna Preserve, ten 1-m² plots beneath and around Oregon white oak were raked with a short-tined garden cultivator 1-3 times weekly during April-November 2003 and March-August 2004. Leaf litter and loose soil were examined for hypogeous sporocarps. Truffles were collected during 20 forays, 10 m²/foray, for a total area sampled of 200 m². We collected at Emigrant Lake and Brickpile Ranch during five forays in April and June 2004.

Specimens were described the same day according to Weber et al. (1997). Sporocarps were photographed in the field (Canon EOS). In the lab, we used a Leica MZ75 dissecting microscope and SPOT RT Color digital camera and software (Diagnostic Instruments, Inc.) and a Leica DMLB

compound microscope with SPOT QE insight camera and software. Hand sections were mounted in KOH and were stained with Melzer's reagent (Castellano et al. 1989). Permanent slides were made with polyvinyl alcohol-lactic acid-glycerol (Brundrett et al. 1996) and archived with dried specimens. Fungi were identified by use of keys and descriptions in Gilkey (1916, 1939, 1954), Trappe (1979, 1989), Arora (1986), Castellano et al. (1989), Trappe and Castellano (1991), Hansen (2001), Fogel and States (2002, 2003) and Frank et al. (2006a, b). We consulted with James Trappe, Nancy Smith Weber, Karen Hansen, and Matthew E. Smith. Collections were deposited in herbaria of Southern Oregon University (SOC), Oregon State University (OSC), and San Francisco State University (SFSU).

Mycorrhizal oak roots were obtained from soil samples at Whetstone Savanna Preserve under mature trees and saplings of Oregon white oak. Saplings, 0.7-4.5 m in height at distances of 3.4 to 191 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 et al. 2005). Mycorrhizas were grouped by morphotype (Agerer 1991, Goodman et al. 1996, Valentine et al. 2004). Characters examined included color, shape, branching pattern, surface texture, hyphal structure and density, and mantle pattern. Terminology follows Goodman et al. (1996). The saplings were sorted into three categorical bins, with equal numbers of saplings, based on their distances from the nearest mature tree: the near saplings (0-13.5 m), the middle saplings (14-17.5 m), and the far saplings (18-191 m).

Analysis of DNA

DNA was analyzed from fresh and dried sporocarps and from mycorrhizal root tips. DNA was extracted with CTAB and amplified in polymerase chain reactions (PCR) with fungal specific primers ITS1F and ITS4 (White et al. 1990, Gardes and Bruns 1993). Molecular data were obtained by sequencing the internal transcribed spacer (ITS) region, including ITS1, the 5.8S ribosomal DNA gene, and ITS2. PCR products were cleaned in Montage PCR Centrifugal Filter Devices (Millipore Corporation), prepared for sequencing with BigDye Terminator Ready Reaction Mix and sequenced in an ABI 310 Genetic Analyzer (Applied Biosystems). Sequences were edited with Chromas, and compared to fungal sequences in

GenBank (www.ncbi.nlm.nih.gov) with BLAST and to each other with ClustalX (Altschul et al. 1990, Thompson et al. 1997). ITS sequences were deposited in GenBank.

Animal Trapping

Sixty Sherman live traps (7.5 x 9 x 23 cm) baited with rolled oats were set in a 5 x 12 grid, spaced approximately 10 m apart at Whetstone Savanna Preserve under and around Oregon white oak and buck brush where truffles had been collected (Frank 2005). From 15 April to 12 June 2003 and 27 April to 6 June 2004, we trapped every other week, checking traps three consecutive days per week. Captured animals were identified and released according to Southern Oregon University Institutional Animal Care and Use Committee guidelines. Fecal pellets were collected and stored at 4°C.

For spore identification, slides of fresh fecal pellets were stained with Melzer's reagent and viewed with a compound microscope. Methods for quantifying fungal spores in fecal pellets were modified from Colgan et al. (1997). Spore density was determined in fecal pellets of ten California voles (*Microtus californicus*), ten deer mice (*Peromyscus maniculatus*), and eight western harvest mice (*Reithrodontomys megalotis*). Fecal pellets were dried at 55°C, weighed, and dispersed in 5% KOH (1 µg fecal pellet/40 µl KOH). Spore density was determined with a hemacytometer. Spores were identified based on comparisons to sporocarps collected at the research sites and to descriptions in Castellano et al. (1989) and in consultation with J. M. Trappe. Spore counts for *Tuber candidum* and *T. quercicola* were combined because spores of these two species could not be distinguished. Remaining fecal pellets were stored at -20°C.

