Distribution and Abundance of Rare Sequestrate Fungi in Southwestern Oregon FY10-15 ISSSSP Project Final Report Darlene Southworth, Department of Biology, Southern Oregon University, Ashland January 2016

Abstract. This project was a strategic survey of rare and little-known sequestrate fungi in hardwood and mixed hardwood-conifer habitats in southwest Oregon. These vegetation types, comprising a significant fraction of BLM lands and mid-elevation USFS lands, are targeted for increased management activities such as fuel reduction, recreation, ATV use, and harvesting of special forest products. In addition, global warming may decrease the area occupied by conifers and increase the area of mixed hardwood-conifer forests and oak savannas.

Objectives were to extend knowledge of sequestrate fungi on federal lands in southern Oregon including (1) distribution in oak and mixed conifer-hardwood vegetation, (2) habitat breadth breadth, (3) rarity, and DNA sequences to verify identification.

We surveyed for sequestrate fungi in oak woodlands, oak-rosaceous chaparral, mixed coniferhardwood forests, and conifer stands, from three geographic regions in Jackson and Josephine Counties: the eastern slope of the Siskiyou Mountains, interior valleys, and the western slope of the southern Cascades.

We collected over 700 specimens in two survey seasons (spring 2010 and 2011). Host species included Oregon white oak, California Black oak, Douglas-fir, manzanita and curl-leaf mountain mahogany. Undisturbed mature stands with thick leaf litter and interspersed with openings yielded the largest collections. Wet years were more productive than dry years. From over 700 specimens, we have identified an estimated 83 species. We have tentatively identified 15 species on the target list. In addition, we described about 10 new species. We submitted xx sequences of the ITS region to GenBank and analyzed sequences in four genera by phylograms.

Case studies. *Geopora cercocarpi* was described as a new species associated with the roots of curl-leaf mountain mahogany (Southworth et al. 2011). We submitted it to ORBIC. We are describing new species of *Balsamia* and creating a key to identification of species. *Rhizopogon subpurpurascens* and *R. atroviolaceous* both are on the ORBIC list, they may be a single species. Three species of *Gymnomyces* are on lists. One was identified *G. fallax* but DNA did not match *G. fallax* in GenBank. In the original description it had remained as *Martellia compactus*. *Hysterangium*, one of our most common genera has no species listed as rare. Of our 60 collections, 6 (10%) do not fit described species. *Melanogaster*, with no listed species, is frequent in southern Oregon. We may have new species under oaks in southern Oregon.

INTRODUCTION

The major tree species in mixed hardwood-conifer forests form obligate symbiotic relationships, ectomycorrhizas, with macrofungi. Virtually every root tip is coated with a fungal mantel. In southern Oregon, tree hosts of ectomycorrhizal fungi include members of the Pinaceae (pine, fir, spruce, hemlock, Douglas-fir), the Fagaceae (oak, tanoak), and some woody Rosaceae (mountain mahogany). Mycorrhizal fungi include basidiomycetes and ascomycetes, with fruiting bodies that are both epigeous (mushrooms), fruiting above ground, and hypogeous (truffles), fruiting below ground. Truffles are described as sequestrate because they have closed fruiting bodies with spores that are dispersed by small mammals.

The seasonality and hypogeous nature of truffles make them difficult to detect. As evidence that they are understudied, within the past 10 years, we have described three new truffle species, *Tuber quercicola, T. whetstonense* and *Pachyphloeus austro-oregonense*, from one oak woodland (Whetstone Savanna) in the Rogue Valley. Sequestrate fungi (truffles) comprise a significant fraction of species in mycorrhizal communities. Of the sensitive species, 39% are sequestrate, as are 34% of strategic species. This is far greater than the proportion of sequestrate species among fungi.

Objectives were to extend knowledge of sequestrate fungi on federal lands in southern Oregon including (1) distribution in oak and mixed conifer-hardwood vegetation, (2) habitat breadth (host species, maturity of stands, ecological conditions), (3) rarity (abundance and frequency), and (4) DNA sequences to facilitate identification of immature sporocarps and mycorrhizas.

METHODS

Reviewof existing occurrences of target species and CVS/FIA plot data in SW Oregon in GeoBob database. We determined which species were considered rare in SW Oregon from BLM and USFS lists (Castellano et al. 1999, Trappe et al. 2009). In addition, because of the understudied nature of sequestrate fungi, their understudied mid-elevation habitats, and their understudied hosts, we examined other sources of information including the ORBIC list, the database of hypogeous fungi at OSU, and published accounts of new southern Oregon species that had not yet been added to the official lists (Table 1).

Field Surveys. We surveyed for sequestrate fungi particularly in hardwood vegetation including oak woodlands, oak-rosaceous chaparral, mixed conifer-hardwood forests, and low elevation conifer stands. Sites on federal lands were selected from three geographic regions primarily in Jackson and Josephine Counties: (1) the eastern slope of the Siskiyou Mountains, (2) the interior valleys of the Klamath Mountains (Applegate and Rogue River drainages), and (3) the western slope of the southern Cascades including the Cascade-Siskiyou National Monument. In addition, other collectors, surveying sites further north, brought us specimens. Working with BLM and USFS botanists (Doug Kendig and Wayne Rolle), we selected sites in each of the geographic regions for sampling (Fig. 1, Table 2). In addition, we sampled Whetstone Savanna Preserve, an oak woodland owned by The Nature Conservancy, to serve as a positive control for valley stands because of the published work on this oak woodland (Frank et al. 2006, Frank et al. 2009). We surveyed 46 sites in 2010, returning to 31 of these in 2011, and several in 2012 and 2013 (Fig. 1, Table 1).

