Rodent dispersal of fungal spores promotes seedling establishment away from mycorrhizal networks on *Quercus garryana*

J.L. Frank, S. Anglin, E.M. Carrington, D.S. Taylor, B. Viratos, and D. Southworth

Abstract: With global warming and the possible decline of conifers, more habitat may be available to oaks, particularly at higher elevations and more northerly latitudes. Whether oaks expand into new habitats will depend on their ability to disperse and establish at the margins of existing woodlands. Because oaks have a symbiotic relationship with ectomycorrhizal fungi, range expansion requires dispersal of both symbionts: the acorns and the mycorrhizal inoculum. Little is known of this dual dispersal. Here we assess the availability of ectomycorrhizal inoculum as a function of the distance from mature oaks. We examined soil cores for ectomycorrhizal roots and rodent fecal pellets for fungal spores along transects away from mature trees of *Quercus garryana* Dougl. ex Hook., and planted acorns as bioprobes. We identified spores by microscopy, and mycorrhizas by DNA sequences of the ITS region. Mycorrhizas were present in soil cores 5 m from parent trees, but not beyond. Spores of hypogeous fungi were found in rodent fecal pellets at distances up to 35 m from mature trees. Hypogeous fungi formed ectomycorrhizas with first-year seedlings within the root zone of mature trees and with second-year seedlings beyond the root zone. These data indicate that for seedlings near mature trees, the source of fungal inoculum was the mycorrhizal network of mature trees, and for seedlings beyond that, rodents dispersed the inoculum. We conclude that rodent dispersal of fungal spores promotes seedling establishment away from mycorrhizal networks in *Q. garryana*.

Key words: ectomycorrhizas, Garry oak, hypogeous fungi, Janzen–Connell hypothesis, Oregon white oak, plant dispersal.

Résumé : Avec le réchauffement global et de déclin possible des forêts coniférienes, on pourrait observer une augmentation des habitats disponibles pour les chênes, particulièrement à haute altitude et aux latitudes plus nordiques. L'expansion possible des chênes dans de nouveaux habitats dépendra de leur capacité à se disperser et à s'établir à la marge des forêts existantes. Parce que les chênes forment des ectomycorhizes, l'expansion de l'aire de distribution nécessite une dispersion des deux symbiotes, soit les glands et les inoculums mycorhiziens. On sait peu de choses sur cette double dispersion; les auteurs ont donc évalué la disponibilité des inoculums mycorhiziens en fonction de la distance des chênes matures. Le long de transects s'éloignant de tiges du *Quercus garryana* Dougl. ex Hook., les auteurs ont examiné des carottes de sol pour y chercher des racines ectomycorrhizées et des féces afin d'y déceler des spores fongiques, et ils ont planté des glands comme sondes biologiques. Ils ont identifié les spores par microscopie et à l'aide des séquences ADN de la région ITS. Ils ont retrouvé des mycorhizes dans les carottes de sols récoltées à 5 m des arbres parents, mais pas au-delà. Ils ont également trouvé les spores de champignons hypogés dans les féces à des distances atteignant 35 m d'arbres matures. Les champignons hypogés ont formé des mycorhizes avec les plantules de première année dans la zone racinaire d'arbres matures et au cours de la deuxième année seulement au-delà de la zone racinaire. Ces données indiquent que chez les plantules près des arbres matures, la source d'inoculum était constituée par le réseau mycorhizien des arbres matures et pour les plantules au-delà de cette zone, les rongeurs y ont dispersé les inoculums. Les auteurs concluent que la dispersion des spores fongiques par les rongeurs favorise l'établissement des plantules au-delà du réseau ectomycorhizien du *Q. garryana*.

Mots-clés : ectomycorhizes, chêne de Garry, champignons hypogés, hypothèse de Janzen–Connell, chêne blanc de l'Oregon, dispersion des plantes.

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Introduction

Seed dispersal and seedling development are required for the establishment of plant populations in new suitable habitats. Factors that influence the long-distance dispersal of plants, particularly foundation species such as oaks, are important for understanding regeneration, habitat restoration, and range expansion (McCreary 2004; Nathan 2006). The warmer and drier conditions predicted by climate change will expand the habitat available to oaks at higher elevations and more northerly latitudes where *Quercus garryana* woodlands adjoin conifers (Hosten et al. 2006; Devine et al.