Data Analyses

Relative fungal species composition and abundance among small mammal fecal pellets were contrasted by Nonmetric Multidimensional Scaling (NMS in PC-ORD, McCune and Mefford 1999, McCune and Grace 2002). We analyzed a matrix of fungal spore counts per mg fecal pellets for 25 animals and 12 fungal taxa. Data were neither transformed nor modified. The NMS was performed with 50 runs of real data along with 100 runs with randomized data for a Monte Carlo test of significance. Incidence of hypogeous fungi among sapling mycorrhizas was analyzed by chi-square.

Results

A total of 21 species of hypogeous sporocarps were collected under Oregon white oak, 14 Ascomycota and 7 Basidiomycota (Table 1). Fifteen species were found only at Whetstone Savanna Preserve, and three species, *Genea arenaria*, *Octaviania* sp. and *Hysterangium* sp., only at Emigrant Lake. *T. candidum* and *Gymnomyces* sp. were collected at two sites, and *G. gardneri* at all three sites (Table 1).

At Whetstone Savanna Preserve, 471 hypogeous sporocarps, with a total dry biomass of 212.2 g, were collected from an area of 200 m², an average density equivalent to 10.6 kg/ha/yr of hypogeous sporocarps in oak woodlands (Table 1). This total excludes *Scleroderma cepa* that fruits at the soil surface and is more easily collected. *Tuber candidum* dominated the hypogeous fungal community with more sporocarps than all other hypogeous taxa combined, comprising 37% of all hypogeous fungi by dry weight.

Most hypogeous fungi were collected in spring and early summer (Figure 1). *Balsamia alba* was the only hypogeous fungus collected in the fall (Table 1). The greatest sporocarp biomass and the greatest species diversity occurred in May. At Whetstone Savanna Preserve, bud break occurs in late March with leaf expansion in early April.

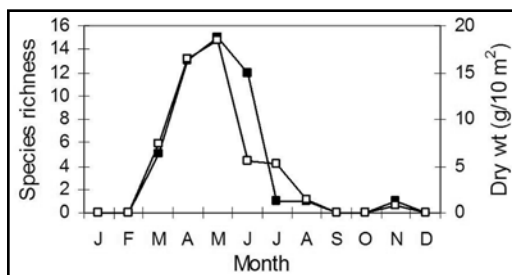


Figure 1. Seasonal sporocarp production by hypogeous fungi at Whetstone Savanna Preserve in 2003-04. Sporocarp biomass (closed squares) and species richness (open squares) peak in May.

We confirmed the mycorrhizal status of four truffle species: *Tuber candidum*, *T. whetstonense*, *Hydnortryopsis setchellii* and *Genea harknessii*, matching ITS sequences of sporocarps to those of mycorrhizas at Whetstone Savanna Preserve (Table 1). Mycorrhizal color often resembled the color of the sporocarps. Mycorrhizas and sporocarps

of *Genea harknessii* were dark brown, those of *Hydnortryopsis setchellii* were white, and those of *T. candidum* and *T. whetstonense* were tan with lighter patches. While mycorrhizas of both *T. candidum* and *T. whetstonense* were tan with light tips, those of *T. whetstonense* had cystidia.

We captured and released 60 small mammals: 28 California voles (16 April - 12 June 2003, 27 April - 4 June 2004), 24 deer mice (27 May - 12 June 2003, 31 May - 6 June 2004), and 8 harvest mice (15 April - 14 May 2003). Neither Botta's pocket gophers (*Thomomys bottae*), observed on site, nor western gray squirrels (*Sciurus griseus*), observed near the site, were caught by the traps. In the fecal pellets, gamma diversity (total hypogeous fungal species richness) was 12; alpha diversity (species richness per sample) was 5.5, and beta diversity (gamma/mean alpha) was 2.2. Species composition by rodent species is listed in Table 2. None of the mammal species consumed all twelve fungi. California voles had the most widely inclusive diet; 11 of the 12 fungal species were found in their fecal pellets. Deer mice consumed 10 of the 12 fungal species, and harvest mice only 3 species. No fecal pellet from any one animal contained all species of fungi consumed by that species. These three rodent species differed in patterns of fungal spore composition and abundance in their diets (Figure 2). The proportion of variance explained, calculated as a proportion of the variation in the reduced matrix in ordination space relative to that in the original data matrix, by NMS ordination axis 1 was 32.6% while axis 2 explained an additional 24%. Major differences in diet patterns were driven by the two species of *Tuber*. We also observed direct evidence of small mammal mycophagy by teeth marks in sporocarps of *Peziza infossa*, *Tuber candidum*, *Cazia flexiascus* and *Gymnomyces* sp.

