FUNGAL ENDOPHYTES ISOLATED FROM LARGE ROOTS OF DOUGLAS-FIR (*PSEUDOTSUGA MENZIESII)* AND PONDEROSA PINE (*PINUS PONDEROSA*)

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of John Richard Goetz III find it satisfactory and recommend that it be accepted.

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Chair

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Abstract

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Little is known about the diversity and ecological roles of endophytes in woody roots of forest trees in the inland northwestern USA, especially as related to fire ecology. To assess fungal endophytes in large woody roots of Douglas-fir (*Pseudotsuga menziesii*) and ponderosa pine (*Pinus ponderosa*), we conducted a mycobiotic survey of root-core samples from 12 sites in north-central Washington State. Each site was subjected to one of the following fuel treatments: 1) untreated control; 2) burn only 3); thin only; and 4) thin and burn. Fungal endophytes were cultured and identified by morphology and sequencing of ITS1, 5.8S, ITS2 rDNA. In total, 1130 surface-disinfected, root-cores pieces were processed and 43% yielded one or more fungal isolates. From the 1130 surface-disinfected, root-cores pieces, 365 (72%) of 509 fungal isolates were identified comprising 20 genera (224 isolates) of hyphomycetes, 5 genera (79 isolates) of Ascomycota, 3 genera (48 isolates) of Zygomycota, and 14 isolates of

Basidiomycota. Among the more interesting findings were 1) yeast taxa usually associated with above-ground bark beetles including two undescribed species of *Candida*; 2) a putatively undescribed eurotiaceous fungus that produces ascomata in culture; and 3) multiple genera of dematiaceous hyphomycetes that have been reported from above-ground plant tissues and grass roots. Fuel treatment, host species and culture media all significantly influenced fungal endophytes recovered. Compared to controls, units subjected to burn-only showed reduced numbers of fungal endophytes; whereas recovery of endophytic fungi increased in the thin and burn units and thin-only units exhibited no change. Some associations are noted among tree root endophytes, grass root endophytes, and above-ground saprobes and facultative parasites, because many of the same species are found across all of these habitats and environments. Possible roles and functions of endophytic fungi of woody coniferous roots are discussed.

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CHAPTER ONE

INTRODUCTION AND REVIEW OF LITERATURE

Definition of fungal endophyte

Fungal endophytes have been defined in various manners, but they are typically considered as fungi that asymptomatically occupy intercellular or intracellular space within a plant. Endophytes may be mutualistic or commensal, and typically exhibit no overtly negative effects to the host. ROGERS (2000) states that an endophyte is a fungus which leads a cryptic lifestyle without disrupting normal host plant functions. SAIKKONEN et al. (1998) define endophytes as fungi that, for at least part of their life cycle, live asymptomatically within the tissues of their host plants. SIAKKONEN et al. consider latent pathogens as endophytes whereas ROGERS (2000) does not. Endophytes are defined by WILSON (1995) as organisms that for all or part of their life cycle invade living plant tissues causing no symptoms of disease. WILSON (1995) excludes mycorrhizal fungi from his definition, but includes fungi that have dormant or latent phases within the host before causing disease symptoms and known pathogenic fungi of a particular host that never cause disease symptoms following infection as a result of mutation (see REDMAN et al. 2001, FREEMAN and RODRIQUEZ 1993). STONE et al. (2004) state that endophytes act as mutualists, which might protect grasses and conifers against defoliating insects, and point out that many endophytes, when grown in culture, produce antagonistic metabolites. From these definitions, plants that are colonized with

endophytes are apparently unaltered morphologically or physiologically; however latent or minor pathogens are not universally accepted as fungal endophytes.

While many regard fungal endophytes as protective mutualists, others note lacking evidence to support the protective mutualist role of some endophytes (FAETH 2002). FAETH (2002) indicates that environment, plant and fungal genotypes, selective herbivore pressure, agronomic cultivation, variable infection frequencies, host population dynamics and alkaloid production might or might not favor the host. This evidence suggests that endophytes should be viewed as opportunistic and only mutualistic when a myriad of biotic and abiotic environmental factors are favorable for this interaction.

As mentioned previously, others consider endophytes as minor, secondary or latent pathogens. Pathogens isolated from asymptomatic hosts can be tested for pathogenic activity; however, endophytes are commonly isolated from apparently healthy plant tissues and the successful completion of Koch's postulates can be quite difficult or unsuccessful (SIEBER et al. 1999). Endophytes are recovered from various apparently healthy plant parts of ages ranging from several years old (HOFF et al. 2004a) to current year buds (SIEBER et al. 1999). As an added complexity, some endophytes might delay their sporulation until host tissue senescence, suggesting a saprophytic or minor pathogenic role (SHERWOOD-PIKE et al. 1986). Other endophytes apparently do not sporulate on

their host but periodically reinfect the host from other substrates (J.D. ROGERS, personal communication).

Ecological Roles of Fungal Endophytes

The ecological roles that fungal endophytes play within their host plants are complex, often not well understood and debatable. Findings from research conducted on endophytes from grasses and other plants may help explain the roles of coniferous root endophytes. It has been reported that fungal endophytes might have biological control potential (ADDY et al. 2005, HOFF et al. 2004a), might increase host heat tolerance (BEUCHAT and PITT 2002), might increase host tolerance to harsh environmental conditions, such as drought and extreme soil temperatures (REDMAN et al. 2002) and might confer resistance to herbivory from insect pests (MILLER et al. 2002) or herbivorous animals. It has also been suggested that fungal endophytes might function in a manner similar to mycorrhizal fungi in the acquisition of nutrients or water (MANDYAM and JUMPPONEN 2005). However, most studies have been limited to identifying endophytic fungi and detailing their presence within their host plants rather than exploring the complex ecological roles of fungal endophytes.

Endophyte surveys have contributed to understanding of fungal endophyte communities of plant tissues and provided insight into their ecological roles within host plants. Most endophyte surveys have focused on above-ground plant tissues, and much less attention has been paid to the

endophyte communities within plant roots. Among root endophyte studies, few have focused on large woody roots of forest trees (HOFF et al. 2004a).

Recent advances in molecular techniques have helped identify and quantify these cryptic fungi (HOFF et al. 2004b). Using culture-mediated and environmental PCR techniques to conduct surveys for endophytic fungi, researchers have revealed a vast array of organisms that occupy ecological niches that are largely understudied (for example, ADDY et al. 2005, SURYANARAYANAN et al. 2005, GANLEY et al. 2004, HOFF et al. 2004a, KERNAGHAN et al. 2003, GRUNIG et al. 2002, VANDENKOORNHUYSE et al. 2002, DECKERT and PETERSON 2000). However, some limitations are associated with culture-based and environmental PCR sampling methods. Culture-based surveys typically yield high proportions of ascomycetous and asexually reproducing fungi and do not yield slower growing basidiomycetes and unculturable organisms (MENKIS et al. 2006, ARNOLD et al. 2005, unpublished data). Environmental PCR surveys have yielded suspected new lineages of fungi (VANDENKOORNHUYSE et al. 2002), but the ability to investigate these findings and characterize the morphology of these suspected new organisms is, in many instances, not yet possible (WINTZINGERODE et al. 1997).

Dematiaceous hyphomycetes are a diverse group typically recovered during endophyte surveys (ADDY et al. 2005) and have been referred to as dark septate endophytes (DSE), dark septate hyphomycetes (DSH) and dark septate fungi (DSF) (ADDY et al. 2000). The designator DSE has been given to sterile

endophytic fungi isolated from roots that produce melanized or pigmented hyphae (ADDY et al. 2000). Culture conditions including prolonged low temperature incubation have allowed the morphological identification of only a few specific genera formerly designated as DSE (ADDY et al. 2000). These DSE are found in both the above-ground plant tissues of conifers, and in the roots and lamina of grasses suggesting an association among unrelated host plants and their endophyte assemblages. The ecological implications and importance of this apparent association between coniferous and grass endophytes are unknown. DSE are of relative importance as they produce multiple antagonistic metabolites (FINDLAY et al. 2003, MILLER et al. 2002) and some species have high levels of heat tolerance (BEUCHAT and PITT 2002). Chlamydospores of *Phialophora* species reportedly isolated from grass roots by ADDY et al. (2005), exhibit tolerance to high temperatures for short periods of time. However, prolonged exposure of *Phialophora* sp. chlamydospores to heated apple juice resulted in an inactivation of the structures. Although DSE represent interesting and important fungi, they are difficult to identify and a comprehensive treatment of this group is currently unavailable.

Protection of host plants

Fungal endophytes may augment host protection by producing antagonistic metabolites. Studies have shown that needles of white spruce (*Picea glauca*) seedlings inoculated with toxigenic fungal endophytes contained the chemical rugulosin in amounts shown *in vitro* to inhibit spruce budworm growth

rates (FINDLEY et al. 2003, MILLER et al. 2002). MILLER et al. (2002) reported that larvae feeding from endophyte-infected needles did not gain as much weight as larvae feeding on endophyte-free needles, suggesting a feeding deterrence in endophyte-infected needles due to the presence of rugulosin.

SABZALIAN et al. (2004) tested the response of mealy bugs (*Phenococcus solani*) and barley aphids (*Sipha maydis*) to tall and meadow fescues inoculated with fungal endophytes under greenhouse conditions. The authors found that mealy bugs were able to survive on and preferred endophyte-free plants versus endophyte-infected plants. Furthermore, barley aphids were unable to survive on endophyte-infected grasses, and aphid damage to endophyte-free plants was so extensive that the plants did not survive the experiments.

Previous studies used tall fescue (*Festuca arundinacea*) naturally and artificially inoculated with the endophyte *Neotyphodium coenophialum* to test the effects on the feeding of bird-cherry-oat aphid (*Rhopalosiphum padi*) in enclosed population and clip cage experiments (HUNT and NEWMAN 2005). These studies showed that aphid populations grew the fastest on the endophyte-free plants, relatively slower on those plants artificially inoculated, and even slower or not at all on plants naturally infected with fungal endophytes. A *Neotyphodium* endophyte has been shown not only to deter feeding upon its host, commonly perennial ryegrass (*Lolium perenne*) or tall fescue, but also to increase mortality from both the first-instar larvae and adult rice leaf bug, *Trigonotylus caelestialium* (SHIBA and SUGAWARA 2005).

Fungal endophytes not only protect the host from herbivorous insects and animals, but can also protect their host from pathogenic fungi (ARNOLD et al. 2003, PARK et al. 2001). Using leaves of the tropical tree *Theobroma cacao*, ARNOLD et al. (2003) demonstrated that infection by endophytes decreased leaf necrosis and mortality when challenged with a major pathogen (*Phytophthora* sp.). It was also found that endophyte amelioration of pathogen damage was stronger in mature leaves than young leaves and, further demonstrated that endophytes of angiosperm leaves have an "unappreciated role in host defense."

Altering host tolerance to the environment

Increasing host tolerance to harsh environmental conditions is attributed to some fungal endophytes (REDMAN et al. 2002). Surface-sterilized seeds of *Dichanthelium lanuginosum* plants collected from geothermal soils from Lassen Volcanic and Yellowstone National Parks were inoculated with a *Curvularia* sp., previously isolated from the roots of *D. lanuginosum*. Plants were grown in the field at soil temperatures of 45°C and in the lab at constant or intermittent soil temperatures ranging from 45-65°C (REDMAN et al. 2002). Those plants inoculated with the *Curvularia* sp. were able to survive the elevated soil temperatures, whereas all of the non-inoculated control plants shriveled and died (REDMAN et al. 2002). It was concluded that infection by *Curvularia* sp., which is not a heat-tolerant fungus when found growing independently of the host, confers thermotolerance to the host plant allowing both plant and fungus to survive in an otherwise intolerable environment. Fungal endophytes might also

confer cold tolerance to host plants growing on sites with constantly near-freezing or freezing temperatures. A survey conducted at the forefront of a retreating glacier found many plants harbored dark septate fungi that were considered to have a neutral or positive effect on the host plants (CAZARES 1992).

Heat tolerant fungi as endophytes

In a recent study HOFF et al. (2004a) frequently isolated the ascomycetous genus *Byssochlamys* from large woody roots of Douglas-fir and ponderosa pine. *Byssochlamys*, and its anamorph *Paecilomyces*, are heat-tolerant fungi capable of producing volatile mycotoxins (ESCOULA 1975). Species of *Byssochlamys* have been shown to withstand high temperatures for extended periods of time (BEUCHAT and PITT 2002, KING et al. 1979, BAUMGART and STOCKSMEYER 1976, BAYNE and MICHENER 1976, KING et al. 1969) and produce volatile mycotoxins in culture, on food and in fruit juices (RICE et al. 1977). ESCOULA (1975) detected the mycotoxin patulin from cultures of *P. variotii* and *B. fulva*. ESCOULA (1975) also detected both patulin and byssochlamic acid from an isolate of *B. nivea*. The culture exudates of *B. nivea* have been shown to inhibit hatching of the eggs of the nematode *Caenorhabditis elegans*, disrupt the cuticle of live *C. elegans* and increase mortality of *C. elegans* (PARK et al. 2001). KING et al. (1979) found that ascospores of *B. nivea*, submerged in grape juice, survived 60 minutes of heating at 88°C. BAUMGART and STOCKSMEYER (1976) submerged ascospores of *B. nivea* in 99°C apple juice and found the ascospores were still viable. *Byssochlamys fulva* is

also able to survive high temperatures (BAYNE and MICHENER 1976, and KING et al. 1969).

Much more information, factual and conjectural, could be cited here, but the above should indicate the breadth and depth of endophyte research.

In addition, some notable reviews of fungal endophytes are also available (ADDY et al. 2005, SCHULZ and BOYLE 2005, STONE et al. 2004, SAIKKONEN et al. 1998).

Previous Investigation

A pretreatment endophyte survey of the large woody roots of Douglas-fir (*Pseudotsuga menziesii* (MIRB.) FRANCO) and ponderosa pine (*Pinus ponderosa* DOUGLAS ex LAWSON and C. LAWSON) was conducted by HOFF et al. (2004a). The survey by HOFF et al. (2004a) was designed to gather baseline data for comparison with subsequent surveys of the same sampling units following the application of prescribed burn treatments.

Using sequence data for fungal identification, HOFF et al. (2004a) found that 50% of the roots sampled yielded fungal endophytes comprising 17 genera. Among the more frequently occurring fungal isolates are species of *Byssochlamys*, *Mucor*, *Penicillium* and several species of *Umbelopsis*. As previously mentioned, *Byssochlamys* is a heat tolerant ascomycete able to produce multiple volatile mycotoxins that has the potential to function as a biological control agent. *Umbelopsis* is purportedly tolerant to elevated temperatures (BOLLEN and VAN DER POL-LUITEN 1975) and is a common forest inhabitant (HOFF et al. 2004a) with

an unknown role as an endophyte. It was determined that host species and plant association did not significantly influence the recovery of fungal endophytes (HOFF et al. 2004a). The authors demonstrated that the large, woody roots of Douglas-fir and ponderosa pine are host to a wide array of fungi and conclude that our knowledge of endophyte diversity might be enhanced by studying the endophyte assemblages of increasingly diverse forest ecosystems using both molecular and culture media techniques.

Present Investigation and Objectives

Little is known about the diversity and ecological roles of endophytes in roots of forest trees in the inland northwestern USA and elsewhere. The objective of this study was to examine the distribution of fungal endophytes within woody roots of Douglas-fir and ponderosa pine subjected to four fuel treatments and to examine potential ecological relationships.

