

FUNGAL DIVERSITY IN WOODY ROOTS OF EAST-SLOPE CASCADE  
PONDEROSA PINE (*PINUS PONDEROSA*) AND DOUGLAS-FIR  
(*PSEUDOTSUGA MENZIESII*)

By

JILL ANNE HOFF

A thesis submitted in partial fulfillment  
of the requirements for the degree of

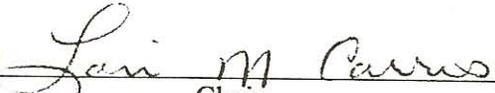
MASTER OF SCIENCE IN PLANT PATHOLOGY

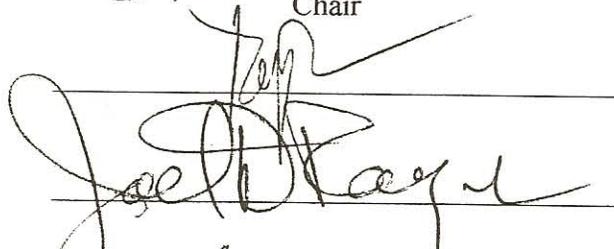
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**Abstract**

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Because the fungal community of large woody roots of conifers is not well documented, a mycodiversity study was conducted using increment cores from the woody roots of Douglas-fir (*Pseudotsuga menziesii*) and ponderosa pine (*Pinus ponderosa*) in the dry forests of the east-slope Cascades. Fungal isolates were identified using molecular techniques in combination with morphological characterization. Fungal species identified in this study will serve as a baseline for fungal diversity. Examination of rDNA internal transcribed spacer sequences and morphology of the cultured fungi delineated 27 fungal genera. Two groups predominated: *Byssochlamys* species (39 % of isolations) and *Umbelopsis/Micromucor* species (27 % of isolations). This is the first report of these fungi present on the large woody roots of conifers. These two species, as well as other species isolated in this study (e.g. *Epicoccum* sp., *Phialophora* sp., and *Phlebiopsis gigantea*) have potential as biological control agents. *Byssochlamys* species are heat-resistant fungi in which the ascospores can withstand heat up to 99 C. The occurrence of this fungus is potentially related to fire regimes. In the confines of this

study, the distribution of these genera was not affected by host or habitat type. More information is needed regarding the ecological role of these fungi, their relationship with fire, and their potential for biological control of forest pathogens.

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## CHAPTER ONE

### LITERATURE REVIEW

#### Fungal Diversity

In recent years, ecologists have increased efforts to understand critical below-ground interactions in forest ecosystems. Dynamic interactions that occur among plant roots, animals, and microorganisms are determining factors for ecosystem processes. Ecologists have barely begun to study the biodiversity that underpins these interactions (Copely 2000).

Fungi of forest ecosystems are tremendously diverse and play wide-ranging roles in forest ecosystem processes (Rossman 1998). Their capacity for growth on various substrates depends on a complex set of metabolic enzymes and the ability to absorb essential nutrients. Because they obtain their nutrients through absorption, many fungi can successfully exploit organic matter of diverse origin. Fungi play a number of key roles within the world's ecosystems; the most important of which may be the cycling of nutrients derived from the breakdown of plant and animal matter, allowing the re-use of limited biotic and abiotic resources (Rossman et al. 1998).

Studies of fungal biodiversity in forest ecosystems could provide baseline information for determining interrelationships among organisms and indicate potential roles of fungi in forest ecosystem dynamics. Understanding the role of fungi in forest ecosystem processes is key to successful characterization of biological resources. In addition, fungal biodiversity studies will contribute to the discovery and characterization of fungal resources, provide insight into their sustainability, and help slow the loss of these biological resources (Rossman et al. 1998)

Due to their widespread distribution and association with diverse organic and inorganic substrates, the actual number of fungal species in existence is difficult to assess (Rossman et al. 1998). A wide range of fungi have been found to occur in wood including certain Mucoraceous species, Ascomycetes, Basidiomycetes, and Fungi Imperfecti (Rayner and Todd 1979).

### *Wood decay fungi*

Species composition estimates of wood decay fungi in coniferous forests are usually based on the distribution of fruiting bodies. Since fruiting bodies are formed in response to specific environmental conditions, such inventories can span several years and still not guarantee accurate reflections of the fungal species present (Johannesson and Stenlid 1999). Decaying wood presents unique opportunities to investigate fungal growth and interactions within populations and communities of the substrata. The primary biotic decomposers of wood are basidiomycetous decay fungi, which can attack and degrade both wood in the forest and wood in commercial service. In the forest ecosystem, wood decay fungi play an important role in carbon and nitrogen cycling and help convert organic debris into the humus layer of the soil. Some fungi attack living trees; others invade down timber and slash on the forest floor, and lumber. Wood decay basidiomycetes colonize and degrade wood using enzymatic and nonenzymatic processes. Brown-rot fungi preferentially attack and rapidly depolymerize the structural carbohydrates (cellulose and hemicellulose) in the cell wall leaving the modified lignin behind. White-rot fungi can progressively utilize all major cell wall components, including both the carbohydrates and the lignin (Jasalavich et al. 2000).

### *Pathogenic fungi*

Species of fungi in the families Polyporaceae, Hymenochaetaceae, Stereaceae, and Hericiaceae, which reside in the Aphyllophorales, are specialized to enter wounds in living trees and penetrate to the heartwood. Some heart-, butt-, and root-rot fungi are pathogenic and can kill living trees by attacking functional vascular tissues (Alexopoulos et al. 1996). Laminated root rot, caused by *Phellinus pini* (Murrill.) R.L. Gilbertson, kills trees in slowly expanding mortality centers, creating gaps in the forest canopy. *P. pini* is widespread, locally abundant, and very long-lived. It is among the most important disturbance agents in the long intervals between stand-replacing events, such as wildfire or harvest, in these ecosystems and shapes the structure and composition of both wild and managed forests (Hanson and Goheen 2000).

Annosus root disease, caused by the basidiomycete *Heterobasidion annosum* Fr. (Bref.), is a root pathogen present in coniferous forests of the Northern Hemisphere. This species complex includes at least three biological species which display a marked host preference. Stumps and wounds can act as infection courts for this fungus, and roots are common routes of secondary spread from tree to tree. Once the pathogen has established itself in a stand, it will often spread progressively to neighboring trees creating mortality centers (Garbelotto et al. 1997.)

### *Endophytic fungi*

Fungal endophytes can live internally, intercellularly or intracellularly, and asymptotically. They usually occur in above-ground plant tissues, but also occur

occasionally in roots, and are distinguished from mycorrhizal fungi by lacking extra radical hyphae or mantles. The definition of endophyte has been modified over the last decade and disagreement continues as to the definition of endophyte. For our purposes, endophytes are fungi that live for all, or at least a significant part, of their life cycle internally and asymptotically in plant parts (Saikkonen et al. 1998.)

While a large body of information is available on pathogens and mycorrhizal fungi, little is known about other symptomless – possibly neutral or mutualistic - endophytic symbionts of aerial plant organs or root associates. Symptomless endophytes can be assembled into two distinct ecological groups: the clavicipitaceous system endophytes, and the endophytes of trees and shrubs including the non-clavicipitaceous grass endophytes (Petrini 1997). Fungal endophytes have been found in all woody plants studied, and numerous species of fungal endophytes have been found in individual plant species and even in individual plants. Trees and shrubs usually contain numerous fungal species. However, there has been little attempt to integrate ecological and evolutionary aspects of fungal endophytes of grasses and woody plants (Saikkonen et al. 1998).

### *Epiphytic fungi*

The external plant provides a suitable environment for diverse microbial flora that vary in kinds, numbers, properties, and local distribution patterns. Surface growth has several implications for microbial structure and function. The plant surface is living and thus establishes several specialized implications depending on the plant species, plant organ, and surrounding biotic and abiotic environment. New areas become progressively

available for colonization as the plant grows (Andrews and Harris 2000). Many researchers have noted the existence of seasonal patterns in the composition of phyllosphere microbial communities. Lindsey and Pugh (1976) reported ascomycetes to be the major group of epiphytic fungi inhabiting leaves. These genera include: *Phoma* spp., *Epicoccum* spp., *Penicillium* spp., and *Cladosporium* spp. Epiphytic fungi of woody roots are not well documented and warrant further study.

### *Ectomycorrhizal fungi*

Ectomycorrhizal (EM) symbioses are important on a global scale because most trees are ectomycorrhizal throughout the world's boreal, temperate, subtropical, and tropical forests. For host trees, these fungi serve as the primary nutrient gathering interface, scavenging nitrogen, phosphorus, and trace elements from both inorganic and organic pools in the soil; this is done in exchange for fixed carbon from the tree (Horton and Bruns 2001). These mutualistic symbioses also provide plants with increased access to other resources, such as water. They also protect plants from pathogens, chemical extremes (e.g., high pH), and heavy-metal contamination (Cullings et al. 2000). Until the middle 1990s, knowledge of EM fungal community structure, and the degree to which species assemblages show predictable colonization patterns in space and time, was almost exclusively based on sporocarp surveys (Dahlberg 2001). EM fungi are generally basidiomycetous fungi (e.g., species of *Russula*, *Cortinarius*, *Chroogomphus*, *Lactarius*, *Suillus*, *Paxillus*) (Cullings et al. 2000; Horton and Bruns 2001).

### *Mycoparasitic fungi*

Interactions between biocontrol fungi and their pathogens are the focus of many continuing research efforts. Some recent examples of fungal-fungal interactions in the rhizosphere and spermosphere include *Trichoderma hamatum* (Bonorden) Bainier with *Rhizoctonia solani* Kuhn, *Phialophora* sp. with *Gaeumannomyces graminis* var. *tritici* (Ggt). Haver, and *Cladorrhinum foecundissimum* Saccardo & Marchal with *Pythium ultimum* Trow (Whipps 2001).

Mycoparasites are extremely common in soil, where they can act as natural regulators of fungal populations (Mulligan and Deacon 1992). The relationships may be biotrophic, with the host showing few symptoms other than reduced growth rate, or necrotrophic, with the host tissue being killed under some conditions. Researchers are actively pursuing the use of mycoparasitic fungi (e.g., *Trichoderma* spp.) as agents of biological control, and several formulations are available for use against certain plant pathogens including *Pythium* spp. and *Heterobasidion annosum* (Alexopoulos et al. 1996).

### *Species concepts and identification*

The species concept is central to biology and has caused considerable debate, yet a universal definition of species has not been found. Much of the discussion has centered on animals and plants, while fungi have received less attention. Species definitions have been based on phenotypic similarity, ecological parameters, reproductive isolation or cohesion, evolutionary principles, and various combinations of the above. Fungal species concepts, in practice, have evolved from strictly morphological descriptions, to a

biological species concept, to phylogenetically based species concepts (Harrington and Rizzo 1999).

In some cases, ecological species have been based on adaptation to a particular niche, or in plant pathology some fungal pathogens have been defined mainly on the basis of disease symptoms or host association. Morphological, ecological, and pathological species are all, therefore, defined from phenetic characters, most of which relate directly to functional and structural attributes. PCR amplification provides information on deoxyribonucleic acid (DNA) sequences, and thereby allows the testing of hypotheses about relationships within and among phenetically defined species (Bridge et al. 1998).

Information on DNA sequences may reinforce existing phenetic species groupings, and in these cases the species can be described as polythetic. But, DNA sequence information can also provide valuable insights into the evolutionary history of phenetic species. This can be especially important in species where the major functional characters available relate to an aspect of their environmental niche, such as in ecological species (Bridge et al. 1998).

Genetic similarities and differences at the molecular level provide a reliable means for assessing phylogenetic relationships. Redecker et al. (1999) reported that single spores of the Glomales contain divergent ribosomal DNA (rDNA) sequences due to the fact these spores contain several thousand nuclei. They examined sequences of *Acaulospora gerdemannii* Schenk & Nicolson and *Glomus leptotichum* Schenck and Smith which possess dimorphic spore types that are divergent to the extent expected for two different genera. The level of sequence divergence found [due to considerable

degree of variation detected among copies of rDNA within single spores of the Glomales (Redecker et al. 1999)] is within the range expected for intraspecific variability. Their phylogenetic analyses indicate that evolutionary patterns in the Glomales are much more complex than previously thought, which makes it difficult to distinguish closely related arbuscular mycorrhizal (AM) fungi by sequence comparisons.

Morphological identification of fungi isolated from wood is difficult and tedious, and many fungal species that grow in wood may not be culturable (Johannesson and Stenlid 1999). Moreover, an additional problem occurs with the identification of cultures of wood-rotting fungi. Even though fungal isolates belong to species that have already been described on the basis of sporocarp morphology, the cultures do not exhibit the characters by which they may be assigned to genera or families (Nobles 1948). Molecular techniques have proven valuable in the identification of fungi from plants and in many disciplines in the biological sciences, including systematics soil (e.g., Vandenkoornhuysen et al. 2002; Horton and Bruns 2001; Johannesson and Stenlid 1999; LanFranco et al. 1998; Liesack et al. 1997), and the elucidation of evolutionary relationships among major groups of fungi (Klich and Mullaney 1992).

Sequences of conserved DNA regions have successfully resolved broad evolutionary relationships among fungi at the family, order, and class levels, but species-level relationships require more variable DNA regions. Ribosomal repeats in rDNA have been regions of choice in identification and phylogenetics for various reasons. Fungal nuclear ribosomal gene clusters are arranged in roughly 200 tandem repeats (Berbee and Taylor 2001). Because each nucleus contains approximately 200 copies of the region, at least one intact copy for molecular analysis can usually be recovered, even from low-

quality DNA preparations (Berbee and Taylor 2001). The transcribed spacer regions in the rDNA operon (internal transcribed spacer regions, ITS1 and ITS2) and the non-transcribed spacer regions between the tandem repeats of the rDNA operon (the intergenic spacer region, IGS) have been successfully used to identify distinct lineages of fungal species and resolve relationships between closely related species (Harrington and Rizzo 1999).

Most of the molecular ecology on EM fungi has involved the restriction analyses of the ITS region. This nuclear region lies between the small subunit (SSU, 17S) and the large subunit (LSU, 25S) rDNA genes and contains two noncoding spacer regions (ITS1 and ITS2) separated by the 5.8S rRNA gene (Horton and Bruns 2001).

In the past, the different methods for identifying fungi used by various authors made it difficult to translate the data supplied by different authors (Nobles 1948). Presently, sequences are published and/or deposited in electronic databases (eg., GenBank, EMBL, and DDBJ), so results from different laboratories can be compared directly. Results can be confirmed or applied to other taxa without obtaining biological strains or clones. However, a limitation to this approach is that there may not be a way to confirm the accuracy of the identification on the sequences deposited. DNA information (e.g., nuclear and mitochondrial DNA sequences), PCR-RFLP, random amplified polymorphic DNA (RAPD), and amplified polymorphic PCR (AP-PCR) have also proved useful for phylogenetic analysis (Takamatsu 1998).

The ITS regions are ideal for identification because they typically have the resolving power to place unknown organisms to the species level or at least within a genus. A fast way to take advantage of the ITS data currently deposited is to search

GenBank or EMBL using only the ITS sequences from unknown samples; sequence variation within spacers is so high that only very closely related taxa are retrieved (Horton and Bruns 2001). For the purposes of the diversity study, we chose to amplify the ITS region of the rDNA to assess species diversity of fungi present in woody roots of Douglas-fir and ponderosa pine. The application of molecular techniques contributes to a more comprehensive assessment of fungal diversity than a diversity study that is based solely on morphology. It allows us to identify fungal species that are non-sporulating and non-sporocarp forming. As the role of fungal diversity in ecosystem processes becomes better understood, information can be used to develop sustainable forest practices (e.g., using prescribed burning to favor fungi that are more heat resistant, with biological control capabilities, and could potentially displace fungal pathogens which are less heat tolerant).

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## CHAPTER TWO

### FUNGAL BIODIVERSITY OF DOUGLAS-FIR (*PSEUDOTSUGA MENZIESII*) AND PONDEROSA PINE (*PINUS PONDEROSA*) WOODY ROOTS

#### Introduction

Evolutionary history of organisms comprising a forest ecosystem is characterized by mortality-causing natural disturbance regimes (e.g., fires, predation, disease, insect attack) that have varied in kind, frequency, intensity, and extent. Transitory phenomena dominate evolutionary history of organisms to the extent that a constant state of instability is considered characteristic of natural systems (Covington and Moore 1994). Abiotic disturbances, such as fire, wind, landslides, and mudflows, have long been recognized for their critical influences on vegetation patterns within a forest ecosystem. In addition, pathogens, mutualists, and saprophytes are starting to be recognized for their key roles in structuring natural forest ecosystems.

Application of molecular techniques can provide a more comprehensive assessment of fungal diversity. They allow us to explore unique niches of fungal diversity that are occupied by non-sporulating fungi. For example, a study conducted by Vandenkoornhuysen et al. (2002) found 49 phylotypes in the roots of the grass *Arrhenatherum elatius* using newly designed fungal-specific polymerase chain reaction (PCR) primers for small subunit ribosomal DNA. The phylotypes represented the four recognized fungal taxa: Ascomycota, Basidiomycota, Chytridiomycota, and Zygomycota. Their study also suggested the existence of previously unknown groups of fungi, with two of these groups containing two deeply branching lineages in the phylogenetic tree

that may represent new phyla. In contrast, the authors noted that similar culture-based studies have yielded primarily ascomycetes (Vandenkoornhuysen et al. 2002).

Our overall objective was to establish baseline information on fungal diversity associated with woody roots of Douglas-fir and ponderosa pine (in relation to host species and habitat type) within a relatively dry forest ecosystem in the Wenatchee National Forest, east of the Washington Cascades. In 5 years, a subsequent diversity survey will follow fire treatments to determine the effects of fire regimes on fungal diversity. As the role of fungal diversity in ecosystem processes becomes better understood, such information can be used to develop sustainable forest practices.

## Materials and Methods

### *Study Design*

Our study was conducted on south, west, and east aspects in the Mission Creek watershed, Wenatchee National Forest, Wenatchee, WA. On these aspects, a mixed-conifer forest is dominated by ponderosa pine (*P. ponderosa*) and Douglas-fir (*P. menziesii*). Generally, this type of dry forest dominates the eastern edge of the Wenatchee National Forest. A fire-return interval of 10-20 years occurs over the 890,308 ha in the Wenatchee National Forest (<http://www.fs.fed.us/pnw/weblab>). The Wenatchee National Forest receives less than 762 mm of annual precipitation.

The study consisted of 12 experimental units (10-20 ha in size) that will undergo different fire treatments (Fig. 1) (Hessburg and Edmonds, unpublished). When the fire treatments are applied, three units will serve as controls, three as burn-only [prescribed fire only, with periodic burns (Agee et al. 2001)], three as thin-only [periodic cutting,

followed by mechanical fuel treatment and/or physical removal of residue; no use of prescribed fire (Agee et al. 2001)], and three as thin-and-burn [initial and periodic cutting, followed by prescribed fire; fire alone could be used one or more times between cutting intervals (Agee et al. 2001)] (Hessburg and Edmonds, unpublished). Units were placed on all aspects except north, on slopes < 40 %, with no more than 10 % rock cover. Areas that included rare plant species were avoided. Units were placed in a narrow range of dry forest environments, and were indicated by dry Douglas-fir potential vegetation types including the PSME-SPBEL (*Pseudotsuga menziesii*/ *Spiraea betulifolia* Pall.), PSME-PUTR (*P. menziesii*/*Purshia tridentata* (Pursh) DC.), PSME-CARU (*P. menziesii*/*Calamagrostis rubescens* Buckley), and PSME-SYAL (*P. menziesii*/*Symphoricarpos albus* (L.) S.F. Blake) plant associations (Williams and Smith 1991).

Pretreatment surveys of existing disease symptoms or mortality associated with root pathogens and fungi associated with woody roots of ponderosa pine and Douglas-fir occurred during the 2000 field season (May-September); thinned units were harvested in 2001 after sample collections, and burn treatment units will be ignited in 2002. Post-treatment surveys (identification and mapping of new symptoms or mortality associated with a root disease pathogen) will occur during the 2003 and 2004 field seasons.

#### *Area and Treatment Units*

The experimental area and units are shown in Fig. 1. During pre- and post-treatment surveys, trees showing new symptoms or mortality associated with a root pathogen were identified. Root pathogens of original interest were 1) *Phellinus weirii*

(Murrill.) (PHWE), the cause of laminated root rot, 2) *Heterobasidion annosum*. (HEAN; both S- and P-groups), the cause of Annosum root disease, 3) *Armillaria* spp., especially *A. ostoyae* (Romagnesi) Herink (AROS), the cause of Armillaria root disease, and 4) *Leptographium wageneri* (Kendrick) M.J. Wingfeld (LEWA), the cause of black stain root disease (Hessburg and Edmonds, unpublished).

