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Pezizalean mycorrhizas and sporocarps in ponderosa pine (*Pinus ponderosa*) after prescribed fires in eastern Oregon, USA

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Abstract Post-fire Pezizales fruit commonly in many forest types after fire. The objectives of this study were to determine which Pezizales appeared as sporocarps after a prescribed fire in the Blue Mountains of eastern Oregon, and whether species of Pezizales formed mycorrhizas on ponderosa pine, whether or not they were detected from sporocarps. Forty-two sporocarp collections in five genera (*Anthracobia*, *Morchella*, *Peziza*, *Scutellinia*, *Tricharina*) of post-fire Pezizales produced ten restriction fragment length polymorphism (RFLP) types. We found no root tips colonized by species of post-fire Pezizales fruiting at our site. However, 15% (6/39) of the RFLP types obtained from mycorrhizal roots within 32 soil cores were ascomycetes. Phylogenetic analyses of the 18S nuclear ribosomal DNA gene indicated that four of the six RFLP types clustered with two genera of the Pezizales, *Wilcoxina* and *Geopora*. Subsequent analyses indicated that two of these mycobionts were probably *Wilcoxina rehmii*, one *Geopora cooperi*, and one *Geopora* sp. The identities of two types were not successfully determined with PCR-based methods. Results contribute knowledge about the above- and below-ground ascomycete community in a ponderosa pine forest after a low intensity fire.

Keywords Ectendomycorrhizas · Pezizales · nrDNA · Prescribed burn · Ponderosa pine

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Introduction

After a wildfire or prescribed burn, a series of fungi appear that typically fruit only after a fire. So-called post-fire fungi, largely belonging to the order Pezizales, commonly start fruiting approximately 6 weeks after a fire and continue to fruit in successional groups for about 2 years before seemingly disappearing from the landscape (Petersen 1970). Most have been reported to be saprotrophic (Visser et al. 1981; Egger 1986). When suggested to be mutualistic, post-fire Pezizales are often referred to as facultatively mycorrhizal. Post-fire fungi may alternate between saprotrophism after a disturbance, and mutualism in stable forests when there is more competition for nutrients from other saprotrophic fungi, a strategy suggested for morels (Buscot 1994). Biotrophism for some Pezizalean fungi is supported by direct observation of the fungi colonizing root tips in vitro (Egger and Paden 1986; Warcup 1990; Dahlstrom et al. 2000) as well as in vivo (Vrålstad et al. 1998).

In our study, we examined the mycorrhizal status of post-fire Pezizales based on samples collected from the field following a prescribed burn. Some fungi in our study formed a type of mycorrhiza known as ectendomycorrhiza (Smith and Read 1997). This unique type of mycorrhiza was originally described on *Pinus* spp. and defined by intracellular penetration of cortex cells, a weakly developed mantle, and wide hyphae in the Hartig net (Laiho

1965; Mikola 1965). Laiho (1965) further applied the label 'E-strain fungus' to isolates that form this type of ectendomycorrhiza. Subsequently, researchers have identified many of the E-strain cultures as *Wilcoxina* (Yang and Wilcox 1984; Yang and Korf 1985; Egger et al. 1991) or other members of the Ascomycota (Yu et al. 2001).

Our study focused on the mycorrhizal fungi that occur after a low intensity fire in ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) forests of eastern Oregon, USA. Knowledge of the above- and below-ground composition of mycorrhizal fungal species in this region and their response to fire is limited. Ponderosa pine forests in this region are historically known for their spacious, park-like appearance, consisting of stands of even-aged trees (Agee 1998). Ponderosa pines are drought-resistant, often associated with cold winters and warm droughty summers (Agee 1998). Their thick bark and deep taproots protect them from low intensity, high frequency fires, which smolder at the base and rarely reach the crown (Agee 1998). These forests are ideal sites to examine mycorrhizas of ponderosa pines in vivo because they are the dominant mycorrhizal host and their root tips are easily distinguished from root tips of other plants in the area.