Currently, research interest is increasing concerning root endophytes of diverse flora. The data compiled during this study may be used for comparison with data from subsequent surveys of the same study area and elsewhere to facilitate monitoring changes in endophyte communities over time. Washington State University and the USDA Forest Service have taken leadership in endophyte surveys to study the effects of disturbance upon forest fungi and to examine the potential ecological roles of fungal endophytes in a forest ecosystem.

MATERIALS AND METHODS

Sampling sites

Sites for this study are included in the National Fire and Fire Surrogates Project (AGEE et al. 2001). The fire surrogate units are dominated by two tree species, ponderosa pine and Douglas-fir. The study consisted of 12 sampling units (Crow 1, Crow 3, Crow 6, Little Camas 11, Pendleton 30, Poison 6, Ruby, Sand 19, Sand 2, Slawson 8, Spromberg 4, and Tripp 9) 10-20 ha in size (HOFF et al. 2004a) (Figure 1). The sampling units contain the following plant associations (WILLIAMS and SMITH 1991; LILLYBRIDGE et al. 1995): PSME/SPBEL (*P. menziesii/Spirea betulifolia* var. *Lucida* (Douglas) C.L. Hitchc., PSME/PUTR (*P. menziesii/Purshia tridentate* (Pursh) DC.), PSME/CARU (*P. menziesii /Calamagrostis rubescens* Buckley), PSME/SYAL (*P. menziesii /Symphoricarpus albus* (L.) S.F. Blake), PSME/AGSP (*P. menziesii /Agropyron spicatum* (Pursh) Scribn. and Smith), PIPO/PUTR (*P. ponderosa /P. tridentate)*, PIPO/CARU (*P. ponderosa / C. rubescens)*, and PIPO/AGSP (*P. ponderosa/ A. spicatum)* (Appendix D). The sampling units used during this study were chosen from preexisting timber sale units thoroughly examined by the USDA Forest Service-Pacific Northwest Research Station-Managing Disturbance Regimes (PNW-4577), Wenatchee Forestry Sciences Laboratory. These fire surrogate units are located on all aspects, except north, in the dry, mixed conifer forest, of the Mission Creek watershed, in the Okanogan-Wenatchee National Forest of north-central Washington State, USA

(AGEE et al. 2001). Sampling units were placed on slopes < 40% with no more than 10% rock cover (HOFF et al. 2004a).

Fuel Treatments

During April and May of 2004, units designated for burn treatment received a relatively low intensity surface fire. During the burn treatments herbaceous plants were emerging from the soil and some areas of the designated burn units contained high amounts of moisture in the litter layer (personal observation). Burn treatments were conducted by helicopter-facilitated aerial ignition and surface strip firing by the Leavenworth Ranger District Initial Attack hand crew.

The thin only treatments were conducted during the same months as the burn treatments and consisted of removing a specified number of trees per acre, followed by mechanical piling and burning the remaining logging slash.

Sample collection

Using the sampling design of HOFF et al. (2004a), sample collection for the current study was conducted from June to August 2004, but no trees sampled in HOFF'S survey were re-sampled for the current research. All field work was performed and root-core samples were collected by the USDA Forest Service Pacific Northwest Research Station-Managing Disturbance Regimes, Wenatchee Forestry Sciences Laboratory.

Root-core samples (ca. 10 cm in length), extracted with a standard increment borer that had been surface-sterilized with 95% ethanol, were taken

for future fungal isolation. Root cores were extracted from one major lateral root excavated to a distance of 1 m from the root collar of randomly selected, asymptomatic Douglas-fir or ponderosa pine. Root-cores were placed into plastic soda straws, flame-sealed at both ends and stored at 4°C at the Forestry Sciences Laboratory in Wenatchee, WA, USA. Weekly packages of cores on dry ice were shipped to the USDA Forest Service Rocky Mountain Research Station-Microbial Process as Ecosystems Regulators in Western Forests in Moscow, ID, USA for fungal isolation, isolate identification, DNA sequencing and archiving.

Fungal isolation and cultures

Root-core samples were maintained at 4°C until processing (within 1 week of sample collection) at the Forestry Sciences Laboratory in Moscow, ID, USA. Before culturing, the outer root periderm and phloem tissue was removed and discarded. The outer 2 cm of xylem tissue was excised, divided into two pieces of similar size and the remaining root-core tissue was placed back in storage at 4°C. Each root-core piece was then surface-sterilized by submerging the sample in 70% ethanol, followed by brief flaming. Paired root-core samples were randomly placed into 60 X 15 mm Petri plates that contained either: 1) a selective benomyl-dichloran-streptomycin (BDS) agar composed of 15g Bacto™ malt extract, 15 g Bacto™ agar, 40 mg benomyl, 20 mg dichloran (2,6-dichloro-4-nitroaniline), and 100 mg streptomycin sulfate per liter (WORRALL and HARRINGTON 1993); or 2) 2% Bacto™ (Becton, Dickinson and Co., Sparks, MD, USA) malt-extract agar (MEA) amended with 100 mg streptomycin sulfate per

liter. Plates were incubated in the dark at 21°C for at least 8 weeks, and periodically examined with a dissecting microscope for fungal growth.

Characterization of fungal morphology

For morphological characterization cultures were grown on several Bacto™ media [(2% Corn Meal Agar (CMA), 2% Malt Extract Agar (2% MEA), Czapek Dox Agar and Potato Dextrose Agar (PDA)] and grouped according to similar cultural characteristics. Root cores in culture were examined for fungal growth using a dissecting microscope and any fungi found were immediately subcultured to 2% MEA. Culture characters used to group similar isolates included colony color, texture, extent and presence of sporulation, associated structures and growth rates. After isolates were grouped, representative cultures were intensively examined and morphological features (e.g. hyphae, conidia, asci/ascospores, sporangiophores/sporangiospores, zygospores and chlamydospores) were measured and recorded. Slides prepared from actively growing cultures were viewed with Differential Interference Contrast (DIC) microscopy. Material for examination was mounted in water, 3% KOH, or Melzer's reagent. Permanent slides were prepared using Shurmount® (Triangle Biomedical Science, Durham, NC, USA), an aqueous mounting medium or glycerine gelatine (J.D. ROGERS, personal communication). A Nikon Cool Pix 4300® digital camera (Nikon, Melville, NY, USA) was used for all photomicrographs.

All fungal isolates recovered are maintained as archival cultures at the Forestry Sciences Laboratory in Moscow, ID at 4°C in labeled 5 ml test tubes that contain 7.5g FisherBiotech Dextrose (Fisher Scientific, Fairlawn, NJ, USA), 7.5g Bacto™ malt extract, 5g Bacto™ peptone and 15g Bacto™ agar per liter.

Root core tissue unused for fungal isolation that had been stored at 4°C was examined for surface-contaminating fungi. Those cores free of contamination were placed in a 2% paraformaldehye/2% gluteraldehyde fixative with 0.01 M phosphate buffer for several weeks at 4°C. Following primary fixation, samples were rinsed three times for 10 min. each with 0.01 M phosphate buffer. A post fixation was conducted using 1% osmium tetroxide (OsO_4) at 4°C for 24 hours, then samples were rinsed three times for 10 min. each with 0.01 M phosphate buffer. To allow for viewing of longitudinal tracheids along natural weak points, samples were submerged in liquid nitrogen until completely frozen and freeze-fractured with a razor blade. Fractured sample pieces were collected and placed into 2-ml tubes for ethanol dehydration. Samples were exposed to a graded ethanol dehydration series at concentrations of 30, 40, 50, 60, 70, and 95% respectively, for 10 min. each. A final triple rinse with 100% ethanol was conducted at 10 min. for each exchange. Each sample was finalized for viewing by Critical Point Drying (CPD) with a Tousimis Samdri-PVT-3B (Tousimis Research Co., Rockville, MD, USA) and sputter coated with gold using a Anatech Technics Hummer V (Anatech, San Jose, CA, USA). Fungal hyphae were present in the longitudinal tracheids of several samples (Figures 2 and 3).

Cultures and root core samples were also viewed with a Hitachi S-570 scanning electron microscope (SEM) (Hitachi Scientific Instruments Nissei Sangyo America, Ltd., Mountain View, CA, USA) at the Franceschi Microscopy and Imaging Center at Washington State University, Pullman, WA, USA.

Fungal cultures selected for SEM work were processed by one of three methods. The first method of processing fungal cultures followed the exact steps used to prepare root-cores for the SEM (see above). Secondly, a "stub culture" method was developed, and lastly a microwave-mediated fixation protocol was developed for rapid sample preparation (ca. 120 min.).

For the "stub culture" method, a small drop of sterile media (1% MEA) was applied to the surface of an autoclaved aluminum examination stub, which is allowed to cool and solidify. A spore suspension of the desired fungal isolate was made by flooding an actively sporulating culture with sterile water then collecting a small amount of the resulting suspension with a pipette. The medium on the stub was inoculated with the spore solution and incubated in glass vials at 25°C for 5-7 days. Stub cultures are processed for viewing with SEM by lyophilizing with a Virtis lyophilizer model 6201-3130 (Virtis Co., Inc, Gardiner, NY) for 24 hours then sputter coating with gold (Figures 4, 5, and 6).

The microwave-mediated fixation protocol was conducted using a Pelco 3450 Laboratory Microwave Processor (Ted Pella, Inc. Redding, CA). Two 500-ml beakers each containing 400 ml of double distilled (dd) H_2O were placed in the microwave processor. A water pump tube was placed within one beaker to

regulate chamber temperature, and an air tube was placed in the other beaker to allow for internal humidity control. The microwave processing chamber was tested for hot spots using a neon bulb array. For processing, samples were placed away from areas identified as hot spots within the microwave processing chamber. A "dummy vial", which is a 5-ml glass scintillation vial with 2 ml of ddH_2O was kept on ice before it was placed into the microwave processing chamber with fungal samples to maintain a stable chamber temperature.

Approximately 1 cm^3 pieces of 2% MEA colonized with the fungus of interest was placed in 5-ml glass scintillation vials containing 1.5 ml of 2% paraformaldehyde / 2% gluteraldehyde in 0.1 M Phosphate buffer. Scintillation vials with fungal samples and fixative are placed in the microwave and the microwave is activated at 100% power for 2.5 min. Some samples were subjected to a post-fixation step in 2% OsO_4 at 25°C for 1 hour. Post-fixation with OsO_4 resulted in no visibly detectable differences in sample preservation when compared to those samples fixed with 2% paraformaldehyde / 2% gluteraldehyde in 0.1 M phosphate buffer only. Following fixation, samples are rinsed three times for 5 min. each with 0.1 M phosphate buffer in a fume hood.

A graded ethanol dehydration series followed the fixation. A 30%, 50%, and 60% ethanol dehydration series was conducted in the microwave processing chamber with one 40 sec. treatment at each concentration. Subsequently an ethanol dehydration series of 70, 80, 90, and 100% was conducted with two 40 sec. treatments at each concentration, using fresh ethanol for each 40 sec.

treatment. Following the graded ethanol dehydration series, samples were either stored at 25°C in 100% ethanol for 24 hours, or critical-point-dried with a Tousimis Samdri-PVT-3B (Tousimis Research Co., Rockville, MD, USA) and gold coated with an Anatech Technics Hummer V (Anatech, San Jose, CA, USA).

This method of processing fungal samples for SEM, to the best of my knowledge has not previously been done with the intent of investigating morphological structures of fungi.

DNA sequencing

In addition to morphological examination Polymerase Chain Reaction (PCR) and DNA sequencing methods, based on HOFF et al. (2004a), were used. DNA sequence data were used to confirm or facilitate morphological identifications. For most reactions, the mycelia of young, actively growing cultures (less than 7 days) were lightly scraped with a sterile pipette tip to obtain the DNA template for PCR amplification of the Internal Transcribed Spacer (ITS1 and ITS2) and 5.8S regions of nuclear ribosomal DNA (rDNA). When mycelia did not provide a suitable template for PCR, subcultures were grown for DNA extractions using DNeasy® Plant Mini Kits (QIAGEN Sciences, Inc., Germantown, MD, USA) following the protocols suggested by the manufacturer.

Each 50-µl reaction mixture contained 5 µl of 10X buffer (Applied Biosystems, Foster City, CA, USA), 4 µM $MgCl_2$, 200 µM dNTP's, 0.5 µM ITS1-F primer, 0.5 µM ITS-4 primer (WHITE et al. 1990), and 1.2 units of AmpiTaq® (Applied Biosystems). Thermocycler (PTC-200, MJ Research, Watertown, MA,

USA) parameters were a modification of those of PIMENTEL et al. (1998), as follows: initial denaturation 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1.5 min, annealing at 50°C for 1.5 min, and extension at 72°C for 2.5 min, and with a 5 min final extension at 72°C. PCR products were subjected to agarose-gel electrophoresis at 120 V for 2 hours, and then viewed under UV light after a 45 min stain with 0.5 µg/ml ethidium bromide.

In preparations for sequencing, PCR products were treated with ExoSap-IT™ (USB, Cleveland, OH, USA) following the manufacturer's protocol of 37°C for 15 min followed by 80°C for 15 min. DNA sequencing was performed by Davis Sequencing (Davis, CA, USA) or University of Wisconsin Biotechnology Center (Madison, WI, USA). BioEdit (Hall 1999; www.mbio.ncsu.edu/BioEdit/bioedit.html) was used to delete ambiguous terminal regions of single-stranded sequences, and create contigs. The National Center for Biotechnology Information's GenBank BLAST search was used to compare DNA sequences (ca. 600 bp, including partial ITS1, 5.8S, and partial ITS2) from fungal isolates with those of known fungal species. If needed, DNA sequences were obtained from previously identified fungal isolates to facilitate comparisons.

Statistical analysis

SAS GLIMMIX procedure for generalized linear mixed models was used for statistical analysis. The GLIMMIX procedure was used to determine overall fuel treatment, culture media, and host plant effects upon recovered fungal endophytes. The overall fuel treatment effect upon fungal endophytes was

conducted by considering the total number of fungal isolates recovered by fuel treatment without considering the taxonomic status of these isolates. The analysis of culture media and host plant effects upon recovered fungal isolates was conducted with consideration for the taxonomic status of recovered fungi.

