Low ectomycorrhizal inoculum potential and diversity from soils in and near ancient forests of bristlecone pine (*Pinus longaeva*)

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Abstract: Intersite variation in ectomycorrhizal (ECM) inoculum potential in soils from 16 sites located in arid subalpine areas of the White Mountains of California was quantified. The study sites included valleys dominated by big sagebrush (*Artemisia tridentata* Nutt.) and mountainsides dominated by ancient Great Basin bristlecone pine (*Pinus longaeva* Bailey). ECM inoculum potential was not detected at three of four valley sites nor in 42% of forest soil samples. Only 10 mycorrhizal species were detected in bioassays, and four of those accounted for 94.5% of all colonized seedlings, in order of decreasing abundance these were Pyronemataceae sp., *Rhizopogon* sp., *Wilcoxina rehmii* Yang & Korf, and *Cenococcum* sp. These species were identified also from in situ mycorrhizal roots. The abundance of the dominant Pyronemataceae sp. was significantly positively correlated with pH, which at all forest sites was high compared with typical conifer forest soils. Our results show that the ECM inoculum potential of soils is low, homogeneous, and spatially restricted in these ancient high-elevation forests.

Key words: fungal community, molecular ecology, spore dispersal.

Résumé : Les auteurs ont quantifié la variation, entre les sites, du potentiel inoculant ectomycorhizien (ECM) de sols provenant de 16 sites isolés des régions subalpines des White Mountains, en Californie. Les sites d'étude incluent des vallées dominées par la grande armoise, *Artemisia tridentata* Nutt., et les flancs de montagnes dominées par le pin des anciens Great Basin, *Pinus longaeva* Bailey. Aucun inoculum ECM n'a été détecté sur trois des quatre sites des vallées, non plus que de 42 % des échantillons de sols forestiers. On n'a retrouvé que 10 espèces mycorrhiziennes dans les bio-essais, et quatre de ces espèces colonisent à elles seules 94,5 % de tous les plants mycorrhizés. Ce sont, en ordre décroissant d'abondance : une Pyronématacée sp., un *Rhizopogon* sp., le *Wilcoxina rehmii* Yang & Korf, et un *Cenococcum* sp. Ces espèces ont également été identifiées à partir de racines mycorrhizées récoltées in situ. L'abondance de la Pyronématacée sp. est significativement et positivement corrélée avec le pH, lequel sur tous les sites forestiers est élevé, comparativement aux sols typiques des forêts conifériennes. Les résultats indiquent que le potentiel ECM inoculant est faible, homogène, et spatialement restreint dans ces vieilles forêts de haute altitude.

Mots clés : communauté fongique, écologie moléculaire, dispersion des spores.

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Introduction

The ancient Great Basin bristlecone pine (*Pinus longaeva* Bailey) has long generated interest among climatologists and plant ecologists (Ferguson 1968; LaMarche 1969; Hiebert and Hamrick 1984). Members of the Pinaceae are known to be obligately ectomycorrhizal (ECM), but the ECM fungal associates of bristlecone pine remain unstudied. In general, belowground diversity in cold environments, including that

of mycorrhizal fungi, has been largely neglected (Gardes and Dahlberg 1996; Smith and Read 1997).

Pinus longaeva is the main subalpine tree of the dry, cold, and barren White Mountains of California. In this high desert range, the distribution of *P. longaeva* is complementary to that of big sagebrush (*Artemisia tridentata* Nutt.) (Wright and Mooney 1965). Although *P. longaeva* can occur in mixed stands with rare limber pine (*Pinus flexilis* James) and *A. tridentata* on more nutrient-rich sites, it is mostly found in scattered stands in harsh high-elevation sites where other plant species are absent (Wright and Mooney 1965; Beasley and Klemmedson 1980). *Artemisia tridentata*, an arbuscular mycorrhiza-dependent shrub, is dominant in all non-forested subalpine areas.