This project was part of a larger, integrative study of the effects of prescribed fires on the ectomycorrhizal fungal community and various components of the ecosystem. This project focused on a small section of the ectomycorrhizal community, a group of ascomycetes. Our objectives were to determine (1) which Pezizales appear as sporocarps after a fire in a ponderosa pine forest in the Blue Mountains of Oregon, (2) whether species of Pezizales that appear as sporocarps form mycorrhizas on ponderosa pine, and (3) whether species of Pezizales not detected from sporocarps form mycorrhizas on ponderosa pine.

Materials and methods

Site description

The study site is located in a ponderosa pine forest on the Emigrant Creek Ranger District of the Malheur National Forest, Harney County, Oregon (43.5° N, 119° W). The oldest ponderosa pines on the site are 100–200 years old. The site, dry in the summer, received an average of 0.12 cm of rain between June and October in 1997 and 1998 (Oregon Climate Service http://www.ocs.orst.edu). Maximum temperatures average 27°C in the summer (June–September 1997–1998), and 4°C in the winter (November–February, 1997–1998) (Oregon Climate Service).

The site is divided into four stands, designated as Kidd Flat, Trout, Driveway 14, and Driveway 17. The distance between Kidd Flat and Trout is about 3.2 km. These two sites are 14.5 km west of the two Driveway sites. The distance between the two Driveway stands is less than 1 km.

Study design

In the autumn of 1997, each stand (8–12 ha) was prescribe-burned, largely reducing the organic layer and smaller diameter trees (Thies et al. 2001). Prior to the fire, one 240 m permanent transect line was established with six permanent plots, approximately 40 m apart, per stand. The ponderosa pine closest to each 40 m mark that would most likely survive the prescribed fire was the point of reference for each plot and was marked with an aluminum tag. Areas within the dripline (tree canopy edge) of the marked trees were designated as plots.

Root tips were obtained from 5 cm soil cores driven into the ground to a depth of 15 cm. Location of cores was determined by presence of post-fire Pezizalean sporocarps on plots. Fungi are patchily distributed over the landscape, and the location of their presence on root tips is difficult to determine (Gardes and Bruns 1996a). Soil cores were collected under Pezizalean sporocarps to increase the chances of finding colonization by these post-fire Pezizales. If no sporocarps were present on a plot, cores were taken directly south of the marked tree, within its dripline. If the area around the marked tree was not burned, then the area around the closest ponderosa pine with burned ground was sampled. Either 1 or 2 soil cores were taken from each plot, 8 cores per stand, 32 cores for the entire site. Plots from which a second core was taken were selected by the presence of more than one post-fire Pezizalean species. Soil cores were placed in plastic bags, stored in an ice chest for no more than 3 days, and transferred to a 4°C refrigerator. Root tips were examined and lyophilized within 2 weeks after collection. Soil cores were collected in June and July 1998.

Sporocarps of post-fire Pezizales were collected from all plots in June 1998. None were found in July 1998 due to dry conditions. For DNA extraction, pieces of sporocarps with a surface area of 1 mm ×1 mm, or single specimens of small sporocarps were stored in 95% ethanol for less than 1 month. Collections belonging to genera not adequately monographed for the western United States were differentiated by microscopic characters and given a type number or letter. Morphological characters of remaining fresh sporocarps were recorded. Specimens were then dried on a food dehydrator and deposited in the Oregon State University Mycological Herbarium (OSC).

Laboratory analysis

Soil cores were soaked in water for at least 2 h, gently rinsed, and poured through a 0.5 mm sieve (No. 35 USA standard testing sieve; Tyler, Edmonton, Canada) to catch root tips. Saved root tips were stored in water at 4–5°C.

Mycorrhizal root tips were categorized into morphotype groups, loosely following the system of Agerer (1987–1998), based on color, branching pattern, thickness of mantle, and presence or absence of rhizomorphs. Root tips were then lyophilized for 24–36 h and stored at −20°C until needed for DNA extraction.