Stands were surveyed beginning in late March at low elevations and ending in early July for high elevations. Field methods followed guidelines in the appendix of Special Considerations for Hypogeous Fungi of Sporocarp Survey Protocol for Macrofungi, version 1.0. 2008. We used an intuitive survey method focusing on areas under the dripline of host trees, moist areas on the forest floor, animal diggings, and humps in leaf litter. We also sampled under curl-leaf mountain mahogany (*Cercocarpus ledifolius*) because a previous survey of ectomycorrhizal roots had reported an unidentified species of *Geopora*, a genus with some hypogeous species. We raked away leaf litter, loosened the soil, and examined closely for hypogeous fungi. Each survey stand received a minimum number of person-hours, e.g., 135 person-minutes (3 people, 45 min). Where hypogeous fungi were found, the time was increased. If no sequestrate fungi were encountered, we moved to another site. Data included GPS coordinates, field tags, and field descriptions.

We collected <u>all</u> hypogeous specimens encountered because we could not identify truffles to species in the field and because we wanted to describe the fungal community associated with rare species.

<u>Comparison of truffles with mycorrhizas</u>. In year 3 (2012), we focused on one site above Limpy Creek in Josephine County, Oregon, (UTM zone 10 Easting 452833 and Northing 4696754; N 42° 25.3' W -123° 34.4', 930 m elevation) where we took soil cores to determine whether *Rhizopogon* species could be collected as mycorrhizal tubercles. Dominant vegetation was *Pinus ponderosa*, *P. lambertiana*, and *Pseudotsuga menziesii*, with an understory of *Notholithocarpus densiflorus*, *Chrysolepis chrysophylla*, *Arbutus menziesii*, *Quercus garryana* var. *breweri*, and *Arctostaphylos viscida*.

At this site, soils were derived from partially weathered metasedimentary rocks of the Jurassic Galice Formation (Ramp 1979). Exposed rock was metamorphosed dark gray to black siltstone and mudstone exhibiting a penetrative and steeply inclined slaty cleavage. The rocks were weathered to pale sand-colored slaty chips mingled with organic material a few centimeters below the duff layer. The Galice Formation consists of fine-grained sediments that were deposited in a back-arc basin (marginal sea) atop the Josephine Ophiolite (oceanic crust and mantle). They were subsequently deformed, metamorphosed to serpentinite (mantle), meta-gabbro (sheeted dike complex), pillow basalt (uppermost crust), and slate and metasiltstone (sedimentary cover over the ophiolite). During deformation, slaty cleavage was imposed on the finer-grained rocks.

On 13 Jun 2012 we surveyed for truffles for 5 person-hours. *Rhizopogon* collections were tested with Melzer's reagent on site to determine whether they were in section Amylopogon which includes two rare species, *R. subpurpurascens* and *R. atroviolaceous*. Where *Rhizopogon* sect. Amylopogon truffles were found, transects were set for soil sampling. Along three 20-m transects, paired soil samples 10 cm apart were taken at 2-m intervals using a soil corer (2.5 cm diameter, 15 cm depth) or trowel when the soil was too rocky for the soil corer.

Soil samples were washed over a sieve (1 mm openings). Roots with clumps of soil and mycorrhizas were picked out and examined under a dissecting microscope. Digital images of mycorrhizas were taken under a dissecting microscope and of mantle peels and emanating

hyphae under a compound microscope using Spot RT cameras and software. Representative mycorrhiza samples were stored in CTAB buffer for DNA sequencing.

Identification of specimens. We identified and photographed specimens in the laboratory at Southern Oregon University. In addition, we consulted with Dr. Michael Castellano and Dr. Jim Trappe at Oregon State University for a better understanding of nomenclature.

For tentative identification, specimens of sequestrate fungi were examined with dissecting and compound microscopes with digital images of fruiting body surface (peridium), interior (gleba), and spores (Castellano et al. 1989, Trappe et al. 2007). For specimens of uncertain identification or known specimens for comparison, we measured 20 spores per collection. We received help with clarification of morphological characters, current references, and unpublished manuscripts from Michael Castellano and Jim Trappe at OSU. Voucher Collection and documentation followed standard guidelines. Selected specimens were sent to Efren Cazares for identification. Voucher specimens are currently in the herbarium at Southern Oregon University (SOC) and will be deposited in the OSU Herbarium (OSC).

DNA sequencing. We sequenced the ITS (Internal Transcribed Spacer) region of ribosomal DNA from selected specimens and compared the sequences to those of type specimens in GenBank. In addition to tissues of fresh specimens, and on advice from Michael Castellano and Jim Trappe, we sampled related species from the hypogeous fungi collection at the US Forest Service lab in Corvallis. Additional type specimens were obtained from other herbaria.

Sequencing methods methods followed Southworth and Frank (2011). Briefly, DNA was extracted in 2% cetyltrimethyl ammonium bromide (CTAB) with chloroform and amplified in polymerase chain reactions (PCR) with fungal primers ITS1F and TW13 (White et al. 1990, Gardes and Bruns 1993). An initial 3 min at 93 C was followed by 30 cycles of 30 s at 95 C, 2 min at 54 C and 3 min at 72 C, with a final cycle for 10 min at 72 C, modified from Frank et al. (2010). PCR products were purified with QIAquick PCR Purification kits, prepared with BigDye Terminator Ready Reaction Mix 3.1 and sequenced in an ABI 310 Genetic Analyzer at 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, and reverse primer ITS4, and part of the 28S ribosomal DNA gene with forward primer ITS4r and reverse primer TW13.