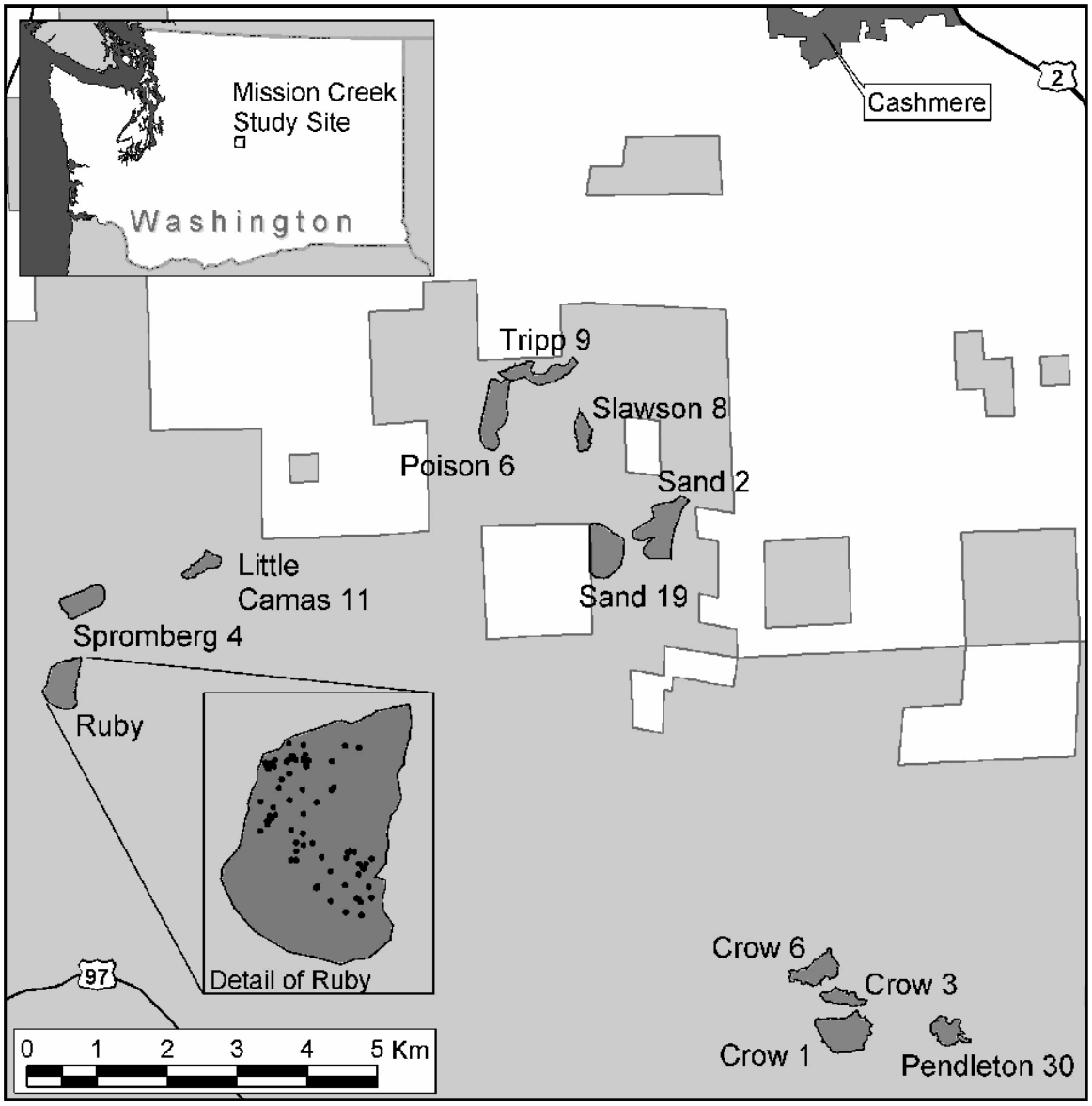

Figure 1. Location of the sampling units at the Okanogan-Wenatchee National Forest North Central WA, USA. Inset: individual trees sampled within the Thin Only Unit "Ruby." Top of map is north.

RESULTS

Overall results

In total, 1130 root-core pieces were processed and 43% of the cores processed yielded one or more fungal isolates (Table 1). From the cores processed, 509 isolates were recovered. Of the 509 isolates, 365 (72%) were identified to the genus or species level (Table 2).

In total, 224 isolates representing 20 genera of hyphomycetes, 79 isolates representing 5 genera of Ascomycota, 48 isolates representing 3 genera of Zygomycota and 14 isolates of the Basidiomycota, were identified.

Within the recovered Ascomycota, 25 isolates represent a putative new eurotiaceous taxon that produces ascomata in culture, but lacks an anamorphic state. In addition, 33 isolates of ascomycetous yeasts were recovered, including two or three undescribed species of *Candida*.

The dematiaceous hyphomycetes were the most frequently recovered taxonomic group of fungi isolated during the current study. A comprehensive list of the fungi isolated and the literature reports of substrates from which they have been isolated can be found in Table 3. Authorities for all fungi discussed are in Table 4.

Identified fungal taxa by treatment

A summary of all identified fungal isolates is presented in Table 2. From the control units, 158 fungal isolates were recovered, representing 20 genera, from 326 root cores. The control units were dominated by 36 isolates (23%)

representing six species *Penicillium*. The dematiaceous hyphomycetes *Ulocladium atrum*, *Hormonema dematioides* and *Rhinocladiella* sp. were the next most common fungal isolates from control units, represented by 14 (9%), 12 (8%) and 4 (3%) isolates, respectively. Members of the Ascomycota include 9 (6%) isolates of *Byssochlamys nivea* and 7 (4%) isolates of a putative new eurotiaceous taxon that produces ascomata in cultures and lacks an anamorphic state. The yeasts are represented by 3 (2%) isolates of *Trichosporon pullulans* and 5 (3%) isolates of *Candida* sp., including two novel species. *Mortierella* sp., *Umbelopsis isabellina*, *Mucor* sp., *Mucor racemosus,* and *Mucor plumbeus* represent the Zygomycota and were recovered 8 (5%), 5 (3%), 2 (1%), 2 (1%), 1 (1%) times, respectively. The Basidiomycota is represented by two (1%) isolates. In addition to the above fungal isolates the following fungi were recovered: 1 (1%) *Alternaria malorum* (Appendix A), 1 (1%) *Alternaria alternata*, 1 (1%) *Alternaria tenuissima*, 2 (1%) *Aureobasidium pullulans*, 1 (1%) *Leptographium* sp., 1 (1%) *Cladosporium herbarum*, 1 (1%) *Sporothrix schenckii*, 1 (1%) *Cylindrocarpon* sp., 1 (1%) *Humicola grisea*, and 4 (3%) *Paecilomyces variotii* (Figure 7).

Root-core samples from the thin-only units yielded 117 isolates representing 21 genera from 290 root cores. The most prevalent fungi from thin-only units are 12 (10%) representing five species of *Penicillium*. There was 11 (9%) representatives of a putative new eurotiaceous taxon that produces ascomata in culture and 10 (9%) of the dematiaceous hyphomycete *Hormonema dematioides*. The dematiaceous hyphomycetes were the most prevalent taxonomic group of

fungi from the thin-only units represented by 10 (9%) *Hormonema dematioides* 3 (3%) *Ulocladium atrum*, 4 (3%) *Cladosporium herbarum*, 1 (1%) *Phialophora* sp., 6 (5%) *Rhinocladiella* sp., 1 (1%) *Alternaria malorum* (Appendix A), 1 (1%) *Exophiala* sp., 1 (1%) *Ulocladium botrytis*, 1 (1%) *Leptographium* sp., 1 (1%) *Alternaria arborescens*, 1 (1%) *Thysanophora penicillioides* and 2 (2%) *Phialophora* cf. *fastigiata*. The Zygomycota was the second most prevalent group of fungi from thin-only units represented by the following numbers: 9 (8%) *Umbelopsis isabellina,* 3 (3%) *Mucor racemosus,* 3 (3%) *Mortierella* sp., 2 (2%) *Mortierella hyalina,* 1 (1%) *Mucor plumbeus* and 1 (1%) *Mucor piriformis.* Recovered yeasts include 3 (3%) isolates of *Candida*, which represented one novel species and two tentatively undescribed species, and 1 (1%) isolate of *Trichosporon pullulans*. There were 3 (3%) isolates of the Basidiomycota, 2 (2%) *Paecilomyces variotii*, 1 (1%) *Sporothrix schenckii*, 1 (1%) *Byssochlamys nivea*, 1 (1%) *Chrysosporium* sp. and 1 (1%) isolate of *Beauveria bassiana*.

Burn-only units yielded 83 isolates from 222 root cores representing 18 genera. The change in recovered fungi from the burn only treatment and taxonomic class can be viewed in Table 5. Compared with the 48% of 326 cores that yielded one or more fungal isolates from the control units, the reduction of fungi from the burn only treatment is evident. Nine (11%) isolates of *Penicillium*, which represented four species, dominated the recovered fungi. Other hyphomycetes included 2 (2%) *Paecilomyces variotii*, 1 (1%) *Fusarium* sp., 1 (1%) *Trichoderma* cf. *saturnisporum*, and 1 (1%) *Sporothrix schenckii*. Dematiaceous

hyphomycetes included 8 (10%) *Hormonema dematioides*, 4 (5%) *Ulocladium atrum*, 2 (2%) *Leptographium* sp., 2 (2%) *Leptodontidium* sp., 1 (1%) *Ulocladium botrytis*, 1 (1%) *Rhinocladiella atrovirens*, 1 (1%) *Rhinocladiella* sp., 1 (1%) *Alternaria* sp. and 1 (1%) *Aureobasidium pullulans*. Fungi of the Ascomycota included 4 (5%) isolates of *Candida* sp., 3 (4%) isolates of *Candida ernobii*, 1 (1%) *Pichia scolyti*, 4 (5%) isolates of *Byssochlamys nivea* and 3 (4%) isolates of a putative new eurotiaceous taxon that produces ascomata in culture. Basidiomycota was represented by 3 (4%) isolates including 1 (1%) *Trichosporon pullulans*, and the Zygomycota compromised 1 (1%) isolate of *Umbelopsis isabellina*. The recovery of *B. nivea* was reduced from 9 isolates on control units to 4 isolates from burn-only units. *Umbelopsis isabellina*, the only member of the Zygomycota from the burn-only units, was reduced from 5 isolates on control units to 1 isolate and *P. variotii* recovery reduced from 4 isolates on controls to 2 isolates from burn only units.

Of the 292 cores processed from the thin and burn units, 151 isolates were recovered including 19 genera. Similar to the other treatment units examined during this study, the dematiaceous hyphomycetes were highly represented. From the thin and burn units, *Leptographium* sp. dominated with 10 (7%) isolates. Other dematiaceous hyphomycetes recovered included 9 (6%) *Hormonema dematioides,* 7 (5%) *Ulocladium atrum*, 2 (1%) *Cladosporium herbarum,* 1 (1%) *Cladosporium* sp., 2 (1%) *Rhinocladiella* sp., 5 (3%) *Aureobasidium pullulans,* 1 (1%) *Alternaria arborescens,* 1 (1%) *Alternaria malorum* (Appendix A) and 1 (1%) *Exophiala* cf. *dermatitidis*. Other hyphomycetes recovered include 8 isolates (5%)

of *Penicillium* represented by 3 species, 7 (5%) *Paecilomyces variotii*, 1 (1%) *Thysanophora* sp., 1 (1%) *Trichoderma* cf. *harzianum*, 1 (1%) *Trichoderma* sp. and 1 (1%) *Sporothrix schenckii*. Ascomycetes isolated include 4 isolates of a putative new eurotiaceous taxon that produces ascomata in culture, 5 (3%) isolates of *Byssochalmys nivea* and 1 (1%) isolate of *Pichia scolyti*. Zygomycetes include 8 (5%) *Umbelopsis isabellina*, 1 (1%) *Mucor piriformis* and 1 (1%) *Mucor racemosus*. The Basidiomycota was represented by 2 (1%) isolates.

Overall affect of fuel treatments upon fungal endophytes

The overall number of fungal isolates recovered by fuel treatment, with no consideration of taxonomic status, was analyzed and is tabulated in Tables 5 and 6. Fuel treatments had an overall significant effect upon fungal endophyte recovery when compared with controls ($p=0.0357$). Proportionally more fungal isolates were recovered from the thin and burn units (thin and burn> control> thin only> burn only). The overall number of fungal isolates recovered by treatment unit, with no consideration of taxonomic status, was not significantly different for control ($p=0.1769$), thin-only ($p=0.2177$), burn-only ($p=0.1167$) and thin-and-burn ($p=0.3940$) units.

Identified fungal taxa by host

Overall, host tree species was highly significant on the total number of fungal isolates recovered, without regard to taxonomic status ($p=0.0001$). When the taxonomic status of fungal isolates was analyzed, it was determined that ponderosa pine (Table 7) yielded more Zygomycota ($p=0.0205$), hyphomycetes

(p=0.0200) and dematiaceous hyphomycetes (p=0.0237), compared to Douglas-fir (Table 8). When dematiaceous hyphomycetes were combined with hyphomycetes, the Ascomycota with ascomycetous yeasts and the Basidiomycota with basidiomycetous yeasts, significant host effects were again observed for the hyphomycetes (p=0.0017) and Zygomycota (p=0.0393) (see Table 9 for Douglas-fir and Table 10 for ponderosa pine). Numbers of recovered isolates of the Ascomycota, Basidiomycota and the isolated yeasts were not significantly different between host tree species influence, with or without combining the Ascomycota with ascomycetous yeasts and combining the Basidiomycota with basidiomycetous yeasts (data not shown).

Identified fungal taxa by culture media

Culture media influence upon recovered fungal isolates was highly significant (p=0.0001), with more fungi recovered on 2% MEA than BDS. Specifically, 2% MEA significantly increased the recovery of hyphomycetous isolates (p=0.0123) and decreased the recovery of the Zygomycota (p=0.0242) (see Table 11 for 2% MEA and Table 12 for BDS). From analyzing the combined data sets, culture media effect was highly significant for hyphomycetes (p=0.0001) only (see Table 13 for 2% MEA and Table 14 for BDS).

Depending upon the analysis, either the Zygomycota and the hyphomycetes, or only the hyphomycetes were recovered more frequently from 2% MEA than BDS. Of the core pieces plated on 2% MEA, 56% (318 of 565)

yielded one or more fungal isolates. Core pieces plated on BDS yielded one or more fungal isolates 33% (189 of 565) of the time.

The use of 2% MEA had non-significant effects upon the recovery of the Ascomycota, Basidiomycota and isolated yeasts with or without combining the Ascomycota with ascomycetous yeasts and the Basidiomycota with basidiomycetous yeasts (data not shown).

Table 1.
Percent of cores by treatment that yielded one or more fungal isolates

Unit	Treatment	Number of cores	Number of isolates	% of cores w/ fungi
Pendlenton 30	Control	106	65	61%
Sand 19	Control	74	38	51%
Sand 2	Control	68	18	26%
Crow 3	Control	78	37	47%
TOTALS		**326**	**158**	**48%**
Crow 6	Thin only	64	33	52%
Slawson 8	Thin only	68	38	56%
Crow 1	Thin only	72	23	32%
Ruby	Thin only	86	23	27%
TOTALS		**290**	**117**	**40%**
Spromberg 4	Burn only	124	36	29%
Poison 6	Burn only	98	47	48%
TOTALS		**222**	**83**	**37%**
Tripp 9	Thin and burn	80	51	64%
Camas 11	Thin and burn	212	100	47%
TOTALS		**292**	**151**	**52%**
	TOTALS	1130	509	

Table 2.
Number of identified fungal species and their frequency (%) of occurrence.