DNA extraction and restriction fragment length polymorphism (RFLP) analyses were conducted for all morphotype groups, a morphotype group being all morphologically similar tips from one soil core. The extraction protocol followed that of Gardes and Bruns (1996b) with minor adjustments. Samples for which this protocol was unsuccessful were re-extracted following Lee and Taylor (1990). Extractions were made from at least 1–2 root tips from each morphotype group, or until amplification was successful. Some samples did not yield PCR product for any primer pair tested. Data are presented only for morphotype groups for which molecular data were collected.

The internal transcribed spacer (ITS) region of the nuclear ribosomal (nr)DNA was amplified from dilutions made from either a root tip or a sporocarp extract. The fungal specific primer ITS-1f (Gardes and Bruns 1993) was used in conjunction with the more universal primer ITS-4 (White et al.1990; Gardes et al. 1991). The primer NL6A, specific for ascomycetes (Egger 1995), was paired with the primer ITS-1f. The results from primer pair ITS-1f and ITS-4 produced cleaner, brighter bands than this latter pair and so ITS-1f and ITS-4 was the main primer pair used. The primer pair ITS-1f and ITS-4b preferentially amplifies basidiomycetes (Gardes and Bruns 1993) and was used to screen out basidiomycete mycobionts from all samples that successfully amplified with primer pair ITS-1f and ITS-4.

PCR amplifications followed the protocol of Gardes and Bruns (1996b) with the following program: 94°C (30 s); [93°C (35 s); 55°C (53 s); 72°C (30+5 s/cycle)] ×35 cycles. Successful amplification was verified by gel electrophoresis with 3% or 2% agarose gels. Gels were run at 110 V for about 40 min, stained in ethidium bromide and rinsed in deionized H_2O for 10 min.

Restriction endonuclease digests were performed on ITS PCR products using *Alu*I, *Hinf*I, and *Dpn*II in single enzyme digests. Restriction fragments were subsequently separated on agarose gels (3% agarose) and visualized with ethidium bromide under UV light. Band sizes were measured against a 100 bp DNA ladder using AlphaImager AlphaEase software, which has an error in size estimation of RFLP bands of around 5–10%. All RFLPs were recorded by AlphaImages, and photos were taken using a Sony Digital Graphic Printer UP-D890. Species level identification was estimated by identical RFLP patterns for all three endonucleases. Sequence analyses were conducted for all root-tip RFLP types, because none matched the patterns from sporocarps.

For sequencing, DNA was amplified by PCR using primers for the gene region of interest (see below). PCR products were cleaned using a QIAquick PCR purification kit (Qiagen, Valencia, Calif.). To verify recovery after purification, 2 µl purified PCR product was gel electrophoresed, and DNA concentrations were estimated by comparison of band intensity to standards. If necessary, the concentration of the purified PCR products was adjusted before the samples were sent to the Center for Gene Research and Biotechnology at Oregon State University for sequencing on an ABI 377 automated sequencer. The resulting sequences were edited in SeqEd (PE Biosystems, Foster City, Calif.), and aligned manually using PAUP 3.1 (Swofford 1993) and PAUP* (Swofford 1999) and a color font.

The primer pair ITS-1f and ITS-4b does not amplify all basidiomycetes. To further differentiate basidiomycete mycobionts from ascomycetes, the ITS region including the 5.8S rRNA gene was amplified and sequenced with primers ITS1f and ITS4. 5.8S rDNA sequences were placed in an existing database designed to differentiate between plant, animal, and fungal DNA, with some resolution between ascomycetes and basidiomycetes (Cullings and Vogler 1998). Neighbor-joining analysis was conducted as suggested by Cullings and Vogler (1998).