Sequences were edited with Chromas 1.45; contigs were assembled with Sequencher 4.7. Sequences were compared with Clustal X and to fungal sequences in GenBank (www.ncbi.nlm.nih.gov) with BLAST. DNA sequences will be deposited in GenBank.

Phylogenetic trees, inferred with parsimony and maximum likelihood with 1000 bootstrap replicates and 1000 jackknife replicates, were generated from 23 ITS sequences with PAUP 4.10B10. Consensus trees with 50% majority rule were generated with a tree bisection reconnection branch-swapping algorithm. All characters were given equal weight; gaps were treated as missing. Consensus trees were examined to confirm branch positions.

PROJECT ACCOMPLISHMENTS

Field Surveys. We surveyed 46 sites in 2010, returning to 31 of these in 2011, and several in 2012 and 2013 (Fig. 1, Table 1). The sites were divided approximately into three general locations: the western Cascades (Jackson County), the eastern Siskiyous (Josephine County), and the interior valley between them (both counties). Elevations ranged from 290 to 2160 m.

It was nearly impossible to find sites that were completely hardwoods or completely conifers. At 44% of sites, oaks, chiefly *Quercus garryana*, were the dominant trees; oaks were present at 65% of sites. *Cercocarpus ledifolius* was present at five sites. The remaining sites were dominated by conifers (*Pseudotsuga menziesii*, *Pinus ponderosa*, or *Abies concolor*). Mixed conifer hardwood vegetation was the norm.

Identification of specimens including target Species. From over 700 specimens, we have identified an estimated 83 sequestrate (hypogeous) species including 15 tentatively identified target species, chiefly in the genus *Rhizopogon*, as well as several species that had been described (published), but not yet entered into the various lists (Tables 3; Appendix 1). The number of species per site ranged from 0 to 18. The most common species occurring at more than 10 sites were *Genabea cerebriformis*, and *Tuber candidum* (Ascomycota), *Gymnomyces compactus*, *Hysterangium separabile*, *Melanogaster tuberiformis*, *Rhizopogon vinicolor* (Basidiomycota), and *Endogone lactiflua* (Zygomycota).

Case studies

 Geopora cercocarpi was described as a new species (Southworth et al. 2011, Appendix 2). Prior work on mycorrhizal fungi associated with roots of *Cercocarpus ledifolius* (curl-leaf mountain mahogany) in the Cascade-Siskiyou National Monument indicated that a species of *Geopora* was a frequent mycobiont, but we could not identify the species by molecular data (McDonald et al. 2010). Since *G. cooperi*, is a relatively common truffle, we decided to look for truffle species under *C. ledifolius*. This is an unusual host species, but some woody species in the Rosaceae were known to be ectomycorrhizal. We found hypogeous specimens of a partially sequestrate *Geopora* under mountain mahogany at five sites on federal lands. The DNA from these specimens matched the sequences that we had found on mycorrhizas. We described a new species, *Geopora cercocarpi*. We submitted it to ORBIC. The species may not be rare, except that its host *C. ledifolius* is uncommon in Jackson and Josephine Counties.

McDonald KR, Pennell J, Frank JL, Southworth D. 2010. Ectomycorrhizas of *Cercocarpus ledifolius* (Rosaceae). Am J Bot 97:1867–1872, doi:10.3732/ajb.0900357 Southworth D, Frank JL. 2011. Linking mycorrhizas to sporocarps: a new species, *Geopora cercocarpi*, on *Cercocarpus ledifolius* (Rosaceae). Mycologia 103:1194-1200.

2. Rhizopogon subpurpurascens and R. atroviolaceous are both target species. They are in the section Amylopogon because spores turn purple with Melzer's reagent (Fig. 4). I initially identified our specimens as R. atroviolaceous based on an unpublished key from the North American Truffling Society, but DNA sequences matched that of R. subpurpurascens deposited in GenBank by M. Gordon (contract with USFS) and DNA from the type specimen (Fig. 6). Then E. Cazares identified the specimens as R. atroviolaceous. Thus morphological

and molecular methods disagree. We have borrowed the Type specimen of *R. atroviolaceous* and will continue the comparison. If they are synonymous, *R. subpurpurascens* will be the priority name.

Rhizopogon subclavatisporus, from the same site, was identified by molecular methods (Fig. 4). In addition we sent specimens for identification by morphology. One *R. subclavatisporus* specimen was identified as *R. vinicolor* and one specimen of *R. vinicolor* as *R. subclavatisporus*. Of 20 *Rhizopogon* specimens sent to E. Cazares for identification, there was agreement between the molecular and morphological identifications on only one specimen. This needs further resolution.

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- 3. Balsamia alba (Harkness 1899) is confusing. We collected two white specimens (small, somewhat immature) on Chiquapin Mountain in the Cascade-Siskiyou National Monument. These sequenced together and distinct from other sequences. White specimens are indeed rare and some are clearly other species parasitized by a second fungus. In addition, some *B. magnata* specimens have been identified as *B. alba*. The Type Specimen of *B. alba* at OSU is in poor condition; no DNA sequences will be forthcoming. Initially, I recommended that it be removed from the ORBIC rare list, however, the molecular data on two specimens suggests that *B. alba* should be retained on the ORBIC rare list.

Balsamia is a small genus with about 5 species described from California chiefly by Harkness (1899). The descriptions are poor and the species uncertain. We have re-examined collections of *Balsamia* species from the OSU herbarium and propose descriptions of six new species, three of which occur in southern Oregon. A draft manuscript with descriptions and proposed names will be submitted in 2016 (Appendix 4). References are in the manuscript.