All sapling roots were mycorrhizal. Epigeous fungi were found on all saplings. Hypogeous fungi, including *Tuber candidum*, were found on Oregon white oak saplings as far away as 72 m from the nearest mature tree (Figure 3). The incidence of hypogeous fungi, chiefly *T. candidum*, on saplings far from mature trees was similar to that of saplings near mature trees (Figure 3). The farthest sapling at 191 m had mycorrhizas of epigeous species only. Spores of *T. candidum* and *P. infossa* were found in fecal pellets.

TABLE 2. Diversity of hypogeous fungal species in dry fecal pellets from three rodent species: *Microtus californicus* (n = 10), *Peromyscus maniculatus* (n = 10) and *Reithrodontomys megalotis* (n = 8). Rodents were trapped near Oregon white oak at Whetstone Savanna Preserve in southern Oregon, USA in 2003 and 2004. Values are mean spore density (s.d.).

Fungus	Spores (x 10 ³) mg ⁻¹ dry wt. fecal pellets		
	<i>Microtus californicus</i>	<i>Peromyscus maniculatus</i>	<i>Reithrodontomys megalotis</i>
Ascomycota			
<i>Cazia flexiascus</i>	236 (22.9)	648 (64.5)	95 (11.3)
<i>Genabea cerebriformis</i>	80 (10.8)	68 (11.9)	0
<i>Genea harknessii</i>	396 (57.2)	0	0
<i>Peziza ellipsospora</i>	76 (9.1)	216 (17.8)	0
<i>Hydnotryopsis setchellii</i>	132 (18.2)	532 (58.0)	0
<i>Pachyphloeus austro-oregonensis</i>	124 (20.3)	220 (37.3)	0
<i>Pachyphloeus citrinus</i>	84 (9.7)	180 (22.8)	0
<i>Peziza infossa</i>	68 (10.3)	204 (20.3)	0
<i>Tuber candidum/T. quercicola</i>	596 (40.5)	0	45 (8.2)
<i>Tuber whetstonense</i>	0	104 (8.0)	0
Basidiomycota			
<i>Gymnomyces</i> sp.	224 (35.3)	288 (22.4)	105 (15.8)
<i>Hymenogaster boozeri</i>	256 (33.7)	0	0

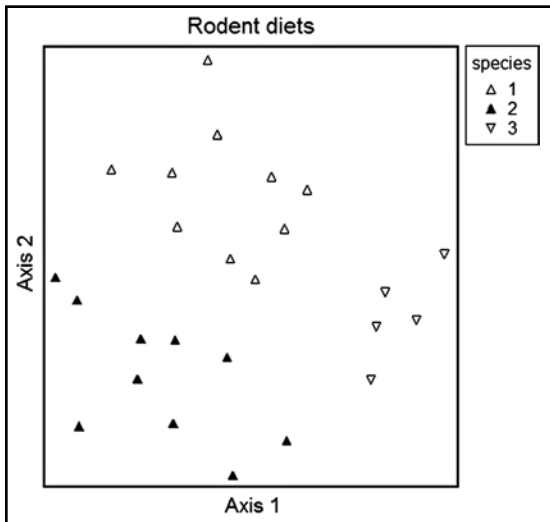


Figure 2. NMS ordination plot of small mammal diets *Microtus californicus* (species 1), *Peromyscus maniculatus* (Species 2), and *Reithrodontomys megalotis* (species 3), showing distinct foraging preferences at Whetstone Savanna Preserve in 2003 and 2004.