Trees with symptoms (e.g., thinning crown, topkill, resinosis) were identified. An increment borer, which was surfaced sterilized with ethanol after each use, was used to extract increment cores (ca. 5 mm diameter) from the tree roots. Then, the root core was placed in a plastic soda straw with both ends of the straw sealed with masking tape, stored at 4 C, and shipped to the Forestry Sciences Laboratory (USDA Forest Service, RMRS, Moscow, ID) for fungal isolation and identification.

In addition to collecting root cores from all visibly symptomatic trees, trees without visible symptoms of root disease or other damaging agents were surveyed. When an apparently healthy appearing tree was randomly sampled, the tree was mapped, site and tree attribute data were recorded, and one major lateral root was examined in the same manner as previously described (Hessburg and Edmonds, unpublished).

#### *Laboratory Processing of Samples*

The samples were stored on ice or at 4 C in a styrofoam cooler for 10 days to 4 weeks after collection in the field. Samples were processed within hours after arrival at the Forestry Sciences Laboratory, Moscow, ID. Depending on the length of the lateral root increment core, it was split into several pieces (ca. 2.5 cm). Each core piece was submerged into 70 % ethanol, flame-sterilized, and placed onto a 60 x 15 mm petri dish

containing a medium selective for basidiomycetous fungi, then placed into an incubator at 21 C in the dark. The selective medium was a benomyl dichloran streptomycin (BDS) agar containing 15 g malt extract, 15 g agar, 40 mg benomyl, 20 mg dichloran (2,6-dichloro-4-nitroaniline), and 100 mg streptomycin per L (Worrall and Harrington 1993).

After 2 months of incubation, the cores were examined for mycelial growth. Subcultures were made on BDS, a nutrient-rich medium (3 % dextrose, 1 % peptone, 1.5 % agarose, and 3 % malt extract), and 2 % malt extract agar (MEA) medium. Cores that supported mycelial growth limited to wood were placed on a non-selective medium (MEA and/or nutrient rich medium) to facilitate growth.

#### *Morphological characterization*

To morphologically characterize fungal isolates, specimens were examined with bright-field and phase-contrast optics using an Olympus BX 60 microscope after staining with Melzer's Reagent (Melzer 1924). Depending on the isolate, general morphological characters, such as hyphae, basidia, basidiospores, asci, ascospores, sporangiophores, sporangiospores, zygospores, and chlamydospores, were observed and measured. Taxonomic keys such as: Barron 1971; Kendrick 1971; O'Donnell 1979; Von Arx 1981; Gilbertson 1986; Ellis 1993; Hanlin 1997, etc. were used to aid in identification of fungal cultures. Photographic images were taken with a Nikon Cool Pix 990<sup>®</sup> digital camera (Melville, NY). Permanent slides were prepared using Shurmount<sup>®</sup> aqueous mounting medium (Triangle Biomedical Sciences, Durham, NC).

### *Use of Polymerase Chain Reaction (PCR) as a Diagnostic Tool*

A mycelial scrape was placed in a 50- $\mu$ l PCR amplification mixture. When direct mycelial scraping did not provide a suitable DNA template for PCR, mycelial samples were treated with Lyse-N-Go™ PCR reagent (Pierce, Rockford, IL) following the protocol of the manufacturer. Each 50- $\mu$ l PCR amplification mixture contained 1.2 U AmpliTaq® (Applied Biosystems, Foster City, CA), 5  $\mu$ l 10X PCR buffer (Applied Biosystems), 200  $\mu$ M dNTPs, 4.0  $\mu$ M MgCl<sub>2</sub>, 0.5  $\mu$ M ITS-1 primer, and 0.5  $\mu$ M ITS-4 primer (White et al. 1990). The cycling parameters were modified from Pimentel et al. (1998): DNA was denatured for 5 min at 94 C, followed by 30 cycles of denaturation at 94 C for 90 s, annealing at 50 C for 1 min 30 s, and extension at 72 C for 2 min 30 s. A final extension was performed at 72 C for 5 min. Amplified products (4  $\mu$ l) were subjected to agarose-gel electrophoresis at 9V/cm for 2 hr, then viewed with UV light after staining in ethidium bromide.

DNA products were prepared using Exosap-IT (USB, Cleveland, OH) PCR product clean up, following the protocol of the manufacturer. The reaction conditions were 37 C for 15 min followed by 80 C for 15 min. The DNA products were sent to Amplicon Express (Pullman, WA) for sequencing.

Single-stranded sequences were trimmed on both ends, then used to generate double-stranded contigs using BioEdit (Hall 2001). The contigs were then entered into the National Center for Biotechnology Information's GenBank nucleotide BLAST search to facilitate species identification.

## Statistical Analyses

Chi-square tests for independence were conducted using Web Chi Square Calculator ([http://www.georgetown.edu/cball/webtools/web\\_chi.html](http://www.georgetown.edu/cball/webtools/web_chi.html)) to determine host and habitat influence on fungal occurrence in the study sites and the relationship of fungal occurrence with symptom expression.

## Results

### *Significance of plant association and host on fungal species occurrence*

Approximately 402 cores were processed, of which 28 cores yielded growth of more than one fungal species, 160 yielded growth of one fungal species, and 214 cores did not yield any fungal growth. Of the 402 increment cores obtained within the two types of plant series (PSME and PIPO), 47 % of the samples yielded growth on the increment cores (see Table 1 for summary of isolated fungal genera). However, plant associations had no significant influence on fungal occurrence ( $P = 0.66 < 1.0$ ; Table 2). Of the tree hosts across all of the units, 52 % yielded fungal isolates. However, host species did not significantly influence fungal occurrence ( $P = 0.18 < 0.10$ ; Table 3).

### *Predominant fungal species occurring in the study sites*

Two different fungal genera predominated across all of the units (Table 1). *Byssochlamys* and a species resembling *Umbelopsis* sp. or *Micromucor* sp. (a definitive identification of these isolates could not be made, thus these fungi will be referred to as Group 1), occurred in all of the plant association and host species. *Byssochlamys* spp. comprised 39 % (74 isolates) of the total fungal isolates cultured (188), [18 % of the total

root cores collected (402)] (Tables 4 and 5), and fungal isolates from Group 1 consisted of 27 % (50 isolates) of the fungal isolates cultured [12 % of the total root cores collected (402)] (Tables 6 and 7). However, no significant interactions were observed among the occurrence of the two genera by plant association (*Byssochlamys* spp.:  $P = 0.08 < 0.10$ ; Table 4); (Group 1:  $P = 0.43 < 1.0$ ; Table 6), or by tree host (*Byssochlamys* spp.:  $P = 0.18 < 1.0$ ; Table 5); (Group 1:  $P = 0.07 < 0.10$ ; Table 7), The increment cores from which fungi were isolated came from apparently healthy wood.

ITS sequences of *Byssochlamys* spp. isolates revealed a 93-95 % sequence identity with *B. nivea* (U18361, GenBank). However, *B. nivea* was the only *Byssochlamys* species with ITS sequence information entered into GenBank.

Morphologically, the asci and ascospores of *Byssochlamys* spp. isolates resembled *Byssochlamys nivea* (Westling), and ascogonial initials were observed twice in culture. Chlamydospores (5-7  $\mu\text{m}$  diam) and conidia [(3.0)-4.0  $\mu\text{m}$  x (1.5)-2.5  $\mu\text{m}$ ] were frequently observed (Fig. 2), and phialides occasionally observed. They resembled a *Penicillium*-type conidiophore, with long, tapering phialides. The conidia were borne in chains. The ascospores of *B. nivea* are approximately 3  $\mu\text{m}$  in diameter at maturity (Hanlin 1998), the ascospores of our *Byssochlamys* sp. isolated were approximately 3-4  $\mu\text{m}$  in diameter; the asci were approximately 11  $\mu\text{m}$  in diameter. The hyphae were irregularly branched and septate. The color of the mycelia in culture (2 % MEA) ranged from olive to brown with areas of white mycelia interspersed throughout in most cultures (Fig. 2).

Group 1 fungal isolates were isolated from the roots of the conifer species and this group showed morphological relationship to zygomycetous fungi. Surprisingly, ITS

sequences of Group 1 had a sequence identity (90-98 %) to sequences attributed to *Acaulospora colossica* Schultz, Bever Morton (AF133780 and AF133780, GenBank) (Pringle et al. 2000), an arbuscular mycorrhizal fungus that cannot be cultured. Consequently, a subset of our Group 1 fungal isolates were sent to A. Schüßler (Institute of Botany, U. of Darmstadt, Germany) for small subunit (SSU) rDNA sequence analyses, which revealed a high sequence identity with *Umbelopsis* spp. and/or *Micromucor* spp. Additional analyses utilized ITS sequences from other Group 1-like fungal isolates provided by R. Vilgalys (Duke University), E. Vandegrift (Oregon State University), and K.L. O' Donnell (USDA ARS, Peoria, IL) (refer to Chapter 3). These ITS analyses corroborated the identification of our isolates as either *Umbelopsis* sp. or *Micromucor* sp. Further morphological characterization of these fungi is found in Chapter 3.

The occurrence of *Byssochlamys* spp. was not significantly associated with symptomatic nor nonsymptomatic trees (*Byssochlamys* spp:  $P = 0.81 < 0.10$ ; Table 9); However, the occurrence of Group 1 was associated with symptomatic trees (Group 1:  $P = 0.03 < 0.05$ ; Table 8).

#### *Fungal taxa present in study units*

The Crow 1 unit, located in the Crow and Pendleton area, consisted of *P. ponderosa*/*Purshia tridentata*, *P. menziesii*/*P. tridentata*/*Agropyron spicatum*, and *P. menziesii*/*Symphoricarpos albus* plant associations. Sixty-eight percent (32 cores) of the increment cores in this unit yielded fungal growth, while 32 % (15 cores) yielded no fungal growth. Five different genera were found, with Group 1 fungal isolates

(69 %) and *Byssochlamys* spp. (28 %) predominating. One *Mucor* sp., one *Cladosporium* sp., one *Mycena* sp., and three unidentified fungal isolates constituted the remaining 19 % of fungal isolates.

The Crow 3 unit is also located in the Crow and Pendleton area. Sixty-two percent of the increment cores in this unit (18 cores) yielded growth, while 38 % (11 cores) of the increment cores did not yield any growth. Five different genera were found, with *Byssochlamys* spp. (55 %) and Group 1 fungal isolates (38 %) predominating. Two *Epicoccum* species, two *Cladosporium* spp., one *Mucor* spp., and one unidentified fungal isolate constituted the remaining 33 % of fungal isolates.

The Crow 6 unit is also located in the Crow and Pendleton area. Fifty-three percent of the increment cores in this unit (28 cores) yielded growth, while 47 % (25 cores) did not yield any fungal growth. Eight different genera were found, with Group 1 fungal isolates (36 %) and *Byssochlamys* spp. (18 %) predominating. Two *Penicillium* spp., one *Alternaria* sp., one *Phialophora* sp., one *Ulocladium* sp., one *Rhinochladella* sp., one unknown zygomycete and nine unidentified isolates constituted the remaining 57 % of fungal isolates.

Pendleton 30 is found in the Crow and Pendleton area. Forty-nine percent (17 cores) of the increment cores yielded fungal growth, while 51 % (18 cores) of the increment cores did not yield any fungal growth. Five different fungal genera were found with *Byssochlamys* spp. (41 %) predominating. Two *Tremella* sp., one *Tyromyces* sp., one *Heterobasidion* sp., one *Verticillium* sp. and four unidentified fungal isolates constituted the remaining 53 % of fungal isolations. Group 1 fungal isolates did not occur in this unit.

The Unit Ruby is located in the Deer Park Springs area, which also included units Little Camas #11 and Spromberg #4. The plant associations in this area are *P. ponderosa/Rosa woodsii*, *P. ponderosa/S. albus*, and *P. ponderosa/S. albus/C. rubescens*. Sixty-five percent (20 cores) of the increment cores in this unit yielded fungal growth, while 35 % (11 cores) of the increment cores did not yield any growth. Eight different genera were found with *Mucor* spp. (30%) and *Byssochlamys* spp. (20 %) predominating. Two *Mortierella* sp., two *Penicillium* spp., one *Ganoderma* sp., one *Pesotum* sp., one *Verticillium* sp., one ericoid mycorrhizal fungus, and four unidentified fungal isolates constituted the remaining (55 %) fungal isolates. Group 1 fungal isolates did not occur in this unit.

The unit Spromberg #4 is found in the Deer Park area. Fifty percent (18 cores) of the increment cores yielded fungal growth, while 50 % (18 cores) of the increment cores did not yield any growth. Three different fungal genera were found with *Mucor* spp. (44 %), Group 1 fungal isolates (28 %), and *Byssochlamys* spp. (28%) predominating. Six unidentified fungal isolates constituted the remaining 33 % of fungal isolates.

The unit Little Camas # 11 is found in the Deer Park area. Thirty percent (7 cores) of the increment cores yielded fungal growth, while 70 % (16 cores) did not yield any growth. Three different genera were found in this unit with *Byssochlamys* spp. (29%) predominating, and Group 1 constituted 14 % of fungal isolates. One *Phlebiopsis* sp. and three unidentified fungal isolates constituted 57 % of the remaining fungal isolates.

The Unit Slawson 8b is located in the Poison, Slawson, and Tripp Canyon area. The plant associations with this unit are *P. menziesii/Holodiscus discolor*, *P.*

*menziesii/Holodiscus discolor/C. rubescens*, and *P. menziesii/Spiraea betulifolia*. Fifty-one percent (18 cores) of the increment cores yielded fungal growth, while 49 % (17 cores) did not yield any fungal growth. Ten different genera were found with *Byssochlamys* spp. predominating (28 %). Two Group 1 fungal isolates, two *Tremella* sp., two *Mucor* spp., two *Penicillium* spp., one *Merimbla* sp., one *Cladosporium* sp., one *Pycnoporus* sp., one *Clavulina* sp., one *Hypocrea* sp., and two unidentified fungal isolates constituted the remaining 83 % of the fungal isolates.

The unit Tripp is located in the Poison, Slawson, and Tripp Canyon area. Thirty-nine percent (13 cores) of the increment cores yielded fungal growth, while 61 % (20 cores) did not yield any fungal growth. Two different genera were found with *Byssochlamys* spp. (77 %) predominating. One *Mucor* sp. and two unidentified fungal isolates constituted the remaining 23 % of the fungal isolates.

The unit Poison is located in the Poison, Slawson, and Tripp Canyon area. Thirty-four percent (10 cores) of the increment cores yielded fungal growth, while 66 % (19 cores) did not yield any fungal growth. Six different genera were found on this site with *Byssochlamys* spp. (30 %) and *Verticillium* sp. (20 %) predominating. One *Eupenicillium* sp., one *Mucor* sp., one *Penicillium* sp., and one unidentified fungal isolate constituted the remaining 60 % of the fungal isolates.

Units Sand 2 and Sand 19 are located in the Sand Creek area. Forty-three percent (13 cores) of the increment cores yielded fungal growth, while 57 % (17 cores) did not yield any fungal growth. Four different genera were found with *Byssochlamys* spp. (77 %) predominating. Two *Penicillium* sp., one *Hormonema* sp., one Group 1 fungal

isolate, and one unidentified fungal isolate constituted the remaining 39 % of the fungal isolates.

In the Sand 19 unit, only 18 % (6 cores) of the increment cores yield fungal growth, while 82 % (27 cores) did not yield any fungal growth. Two different genera were found in this unit, with *Byssochlamys* spp. (67 %) predominating. Two *Penicillium* sp. and one unidentified fungal isolate constituted the remaining 50 % of the fungal isolates.

### **Discussion**

Results revealed woody roots of Douglas-fir and ponderosa pine were colonized by a range of fungi including *Byssochlamys* spp., Group 1 fungal isolates, *Mucor* spp., *Tremella* sp., *Penicillium* spp., *Verticillium* sp., *Ganoderma* sp., and other species. If these fungi are actively growing in woody roots, their ecological role is unknown and warrants further investigation.

Originally, this study was designed to survey for *Heterobasidion annosum*, which is a basidiomycete affecting a wide array of coniferous tree species worldwide. Only one isolate (Pendleton G10-1-1) was found across the units in this study. Studies on the etiology of this pathogen in *Pinus* spp. and *Picea* spp. have indicated that stumps are important sites for fungal primary infections, presumably by basidiospores (Garbelotto et al. 1999). The creation of stumps, by extensive logging of conifer forests, have favored the establishment of this pathogen in stands where originally it may have been rare (Garbelotto et al. 1999).

*Phlebiopsis gigantea* was another species isolated from woody roots, and is a common colonizer of fresh conifer wood in boreal and temperate forests throughout the world. This fungus typically causes a white rot of coniferous timber and is a primary colonizer of wood, requiring high moisture content for its growth (Holdenreider and Greig 1998). It is a primary colonizer of conifer logs, and, over time, can cause significant deterioration in the forest. Basidiomata of *P. gigantea* frequently form on stumps, fallen trunks, and log piles (Holdenrieder and Greig 1998). Fructification occurs normally within 1 yr after infection and continues for up to 3 or 4 yrs.

In studies on *H. annosum*, Rishbeth (1963) found that *P. gigantea* colonized stumps and showed some ability to replace *H. annosum* in roots. The level of natural colonization of pine stumps by *P. gigantea* can be high. The strong ability of *P. gigantea* to colonize pine stumps indicates that natural infection alone could potentially protect stumps against invasions by *H. annosum* (Holdenrieder and Greig 1998). Treating stumps with *P. gigantea* to control *H. annosum* is an example of successful biological control of a fungal disease in forestry.

The isolation of *P. gigantea* from the woody roots of conifers raises questions about its ecological relationship with the roots. Perhaps its mycelium can colonize the woody root system in addition to stumps of pine. Woody roots may represent the initial colonization site for mycelium that eventually grows to the stump surface following tree cutting. Because only one isolate of *P. gigantea* was isolated, the role that *P. gigantea* played in limiting the occurrence of *H. annosum* cannot be determined.

*Verticillium* species are commonly found in soil or in decaying plant matter (Gams 1971). *Verticillium* spp. invade plant roots as they grow through the soil.

(Gleason and Flynn 1998). However, the occurrence of *Verticillium* spp. in roots of conifers is not well-documented, and thus the occurrence of *Verticillium* sp. needs to be examined further.

Species of Mortierellaceae (Zygomycota) commonly are isolated from soil, but can also be found in wood, leaves, fungi, and other organic material (Alexopoulos et al. 1996). Members of the genus *Mortierella* are typically found in soil and are thought to play significant roles in the ecology of forest soils (Carreiro and Koske 1992). Reports of the occurrence of *Mortierella* spp. with woody roots were not found in existing literature, and the nature of the association between the fungus on conifer roots remains unknown.

*Epicoccum* species are widely distributed and commonly isolated from air, soil, and foodstuff (Domsch et al. 1980). This genus occurs in association with some animals and textiles, although it is not known to cause symptoms of infection in animals.

*Epicoccum nigrum* Link. is the common causative agent of leaf spots of various plants (Pritchard and Muir 1987), and is common on dead or dying plants (Domsch 1980).

*Epicoccum* spp. can also serve as a biocontrol agent to control *Sclerotium sclerotiorum* (Lib.) de Bary (Huang et al. 2000). Piirto et al. (1998) reported the occurrence of *Epicoccum nigrum* on giant sequoias [*Sequoiadendron giganteum* (Lindl.) Buchholz] and it is also known as a soft rotter of wood (Piirto et al. 1998).

*Byssochlamys* species and Group 1 fungal isolates were the predominant fungi occupying the study sites (Tables 4, 5, 6, and 7) (see Chapter 3 for further discussion on Group 1 fungal isolates). *Byssochlamys* spp. is an ascomycete genus characterized by rudimentary cleistothecia, which envelop asci during development in most ascomycetes.

Asci in *Byssochlamys* spp. are associated with unstructured wefts of white fine hyphae (Beuchat and Pitt 2002).

Piirto et al. (1998) reported a high incidence (38 %; 34 out of 90 fire scars) of *Byssochlamys fulva* isolated from fire scars of giant sequoia. *B. fulva* was found most commonly in fire scars in the 5-year burn group (85 %) versus unburned group (29 %) and 1-year burned (8 %). *Byssochlamys* spp. may have a great potential to serve as biological control agents for forest pathogens such as *Heterobasidion annosum* and *Armillaria* spp. Further study is required to determine what interactions occur between *Byssochlamys* spp. and *H. annosum*, *Armillaria* spp, or other forest pests. At this point, it remains unknown if *Byssochlamys fulva* was isolated from the roots of Douglas-fir and ponderosa pine in our study, ITS sequence information for *B. fulva* is unavailable in either GenBank or EMBL to allow more definitive comparisons.