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J. Frank, S. Anglin, D. Taylor, B. Viratos, and D. Southworth.[1] Department of Biology, Southern Oregon University, Ashland, OR 97520, USA.
E. Carrington. Department of Biology, Southern Oregon University, Ashland, OR 97520, USA; Department of Land, Air and Water Resources, University of California, Davis, CA 95606, USA.

[1]Corresponding author (e-mail: southworth@sou.edu).

2007; Intergovernmental Panel on Climate Change 2007; Thompson 2007). Whether oaks, especially those adapted to seasonally dry Mediterranean climates, expand into new habitats will depend on their ability to establish beyond the margins of existing woodlands (Crawford 2008). The importance of mycorrhizas in seedling success has largely been ignored. Recent studies on oak seedling survival and response to drought did not consider any mycorrhizal influence (Fuchs et al. 2000; Quero et al. 2006; Tyler et al. 2006; Valladares and Gianoli 2007).

Fungal symbionts are essential components of healthy oak woodland ecosystems (Valentine et al. 2002, 2004; McCreary 2004; Dickie and Reich 2005; Dickie et al. 2007; Frank et al. 2008; Morris et al. 2008; Moser et al. 2009). In savannas and woodlands, virtually all fine root tips of Oregon white oak saplings and mature trees are ectomycorrhizal, with a single tree connected to as many as nine fungal species (Valentine et al. 2004; Moser et al. 2005, 2009; Southworth et al. 2005; Frank et al. 2006*a*). Range expansion of obligate mutualists requires that both partners disperse to the same place within a limited time frame. Possible mechanisms for the dispersal of mycorrhizal inoculum include seedling root contact with the mycorrhizal network of mature trees, dispersal of fungal spores via animal feces, airborne dispersal of propagules (spores, hyphae, sclerotia), and codispersal of plant and fungal propagules.

If the mechanism for mycorrhizal dispersal is seedling contact with the mycorrhizal network of mature trees, then the assemblage of mycorrhizas on seedlings should be a subset of the ectomycorrhizas on mature trees with similar ratios of epigeous to hypogeous species and with the mycorrhizal fungus *Cenococcum*. Species richness on seedlings would be relatively high, and seedlings would succeed chiefly near mature trees. Seedlings of red oaks near the edge of a mature stand and conifer seedlings near the canopy dripline of retention trees developed mycorrhizas; the influence of mature trees declined in mycorrhizal colonization beyond that distance (Cline et al. 2005; Dickie and Reich 2005; Dickie et al. 2007).

If the dispersal of mycorrhizal fungi occurs via small mammal mycophagy of hypogeous sporocarps, then seedlings beyond the root zone of mature trees could develop mycorrhizas: these fungi would be predominately hypogeous, with *Cenococcum* being less prevalent. Because this requires two vectors, mycorrhizal associations might be rare. Many mycorrhizal fungi associated with oaks in dry habitats are hypogeous, producing spores underground (Valentine et al. 2004; Frank et al. 2006*a*; Moser et al. 2009). Interactions among ectomycorrhizal trees, hypogeous fungi, and small mammals are found in many dry, temperate habitats, including the coniferous forests of western North America (Fogel and Trappe 1978; Maser et al. 1978; Hayes et al. 1986; Johnson 1996; Colgan et al. 1997; North et al. 1997; Meyer et al. 2005). The hypogeous fruiting habit, in which fruiting bodies do not readily open to shed spores, is considered an adaptation to dry environments (Trappe 1979; Thiers 1984; Bruns et al. 1989; Fogel 1992; Castellano et al. 2004).

By contrast, if mycorrhizal inoculum disperses as airborne spores, then epigeous fungi would predominate on seedlings.

Fig. 1. Availability of mycorrhizal inoculum, and response by bioprobe seedlings at distances from mature *Quercus garryana* in *Ceanothus cuneatus* shrublands (diamonds) and in grasslands (squares) at Whetstone Savanna in southern Oregon. (*a*) Germination of acorns. Differences in germination rates among distances were not significant (χ^2, $P > 0.05$); overall germination between grasslands and shrublands differed significantly (χ^2, $P = 0.001$). (*b*) Total species richness of mycorrhizas on tree roots from soil cores taken at 0.5 m from seedlings. (*c*) Total species richness of mycorrhizas on lateral roots of first-year (solid symbols) and second-year (open diamonds) seedlings. (*d*) Total numbers of rodents (*Microtus californicus*, *Peromyscus maniculatus*, and *Reithrodontomys megalotis*) that entered Sherman live traps. Ten traps were set at each distance in both shrublands and grasslands. (*e*) Frequency of spores of hypogeous fungi in fecal pellets of mammals ($n = 55$) trapped in shrublands. In grasslands, 100% of fecal pellets of the few animals ($n = 6$) trapped also had spores.