Fungal species	Number of isolates	Percentage of Total
Hormonema dematioides	39	11%
Ulocladium atrum	29	8%
Paecilomyces variotii	15	4%
Leptographium sp.	14	4%
Rhinocladiella sp.	13	4%
Aureobasidium pullulans	8	2%
Cladosporium herbarum	7	2%
Sporothrix schenckii	5	1%
Alternaria malorum	3	1%
Alternaria arborescens	3	1%
Ulocladium botrytis	2	1%
Phialophora cf. fastigiata	2	1%
Phoma herbarum	2	1%
Phialophora sp.	1	0%
Leptodontium sp.	1	0%
Leptodontium elatius	1	0%
cf. *Exophiala*	1	0%
Exophiala cf. dermatidis	1	0%
Trichoderma sp.	1	0%
Alternaria tenuissima	1	0%
Cylindrocarpon sp.	1	0%
Rhinocladiella atrovirens	1	0%
Trichoderma cf. *harzium*	1	0%
Trichoderma cf. *saturnisporium*	1	0%
Thysanophora penicillioides	1	0%
Thysanophora sp.	1	0%
Cladosporium sp.	1	0%
Beauveria bassiana	1	0%
Chrysosporium sp.	1	0%
Fusarium sp.	1	0%
Gliocladium sp.	1	0%
Humicola grisea	1	0%
Alternaria sp.	1	0%
Alternaria alternata	1	0%
Eurotiaceous sp. nov.	25	7%
Byssochlamys nivea	19	5%

Ascomycetous yeasts indet.	13	4%
Trichosporon pullulans	6	2%
Candida sp.	6	2%
Candida ernobii	4	1%
Candida sp. Nov.	4	1%
Ascomycetous yeasts sp. Nov.	3	1%
Pichia scolyti	2	1%
Eurotium sp.	2	1%
Candida sp. Nov. # 2	1	0%
Basidiomycete isolates	8	2%
Umbelopsis isabellina	20	5%
Mortierella sp.	11	3%
Mucor racemosus	6	2%
Umbelopsis sp.	3	1%
Mucor sp.	2	1%
Mortierella cf. *hyalina*	2	1%
Mucor plumbeus	2	1%
Mucor piriformis	2	1%
Penicillium sp.	16	4%
Penicillium spinulosum	13	4%
Penicillium restrictum	12	3%
Penicillium canescens	5	1%
Penicillium waksmanii	4	1%
Penicillium diversum	3	1%
Penicillium janczewskii	3	1%
Penicillium decumbens	2	1%
Penicillium raistrickii	2	1%
Penicillium cf. *corylophilum*	1	0%
Penicillium cf. *waksmanii*	1	0%
Penicillium cf. *decumbens*	1	0%
Penicillium citrium	1	0%

Table. 3

Identified fungal species, their frequency (%) of occurrence and common substrate reported in the literature.

Fungal species	# of isolates	Substrate	% of Total
Hormonema dematioides	39	Blue stained timber, wood pulp, utility poles, conifer needles and buds, human infections	11%
Ulocladium atrum	29	Plant tissue, soil	8%
Paecilomyces variotii	15	Soil, air, utility poles, food, plant material, human infections	4%
Letpotgraphium sp.	14	Black stain of conifers	4%
Rhinocladiella sp.	13	Forest litter layers, soil, utility poles, soil	4%
Aureobasidium pullulans	8	Grapes, cacti, human infections, leaf surfaces, seeds, soil	2%
Cladosporium herbarum	7	Plant tissue, air, soil, food products, paint, textiles, etc.	2%
Sporothrix schenckii	5	Human infections, soil, conifer wood, straw, living plants	1%
Alternaria malorum	3	Conifer roots, grape seed, wheat kernel, chickpea	1%
Alternaria arborescens	3		1%
Ulocladium botrytis	2	Herbaceous plants, rotten wood, paper, textiles, soil, strawberry roots, human infections	1%

Phialophora cf. *fastigiata*	2	Air, soil, water, wood	1%
Phoma herbarum	2	Herbaceous and woody plants, soil, water	1%
Phialophora sp.	1	Air, soil, water, wood and wood pulp	0%
Leptodontidium sp.	1		0%
Leptodontidium elatius	1		0%
cf. *Exophiala*	1	Animal infections, decaying wood, soil	0%
Exophiala cf. dermatitidis	1	Animal infections, decaying wood, soil	0%
Trichoderma sp.	1		0%
Alternaria tenuissima	1	Plant tissues	0%
Cylindrocarpon sp.	1		0%
Rhinocladiella atrovirens	1	Forest liter layers, soil, utility poles	0%
Trichoderma cf. *harzianum*	1	Soil, seeds, paper, textiles, sewage sludge, jet fuel	0%
Trichoderma cf. *saturnisporum*	1		0%
Thysanophora penicillioides	1	Soil, conifers	0%
Thysanophora sp.	1	Soil, conifers	0%
Cladosporium sp.	1	Plant tissue, air, soil, food products, paint, textiles, etc.	0%
Beauveria bassiana	1	Soil, insect infections	0%
Chrysosporium sp.	1	Soil, animal infections,	0%
Fusarium sp.	1	Soil	0%
Gliocladium sp.	1	Soil, plant tissues	0%
Humicola grisea	1	Soil, wood, plant tissues	0%
Alternaria sp.	1	Various substrates	0%

Alternaria alternata	1	Plant tissue, soil, food products, textiles, utility poles, human infection	0%
Eurotiaceous sp. nov.	25	Conifer roots	7%
Byssochlamys nivea	19	Soil, rotting vegetation and fruit, fire scars from California coast redwood, conifer roots	5%
Ascomycetous yeasts indet.	13	Conifer roots	4%
Trichosporon pullulans	6	Soil	2%
Candida sp.	6	Bark beetles	2%
Candida ernobii	4	Bark beetles, human infections	1%
Candida sp. Nov.	4	Conifer roots	1%
Ascomycetous yeasts sp. Nov.	3	Conifer roots	1%
Pichia scolyti	2	Bark beetles, other insects	1%
Eurotium sp.	2	Soil	1%
Candida sp. Nov. # 2	1	Conifer roots	0%
Basidiomycete isolates	8	Conifer roots	2%
Umbelopsis isabellina	20	Conifer roots, decaying vegetation, forest soils	5%
Mortierella sp.	11	Soil	3%
Mucor racemosus	6	Soil, dung, decaying vegetation, stored grains, causing zygomycosis	2%
Umbelopsis sp.	3	Conifer roots, forest soils	1%
Mucor sp.	2	Soil, dung, vegetation, stored grains, causing zygomycosis	1%
Mortierella cf. *hyalina*	2	Soil	1%

Mucor plumbeus	2	Soil, dung, decaying vegetation, stored grains, causing zygomycosis in humans	1%
Mucor piriformis	2	Soil, dung, decaying vegetation, stored grains, causing zygomycosis in humans	1%
Penicillium sp.	16	Many substrates	4%
Penicillium spinulosum	13	Soil, plant tissue, food products, textiles, substrates containing heavy metals, acids and tannins	4%
Penicillium restrictum	12	Soil	3%
Penicillium canescens	5	Soil	1%
Penicillium waksmanii	4	Forest soil	1%
Penicillium diversum	3	Deteriorating military equipment, dried egg powder, soil, moldy leather	1%
Penicillium janczewskii	3	Soil	1%
Penicillium decumbens	2	Soil, deteriorating military equipment	1%
Penicillium raistrickii	2	Moldy cotton	1%
Penicillium cf. *corylophilum*	1	Many substrates	0%
Penicillium cf. *waksmanii*	1	Forest soil	0%
Penicillium cf. *decumbens*	1	Soil, deteriorating military equipment	0%
Penicillium citrinum	1	Soil, plants, food products, textiles, biodegrading materials	0%

Table. 4

Identified fungal species and their authorities **Fungal species**	**Authority**
Hormonema dematioides	Lagerb. & Melin
Ulocladium atrum	Preuss
Paecilomyces variotii	Bainier
Leptographium sp.	
Rhinocladiella sp.	
Aureobasidium pullulans	(de Bary) G. Arnaud
Cladosporium herbarum	(Pers.) Link
Sporothrix schenckii	Hektoen & C.F. Perkins
Alternaria malorum	(Rühle) U. Braun, Crous & Dugan
Alternaria arborescens	E.G. Simmons
Ulocladium botrytis	Preuss
Phialophora cf. fastigiata	(Lagerb. & Melin) Conant
Phoma herbarum	
Phialophora sp.	
Leptodontidium sp.	
Leptodontidium elatius	(F. Mangenot) de Hoog
cf. *Exophiala*	
Exophiala cf. dermatitidis	(Kano) de Hoog
Trichoderma sp.	
Alternaria tenuissima	(Kunze) Wiltshire
Cylindrocarpon sp.	
Rhinocladiella atrovirens	Nannf.
Trichoderma cf. *harzianum*	Rifai
Trichoderma cf. *saturnisporum*	Hammill
Thysanophora penicillioides	(Roum.) W.B. Kendr.
Thysanophora sp.	
Cladosporium sp.	
Beauveria bassiana	(Bals.-Criv.) Vuill..
Chrysosporium sp.	
Fusarium sp.	
Gliocladium sp.	
Humicola grisea	Traaen
Alternaria sp.	
Alternaria alternata	(Fr.) Keissl.
Eurotiaceous nov. sp.	
Byssochlamys nivea	Westling
Ascomycetous yeasts indet.	
Trichosporon pullulans	(Lindner) Diddens & Lodder

Candida sp.	
Candida ernobii	(Lodder & Kreger) S.A. Mey. & Yarrow
Candida sp. Nov.	
Ascomycetous yeasts sp. Nov.	
Pichia scolyti	(Phaff & Yoney.) Kreger
Eurotium sp.	
Candida sp. Nov. # 2	
Basidiomycete isolates	
Umbelopsis isabellina	(Oudem.) W. Gams
Mortierella sp.	
Mucor racemosus	Fresen.
Umbelopsis sp.	
Mucor sp.	
Mortierella cf. *hyalina*	(Harz) W. Gams
Mucor plumbeus	Bonord.
Mucor piriformis	Scop.
Penicillium sp.	
Penicillium spinulosum	Thom
Penicillium restrictum	J.C. Gilman & E.V. Abbott
Penicillium canescens	Sopp
Penicillium waksmanii	K.M. Zalessky
Penicillium diversum	Raper and Fennel
Penicillium janczewskii	K.M. Zalessky
Penicillium decumbens	Thom
Penicillium raistrickii	G. Sm.
Penicillium cf. *corylophilum*	Dierckx
Penicillium cf. *waksmanii*	K.M. Zalessky
Penicillium cf. *decumbens*	Thom
Penicillium citrinum	Thom

Table 5. Total number of fungal isolates listed by treatment unit, combined taxonomy.

Unit	Treatment	Number of cores	Number of isolates	HYPHO	ASCO	BASIDIO	ZYGO	INDET.
Pendlenton 30	Control	106	65	28	13	2	10	12
Sand 19	Control	74	38	22	6	2	2	6
Sand 2	Control	68	18	12	2	1	0	3
Crow 3	Control	78	37	19	6	0	6	6
TOTALS		**326**	**158**	**81**	**27**	**5**	**18**	**27**
Crow 6	Thin only	64	33	16	5	1	8	3
Slawson 8	Thin only	68	38	14	6	2	7	9
Crow 1	Thin only	72	23	9	4	0	2	8
Ruby	Thin only	86	23	10	3	2	2	6
TOTALS		**290**	**117**	**49**	**18**	**5**	**19**	**26**
Spromberg 4	Burn only	124	36	18	7	2	1	8
Poison 6	Burn only	98	47	19	11	1	0	16
TOTALS		**222**	**83**	**37**	**18**	**3**	**1**	**24**
Tripp 9	Thin and burn	80	51	27	5	1	4	14
Camas 11	Thin and burn	212	100	33	13	0	6	48
TOTALS		**292**	**151**	**60**	**18**	**1**	**10**	**62**
	TOTALS	1130	509	227	81	14	48	139
	% of total			45%	16%	3%	9%	27%

ASCO – Ascomycota
BASIDIO – Basidiomycota
ZYGO – Zygomycota
HYPHO – Hyphomycetes
INDET – Undetermined identification

Table 6. Total number of fungal isolates listed by treatment unit.

Unit	Treatment	Number of cores	Number of isolates	DH	ASCO	BASIDIO	ZYGO	YEASTS	HYPHO	INDET.
Pendlenton 30	Control	106	65	14	7	2	10	6	14	12
Sand 19	Control	74	38	9	4	0	2	4	13	6
Sand 2	Control	68	18	7	2	0	0	1	5	3
Crow 3	Control	78	37	9	5	0	6	1	10	6
TOTALS		**326**	**158**	**39**	**18**	**2**	**18**	**12**	**42**	**27**
Crow 6	Thin only	64	33	12	1	0	8	4	4	4
Slawson 8	Thin only	68	38	8	6	1	7	1	6	9
Crow 1	Thin only	72	23	6	3	0	2	1	3	8
Ruby	Thin only	86	23	6	2	2	2	1	4	6
TOTALS		**290**	**117**	**32**	**12**	**3**	**19**	**7**	**17**	**27**
Spromberg 4	Burn only	124	36	12	0	2	1	7	6	8
Poison 6	Burn only	98	47	11	7	0	0	5	8	16
TOTALS		**222**	**83**	**23**	**7**	**2**	**1**	**12**	**14**	**24**
Tripp 9	Thin and burn	80	51	22	3	1	4	2	5	14
Camas 11	Thin and burn	212	100	19	7	0	6	6	14	48
TOTALS		**292**	**151**	**41**	**10**	**1**	**10**	**8**	**19**	**62**
	TOTALS	1130	509	135	47	8	48	39	92	140
	% of total			27%	9%	2%	9%	8%	18%	28%

DH – Dematiaceous Hyphomycetes
ASCO – Ascomycota
BASIDIO – Basidiomycota
ZYGO – Zygomycota
YEASTS – Ascomycetous and Basidiomycetous yeasts
HYPHO – Hyphomycetes
INDET – Undetermined identification

Table 7. Total number of fungal isolates recovered from ponderosa pine.

Unit	Treatment	Host Ponderosa pine	Number of cores	Total Isolated	DH	ASCO	BASIDIO	ZYGO	YEASTS	HYPHO	INDET.
Pendlenton 30	Control	45	90	64	13	7	2	10	6	14	12
Sand 19	Control	10	20	19	5	2	0	1	2	6	3
Sand 2	Control	5	10	2	1	0	0	0	0	0	1
Crow 3	Control	28	56	30	9	2	0	4	1	9	5
TOTALS		**88**	**176**	**115**	**28**	**11**	**2**	**15**	**9**	**29**	**21**
Crow 6	Thin only	31	62	32	12	1	0	8	4	4	3
Slawson 8	Thin only	11	22	18	7	1	1	6	0	3	0
Crow 1	Thin only	25	50	19	7	3	0	1	1	3	4
Ruby	Thin only	19	38	15	3	1	2	2	1	3	3
TOTALS		**86**	**172**	**84**	**29**	**6**	**3**	**17**	**6**	**13**	**10**
Spromberg 4	Burn only	35	70	22	6	0	2	0	5	4	5
Poison 6	Burn only	37	74	37	8	7	0	0	3	6	13
TOTALS		**72**	**144**	**59**	**14**	**7**	**2**	**0**	**8**	**10**	**18**
Tripp 9	Thin and burn	9	18	14	7	1	0	1	2	2	1
Camas 11	Thin and burn	86	172	87	18	5	0	5	4	13	42
TOTALS		**95**	**190**	**101**	**25**	**6**	**0**	**6**	**6**	**15**	**43**
	TOTALS	341	682	359	96	30	7	38	29	67	92
	percentage of total				27%	8%	2%	11%	8%	19%	26%

DH – Dematiaceous Hyphomycetes
ASCO – Ascomycota
BASIDIO – Basidiomycota
ZYGO – Zygomycota
YEASTS – Ascomycetous and Basidiomycetous yeasts
HYPHO – Hyphomycetes
INDET – Undetermined identification

Table 8. Total number of fungal isolates recovered from Douglas-fir.