The high frequency of stem clumps observed in ancient bristlecone pine stands indicates that establishment of these winged-seeded conifers is actually mediated by seed-caching nutcrackers (*Nucifraga* spp., Corvidae) particularly at high-elevation sites (Lanner 1988; Torick et al. 1996). In contrast, the Colorado bristlecone pine (*Pinus aristata* Bailey), the closest relative of *P. longaeva*, is a shade-intolerant pioneer

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species that regenerates following fires, with fire intervals of a thousand or more years (Baker 1992). Regardless of the specific mechanisms for seed dispersal, conifer seedling performance in nature is affected by ECM inoculum availability (Read 1998; Horton et al. 1999).

Both ECM inoculum and diversity appear to increase with time along primary and secondary successional chronosequences, but they remain spatially uneven (Visser 1995; Boerner et al. 1996; Helm et al. 1996). For instance, establishment may be sporadic and limited to forest edges (Boerner et al. 1996) or places close to an inoculum source. The patchy nature of ECM inoculum may interact with seed dispersal and competition between seedlings of ECM-dependent species and established individuals of arbuscular mycorrhizae dependent species. This can ultimately regulate the rate and spatial pattern of ECM-dependent species establishment (Boerner et al. 1996).

How the complementary distribution of *P. longaeva* and *A. tridentata* interacts with ECM community structure across soil types is not known. To begin to address this question, we used *P. longaeva* seedling bioassays to quantify intersite variation in ECM inoculum potential of soils among 16 sites at the White Mountains. We examined whether ECM inoculum potential differs (i) among *P. longaeva* forest patches because of substrate type and (ii) between forests and valleys. We predicted that ECM inoculum reflects mycorrhizal adaptation to substrate type and that there is limited ECM inoculum dispersal from mountainside *P. longaeva* forests to valleys of *A. tridentata*. If either were true, mycorrhizal inoculum can reinforce the spatial patterning characteristic of *P. longaeva* and *A. tridentata* communities.

Materials and methods

Study areas

In July 1997, sixteen sites were chosen for this study, of which 12 were located in different forested mountainsides dominated by Great Basin bristlecone pine (*Pinus longaeva* Bailey, Pinaceae), with scattered limber pine (*Pinus flexilis* James), big sagebrush (*Artemisia tridentata* Nutt., Compositae), lupine (*Lupinus* sp., Leguminosae), buckwheat (*Eriogonum* spp., Polygonaceae), and bluegrass (*Poa* spp., Poaceae). Four sites were located in different treeless valleys (V), dominated by *A. tridentata* with scattered *Lupinus* spp., in concave positions between forested mountainsides. All sites were located within the Ancient Bristlecone Pine Forest of the Mount Barcroft-Blanco Mountain Area, Inyo National Forest, southeastern California, U.S.A. (Fig. 1), and all stands of *P. longaeva* were approximately 1000 years old. This area is in cold uplands, where the mean annual precipitation is 23 cm and the mean annual temperature is 1°C. The soils are Cryoborolls on colluvial and alluvial positions on mountains at elevations from 2800 to 3500 m. Of the 12 forest sites, four were dolomite (D) outcrops (light gray to white calcareous rock), four were sandstone (S) outcrops of the Campito Formation (greenish to brownish quartzitic sandstone), and four were granitic (G) outcrops (medium gray monzonite – diorite granites). These were the three geologic substrates examined in detail by Wright and Mooney (1965), who first studied the complementary distributions of *P. longaeva* and *A. tridentata* in this area. The trees are widely spaced, surrounded by their own litter shadow and separated from neighboring trees by little or no vegetation amidst the gravel and bare rock. This pattern is most pronounced at D sites where conditions are the most arid, *P. flexilis* and *A. tridentata* are

Fig. 1. Location of study sites (solid squares) within the Ancient Bristlecone Pine Forest and within California. S, sandstone; D, dolomite; G, granite; V, valley.

absent, the spread of fire from lightning is most unlikely, and pure stands of *P. longaeva* can reach their greatest age (up to 4700 years) (Billings and Thompson 1957).