Both *B. fulva* and *B. nivea* are heat-resistant fungi; ascospores can survive heat exposure up to 99 C (Beauchat and Pitt 2002). *B. nivea* has been shown to be a potential biocontrol species. It is known to inhibit *in vitro* growth of plant pathogenic fungi *Fusarium oxysporum* Schlecht, *Gaeumannomyces graminis* (Sacc.) von Arx and Olivier var. *tritici* J. Walker, *Phytophthora cinnamoni* Rands, *Pythium irregulare* Buisman, and *Rhizoctonia solani* Kuhn (Park et al. 2001). In this study, *Byssochlamys* spp. were not associated with obvious signs of pathogenicity ( $P = 0.8138$ ; Table 8), but a pathogenic role of this fungus cannot be ruled out. *Byssochlamys nivea* also has been found to cause cuticular disruption, inhibit hatching of eggs, and cause death in nematodes (Park et al. 2001).

Because of its heat resistant nature, fire could potentially be used to favor *Byssochlamys* spp., and could displace root pathogens such as *H. annosum* and *Armillaria ostoyae*. After determining the heat tolerance of root pathogens, a prescribed burn of particular heat intensity above the tolerance level of the pathogen could be used to destroy pathogenic inoculum and favor *Byssochlamys* spp., as long as the heat threshold remains under the tolerance range of *Byssochlamys* spp.

The presence of *Byssochlamys* spp. may remain relatively the same after the 2002 burn. Alternatively, the occurrence of *Byssochlamys* spp. may increase in years following the fire. Other fungal species, which are not heat-resistant, should decline in number or be completely eliminated. This process could allow *Byssochlamys* spp. to establish itself in a manner that could prevent or suppress the establishment of root pathogens and insect pathogens. Such interactions would be especially beneficial in dry forests since they are more prone to pest damage (Wenatchee National Forest 2000). These fungi could also aid in recycling nutrients into the soil, and also could also act as a catalyst for fungal succession after a burn.

Further research (e.g., *in vitro* tests studying interactions between *Byssochlamys* spp. and pathogenic fungi such as *Armillaria* spp. and *H. annosum*; inoculating insect larvae such as pine sawflies, western pine beetle, Douglas-fir tussock moth, Douglas-fir beetle, spruce budworm, and inoculating various conifer species to screen for pathogenic tendencies of *Byssochlamys* spp.) is needed to fully understand potential biological control capabilities of *Byssochlamys* spp., and how to manipulate its presence in a forest ecosystem on the basis of its thermophilic nature. The diversity of the fungal community in large woody roots after the burn treatment should reveal a composition of fungi that

have ruderal characteristics, including effective dispersal, rapid uptake in nutrients, rapid extension for resource capture, and stress tolerance. These characteristics are representative of fungi that favor disturbance (Pugh and Boddy 1988). Because surveys on fungi inhabiting older sections of woody roots have not been previously reported, it cannot be determined if the fungi found in this study are representative of fungi that favor non-stressed environments.

Neither host nor habitat type had any apparent influence on any of the fungal species isolated (Table 2). This may be due to closely related habitat types found across all of the study units. Although plant association did not significantly influence *Byssochlamys* spp. occurrence statistically, *Byssochlamys* spp. occurred in a higher percentage in the PSME series. This trend could be due to the fact that the PSME association is in a more moist environment versus the PIPO association, where the environment is drier.

The overall lack of association between host, plant series, and fungal occurrence could be due to relative similarity in the environmental conditions of Douglas-fir and ponderosa pine associations, or that these fungi are nonspecific. Another possibility is that the fungi found in this study inhabit a micro-environment that is more protected from disturbances, than many ectomycorrhizal fungi. Fungi that colonize fine woody roots are more exposed to the surrounding environment and are easily influenced by varying disturbance regimes (moisture, temperature, logging, fire, etc.). Stendall et al. (1999), found significantly less ectomycorrhizal biomass in soil cores from the top layer (litter and organic horizons) after fire; no significant differences were detected for the middle

and bottom layers of the fire plot cores. In the unburned, control plots, no significant change in ectomycorrhizal biomass was observed between years for any core layer.

The fungal community occupying older sections of woody roots may be less prone to dynamic change after a disturbance such as fire. Further investigations are needed to identify and determine the ecological roles of fungal species that occupy specialized niches that have not been previously known to be inhabited by fungi.

A more extreme variation in environmental conditions, such as moist habitats versus dry habitats, would more likely exhibit a significant influence on the types and number of fungal species isolated. This study was originally designed to determine the amount of *Armillaria* spp. and *Heterobasidion annosum* occupying the study sites before a scheduled 2002 burn. Because of the dry habitats, *Armillaria* spp. (McDonald et al. 1999) and *H. annosum* (P. Hessburg, per. comm.) were not expected to predominate. In 5 yrs, another survey is planned to examine the presence of these pathogens and other fungi to determine how fire treatments impacted the fungal communities inhabiting these sites, and determine if fungal diversity increased or decreased after fire treatments. It would be of further interest to study how fungi occupying these recently discovered niches influence the community dynamics of other fungi in the rhizosphere of forest trees.

#### *Considerations for future diversity studies*

To fully encompass the diversity of fungal communities within a geographic region, diverse sites are required that vary in moisture, elevation and aspect. To fully understand diversity of fungal flora in forest ecosystems, other parts of the host must be

examined, such as its bole, branches (upper and lower crown), leaves (upper and lower crown), fine roots, and rhizosphere. Such studies should span time and seasons. Also, a selective medium should be used for each class of fungi (e.g., basidiomycetes, ascomycetes, zygomycetes, etc.). Different methods of sterilization may be necessary and sample processing needs to be done as quickly as possible to avoid loss of fungal isolates. When using an increment borer, effective sterilization must be employed after each use. Ethanol may not be the most effective decontaminating agent since some fungi could be resistant to alcohol, e.g., *Byssochlamys* spp. (Brown and Smith 1957). The numerous fungal isolates that were obtained from such dry locations is indicative of the potential diversity of fungi that could be obtained from other environments that have not been well studied previously. The use of molecular tools can aid in faster identification in a culture-based study. These tools should save time in keying out species versus using morphology alone, tentative identification can be facilitated through GenBank, followed by morphological verification. Using molecular techniques to identify fungi directly from the substrate (which is beneficial for fungal species that cannot be cultured or are non-sporulating) can yield in a higher percentage of diversity, but the disadvantage is that there is no culture available for further identification of taxonomic status. We have not yet reached the point where identifications can be based solely on molecular data available in databanks, but the utility of molecular data is continually increasing.

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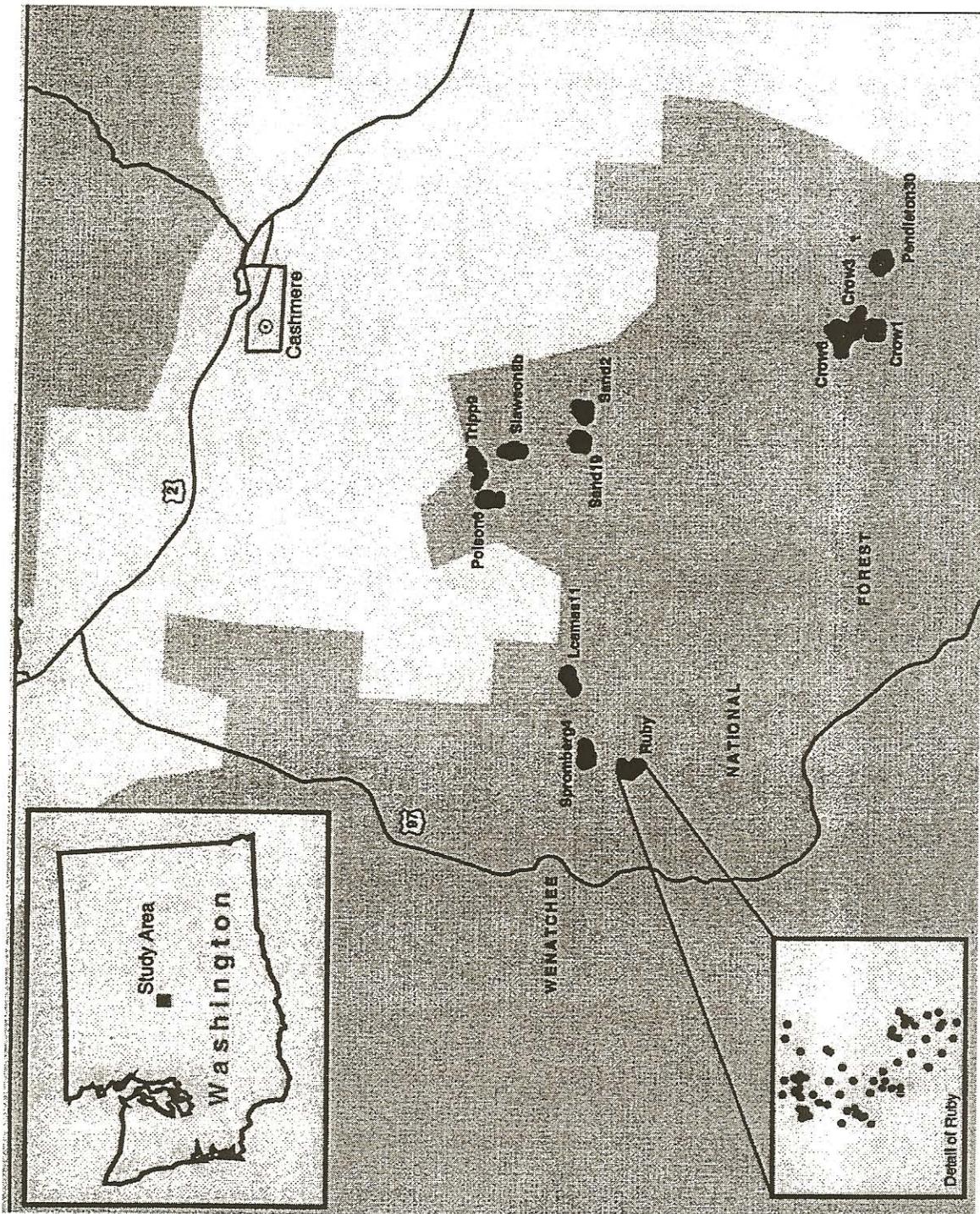


Fig. 1. Experimental plot locations in Wenatchee National Forest. Inset: individual trees sampled within unit Ruby.

Table 1: Fungal genera isolated from woody roots of ponderosa pine and Douglas-fir in decreasing order of frequency.

Taxon	# Isolations	% of Total	% Sequence identity with taxa from GenBank
<i>Byssochlamys</i> spp.	74	39	93-95 % U18361
Group 1	50	27	90-98 % AF133780, AF133782
<i>Mucor</i> sp. 1	11	5.9	Morphologically identified only
<i>Mucor</i> sp. 2	5	2.7	97-98 % AF412291
<i>Verticillium</i> sp.	4	2.1	99-100 % AF033410
<i>Tremella</i> sp.	4	2.1	96 % AF042453
<i>Penicillium</i> sp. 1	3	1.6	100 % AF034448
<i>Cladosporium</i> sp.1	2	1.1	98 % AF393714
<i>Epicoccum</i> sp.	2	1.1	100 % AF279486
<i>Mortierella</i> sp.	2	1.1	100 % AJ 271360
<i>Penicillium</i> sp. 2	2	1.1	99 % AJ279476
unknown zygomycete (oat-root assoc. fungus))	2	1.1	97 % AJ246162
<i>Cladosporium</i> sp. 2	1	1.1	100 % AF393712
<i>Penicillium</i> sp. 3	1	.005	99 % AF033410
<i>Penicillium</i> sp. 4	1	.005	99 % AF034450
<i>Penicillium</i> sp. 5	1	.005	100 % AF033493
<i>Penicillium</i> sp. 6	1	.005	99 % AF033459

<i>Penicillium</i> sp. 7	1	.005	97 % AF033445
<i>Penicillium</i> sp. 8	1	.005	99 % AF033489
<i>Eupenicillium</i> sp.	1	.005	94 % U18358
<i>Rhinoctadiella</i> sp.	1	.005	98 % AF229487
<i>Phlebiopsis</i> sp.	1	.005	99 % AF087488
<i>Ganoderma</i> sp.	1	.005	95 % AF255097
<i>Heterobasidion</i> sp.	1	.005	99 % X70023
<i>Phialophora</i> sp.	1	.005	94 % AF083200
<i>Hormonema</i> sp.	1	.005	98 % AF013228
<i>Merimbla</i> sp.	1	.005	93 % AF368298
<i>Pycnoporus</i> sp.	1	.005	94 % AF363772
<i>Alternaria</i> sp.	1	.005	98 % AF314587
<i>Hypocrea</i> sp.	1	.005	97 % AF275330
<i>Clavulina</i> spp.	1	.005	99 % AF335456
<i>Mycena</i> sp.	1	.005	92 % AF335444
<i>Pesotum</i> sp.	1	.005	95 % AF198248
<i>Mucor</i> sp. 3	1	.005	93 % AF474242
<i>Chrysosporium</i> sp.	1	.005	99 % AJ131680
<i>Ulocladium</i> sp.	1	.005	98 % AF229487
unidentified white mycelium	1	.005	93 % AB041994
unknown genus (Ericoid mycorrhizal)	1	.005	94 % AF072297

Table 2. Chi-square results for significance of plant association on fungal occurrence.

HOST	FUNGAL GROWTH		NO FUNGAL GROWTH		TOTAL (cores/series)		CHI-SQUARE
	# cores	%	# cores	%	# cores	%	
PSME	32	49	33	50	65	100	0.189
PIPO	156	46	181	54	337	100	
TOTAL	188	47	214	53	402	100	

df = 1,  $\alpha = 0.05$ ,  $P = 0.66 < 1.0$ .

PIPO = ponderosa pine  
 PSME = Douglas-fir

Table 3. Chi-square results for significance of host/plant association on fungal occurrence.

HOST/PLANT ASSOCIATION	FUNGAL GROWTH		NO FUNGAL GROWTH		TOTAL TREES (cores/host)		CHI-SQUARE
	# cores	%	# cores	%	# cores	%	
PIPO/PIPO	32	49	33	50	65	100	0.734
PIPO/PSME	83	48	89	51	172	100	
PSME/PSME	73	44	92	56	165	100	
TOTAL	188	47	214	53	402	100	

df = 2,  $\alpha = 0.05$ ,  $P = 0.18 < 1.0$ .

PIPO = ponderosa pine  
 PSME = Douglas-fir

Table 4. Chi-square results for occurrence of *Byssochlamys* spp. within a plant association.

HOST	<i>Byssochlamys</i> spp.		No <i>Byssochlamys</i> spp.		TOTAL (cores/series)		CHI-SQUARE
	# cores	%	# cores	%	# cores	%	
PSME	7	11	58	89	65	100	3.012
PIPO	67	20	270	80	337	100	
TOTAL	74	18	328	82	402	100	

df = 1,  $\alpha = 0.05$ , P = 0.08

PIPO = ponderosa pine  
PSME = Douglas-fir

Table 5. Chi-square results for occurrence of *Byssochlamys* spp. on host.

HOST/PLANT ASSOCIATION	<i>Byssochlamys</i> spp.		No <i>Byssochlamys</i> spp.		TOTAL (cores/host)		CHI-SQUARE
	# cores	%	# cores	%	# cores	%	
PIPO/PIPO	7	11	58	89	65	100	3.394
PIPO/PSME	32	19	140	81	172	100	
PSME/PSME	35	21	130	79	165	100	
TOTAL	74	18	328	82	402	100	

df = 2,  $\alpha = 0.05$ , P = 0.18 < .20.

PIPO = ponderosa pine  
PSME = Douglas-fir

Table 6. Chi-square results for occurrence of Group 1 within plant association.

HOST/PLANT ASSOCIATION	Group 1		No Group 1		TOTAL (cores/series)		CHI-SQUARE
	# cores	%	# cores	%	# cores	%	
PSME	10	15	55	85	65	100	0.618
PIPO	40	12	297	88	337	100	
<b>TOTAL</b>	50	12	352	88	402	100	

df = 1,  $\alpha = 0.05$ ,  $P = 0.43 < 1.0$ .

PIPO = ponderosa pine

PSME = Douglas-fir

Table 7. Chi-square results for occurrence of Group 1 on host.

HOST	Group 1		No Group 1		TOTAL (cores/series)		CHI-SQUARE
	# cores	%	# cores	%	# cores	%	
PIPO/PIPO	10	15	55	85	65	100	5.346
PIPO/PSME	27	16	145	84	172	100	
PSME/PSME	13	8	152	92	165	100	
<b>TOTAL</b>	50	12	352	88	402	100	

df = 2,  $\alpha = 0.05$ ,  $P = 0.07 < 0.10$ .

PIPO = ponderosa pine

PSME = Douglas-fir

Table 8. Chi-square results: Group 1 in symptomatic versus nonsymptomatic trees.

	Group 1 +		Group 1 -		TOTAL		CHI-SQUARE
	#	%	#	%	#	%	
<b>NONSYP</b>	26	9	268	91	294	100	*4.568
<b>SYMP</b>	14	17	68	83	82	100	
<b>TOTAL</b>	50	13	336	89	376	100	

df = 1,  $\alpha = 0.05$ ,  $P = 0.03 < 0.05$ .

Table 9. Chi-square results: *Byssochlamys* spp. in symptomatic versus nonsymptomatic trees.

	<i>Byssochlamys</i> spp. +		<i>Byssochlamys</i> spp. -		TOTAL		CHI-SQUARE
	#	%	#	%	#	%	
<b>NONSYP</b>	54	18	240	82	294	100	0.0555
<b>SYMP</b>	16	20	66	80	82	100	
<b>TOTAL</b>	70	19	306	81	376	100	

df = 1,  $\alpha = 0.05$ ,  $P = 0.81 < 1.0$

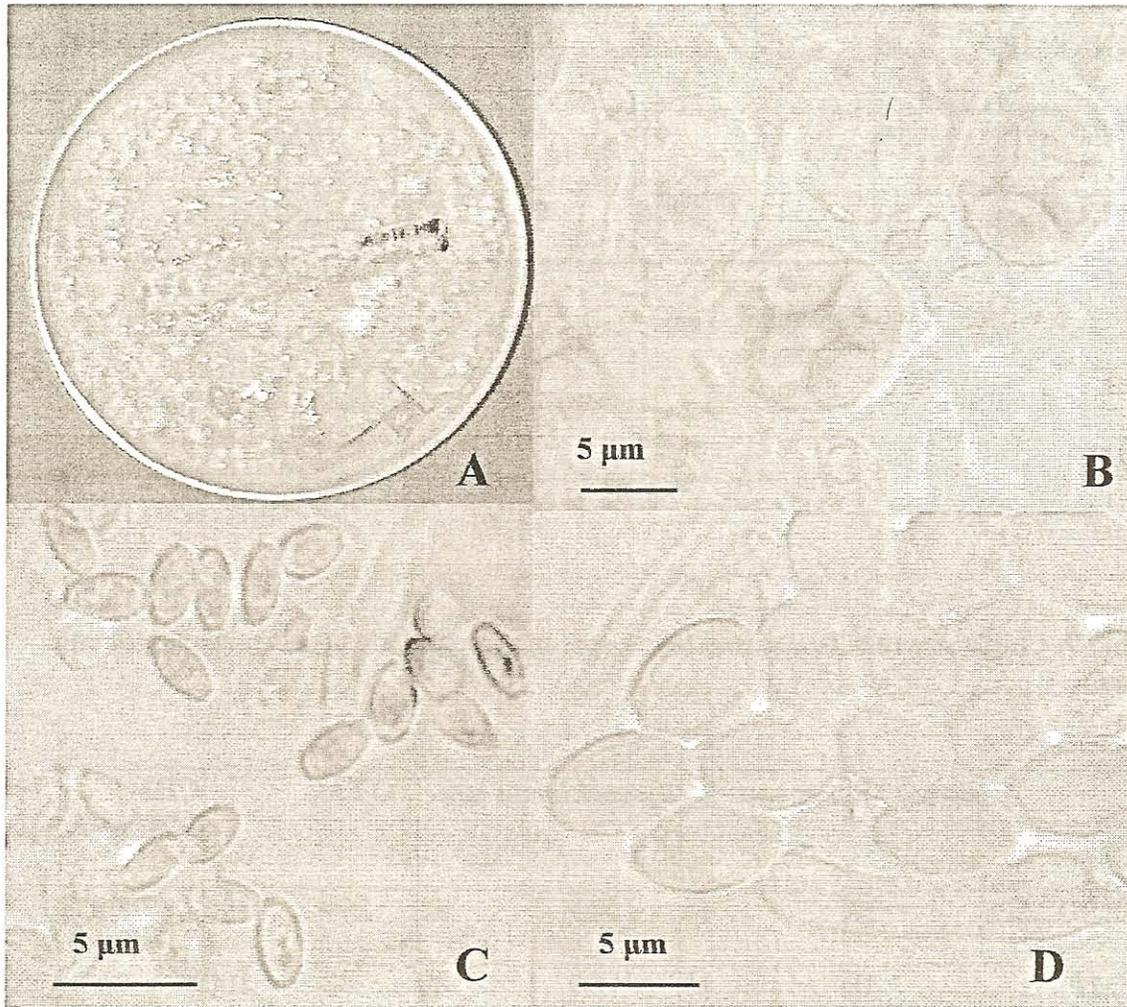


Fig. 2 A. culture of *Byssochlamys* sp.; B. asci containing ascospores; C. conidia; D. chlamydospores

## CHAPTER THREE

### DESCRIPTION AND POSSIBLE ECOLOGICAL SIGNIFICANCE OF GROUP 1 FUNGAL ISOLATES

#### Introduction

Ecologists are becoming increasingly aware that many critical interactions with plants occur below ground. In the soil, dynamic interactions among plant roots, animals, and microbes help determine composition of plant communities. The biodiversity that drives these interactions is poorly understood. As ecologists attempt to understand these interactions, a primary hindrance is that soil and roots can contain thousands of unidentified and potentially unculturable species of microorganisms and invertebrates (Copely 2000).