Discussion

Our results demonstrate a complex mutualism among Oregon white oaks, hypogeous fungi and small mammals. Oaks have mycorrhizal associations with hypogeous fungi that form sporocarps belowground. Small mammals eat the sporocarps and disperse the spores in their fecal pellets. Saplings beyond the root-hyphal zone of mature

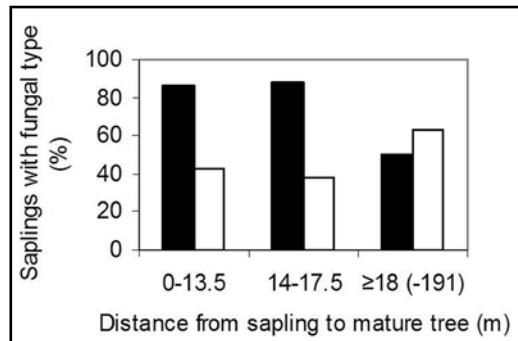


Figure 3. Incidence of hypogeous species among the mycorrhizas of Oregon white oak saplings does not differ significantly at distances from mature trees at Whetstone Savanna Preserve. Black bars, hypogeous fungi (*Tuber* spp., *Peziza infossa*, *Scleroderma cepa*); white bars, epigeous and resupinate fungi. Differences among saplings at distance categories are not significant by chi square (n = 7-8; $P > 0.05$).

trees have hypogeous species in their mycorrhizal communities.

Hypogeous fungal communities of oaks differ from those of conifers with a greater proportion of Ascomycota and with different species. In conifer forests, Basidiomycota, e.g., *Rhizopogon*, *Gautieria*, *Melanogaster*, *Hysterangium*, and *Gymnomyces*, dominate based on sporocarp

biomass, number of species, and relative mycorrhizal frequency in the hypogeous fungal community (Luoma et al. 1997, Smith et al. 2002, North 2002, Izzo et al. 2005) while Ascomycota, e.g., *Tuber*, *Elaphomyces*, *Geopora*, and *Hydnotrya*, occur to a lesser extent. No species in the most common genera of hypogeous fungi associated with conifers in the Pacific Northwest, *Rhizopogon* and *Gautieria*, were found with Oregon white oak (Luoma et al. 1997, Colgan et al. 1999, Smith et al. 2002, North 2002). Three hypogeous species (*Genea harknessii*, *Genabea cerebriformis*, and *Melanogaster euryspermus*) found with Oregon white oak also occur with Douglas-fir (Colgan et al. 1999). Those that appear unique to oaks include *Tuber candidum*, *T. whetstonense*, *T. quercicola*, *Cazia flexiascus*, *Peziza infossa*, *Pe. ellipsospora*, *Pachyphloeus austro-oregonensis* and *Pa. citrinus*.

The apparent rareness of hypogeous fungi in oak woodlands may be a function of several factors (McDonald 2004). Species may not have been detected because they fruit seasonally or because sporocarps below ground are not visible to mushroom hunters or casual observers. Previously no hypothesis has led to methodical truffling in oak woodlands, particularly with attention to seasonal fruiting. Although we collected large numbers of some species, e.g., *Tuber candidum* and *Peziza infossa*, their distribution is clumped in close association with oaks. Other species, e.g., *Tuber whetstonense* and *Cazia flexiascus*, actually may be rare, in terms of having small numbers.

Tuber species vary in importance in mesic and xeric environments. In North Carolina, a putative *Tuber* species dominated the mycorrhizal communities of *Quercus rubra* and *Q. prinus* (Walker et al. 2005). In Minnesota, only two hypogeous taxa occurred among 54 fungal species on roots of *Quercus rubra*; both were species of *Tuber* (Avis et al. 2003). The dominant ectomycorrhizal genera were *Cenococcum*, *Russula*, and *Cortinarius*. In southern Oregon, *T. candidum* was the hypogeous species most frequently collected as sporocarps and as ectomycorrhizas; it also fruits under other oak species.

Seasonal production of sporocarps by most hypogeous species parallels Oregon white oak phenology. The flush of hypogeous sporocarps in spring coincides with leaf expansion and the onset of photosynthesis. By spring, acorns that

germinated and formed deep tap roots in winter have begun to develop lateral roots that form mycorrhizas. At a time when other foods such as grass seeds and acorns are scarce, hypogeous sporocarps are available to small mammals. The rodents benefit from fungi as food source and potentially contribute to the regeneration success of Oregon white oak by spreading mycorrhizal inocula.