Partial 18S (nuclear small subunit) rRNA gene was sequenced for ascomycete RFLP types. This region was used to identify mycobionts to family and, if possible, genus. Primer pairs NS1/NS2, NS3/NS4, and NS5/NS8 (White et al. 1990) were used for amplification and sequencing. Primer pairs NS3/NS4 and NS5/NS8 amplified most fungal DNA from root tips. In a few instances, however, they also amplified plant DNA as indicated when the sequences were compared to sequences in GenBank (http:// www.ncbi.nlm.nih.gov, blast search). In these cases, fungal-specific primer pairs NS21 and NS24 (Gargas and DePriest 1996) were used for amplification and sequencing of the 18S region as well. Blast searches were conducted for all sequences to verify that sequences were from fungal DNA and not plant DNA.

Sequences from the 18S region of nrDNA of the mycobionts were added to the database of sequences included in Spatafora et al. (1998) with some changes. Our database continued for 576 bp beyond the 1,150 bp used by Spatafora et al. (1998), for a total of 1,726 bp. Sequences of other ascomycetes, primarily members of the Pezizales, which were added to those in Spatafora et al. (1998), were either downloaded from GenBank (*Aleuria aurantia* U53371, *Balsamia vulgaris* AF054905, *Barssia oregonensis* U42657, *Geopyxis carbonaria* U62011, *Inermisia aggregata* Z30241, *Lamprospora maireana* AF061719, *Leucoscypha oroarctica* AF061724, *Morchella elata* U42641, *Neottiella rutilans* AF061720, *Octospora wrightii* AF061722, *Orbilia delicatula* U72603, *Otidea leporina* U53381, *Phialocephela fortinii* L76626, *Plectania rhytidia* AF061723, *Pseudorhizina californica* U42650, *Pyronema domesticum* U53385, *Rhizina undulata* U42664, *Sarcosoma globosum* U53386, *Sarcosphaera coronaria* AF133157, *Scutellinia scutellata* U53387, *Sphaerosporella brunnea* U53388, *Tarzetta catinus* U53389, *Trichophaea hybrida* U53390, *Underwoodia columnaris* U42658, *Wilcoxina mikolae* U62014, and *Wynnella sylvicola* U42655) or produced for this study (*Cheilymenia* sp. AF260409/AF260410, accession NSW 7552; *Genea intermedia* AF260411/AF260412, accession NSW 8187; *Geopora cooperi* AF260413/AF260414, accession NSW 7527; and *Lasiobolus* sp. AF260407/AF260408, accession NSW 8223).

Table 1 Number of collections of sporocarps at each stand, and number of cores taken beneath sporocarps of each fungal genus. Number of cores taken beneath each genus is in parentheses

Stand	Genus				
	Anthracobia	*Morchella*	*Peziza*	*Scutellinia*	*Tricharina*
Driveway 14	0	0	0	1(1)	5(5)
Driveway 17	6(4)	0	0	0	10(1)
Kidd Flat	0	1(1)	2(2)	0	8(4)
Trout	3(2)	2(2)	1(0)	0	3(3)
Total number of collections and cores	9(6)	3(3)	3(2)	1(1)	26(13)

Maximum parsimony analysis was performed in PAUP 3.1.1 (Swofford 1993) and PAUP* (Swofford 1999) according to Spatafora et al. (1998). Only parsimony-informative characters were used. Gaps were treated as missing characters; 100 heuristic searches were performed with random sequence addition using the tree-bisection-reconnection algorithm for branch swapping. Weighted parsimony analysis using a step matrix (Spatafora et al. 1998), favoring transitions over transversion was also conducted [1:1.4 (TS:TV)]. Bootstrap analysis using a full heuristic search with 100 replicates was performed to estimate relative support for individual nodes.

To improve genus-level identification of some RFLP types, sequences from the 3′ end of the 18S rRNA gene and from the ITS 1 region were obtained. The ITS region is more variable than the 18S region and is helpful for inter- and intra-generic studies (Gardes and Bruns 1993; Norman and Egger 1996; Cullings and Volger 1998; Vrålstad et al. 1998; Horton 2002). The 3′ end was amplified and sequenced with NS5 and NS8 (as described above); the ITS 1 region was amplified with ITS-1f and ITS-4 and sequenced with ITS-1f and ITS-2.