Sampling design

In this study there were 16 study sites $\times$ 1 sampling area $\times$ 4 sampling plots $\times$ 4 soil samples = 256 soil samples. In each of the 16 study sites (Fig. 1), one square sampling area of 20 $\times$ 20 m was established. In the 12 forested sites, each of these areas was chosen to have at each edge one mature *P. longaeva* and to be at least 20 m away from the edge to the nearest *P. flexilis* (present only at S and G sites). Within each 20 $\times$ 20 m area, four sampling plots were chosen on a tree-biased fashion, since the litter shadows of each tree represented the only comparatively mesic areas at the sites. At V sites, a similar procedure was followed, but the sampling plots were located at each corner of the sampling area. All study sites were located with a global positioning system (GPS) Pathfinder Basic⁺ (Trimble, Sunnyvale, Calif.), and the distance from V sites to the nearest forest edge was estimated in situ and checked with topographic maps. At each sampling plot, four samples of approximately 0.2 L of soil were collected with a hand trowel from among the rocks and gravel from points chosen at random within a 1-m² area adjacent to the tree trunk and sealed in-

dividually in plastic bags. To prevent cross contamination, the trowel used for sampling was rinsed with dilute sodium hypochlorite between sampling plots. At site D7 we also removed five soil cores (4.6 cm diameter, 15–25 cm depth) at arbitrary locations within the sampling area to estimate in situ ectomycorrhizal diversity. Unfortunately, because of the abundance of rocks, soil coring was not possible at other sites. Care was taken to minimize disruption in the areas sampled. All soil samples and soil cores were transported to the laboratory under refrigeration.

Mycorrhizal inoculum potential

Pinus longaeva seeds, obtained from the USDA Forest Service Nursery, Placerville, Calif., were surface sterilized in 30% hydrogen peroxide for 30 min and planted in Cone-tainer pots (RLC-4 Pine Cell, 2.5×16 cm; Stuewe and Sons, Corvallis, Oreg.) filled with the contents of each soil sample. Within 4 days after field collection, one seed was planted in each soil pot. Seeds were handled aseptically to avoid cross contamination during planting. In addition, 20 pots were filled with soil from randomly selected soil samples (approximately 90 mL) and steam-sterilized for 2 h twice with a 2-day interval between sterilizations. These were planted as controls to assay for laboratory contamination. The seeds had over 90% germination rate, and for those that failed to germinate, a second seed was planted 10 days after the first planting. Seedlings that died after the second planting were not replaced. Pots were maintained under fluorescent lights in the laboratory, isolated with plastic sheets to prevent airborne spore contamination, and watered twice weekly with tap water. Ten months after planting, the 236 surviving seedlings (217 from the 256 field samples and 19 from 20 controls) were harvested within a 1-month period. By then, the root systems were densely branched, they strongly maintained the soil structure when removed from the pots, and taproots were emerging from the bottom. Each root system was washed by spraying with water and examined using a dissecting microscope to sample all the morphological types of live mycorrhizal root tips present in each seedling. The mycorrhizal root tip types were subsequently preserved by lyophilization.

The soil cores were examined by placing each in a 500 μ m opening sieve and spraying with water. All the washed soil and roots were then spread thinly in Petri dishes and examined using dissecting scopes, sorted into morphotypes for each core, and lyophilized.

Molecular identification

ECM fungi were identified using methods described by Gardes and Bruns (1996). Briefly, we extracted DNA from individual ECM roots of each morphotype, and we amplified the internal transcribed spacer (ITS) of the nuclear ribosomal repeat by the polymerase chain reaction (PCR) with the fungal-specific primers ITS1F and ITS4 (White et al. 1990; Gardes and Bruns 1993). PCR products were then screened by restriction fragment length polymorphisms (RFLP) using the restriction endonucleases *AluI* and *HinfI* (New England Biolabs Inc., Beverly, Mass.). We estimated the molecular size of the restriction fragments using GelReader version 2.0.5 (National Center for Supercomputing Applications, Champaign, Ill.) and sorted the morphotype fragment sizes in various combinations to identify groups of morphotypes that matched for both restriction endonucleases. We checked that matching ITS–RFLP types were compatible with our morphotype descriptions of their gross morphology.