Both *Microtus californicus* and *Peromyscus maniculatus* consumed significant amounts of hypogeous fungi as evidenced by the density of spores and the diversity of species in their fecal pellets. *Reithrodontomys megalotis* consumed fewer species and fewer spores possibly because *R. megalotis* were trapped earlier in the season and only in one year. *M. californicus* and *P. maniculatus* were trapped over a longer time period, later in the season, and over two years. Patterns of species composition in fecal pellets suggest variations in dietary preferences or in foraging behavior.

While evidence of improved spore germination remains elusive, germination of hypogeous fungal spores may be improved by passage through the mammalian gut where spores are subject to enzymes that break down spore walls or elicit a germination response (Fogel and Trappe 1978, Pacioni 1989). Additionally, mycophagy disperses spores to areas relatively lacking in fungal inoculum in non-ectomycorrhizal woody vegetation where sapling root exudates may promote spore germination (Pacioni and Comandini 1999).

We studied the ectomycorrhizal communities of saplings instead of seedlings because saplings represent those few successful seedlings that formed mycorrhizas and survived environmental stresses. Seedlings cluster under mature trees, but rarely survive and tell us little of regeneration potential. Our observation that the mycorrhizal community of Oregon white oak saplings up to 72 meters from the nearest mature tree included hypogeous fungi demonstrates that inoculum reaches roots beyond the likely root-hyphal zone of mature trees and suggests that small mammals disperse spores.

These findings have implications for restoration. Throughout the US, oak woodlands have steadily declined as they have been cleared for agriculture and development and cut for fire wood (Reed and Sugihara 1987, Stein 1990). Also predation from deer, turkey, and other wildlife in human-modified

landscapes has contributed to a decrease in oak regeneration. Saplings of Oregon white oak are uncommon in grasslands surrounding large trees; survival success of seedlings for restoration projects is poor (McCreary 2004, Frank 2005).

Some form of inoculum appears essential for oak seedlings to become established. Commercial mycorrhizal inocula have been assembled from *Rhizopogon* spp., and *Pisolithus tinctorius* (<http://www.fungi.com/mycogrow>, Castellano and Trappe 1985, Amaranthus and Perry 1987). Mycorrhizal inoculum designed specifically for oaks is limited to three species of *Scleroderma* (Michael P. Amaranthus, Mycorrhizal Applications, Inc., personal communication). While inoculum of such limited diversity may help seedlings become established, the ectomycorrhizal community that we observed on saplings was much more diverse. Small mammal fecal pellets themselves may be useful for assessing fungal biodiversity and for obtaining a diverse mycorrhizal inoculum.

Fungal spores may be dispersed by a variety of agents in addition to small mammals. Deer have been implicated in dispersal of spores via fecal

pellets (Ashkannejhad and Horton 2006). Arthropods and their predators may disperse spores either via digestive tracts or on body surfaces (Lilleskov and Bruns 2005). Wind may also be a vector for spore dispersal depending on wind dynamics and the topography of specific habitats (Allen 1991). In natural regeneration and expansion of oak woodlands, small mammals seem particularly well suited for dispersal of mycorrhizal inoculum.

Acknowledgements

This study was supported by National Science Foundation Grants DEB-9981337, DEB-0516229, and DBI-0115892 to D. S. and by a North American Truffling Society award to J. L. F. We thank The Nature Conservancy for access to Whetstone Savanna Preserve; Karen Stone, Steve Cross, and Michael Parker for advice on animal trapping and identification; and James Trappe, Nancy Smith Weber (Oregon State University), Matthew E. Smith (University of California, Davis) and Karen Hansen (Harvard University) for confirmation of hypogeous fungal identifications.