Maximum parsimony analyses with the same conditions described above, with the exception that all characters were weighted equally, were performed on the partial 18S and ITS 1 sequences for the *Wilcoxina*/*Trichophaea* clade. Sequences for *Wilcoxina mikolae* var. *mikolae* (Yang and Wilcox) Yang and Korf (GenBank U38635), *W. rehmii* (Yang and Wilcox) Yang and Korf (U38567), *W. alaskana* Kempton, Yang, & Korf (U38583.1), *Tricharina gilva* (Boud. in Cooke) Eckblad (U38629), and *Sphaerosporella brunnea* (U38587) were downloaded from GenBank. The sequence for *Trichophaea hybrida* (NSW 7162) was determined for this analysis. *Sphaerosporella brunnea* was used as the outgroup.

Following the sequence analysis, ITS-RFLPs were produced from dried sporocarps of selected fungi we thought might be related to the unknown mycobionts. Dried sporocarp materials for five putative species of *Geopora* (*Geopora* sp. A (NSW 7225), *Geopora* sp. B (NSW 7341), *Geopora* sp. C (NSW 7342), *Geopora clausa* 1 (JMT 5438), and *Geopora clausa* 2 (JMT 7420), were obtained from the collections of N.S. Weber and J.M. Trappe. DNA was extracted from *W. rehmii* cultures provided by K.F. LoBuglio.

Results

Forty-two collections of sporocarps within five genera (*Anthracobia*, *Morchella*, *Peziza*, *Tricharina*, *Scutellinia*) of post-fire Pezizales represented ten RFLP types from our site (Table 1). *Tricharina* occurred in all four stands, *Anthracobia*, *Morchella* and *Peziza* were each found in two stands, and *Scutellinia* was found in only one stand (Table 1).

A total of 25 soil cores were collected directly beneath sporocarps (Table 1). Seven cores were collected from plots where no sporocarps were found, two plots at Driveway 14, three plots at Driveway 17, and one plot each from Kidd Flat and Trout. A total of 91 morphotype root tip groups were found in the 32 soil cores. Of those, 42% (38/91) were good candidates for ascomycetes, as they amplified with ITS-1f and ITS-4, but not with ITS-1f and ITS-4b. Twenty-nine morphotypes did not amplify with any primer pairs tested, and 24 were determined as likely to be basidiomycetes based on successful PCR amplification with the primer pair ITS-1f and ITS-4b.

Among the 38 morphological groups that were possible ascomycetes, 15 unique RFLP types were observed. Sequence analysis of the 5.8S region showed 40% (6) of the 15 RFLP types were ascomycetes and the remaining were basidiomycetes. Distribution of the 15 RFLP types among stands is shown in Table 2. Five of the ascomycete RFLP types [Type 6 (=*Geopora* sp.), Type 10 (=*W. rehmii*), Type 11 (=*Geopora cooperi*), Type 12, and Type 14] were found in Kidd Flat or Driveway 14, Type 8 (=*W. rehmii*) was found in both stands (Table 2). RFLP band sizes and descriptions of the mycorrhizal morphology of the six ascomycetes are reported in Tables 3 and 4. No RFLP types from the mycorrhizas matched RFLPs from sporocarps collected from our site.

Table 2 Locality of restriction fragment length polymorphism (RFLP) types. RFLP types: *O* Mycorrhizal ascomycetes, *X* mycorrhizal basidiomycetes

Stand	RFLP type														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Driveway 14	X						X	O					X	O	X
Driveway 17		X	X				X								
Kidd Flat					X	O	X	O	X	O	O	O			
Trout				X											

Table 3 RFLP band sizes for six Pezizalean mycobionts, Pezizalean sporocarps found on site, and dried sporocarps of *Wilcoxina rehmii* and *Geopora* spp.