Unit	Treatment	Host Douglas-fir	Number of cores	Total Isolated	DH	ASCO	BASIDIO	ZYGO	YEASTS	HYPHO	INDET.
Pendlenton 30	Control	2	4	1	1	0	0	0	0	0	0
Sand 19	Control	27	54	19	4	2	0	1	2	7	3
Sand 2	Control	29	58	16	6	2	0	0	1	5	2
Crow 3	Control	11	22	7	0	3	0	2	0	1	1
TOTALS		**69**	**138**	**43**	**11**	**7**	**0**	**3**	**3**	**13**	**6**
Crow 6	Thin only	1	2	1	0	0	0	0	0	0	1
Slawson 8	Thin only	23	46	21	1	5	0	1	1	3	10
Crow 1	Thin only	11	22	2	0	0	0	1	0	0	1
Ruby	Thin only	22	44	8	3	1	0	0	0	1	3
TOTALS		**57**	**114**	**32**	**4**	**6**	**0**	**2**	**1**	**4**	**15**
Spromberg 4	Burn only	27	54	14	6	0	0	1	2	2	3
Poison 6	Burn only	14	28	10	3	0	0	0	2	2	3
TOTALS		**41**	**82**	**24**	**9**	**0**	**0**	**1**	**4**	**4**	**6**
Tripp 9	Thin and burn	31	62	37	15	2	1	3	0	3	13
Camas 11	Thin and burn	26	52	12	1	2	0	1	2	1	5
TOTALS		**57**	**114**	**49**	**16**	**4**	**1**	**4**	**2**	**4**	**18**
	TOTALS	224	448	148	40	17	1	10	10	25	45
	percentage of total				27%	11%	1%	7%	7%	17%	30%

DH – Dematiaceous Hyphomycetes
ASCO – Ascomycota
BASIDIO – Basidiomycota
ZYGO – Zygomycota
YEASTS – Ascomycetous and Basidiomycetous yeasts
HYPHO – Hyphomycetes
INDET – Undetermined identification

Table 9. Total number of fungal isolates recovered from Douglas-fir.

Unit	Treatment	Host Douglas-fir	Number of cores	Total Isolated	HYPHO	ASCO	BASIDIO	ZYGO	INDET.
Pendlenton 30	Control	2	4	1	1	0	0	0	0
Sand 19	Control	27	54	19	11	4	0	1	3
Sand 2	Control	29	58	16	11	2	1	0	2
Crow 3	Control	11	22	7	1	3	0	2	1
TOTALS		**69**	**138**	**43**	**24**	**9**	**1**	**3**	**6**
Crow 6	Thin only	1	2	1	0	0	0	0	1
Slawson 8	Thin only	23	46	21	4	5	1	1	10
Crow 1	Thin only	11	22	2	0	0	0	1	1
Ruby	Thin only	22	44	8	4	1	0	0	3
TOTALS		**57**	**114**	**32**	**8**	**6**	**1**	**2**	**15**
Spromberg 4	Burn only	27	54	14	8	2	0	1	3
Poison 6	Burn only	14	28	10	5	1	1	0	3
TOTALS		**41**	**82**	**24**	**13**	**3**	**1**	**1**	**6**
Tripp 9	Thin and burn	31	62	37	18	2	1	3	13
Camas 11	Thin and burn	26	52	12	2	4	0	1	5
TOTALS		**57**	**114**	**49**	**20**	**6**	**1**	**4**	**18**
	TOTALS	224	448	148	65	24	4	10	45
	percentage of total				44%	16%	3%	7%	30%

ASCO – Ascomycota
BASIDIO – Basidiomycota
ZYGO – Zygomycota
HYPHO – Hyphomycetes
INDET – Undetermined identification

Table 10. Total number of fungal isolates recovered from ponderosa pine.

Unit	Treatment	Host Ponderosa pine	Number of cores	Total Isolated	HYPHO	ASCO	BASIDIO	ZYGO	INDET.
Pendlenton 30	Control	45	90	64	27	13	2	10	12
Sand 19	Control	10	20	19	11	2	2	1	3
Sand 2	Control	5	10	2	1	0	0	0	1
Crow 3	Control	28	56	30	18	3	0	4	5
TOTALS		**88**	**176**	**115**	**57**	**18**	**4**	**15**	**21**
Crow 6	Thin only	31	62	32	16	5	1	8	2
Slawson 8	Thin only	11	22	17	9	1	1	6	0
Crow 1	Thin only	25	50	19	9	4	0	1	5
Ruby	Thin only	19	38	15	6	2	2	2	3
TOTALS		**86**	**172**	**83**	**40**	**12**	**4**	**17**	**10**
Spromberg 4	Burn only	35	70	22	10	5	2	0	5
Poison 6	Burn only	37	74	37	14	10	0	0	13
TOTALS		**72**	**144**	**59**	**24**	**15**	**2**	**0**	**18**
Tripp 9	Thin and burn	9	18	14	9	3	0	1	1
Camas 11	Thin and burn	86	172	87	31	9	0	5	42
TOTALS		**95**	**190**	**101**	**40**	**12**	**0**	**6**	**43**
	TOTALS	341	682	358	161	57	10	38	92
	percentage of total				45%	16%	3%	11%	26%

ASCO – Ascomycota
BASIDIO – Basidiomycota
ZYGO – Zygomycota
HYPHO – Hyphomycetes
INDET – Undetermined identification

Table 11. Total number of fungal isolates recovered from 2% MEA culture media

Unit	Treatment	MEDIA 2% MEA	Number of cores	DH	ASCO	BASIDIO	ZYGO	YEASTS	HYPHO	INDET
Pendlenton 30	Control	39	53	9	4	1	5	3	12	5
Sand 19	Control	27	37	4	4	0	1	2	11	5
Sand 2	Control	14	34	5	1	0	0	1	5	2
Crow 3	Control	22	39	3	5	0	2	0	8	4
TOTALS		**102**	**163**	**21**	**14**	**1**	**8**	**6**	**36**	**16**
Crow 6	Thin only	20	32	6	1	0	3	2	3	5
Slawson 8	Thin only	19	34	7	4	0	1	0	4	3
Crow 1	Thin only	15	36	4	3	0	2	0	2	4
Ruby	Thin only	15	43	4	2	1	2	0	3	3
TOTALS		**69**	**145**	**21**	**10**	**1**	**8**	**2**	**12**	**15**
Spromberg 4	Burn only	23	62	10	0	1	0	3	4	5
Poison 6	Burn only	31	49	10	6	0	0	2	6	7
TOTALS		**54**	**111**	**20**	**6**	**1**	**0**	**5**	**10**	**12**
Tripp 9	Thin and burn	31	40	16	2	0	1	0	3	9
Camas 11	Thin and burn	62	106	12	6	0	3	2	11	28
TOTALS		**93**	**146**	**28**	**8**	**0**	**4**	**2**	**14**	**37**
	TOTALS	318	565	90	38	3	20	15	72	80
percentage of total				28%	12%	1%	6%	5%	23%	25%

DH – Dematiaceous Hyphomycetes
ASCO – Ascomycota
BASIDIO – Basidiomycota
ZYGO – Zygomycota
YEASTS – Ascomycetous and Basidiomycetous yeasts
HYPHO – Hyphomycetes
INDET – Undetermined identification

Table 12. Total number of fungal isolates recovered from BDS culture media

Unit	Treatment	MEDIA BDS	Number of cores	DH	ASCO	BASIDIO	ZYGO	YEASTS	HYPHO	INDET
Pendlenton 30	Control	26	53	5	3	1	5	3	2	7
Sand 19	Control	11	37	5	0	0	1	2	2	1
Sand 2	Control	4	34	2	1	0	0	0	0	1
Crow 3	Control	15	39	6	0	0	4	1	2	2
TOTALS		**56**	**163**	**18**	**4**	**1**	**10**	**6**	**6**	**11**
Crow 6	Thin only	13	32	3	0	0	5	2	1	2
Slawson 8	Thin only	19	34	1	2	1	6	1	2	6
Crow 1	Thin only	7	36	3	0	0	0	1	1	2
Ruby	Thin only	7	43	2	0	1	0	1	1	2
TOTALS		**46**	**145**	**9**	**2**	**2**	**11**	**5**	**5**	**12**
Spromberg 4	Burn only	13	62	2	0	1	1	4	2	3
Poison 6	Burn only	16	49	1	1	0	0	3	2	9
TOTALS		**29**	**111**	**3**	**1**	**1**	**1**	**7**	**4**	**12**
Tripp 9	Thin and burn	20	40	6	1	1	2	2	2	6
Camas 11	Thin and burn	38	106	7	1	0	3	4	3	20
TOTALS		**58**	**146**	**13**	**2**	**1**	**5**	**6**	**5**	**26**
	TOTALS	189	565	43	9	5	27	24	20	61
percentage of total				23%	5%	3%	14%	13%	11%	32%

DH – Dematiaceous Hyphomycetes
ASCO – Ascomycota
BASIDIO – Basidiomycota
ZYGO – Zygomycota
YEASTS – Ascomycetous and Basidiomycetous yeasts
HYPHO – Hyphomycetes
INDET – Undetermined identification

Table 13. Total number of fungal isolates recovered from 2% MEA culture media.

Unit	Treatment	MEDIA 2% MEA	Number of cores	HYPHO	ASCO	BASIDIO	ZYGO	INDET
Pendlenton 30	Control	39	53	21	7	1	5	5
Sand 19	Control	27	37	15	4	2	1	5
Sand 2	Control	14	34	10	1	1	0	2
Crow 3	Control	22	39	11	5	0	2	4
TOTALS		**102**	**163**	**57**	**17**	**4**	**8**	**16**
Crow 6	Thin only	20	32	12	3	1	3	1
Slawson 8	Thin only	19	34	11	4	0	1	3
Crow 1	Thin only	15	36	5	3	0	2	5
Ruby	Thin only	15	43	7	2	1	2	3
TOTALS		**69**	**145**	**35**	**12**	**2**	**8**	**12**
Spromberg 4	Burn only	23	62	14	3	1	0	5
Poison 6	Burn only	31	49	16	7	1	0	7
TOTALS		**54**	**111**	**30**	**10**	**2**	**0**	**12**
Tripp 9	Thin and burn	31	40	19	2	0	1	9
Camas 11	Thin and burn	62	106	23	8	0	3	28
TOTALS		**93**	**146**	**42**	**10**	**0**	**4**	**37**
	TOTALS	318	565	164	49	8	20	77
percentage of total				52%	15%	3%	6%	24%

ASCO – Ascomycota
BASIDIO – Basidiomycota
ZYGO – Zygomycota
HYPHO – Hyphomycetes
INDET – Undetermined identification

Table 14. Total number of fungal isolates recovered from BDS culture media

Unit	Treatment	MEDIA BDS	Number of cores	HYPHO	ASCO	BASIDIO	ZYGO	INDET.
Pendlenton 30	Control	26	53	7	6	1	5	7
Sand 19	Control	11	37	7	2	0	1	1
Sand 2	Control	4	34	2	1	0	0	1
Crow 3	Control	15	39	8	1	0	4	2
TOTALS		**56**	**163**	**24**	**10**	**1**	**10**	**11**
Crow 6	Thin only	13	32	4	2	0	5	2
Slawson 8	Thin only	19	34	3	2	2	6	6
Crow 1	Thin only	7	36	4	1	0	0	2
Ruby	Thin only	7	43	3	1	1	0	2
TOTALS		**46**	**145**	**14**	**6**	**3**	**11**	**12**
Spromberg 4	Burn only	13	62	4	4	1	1	3
Poison 6	Burn only	16	49	3	4	0	0	9
TOTALS		**29**	**111**	**7**	**8**	**1**	**1**	**12**
Tripp 9	Thin and burn	20	40	8	3	1	3	5
Camas 11	Thin and burn	38	106	10	5	0	3	20
TOTALS		**58**	**146**	**18**	**8**	**1**	**6**	**25**
	TOTALS	189	565	63	32	6	28	60
percentage of total				33%	17%	3%	15%	19%

ASCO – Ascomycota
BASIDIO – Basidiomycota
ZYGO – Zygomycota
HYPHO – Hyphomycetes
INDET – Undetermined identification

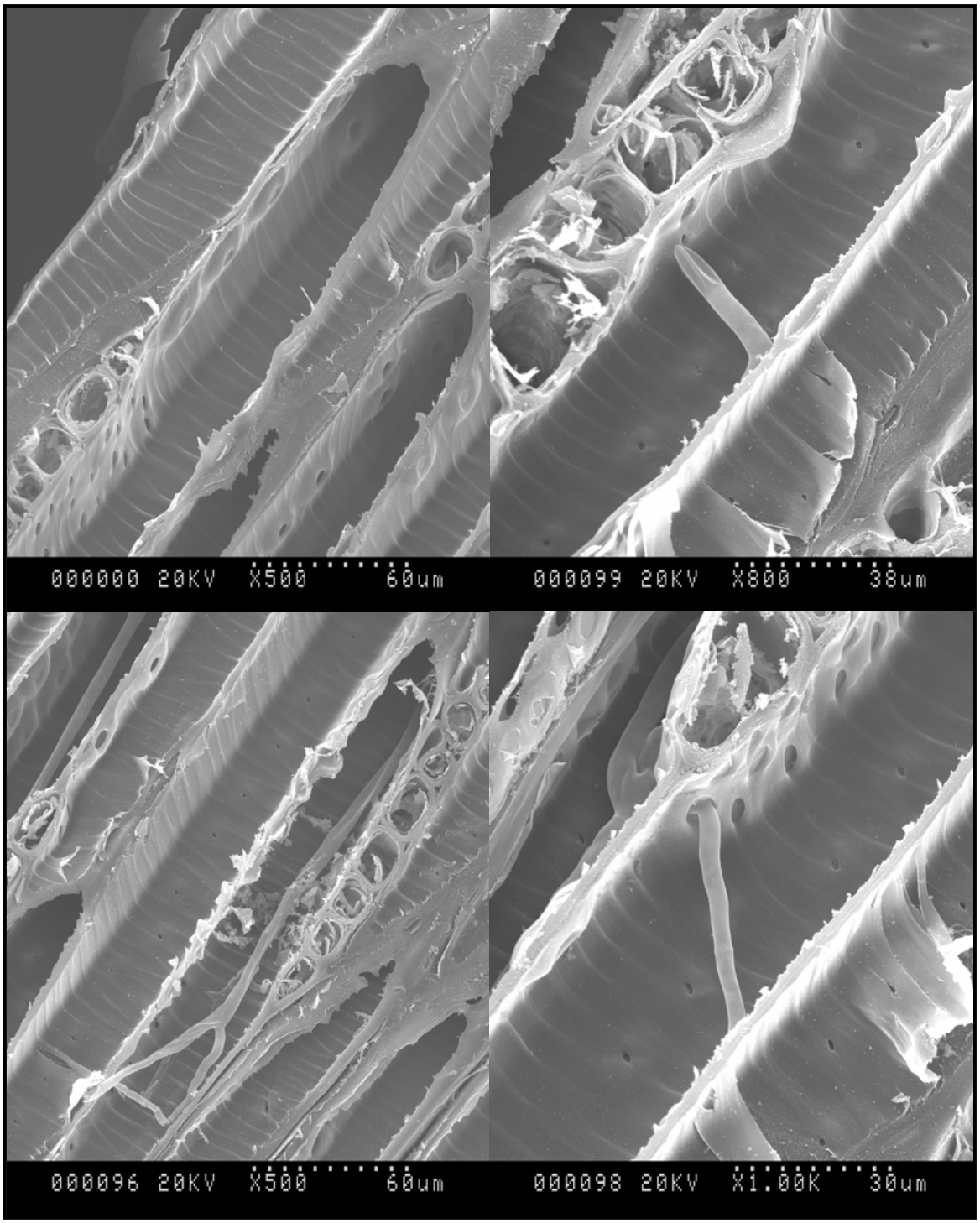

Figure 2. Clockwise from upper left: Longitudinal tracheids and ray cells free of fungal hyphae. A hyphae growing within a longitudinal tracheid. Branched hyphae growing within a longitudinal tracheid. Hyphae passing between longitudinal tracheids via a pit adjacent to ray cells.