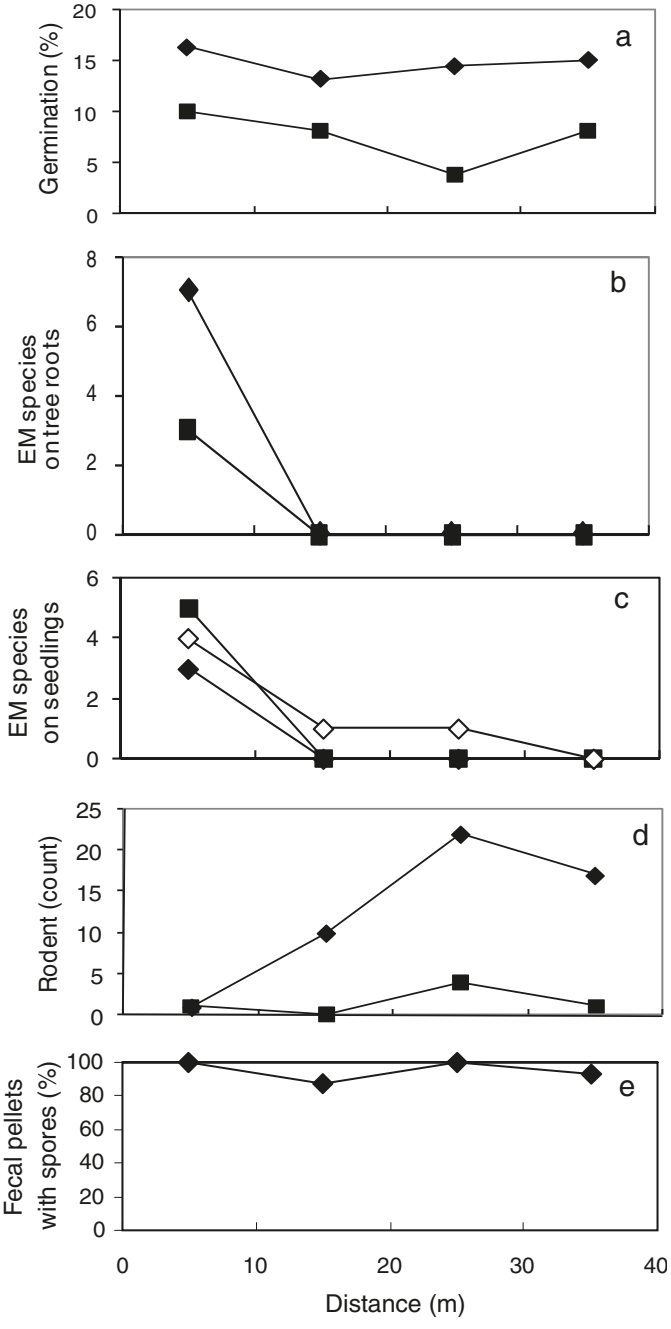

Table 1. Identification of ectomycorrhizal fungi from seedlings of *Quercus garryana*, from roots in soil cores, and from fecal pellets of *Peromyscus maniculatus* based on BLAST matches from GenBank.