The phylogenetic distribution of the most frequent ITS–RFLP types obtained was also examined. Because herbarium collections of ectomycorrhizal fungal sporocarps from the area were not available and we did not find any sporocarps while field sampling, direct ITS–RFLP matching to fungal sporocarps was not possible. Instead, we used PCR amplification and sequencing of one or more

of the following fungal rDNA regions: (i) a fragment of the mitochondrial large subunit (mtLSU) (Bruns et al. 1998), (ii) the 5' end of the 28S nuclear gene between primers ctb6 (GCATATCAATAAGCGGAGG) and tw14 (GAAGTTTCCCTCAGGATAGC), and (iii) the ITS+5.8S nuclear genes. Sequencing of both strands was performed with an ABI model 377 sequencer (Applied Biosystems Co., Foster City, Calif.) using an ABI prism dye terminator cycle sequencing core kit (Perkin Elmer Co., Foster City, Calif.) or a Thermo Sequenase dye terminator cycle sequencing premix kit (Amersham Pharmacia Biotech, Piscataway, N.J.). We used DNA Sequencing Analysis version 2.1.2 and Sequence Navigator version 1.0.1 (Applied Biosystems Co., Foster City, Calif.) software for processing raw data. Sequences from the mtLSU were manually aligned to the data base of Bruns et al. (1998). Sequences from the 28S and ITS+5.8S genes were used to query the GenBank data base via the basic local alignment search tool (BLAST). The nearest relatives of each mycorrhizal type were downloaded, aligned using CLUSTAL X (Windows interface for CLUSTAL W; Thompson et al. 1994), and the alignment checked manually. To infer relationships, we used the neighbor-joining algorithm implemented in the program PAUP, version beta 4a (Swofford 2000).

Soil parameters

The soil pH and organic matter on samples from all plots was measured. Three grams of fresh soil per sample plot (pooled from the four soil samples taken at each plot) were placed in a 125-mL beaker to which we added 37.5 mL of double distilled water (ddH₂O) to obtain a 1:12.5 (w/v) soil–ddH₂O dilution as recommended for calcareous soils by Nilsson et al. (1995). The soil and water were shaken for 2 h, left to settle overnight, and the pH in H₂O (pH_w) determined using a Corning pH Meter 220 (Corning Instruments, Corning, N.Y.). Then, 2.8 g of KCl were added and the procedure repeated to determine pH in salt solution (pH_K). To estimate organic matter content, percent loss on ignition (%LOI) was determined at 360°C for 1–2 g of each soil sample. The %LOI was converted to standard Walkley–Black percent soil organic matter (%OM) using the equation: %OM = %LOI – 0.30 (Sims and Heckendorn 1991).

Data analysis

Because pH and %OM had significantly unequal variances, we log transformed the data and used Kruskal–Wallis tests and Tukey–Kramer honestly significant difference tests for comparing pH and %OM among sites and between pairs of sites, respectively. We also tested the ability of pH and %OM to predict the abundance of mycorrhizal types using nominal logistic regressions between the soil parameters and each mycorrhizal type across all sites. For statistical tests, we used JMP (SAS Institute Inc., Cary, N.C.).

The dissimilarity among sites was calculated in terms of the composition of their mycorrhizal assemblages using the Bray–Curtis distance measure (Bray and Curtis 1957), which is a robust measure of the ecological distance between sites (Faith et al. 1987). The distance between two sites was calculated using the site counts of mycorrhizal seedlings of each mycorrhizal type as follows: $(\sum_k |X_{ik} - X_{jk}|) / (\sum_k X_{ik} + X_{jk})$ where X_{ik} is the abundance of the k th mycorrhizal type at site i , and X_{jk} is the abundance of the k th mycorrhizal type at site j . Sites supporting identical assemblages have a dissimilarity of 0, and sites with no shared types have a dissimilarity of 1. We used the counts of mycorrhizal types that were detected in more than one seedling (Pyrenomataceae sp., *Rhizopogon* sp., *Wilcoxina rehmlii* Yang & Korf, and *Cenococcum* sp.). The sites were then clustered on the basis of the site by site dissimilarity matrix of their mycorrhizal assemblages by neighbor-joining using PHYLIP (Felsenstein 1981) and cluster support was assessed with 1000 bootstrap runs.

Table 1. GenBank sequence accession numbers or restriction fragment sizes for eight identified mycorrhizal taxa of *Pinus longaeva* detected in situ at site D7 including four taxa detected in more than one seedling in bioassays from all sites.