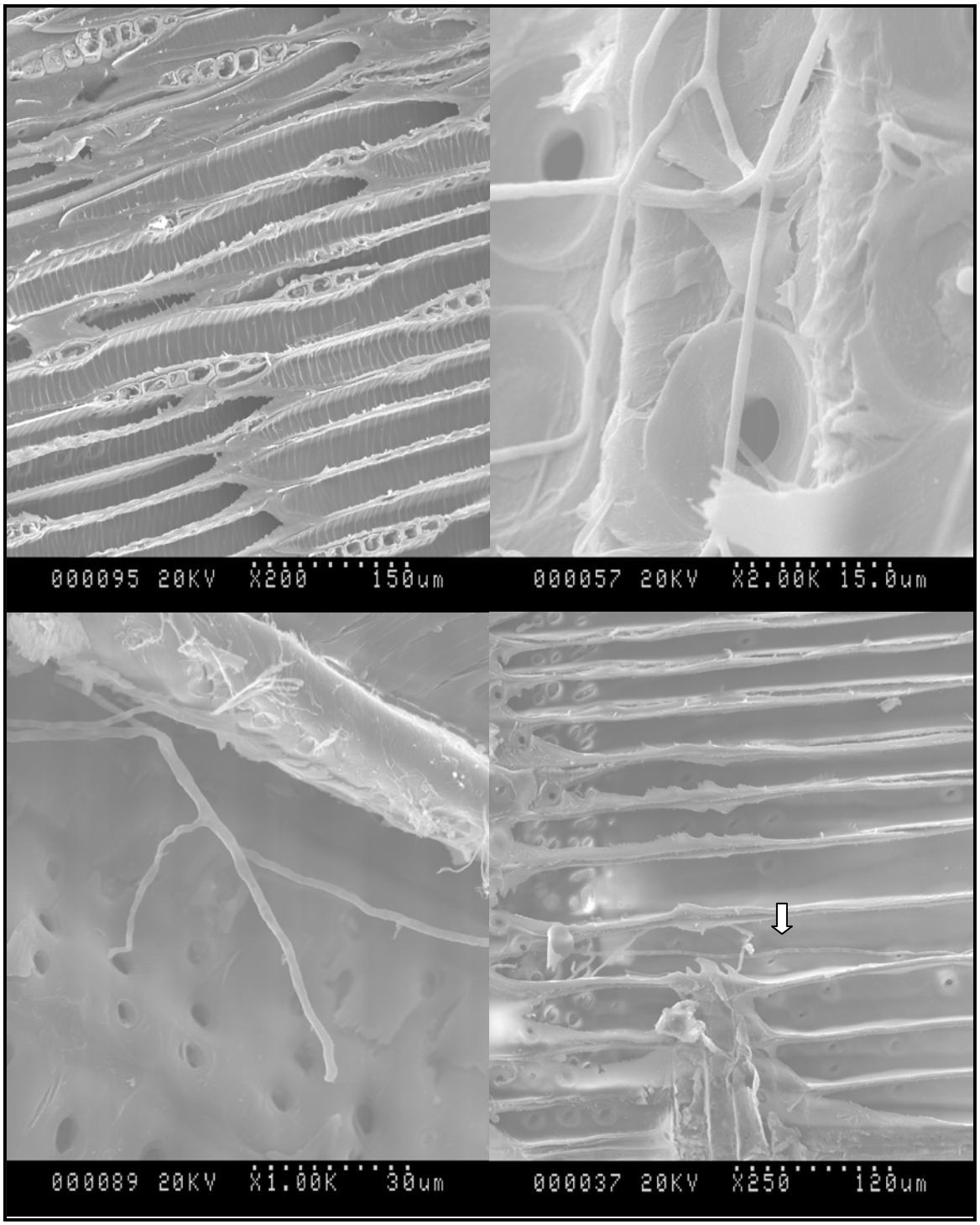

Figure 3. Clockwise from upper left: Longitudinal tracheids and ray cells free of fungal hyphae. Hyphae growing within longitudinal tracheids and pits. Hyphae growing with a longitudinal tracheid. Hyphae within longitudinal tracheid (white arrow).

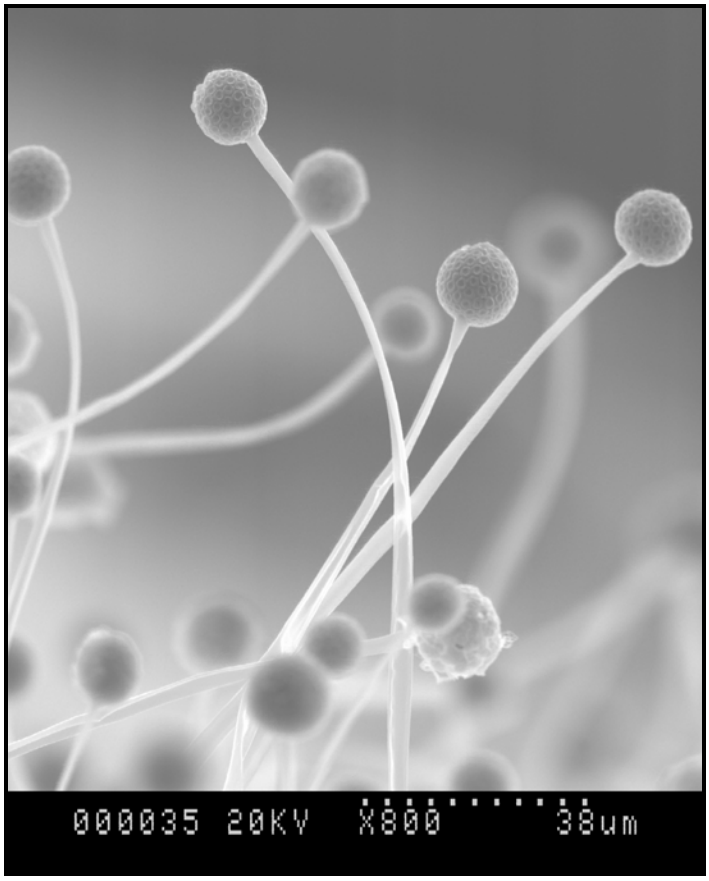

Figure 4. SEM photomicrograph of an *Umbelopsis isabellina* stub culture showing sporangia and sporangiophores.

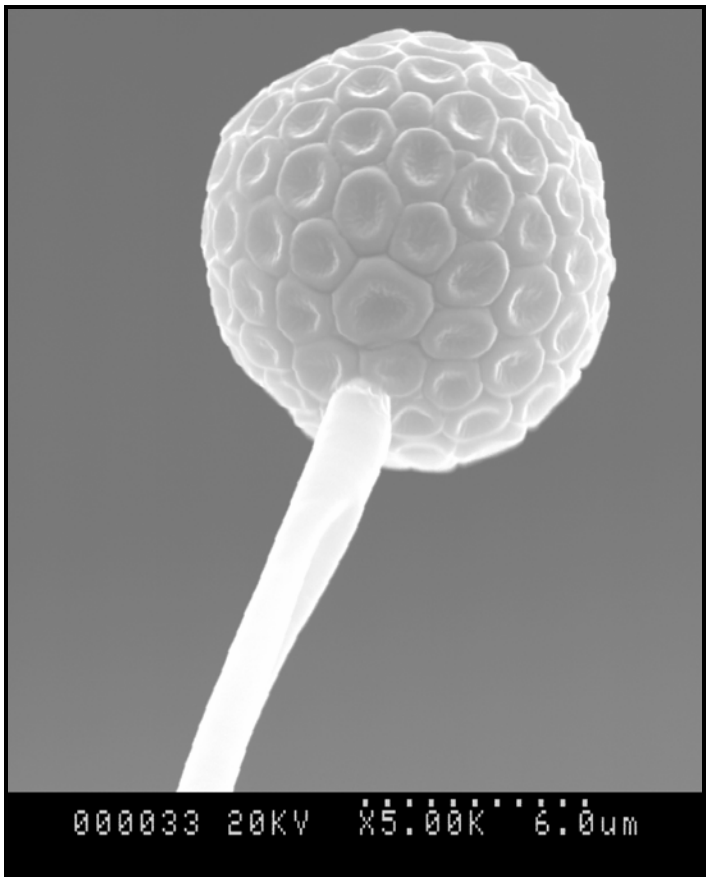

Figure 5. Close-up SEM photomicrograph of an *Umbelopsis isabellina* stub culture showing sporangia and sporangiophore.

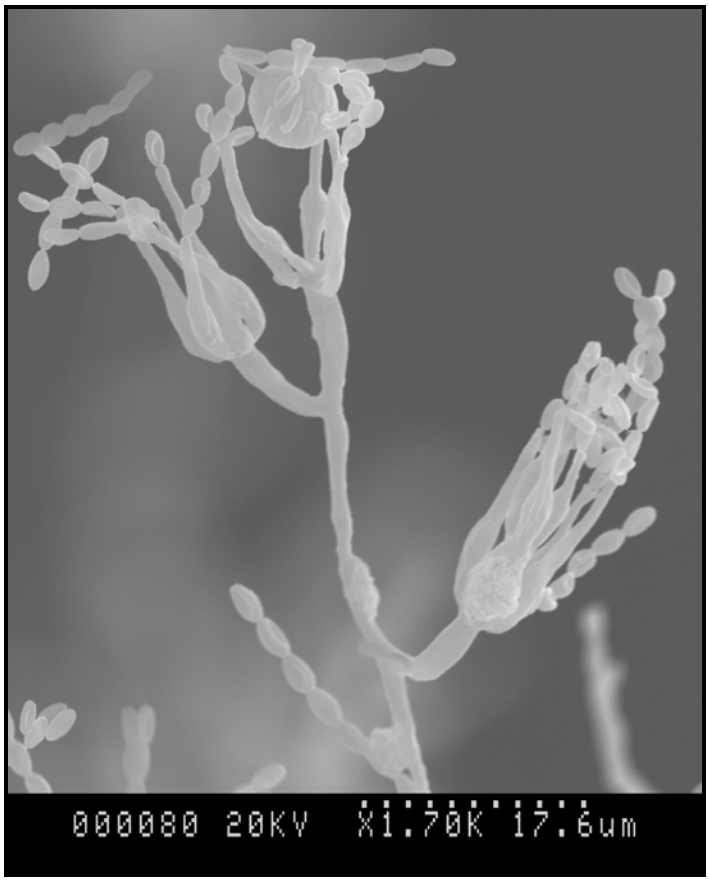

Figure 6. SEM photomicrograph of a *Paecilomyces variotii* stub culture showing branching stipes, phialides and conidial chains.

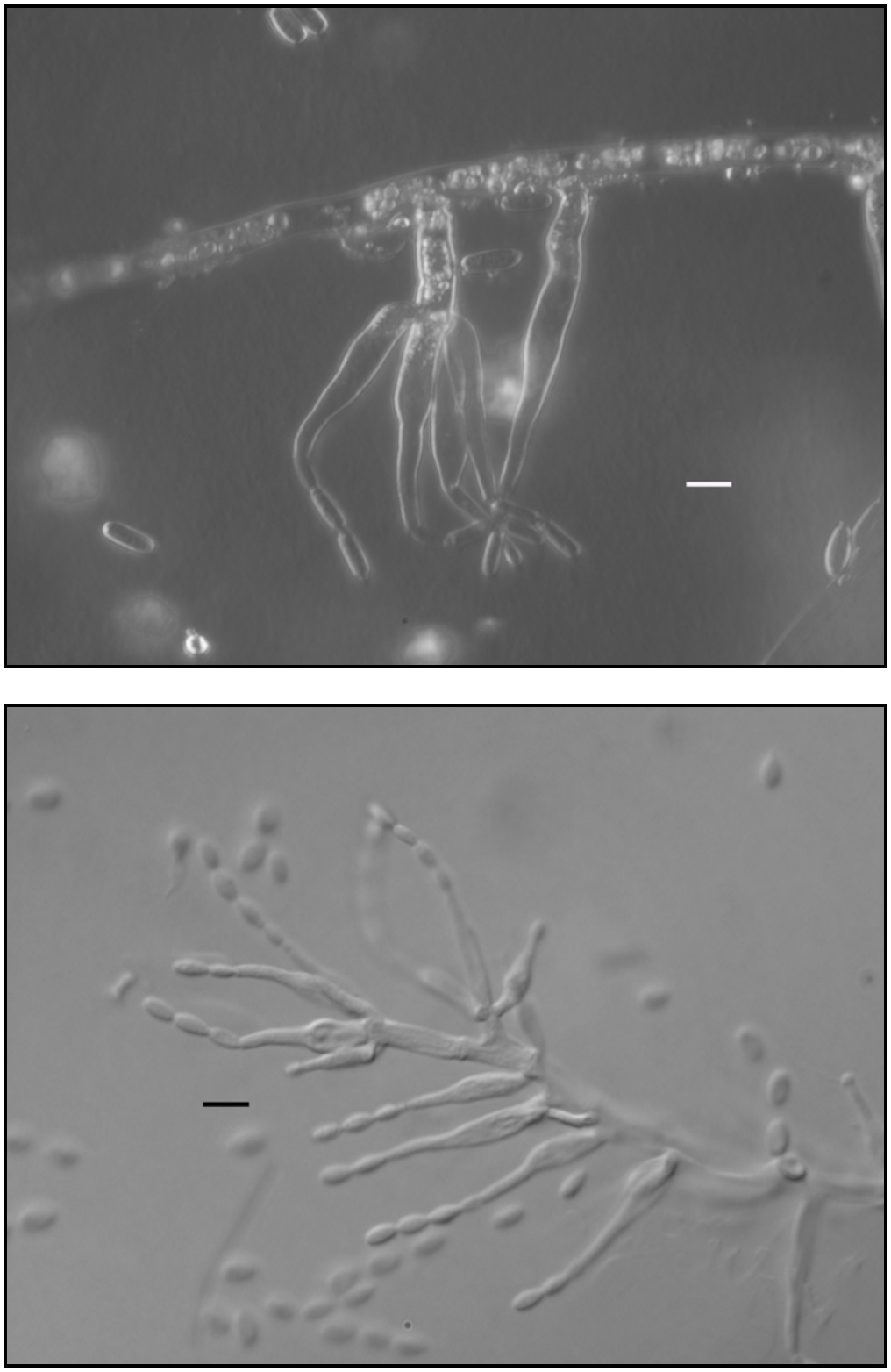

Figure 7. *Paecilomyces variotii* photomicrographs showing branched stipes, phialides and conidial chains. Image: DIC. Bar = 5 µm