Consensus taxon	GenBank accession No.	Source	Vegetation type	Dist. (m)	Length (bp)	Closest vouchered BLAST match	Max. score	Query coverage (%)	E value	Max. ident. (%)
Astraeus	EU334888	Soil	Shrub	5	442	*Astraeus pteridis* AY629407	754	99	0.0	97
Balsamia	EU334902	Feces	Shrub	35	294	*Balsamia* sp. DQ974730	468	99	6E-129	95
Cazia flexiascus	EU334889	Seedling	Grass	5	530	*Cazia flexiascus* AY830852	935	95	0	100
Geopora	EU334903	Feces	Shrub	35	461	*Geopora cooperi* DQ974731	513	85	2E-142	89
Geopora	EU334890	Seedling	Shrub	5	524	*Geopora cooperi* DQ974731	524	97	23–145	84
Geopora	FJ235158	Seedling	Shrub	25	444	*Geopora cooperi* DQ974731	311	84	2E-81	80
Hebeloma	EU334891	Soil	Shrub	5	558	*Hebeloma bruchetii* AY948195	913	100	0.0	96
Tarzetta	EU334892	Soil	Shrub	u	605	*Tarzetta* cf. *cupularis* FJ235145	801	74	0	99
Trichophaea	EU334893	Soil	Grass	u	496	*Trichophaea woolhopeia* DQ200835	405	88	1E-109	81
Scleroderma	EU334894	Seedling	Grass	5	428	*Scleroderma cepa* DQ453694	769	99	0	99
Tomentella	EU334896	Seedling	Grass	5	513	*Tomentella* sp. AJ534912	839	99	0	97
Tomentella	EU334897	Soil	Shrub	u	439	*Tomentella* sp. EF372408	719	99	0	96
Tomentella	EU334895	Soil	Shrub	5	351	*Tomentella ferruginea* AF272909	506	100	3E-140	94
Tuber candidum	EU334898	Seedling	Grass	5	512	*Tuber candidum* AY30856	843	100	0	96
Tuber candidum	EU334899	Seedling	Shrub	5	463	*Tuber* cf. *candidum* DQ974807	721	100	0	95
Tuber candidum	EU334901	Seedling	Shrub	5	454	*Tuber candidum* AY30856	852	99	0	97
Tuber candidum	EU334900	Soil	Grass	u	445	*Tuber candidum* AY30856	773	100	0	97
Tuber candidum	FJ235159	Seedling	Grass	5	500	*Tuber candidum* AY30856	733	99	0	93

Note: Distance (Dist.) was measured from canopy edge; some soil cores were taken from under the canopy (u).

This appears to be the case with conifers for which the occurrence of epigeous fungi as mycorrhizal symbionts, particularly as generalist species, suggests airborne dispersal of the fungi (Molina et al. 1992; Cairney and Chambers 1999).

Codispersal of plant and mycorrhizal propagules has been observed in a coastal strand plant in which rhizomes containing arbuscular mycorrhizal fungi break off and disperse (Koske and Gemma 1990). Several features of acorn dispersal and germination make codispersal of propagules unlikely: oaks lack vegetative propagules, and the surfaces of acorns do not appear to contain mycorrhizal spores. Also the timing is incompatible — acorns disperse in the fall, at which time birds and small mammals carry and cache the seeds, but hypogeous fungi typical of *Q. garryana* mycorrhizal communities fruit in the spring (Frank et al. 2006*a*).

The goal of this study was to determine the availability of mycorrhizal inoculum beyond the root zone and mycorrhizal network of mature trees. Our objectives were (*i*) to assess the availability of ectomycorrhizal inoculum as a function of distance from mature oaks through the use of seedlings as bioprobes, (*ii*) to determine the potential of small mammals to disperse mycorrhizal inoculum, and (*iii*) to compare availability of mycorrhizal inoculum in shrublands with that in grasslands. We predict that seedlings nearer mature trees will have greater ectomycorrhizal fungal diversity and that mycorrhizas on seedlings beyond the root zone of mature trees will include more hypogeous fungi dispersed by rodents.

Materials and methods

Study site

We conducted the study at Whetstone Savanna Preserve, a 58 ha site owned by The Nature Conservancy in Jackson County, Oregon, USA (42°25′N, 122°54′W) (Valentine et al. 2004). *Quercus garryana* Dougl. ex Hook. savanna and woodlands adjoin *Ceanothus cuneatus* (Hook.) Nutt. shrublands with scattered *Q. garryana* saplings to the north and grasslands of exotic annual grasses (e.g., *Taeniatherum caput-medusae* (L.) Nevski and *Poa bulbosa* L.) to the south. The climate is Mediterranean with dry summers; average annual rainfall is 48 cm falling primarily during October and through May (Western Regional Climate Center 2002).