4. Three species of *Gymnomyces* are on various lists. Although we did not find these, we found one that Jim Trappe and later Efren Cazares identified as *G. fallax* based on a key in Whitbeck's unpublished master's thesis from OSU. The identification seemed forced. However, the DNA match was not to *G. fallax*, which was in GenBank. We went back to the original description and to subsequent species revisions and found that it had remained longer as *Martellia compactus* and thus was not in the unpublished *Gymnomyces* key. Whether or not *G. compactus* is rare in southern Oregon remains to be determined. I requested that it be added to the list.

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5. Hysterangium, one of our most commonly collected genera has no species listed as rare. Of our 60 Hysterangium collections, 54 fit into described species; 6 (10%) do not. One has spores resembling the Mexican species H. velatisporum found under oaks; others are most likely undescribed species. Michael Castellano did his Ph.D. thesis on Hysterangium in 1988, and we have sent our specimens to him at the Forest Service Research Center in Corvallis. He is working with Kentaro Hosaka on DNA sequencing and molecular analysis to determine the number of species, their rarity and ranges, and to describe new species.

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6. Melanogaster, with no listed species, is frequent in southern Oregon. An unpublished report to the USDA Forest Service by Wang et al. (1986) from OSU proposed new species, but this has not been published. Only one species from that project has been published (*M. natsii*) in an obscure Chinese journal. Furthermore, many common *Melanogaster* specimens are assigned to *M. tuberiformis*, a European species. Our DNA work shows that specimens from the PNW differ from *M. tuberiformis*, thus a new description is required. We may also have a new species under oaks in southern Oregon, and it may not be rare. We will develop this manuscript in spring 2016.

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Communities of Rare Fungi. Rare species were collected from diverse assemblages. *Cazia flexiascus,* collected at Neathammer Gulch and also near the Jacksonville Cemetery, occurred with many other truffle species and specimens. *Geopora cercocarpi* occurred on a singular host (*Cercocarpus ledifolius*) at sites with few other truffles. *Rhizopogon subpurpurascens* occurred in abundance at particular habitats in higher elevation conifer stands in the Klamath-Siskiyous, along with a few other truffle species. We have begun to analyze these relationships between rarity and fungal communities and presented the following talk at the Ecological Society of America meeting in Portland, OR, in 2012.

Rarity in sequestrate fungi (truffles): A function of niche width or productivity (ESA Abstract. For PowerPoint, see Appendix 3.)

Sequestrate fungi (truffles) develop in an enclosed form underground where the mature fruiting bodies are eaten by small mammals that disperse the spores. Although they are not easily observed, truffles are seasonally abundant in association with their mycorrhizal hosts. Among all truffle species, however, some are common while others are rare, as defined by frequency of occurrence. We tested two hypotheses to explain rareness. H1: Rare truffle species may have a narrow niche that is highly specific to particular host trees or to certain abiotic conditions for the host (e.g., elevation and precipitation). A truffle species with a narrow niche may be locally abundant. H2: Rare truffles may occur under environmental conditions of high truffle productivity as indicated by high truffle biomass, numerous fruiting bodies, or high species richness. We surveyed for sequestrate fungi at 40 sites in the eastern Siskiyou Mountains, the western Cascades, and the interior valleys between them in southern Oregon, with vegetation ranging from predominantly conifer through mixed conifer-hardwood to hardwood. We identified species by morphology and DNA sequences.

Species of rare truffles differed in niche preference and in response to productivity. Rhizopogon subpurpurascens (Basidiomycota) occurred at three sites in the Siskiyou Mountains at 900-1300 m in predominantly conifer vegetation (Pinus ponderosa, P. lambertiana, Pseudotsuga menziesii), with 1-9 fruiting bodies per site. Nine species of sequestrate fungi were present at these sites. These data indicate support for H1—a limited niche consisting of a narrow host range and environmental conditions, limited truffle diversity, and local abundance. High elevation conditions may separate potential sites and limit dispersal. In contrast, Cazia flexiascus (Ascomycota) occurred at three sites in the interior valley at 500-600 m in mixed conifer-hardwood vegetation, chiefly Quercus garryana but also Pinus ponderosa and Pseudotsuga menziesii, with 1-2 fruiting bodies per site. These sites were among the most species-rich with 15 species of sequestrate fungi. These data support H2—occurrence of the rare species in a widespread niche when truffle biodiversity was high. These results indicate that no single explanation can account for rarity among truffles. Although, their similarity of habitat (belowground), morphology (sequestrate), and functional guild (mycorrhizal) suggests that they might respond to common environmental factors, that is not the case. Truffle rarity must be investigated on a species-by-species basis.

Comparison of truffles with mycorrhizas. In three 30-m transects at the Limpy Creek siltstone site, we found mycorrhizal roots, particularly tuberculate mycorrhizas characteristic of *Rhizopogon* species along with sporocarps of *R. subpurpurascens* and *R. truncatus*. The

tubercles were up to 1 cm in diameter and resembled small sporocarps (Fig. 5); however, crosssections of tubercles show hyphae-coated mycorrhizal roots and not spores. Tubercles were identified as *R. subclavatisporus* and *R. truncates*. This indicates that soil surveys of mycorrhizal fungi, identified by DNA sequences, could generate additional information about species presence.

CONCLUSIONS

Rare sequestrate fungi definitely occur on federal lands in southern Oregon. They are distributed in the full range of habitats: oak savannas, mixed conifer-hardwood vegetation, conifer forests, and mountain mahogany shrublands—wherever host species occur. Spring warming with moisture provided the best climatic conditions and relatively deep, undisturbed leaf litter and forest duff yielded the most sporocarps. Host preferences of rare species were difficult to verify in habitats of mixed host species. It appears that they had somewhat narrow host preferences ranging from a single host species to apparently several conifer species.