	RFLP type or species	Restriction enzyme		
		AluI	*Dpn*II	*Hinf*I
Mycobiont	Type 6	695	235 325	370
	Type 8	205 443	216 322	131 220 289
	Type 10	216 323	208 303	133 222 288
	Type 11	623	234 325	99 184 379
	Type 12	676	213 332	138 220 290
	Type 14	194 420	178 243 275	141 233 297
Fresh sporocarp	*Anthracobia* type 1	209 442	212 257	94 104 338
	Anthracobia type 2	608	172 257	279 352
	Anthracobia type 3	596	149 320	267 317
	Anthracobia type 4	601	173 257	387
	Morchella type 1	112 619	247 421	101 112 264
	Morchella type 2	121 655	259 450	103 299 387
	Peziza type 1	93 116 183 238	228 346	319 274
	Scutellinia type 1	656	102 145	158 355
	Tricharina type 1	207 430	215 318	356
	Tricharina type 2	209 417	221 305	126 142 275
Dried sporoacarp	*Geopora clausa* 1	625	245 340	119 171 213
	G. clausa 2	630	257 340	144 171 195
	Geopora collection A	551	236 326	112 198 398
	Geopora collection B	634	151 170 236	110 205 398
	Geopora collection C	642	241 334	114 209 417
	Geopora cooperi	623	234 325	99 184 379
	W. rehmii	205 443	216 322	131 220 289

Maximum parsimony analysis resulted in four most parsimonious trees, all of which were 921 steps long with a consistency index (CI) of 0.439 and a retention index (RI) of 0.686. Primers NS1 and NS2, which amplify the 5′ end of the small subunit rRNA gene, did not provide clean sequence reads. Thus, the first 600 characters of the 5′ end of the small subunit were not used in the final sequence analysis. Of the remaining 1,126 characters, 35 were excluded because of hypervariability, leaving 1,091 included characters, of which 257 were parsimony-informative. RFLP Type 14 was not included in the analysis because high quality sequences were unattainable using primers NS-3, -4, -5, -8, -21, and -24. Although successful sequences were attained using NS-21 and -24 for RFLP Type 12, there were still too few informative characters for successful analysis. Strong support was

Table 4 Morphological description of ascomycetous mycobionts

RFLP type and accession number	Color	Mantle	Intracellular penetration and Hartig net	General shape	Hyphal description and measurements
Type 6 AF260423 AF260424	Yellow to orange-brown	Thin, net prosenchyma to net synenchyma	Yes	Bifurcate	Hyaline, thick-walled, 4.2–13.3 μm diameter
Type 8 AF351580	Bright orange to red-brown	Thin (21–84 μm) to none, net synenchyma	Yes	Both monopodial and bifurcate	Hyaline, 4.9–7.7 μm diameter
Type 10 AF260417 AF260418 AF351581	Red-brown	Thin (21–84 μm)	Yes	Bifurcate	Hyaline, 2.1–8.4 μm diameter
Type 11 No sequence	Red-brown	Thin, net prosenchyma	Yes	Multiple branched, club-shaped	Hyaline
Type 12 No sequence	Root tips similar to Type 11, red-brown	Thin (14–70 μm), net synenchyma	Yes	Multiple branched, club-shaped	Brown, 3.5–6.3 μm diameter
Type 14 No sequence	Orange-brown	Thin (28–70 μm) to none, net prosenchyma	Yes	Bifurcate, hyphae bulbous	Hyaline, 3.5–9.8 μm diameter

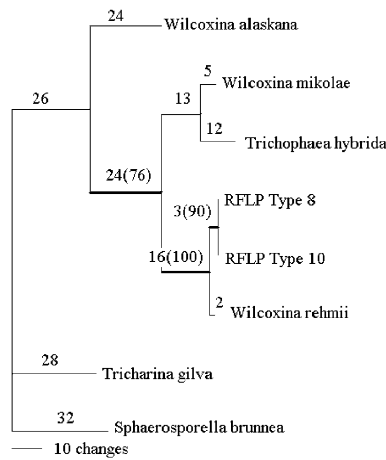

Fig. 1 Maximum parsimony analysis, based on the partial 18S region and internal transcribed spacer (ITS) 1 region of nuclear ribosomal (nr)DNA. Branch length indicates number of changes. Branches with >70% bootstrap support are shown in *bold* with bootstrap value in *parenthesis*

evident for RFLP Types 6 and 11 being closely related to *Geopora cooperi* (bootstrap value =95%). The closest affiliations for RFLP Types 8 and 10 were *Wilcoxina* and *Trichophaea*.