To determine the extent of available mycorrhizal inoculum, we selected 20 trees of *Q. garryana* at 40 m intervals along the edge of the woodland: 10 trees adjacent to *Ceanothus* shrublands and 10 adjacent to grasslands. From each tree, we set a transect radiating away from the woodland for 35 m in a direction that would allow all points to remain >35 m from neighboring trees. Each transect had sampling points at 5, 15, 25, and 35 m for animal traps, soil cores, and seedling bioprobes as in situ bioassays for mycorrhizal inoculum.

Field collections

To determine which mycorrhizal fungi seedlings might encounter, we extracted four soil cores (2 cm diameter × 25 cm length) and combined them as a single sample at distances of 0.5 m from the seedlings along each transect, at each sampling point, and from under the canopy of the mature trees (n = 100). Soil samples were washed over a

Table 2. Presence of mycorrhizal inoculum at distances (metres) from the canopy edge of mature oaks in shrublands and grasslands detertrees in soil cores, and from mycorrhizas on roots of seedling bioprobes in two years of growth at Whetstone Savanna in southern Oregon.

	Shrublands											
	Fecal pellets				Tree roots				Seedlings			
	5	15	25	35	5	15	25	35	5	15	25	35
Astraeus					+							
Balsamia			+	+								
Cazia flexiascus			+	+								
Cenococcum					+							
Genabea		+	+									
Genea			+	+								
Geopora			+	+					+		+*	
Gymnomyces			+									
Hebeloma					+							
Hydnobolites			+	+								
Hydnotryopsis		+	+	+								
Melanogaster			+									
Pachyphloeus				+								
Peziza		+	+	+								
Scleroderma					+							
Tarzetta					+				+			
Tomentella					+				+			
Trichophaea												
Tuber candidum					+				+	+*		
Tuber whetstonense	+	+	+	+								
Zelleromyces				+								

*On second-year seedlings only.

1.0 mm mesh sieve and the roots picked out and examined under a dissecting microscope. Ectomycorrhizas were photographed and described according to Agerer 1991, with representative samples stored for DNA sequencing.

To determine the characteristics of spore dispersal by rodents, we set Sherman live traps (7.5 cm × 9 cm × 23 cm) baited with rolled oats at all sampling points in the grasslands and shrublands (n = 80) and opened the traps for three consecutive trapping days every other week from 17 May to 15 June 2006. Captured animals were identified and released. Fecal pellets from each trapped animal were stored at 4 °C in 70% ethanol. Three fecal pellets from each collection were examined. Single pellets were dispersed on a slide, stained with Melzer's reagent, and examined under a Leica DM LB compound microscope (Castellano et al. 1989; Colgan et al. 1997). Spores were identified by comparison with spores of hypogeous fungi collected from the same site (Frank 2005; Frank et al. 2006*a*, 2006*b*, 2006*c*) and to images in Castellano et al. (1989). Voucher images were captured with a SPOT RT camera (Diagnostic Instruments, Sterling Heights, Michigan, USA).

In situ bioassay for mycorrhizal inoculum

To assess potential mycorrhizal inoculum in the soil and on acorns, we planted seeds as bioprobes. We collected acorns from mature oaks, immediately carried them along transects to sampling points, and planted 16 acorns at a depth of 2–4 cm at each sampling point in October and November 2006. In May 2007, around the time of leaf expansion, we excavated first-year seedlings from 10 transects, 5 each in grasslands and shrublands, at four sampling points per transect, for a total of 71 seedlings (32 in grasslands, 39 in shrublands). In May 2008, we excavated all remaining second-year seedlings from all transects, 6 seedlings in grasslands and 67 in shrublands. Seedling roots were rinsed and the mycorrhizas sorted by morphological features. Ectomycorrhizal morphotypes were described by color, shape, branching pattern, surface texture, hyphal structure and density, and mantle pattern, and voucher images recorded (Agerer 1991; Goodman et al. 1996). Representative mycorrhizas for DNA sequencing were stored in buffer (0.1 mol·L^{-1} Tris, 0.3 mol·L^{-1} NaCl, 0.04 mol·L^{-1} EDTA) at 4 °C.