Literature Cited

- Agerer, R. 1991. Characterization of ectomycorrhizae. *Methods in Microbiology* 23:25-73.
- Allen, M. F. 1991. *The ecology of mycorrhizae*. Cambridge University Press, NY.
- Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215:403-410.
- Amaranthus, M. P., and D. A. Perry. 1987. The effect of soil transfers on ectomycorrhizal formation and the survival and growth of conifer seedlings on old, nonreforested clearcuts. *Canadian Journal of Forestry Research* 17:944-950.
- Arora, D. 1986. *Mushrooms Demystified*, 2nd edition. Ten Speed Press, Berkeley, CA.
- Ashkannejhad, S., and T. R. Horton. 2006. Ectomycorrhizal ecology under primary succession on coastal sand dunes: interactions involving *Pinus contorta*, suilloid fungi and deer. *New Phytologist* 169:345-354.
- Avis, P. G., D. J. McLaughlin, B. C. Dentinger, and P. B. Reich. 2003. Long-term increase in nitrogen supply alters above-ground and below-ground ectomycorrhizal communities and increases the dominance of *Russula* spp. in a temperate oak savanna. *New Phytologist* 160:239-253.
- Brundrett, M., N. Bougher, B. Dell, T. Grove, and N. Malajczuk. 1996. *Working with mycorrhizas in forestry and agriculture*. ACIAR Monograph 32, Pirie Printers, Canberra, Australia.
- Bruns, T. D., R. Fogel, T. J. White, and J. Palmer. 1989. Accelerated evolution of a false truffle from a mushroom ancestor. *Nature* 339:140-142.
- Castellano, M. A., and J. M. Trappe. 1985. Ectomycorrhizal formation and plantation performance of Douglas-fir nursery stock inoculated with *Rhizopogon* spores. *Canadian Journal of Forest Research* 15:613-617.
- Castellano, M. A., J. M. Trappe, Z. Maser, and C. Maser. 1989. Key to spores of the genera of hypogeous fungi of north temperate forests with special reference to animal mycophagy. Mad River Press, Eureka, CA.
- Castellano, M. A., J. M. Trappe, and D. L. Luoma. 2004. Sequestrate Fungi. Pages 197-213 *In* G. M. Mueller, G. F. Bills, and M. S. Foster (editors), *Biodiversity of Fungi*. Elsevier Academic Press, San Francisco.
- Chalot, M., A. Javelle, D. Blaudez, R. Lambilliotte, R. Cooke, H. Sentenac, D. Wipf, and B. Botton. 2002. An update on nutrient transport processes in ectomycorrhizas. *Plant and Soil* 244:165-175.
- Claridge, A. W., and T. W. May. 1994. Mycophagy by Australian mammals. *Australian Journal of Ecology* 19:251-275.
- Colgan, W. III, A. B. Carey, and J. M. Trappe. 1997. A reliable method of analyzing dietaries of mycophagous small mammals. *Northwestern Naturalist* 78:65-69.
- Colgan, W. III, A. B. Carey, J. M. Trappe, R. Molina, and D. Thysell. 1999. Diversity and productivity of hypogeous fungal sporocarps in a variably thinned Douglas-fir forest. *Canadian Journal of Forestry Research* 29:1259-1268.