Rare species differed in relative abundance at preferred sites, from single sporocarp collections that were not repeated in subsequent years (e.g., *Cazia flexiascus*) to six or more sporocarps reliably found over three years but at very specific sites (e.g., *Rhizopogon subpurpurascens* and *Geopora cercocarpi*).

DNA sequences were essential for identification of most rare species. Furthermore, the sequences needed to be compared to sequences from *type* specimens. Molecular data in GenBank is not always based on type specimens, but on specimens that may be misidentified. A single entry in GenBank with a BLAST match is not sufficient. Sequences need to be compared in phylograms to determine relationships among collections and species.

Morphological identification of rare sequestrate species depends on original descriptions which do not include molecular data. Many common species collected in the Pacific Northwest are poorly described and often have specific names from European collections. Much of the literature is outdated, imprecise, or unpublished (and hence not reviewed). It is essential that morphological and molecular data be linked and that rare species be compared to other closely related and highly similar species.

Among the common species in genera with <u>no</u> listed species, we found undescribed or unexpected species, and range extensions for species unknown from Oregon or unknown from Jackson and Josephine Counties. These common genera need much more systematic analysis.

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Fig. 1. Sites in southern Oregon (Jackson and Josephine Counties) surveyed for sequestrate fungi in 2010 and 2011 (Table 2).



Figs. 2-5 *Rhizopogon subpurpurascens* collected at Limpy Creek siltstone site. 2. Cross section of mature sporocarp. 3. Dark purple-black peridium of mature sporocarp. 4. Spores stained purple in Melzers reagent. Fig. 4. Tubercle of *Rhizopogon* sp. with peridium removed. Fig. 5. Spores of *R. subclavatisporus* in Melzers reagent.



Fig. 6. Phylogram (working draft) of ITS DNA sequences extracted from *Rhizopogon* specimens collected for this project and from GenBank. Holotype, H or HT; paratype P or PT. Collection numbers beginning with SOC were collected on federal lands in southern Oregon.

Table 1. Target list of sensitive or special status species of sequestrate fungi that might occur in Jackson (JA) or Josephine (JO) County, Oregon.

Phylum	Species	ISSSSP	Medford	Global	ORNHIC	OR
		Status	Dist	Rank	List	County
Ascomycota	Balsamia alba	OR-STR	*	G2?	3	
Ascomycota	Balsamia nigrans	OR-STR	D	G3	3	OLAL
Ascomycota	Balsamia platyspora	OR-STR		G2?	3	
Ascomycota	Cazia flexiascus	OR-STR	S*	G2?	3	JO
Ascomycota	Chamonixia caespitosa	OR-SEN		GU	2	JA
Ascomycota	Choiromyces alveolatus			G3	3	JA
Ascomycota	Choiromyces venosus	OR-SEN		G4	2	
Ascomycota	Elaphomyces			G3G4	3	
	anthracinus					
Ascomycota	Elaphomyces asperulus	OR-STR		G3?	3	
Ascomycota	Elaphomyces decipiens	OR-STR	S	G2?	3	JO
Ascomycota	Elaphomyces reticulatus	OR-STR	S	G3?	3	JO
Ascomycota	Elaphomyces	OR-STR	S	G2G3	3	JA
	subviscidus					
Ascomycota	Genea compacta	OR-STR	*	G2?	3	
Ascomycota	Helvella crassitunicata	OR-SEN	S	G3	2	
Ascomycota	Helvella elastica	OR-STR	D	G4	3	
Ascomycota	Hydnotrya inordinata	OR-STR		G2	3	
Ascomycota	Hydnotrya michaelis	OR-STR		G3	3	
Ascomycota	Pachyphloeus austro-		*			JA
	oregonense					
Ascomycota	Tuber asa	OR-STR		G3	3	
Ascomycota	Tuber pacificum	OR-STR		G2	3	
Ascomycota	Tuber quercicola		*			JA
Ascomycota	Tuber whetstonense		*			JA
Basidiomycota	Alpova alexsmithii	OR-SEN		G2	1	
Basidiomycota	Alpova olivaceotinctus	OR-STR	D	G2G3	3	JA
Basidiomycota	Amogaster viridigleba			G2S2	1	
Basidiomycota	Arcangeliella	OR-SEN		G2	1	JO
	camphorata					
Basidiomycota	Arcangeliella crassa	OR-STR		G2G4	3	
Basidiomycota	Arcangeliella			G2G3S	3	
	lactarioides			1		
Basidiomycota	Brauniellula albipes	OR-STR	S	G3	3	JO
Basidiomycota	Cystangium idahoensis	OR-SEN		G2G3	1	
Basidiomycota	Destuntzia fusca	OR-STR		G2	3	
Basidiomycota	Destuntzia rubra	OR-SEN		G2	1-ex	
Basidiomycota	Gastroboletus imbellus	OR-SEN		GH	1-X	
Basidiomycota	Gastroboletus ruber	OR-STR		G3	3	
Basidiomycota	Gastroboletus vividus	OR-SEN	S	G2?	1	JA
Basidiomycota	Gautieria magnicellaris	OR-STR		G3G5	3	