Taxon	Bioassay	nr28S	mtLSU	nrITS
Pyronemataceae sp.	Yes	AF266709		AF266709
<i>Rhizopogon</i> sp.	Yes	AF266705		AF266710
<i>Wilcoxina rehmii</i>	Yes	AF266708		AF266708
<i>Cenococcum</i> sp.	Yes			<i>AluI</i> : 380, 182 <i>HinfI</i> : 148, 110
Tricholomatoid sp.	No		AF266703	<i>AluI</i> : 500, 123 <i>HinfI</i> : 282, 225, 116
Thelephoroid sp.	No		AF266702	<i>AluI</i> : 235, 112, 92, 89 <i>HinfI</i> : 372, 151
Suilloid sp.	No		AF266704	<i>AluI</i> : 436, 209 <i>HinfI</i> : 250, 116, 105, 100
Cortinarioid sp.	No	AF266711		<i>AluI</i> : 574, 111, 89 <i>HinfI</i> : 320, 250, 106, 101

Note: Six additional unidentified mycorrhizal taxa were detected in single bioassay seedlings (not listed).

Results

Mycorrhizal community

The four mycorrhizal taxa from bioassays identified in this study are listed in Table 1. Four additional taxa identified from roots at site D7 but not detected in bioassays are also listed in Table 1. Six additional mycorrhizal types that were present only in single bioassay seedlings (5.5% of all mycorrhizal seedlings) were not identified further or included in our analyses. The dominant mycorrhizal taxon across seedling bioassays from forest sites shows weak ITS and 28S sequence similarity to Pezizales species (Ascomycota) available in GenBank. It is likely to represent a novel mycorrhizal lineage within the family Pyronemataceae (see Landvik et al. 1997) of the order Pezizales based on comparing its ITS sequence to *Sphaerospora*, *Scutellinia*, *Tricharina*, *Geopyxis*, *Tarsetta*, *Pyropyxis*, *Rhizina*, and other Pezizalean and Helotialean genera (T. Schumacher, University of Oslo, Oslo, Norway, personal communication) and its 28S sequence to members of Pezizaceae (51 species), *Ascobolus*, *Verpa*, and Pyronemataceae (*Aleurina*, *Otidea*, *Smardaea*, *Marcellina*) (K. Hansen, University of Copenhagen, Copenhagen, Denmark, personal communication). For the purposes of this study, we labeled it "Pyronemataceae sp." Another recurring mycorrhizal taxon in the bioassays produced an ITS sequence that differs from *W. rehmii* (GenBank accession U38565) by only one transition (K.N. Egger, personal communication); thus, we labeled it *W. rehmii*. A *Rhizopogon* sp. (Rhizopogonaceae, Basidiomycota) ITS sequence from the bioassays was found to be most closely related to *Rhizopogon* section *Rhizopogon* (L. Grubisha, University of California, Berkeley, personal communication; Grubisha 1998). The final taxon to be detected in the bioassays was *Cenococcum* sp. (Loculoascomycetes, Ascomycota), which is distinguished morphologically by black, club-shaped mycorrhizas and thick emanating hyphae. All control seedlings appeared non-mycorrhizal; they showed abundant root hairs and no hyphal mantles. This criterion was consistent with negative ITS1F–ITS4 PCR amplification from roots of

a subset of seedlings grown in fresh soil ($n = 15$) that were classified as non-mycorrhizal because of root morphology.

Mycorrhizal community and soil parameters

A nominal logistic regression model with pH_K as a factor and Pyronemataceae sp. as a response had a significant likelihood ratio chi-square ($p < 0.0001$, $r^2 = 0.19$), although the percentage of variation explained was low. The pH_w behaved similarly. The pH_K accurately predicted the presence of Pyronemataceae sp. in approximately 20% of the 64 sample plots, while the standard correlation between Pyronemataceae sp., and pH_w was 63%.

The Kruskal–Wallis tests indicated that pH_w , pH_K , and %OM were significantly different among sites ($p < 0.0001$, 0.0001, and 0.0006, respectively). The Tukey–Kramer tests indicated that pH_w and pH_K were significantly higher at the D sites compared with all other sites and that pH_K was significantly higher at the G sites than at the V sites.