In this study, 50 isolates of a predominantly nonsporulating fungus were isolated from the woody roots of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] and ponderosa pine (*Pinus ponderosa* Douglas ex Lawson & C. Lawson). Twenty-seven different genera were isolated out of 188 total increment cores yielding fungal isolates (402 total increment cores processed), with the unknown group encompassing approximately 27 % of the fungal isolates collected. Sequence data of the small subunit (SSU) indicate this group of fungi belong to *Umbelopsis* or *Micromucor* (A. Scheußler, per. com.). Morphological features were insufficient for identification thus we will refer to these fungal isolates as Group 1 for the purposes of this study. *Umbelopsis* might be common in forest ecosystems: they been found in soil (K.L. O'Donnell per. comm.; R. Vilgalys, per. comm.), in large woody roots and boles of various conifer species (E.Vandegrift, per. comm.; J.W. Hanna, per. comm.; J.A. Hoff, unpublished), in the roots

of deciduous trees (Amos and Barnett 1966), in association with other fungi [e.g., *Peziza* sp.; (Kendrick et al. 1994)], and in association with tree diseases [e.g., blister rust canker, oak wilt; (B.A. Richardson, per. comm.; Amos and Barnett 1966)].

## **Objectives**

The specific objectives of this study were to:

- 1) Demonstrate the limitations of morphology and sequence data from the internal transcribed spacer region (ITS) of ribosomal DNA (rDNA) for identification of certain groups of fungi.
- 2) Determine effects of habitat type, host tree, and geographic location of Group 1 and distribution.

## **Materials and Methods**

### *Study Design*

Our study was conducted on south, west, and east aspects in the Mission Creek watershed, Wenatchee National Forest, Wenatchee, WA. On these aspects, a mixed-conifer forest is dominated by ponderosa pine and Douglas-fir. Generally, this type of dry forest dominates the eastern edge of the Wenatchee National Forest. A fire return interval of 10-20 years occurs over the 890,308 ha in the Wenatchee National Forest (<http://www.fs.fed.us/pnw/weblab>). Wenatchee National Forest receives less than 762 mm of annual precipitation.

The study consisted of 12 experimental treatment units ranging in size from 10-20 ha (Fig. 1) (Hessburg and Edmonds, unpublished). When the fire treatments are applied,

three units will serve as controls, three as burn-only [prescribed fire only, with periodic burns (Agee et al. 2001)], three as thin-only [periodic cutting, followed by mechanical fuel treatment and/or physical removal of residue; no use of prescribed fire (Agee et al. 2001)], and three as thin-and-burn [initial and periodic cutting, followed by prescribed fire; fire alone could be used one or more times between cutting intervals (Agee et al. 2001)] (Hessburg and Edmonds, unpublished). Units were placed on all aspects except north, on slopes < 40 %, with no more than 10 % rock cover. Areas that included rare plant species were avoided. Units were placed in a narrow range of dry forest environments, and were indicated by dry Douglas-fir potential vegetation types including the PSME-SPBEL (*Pseudotsuga menziesii*/ *Spiraea betulifolia* Pall.), PSME-PUTR (*P. menziesii*/*Purshia tridentata* (Pursh) DC.), PSME-CARU (*P. menziesii*/*Calamagrostis rubescens* Buckley), and PSME-SYAL (*P. menziesii*/*Symphoricarpos albus* (L.) S.F. Blake) plant associations (Williams and Smith 1991).

Pretreatment surveys of existing disease symptoms or mortality associated with root pathogens and fungi associated with woody roots of ponderosa pine and Douglas-fir occurred during the 2000 field season (May-September); thinned units were harvested in 2001 after sample collections, and burn treatment units will be ignited in 2002. Post-treatment surveys (identification and mapping of new symptoms or mortality associated with a root disease pathogen) will occur during the 2003 and 2004 field seasons.

#### *Area and Treatment Units*

The experimental area and treatment units are shown in Fig. 1. During pre- and post-treatment surveys, trees showing new symptoms or mortality associated with a root

pathogen were identified. Root pathogens of original interest were 1) *Phellinus weirii* (PHWE), the cause of laminated root rot, 2) *Heterobasidion annosum* (Fr.) Bref. (HEAN; both S- and P-groups), the cause of Annosum root disease, 3) *Armillaria* spp., especially *A. ostoyae* (Romagnesi) Herink (AROS), the cause of Armillaria root disease, and 4) *Leptographium wageneri* (Kendrick) M.J. Wingfeld (LEWA), the cause of black stain root disease (Hessburg and Edmonds, unpublished).

Trees with symptoms (e.g., thinning crown, topkill, resinosis) were identified. An increment borer, which was surfaced sterilized with ethanol after each use, was used to extract increment cores (ca. 5 mm in diameter) from the tree roots. Then, the root core was placed in a plastic soda straw with both ends of the straw sealed with masking tape, stored at 4 C, and shipped to the Forestry Sciences Laboratory (USDA Forest Service, RMRS, Moscow, ID) for fungal isolation and identification.

In addition to collecting root cores from all visibly symptomatic trees, trees without visible symptoms of root disease or other damaging agents were surveyed. When a healthy appearing tree was randomly sampled, the tree was mapped, site and tree attribute data were recorded, and one major lateral root was examined in the same manner as previously described (Hessburg and Edmonds, unpublished).

#### *Laboratory Processing of Samples*

The samples were stored on ice or at 4 C in a styrofoam cooler for 10 days to 4 weeks after collection in the field. Samples were processed within hours after arrival at the Forestry Sciences Laboratory, Moscow, ID. Depending on the length of the lateral root increment core, it was split into several pieces (ca. 2.5 cm). Each core piece was

submerged into 70 % ethanol, flame sterilized, and placed onto a 60 x 15 mm petri dish containing a medium selective for basidiomycetous fungi, then placed into an incubator at 21 C in the dark. The selective medium was a benomyl dichloran streptomycin (BDS) agar containing 15 g malt extract, 15 g agar, 40 mg benomyl, 20 mg dichloran (2,6-dichloro-4-nitroaniline), and 100 mg streptomycin per L (Worrall and Harrington 1993).

After 2 months of incubation, the cores were examined for mycelial growth. Subcultures were made on BDS, a nutrient-rich medium (3 % dextrose, 1 % peptone, 1.5 % agarose, and 3 % malt extract), and 2 % malt extract agar (MEA) medium. Cores that supported mycelial growth limited to wood were placed on a non-selective medium (MEA and/or nutrient rich medium) to facilitate growth.

#### *Morphological characterization*

To morphologically characterize fungal isolates, specimens were stained with Melzer's Reagent (Melzer 1924), and examined with bright-field and phase-contrast optics using an Olympus BX 60 microscope. Each isolate was examined for morphological characters, such as hyphae, sporangiophores, sporangiospores, and chlamydospores. Also, variations in colony color were noted. Photos were taken with a Nikon Cool Pix<sup>®</sup> digital camera (Melville, NY). Permanent slides were prepared using Shurmount<sup>®</sup> aqueous mounting medium (Triangle Biomedical Sciences, Durham, NC). Representative cultures will be deposited into American Type Culture Collections (ATCC) (Manassas, VA), and representative cultures will also be archived at the USDA Forest Service, Forestry Sciences Laboratory (Moscow, ID).

### *Use of Polymerase Chain Reaction (PCR) as a Diagnostic Tool*

A mycelial scrape was placed in a 50- $\mu$ l PCR amplification mixture. When direct mycelial scraping did not provide a suitable DNA template for PCR, mycelial samples were treated with Lyse-N-Go™ PCR reagent (Pierce, Rockford, IL) following the protocol of the manufacturer. Each 50- $\mu$ l PCR amplification mixture contained 1.2 U AmpliTaq® (Applied Biosystems, Foster City, CA), 5  $\mu$ l 10X PCR buffer (Applied Biosystems), 200  $\mu$ M dNTPs, 4.0  $\mu$ M MgCl<sub>2</sub>, 0.5  $\mu$ M ITS-1 primer, and 0.5  $\mu$ M ITS-4 primer (White et al. 1990). The cycling parameters were modified from Pimentel et al. (1998): DNA was denatured for 5 min at 94 C, followed by 30 cycles of denaturation at 94 C for 90 s, annealing at 50 C for 1 min 30 s, and extension at 72 C for 2 min 30 s. A final extension was performed at 72 C for 5 min. Amplified products (4  $\mu$ l) were subjected to agarose-gel electrophoresis at 120 V for 2 hr, then viewed with UV light after staining in ethidium bromide.

DNA products were prepared using Exosap-IT (USB, Cleveland, OH) PCR product clean up, following the protocol of the manufacturer. The reaction conditions were 37 C for 15 min followed by 80 C for 15 min. The DNA products were sent to Amplicon Express (Pullman, WA) for sequencing on an ABI 377 sequencer (Applied Biosystems, Foster City, CA).

Single-stranded sequences were trimmed on both ends, then used to generate double-stranded contigs using BioEdit (Hall 2001). Multiple sequences were aligned in ClustalX (Higgins et al. 1996) using default settings and checked visually. Cluster analysis was then performed to demonstrate the diversity of phylogenetic clades where these fungi are found. Phylogenetic analysis was performed using MEGA2: Molecular

Evolutionary Genetics Analysis software (Sudhir et al. 2001). Representative of rDNA (ITS-1, 5.8S, ITS-2) sequences will be deposited at GenBank.

## Results

A total of 402 increment cores were processed. Of the 402 cores processed, 188 of the cores yielded fungal growth, and Group I constituted 27 % of those isolates (Refer to Chapter 2).

### *Host and Habitat Influence on Group 1 Occurrence*

Group 1 was not significantly associated with plant association ( $P = 0.43 < 1.0$ ) or host species ( $P = 0.07 < 0.10$ ), and were found across all of the sites (Tables 6 and 7, Chapter 2). They were isolated from 37 ponderosa pines out of a total of 144, in PIPO and PSME series, and isolated from 13 Douglas-fir (in PSME series) out of a total 165 (Table 6, Chapter 2). In addition, Group 1 isolates showed a significant association with symptomatic trees ( $P = 0.0326 < 0.05$ ) (Table 8, Chapter 2).

### *Sequence analysis*

The ITS-1, 5.8S, ITS-2 rDNA sequences of Group 1 fungal isolates were entered into GenBank for species identification. BLAST results showed that sequences from several of Group 1 isolates had high sequence identity with *Acaulospora colossica* Schultz, Bever and Morton rDNA sequences deposited into GenBank by Pringle et al. (2000), specifically, the Type 2 cluster they acquired from spore types IVB.10 and IVB.12 collected from a site in Durham, NC. These results were unexpected because, unlike arbuscular mycorrhizal (AM) fungi, our Group 1 isolates were derived from

woody roots of conifers, and the fungi were able to grow independently in culture, without the aid of plant material. An isolate subset (CROW1 G 8-2-2, SI.8b A3-3-6, CROW1 F5-3-3, CROW3 C7-2-4 ) was sent to A. Schüßler (Institute of Botany TU Darmstadt, Germany) for rDNA small subunit (SSU; 17S) sequencing.

Due to high variability within the ITS regions of zygomycetes, we compared the 5.8S rDNA sequence with the database of Redecker et al. (1999). Cluster analysis with the 5.8S rDNA sequences of Glomalean fungi, basidiomycetes, and ascomycetes (Redecker et al. 1999) defined one group of our fungal isolates (Group 1) (Fig. 2). They separated into a distinct cluster in comparison with the other groups of fungi, but also included in this cluster were *A. colossica* spore types IVB.10 and IVB.12 (Pringle et al. 2000), which also displayed the highest ITS sequence identity (95-98 %) to Group 1. ITS sequences of *Umbelopsis* spp. and *Micromucor* spp. were not available in GenBank or EMBL for comparison with Group 1. However, culture isolates or ITS sequences of *Umbelopsis* spp. and *Micromucor* spp. were provided by E. Vandegrift, (Oregon State University), R. Vilgalys (Duke University), and K.L. O'Donnell (USDA-ARS) for incorporation and comparison with the 5.8S sequence dataset of Redecker et al. (1999). These sequence data were compared with our Group 1 isolates and other fungal groups. Bootstrap values (65 %) do not support the distinction between *Umbelopsis* spp. or *Micromucor* spp (Fig. 2).

ITS phylogenetic analysis indicates that there is sequence variability among Group 1 fungal isolates (Fig. 3). Within the clade that constitutes Group 1 (100 % bootstrap support), 2 distinct clusters formed, with three of the ITS sequences from Group 1 clustering closely with *Umbelopsis isabellina*, while the other sequences are not

associated directly with either *Umbelopsis isabellina* or *Micromucor rammanianus* Möller. Thus, it is difficult to conclusively determine the taxonomic identity of Group 1.

### *Morphology*

Group 1 isolates produced abundant growth on BDS, a nutrient-rich medium, and 2% (MEA). Culture colonies ranged from cream to tan in color, and some exhibited a grayish color. Three isolates had colonies which were pink (Fig. 3). The mycelia and chlamydospores were filled with lipid droplets, that were visible microscopically (Fig. 3). Hyphae were coenocytic, with an apparently random branching pattern. Intercalary and terminal chlamydospores were formed that were 20-(49) x 22-(49)  $\mu\text{m}$  in diameter (Fig. 3). Sporangia, with sporangiospores were occasionally observed in some of the isolates (Fig. 3).

### **Discussion**

Morphologically, it is difficult to conclusively determine whether our Group 1 fungal isolates are identical to *Umbelopsis* or *Micromucor*. These isolates tend to demonstrate morphological variability among chlamydospores within isolates (Fig. 4). Sporangiospores have been reported that were seemingly abundant and highly variable in *Umbelopsis versiformis* (Amos and Barnett 1966; Kendrick et al. 1994). Our Group 1 fungal isolates and *Umbelopsis versiformis* have both been isolated from woody roots of trees. However, Group 1 was isolated from conifers, whereas *Umbelopsis* species were previously isolated from deciduous trees and from a *Peziza* species (Amos and Barnett 1966; Kendrick et al. 1994).

Variability of the ITS sequences within Group 1 fungal isolates may indicate that ITS sequences alone may be insufficient for taxonomic classification of this particular fungal group. Perhaps a more in depth sequence analysis of other regions of the genome (i.e., SSU or LSU) may give more definitive insights into the taxonomic classification of these isolates.

Morphologically, Group 1 isolates are of zygomycetous nature based on the morphology of the sporangia and chlamydozoospores (Fig. 4). However, phylogenetically, it is difficult to assess exactly where Group 1 fits (Figs. 2 and 3) within the Zygomycota, and separation of *Micromucor* and *Umbelopsis* is not well-supported by bootstrap values in the phylogenetic analysis of 5.8S sequences (65 %, Fig. 2). However, phylogenetic analysis of ITS, 5.8S, and ITS 2 sequences indicates a separation of these two species (83 %, Fig. 3). Neither morphological data, 5.8S sequence data, nor ITS sequence data were sufficient for definitive classification of Group 1 within the zygomycetes, although SSU data reveal a similarity to *Umbelopsis isabellina* (A. Schüßler, per. com.). This ambiguity is partially due to the current state of uncertainty regarding zygomycete taxonomy. Voigt and Wöstemeyer (2001) examined the taxonomic placement of *Umbelopsis* spp. and *Micromucor* spp. using sequence data from the actin and translation elongation factor (EF-1 $\alpha$ ) genes, and considered these genera as members of the Mucorales rather than the Mortierellales where they have been placed. Based on 18S and 28S rDNA, Voigt et al. (1999) stated that *Micromucor ramannianus* represents one of the basal divergences within the Mucorales. O'Donnell et al. (2001) using 18S rDNA, 28S rDNA, and EF-1 $\alpha$  gene exons showed that a *Micromucor-Umbelopsis* clade, traditionally included in the Mortierellaceae, was the basal sister-group to all other Mucorales. Their

report showed that traditional family-level classification schemes for this order appear to be highly ambiguous as evidenced by polyphyly of four of the seven families containing two or more genera.

The fungal kingdom has been delineated by the use of morphological, biochemical, and molecular studies. However, phylogenetic groupings at the more 'primitive' end of the clade, including Zygomycota, have yet to be defined. Ascomycetes and basidiomycetes are monophyletic (Schüßler et al. 2001), but according to Voigt and Wöstemeyer (2001), a complete revision of the classification of zygomycetes is needed.

#### *Considerations of using ITS for determining fungal phylogenies and identification*

The results in this study indicate limitations of internal transcribed spacer region (ITS) and 5.8S rDNA for phylogeny and identification, related to high variability of ITS in some fungal taxa (Schüßler 1999). Apparent similarity of ITS sequences may indicate a potential affiliation of this fungal group with Glomalean fungi. Alternatively, relationships among ITS sequences may reflect high ITS sequence divergence found within Glomales and Group 1. Sequence data from other regions of the genome could provide more information for discerning species and evaluating phylogenetic relationships. Commonly, the SSU region of rDNA is used in Glomalean fungal diversity and phylogeny (Simon et al. 1992; Clapp et al. 1995; Sanders et al. 1996; Bago et al. 1998; Sawaki et al. 1999; Schüßler 1999; Redecker et al. 1999; O'Donnell et al. 2001).

This and other studies are hampered by uncertainty in the reliability of taxonomic identification of sequences entered into databases. This issue is particularly relevant for fungi that cannot be cultured, have non-sporulating strains, or fungi for which taxonomic

status is tenuous. Glomalean fungi are obligate symbionts and must be cultured in association with a host plant. As a consequence, these fungi are difficult to maintain free from contaminating organisms. In a recent study on a number of Glomalean fungi, about 30 % of the cleaned single spores investigated gave rise to sequences from plant, animal, or ascomycetous contaminants (Schüßler 1999). The questionable validity of Glomalean sequences was further emphasized by Redecker et al. (1999) who reported that a published SSU r RNA sequence for *Scutellospora castanea* did not cluster within the Glomaceae, but within the Ascomycetes, with very high bootstrap support. The published sequence was probably derived from an ascomycetous contaminant. Another possibility they proposed was that *S. castanea* contains Ascomycete-related SSU rRNA genes, but that hypothesis is much less probable when compared with the assumption that the DNA isolated from some of the spores investigated was simply contaminated.

This study supports that molecular, morphological, and physiological characters are necessary to assess the taxonomic relatedness and ecology of fungal organisms.

#### *Ecological significance of Group 1*

Group 1 isolates are fungi that can inhabit living roots of woody plants or function as hyperparasites of other fungi that are inhabiting woody roots; however, their exact ecological function remains unknown. Group 1 isolates were found on both ponderosa pine and Douglas-fir on dry sites. Thus, neither habitat type nor host species have an apparent strong influence on the presence of this group of fungi (Tables 6 and 7, Chapter 2). In this study, Group 1 isolates were correlated with damage symptoms (Table 8, Chapter 2). At this point it is difficult to assess what factors are driving this relationship.

One possibility is that Group 1 is carried in with insects and establishes itself in trees that are already stressed. This fungus could be occurring in association with pathogens that are actually responsible for pathogenicity. Further research, including a test of Koch's postulates, is warranted to arrive at a more definitive conclusion on the role of pathogenicity, if any, of this fungus.

Other occurrences indicate that these fungi may commonly occur in forest ecosystems. A similar fungus was isolated from wood of Douglas-fir located in a mortality center near Chewelah, WA (J.W. Hanna, USDA Forest Service, Moscow, ID, per. comm.) and from a white pine blister rust (*Cronartium ribicola* Fischer) canker on white pine (*Pinus monticola* Douglas ex D. Don) near Bovill, ID (B.A. Richardson, USDA Forest Service, Moscow, ID, per. comm.). Amos and Barnett (1966) isolated an *Umbelopsis* species while isolating *Ceratocystis fagacearum* (Bretz) Hunt in the roots of naturally infected oak trees.