Basidiomycota	Gautieria otthii	OR-STR	S	G3G5	3	JO
Basidiomycota	Gymnomyces fragrans	OR-SEN	S	G2G3	1	JA
Basidiomycota	Gymnomyces	OR-STR		G1	3	
	monosporus					
Basidiomycota	Gymnomyces	OR-SEN		G1	1	
	nondistincta					
Basidiomycota	Leucogaster citrinus	OR-SEN	D	G3G4	3	JA
Basidiomycota	Leucogaster			G3	4	
	microsporus					
Basidiomycota	Leucogaster odoratus	OR-STR		G2	3	
Basidiomycota	Macowanites	OR-STR		G3?	3	
	chlorinosmus					
Basidiomycota	Macowanites mollis			G1G2	1	
Basidiomycota	Martellia medlockii	OR-STR		G1	3	
Basidiomycota	Octaviania cyanescens			G2?	3	
Basidiomycota	Octaviania macrospora	OR-SEN		GH	1-X	
Basidiomycota	Rhizopogon abietis	OR-STR		G2G4	3	
Basidiomycota	Rhizopogon	OR-STR		G2G3	3	
	atroviolaceus					
Basidiomycota	Rhizopogon	OR-STR		G2G3	3	
	bacillisporus					
Basidiomycota	Rhizopogon	OR-STR		G2G3	2-ex	
	brunneifibrillosus					
Basidiomycota	Rhizopogon	OR-STR	S	G2G3	3	
	brunneiniger					
Basidiomycota	Rhizopogon	OR-SEN	S	G2G3	2	JO
	chamaleontinus					
Basidiomycota	Rhizopogon	OR-STR	S	G2G3	2	JAJO
	clavitisporus					
Basidiomycota	Rhizopogon	OR-SEN	D	G2G3	2	JAJO
	ellipsosporus					
Basidiomycota	Rhizopogon exiguus	OR-SEN	S	G2G3	2	JO
Basidiomycota	Rhizopogon	OR-STR	S	G2G3	3	JO
	flavofibrillosus					
Basidiomycota	Rhizopogon inquinatus	OR-SEN		G2G3	2	
Basidiomycota	Rhizopogon masoniae	OR-STR		GH	1-X	
Basidiomycota	Rhizopogon oswaldii	OR-STR		G2G3	2-ex	
Basidiomycota	Rhizopogon quercicola	OR-STR		G2G3	2-ex	
Basidiomycota	Rhizopogon rogersii	OR-STR	S	G2G3	3	JA
Basidiomycota	Rhizopogon	OR-STR	S	G2G3	3	10
	semireticulatus					
Basidiomycota	Rhizopogon semitectus	OR-STR		G2G3	3	
Basidiomycota	Rhizopogon	OR-STR		G2G3	3	
-	subcinnamomeus					
Basidiomycota	Rhizopogon	OR-STR		G2G3	3	
	subclavitisporus					

Basidiomycota	Rhizopogon subpurpurascens	OR-STR		G2G3	3	
Basidiomycota	Rhizopogon subradicatus	OR-STR		G2G3	2-ex	
Basidiomycota	Rhizopogon truncatus			G4	4	
Basidiomycota	Rhizopogon variabilisporus	OR-STR	S	G2G3	3	JA
Basidiomycota	Thaxterogaster pavelekii	OR-SEN		G2	1	
Zygomycota	Endogone oregonensis	OR-STR		G2G3	3	
Glomeromycota	Glomus pubescens	OR-STR		G2?	3	
Glomeromycota	Glomus radiatum	OR-STR		G2G4	3	

*Collected by D. Southworth prior to the start of this study.

Table 2. Sites surveyed for sequestrate fungi in Jackson and Josephine Counties, Oregon. Regions included the interior valleys of the Applegate and Rogue Rivers (Interior), the eastern Klamath-Siskiyou Mountains surrounding the Illinois Valley (Siskiyous), and the western Cascades including the Cascade-Siskiyou National Monument (Cascades). Collections (Coll) refer to the number of sequestrate sporocarps collected.

REGION	SITE	DATE_1	Coll1	DATE_2	Coll2	DATE_3	Coll	Easting	Northing	Elev	Host_1	Host_2
							3			(m)		
Interior	Whetstone	3/31/2010	1	5/6/2010	11	6/22/2010	38	508228	4696045	400	QUGA	
Interior	Table Rock	4/2/2010	3	5/6/2011	5			508357	4702337	520	QUGA	
Interior	Neathammer	5/2/2010	14	6/11/2010	15	5/8/2011	18	496718	4714455	570	QUGA	PSME
Interior	Thunderbird Rd	5/2/2010	7	5/8/2011	8			504580	4716954	530	PSME	QUGA
Interior	Meadows Rd	5/2/2010	18	5/8/2011	6			505405	4711402	480	QUGA	PSME
Interior	China Gulch	4/29/2010	13	1/26/2011	12	5/2/2011	25	494502	4679572	690	QUGA	PSME
Interior	Cantrall Buckley	4/29/2010	13	5/2/2011	14			494978	4671892	580	QUGA	PSME
Interior	Sterling Mine	4/29/2010	6	2/12/2011	1	5/13/2011	11	507297	4667360	720	QUGA	PSME
Interior	Wellington Saddle	5/7/2010	10	5/10/2011	3	5/26/2011	10	490657	4682537	1140	PSME	QUKE
Interior	Wellington Butte	5/7/2010	3			5/26/2011	6	492856	4682442	840	PSME	QUKE
Interior	Enchanted Forest	5/6/2010	9	5/7/2010	16	5/26/2011	10	484339	4683381	620	PIPO	QUGA
Interior	Jacksonville	5/6/2010	1	5/10/2011	0			502197	4685214	510	QUKE	QUGA
Interior	Logtown	5/6/2010	1					497839	4678500	540	PSME	QUGA
Siskiyous	Josephine Camp	4/23/2010	4	5/1/2011	14			443146	4677394	400	PIPO	QUKE
Siskiyous	Green Bridge	4/23/2010	3	5/1/2011	3			443009	4677488	370	PSME	QUKE
Siskiyous	Star Flat	4/23/2010	4	5/1/2011	18			444276	4681179	400	PIPO	QUGA
Siskiyous	Deer Creek	4/23/2010	7	5/1/2011	7			446966	4682546	530	QUGA	PIPO
Siskiyous	Waldo Saddle	5/28/2010	15					445369	4653780	720	QUGA	
Siskiyous	French Flat	5/28/2010	6					447555	4660130	450	QUGA	PIPO
Siskiyous	Shan Overlook	6/23/2010	2					450793	4699174	1140	ABCO	PSME
Siskiyous	Onion Mtn	6/23/2010	13					449134	4700389	1290	ABCO	PSME
Siskiyous	Peavine lo	6/16/2010	5					450086	4715281	290	PIPO	
Siskiyous	Peavine hi	6/16/2010	1					447689	4714743	940	PIPO	
Siskiyous	Hog Ck Rd	6/16/2010	18	5/9/2011	11			451727	4715269	630	QUKE	PIPO