RFLP patterns for Type 11 and *Geopora cooperi* matched with all three restriction enzymes (Table 3). RFLP patterns did not match between Type 6 and any of the *Geopora* species analyzed (see Table 3); interestingly, the two collections of *Geopora clausa* (Gilkey) Burds. yielded different RFLP patterns. Despite the lack of RFLP matches between Type 6 and any of the *Geopora* collections analyzed, we hypothesize that Type 6 is probably a *Geopora* species, as indicated by our analysis of the 18S rRNA gene.

To determine if the mycobionts with RFLP Types 8 and 10 were *Wilcoxina* or *Trichophaea*, the partial 18S and ITS 1 regions were analyzed together with known sequences obtained from GenBank. All 66 parsimony informative characters were used, and one most parsimonious tree (129 steps long, CI =0.738, RI =0.692) was recovered (Fig. 1). Types 8 and 10 are likely *W. rehmii* (bootstrap value =100%) (Fig. 1). RFLP patterns were matched between a culture collection of *W. rehmii* and Type 8 (Table 3). Type 10 and the *W. rehmii* culture showed a slight variation with restriction enzyme *Alu*I (Table 3).

Discussion

We observed ten RFLP types of sporocarps within five genera (*Anthracobia*, *Morchella*, *Peziza*, *Scutellinia*, *Tricharina*) of post-fire Pezizales in our ponderosa pine site in the Blue Mountains of Oregon. Species belonging to the genera found in our study are commonly found following burning of forest habitats (Petersen 1970; Egger and Paden 1986). Some species of post-fire Pezizales belonging to genera found in our study are reported to form ectendomycorrhizal associations with members of the Pinaceae (Danielson 1984; Egger and Paden 1986; Dahlstrom et al. 2000).

Assessing whether post-fire Pezizales were mycorrhizal was an objective of this study. We extracted multiple soil cores from below sporocarps of the fungal genera found, but found no root tips colonized by species of post-fire Pezizales observed fruiting at our site. Mycorrhizal fungi may (1) produce sporocarps a greater distance from the colonized roots than our sampling method detected (Gardes and Bruns 1996a), (2) colonize roots deeper than our 15 cm soil core, which is a suggested strategy for post-fire fungi (Mikola et al. 1964; Vrålstad et al. 1998), (3) be distributed patchily and at so fine scale that our sampling method did not retrieve their roots by chance alone (Horton and Bruns 2001), (4) form mycorrhiza or fruit at a later date than the time we collected our roots. Another possibility is that these post-fire Pezizales are not mycorrhizal.

Recent research on the ecosystem function of *Morchella* indicates that some species fruiting in the absence of fire may be mycorrhizal (Dahlstrom et al. 2000; Hobbie et al. 2001). *Morchella* species from burned sites may not form mycorrhizas or, if they do, they may revert to being saprobic (Buscot 1994; Dahlstrom et al. 2000). Isotopic evidence (Hobbie et al. 2001, 2002) or bioassays using inocula from *Morchella* species from burned and non-burned sites may help distinguish between mycorrhizal and saprobic functions of post-fire *Morchella* species.

We detected six ascomycete RFLP types; all formed ectendomycorrhizal morphology with the roots of ponderosa pine. Two RFLP types were determined to be species of *Wilcoxina*, two were species of *Geopora*, and the identities of two were not confirmed. Ten species have been reported to form ectendomycorrhizas: *Wilcoxina mikolae*, *W. rehmii*, *Sphaerosporella brunnea*, *Chloridium paucisporum*, *Tricharina mikolae*, *Phialophora finlandia*, *Terfezia arenaria*, *Terfezia claveryi*, *Tirmania pinoyi* (Yu et al. 2001) and *Morchella* sp. (Dahlstrom et al. 2000). Our findings on *Geopora* supports the hypothesis of Danielson (1982) and Egger (personal communication) that *Geopora* also forms ectendomycorrhizas.