Recently, E. Vandegrift (Oregon State University, per. comm.) isolated a similar fungus from woody roots of Douglas-fir, western hemlock [(*Tsuga heterophylla* (Raf.) Sarg.], Sitka spruce [(*Picea sitchensis* (Bong.) Carrière), ponderosa pine, and lodgepole pine (*Pinus contorta* Louden). Her study area covers three geographic regions: Oregon coast, Washington Cascades, and high desert in Oregon. She also observed a high ITS sequence similarity with *Acaulospora colossica* (Pringle et al. 2000) (E. Vandegrift, per. comm.) An ITS sequence obtained from Vandegrift clustered with Group 1 (Figs. 2 and 3). Additionally, the SSU and LSU sequence data of her isolates demonstrated high sequence similarity with *Umbelopsis isabellina* (E. Vandegrift, per. comm.).

Alternatively, Group 1 fungi may function as mycorrhizal symbionts, endophytes, or possible agents of biological control. One goal of this study was to survey for *Heterobasidion* spp. and *Armillaria* spp. However, the occurrence of these two pathogens and other pathogenic fungi was limited in the study sites. Because Group 1 fungi were among one of the predominant fungi isolated, further studies are needed to determine if interactions are occurring among Group 1, *Heterobasidion* spp., *Armillaria* spp., and other root pathogens, and bole pathogens.

Group 1 fungi may impact ecosystem processes in an indirect manner. For example, these fungi may alter the rhizosphere to allow colonization of other fungi that can compete with pathogenic fungi. A number of questions remain to be answered: How do these fungi enter woody roots? What sort of symbioses (e.g., competitive, mutualistic, commensalistic, or parasitic) are occurring with this group and other fungi in the rhizosphere? Is competition occurring among them, or is mutualistic symbiosis occurring? And, how do these biological interactions affect the surrounding plant community? Thus, ecological interactions must be considered at multiple levels to determine potential ecological roles of Group 1 fungi.

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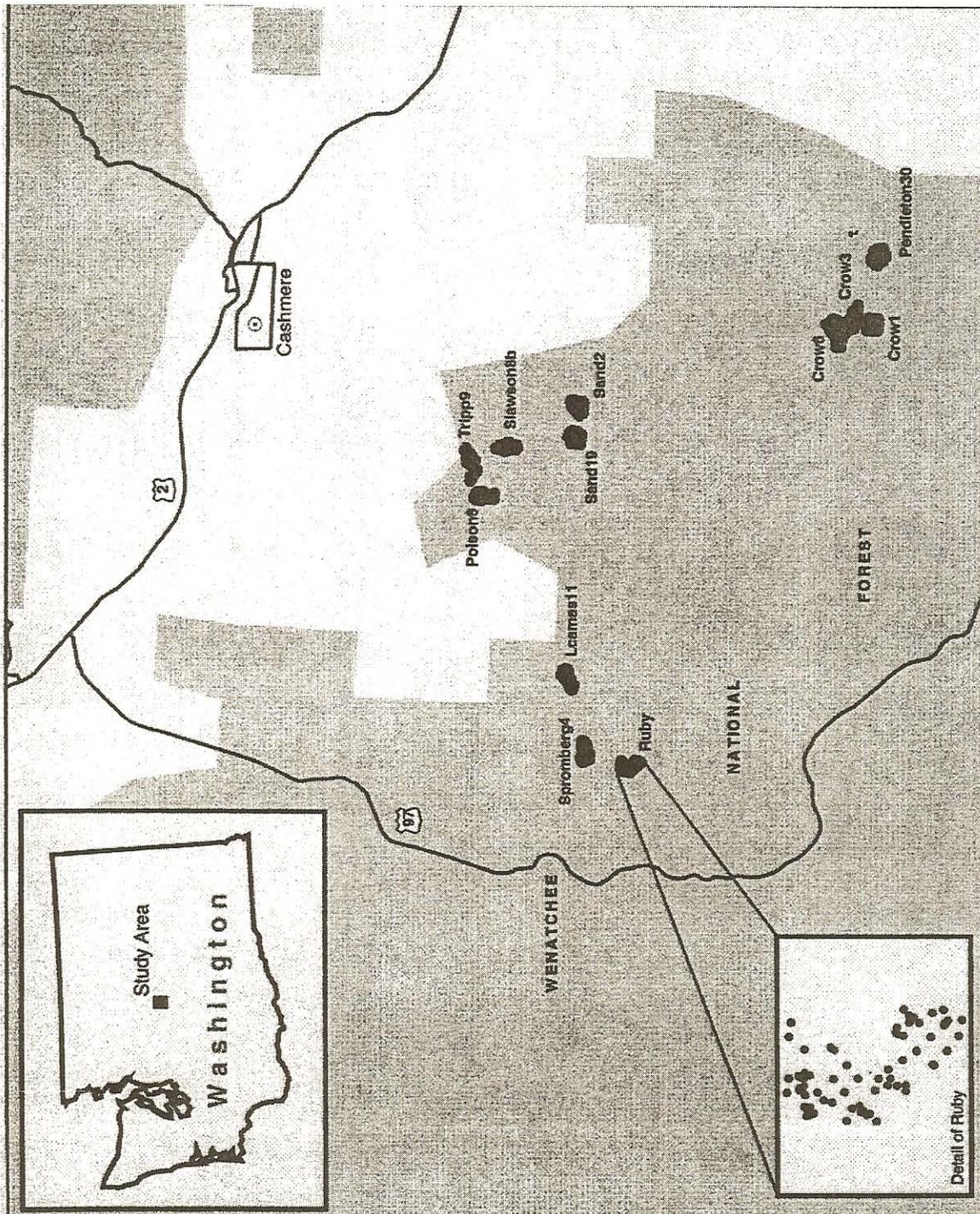


Fig. 1. Experimental plot locations in Wenatchee National Forest. Inset: individual trees sampled within unit Ruby.

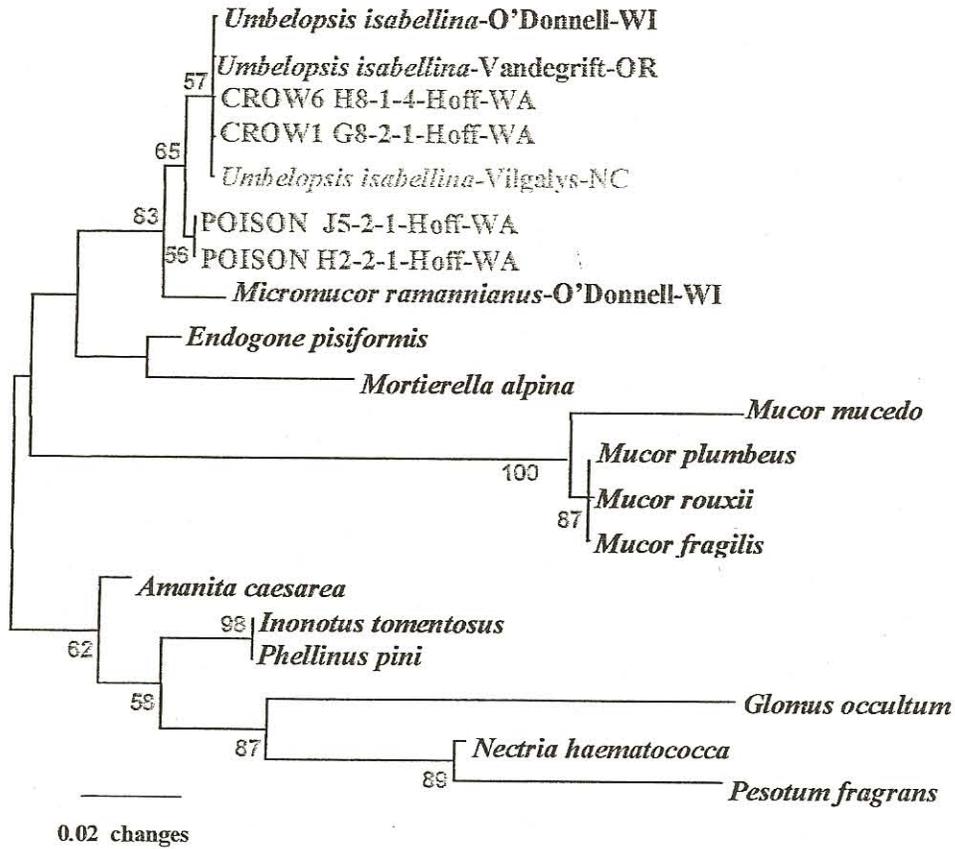
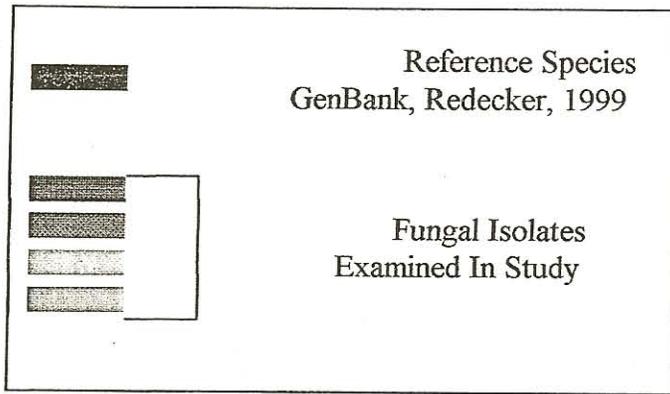


Fig.2. Neighbor-joining tree (1000 bootstraps) of fungal isolates and reference species based on 5.8 S rDNA. Bootstrap values under 50 % were not included in the tree.

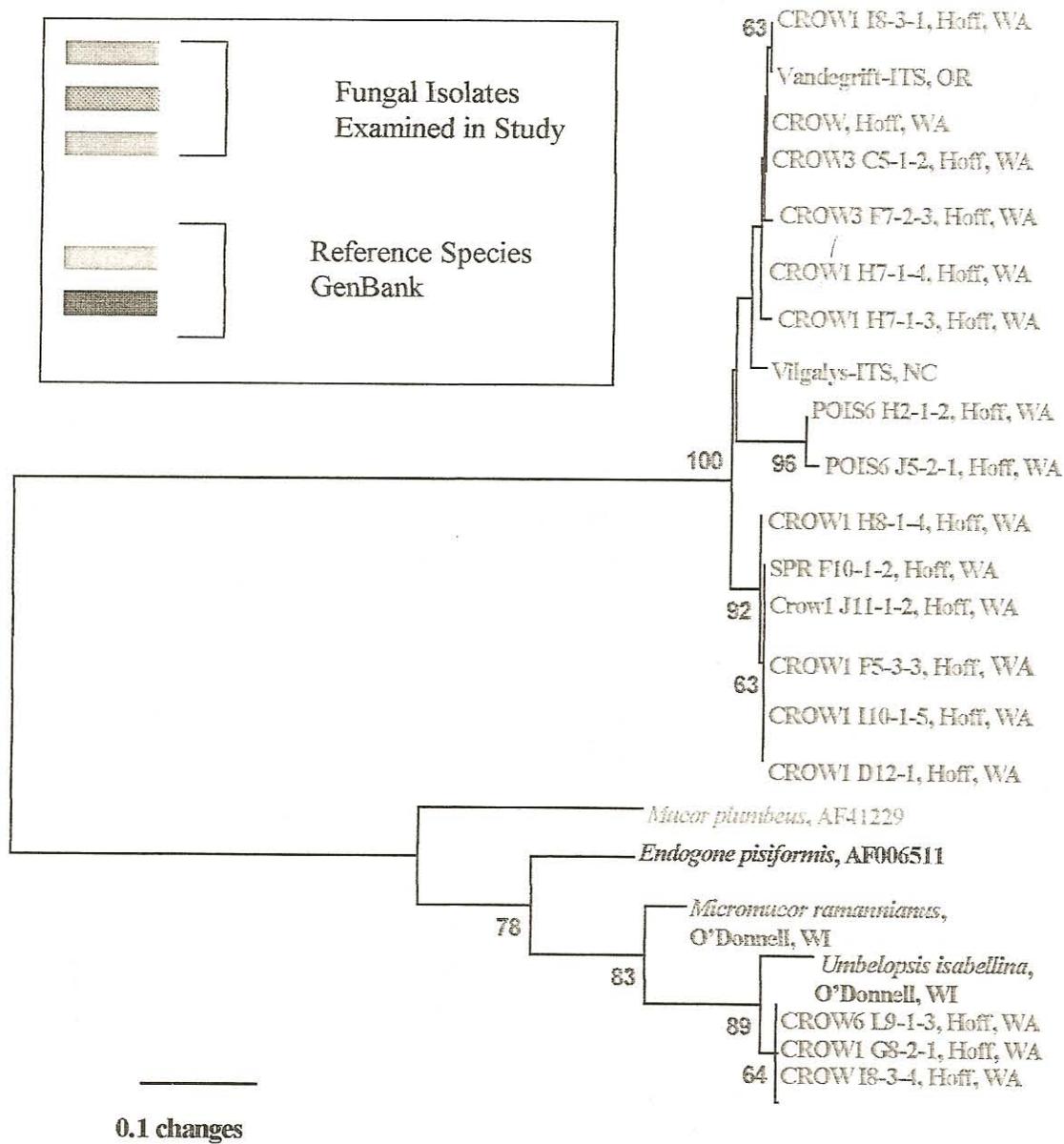


Fig. 3. Neighbor-joining tree (1000 bootstraps) of ITS-1 and ITS-4 rDNA fungal sequences examined and reference sequences from genBank. Bootstrap values under 50 % were not included in tree.

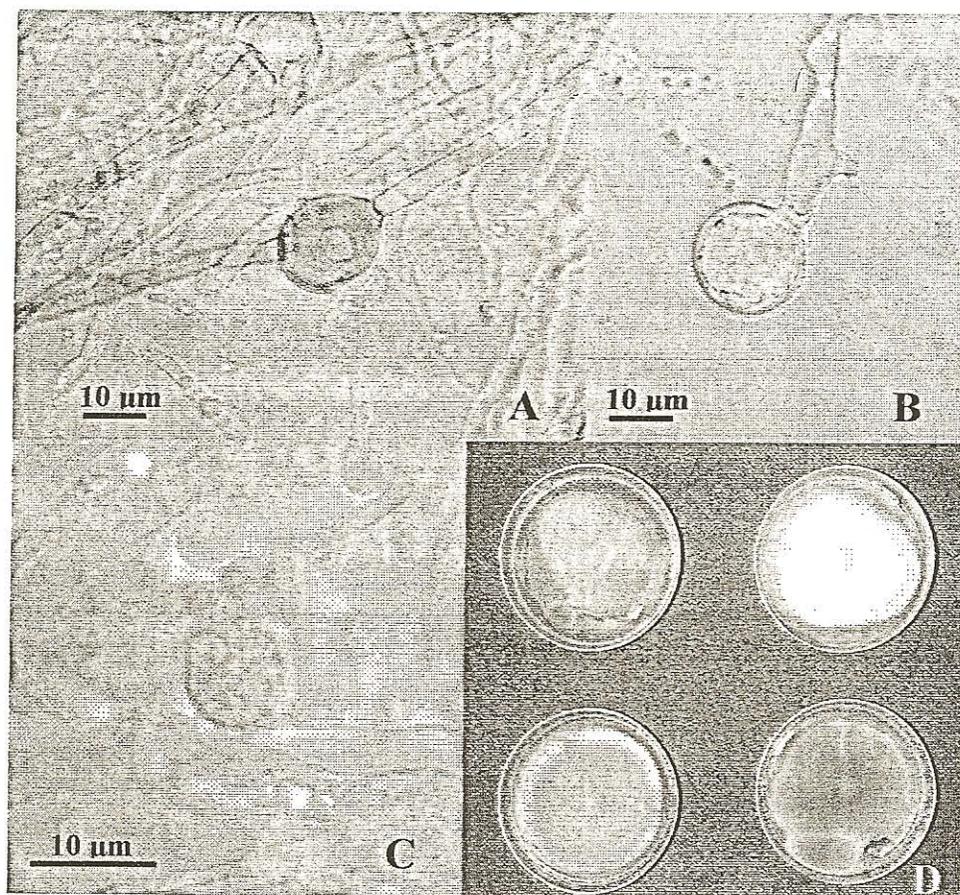


Fig. 3. Morphology of Group 1 fungal isolates A: Intercalary chlamydospores; B: Terminal chlamydospores; C: Sporangia; D. Color variations in colonies

**APPENDIX**

B. Summary Table of Fungal Taxa Found Within Each Study Unit.

UNITS	NO GROWTH	BYSSOCHLAMYS SPP.	GROUP 1	ALTERNARIA SP.	CHRYSOSPORIUM SP.	CLADOSPORIUM SPP.	CLAVULINA SP.	EPICOCUM SPP.	EUPENICILLIUM SP.	GANODERMA SP.	HETEROBASIDIUM SP.	HORMONEMA SP.	HYPOCREA SP.	MERIMBLA SP.	MUCOR SPP.	MORTIERELLA SP.	MYCENA SP.	PENICILLIUM SPP.	PESOTUM SP.	PHLEBIOPSIS SP.	PHIALOPHORA SP.	PYCNOPORUS SP.	RHINOCADIELLA SP.	TREMELLA SP.	TYROMYCES SP.	ULOCADIUM SP.	VERTICILLIUM SP.	ERICOID MYCORRHIZAL	UNIDENTIFIED WHITE MYCELIUM	UNKNOWN ZYGOMYCETE	UNIDENTIFIED	
CROW 1	15	9	22	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	
CROW 3	11	10	7	0	0	2	0	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
CROW 6	25	5	10	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	1	0	0	0	1	9	
L. CAMUS	16	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	
PENDLETON 30	18	7	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	
POISON 6	19	3	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
RUBY	11	4	0	0	0	0	0	0	0	1	0	0	0	0	6	1	0	2	1	0	0	0	0	0	0	0	0	0	0	0	4	
SAND 19	27	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	
SAND 2	17	10	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	
SLAWSON	17	5	2	0	0	1	1	0	0	0	0	0	1	1	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SPROMBERG 4	18	5	5	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	
TRIPP 9	20	10	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	