Siskiyous	Limpy serpentine	6/15/2010	5			455801	4705433	460	QUGA	PIJE
Siskiyous	Limpy siltstone	6/15/2010	4			452833	4696754	930	PIPO	PSME
Siskiyous	Limpy PILA hill	5/9/2011	12			457187	4910934	444	PILA	QUKE
Siskiyous	Wagner Butte	7/8/2010	4			519294	4660906	2010	CELE	PSME
Siskiyous	Dutchman Peak	7/15/2010	1			509656	4654132	2160	CELE	ABCO
Cascades	Emigrant Ck	5/14/2010	9	5/27/2011	24	538021	4663850	720	QUGA	
Cascades	Buckhorn Rd	5/14/2010	2			538027	4662740	760	QUGA	PSME
Cascades	Tyler Creed Rd hi	5/14/2010	6	5/27/2011	12	540781	4662940	1010	QUGA	
Cascades	Tyler Creek Rd lo	5/14/2010	0	5/27/2011	2	538027	4662721	760	QUGA	
Cascades	Burnt Prairie Rd	5/19/2010	0	6/10/2010	1	540334	4676971	1540	QUGA	
Cascades	Cove Rd	6/10/2010	6			535905	4673554	1050	QUGA	
Cascades	CSNM Mariposa	6/4/2010	21			532012	4652162	1030	QUGA	
Cascades	CSNM Pilot Rock	6/4/2010	0			509660	4651633	1930	ABCO	
Cascades	Grizzly Pk Rd	5/19/2010	0			533808	4680484	1390	PIPO	PSME
Cascades	CSNM Table Mtn	7/1/2010	2			541900	4671830	1830	CELE	QUGA
Cascades	CSNM Buck Prairie	6/17/2010	0	7/1/2010	6	544152	4674804	1640	QUGA	PSME
Cascades	CSNM Hobart	6/30/2010	3			543345	4660550	1680	CELE	QUGA
Cascades	Little Hyatt Lake	6/24/2010	1			540898	4666642	1450	PIPO	ABCO
Cascades	Little Hyatt Lake E	6/24/2010	4			541098	4667661	1390	PIPO	ABCO
Cascades	CSNM Chinquapin	6/17/2010	5	6/24/2010	4	546828	4665383	1830	ABCO	CELE

Table 3. Rare (target list, Table 2) species of sequestrate fungi collected in Jackson and Josephine Counties, Oregon, 2010 and 2011. SITE, see Table 2.

TARGET_SPP	HOST	SITE	ADMIN
Alpova olivaceotinctus	PIPO/PIJE/PSME	Deer Creek	BLM
Balsamia alba	PIPO/PIJE/PSME/QUKE	Deer Creek, Josephine	BLM
		Camp	
Balsamia nigrans	АВСО	Chinquapin	BLM
Cazia flexiascus	QUGA/PSME	Neathammer, Jacksonville	BLM
		Cemetery	
Geopora cercocarpi ¹	CELE	Chinquapin, Hobart, Table	BLM
		Mtn, Wagner	
Rhizopogon	QUGABR/PIPO//PIJE/PILA/	Limpy Creek serpentine,	USFS
atroviolaceus ²	PSME/CHCH/ARME	Limpy Creek siltstone,	
		Onion Mtn	
Rhizopogon abietis	PIPO/PIJE/PSME/QUKE	Josephine Camp	USFS
Rhizopogon	PIPO/PIJE/QUKE/PSME	Deer Creek, Peavine	BLM,
bacillisporus			USFS
Rhizopogon exiguus	PSME	Hobart Bluff	BLM
Rhizopogon rogersii	QUGA/ PIPO/PSME/ARME	China Gulch, Enchanted	BLM
		Forest	
Rhizopogon	PIPO/PIJE/PSME/QUKE	Josephine Camp	
semireticulatus			
Rhizopogon	PIPO/PSME/COCO/QUKE	Hog Creek, Little Hyatt W	BLM
subclavitisporus			
Rhizopogon	QUGABR/PIPO//PIJE/PILA/	Limpy Creek serpentine,	USFS
subpurpurascens	PSME/CHCH/ARME	Limpy Creek siltstone,	
		Onion Mtn	
Rhizopogon truncatus	PILA/PIPO/PSME/CHCH/		
	ARME		
Tuber quercicola	QUGA	Neathammer, Buckhorn,	BLM
		Mariposa	

¹Geopora cercocarpi is a newly described species that has been added to the list.

²*Rhizopogon atroviolaceus* may be synonymous with *R. subpurpurascen,* also on the target list.