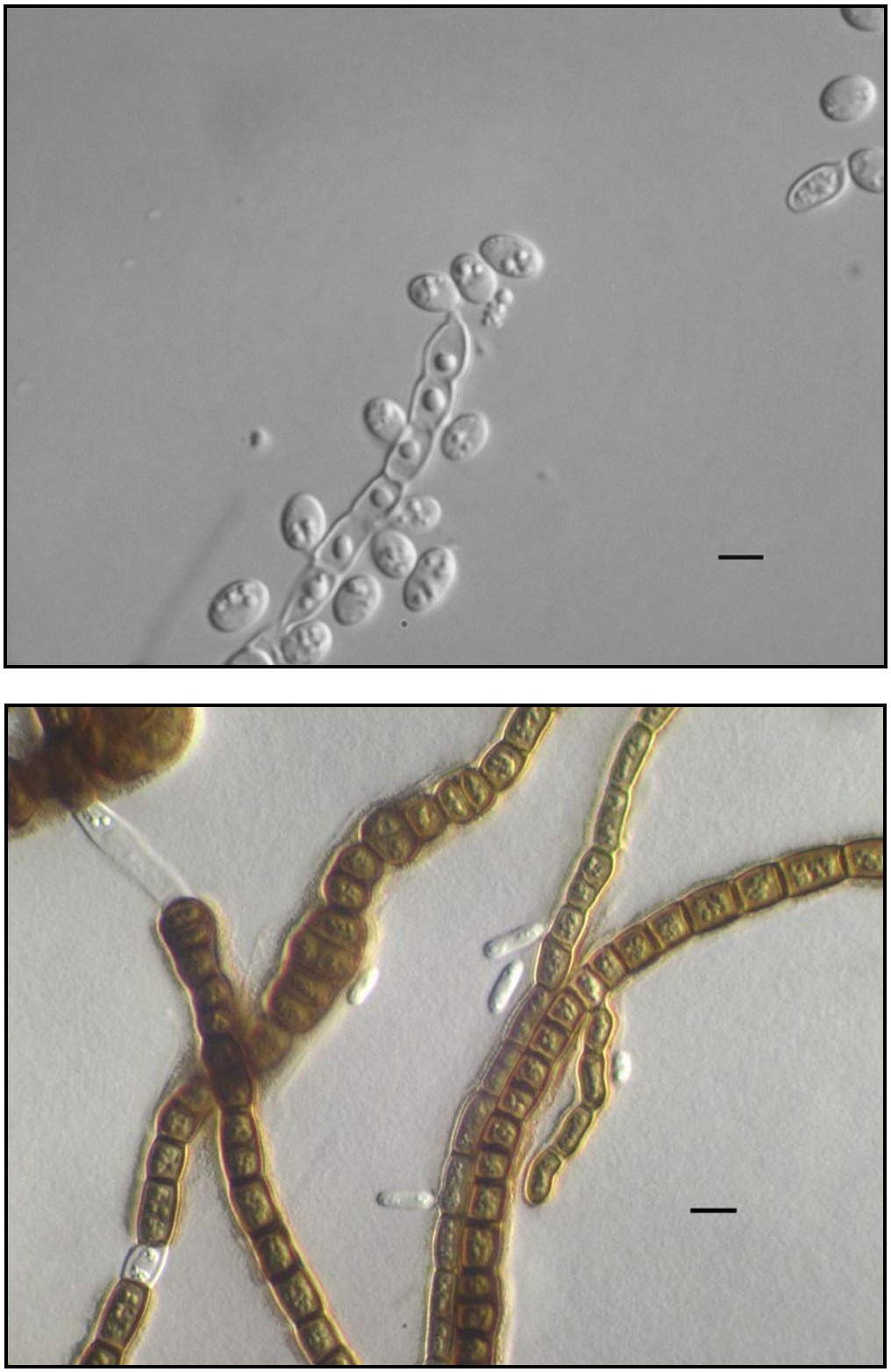

Figure 8. Light photomicrographs of *Hormonema dematioides*. Image: Differential Interference Contrast (DIC). Bar = 5 µm

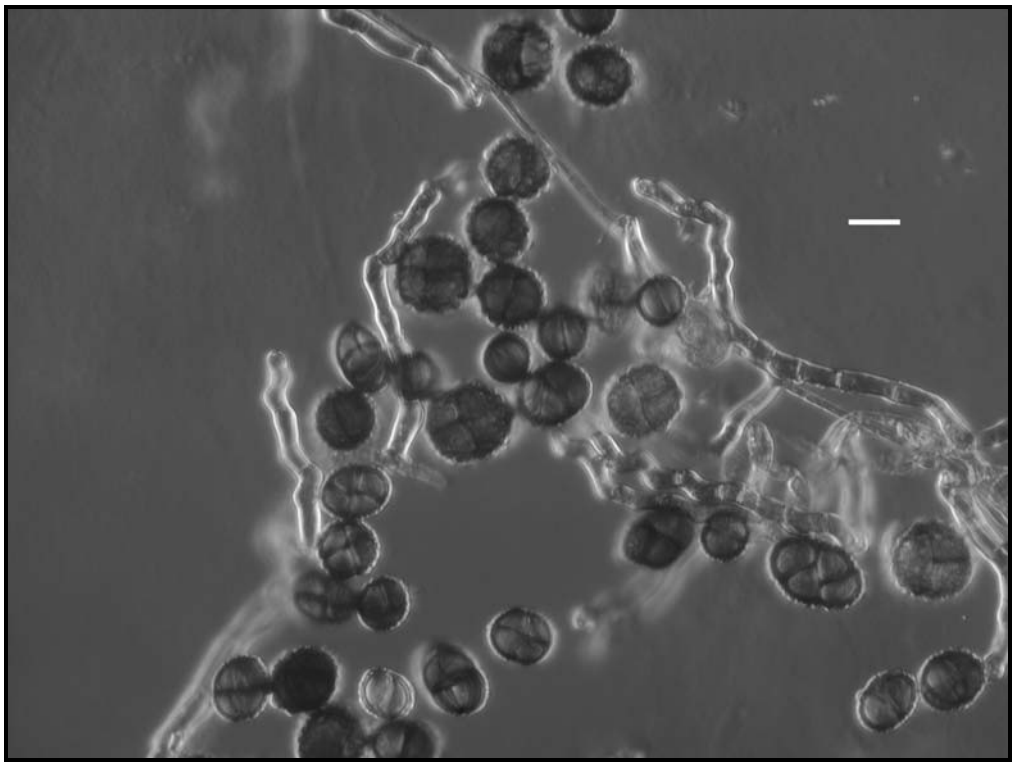

Figure 9. *Ulocladium atrum* photomicrograph showing conidiophores and cruciately septate conidia. Image: DIC. Bar = 10 µm

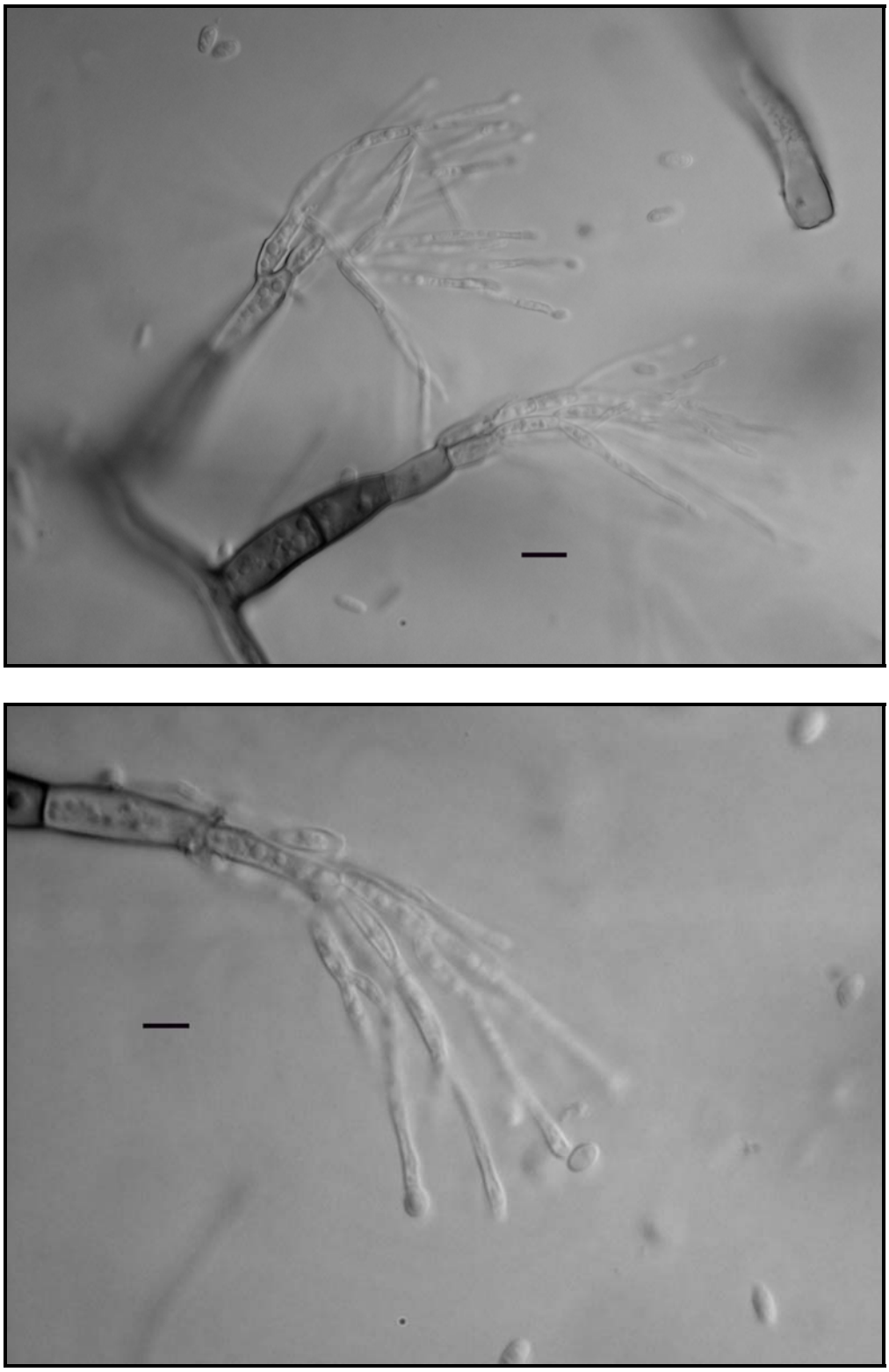

Figure 10. *Leptographium* sp. photomicrographs. Image: DIC. Bar = 10 µm

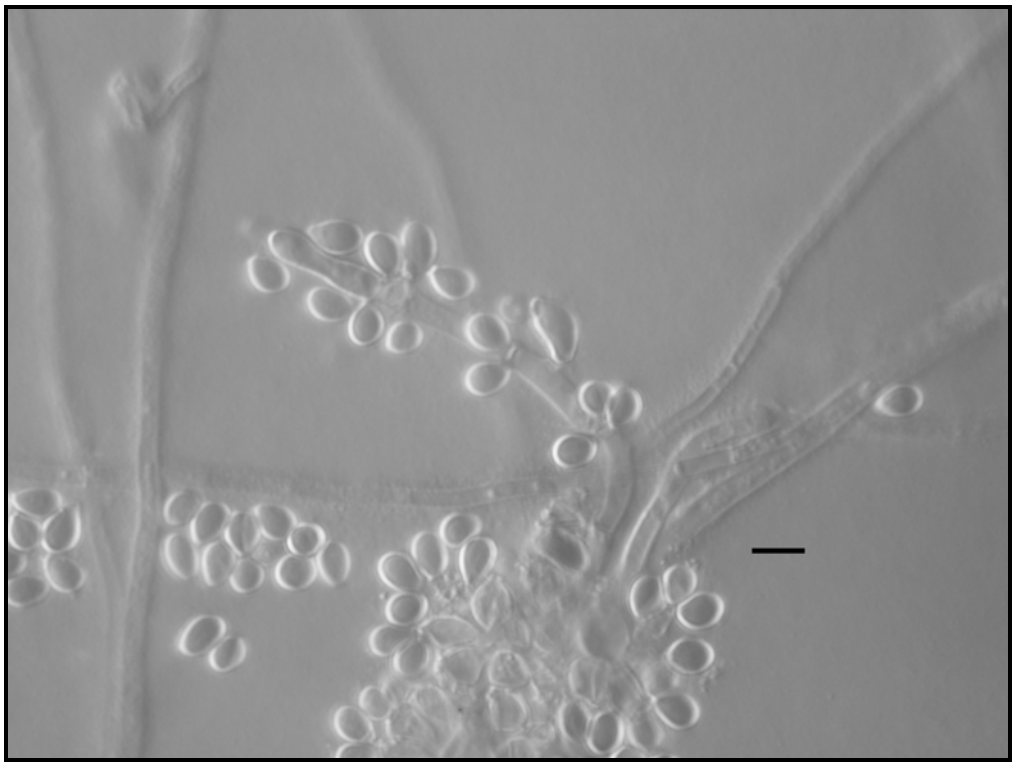

Figure 11. *Rhinocladiella* sp. photomicrograph. Image: DIC. Bar = 10 µm

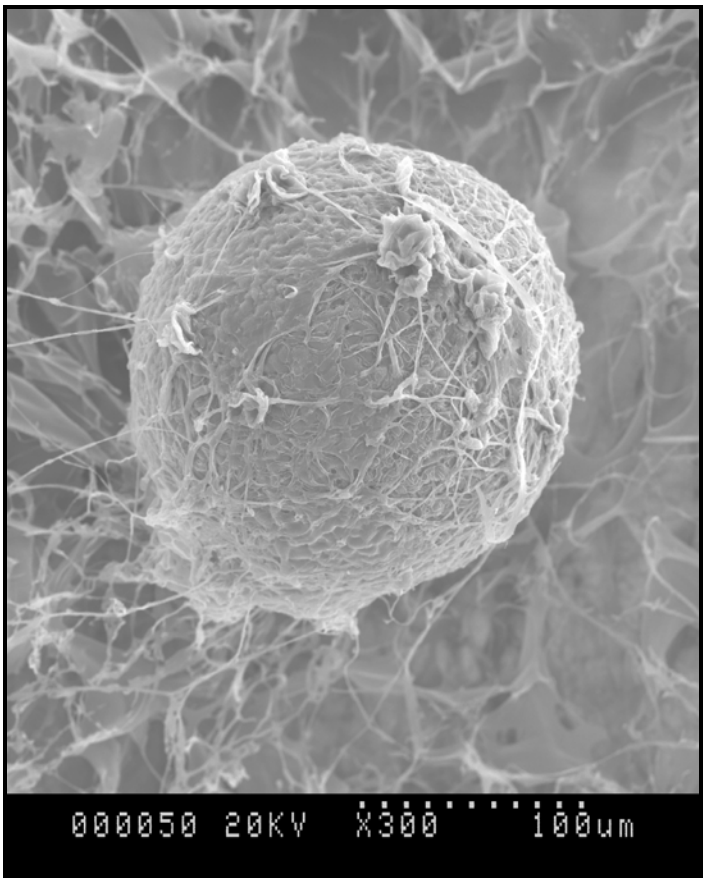

Figure 12. SEM photomicrograph of a putative new eurotiaceous taxon that produces ascomata in culture, showing cleistothecium grown on 2% MEA.

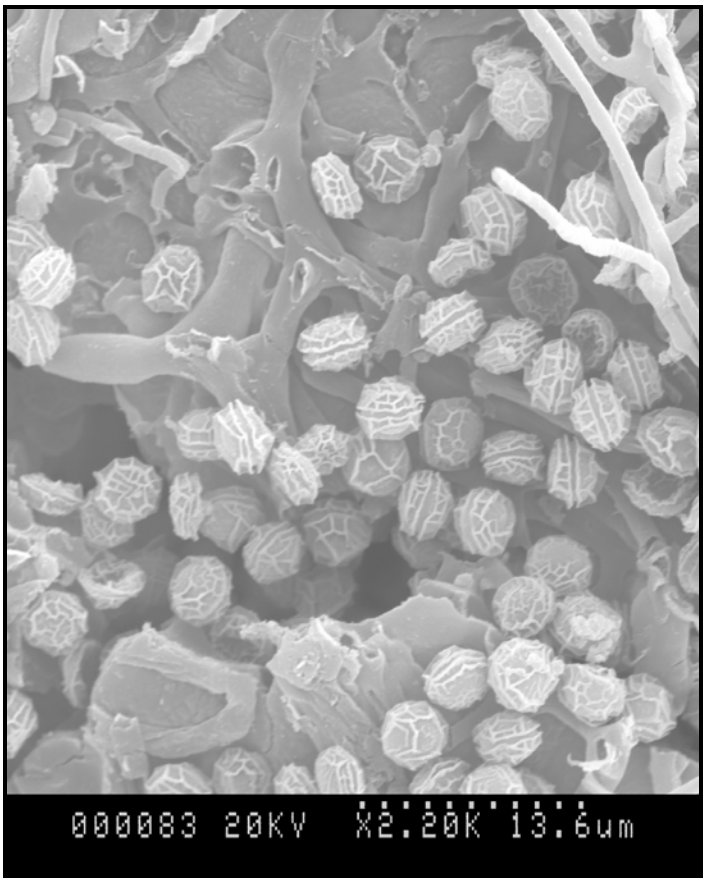

Figure 13. SEM photomicrograph of a putative new eurotiaceous taxon that produces ascomata in culture, showing ascospores grown on 2% MEA.