A. Table of Fungal Isolates Containing Host and Habitat Information.

SITE	UTM cell	TREE NUMBER	UNIT ID	PLANT ASSOCIATION	UNDERSTORY	PHASE	TREE SPECIES	STATUS	DBH	BRIGHT	Radial Growth	LCR	ELEVATION	ASPECT	ITS B	ITS % IDENTITY/ACCESSION NUMBERS	MORPHOLOGICAL IDENTIFICATION
CROW1	C8	1	C8-1	FSME SVAL	AGSF	PIPO	NONS	17.1	74	8	50	2860	30			<i>Mycos. sp. - Byssochlamys sp.</i>	
CROW1	D10	1	D10-1	FSME SVAL	AGSF	PIPO	TPKL	16.7	67	7	20	2920	277			<i>Mycos. 92 % AF135444</i>	
CROW1	D12	1	D12-1	FSME PUTR	AGSF	PIPO	NONS	14.5	53	10	70	2930	125			<i>Umb. dep. sp. - Microascus</i>	
CROW1	D5	1	D5-1	FSME SVAL	AGSF	FSME	NONS	19.4	91	11	80	2600	152			<i>Umb. dep. sp. - Microascus</i>	
CROW1	D7	1	D7-1	FSME SVAL	AGSF	PIPO	NONS	14.4	74	8	40	2850	174			<i>Umb. dep. sp. - Microascus</i>	
CROW1	D9	1	D9-1	PIPO PUTR	AGSF	PIPO	NONS	27.6	71	9	75	2860	226			<i>Umb. dep. sp. - Microascus</i>	
CROW1	E10	1	E10-1	FSME SVAL	AGSF	PIPO	NONS	11.8	34	5	50	2850	245			<i>Umb. dep. sp. - Microascus</i>	
CROW1	E11	1	E11-1	FSME PUTR	AGSF	PIPO	TPKL	17.5	76	5	25	2900	194			<i>Umb. dep. sp. - Microascus</i>	
CROW1	E12	1	E12-1	FSME SVAL	AGSF	PIPO	NONS	11.6	62	8	20	2910	130			<i>Umb. dep. sp. - Microascus</i>	
CROW1	E6	1	E6-1	FSME SVAL	CARU	PIPO	NONS	10.8	61	6	40	2530	146			<i>Umb. dep. sp. - Microascus</i>	
CROW1	E8	1	E8-1	FSME SVAL	AGSF	PIPO	DEAD	22.1	9	NA	NA	2830	188			<i>Umb. dep. sp. - Microascus</i>	
CROW1	E8	11	E8-11	FSME SVAL	AGSF	PIPO	NONS	11.9	71	10	50	2810	190			<i>Umb. dep. sp. - Microascus</i>	
CROW1	E8	12	E8-12	FSME SVAL	AGSF	PIPO	SYMP	11.4	64	9	50	2810	250			<i>Umb. dep. sp. - Microascus</i>	
CROW1	F11	2	F11-2	FSME SVAL	AGSF	PIPO	NONS	11.8	71	8	45	2880	204			<i>Umb. dep. sp. - Microascus</i>	
CROW1	F5	3	F5-3	FSME PUTR	CARU	FSME	NONS	19.7	92	19	70	2540	148			<i>Umb. dep. sp. - Microascus</i>	
CROW1	F6	5	F6-5	FSME SVAL	PIPO	TPKL	21.9	86	5	15	2500	150				<i>Umb. dep. sp. - Microascus</i>	
CROW1	F6	6	F6-6	PIPO SVAL	PIPO	DEAD	10.7	69	4	0	2540	146				<i>Umb. dep. sp. - Microascus</i>	
CROW1	F7	1	F7-1	FSME SVAL	CARU	PIPO	NONS	19.7	89	14	55	2820	167			<i>Umb. dep. sp. - Microascus</i>	
CROW1	F9	1	F9-1	FSME SVAL	AGSF	PIPO	NONS	9.6	60	10	25	2810	195			<i>Umb. dep. sp. - Microascus</i>	
CROW1	G10	1	G10-1	FSME SVAL	AGSF	PIPO	NONS	11.8	42	16	60	2780	167			<i>Umb. dep. sp. - Microascus</i>	
CROW1	G12	1	G12-1	PIPO SVAL	PIPO	NONS	10.2	55	11	35	2490	168				<i>Umb. dep. sp. - Microascus</i>	
CROW1	G6	1	G6-1	FSME SVAL	CARU	PIPO	TPKL	14.0	66	4	20	2810	224			<i>Umb. dep. sp. - Microascus</i>	
CROW1	G8	1	G8-1	FSME SVAL	CARU	PIPO	TPKL	14.0	66	4	20	2810	224			<i>Umb. dep. sp. - Microascus</i>	
CROW1	G8	2	G8-2	FSME SVAL	AGSF	PIPO	NONS	20.1	91	11	55	2800	218			<i>Umb. dep. sp. - Microascus</i>	
CROW1	H11	1	H11-1	PIPO SVAL	CARU	PIPO	NONS	16.1	98	17	55	2470	274			<i>Umb. dep. sp. - Microascus</i>	
CROW1	H5	4	H5-4	FSME SVAL	FSME	NONS	19.0	98	13	45	2530	60				<i>Umb. dep. sp. - Microascus</i>	
CROW1	H7	1	H7-1	FSME SVAL	CARU	PIPO	TPKL	21.5	77	4	20	2790	167			<i>Umb. dep. sp. - Microascus</i>	
CROW1	H7	2	H7-2	FSME SVAL	CARU	PIPO	NONS	8.1	39	5	30	2790	150			<i>Umb. dep. sp. - Microascus</i>	
CROW1	H8	1	H8-1	PIPO PUTR	AGSF	PIPO	DEAD	9.1	42	NA	NA	2800	234			<i>Umb. dep. sp. - Microascus</i>	
CROW1	H9	1	H9-1	FSME SVAL	AGSF	PIPO	NONS	8.3	50	3	10	2750	233			<i>Umb. dep. sp. - Microascus</i>	
CROW1	H10	1	H10-1	FSME SVAL	AGSF	PIPO	NONS	10.4	63	10	50	2720	244			<i>Umb. dep. sp. - Microascus</i>	
CROW1	H12	1	H12-1	PIPO SVAL	AGSF	PIPO	NONS	12.9	50	10	50	2420	155			<i>Umb. dep. sp. - Microascus</i>	
CROW1	I8	1	I8-1	FSME SVAL	CARU	PIPO	SYMP	10.6	69	7	30	2780	110			<i>Umb. dep. sp. - Microascus</i>	
CROW1	I8	2	I8-2	FSME SVAL	CARU	PIPO	SYMP	16.0	66	4	10	2780	192			<i>Umb. dep. sp. - Microascus</i>	
CROW1	I8	3	I8-3	FSME SVAL	CARU	PIPO	SYMP	19.5	67	3	35	2800	247			<i>Umb. dep. sp. - Microascus</i>	
CROW1	J11	1	J11-1	PIPO SVAL	AGSF	PIPO	SYMP	19.0	80	10	20	2720	200			<i>Umb. dep. sp. - Microascus</i>	
CROW1	J11	2	J11-2	FSME SVAL	PUTR	PIPO	NONS	18.1	73	10	70	2430	158			<i>Umb. dep. sp. - Microascus</i>	
CROW1	J7	2	J7-2	FSME SVAL	PIPO	NONS	8.8	62	2	NA	NA	95				<i>Umb. dep. sp. - Microascus</i>	
CROW1	J9	1	J9-1	FSME SVAL	CARU	PIPO	NONS	11.2	69	6	25	2770	161			<i>Umb. dep. sp. - Microascus</i>	
CROW1	K10	1	K10-1	FSME SVAL	CARU	PIPO	NONS	12.5	44	4	45	2740	184			<i>Umb. dep. sp. - Microascus</i>	
CROW1	K12	1	K12-1	FSME SVAL	CARU	PIPO	SYMP	8.9	46	5	10	2430	316			<i>Umb. dep. sp. - Microascus</i>	
CROW1	K12	2	K12-2	FSME SVAL	CARU	PIPO	NONS	10.0	50	6	35	2430	316			<i>Umb. dep. sp. - Microascus</i>	
CROW1	K5	2	K5-2	FSME PUTR	AGSF	PIPO	SYMP	23.4	95	4	40	2570	110			<i>Umb. dep. sp. - Microascus</i>	
CROW1	K6	1	K6-1	FSME SVAL	PIPO	NONS	20.3	67	5	60	2540	120				<i>Umb. dep. sp. - Microascus</i>	
CROW1	K6	6	K6-6	PIPO SVAL	PIPO	NONS	12.2	63	NA	NA	2500	124				<i>Umb. dep. sp. - Microascus</i>	

SITE	UTM cell	TRK NUMBER	UNIT ID	PLANT ASSOCIATION	UNDERSTORY	PHASE	TREE SPECIES	STATUS	DBH	HEIGHT	Radial Growth	LGR	ELAVATION	ASPECT	ITS ID	ITS % IDENTITY/ACCESSION NUMBERS	MORPHOLOGICAL IDENTIFICATION
CROW1	K9	12	K9-12	FSME SYAL	CARU	PIPO	DEAD	10.5	25	3	0	2750	187			NO GROWTH	<i>Umbelopsis/Micromacer</i> ??? NO GROWTH
CROW1	K9	2	K9-2	FSME SYAL													
CROW3	B8	1	B8-1	FSMESPEL	AGSP	FSME	NONS	15.5	86	11	45	2410	241				<i>Bischofia sp.</i> NO GROWTH
CROW3	C10	1	C10-1	FSME PUTR	AGSP	PIPO	DEAD	10.3	38	NA	NA	2550	240				<i>Bischofia sp.</i> NO GROWTH
CROW3	C3	1	C3-1	FSME SYAL													
CROW3	C5	1	C5-1	FSME SYAL													
CROW3	C7	1	C7-1	FSME SYAL													
CROW3	C7	2	C7-2	FSME SYAL													
CROW3	D10	2	D10-2	FSME PUTR	AGSP	FSME	NONS	8.3	42	18	80	2430	355				<i>Umbelopsis/Micromacer</i> <i>Epicecum sp. and Bischofia sp.</i>
CROW3	D11	1	D11-1	PIFO PUTR	AGSP	PIPO	NONS	17.0	58	7	65	2570	207				<i>Epicecum sp.</i> <i>Umbelopsis/Micromacer</i> 98 % AF133782
CROW3	D2	1	D2-1	FSMESPEL													
CROW3	D4	3	D4-3	FSME PUTR													
CROW3	D7	1	D7-1	FSME AGSP													
CROW3	E1	1	E1-1	FSME SYAL	AGSP	PIPO	TPKL	10.8	43	2	25	2540	208				<i>Umbelopsis/Micromacer</i> UNIDENTIFIED 95 % AF133780
CROW3	E1	2	E1-2	FSME SYAL													
CROW3	E3	1	E3-1	FSME SYAL	CARU	PIPO	DEAD	8.9	42	NA	NA	2330	265				<i>Umbelopsis/Micromacer</i> NO GROWTH
CROW3	E7	1	E7-1	FSME SYAL													
CROW3	E7	2	E7-2	PIFO PUTR													
CROW3	E8	1	E8-1	FSME PUTR													
CROW3	F10	3	F10-3	FSME PUTR	AGSP	PIPO	TPKL	12.9	57	2	10	2470	239				<i>Epicecum sp.</i> <i>Micror sp. and Bischofia sp.</i> 100 % AF279486
CROW3	F12	1	F12-1	FSME SYAL	AGSP	PIPO	NONS	25.6	95	6	80	2520	240				<i>Micror sp. and Bischofia sp.</i> <i>Umbelopsis/Micromacer</i>
CROW3	F16	1	F16-1	FSME PUTR	AGSP	PIPO	NONS	11.5	38	12	75	2650	234				<i>Bischofia sp.</i> <i>Umbelopsis/Micromacer</i>
CROW3	F4	2	F4-2	FSME SYAL													
CROW3	F7	2	F7-2	PIFO PUTR													
CROW3	G10	1	G10-1	FSME SYAL	CARU	PIPO	DEAD	19.1	58	NA	NA	2440	109				<i>Umbelopsis/Micromacer</i> NO GROWTH
CROW3	G11	1	G11-1	FSME SYAL	CARU	PIPO	NONS	19.4	78	10	50	2440	234				<i>Umbelopsis/Micromacer</i> 95 % AFO133782
CROW3	G13	1	G13-1	FSME SYAL	CARU	FSME	NONS	16.9	82	17	50	2460	252				<i>Umbelopsis/Micromacer</i> <i>Bischofia sp.</i>
CROW3	G15	4	G14-4	FSME PUTR	AGSP	PIPO	NONS	16.6	77	7	35	2450	239				<i>Cladopetrum maderum</i> NO GROWTH
CROW3	G15	3	G15-3	FSME PUTR	CARU	PIPO	CURA	11.8	66	12	30	2630	280				NO GROWTH
CROW3	G15	5	G15-5	FSME PUTR	CARU	PIPO	NONS	8.0	54	4	60	2620	283				NO GROWTH
CROW3	G15	1	G15-1	FSME PUTR	AGSP	PIPO	SYMP	16.4	61	5	45	2650	280				NO GROWTH
CROW3	H15	1	H15-1	FSME PUTR	AGSP	PIPO	SYMP	10.7	60	7	25	2650	280				98 % AF393714 NO GROWTH
CROW6	A16	1	A16	PIFO PUTR	CARU	PIPO	NONS	20.1	92	7	50	2530	79				NO GROWTH
CROW6	B15	1	B15-1	PIFO PUTR	CARU	PIPO	NONS	15.0	42	14	70	2550	240				NO GROWTH
CROW6	C14	1	C14-1	FSME PUTR	AGSP	PIPO	NONS	12.8	50	11	60	2530	116				<i>Umbelopsis/Micromacer</i> 97 % AJ246162 NO GROWTH
CROW6	D12	1	D12-1	PIFO PUTR	AGSP	PIPO	NONS	15.7	48	9	80	2480	240				<i>Umbelopsis/Micromacer</i> 92 % AFO133782 UNIDENTIFIED
CROW6	D17	5	D17-5	PIFO PUTR	AGSP	PIPO	TPKL	10.4	55	2	5	2480	54				NO GROWTH
CROW6	D17	6	D17-6	PIFO PUTR	AGSP	PIPO	TPKL	10.4	55	2	5	2480	54				NO GROWTH
CROW6	E1	1	E1-1	FSMESPEL	CARU	PIPO	NONS	9.4	42	6	35	2480	82				NO GROWTH
CROW6	E1	2	E1-2	FSMESPEL	CARU	PIPO	NONS	10.0	48	7	75	2480	184				NO GROWTH
CROW6	E3	1	E3-1	FSME SYAL	AGSP	PIPO	NONS	14.7	49	8	65	2430	230				<i>Umbelopsis/Micromacer</i> NO GROWTH
CROW6	E4	3	E4-3	PIFO													
CROW6	E4	4	E4-4	PIFO													
CROW6	E15	4	E15-4	PIFO													
CROW6	E16	1	E16-1	PIFO PUTR	CARU	PIPO	CURA	22.9	57	5	20	2470	128				NO GROWTH
CROW6	E16	2	E16-2	PIFO PUTR	CARU	PIPO	TPKL	24.9	72	5	40	2470	230				NO GROWTH
CROW6	E18	4	E18-4	PIFO CARU													94 % AF083200 <i>Philodora sp.</i> <i>Umbelopsis/Micromacer</i>

SITE	UTM east	TREE NUMBER	UNIT ID	PLANT ASSOCIATION	UNDERSTORY	PHASE	TREE SPECIES	STATUS	DBH	HEIGHT	Radial Growth	L:CR	ELEVATION	ASPECT	ITS ID	ITS % IDENTITY/ACCESSION NUMBERS	MORPHOLOGICAL IDENTIFICATION
CROW 6 F10	1	F10-1	FSME PUTR	CARU	PIPO	NONS	13.0	44	15	75	2440	138			98 % AF314587	<i>Alternaria lentiginosa</i>	
CROW 6 F10	2	F10-2	FSME PUTR	CARU	PIPO	DEAD	12.8	48	NA	NA	2440	172				UNIDENTIFIED	
CROW 6 F12	1	F12-1	FSME PUTR	CARU	PIPO	DEAD	19.3	53	NA	NA	2390	254				<i>Umbelopsis/Micromacer</i>	
CROW 6 F14	1	F14-1	PIPO PUTR	AGSF	PIPO	SYMP	17.0	52	5	50	2490	183				<i>Byssochlamys sp.</i>	
CROW 6 F14	2	F14-2	PIPO PUTR	AGSF	PIPO	SYMP	24.0	62	4	15	2480	206				NO GROWTH	
CROW 6 F17	1	F17-1	PIPO SYAL	CARU	PIPO	NONS	17.3	63	12	70	2470	196				NO GROWTH	
CROW 6 G13	2	G13-2	PIPO PUTR	AGSF	PIPO	NONS	19.6	75	12	55	2370	230				NO GROWTH	
CROW 6 G14	2	G14-2	FSME CARU				14.2	66	7	65	2470	180				NO GROWTH	
CROW 6 G16	1	G16-1	PIPO PUTR	CARU	PIPO	NONS	13.0	43	8	55	2470	307				UNIDENTIFIED	
CROW 6 G16	2	G16-2	FSME CARU				14.2	66	7	65	2470	180				NO GROWTH	
CROW 6 G9	1	G9-1	PIPO PUTR	CARU	PIPO	NONS	13.0	43	8	55	2470	307				UNIDENTIFIED	
CROW 6 H10	9	H10	PIPO PUTR				16.9	56	7	80	2420	196				NO GROWTH	
CROW 6 H11	1	H11-1	PIPO AGSF				10.3	46	9	70	2360	160				UNIDENTIFIED	
CROW 6 H13	1	H13-1	PIPO PUTR	AGSF	PIPO	NONS	25.0	80	6	50	2320	220				NO GROWTH	
CROW 6 H15	2	H15-2	FSME PUTR	CARU	PIPO	NONS	10.1	42	13	35	2560	226				<i>Umbelopsis/Micromacer</i>	
CROW 6 H17	1	H17-1	PIPO PUTR	AGSF	PIPO	NONS	14.8	56	NA	NA	2450	108				<i>Utricularia botrytis</i>	
CROW 6 H6	1	H6-1	FSME SYAL	CARU	PIPO	NONS	13.3	63	9	45	2340	178				<i>Mitrocladia abortiva</i>	
CROW 6 H8	1	H8-1	PIPO PUTR				20.1	61	16	75	2380	234				UNIDENTIFIED	
CROW 6 H8	2	H8-2	PIPO PUTR				15.5	55	5	55	2390	150				UNIDENTIFIED	
CROW 6 H11	3	H11-3	FSME CARU				11.2	52	NA	NA	2320	100				NO GROWTH	
CROW 6 H11	4	H11-4	FSME CARU				9.8	25	NA	NA	2320	160				<i>Umbelopsis/Micromacer</i>	
CROW 6 H12	1	H12-1	FSME PUTR	CARU	PIPO	NONS	8.8	52	13	35	2280	149				NO GROWTH	
CROW 6 H6	1	H6-1	PIPO PUTR	CARU	PIPO	NONS	14.5	51	13	70	2450	201				NO GROWTH	
CROW 6 H7	1	H7-1	PIPO PUTR	CARU	PIPO	TPKL	8.7	47	7	35	2410	130				UNIDENTIFIED	
CROW 6 H7	5	H7-5	FSME PUTR				11.5	64	6	20	2410	97				UNIDENTIFIED	
CROW 6 H5	1	H5-1	FSME SYAL	CARU	PIPO	NONS	13.0	42	9	55	2320	156				NO GROWTH	
CROW 6 H6	1	H6-1	FSME PUTR	CARU	PIPO	SYMP	8.3	50	8	25	2310	213				<i>Penicillium thomii</i>	
CROW 6 H6	2	H6-2	FSME PUTR				11.6	42	NA	NA	2100	204				UNIDENTIFIED	
CROW 6 H9	1	H9-1	FSME PUTR	CARU	PIPO	NONS	13.2	75	6	30	2360	185				NO GROWTH	
CROW 6 H12	1	H12-1	FSME PUTR	CARU	PIPO	NONS	15.1	58	16	60	2240	162				NO GROWTH	
CROW 6 H14	3	H14-3	PIPO SYAL	CARU	PIPO	SYMP	12.6	53	5	20	2400	240				NO GROWTH	
CROW 6 H15	2	H15-2	PIPO PUTR	AGSF	PIPO	NONS	11.5	53	5	15	2490	210				NO GROWTH	
CROW 6 H17	1	H17-1	FSME SYAL	CARU	PIPO	NONS	8.5	48	12	70	2410	145				NO GROWTH	
CROW 6 H4	1	H4-1	FSME PUTR	CARU	PIPO	NONS	9.7	37	6	45	2370	210				NO GROWTH	
CROW 6 H8	2	H8-2	FSME PUTR	CARU	PIPO	NONS	14.8	78	6	35	2270	166				NO GROWTH	
CROW 6 K10	5	K10-5	FSME CARU				11.3	50	NA	NA	2390	246				UNIDENTIFIED	
CROW 6 K7	7	K7-7	FSME PUTR	CARU	PIPO	NONS	17.1	54	9	75	2280	142				NO GROWTH	
CROW 6 K9	2	K9-2	FSME PUTR	CARU	PIPO	NONS	19.8	80	6	65	2270	180				UNIDENTIFIED	
CROW 6 L8	1	L8-1	PIPO PUTR	CARU	PIPO	NONS	12.8	55	9	25	2240	220				<i>Byssochlamys sp.</i>	
CROW 6 L9	1	L9-1	FSME CARU				22.9	82	2	10	2320	168				UNIDENTIFIED	
CROW 6 M11	2	M11-2	FSME SYAL	CARU	FSME	NONS	14.2	60	14	85	2290	323				98 % AF133780 100 % AF034448	<i>Umbelopsis/Micromacer</i> <i>Penicillium thomii</i>
L. CAMUS B10	1	B10-1	ABGR/PBEL	CARU	ABGR	TPKL	11.3	58	7	10	4170	78					NO GROWTH
L. CAMUS E10	1	E10-1	ABGR HODI	CARU	ABGR	NONS	12.0	41	6	75	3800	65					NO GROWTH
L. CAMUS F7	1	F7-1	FSME/PBEL	CARU	FSME	NONS	8.2	51	10	60	3770	198				UNIDENTIFIED	
L. CAMUS F9	1	F9-1	ABGR ACCI				13.8	84	NA	NA	3710	170				NO GROWTH	
L. CAMUS F9	3	F9-3	ABGR ACCI				12.0	78	NA	NA	3700	125				<i>Phialopsis elegantis</i>	
L. CAMUS G6	1	G6-1	FSME/PBEL	CARU	FSME	NONS	10.6	98	19	70	33740	168				UNIDENTIFIED	
L. CAMUS G8	1	G8-1	ABGR ACCI				17.0	102	15	55	3710	173				UNIDENTIFIED	
L. CAMUS H5	1	H5-1	FSME/PBEL	CARU	FSME	NONS	9.9	56	8	75	3780	90				NO GROWTH	