As shown in Table 2, three of the four V sites were devoid of mycorrhizal inoculum potential in our assay. Site V16 represents the only exception; it yielded a higher percentage of seedlings colonized by *Rhizopogon* sp. than any forest. *Rhizopogon* sp. was the only source of mycorrhizal inoculum detected at V16. Site V16 was located on a saddle approximately 30 m away from trees growing on dolomitic soil and 100 m away from trees growing on granitic soil. All other V sites were at least 300 m away from the nearest trees.

Clustering based on Bray–Curtis distances placed all valley sites (except V16, which was an outlier) together with two sandstone sites to the exclusion of all dolomite, all granite, and two other sandstone sites, which formed another cluster. Bootstrap support greater than 50% was not observed for any cluster (data not shown) because of the high similarity observed across sites.

Discussion

The inoculum potential and species richness documented in this study are dramatically lower than those previously reported from coastal California *Pinus muricata* D. Don forest

Table 2. Characterization of soil samples from 16 sampling sites in four substrate types (S, sandstone; D, dolomite; G, granite; V, valley) for water pH ($n = 4$), potassium salt pH ($n = 4$), percent organic matter ($n = 16$), and number of bioassay seedlings of *Pinus longaeva* colonized by different mycorrhizal fungi.

Site	Water pH	Salt pH	Organic matter (%)	No. of seedlings	Pyronemataceae sp.	<i>Rhizopogon</i> sp.	<i>Wilcoxina rehmlii</i>	<i>Cenococcum</i> sp.	Other types	Non-mycorrhizal (%)
S1	5.99±0.24	5.42±0.25	34.0±17	4	1	1	0	0	0	75
S2	6.36±0.12	5.77±0.13	21.0±11	12	6	0	0	0	0	50
S3	6.86±0.08	6.14±0.08	31.0±18	11	2	1	3	0	1	55
S4	6.76±0.18	6.07±0.19	14.0±11	13	4	2	0	0	1	46
D5	7.91±0.21	7.74±0.21	7.5±4	14	7	1	2	0	0	29
D6	7.56±0.13	7.32±0.18	18.0±2.5	15	10	1	1	0	0	27
D7	7.69±0.18	7.28±0.16	6.8±1.2	16	10	1	1	0	1	19
D8	7.68±0.10	7.40±0.15	8.8±3.1	15	7	1	0	0	2	47
G9	6.60±0.14	6.04±0.18	12.0±6	15	2	1	2	2	1	47
G10	6.68±0.20	6.01±0.18	6.1±1.3	16	10	4	2	1	0	6
G11	7.12±0.16	6.45±0.22	2.8±0.7	12	6	1	0	0	0	50
G12	6.58±0.23	6.00±0.21	20.0±12	15	1	1	4	2	0	60
V13	6.62±0.06	5.63±0.04	7.3±1.8	16	0	0	0	0	0	100
V14	6.37±0.01	5.46±0.02	5.6±0.8	15	0	0	0	0	0	100
V15	6.02±0.08	5.82±0.06	8.0±3	14	0	0	0	0	0	100
V16	6.62±0.17	5.61±0.20	2.7±1.3	14	0	8	0	0	0	43

Note: Values for pH and organic matter are mean ± SD.

soils. Taylor and Bruns (1999) reported that 15 soil cores from a single 40-year-old *P. muricata* site produced 37 mycorrhizal types in bioassays and 4 (5.3%) non-mycorrhizal seedlings. Furthermore, only one (1.4%) non-mycorrhizal seedling was found among 70 seedlings planted in soil from nine soil cores removed in a *P. muricata* forest following a stand-replacing wildfire (M.I. Bidartondo, unpublished data). While the bioassay inoculum potential of forest soil does not reflect in situ inoculum exactly, both measures can show similar patterns (Baar et al. 1999). Thus, we infer that the mycorrhizal community associated with ancient *P. longaeva* at the White Mountains has produced a very poorly developed mycorrhizal inoculum bank that is largely dominated by a Pyronemataceae sp. (Ascomycota). Our sixty-four soil samples from 16 different sites produced only 10 mycorrhizal types in bioassays and 67 (42%) of 158 surviving seedlings were non-mycorrhizal when planted in fresh forest soil. Most of the fungi we retrieved from bioassays belong to groups that are also common during the first growing seasons following forest fires (Baar et al. 1999).