Molecular methods

Mycorrhizas and fecal pellets were macerated in microcentrifuge tubes with a micropestle. DNA was extracted in 2% cetyltrimethyl ammonium bromide (CTAB) with chloroform and amplified in polymerase chain reactions (PCR) with fungal primer ITS1F (5′-GGTCATTTAGAGGAAGTAA-3′) and universal eukaryote primer TW13 (5′-GGTCCGTGTTTCAAGACG-3′) (White et al. 1990; Gardes and Bruns 1993, 1996). PCR reactions were performed in 20 μL volumes using 0.6 U of GoTaq and 4 μL 5× colorless buffer (Promega, Madison, Wisconsin, USA), 200 μmol·L^{-1} each dNTP, 0.3 μmol·L^{-1} each primer, 2.5 mmol·L^{-1} $MgCl_2$ and 2 μL undiluted of DNA template. An initial 3 min at 93 °C was followed by 30 cycles of 30 s at 95 °C, 2 min at 56 °C, and 3 min at 72 °C, with a final cycle for 10 min at 72 °C. PCR products were eletrophoresced on 1.5% agarose gels, stained with ethidium bromide (1 mg·mL^{-1}), and visualized under a Kodak EDAS 290 UV transilluminator.

PCR products were purified with QIAquick PCR Purifica-

mined from spores in fecal pellets from trapped rodents, from mycorrhizas on roots of mature

Grasslands											
Fecal pellets				Tree roots				Seedlings			
5	15	25	35	5	15	25	35	5	15	25	35
								+			
				+				+			
		+									
								+			
								+			
								+			
				+							
				+				+			
		+									

tion kits (QIAGEN, Valencia, California, USA), prepared with BigDye Terminator Ready Reaction Mix version 3.1 and sequenced in an ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, Calif.) in the Biotechnology Center at Southern Oregon University. Molecular data were obtained by sequencing the internal transcribed spacer (ITS) region, including ITS1, the 5.8S ribosomal DNA gene and ITS2, with forward primers ITS1F and ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′), and reverse primers ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). Sequences were edited with Chromas 1.45 (McCarthy 1998) and compared with other fungal ITS sequences in GenBank (www.ncbi.nlm.nih.gov) with BLAST and to fruiting bodies collected at the same site with ClustalX (Altschul et al. 1990; Thompson et al. 1997). Fungal DNA sequences from mycorrhizas and fecal pellets have been deposited in GenBank.

Data analysis

Percent germination and percent of fecal pellets containing hypogeous fungal spores versus distance were tested by χ^2, and numbers of ectomycorrhizal (EM) species on tree roots and on seedlings, and of rodents per trap versus distance were analyzed by analysis of variance (ANOVA) using Minitab version 14 (Minitab Inc., State College, Pennsylvania, USA) with significance determined at $P < 0.05$.

Results

Acorns germinated equally well at all distances from mature trees (Fig. 1*a*); differences in germination rates among distances were not significant (χ^2, $P > 0.05$). Acorns planted in October germinated through the winter; by March, seedlings had developed tap roots; by May, lateral roots were available for mycorrhizal inoculation.

Molecular identification of fungi

Ten fungal species from ectomycorrhiza samples and fecal pellets were identified by DNA analysis, and one species, *Cenococcum geophilum*, by morphology (Table 1). Fruiting bodies of all mycorrhizal fungal genera identified by DNA, except *Trichophaea* and *Tomentella*, have been collected at the site (Frank 2005; Frank et al. 2006*b*; J. Frank, unpublished data). *Tuber candidum* was identified to species based on mycorrhizal morphology and consistent BLAST matches of DNA sequences compared with those of fruiting bodies collected on site (Table 1). *Cazia flexiascus* was identified to species based on a 100% BLAST match (Table 1).

Ectomycorrhizas in soil cores and on seedling bioprobes

Ectomycorrhizal roots were detected in soil cores taken under tree canopies and at 5 m beyond the canopy edge, but not at greater distances (Tables 1 and 2; Fig. 1*b*). Eight species were present: four epigeous, two hypogeous, one invertebrate-dispersed (*Tomentella*), and *Cenococcum*.

First-year seedlings just outside the canopy (at 5 m) were mycorrhizal, but those at 15 m and beyond were not (Table 2; Fig. 1*c*). The most frequent fungal species on seedling bioprobe roots were *Tuber candidum* and *Tomentella* sp. Two second-year seedlings acquired mycorrhizas: *Tuber candidum* at 15 m and *Geopora* at 25 m. *Geopora*

and *Cazia flexiascus*, hypogeous fungal spores also found in fecal pellets, occurred on seedling roots near mature trees, but not in soil cores (Tables 1 and 2). Bioprobe seedlings shared four taxa with soil-core mycorrhizas: *Tuber candidum*, *Tomentella* sp., *Scleroderma* sp., and *Cenococcum geophilum* (Tables 1 and 2). *Astraeus*, *Hebeloma*, *Tarzetta*, and *Trichophaea* were found on roots of mature trees in soil cores, and not on seedling roots (Table 2).