	JCK	JPR	NT	łgU	N	DVE	XB	AYS	ER	EVI	ЛС	лск	۸LA	ICH	IT	EN	RE	DBA	DGC	'AT	CK	SE	3E	10	EAD
SPECIES	BL	BL	CA	Ъ	Ъ	8	S	ΔA	DE	DE	Ы	∠ ⊔	2 Ш	EN	EX	FR	GР	Н	Н	Η	ÌĄ	9	ris	LIS	Σ
Alpova olivaceotinctus									1																
Alpova trappei							1																		
Arcangeliella gardneri												1													
Balsamia alba									1													1			
Balsamia magnata				2		1			1						2	2						1			
Balsamia nigrans src395					2																				
Barssia oregonensis																									
Cazia flexiascus																					1				
Endogone flammicorona																						1			
Endogone lactiflua						2								2					1				1		
Endogone sp		2																							
Gautieria candida																				1					
Gautieria monticola							1															1			
Gautieria monticola							1																	1	
Gautieria pterosperma																			1	8					
Gautieria sp2		1																							
Genabea cerebriformis			4	2					1			1		2			1		8			4			
Genea arenaria				3		1																			
Genea gardneri																									
Genea harknessii						1									1				1						1
Genea		1										1													
Geopora cercocarpi					4													3							
Geopora cooperi									1								2					2			
Gilkey compacta						2						13										7			
Glomus sp.						4																			
Gymnomyces			3	5					1			1		1			2					2			
Gymnomyces compactus								1																	
Gymnomyces ellipsosporus	1																								
Hydnobolites														2											
Hydnotryopsis	1														5										
			-	-		-										-	-			-	-	-		//	

-																		
Hymenogaster cf. boozeri																		
Hymenogaster diabolus							1											
Hymenogaster parksii													1					
Hymenogaster												2			1			
Hysterangium crassirachis											1							
Hysterangium separabile		16	7	1	1	2								2	1	11	1	
Hysterangium setchellii	3		5			1		2				5		1				3
Hysterangium sp2																		
Hysterangium sp3		1																
Hysterangium sp4										1								
Hysterangium velatisporum																		
Leucogaster rubescens								1						1				
Leucophleps sp1																	1	
Leucophleps sp2																	1	
Leucophleps spinispora				2											2			
Macowanites luteolus														2				
Melanogaster euryspermus		1	2											1				
Melanogaster cf natsii	1								1									
Melanogaster cf thiersii	2								3									
Melanogaster tuberiformis		2	1	1	1						2		1			1		
Melanogaster									3	1								
Pachyphloeus citrinus																		
Pachyphloeus virescens																		
Peziza ellipsospora											1							
Peziza infossa		6	2			1					1							19
Rhizopogon abietis											1							
Rhizopogon arctostaphyli		4						1			1							
Rhizopogon bacillosporus						2												
Rhizopogon evadens										1								
Rhizopogon occidentalis											1							
Rhizopogon parksii										1								
Rhizopogon rogersii			1							1								
Rhizopogon rubescens																1		
Rhizopogon salebrosus															1			

Rhizopogon semireticulatus																						1			
Rhizopogon subclavatisporus																		2	7	1			1	28	
Rhizopogon subpurpurascens																							2		
Rhizopogon truncatus																			11				2	13	1
Rhizopogon villosulus									3					1											
Rhizopogon vinicolor		3	1						1	1		1							1			1	1		2
Rhizopogon sp				1		6			3			3		2		1	1		1	3	1	4		3	1
Rhopalogaster transversarius																1									
Sarcosphaera coronaria					1																				1
Schenella pityophylus				1								2					3							1	
Scleroderma					1																				
Trappea darkeri					1																				
Tuber californicum				3										3					2						1
Tuber candidum	1			15					1			10		5	10		2								16
Tuber lauryi																5			1						
Tuber quercicola	1					5						2			1		1								1
Tuber src709			3											1											
Tuber whetstonense		1																							
Tuber sp			5	1		6			3					6	3	12			5		1			1	29
	2	14	46	51	13	28	5	1	23	1	1	39	7	30	30	28	14	5	46	18	3	38	7	50	70

NEAT	ONO	PEAV	SHAN	STAR	STER	TAMT	TARO	THUN	ТУЦЕ	WAGN	WALD	WELL	WHET	Species total
														1
														1
													2	3
														2
												1		11
												1		3
												1		1
1														2
														1
	2	4	2	2	2						2			20
	1													3
														2
	1	1	1	1							1			9
														2
	2		2	1										14
														1
2					2						3	7	7	44
												1	1	6
													1	1
					1				6			1		12
1					2			1	1				2	12
						10				7				24
										1				6
9								6	7		2	1		47
					1									5
6		4									2	1	2	30
				1										2
									1					1
														2
1													4	10

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											1	1
												1
												3
1							1					5
							1					2
	4			20			1		5	2		81
				9					1			27
											1	1
												1
												1
3												3
	2	1	1									6
	2											3
												1
									2			6
		1			1				4			8
												4
1											12	16
											4	9
		1					1	1				15
										1	2	7
											3	3
											2	2
											2	3
1					2			4			9	45
												1
								3				9
		1										3
												1
												1
												1
												2
				1								2
												1

														1
														39
	2													4
	2			1							10			39
														4
		1	2	2				2	1		3			25
2		1		4	1				1		8			46
														1
														1
				1							1	2		15
														1
												1		2
1														9
4				10	3		2		1			2	56	138
														6
7							1						8	26
														4
														1
6		3			4			4				9	4	102
46	18	18	8	53	19	10	3	17	26	8	44	31	123	1021