SITE	UTM cell	TREE NUMBER	PLANT ASSOCIATION	UNDERSTORY	PHASE	TREE SPECIES	STATUS	DBH	BRIGHT	Radial Growth	LCR	ELEVATION	ASPECT	T3 B	ITS % IDENTITY/ACCESSION NUMBERS	MORPHOLOGICAL IDENTIFICATION
L-CAMUS	H5	2	H5-2	FSME/SPBEL	CARU/FSME	DEAD	14.8	67	NA	NA	3740	75			<i>Umb. depala/ Micrococor</i>	
L-CAMUS	H5	3	H5-3	FSME/SPBEL	CARU/FSME	DEAD	23.0	81	NA	NA	3740	75			NO GROWTH	
L-CAMUS	H6	2	H6-2	FSME/SPBEL	CARU/FSME	NONS	11.8	52	11	65	3730	92				
L-CAMUS	H9	1	H9-1	ABGR/SPBEL	CARU/FSME	NONS	14.8	102	6	45	3650	105				
L-CAMUS	I5	1	I5-1	FSME/SPBEL	CARU/PIPO	NONS	8.8	42	3	60	3740	120			NO GROWTH	
L-CAMUS	I6	1	I6-1	FSME/SPBEL	CARU/FSME	NONS	12.3	58	12	35	3720	79			NO GROWTH	
L-CAMUS	I8	2	I8-2	FSME/PUTR	CARU/PIPO	NONS	11.7	35	11	60	3680	111			UNIDENTIFIED	
L-CAMUS	I9	3	I9-3	ABGR/ HODI	CARU/ABGR	TPKL	13.9	82	2	18	3600	79			UNIDENTIFIED	
L-CAMUS	J1	1	J1-1	FSME/SPBEL	CARU/ABGR	DEAD	12.7	48	NA	NA	3580	79			NO GROWTH	
L-CAMUS	I3	1	I3-1	FSME/SPBEL	CARU/PIPO	NONS	22.2	82	6	75	3600	165			NO GROWTH	
L-CAMUS	L5	1	L5-1	FSME/SPBEL	CARU/FSME	SYMPT	12.0	63	2	15	3550	90			NO GROWTH	
L-CAMUS	L7	2	L7-2	ABGR/ HODI	CARU/PIPO	NONS	17.4	89	6	70	3560	90			NO GROWTH	
L-CAMUS	M6	1	M6-1	FSME/SPBEL	CARU/FSME	NONS	20.3	89	8	65	3560	75			NO GROWTH	
L-CAMUS	N3	2	N3-2	PIPO/ AGSF	CARU/FSME	NONS	13.4	66	19	90	3540	112			NO GROWTH	
L-CAMUS	O4	1	O4-1	ABGR/ HODI	CARU/ABGR	NONS	9.3	87	28	95	3460	116			NO GROWTH	
PEND 30	I8	2	I8-2	PIPO/ PUTR	CARU/PIPO	NONS	16.0	64	7	40					UNIDENTIFIED	
PEND 30	I6	3	I6-3	FSME/ PUTR	AGSF/ PIPO	NONS	21.1	79	6	60					UNIDENTIFIED	
PEND 30	B7	1	B7-1	FSME/ SYAL	CARU/PIPO	NONS	10.8	33	4	60					NO GROWTH	
PEND 30	C4	1	C4-1	FSME/ SYAL	CARU/PIPO	NONS	16.7	80	9	30					NO GROWTH	
PEND 30	D3	1	D3-1	FSME/ PUTR	PIPO/ NONS	17.7	70	8	40						NO GROWTH	
PEND 30	D5	1	D5-1	FSME/ PUTR	PIPO/ NONS	17.7	70	8	40						NO GROWTH	
PEND 30	D7	1	D7-1	FSME/ PUTR	CARU/PIPO	NONS	24.0	105	7	50					UNIDENTIFIED	
PEND 30	E10	1	E10-1	PIPO/ PUTR	AGSF/ PIPO	NONS	11.6	206	NA	NA					<i>Tremella ghriffa</i>	
PEND 30	E4	3	E4-3	PIPO/ PUTR	CARU/PIPO	NONS	8.8	60	10	15					96 % AF042453	
PEND 30	E6	1	E6-1	FSME/ SYAL	PIPO/ NONS	11.4	63	8	25						NO GROWTH	
PEND 30	E8	3	E8-3	FSME/ SYAL	PIPO/ NONS	13.0	65	3	50						UNIDENTIFIED	
PEND 30	F3	6	F3-6	PIPO/ PUTR	CARU/PIPO	NONS	9.4	65	9	30					NO GROWTH	
PEND 30	F5	1	F5-1	PIPO	CARU/PIPO	NONS	12.3	80	9	30					NO GROWTH	
PEND 30	F7	1	F7-1	FSME/ SYAL	AGSF/ PIPO	NONS	10.3	62	8	45					NO GROWTH	
PEND 30	F9	3	F9-3	FSME/ SYAL	PIPO/ DEAD	13.6	62	NA	NA						NO GROWTH	
PEND 30	F9	4	F9-4	FSME/ SYAL	PIPO/ NONS	13.1	75	5	45						NO GROWTH	
PEND 30	G10	1	G10-1	FSME/ PUTR	CARU/PIPO	NONS	13.4	174	NA	NA					<i>Heterobasidium anatum</i>	
PEND 30	G6	1	G6-1	PIPO/ PUTR	CARU/PIPO	NONS	12.0	48	12	55					99 % X70023	
PEND 30	G8	1	G8-1	FSME/ PUTR	AGSF/ PIPO	NONS	12.8	45	5	55					NO GROWTH	
PEND 30	H11	2	H11-2	FSME/ SYAL	CARU/PIPO	NONS	10.9	70	8	30					<i>Heterobasidium anatum</i>	
PEND 30	H5	1	H5-1	PIPO/ PUTR	CARU/PIPO	NONS	8.9	36	7	50					NO GROWTH	
PEND 30	H7	1	H7-1	FSME/ PUTR	AGSF/ PIPO	NONS	11.8	64	6	45					NO GROWTH	
PEND 30	H9	1	H9-1	FSME/ SYAL	PIPO/ NONS	16.1	62	16	70						NO GROWTH	
PEND 30	I10	2	I10-2	FSME/ SYAL	CARU/PIPO	TPKL	13.7	212	NA	NA					<i>Pyrenopeziza cf. sp.</i>	
PEND 30	I10	4	I10-4	FSME/ SYAL	CARU/PIPO	SYMPT	10.1	228	NA	NA					95 % U18361	
PEND 30	H1	1	H1-1	FSME/ SYAL	CARU/PIPO	NONS	12.3	78	16	40					95 % AF35447	
PEND 30	I6	10	I6-10	PIPO/ PUTR	CARU/PIPO	NONS	14.0	60	9	40					NO GROWTH	
PEND 30	I8	1	I8-1	PIPO/ PUTR	AGSF/ PIPO	NONS	8.8	188	NA	NA					NO GROWTH	
PEND 30	J6	2	J6-2	FSME/ PUTR	PIPO/ DEAD	18.3	79	NA	NA						NO GROWTH	
PEND 30	J7	3	J7-3	FSME/ SYAL	PIPO/ NONS	12.2	64	6	55						<i>Penicillium cf. 277</i>	
PEND 30	J8	1	J8-1	FSME/ SYAL	PIPO/ SYMP	15.0	84	3	35						NO GROWTH	
PEND 30	J8	2	J8-2	FSME/ SYAL	PIPO/ SYMP	17.9	92	6	50						NO GROWTH	
PEND 30	K10	1	K10-1	FSME/ SYAL	CARU/PIPO	NONS	12.5	75	8	25					NO GROWTH	
PEND 30	K6	5	K6-5	FSME/ PUTR	PIPO/ NONS	15.5	73	16	30						96 % AF042453	

SITE	UTM cell	TREE NUMBER	UNIT ID	PLANT ASSOCIATION	UNDERSTORY	PHASE	TREE SPECIES	STATUS	DBH	HEIGHT	Radial Growth	LGR	ELEVATION	ASPECT	ITS ID	ITS % IDENTITY/ACCESSION NUMBERS	MORPHOLOGICAL IDENTIFICATION	
PEND 30	L10	1	L10-1	FSME SYAL	CARU	PIFO	DEAD	10.2	21	NA	NA	NA					UNIDENTIFIED	
POISON 6	F3	1	F3-1	FSME SYAL	CARU	PIFO	NONS	17.2	97	17	45						NO GROWTH	
POISON 6	F7	1	F7-1	PIFO SYAL	CARU	PIFO	NONS	25.1	111	8	20						UNIDENTIFIED	
POISON 6	G11	1	G11-1	FSME CARU	AGSF	FSME	NONS	9.5	75	7	40					99 % AFI10531	<i>Penicillium</i> spp.?	
POISON 6	G14	1	G14-1	PIFO PUTR	AGSF	PIFO	NONS	26.7	105	7	55						<i>Verdictium coccozporium</i>	
POISON 6	G2	1	G2-1	FSME SYAL	CARU	FSME	NONS	13.9	80	18	70						NO GROWTH	
POISON 6	G4	2	G4-2	PIFOROWO	PIFO	SYMP	NONS	10.0	86	11	50						NO GROWTH	
POISON 6	G4	3	G4-3	PIFOROWO	PIFO	NONS	12.5	86	11	40							<i>Verdictium coccozporium</i>	
POISON 6	G6	1	G6-1	PIFO SYAL	AGSF	PIFO	NONS	28.7	117	7	55					99 % AFI10531	<i>Verdictium coccozporium</i> ?	
POISON 6	G6	2	G6-2	PIFO SYAL	PIFO	TPKL	35.8	103	2	40						94 % U18358	<i>Epinephelium javanicum</i>	
POISON 6	G8	1	G8-1	FSME SYAL	CARU	FSME	NONS	10.6	88	10	15						NO GROWTH	
POISON 6	G9	1	G9-1	FSME SYAL	CARU	PIFO	TPKL	32.2	115	7	35						NO GROWTH	
POISON 6	H0	1	H0-1	PIFO PUTR	AGSF	PIFO	NONS	17.9	85	18	75						99 % -AF033410	<i>Penicillium spinulosum</i>
POISON 6	H2	1	H2-1	PIFO PUTR	AGSF	PIFO	NONS	15.3	69	11	70						NO GROWTH	
POISON 6	H6	1	H6-1	FSME SYAL	CARU	PIFO	NONS	10.0	62	12	50						NO GROWTH	
POISON 6	H7	1	H7-1	FSME SYAL	CARU	PIFO	NONS	11.1	78	14	50						NO GROWTH	
POISON 6	H1	1	H1-1	FSME SYAL	CARU	FSME	NONS	17.7	110	6	15						NO GROWTH	
POISON 6	H3	2	H3-2	FSME SYAL	PIFO	NONS	32.1	150	6	65							NO GROWTH	
POISON 6	H3	3	H3-3	FSME SYAL	CARU	PIFO	CURA	14.5	65	17	25						NO GROWTH	
POISON 6	J3	1	J3-1	PIFO CARU	AGSF	PIFO	NONS	13.7	57	18	45						NO GROWTH	
POISON 6	J5	2	J5-1	PIFO SYAL	PIFO	TPKL	35.8	130	2	20							NO GROWTH	
POISON 6	J5	2	J5-2	PIFO SYAL	PIFO	NONS	13.0	67	13	40							NO GROWTH	
POISON 6	J7	1	J7-1	FSME CARU	FSME	NONS	19.2	105	9	45							<i>Umbelopsis/Micromycor</i>	
POISON 6	J8	1	J8-1	FSME SYAL	FSME	TPKL	18.5	108	6	10							NO GROWTH	
POISON 6	J9	1	J9-1	FSME PUTR	CARU	PIFO	TPKL	29.4	78	7	45						NO GROWTH	
POISON 6	K14	1	K14-1	FSME SYAL	AGSF	FSME	NONS	18.3	98	22	65						NO GROWTH	
POISON 6	K3	1	K3-1	FSME SYAL	AGSF	PIFO	NONS	8.6	51	5	50						NO GROWTH	
POISON 6	K4	2	K4-2	FSME SYAL	AGSF	FSME	NONS	14.9	71	21	95					98 % -AF412291	<i>Micror rhombeus</i>	
POISON 6	K5	1	K5-1	FSME SYAL	AGSF	FSME	TPKL	16.2	63	14	50						NO GROWTH	
POISON 6	K6	1	K6-1	FSME SYAL	AGSF	FSME	NONS	18.0	94	21	85						NO GROWTH	
RUBY	D10	6	D10-6	FSME SYAL	CARU	FSME	NONS	12.6	108	9	70	2820	232				UNIDENTIFIED	
RUBY	D6	3	D6-3	FSME SYAL	CARU	FSME	SYMP	18.7	88	5	45	3200	148				<i>Micror</i> sp.	
RUBY	D6	4	D6-4	FSME SYAL	CARU	FSME	SYMP	14.3	93	8	30	3190	170				<i>Penicillium</i> sp.	
RUBY	D6	5	D6-5	FSME SYAL	CARU	FSME	SYMP	25.1	126	6	90	3170	53			Micror-98 % af412291/ <i>Penicillium</i> -99 % AJ279476	<i>Micror</i> sp. **	
RUBY	E9	1	E9-1	FSME SYAL	CARU	FSME	NONS	24.2	110	9	55	3000	238				UNIDENTIFIED	
RUBY	F10	1	F10-1	FSME PBEL	FSME	NONS	15.0	97	17	80	2950	147					NO GROWTH	
RUBY	F12	3	F12-3	FSME PBEL	CARU	FSME	NONS	24.0	124	13	75	2890	228				<i>Verdictium coccozporium</i>	
RUBY	F4	1	F4-1	FSME SYAL	CARU	PIFO	NONS	13.1	38	5	85	3290	209				NO GROWTH	
RUBY	F5	4	F5-4	FSME SYAL	CARU	PIFO	DEAD	9.9	60	NA	3220	240					<i>Micror plumbens</i>	
RUBY	F8	1	F8-1	FSME SYAL	CARU	FSME	NONS	10.8	63	13	80	3100	209				UNIDENTIFIED	
RUBY	G11	3	G11-3	FSME PBEL	CARU	FSME	NONS	19.2	115	13	60	2960	263				<i>Penicillium</i> sp.?	
RUBY	G4	1	G4-1	FSME SYAL	CARU	PIFO	NONS	16.5	90	20	70	3310	232				UNIDENTIFIED	
RUBY	G5	1	G5-1	FSME SYAL	CARU	FSME	NONS	17.7	99	24	60	3270	229				NO GROWTH	
RUBY	G7	1	G7-1	FSME SYAL	CARU	FSME	NONS	13.5	52	12	45	3180	146				NO GROWTH	
RUBY	G9	1	G9-1	FSME HODI	CARU	FSME	NONS	14.0	93	21	90	3050	175				<i>Penicillium fragrans</i>	
RUBY	H2	1	H2-1	FSME PBEL	CARU	FSME	NONS	8.2	57	16	85	3050	237			95 % -AF198248	<i>Penicillium fragrans</i>	
RUBY	H4	1	H4-1	FSME PBEL	CARU	FSME	NONS	16.9	85	26	55	3000	270				NO GROWTH	
RUBY	H8	1	H8-1	FSME SYAL	CARU	FSME	NONS	22.7	135	15	50	3110	192				NO GROWTH	

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RUBY H3		1	H3-1	FSME/SPBL	CARU	PIPO	NONS	16.2	157	4	40	3050	231		NO GROWTH
RUBY H5		1	H5-1	FSME/SPBL	CARU	PIPO	NONS	16.9	79	9	40	2990	233		NO GROWTH
RUBY H5		1	H5-1	FSME/SPBL	CARU	PIPO	NONS	17.4	81	16	75	3360	161		NO GROWTH
RUBY H7		1	H7-1	FSME/SPBL	CARU	PIPO	NONS	17.2	80	13	65	3200	195		NO GROWTH
RUBY H12		3	H12-1	FSME/SPBL	CARU	PIPO	DEAD	11.0	46	NA	NA	3200	284		95 % - UI18361
RUBY H14		1	H14-1	FSME/SPBL	CARU	PIPO	NONS	24.8	112	6	40	3080	245		94 % - AF072297
RUBY H15		1	H15-1	FSME/SPBL	CARU	PIPO	DEAD	14.6	85	NA	NA	3100	221		99 % - AJ034450
RUBY H15		1	H15-1	FSME/SPBL	CARU	PIPO	NONS	11.3	82	8	75	3450	186		NO GROWTH
RUBY H15		1	H15-1	FSME/SPBL	CARU	PIPO	NONS	27.1	150	8	65	3200	232		NO GROWTH
RUBY H16		1	H16-1	FSME/SPBL	CARU	PIPO	NONS	15.5	92	8	70	3100	250		NO GROWTH
RUBY H4		1	H4-1	FSME/SPBL	CARU	PIPO	CURK	11.8	30	NA	NA	3050	174		UNIDENTIFIED
RUBY H4		1	H4-1	FSME/SPBL	CARU	PIPO	NONS	15.0	82	21	65	3460	165		NO GROWTH
RUBY H12		1	H12-1	FSME/SPBL	CARU	PIPO	NONS	14.7	71	14	80	3300	234		NO GROWTH
SAND 19 D11		1	D11-1	FSME/SPBL	AGSP	FSME	NONS	9.5	67	9	50				NO GROWTH
SAND 19 D13		1	D13-1	FSME/SPBL	AGSP	FSME	NONS	15.9	68	10	85				NO GROWTH
SAND 19 D7		1	D7-1	FSME/SPBL	AGSP	FSME	TPKL	30.4	74	6	15				NO GROWTH
SAND 19 D7		2	D7-2	FSME/SPBL	AGSP	FSME	NONS	13.0	70	12	70				NO GROWTH
SAND 19 E8		1	E8-1	FSME/SPBL	AGSP	FSME	NONS	13.8	74	9	80				NO GROWTH
SAND 19 E9		1	E9-1	FSME/SPBL	AGSP	FSME	TPKL	26.3	66	1	10				NO GROWTH
SAND 19 F11		1	F11-1	FSME/SPBL	AGSP	FSME	NONS	25.6	110	10	60				NO GROWTH
SAND 19 F13		1	F13-1	FSME/SPBL	AGSP	FSME	NONS	11.8	87	8	25				NO GROWTH
SAND 19 F7		2	F7-2	FSME/SPBL	AGSP	FSME	NONS	10.0	41	6	85				NO GROWTH
SAND 19 F9		1	F9-1	FSME/SPBL	AGSP	FSME	NONS	20.2	68	13	55				NO GROWTH
SAND 19 G10		1	G10-1	FSME/SPBL	AGSP	FSME	NONS	14.6	92	10	30				NO GROWTH
SAND 19 G12		1	G12-1	FSME/SPBL	AGSP	FSME	NONS	11.5	80	10	75				NO GROWTH
SAND 19 H11		2	H11-2	FSME/SPBL	AGSP	FSME	NONS	17.1	99	12	85				NO GROWTH
SAND 19 H6		1	H6-1	FSME/SPBL	AGSP	FSME	NONS	15.3	43	4	40				NO GROWTH
SAND 19 H7		1	H7-1	FSME/SPBL	AGSP	FSME	NONS	10.8	35	3	40				NO GROWTH
SAND 19 H9		1	H9-1	FSME/SPBL	AGSP	FSME	NONS	9.8	64	11	35				NO GROWTH
SAND 19 H10		2	H10-2	FSME/SPBL	AGSP	FSME	NONS	11.9	53	9	90				NO GROWTH
SAND 19 H11		1	H11-1	FSME/SPBL	AGSP	FSME	TPKL	50.6	105	5	70				NO GROWTH
SAND 19 H12		1	H12-1	FSME/SPBL	AGSP	FSME	NONS	29.1	97	7	90				NO GROWTH
SAND 19 H13		1	H13-1	FSME/SPBL	AGSP	FSME	NONS	47.5	84	NA	NA				NO GROWTH
SAND 19 H14		1	H14-1	FSME/SPBL	AGSP	FSME	NONS	14.3	58	7	60				NO GROWTH
SAND 19 H15		2	H15-2	FSME/SPBL	AGSP	FSME	NONS	14.8	71	9	70				NO GROWTH
SAND 19 J8		1	J8-1	FSME/SPBL	AGSP	FSME	NONS	18.6	56	6	80				NO GROWTH
SAND 19 J8		1	J8-1	FSME/SPBL	AGSP	FSME	NONS	49.0	104	5	55				NO GROWTH
SAND 19 K0		1	K0-1	FSME/SPBL	AGSP	FSME	NONS	8.6	43	2	30				NO GROWTH
SAND 19 K1		1	K1-1	FSME/SPBL	AGSP	FSME	NONS	20.9	98	12	50				NO GROWTH
SAND 19 K2		1	K2-1	FSME/SPBL	AGSP	FSME	NONS	15.3	73	14	90				NO GROWTH
SAND 19 K6		1	K6-1	FSME/SPBL	AGSP	FSME	NONS	18.3	83	15	45	3570	174		NO GROWTH
SAND 19 K9		3	K9-3	FSME/SPBL	AGSP	FSME	TPKL	47.1	108	6	65				NO GROWTH
SAND 19 L9		1	L9-1	FSME/SPBL	AGSP	FSME	NONS	18.7	88	7	75				NO GROWTH
SAND 19 L5-1		1	L5-1	FSME/SPBL	AGSP	FSME	NONS	18.7	88	7	75				NO GROWTH
SAND 2 H18		1	H18-1	FSME/SPBL	AGSP	FSME	NONS	18.0	120	19	45				NO GROWTH
SAND 2 H20		1	H20-1	FSME/SPBL	AGSP	FSME	NONS	11.7	63	10	50				UNIDENTIFIED
SAND 2 H21		2	H21-2	FSME/SPBL	AGSP	FSME	NONS	17.9	60	9	55				NO GROWTH