Dispersal of fungal spores by rodents

Significantly more (χ^2, $P = 0.01$) small mammals were trapped at sampling points 25 and 35 m from mature trees than at closer distances (Fig. 1*d*). Rodents entered traps more frequently in shrublands (26 *Microtus californicus*, 26 *Peromyscus maniculatus*, and 3 *Reithrodontomys megalotis*) than in grasslands (3 *M. californicus*, 2 *P. maniculatus*, and 1 *R. megalotis*).

Nearly all fecal pellet samples contained spores of hypogeous fungi (Fig. 1*e*). Spores of 14 species of hypogeous fungi were identified by morphology; two were confirmed by DNA (Tables 1 and 2). The most frequent were the reticulate spores of *Tuber whetstonense* found at all distances. DNA sequences were obtained from fecal pellets where spores were present as dense masses. Overall success in amplifying fungal spore DNA from fecal pellets was poor with only two sequences, *Balsamia* and *Geopora*, out of 80 extractions from 40 fecal pellet collections.

Comparison of mycorrhizal infection in shrublands and grasslands

The rate of acorn germination in grasslands was about half that in shrublands (Fig. 1*a*); overall germination between grasslands and shrublands differed significantly (χ^2, $P = 0.001$). Differences in mycorrhizal fungal species richness between grasslands and shrublands were not significant on seedlings ($P = 0.242$) and marginally significant on roots in soil cores ($P = 0.070$) at 5 m sampling points and under trees ($P = 0.071$) in the first growing season (Table 2). Differences in mycorrhizal fungal species richness among distances of 5, 15, 25, and 35 m were significant ($P = 0.003$) given the absence of mycorrhizas on all first-year seedlings at 15 m and beyond in both grasslands and shrublands. Second-year seedlings at 15 and 25 m developed mycorrhizas only in shrublands; no seedlings beyond 5 m formed mycorrhizas in grasslands although nearly all fecal pellet samples contained spores of hypogeous fungi in both shrublands and grasslands (Fig. 1*e*).

Discussion

The most abundant mycorrhizal inoculum, that which is richest in species and most frequently accessed by seedlings, was found closest to mature oaks. However, seedlings beyond the mycorrhizal network of parent trees formed mycorrhizas with hypogeous fungal species that were dispersed by rodents in fecal pellets.

Seedlings near mature trees have the advantage of early mycorrhizal inoculation, with potential transfer of carbon from the parent tree via the mycorrhizal network (Simard et al. 2002; Southworth et al. 2005; Nara 2006). The mycorrhizal network might enable seedlings to obtain water via hydraulic lift from the deeper roots of parent trees, particularly as the ground dries out in summer (Egerton-Warburton et al. 2007). In *Quercus macrocarpa* and *Q. ellipsoidalis*, seedlings benefitted from proximity to mature trees as mycorrhizal inoculum declined with distance (Dickie and Reich 2005; Dickie et al. 2007). In Douglas-fir seedlings, mycorrhizal species richness also declined at greater distances from mature trees (Cline et al. 2005). Seedlings of *Pinus virginiana* invading grassland obtained mycorrhizal inoculum chiefly from existing tree roots (Thiet and Boerner 2007).

The disadvantages of proximity to the parent trees and attachment to the mycorrhizal network are competition with much larger trees for water and sunlight, and density-dependent seedling predation as predicted by Janzen (1970) and Connell (1971). According to the Janzen–Connell hypothesis, seeds dispersed greater distances from parent plants have a better chance of survival because they escape seed and seedling predators (Janzen 1970; Connell 1971). However, a meta-analysis by Hyatt et al. (2003) found that while seedlings of some species survived better farther from parent plants, others did not. Although the logic of the Janzen–Connell hypothesis seems compelling, unresolved issues remain, and some unrecognized factor might influence regeneration (Howe and Miriti 2000). That factor may be mycorrhizal inoculum.