- Fogel, R. 1992. Evolutionary processes in truffles and false-truffles: evidence from distribution of hypogeous fungi in the Great Basin, USA. *Micologia e Vegetazione Mediterranea* 7:13-30.
- Fogel, R., and J. S. States. 2002. Materials for a hypogeous mycoflora of the Great Basin and adjacent cordilleras of the western United States. VII. A new truffle-like *Peziza* (Ascomycota, Pezizales). *Mycotaxon* 81:75-82.
- Fogel, R., and J. S. States. 2003. Materials for a hypogeous mycoflora of the Great Basin and adjacent cordilleras of the western United States. IX. A new name for *Peziza quercicola* (Ascomycota, Pezizales). *Mycotaxon* 88:155-156.
- Fogel, R., and J. M. Trappe. 1978. Fungus consumption (mycophagy) by small animals. *Northwest Science* 52:1-31.
- Frank, J. L. 2005. Complex mutualism in an Oregon white oak woodland: hypogeous fungi, mycorrhizas and small mammal mycophagy. M.S. Thesis, Southern Oregon University, Ashland.
- Frank, J. L., D. Southworth, and J. M. Trappe. 2006. NATS truffle and truffle-like fungi 13: *Tuber quercicola* and *Tuber whetstonense*, new species from Oregon, and *Tuber candidum* redescribed. *Mycotaxon* 95:229-240.
- Frank, J. L., D. Southworth, and J. M. Trappe. 2007. NATS truffle and truffle-like fungi 14: *Pachyphloeus austro-oregonensis*, a new species from southern Oregon. *Mycotaxon* 97: in press.
- Gardes, M., and T. D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2:113-118.
- Gilkey, H. M. 1916. A revision of the Tuberales of California. University of California Publications in Botany 6:275-356, plates 26-30.
- Gilkey, H. M. 1939. Tuberales of North America. Oregon State Monographs in Botany 1:1-63.
- Gilkey, H. M. 1954. Taxonomic notes on Tuberales. *Mycologia* 46:783-793.
- Goodman, D. M., D. M. Durall, J. A. Trofymow, S. M. Berch. 1996. A manual of concise descriptions of North American ectomycorrhizas. Mycologue Publications, Sidney, Australia.
- Hansen, K. 2001. Phylogenetics of the Pezizaceae, with an emphasis on *Peziza*. *Mycologia* 93:958-990.
- Hayes, T. P., S. P. Cross, and P. W. McIntire. 1986. Seasonal variation in mycophagy by the western red-backed vole, *Clethrionomys californicus*, in Southwestern Oregon. *Northwest Science* 60:250-257.
- Izzo, A. D., M. Meyer, J. M. Trappe, M. North, and T. D. Bruns. 2005. Hypogeous ectomycorrhizal fungal species on roots and in small mammal diet in a mixed-conifer forest. *Forest Science* 51:243-254.
- Johnson, C. N. 1996. Interactions between mammals and ectomycorrhizal fungi. *Trends in Ecology and Evolution* 11:503-507.
- Lilleskov, E. A., and T. D. Bruns. 2005. Spore dispersal of a resupinate ectomycorrhizal fungus, *Tomentella subulacina*, via soil food webs. *Mycologia* 97:762-769.
- Luoma, D. L., J. L. Eberhart, and M. P. Amaranthus. 1997. Biodiversity of ectomycorrhizal types from southwest Oregon. Pages 249-253 *In* T. N. Kaye, A. Liston, R. M. Love, D. L. Luoma, R. J. Meinke, and M. V. Wilson (editors), Conservation and management of native plants and fungi. Native Plant Society of Oregon, Corvallis.
- Malajczuk, N., J. M. Trappe, and R. Molina. 1987. Interrelationships among some ectomycorrhizal trees, hypogeous fungi and small mammals: western Australia and northwestern American parallels. *Australian Journal of Ecology* 12:53-55.
- Maser, C., J. M. Trappe, and R. A. Nussbaum. 1978. Fungal-small mammal interrelationships with emphasis on Oregon coniferous forests. *Ecology* 59:799-809.
- McCreary, D. D. 2004. Managing and restoring California's oak woodlands. *Natural Areas Journal* 24:269-275.
- McCune, B., and M. J. Mefford. 1999. PC-ORD, Multivariate analysis of ecological data, version 4. MjM Software Design, Gleneden Beach, OR.
- McCune, B., and J. B. Grace. 2002. Analysis of ecological communities. MjM Software Design, Gleneden Beach, OR.
- McDonald, L. L. 2004. Sampling rare populations. Pages 11-42 *In* W. L. Thompson (editor), Sampling rare or elusive species. Island Press, Washington, DC.
- McShea, W. J., and W. M. Healy. 2002. Oak forest ecosystems: ecology and management for wildlife. Johns Hopkins University Press, Baltimore.
- Meyer, M. D., M. P. North, and D. A. Kelt. 2005. Short-term effects of fire and forest thinning on truffle abundance and consumption by *Neotamias speciosus* in the Sierra Nevada of California. *Canadian Journal of Forestry Research* 35:1061-1070.
- Molina, R., H. Massicotte, and J. M. Trappe. 1992. Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. Pages 357-423 *In* M. F. Allen (editor), Mycorrhizal Functioning—An Integrative Plant-Fungal Process. Chapman and Hall, London, UK.
- Montecchi, A., and M. Sarasini. 2000. Funghi ipogei d'Europa. Fondazione Centro Studi Micologici dell'AMB, Vicenza, Italy.
- Moser, A. M., C. A. Petersen, J. A. D'Allura, and D. Southworth. 2005. Comparison of ectomycorrhizas of *Quercus garryana* (Fagaceae) on serpentine and non-serpentine soils in southwestern Oregon. *American Journal of Botany* 92:224-230.
- North, M. 2002. Seasonality and abundance of truffles from oak woodlands to red fir forests. *In* J. Verner (editor), Proceedings—Kings River sustainable forest ecosystems project. USDA Forest Service General Technical Report PSW-183, Pacific Southwest Research Station, Berkeley, CA. Pp. 91-97.
- North, M., J. M. Trappe, and J. Franklin. 1997. Standing crop and animal consumption of fungal sporocarps in Pacific Northwest forests. *Ecology* 78:1543-1554.
- Pacioni, G. 1989. Biology and ecology of the truffles. *Acta Medica Romana* 27:104-117.
- Pacioni, G. and O. Comandini. 1999. *Tuber*. Pages 163-186 *In* J. W. G. Cairney and S. M. Chambers (editors), Ectomycorrhizal fungi key genera in profile. Springer, New York.