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SAND 2	H22	1	H22-1	FSME/SPBEL	CARU	PIFO	NONS	26.2	81	7	50					NO GROWTH	NO GROWTH
SAND 2	H7	1	H7-1	FSME/PUTR	AGSP	PIFO	NONS	15.8	44	6	50						<i>Byssochlamys</i> sp.
SAND 2	H19	1	H19-1	FSME/SPBEL	CARU	FSME	NONS	10.2	60	9	40					98% AF013228	NO GROWTH
SAND 2	J17	1	J17-1	FSME/PUTR	AGSP	FSME	NONS	12.4	65	11	90						<i>Byssochlamys</i> sp.
SAND 2	J20	1	J20-1	FSME/SPBEL	CARU	FSME	NONS	14.5	91	7	30						<i>Byssochlamys</i> sp.
SAND 2	J21	1	J21-1	FSME/SPBEL	CARU	FSME	NONS	15.1	132	7	25						NO GROWTH
SAND 2	J22	1	J22-1	FSME/SPBEL	CARU	FSME	NONS	16.4	108	6	20						UNIDENTIFIED
SAND 2	K15	3	K15-3	FSME/CARU	AGSP	FSME	NONS	17.7	76	7	75						NO GROWTH
SAND 2	K19	1	K19-1	FSME/SYAL													yellow fungus
SAND 2	L16	1	L16-1	FSME/CARU													NO GROWTH
SAND 2	L17	1	L17-1	FSME/SPBEL	CARU	FSME	NONS	15.8	92	10	40						NO GROWTH
SAND 2	L18	1	L18-1	FSME/SPBEL	CARU	FSME	NONS	14.5	119	10	20						<i>Byssochlamys</i> sp.
SAND 2	L20	1	L20-1	FSME/SPBEL	CARU	PIFO	SYMP	25.1	113	7	20						<i>Byssochlamys</i> sp.
SAND 2	L20	2	L20-2	FSME/CARU													<i>Byssochlamys</i> sp.
SAND 2	L21	1	L21-1	FSME/SPBEL	CARU	FSME	DEAD	30.2	109	NA	NA						<i>Byssochlamys</i> sp.
SAND 2	L22	1	L22-1	FSME/SPBEL	CARU	FSME	DEAD	30.2	109	NA	NA						<i>Umbelopsis/Microascus</i>
SAND 2	M17	4	M17-4	FSME/SPBEL	CARU	FSME	NONS	13.3	85	7	25						NO GROWTH
SAND 2	M19	1	M19-1	FSME/SPBEL	CARU	FSME	NONS	11.0	81	7	40						NO GROWTH
SAND 2	M22	1	M22-1	FSME/SPBEL	CARU	PIFO	TPKL	22.9	78	2	10						NO GROWTH
SAND 2	N14	1	N14-1	FSME/SYAL													NO GROWTH
SAND 2	N16	1	N16-1	FSME/CARU													NO GROWTH
SAND 2	N21	1	N21-1	FSME/SPBEL	CARU	FSME	DEAD	27.5	88	NA	NA						NO GROWTH
SAND 2	N22	1	N22-1	FSME/SPBEL	CARU	FSME	DEAD	20.2	86	NA	NA						NO GROWTH
SAND 2	O14	1	O14-1	FSME/SPBEL	CARU	FSME	NONS	10.5	86	5	25						NO GROWTH
SAND 2	O15	1	O15-1	FSME/SYAL	CARU	PIFO	NONS	24.0	84	6	30						NO GROWTH
SAND 2	O16	2	O16-2	FSME/SPBEL	CARU	PIFO	NONS	16.2	79	8	50						<i>Byssochlamys</i> sp.
SAND 2	O17	1	O17-1	FSME/CARU													NO GROWTH
SLAWSON	A8	1	A8-1	FSME/PUTR	AGSP	FSME	DEAD	43.1	89	NA	NA						NO GROWTH
SLAWSON	B3	1	B3-1	FSME/SPBEL	CARU	FSME	SYMP	26.1	106	3	20						<i>Penicillium chrysogenum</i>
SLAWSON	B4	1	B4-1	FSME/SPBEL	CARU	PIFO	DEAD	19.0	65	NA	NA						<i>Umbelopsis/Microascus</i>
SLAWSON	B6	2	B6-2	FSME/SPBEL	CARU	PIFO	DEAD	26.0	92	NA	NA						<i>Umbelopsis/Microascus</i> and <i>Byssochlamys</i> sp.
SLAWSON	B7	1	B7-1	FSME/SYAL	CARU	FSME	NONS	10.1	80	12	40						NO GROWTH
SLAWSON	B9	1	B9-1	FSME/SYAL	CARU	FSME	NONS	9.3	70	11	20						NO GROWTH
SLAWSON	B9	2	B9-2	FSME/SYAL	CARU	FSME	TPKL	19.6	90	3	10						UNIDENTIFIED
SLAWSON	C10	1	C10-1	FSME/SYAL	CARU	FSME	NONS	13.1	109	16	40						UNIDENTIFIED
SLAWSON	C12	1	C12-1	FSME/SYAL	CARU	FSME	NONS	11.1	95	10	40						NO GROWTH
SLAWSON	C13	1	C13-1	FSME/SYAL	CARU	FSME	TPKL	27.3	83	2	25						<i>Penicillium resiliens</i>
SLAWSON	C2	1	C2-1	FSME/SPBEL	CARU	FSME	DEAD	33.3	165	NA	NA						<i>Mucor plumbeus</i>
SLAWSON	C4	1	C4-1	FSME/SPBEL	CARU	PIFO	NONS	19.4	41	6	10						NO GROWTH
SLAWSON	C5	1	C5-1	FSME/SPBEL	CARU	FSME	NONS	8.0	70	8	45						NO GROWTH
SLAWSON	C6	1	C6-1	FSME/SPBEL	CARU	FSME	NONS	14.2	89	12	55						NO GROWTH
SLAWSON	C8	1	C8-1	FSME/SYAL	CARU	FSME	NONS	11.2	71	6	30						NO GROWTH
SLAWSON	D11	1	D11-1	FSME/HODI	CARU	FSME	NONS	14.0	82	13	50						NO GROWTH
SLAWSON	D13	1	D13-1	FSME/SYAL	CARU	FSME	NONS	9.8	82	16	55						<i>Mucor plumbeus</i>
SLAWSON	D15	1	D15-1	FSME/SYAL	CARU	FSME	TPKL	45.7	105	3	45						NO GROWTH
SLAWSON	D5	1	D5-1	FSME/SYAL	CARU	FSME	NONS	14.3	66	14	25						<i>Penicillium canescens</i>
SLAWSON	D5	2	D5-2	FSME/SPBEL	CARU	FSME	NONS	18.8	85	7	40						<i>Metabolia humicola</i>
SLAWSON	D5	2	D5-2	FSME/SPBEL	CARU	FSME	SYMP	14.3	80	6	30						<i>Umbelopsis/Microascus</i>
SLAWSON	D7	2	D7-2	FSME/SYAL	NONS	FSME	NONS	12	70								<i>Tremella gura/ja</i>

SITE	UTM cell	TREE NUMBER	UNIT ID	PLANT ASSOCIATION	UNDERSTORY	PHASE	TREE SPECIES	STATUS	DBH	HEIGHT	Radial Growth	LCR	ELAVATION	ASPECT	ITS ID	ITS % IDENTITY/ACCESSION NUMBERS	MORPHOLOGICAL IDENTIFICATION	
SLAWSON	D9	1	D9-1	FSME SVAL	CARUF	FSME	NONS	13.5	73	7	50					NO GROWTH	NO GROWTH	
SLAWSON	E10	1	E10-1	FSME SVAL	CARUF	FSME	NONS	19.8	87	5	50					NO GROWTH	NO GROWTH	
SLAWSON	E13	1	E13-1	FSME SVAL	CARUF	FSME	NONS	20.3	95	14	50					ost root assoc. fungus	NO GROWTH	
SLAWSON	E14	1	E14-1	FSME SVAL	CARUF	FSME	NONS	10.0	75	9	20						NO GROWTH	
SLAWSON	E7	1	E7-1	FSME HODI	CARUF	FSME	NONS	33.3	142	4	25						NO GROWTH	
SLAWSON	E8	1	E8-1	FSME HODI	CARUF	FSME	NONS	13.0	70	6	50						<i>Tremella glauca</i>	
SLAWSON	F11	1	F11-1	FSME SVAL	CARUF	FSME	NONS	26.9	96	5	50						<i>Pysechlamys</i> sp.	
SLAWSON	F14	1	F14-1	FSME SVAL	CARUF	FSME	NONS	18.3	95	NA	NA						UNIDENTIFIED	
SLAWSON	F8	2	F8-2	FSME SVAL	CARUF	FSME	NONS	32.9	102	3	10						<i>Pysechlamys</i> sp.	
SLAWSON	F9	1	F9-1	FSME SVAL	CARUF	FSME	NONS	13.4	61	3	45						NO GROWTH	
SLAWSON	G10	1	G10-1	FSME SVAL	CARUF	FSME	DEAD	14.6	51	NA	NA						NO GROWTH	
SLAWSON	G10	2	G10-2	FSME SVAL	CARUF	FSME	NONS	36.0	111	6	50						NO GROWTH	
SLAWSON	G8	1	G8-1	FSME SVAL	CARUF	FSME	NONS	10.4	72	13	50						NO GROWTH	
SPROM 4	C7	1	C7-1	FSME SVAL	CARUF	FSME	NONS	15.9	89	25	75	3670	190				NO GROWTH	
SPROM 4	C9	1	C9-1	FSME SVAL	CARUF	FSME	TPKL	11.2	47	2	25	3460	190				NO GROWTH	
SPROM 4	C9	2	C9-2	FSME SVAL	CARUF	FSME	TPKL	9.7	45	2	15	3460	190				NO GROWTH	
SPROM 4	C9	3	C9-3	FSME SVAL	CARUF	FSME	TPKL	17.0	19	3	70	3460	216				NO GROWTH	
SPROM 4	D11	1	D11-1	FSME HODI	CARUF	FSME	NONS	14.0	82	13	50						NO GROWTH	
SPROM 4	D8	1	D8-1	FSME SVAL	CARUF	FSME	NONS	17.0	65	9	40	3550	210				NO GROWTH	
SPROM 4	D9	1	D9-1	FSME HODI	CARUF	FSME	TPKL	10.7	47	2	15	3450	208				NO GROWTH	
SPROM 4	D9	2	D9-2	FSME HODI	CARUF	FSME	TPKL	11.2	64	3	55	3450	208				NO GROWTH	
SPROM 4	E11	1	E11-1	FSME FBEL	CARUF	FSME	NONS	11.7	57	13	35	3450	370				UNIDENTIFIED	
SPROM 4	E6	1	E6-1	FSME SVAL	CARUF	FSME	NONS	16.4	56	6	65	3790					UNIDENTIFIED	
SPROM 4	E9	1	E9-1	FSME HODI	CARUF	FSME	NONS	11.9	53	21	85	3480	256				<i>Umbdopsis/Micromucor</i> and <i>Micor</i> sp.	
SPROM 4	F10	1	F10-1	FSME AGSP	CARUF	FSME	PIPO	NONS	10.4	25	9	65	3540	280				<i>Umbdopsis/Micromucor</i> and <i>Micor</i> sp.
SPROM 4	F6	2	F6-2	FSME FBEL	CARUF	FSME	PIPO	NONS	25.4	99	10	50	3790	198				<i>Micor</i> sp.
SPROM 4	F8	1	F8-1	FSME SVAL	CARUF	FSME	NONS	23.6	80	6	75	3620	238				NO GROWTH	
SPROM 4	G11	1	G11-1	FSME SVAL	CARUF	FSME	NONS	12.1	42	10	45	3560	224				NO GROWTH	
SPROM 4	G9	1	G9-1	FSME SVAL	CARUF	FSME	NONS	12.1	42	16	60	3620	265				UNIDENTIFIED	
SPROM 4	H10	1	H10-1	FSME SVAL	CARUF	FSME	NONS	12.1	42	16	60	3620	265				UNIDENTIFIED	
SPROM 4	H6	1	H6-1	FSME CARU	CARUF	FSME	NONS	15.0	69	7	60	3570	175				NO GROWTH	
SPROM 4	H8	1	H8-1	FSME BASA	AGSP	FSME	PIPO	NONS	12.6	35	5	15	3770	140				<i>Umbdopsis/Micromucor</i> and <i>Micor</i> sp.
SPROM 4	D9	1	D9-1	FSME SVAL	CARUF	FSME	NONS	15.7	45	9	85	3660	176				<i>Pysechlamys</i> sp.	
SPROM 4	J10	1	J10-1	FSME SVAL	CARUF	FSME	NONS	12.8	33	11	30	3560	180				<i>Pysechlamys</i> sp.	
SPROM 4	J7	1	J7-1	FSME CARU	BASA	FSME	NONS	18.3	83	15	45	3570	174				NO GROWTH	
SPROM 4	J8	1	J8-1	FSME CARU	BASA	FSME	NONS	26.4	98	8	65	3780	180				UNIDENTIFIED	
SPROM 4	K10	1	K10-1	FSME SVAL	CARUF	FSME	NONS	8.9	54	12	65	3690	155				NO GROWTH	
SPROM 4	K6	1	K6-1	FSME BASA	AGSP	FSME	PIPO	NONS	21.6	94	11	90	3470	194				<i>Umbdopsis/Micromucor</i> and <i>Micor</i> sp.
SPROM 4	K8	1	K8-1	FSME HODI	AGSP	FSME	TPKL	10.6	62	9	25	3480	192				<i>Umbdopsis/Micromucor</i> and <i>Micor</i> sp.	
SPROM 4	K8	2	K8-2	FSME CARU	BASA	FSME	NONS	11.7	75	14	55	3650	180				UNIDENTIFIED	
SPROM 4	K9	1	K9-1	FSME SVAL	CARUF	FSME	NONS	18.3	83	15	45	3570	174				<i>Micor plumbeus</i>	
SPROM 4	L6	1	L6-1	FSME FBEL	CARUF	FSME	NONS	24.0	92	29	90	3810	238				NO GROWTH	
SPROM 4	M7	1	M7-1	FSME BASA	CAGE	FSME	PIPO	NONS	14.7	68	14	75	3730	225				NO GROWTH
SPROM 4	M8	1	M8-1	FSME BASA	CAGE	FSME	NONS	15.7	77	13	80	3700	165				NO GROWTH	
SPROM 4	M9	1	M9-1	FSME SVAL	CARUF	FSME	NONS	14.1	37	20	90	3510	185				<i>Umbdopsis/Micromucor</i> and <i>Micor</i> sp.	
SPROM 4	N6	1	N6-1	FSME SVAL	CARUF	FSME	NONS	17.1	53	26	70	3840	178				NO GROWTH	
SPROM 4	O6	1	O6-1	FSME SVAL	CARUF	FSME	NONS	17.1	53	26	70	3840	178				NO GROWTH	
SPROM 4	O6	1	O6-1	FSME SVAL	CARUF	FSME	PIPO	NONS	8.9	52	8	25	3800	164				UNIDENTIFIED

SITE	UTM cell	TREE NUMBER	UNIT ID	PLANT ASSOCIATION	UNDERSTORY	PHASE	TREE SPECIES	STATUS	DBH	HEIGHT	Radial Growth	LCR	ELEVATION	ASPECT	ITS ID	ITS % IDENTITY/ACCESSION NUMBERS	MORPHOLOGICAL IDENTIFICATION
SFROM4	Q7	1	07-1	FSME SYAL	CARU	PIPO	NONS	8.5	44	7	40	3770	126			NO GROWTH	Miscor sp.
SFROM4	F7	1	F7-1	FSME SYAL		PIPO	NONS	15.9	70	23	75	3720	226				
TRIPP9	AA2	1	AA2-1	FSME CARU		PIPO	NONS	11.5	59	4	20					NO GROWTH	
TRIPP9	AA4	1	AA4-1	FSME CARU	AGSF	PIPO	NONS	20.0	91	10	45					NO GROWTH	<i>Esseschlamys</i> sp.
TRIPP9	BB3	1	BB3-1	FSME SYAL	AGSF	PIPO	DEAD	18.9	69	NA	NA					NO GROWTH	
TRIPP9	I7	1	I7-1	FSMEFBEL	CARU	FSME	NONS	11.4	78	2	20					NO GROWTH	
TRIPP9	18	2	18-2	FSME CARU	AGSF	FSME	TPKL	19.0	81	11	15					UNIDENTIFIED	
TRIPP9	18	3	18-3	FSME CARU	AGSF	FSME	DEAD	9.6	52	NA	NA						Miscor sp.
TRIPP9	18	4	18-4	FSME CARU	AGSF	FSME	TPKL	17.3	89	15	25						<i>Esseschlamys</i> sp. ?
TRIPP9	L7	1	L7-1	FSME CARU		FSME	NONS	22.5	125	14	30					NO GROWTH	<i>Esseschlamys</i> sp.
TRIPP9	L8	1	L8-1	FSME SYAL	CARU	PIPO	NONS	24.1	91	7	40					NO GROWTH	
TRIPP9	M10	1	M10-1	FSME SYAL	FUTR	CARU	PIPO	TPKL	31.3	133	3	25				NO GROWTH	
TRIPP9	M8	1	M8-1	FSME CARU		FSME	TPKL	37.4	133	3	30						<i>Esseschlamys</i> sp.
TRIPP9	M8	2	M8-2	FSMEFBEL	CARU	FSME	NONS	12.4	92	12	60					NO GROWTH	
TRIPP9	M9	1	M9-1	FSME CARU		FSME	NONS	16.1	76	7	30					NO GROWTH	
TRIPP9	M9	2	M9-2	FSME CARU		PIPO	TPKL	29.8	122	6	40					NO GROWTH	
TRIPP9	N10	1	N10-1	FSME PUTR	CARU	PIPO	NONS	21.2	87	12	45					NO GROWTH	
TRIPP9	N9	1	N9-1	FSMEFBEL	CARU	FSME	NONS	17.6	94	11	45					NO GROWTH	
TRIPP9	F11	1	F11-1	FSMEFBEL	CARU	FSME	TPKL	38.5	70	2	10					NO GROWTH	
TRIPP9	F9	2	F9-2	FSME CARU		FSME	NONS	11.7	81	12	70					93 % 118361	<i>Esseschlamys</i> sp.
TRIPP9	Q10	1	Q10-1	FSME CARU	AGSF	FSME	NONS	8.9	50	8	55					NO GROWTH	<i>Esseschlamys</i> sp.
TRIPP9	Q10	2	Q10-2	FSMEFBEL	CARU	FSME	TPKL	40.5	88	6	10					NO GROWTH	
TRIPP9	R8	1	R8-1	FSMEFBEL	CARU	PIPO	TPKL	30.4	114	10	25					NO GROWTH	
TRIPP9	B9	1	B9-1	FSME SYAL	AGSF	FSME	NONS	8.5	49	18	75					NO GROWTH	
TRIPP9	S7	2	S7-2	FSMEFBEL	CARU	FSME	NONS	11.2	80	8	40					NO GROWTH	
TRIPP9	S8	1	S8-1	FSMEFBEL	CARU	FSME	NONS	10.9	82	15	35						<i>Esseschlamys</i> sp.
TRIPP9	T4	1	T4-1	FSME SYAL	CARU	FSME	NONS	11.5	54	7	60						<i>Esseschlamys</i> sp.
TRIPP9	V7	1	V7-1	FSME HODI	CARU	FSME	TPKL	26.6	87	2	10						<i>Esseschlamys</i> sp.
TRIPP9	W5	1	W5-1	FSMEFBEL	CARU	PIPO	TPKL	31.5	118	3	20					NO GROWTH	
TRIPP9	W6	1	W6-1	FSME SYAL	CARU	PIPO	TPKL	32.2	63	4	10						<i>Esseschlamys</i> sp.
TRIPP9	W8	1	W8-1	FSME SYAL	AGSF	PIPO	NONS	34.2	67	3	60					NO GROWTH	
TRIPP9	X4	1	X4-1	FSME SYAL	CARU	PIPO	TPKL	24.1	153	4	25					NO GROWTH	
TRIPP9	X7	1	X7-1	FSME HODI	CARU	PIPO	NONS	23.8	108	2	40					NO GROWTH	
TRIPP9	Y4	1	Y4-1	FSME SYAL	CARU	FSME	NONS	20.5	111	11	45					NO GROWTH	
TRIPP9	Z4	1	Z4-1	FSME SYAL	CARU	PIPO	NONS	25.9	115	7	60					NO GROWTH	