Although oak seedlings can survive for one year without mycorrhizas, by the time oak seedlings grow into saplings, all were ectomycorrhizal (Frank et al. 2008). Ectomycorrhiza communities on saplings of *Quercus garryana* included hypogeous fungi likely dispersed by small mammals (*Tuber*, *Peziza infossa*), invertebrates (*Tomentella*, Lilleskov and Bruns 2005), and epigeous fungi dispersed by wind (Tricholomataceae), but excluded those spread by direct hyphal growth or by water (*Cenococcum*, Trappe 1969).

Because acorns moved directly to planting sites without washing or sterilization did not frequently develop mycorrhizas, we conclude that codispersal of seeds and spores did not occur.

Potential of small mammals to disperse mycorrhizal inoculum

Beyond the root zone of trees and their mycorhizal network, fungal spore dispersal may assume greater importance. Inoculum becomes sparse and infection of seedling roots less frequent, but not zero. In the second spring, two seedlings beyond the mycorrhizal network of mature trees developed mycorrhizas, both with hypogeous fungi (*Tuber* and *Geopora*). Spores of *Tuber candidum* were not found in fecal pellets in this study, but have been observed in fecal pellets of rodents at this site (Frank et al. 2006*a*).

In this study, small mammals ate hypogeous fruiting bodies and dispersed mycorrhizal inocula via fecal pellets at distances up to 35 m from trees where fruiting bodies occurred, yet the seedlings rarely formed mycorrhizas at locations where fecal pellets were collected. The majority of spores in fecal pellets might not reach seedling roots. A low rate of seed dispersal and inoculum transfer may be adequate to maintain a population and allow for expansion at the margin of oak woodlands. For example, oak woodlands may regenerate by having one seedling per tree, over the life of that tree, survive to become a sapling. Given the

seed productivity of oaks, the rate of seedling survival may be less than one in a million. This low threshold of requisite seedling survival contributes to the difficulty of measuring oak regeneration dynamics.

Other animals disperse fungal inoculum away from the stand margin of ectomycorrhizal plants. Deer ate the fruiting bodies of *Rhizopogon* and dispersed spores in fecal pellets into sand dunes for several hundred metres around (Ashkannejhad and Horton 2006). The greatest diversity of fungal species was found near forested zones. Dispersal by animals contributed to re-establishment of mycorrhizas on Mount St. Helens following the volcanic eruption (Allen et al. 1992). At our site, other animals may be involved. Pocket gophers (*Thomomys bottae*) did not enter the live traps, but they too eat hypogeous fungi and defecate spores (Taylor et al. 2009).

Comparison of mycorrhizal inoculum in shrublands and grasslands

We found that shrub cover of nonectomycorrhizal species promoted ectomycorrhizal sapling survival. Where grasslands and shrublands adjoin oak woodlands, saplings survived only in shrublands. Although removal of herbaceous vegetation has benefitted oak seedlings, suggesting some interference by fibrous roots, shrub facilitation of seedling establishment is well-known in oaks (Callaway 1992; Dickie and Reich 2005; Williams et al. 2006; Dickie et al. 2007). The richness of potential inoculum (spores in fecal pellets) in shrublands correlated with the 10-fold greater number of animals and consequent greater volume of fecal pellets. Shrubs provide cover for small mammals, thus increasing rodent activity and fecal pellet deposition. Because *Ceanothus* species are not ectomycorrhizal, sapling success in shrublands would not result from direct provision of mycorrhizal inoculum. Shrubs provide more favorable abiotic factors (e.g., shade, lower temperatures, less evapo-transpiration) and biotic factors (e.g., less competition from grasses, protection from deer browse) than grasses. *Ceanothus*, with its nitrogen-fixing nodules, might provide increased nutrients (Schwintzer and Tjepkema 1990).

We conclude that while mycorrhizal inoculum for seedlings is most accessible in the existing mycorrhizal network on roots near mature trees, beyond that, mycorrhizal inoculum arises from spores of hypogeous fungi in rodent fecal pellets, as well as from airborne spores of epigeous fungi (Frank et al. 2006*a*, 2008). Our work contributes to an understanding of oak regeneration that may be useful for conservation and management of oak habitat, oak restoration, and reforestation, and may lead to development of an inoculation protocol for oak seedlings. If oaks are to expand into newly available habitat created by climate change, dispersal of mycorrhizal inoculum will be critical.

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