- Pyare, S., and W. S. Longland. 2001. Mechanisms of truffle detection by northern flying squirrels. *Canadian Journal of Zoology* 79:1007-1015.
- Querejeta, J. I., L. M. Egerton-Warburton, and M. F. Allen. 2003. Direct nocturnal water transfer from oaks to their mycorrhizal symbionts during severe soil drying. *Oecologia* 134:55-64.
- Reed, L. J., and N. G. Sugihara. 1987. Northern oak woodlands—ecosystems in jeopardy or is it already too late? Pages 59-63 *In* Proceedings of the Symposium on Multiple-use Management of California's Hardwood Resources. USDA Forest Service General Technical Report PSW-100. Pacific Southwest Research Station, Berkeley, CA.
- Smith, J. E., R. Molina, M. M. P. Huso, D. L. Luoma, D. McKay, M. A. Castellano, T. Lebel, and Y. Valachovic. 2002. Species richness, abundance, and composition of hypogeous and epigeous ectomycorrhizal fungal sporocarps in young, rotation-age, and old-growth stands of Douglas-fir (*Pseudotsuga menziesii*) in the Cascade Range of Oregon. *Canadian Journal of Botany* 80:186-204.
- Smith, D. R., J. A. Brown, and N. C. H. Lo. 2004. Application of adaptive sampling to biological populations. Pages 77-122 *In* W. L. Thompson (editor), *Sampling rare or elusive species*. Island Press, Washington, DC.
- States, J. S. 1990. Mushrooms and truffles of the Southwest. University of Arizona Press, Tucson.
- Stein, W. I. 1990. Oregon white oak. Pages 650-660 *in* R. M. Burns and B. H. Honkala (technical coordinators), *Silvics of North America: Hardwoods*. USDA Agricultural Handbook No. 654. USDA Forest Service, Washington, DC.
- Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 24:4876-4882.
- Trappe, J. M. 1979. The orders, families, and genera of hypogeous Ascomycotina (truffles and their relatives). *Mycotaxon* 9:297-340.
- Trappe, J. M. 1989. *Cazia flexiascus* gen. et sp. nov., a hypogeous fungus in the Helvellaceae. *Memoirs of the New York Botanical Garden* 49:336-338.
- Trappe, J. M., and M. A. Castellano. 1991. Keys to the general of truffles (Ascomycetes). *McIlvainea* 10:47-65.
- Valentine, L. L., T. L. Fiedler, A. N. Hart, C. A. Peterson, H. K. Berninghausen, and D., Southworth. 2004. Diversity of ectomycorrhizas associated with *Quercus garryana* in southern Oregon. *Canadian Journal of Botany* 82:123-135.
- Vesely, D., and G. Tucker. 2004. A landowner's guide for restoring and managing Oregon white oak habitats. U.S. Bureau of Land Management, Salem, OR.
- Walker, J. F., O. K. Miller, and J. L. Horton. 2005. Hyperdiversity of ectomycorrhizal fungus assemblages on oak seedlings in mixed forests in the southern Appalachian Mountains. *Molecular Ecology* 14:829-838.
- Weber, N. S., J. M. Trappe, and W. C. Denison. 1997. Studies on western American Pezizales. Collecting and describing ascomata—macroscopic features. *Mycotaxon* 61:153-176.
- White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (editors), *PCR Protocols: a guide to methods and applications*, Academic Press, New York.

Received 5 June 2006

Accepted for publication 12 September 2006

Note added in proof:

On more species, *Hydnobolites californicus* (Ascomycota), was identified from the 2004 Whetstone Savanna collection.