Wilcoxina rehmii was the most common ascomycete mycobiont detected in our study. Analysis of 18S rDNA sequence data and further analysis with the more variable gene region ITS 1, revealed that two of our RFLP types are likely *W. rehmii* (Fig. 1). Although the sequence analyses suggested that our two RFLP types are related to *W. rehmii* (Fig. 1), the RFLP pattern for one was clearly different from that obtained from our *W. rehmii* culture with the restriction enzyme *Alu*I. The difference could be due to intraspecific variation in the ITS region (Gardes and Bruns 1993; Kårén et al. 1997; Horton 2002).

Interestingly, we did not observe *W. rehmii* as sporocarps during the study. *W. rehmii* may form mycorrhizas on burned soils but fruit only rarely. Alternatively, it may colonize root tips during the first year after a fire, when our study was conducted, but not fruit during this period. *W. rehmii*, when associated with

white spruce [*Picea glauca* (Moench) Voss] and jack pine (*Pinus banksiana* Lamb.), is typically found in unburned soils with high organic matter or in soils amended with peat, although it can be found on disturbed soils (Egger 1986, 1996; Egger et al. 1991). The soils in our study site were not amended with peat but were burned.

The Pezizalean species *W. rehmii* and *G. cooperi* are not reputed post-fire fungi, although *Wilcoxina* sp. was common on post-fire bishop pine (*Pinus muricata* D. Don) seedlings (Baar et al. 1999). *G. cooperi* is widely distributed with species of the Pinaceae in undisturbed forest conditions (Burdsall 1968; Maia et al. 1996; States and Gaud 1997) as well as in nutrient-poor, volcanic cinder soils (Gehring et al. 1998). Our results suggest *W. rehmii* and *G. cooperi* either withstood prescribed burning, or propagules were rapidly introduced after the fire. RFLP patterns and ITS sequence comparisons from mycorrhizal root tips collected both pre- and post-treatment from the same marked trees sampled in our study match those of *W. rehmii* from our study (J.E. Smith, unpublished data). The probability of recovery of these two species in our study may have been increased because the fire removed most of the speciose mycorrhizal fungal community that typically occurs in undisturbed habitats (Horton et al. 1998; Baar et al. 1999; Stendell et al. 1999).

Our findings concur with earlier observations by Yang and Korf (1985) who suggested that *W. rehmii* is the most common species of *Wilcoxina* and is found on unspecified soils and coniferous litter. We expected our mycobionts from burned soils to be *W. mikolae*, because it has repeatedly been reported from burned soils (Yang and Korf 1985; Egger et al. 1991; Egger 1996; Baar et al. 1999). However, only one paratype of *W. mikolae* for North America is available (Yang and Korf 1985) and few collections of *Wilcoxina* have been deposited in herbaria, suggesting it may not be as common as once assumed.

This study focused on identifying the mycorrhizal ascomycetes associated with ponderosa pine, using molecular tools. The section of the 18S rDNA used for our phylogenetic analysis was sufficient to identify most of our mycobionts. The most troublesome difficulty in studies such as this is that mycorrhizal fungi are patchily distributed and occur at a fine scale below ground (Horton and Bruns 2001). This makes it extremely difficult to design a sampling scheme in which an accurate picture of species richness is ensured (Horton and Bruns 2001; Taylor 2002). Another difficulty we encountered was that two of our RFLP types remained unknown because of insufficient sequence data in GenBank for comparison to our unknowns using the blast search approach. Success in diagnostic use of sequences will increase over time as more sequences are submitted to GenBank (Horton and Bruns 2001). Overcoming these difficulties is important for gaining a comprehensive understanding of the ecto- and ectendomycorrhizal fungal community.

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