We hypothesize that the divergent inoculum soil banks of *P. longaeva* and *P. muricata* are due to climatic differences between both biomes, because fungal sporocarp production is strongly controlled by climatic factors (Petersen 1977; Vogt et al. 1992). Temperate coastal forests are well known to support a diverse and voluminous annual mycorrhizal sporocarp production, while sporocarp production in arid subalpine forests may be negligible or altogether absent in most years because of drought and nightly freezing during the short summer season.

The consistent presence of Pyronemataceae sp. mycorrhizal inoculum in soils from all forest sites is quite notable. Within single conifer forest stands, highly spatially variable mycorrhizal assemblages have been reported (Gardes and Bruns 1996; Baar et al. 1999; Taylor and Bruns 1999; Stendell et al. 1999), and this is apparently reflected in uneven localized inoculum distributions (Baar et al. 1999; Taylor and Bruns 1999). Conifer forest soils are typically acidic (pH 4.0–6.5; Barnes et al. 1998), but the cold, dry, and comparatively alkaline soils of the White Mountains' forests (pH 6–7.9) may lead to more uniform ectomycorrhizal communities that allow conifers to obtain nutrients from such environments. Our data provide evidence for a weak positive correlation between the abundance of Pyronemataceae sp. inoculum and soil pH. This may reflect adaptation of this fungus to relatively alkaline soils. Nonetheless, this study suggests weak overall mycorrhizal inoculum structuring according to substrate type or site among the forest sites examined. *Rhizopogon* sp. was widespread but considerably less common and abundant than Pyronemataceae sp. This may reflect the distribution of the field inoculum of *Rhizopogon* sp. or its poor competitive ability relative to Pyronemataceae sp. in bioassays.

The absence of the Ascomycete fungi Pyronemataceae sp., *W. rehmlii*, and *Cenococcum* sp. in all four valley sites suggests that these species have a very limited ability to disperse outside forest edges. These fungi produce asexual chlamydospores or sclerotia in the soil, which are expected to undergo little if any dispersal, instead relying on contact with growing host root tips. Most Pezizales species, perhaps including this study's Pyronemataceae sp., can also produce

airborne ascospores on epigeous cup-shaped ascocarps, but production of these would be limited to rare periods of high humidity. Fewer Pezizales species produce hypogeous ascocarps. The Basidiomycete *Rhizopogon* sp. may have a higher ability to escape the forest edge (as indicated by its comparatively high incidence on site V16) by producing sexual basidiospores, but it was not found in valley sites over 300 m from forest edges. This is explicable by the basidiocarp production and dispersal behavior of *Rhizopogon*. Its underground basidiocarps may be less sensitive to short drying and freezing events, and its small-mammal-dispersed basidiospores should most effectively disperse over short distances (Molina et al. 1999). In the case of *P. muricata*, *Cenococcum* spp. (which produces only asexual soil-borne sclerotia) mycorrhizal inoculum potential could not be detected at 5 and 35 m from the forest edge in an arbuscular mycorrhizal plant community, while *Rhizopogon* spp. inoculum was equally abundant within the edge of the forest as 35 m away from it after a stand-replacing wildfire (M.I. Bidartondo, unpublished data). *Cenococcum* sp. was found to be common in conifer roots at tree line and on exposed glacial moraines in the Oregon Cascades (Trappe 1988). The dispersal range of *Rhizopogon* below 300 m away from *P. longaeva* forest edges remains to be tested, but we predict that it is spatially patchy and quite restricted.

This is the first systematic study of the mycorrhizal community of a high-elevation species, and it suggests that, under harsh environmental conditions, the mycorrhizal community is severely limited in its development. Despite the great age of *P. longaeva* forests, we have shown overall low diversity and strict spatial constraints associated with ectomycorrhizal inoculum potential in the White Mountains. These are likely results of the cold arid environment in which *P. longaeva* grows. A limited mycorrhizal inoculum could interact with seed dispersal and competition with *A. tridentata* to produce and reinforce the landscape-level distribution observed for *P. longaeva*. Future studies of in situ mycorrhizal diversity and mycorrhizal inoculum adjacent to forest edges have the potential to show mycorrhizal-mediated limitations imposed on *P. longaeva* distribution by both substrate and competition.

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