DISCUSSION

Dematiaceous hyphomycetes or Dark Septate Endophytes (DSE)

The high-frequency recovery of dematiaceous hyphomycetes was somewhat unexpected. Previous studies of fungi conducted on progressively deeper soil layers generally return dematiaceous fungi at or near the surface layers with less pigmented and more hyaline fungi found as soil depth increases (J.D. ROGERS, personal communication). The frequency of dematiaceous hyphomycetes from the large roots of Douglas-fir and ponderosa pine has not been previously noted, and little data on their occurrence are available. However, their occurrence in the roots of grasses and described as Dark Septate Endophytes (DSE) (ADDY et al. 2005), fine feeder roots of forest trees and shrubs (AHLICH and SIEBER 1996), above ground conifer tissues (PIRTTILÄ et al. 2003, GRÜNIG et al. 2002, MÜLLER and HALLAKSELA 2000, SIEBER et al. 1999) cacti (SURYANARAYANAN et al. 2005) and now conifer roots reveal an association between the endophytes of unrelated plants and suggests that this group is of high ecological importance. It has been noted that melanized structures are common among fungi occupying environmental extremes (ADDY et al. 2005). There appears to be an association between the endophytes from above and below ground conifer tissues and unrelated plants. That is, a number of common dematiaceous hyphomycetes long known as above-ground inhabitants are common root endophytes. This is a trend that is of potential ecological importance and a major result of this study.

For example, ADDY et al. (2005) studied the microfungal endophytes of grass roots and identified fungi from genera including *Exophiala*, *Leptodontidium*, and *Phialophora*. The recovery of these same genera (*Exophiala*, *Leptodontidium*, and *Phialophora* among others) from the roots of Douglas-fir and ponderosa pine reveal an association among the endophytes of grasses and conifer roots. Phylogenetic studies of these genera isolated from diverse hosts could reveal further evidence of evolutionary relationships among these endophytes.

Hormonema dematioides (Figure 8) was originally described by LAGERBERGER AND MELIN (LAGERBERGER et al. 1928) from blue-stained timbers in Sweden. It has also been isolated from wood pulp (NANNFELDT 1932), utility poles (WANG et al. 1990), discolored conifer needles (DE HOOG 1977), as an endophyte from surface disinfested Scots pine (*Pinus sylvestris*) buds (PIRTTILA et al. 2003) and from a fatal human infection (SHIN et al. 1998). *Hormonema dematioides* has been isolated as an endophyte of symptomless needles of *Pinus mugo* ssp. *uncinata* that were approximately 2.5 years old (SIEBER 1999, ROSSI 1982). *Hormonema dematioides* is the most frequently isolated fungus from this study and this study apparently represents the first report of *H. dematioides* from large roots of conifers. Similar to reported properties of some fungal endophytes, it has been shown that some isolates of *H. dematioides* produce the mycotoxin rugulosin (CALHOUN *et al.* 1992). The ability of *H. dematioides* to produce rugulosin is of particular interest because rugulosin has been shown to inhibit growth rates of the spruce budworm (FINDLEY et al. 2003, MILLER et al.

2002), which is a native defoliating insect of interest on the Okanogan-Wenatchee National Forest. The ability to produce rugulosin seems important because *H. dematioides* has been reported from spruce trees (CAMACHO et al. 1997), which is a host tree to the spruce budworm.

Ulocladium atrum (Figure 9) is a cosmopolitan fungus that has been isolated from plant materials and soil (FARR et al. 1989). *Ulocladium atrum* is closely related to another fungal isolate recovered during this study, *Ulocladium botrytis*, and I believe these isolates have not previously been reported from the roots of conifers. *Ulocladium botrytis* has been isolated from multiple substrata including dead herbaceous plants, rotten wood, paper, textiles and soil (ELLIS 1971). It has also been isolated from strawberry roots, seeds (WATANABE 2002) and rarely from clinical cases (DE HOOG et al. 2000). *Ulocladium botrytis* has been reported from plants of the genera *Alnus*, *Pseudotsuga*, and *Sphaeralcea* (FARR et al. 1989).

Aureobasidium pullulans is a cosmopolitan fungus and saprophyte originally isolated from golden spots on grapes by DE BARY (1866). It was recovered during this survey and may represent the first report of this fungus inhabiting roots of conifers. In recent studies of fungal endophytes, *A. pullulans* was recovered from cacti (SURYANARAYANAN 2005). In addition, *A. pullulans* has been previously isolated from human skin and nails (DE HOOG et al. 2000), leaf surfaces (DOMSCH AND GAMS 1980), seeds and soil (WANTANABE 2002) and many plants (FARR et al. 1989). *Aureobasidium pullulans* is morphologically similar to

Hormonema dematioides and can be isolated simultaneously with *H. dematioides* and can be difficult to identify. Like *H. dematioides*, *A. pullulans* possesses potential biological control properties. Among many examples, *A. pullulans* has been shown to inhibit post harvest strawberry rot (LIMA et al. 1997), Penicillium rots of citrus (WILSON and CHALUTZ 1989) and soil-borne plant pathogens (EL-TARABILY and SVASITHAMPARAM 2006).

Alternaria tenuissima, *A. alternata*, *A. arborescens*, and *A. malorum* were all isolated during the current survey. *Alternaria alternata* is an extremely common saprophyte that can be found on many plants, food products, and textiles (ELLIS 1971). *Alternaria alternata* has also been isolated from various seeds (WATANABE 2002), soil (DOMSCH and GAMS 1980), and utility poles (WANG et al. 1990), it also causes skin lesions in humans (DE HOOG et al. 2000). *Alternaria tenuissima* is a cosmopolitan fungus that is extremely common in the environment, and it has been isolated from a wide variety of plant parts as a secondary invader (ELLIS 1971). It was especially noteworthy that the fungus *A. malorum* was isolated as a root endophyte. *Alternaria malorum*, previously classified as *Cladosporium malorum*, is a minor pathogen on cherry tomato as determined by pathogenicity tests conducted on cherry tomato, table grapes and blueberries (GOETZ and DUGAN 2005); (Appendix A).

A *Leptographium* (Figure 10) species was isolated 14 times during the current survey. These fungi are generally not considered as endophytes. *Leptographium* species cause black stain root disease of conifers in the western

United States and British Columbia and they might be associated with wilts and black stain root disease of conifers (HARRINGTON 1998).

Species of *Rhinocladiella* (Figure 11) were isolated during the current survey. *Rhinocladiella* species have previously been isolated from wood, forest litter layers, and soil. The presence of *Rhinocladiella* species in the large roots of Douglas-fir and ponderosa pine is interesting given that the fungus has typically been isolated from soil (BARRON 1968), Japanese black pine seeds (WATANABE 2002) and utility poles (WANG et al. 1990).

Cladosporium herbarum was isolated during this study. It is a cosmopolitan fungus that is commonly isolated from dead plant tissues, air, soil, food products, paint, textiles and many other substrata (ELLIS 1971).

Phialophora fastigiata and *Phialophora* sp. isolated during this study have also been frequently isolated from air, soil, water, wood and wood pulp (ELLIS 1971). Similarly, *Leptodontidium elatius* has a worldwide distribution on conifers, hardwoods and utility poles (WANG et al. 1990).

These dematiaceous hyphomycetes are of future ecological interest because of their possible roles within their host plant. *Dichanthelium lanuginosum* plants collected from geothermal soils from Lassen Volcanic and Yellowstone National Parks were found to be colonized with a dematiaceous hyphomycete species of *Curvularia* (REDMAN et al. 2002). The *D. lanuginosum* plants were grown in the field at soil temperatures of 45°C and in the lab at constant or intermittent soil temperatures ranging from 45-65°C (REDMAN et al. 2002). Those

plants inoculated with the *Curvularia* sp. were able to survive the elevated soil temperatures that caused mortality in all non-inoculated control plants (REDMAN et al. 2002). REDMAN *et al.* (2002) concluded that infection with *Curvularia* sp. confers thermotolerance to the host plant allowing both plant and fungus to survive in an otherwise intolerable environment. It is unknown whether the prevalence of dematiaceous hyphomycetes in coniferous roots are associated with similar relationships, and these potential ecological roles warrant further investigation.

Ascomycetous and Basidiomycetous yeasts

Of additional interest from this survey is the isolation of filamentous ascomycetous and basidiomycetous yeasts, many of which are strictly associated with the fermentation chambers of beetle guts (BOEKHOUT 2005, SUH et al. 2005). Isolated yeasts include *Candida* spp., *Pichia scolyti* and *Trichosporon pullulans*. *Trichosporon pullulans* is a basidiomycetous yeast, and the only basidiomycetous yeast isolated during this survey. Among the *Candida* spp. isolated, two are novel species. Several representatives of an isolate identical to one recovered from the gut of a beetle (SUH et al. 2005) were identified with the gracious help of Vincent Robert (Centraalbureau voor Schimmelcultures). These *Candida* spp. are typically associated with bark beetles (SUH et al. 2005). Previous reports of yeast-insect associations raise questions as to whether the yeasts are vectored to roots by weevils, bark beetles or perhaps translocated throughout the host tree. Further research is needed to address the ingress of filamentous yeasts into the

large roots of Douglas-fir and ponderosa pine, and assess ecological relationships with insects.

Penicillia as potential endophytes

During my study, 64 isolates of *Penicillium* were recovered. *Penicillium* spp. are encountered as common laboratory contaminants; however, *Penicillium* spp. isolated and identified in this study are considered as true endophytes. Care was taken when root-core samples were extracted and stored to minimize establishment of surface contaminating fungi and bacteria. Root-cores were promptly processed, subjected to surface sterilization and cultured within a laminar flow hood (see materials and methods). These cumulative steps favored the isolation of true endophytes. That recovered fungi should be considered as endophytes is further supported by the fact that only 43% of all cores yielded one or more fungal isolates, which indicates effective surface sterilization and fungal isolation conditions.

Using modified Hagem agar, SUMMERBELL (2005) also recovered *Penicillium waksmanii*, *P. spinulosum*, and *P. citrinum*, among others, from washed *Picea mariana* mycorrhizae, proximal feeder root bark and soil. Thus, it appears that *Penicillium* spp. should be considered as endophytes and not solely common laboratory contaminants. However, it is important to note that *Penicillium* spp. are reported as laboratory contaminants or common soil inhabitants (Table 3).

Novel eurotiaceous taxon and associated questions

A putative new eurotiaceous taxon that produces ascomata in culture (Figures 12 and 13), but lacks an anamorphic state was recovered 25 times. This notable fungus is difficult to identify at any taxonomic level. Prolonged culturing and examination have yielded no evidence of an anamorph. However, the sequence homology of rDNA (ITS and 5.8S) between this eurotiaceous sp. nov. and *Penicillium commune* (AF455544.1), *P. canescens* (AY773331.1), *P. chrysogenum* (AY373903.1) or an uncultured *Penicillium* clone (DQ767595.1) is 98-100%, based on sequences in Gen Bank. In this case, DNA sequence data blasted in Gen Bank was used to facilitate the identification of the eurotiaceous taxon. *Penicillium canescens* was isolated five times during this study. It is known as an anamorphic fungus that lacks a teleomorph. Additionally, *P. commune*, which was not isolated during this study, is not known to have a teleomorph. *Penicillium chrysogenum*, a ubiquitous and commonly isolated fungus (PITT 2000), was not isolated during this study. Perhaps the putative new eurotiaceous taxon represents the teleomorphic state of *P. commune* or *P. canescens*; however the formation of an anamorph is apparently suppressed, lost or absent under our culture conditions. The sequence homology between this eurotiaceous sp. nov. and *P. commune*, *P. canescens*, *P. chrysogenum* and the uncultured *Penicillium* clone is based on ITS sequence data. The molecular congruence between these isolates is problematic and might be clarified by sequencing the β-tubulin region and conducting a search of Gen Bank (GLASS and DONALDSON 1995). The recovery of

the putative new eurotiaceous taxon represents an interesting phenomenon that will be further investigated.

Vertical or horizontal transmission of fungal endophytes

Understanding transmission routes is critical to understanding fungal endophyte ecology. Of particular interest is if endophytic fungi can be transmitted from parent to progeny or between unrelated species (e.g. grasses to trees). More studies are needed to determine whether horizontal (between plants) or vertical (through seeds or clonally propagated plants) transmission occurs among diverse fungal endophytes. Infection of seeds by fungal endophytes (vertical transmission) might allow the seeds and/or seedlings of various plant species to persist within environments that would otherwise be inhospitable (REDMAN et al. 2002), or might protect seeds and/or seedlings from insects and rodent predation or, perhaps, pathogen infection (PARK et al. 2001). Vertically transferred endophytes might also alter physiological, developmental, or morphological characteristics in a way that enhances a host's competitive abilities, especially in stressful environments (e.g., dry forest habitat types, short-interval disturbance regimes, or extreme temperatures), in turn ensuring the survival of the endophyte (FAETH 2002).

Elucidating the role of conifer root endophytes will be a complex process, because it is difficult to control diverse environmental variables within forest ecosystems and greenhouse experiments may not always reflect ecological interactions in natural ecosystems. Nonetheless, common understory plants of

the Okanogan-Wenatchee National Forest, such as pine grass (*Calamagrostis rubescens*) or seedlings of ponderosa pine and Douglas-fir, could be grown under sterile conditions and inoculated with fungi isolated during this study. Such inoculated plants could provide a basis to determine the ecological roles of fungal endophytes in a forest ecosystem. Initial tests should confirm the establishment of the inoculated endophytes. Following successful endophyte establishment, endophyte effects on host tolerance/resistance to drought, elevated temperatures, pathogens, defoliating insects, CO_2 levels and fire could be evaluated.

Additional ecological information could be gained from continued sampling of the large roots of Douglas-fir and ponderosa pine. Subsequent surveys will serve to monitor changes in the fungal endophyte communities. Likewise, understory forest plants should be examined for fungal endophytes, for comparison to those isolated from conifers. Fungal endophytes from understory species could be inoculated to conifer seedlings to examine ecological relationships among endophytes of diverse host plants.

Fungi isolated by overall fuel treatments

Overall fuel treatments significantly affected fungal endophyte recovery when compared with controls ($p=0.0357$). However, when individual treatment units, without considering taxonomic status, were compared to the control units the changes in fungal endophyte recovery were non-significant. See Table 6 for the numbers of isolated fungi by unit and fuel treatments.

Fungi isolated by host

Host tree species had a highly significant effect upon the recovered fungi ($p=0.0001$) (Tables 8 and 9 for Douglas-fir and Tables 7 and 10 for ponderosa pine) with more fungi recovered from ponderosa pine than Douglas-fir.

From the 341 ponderosa pine sampled, 358 fungal isolates were recovered and 148 fungal isolates were recovered from 224 sampled Douglas-fir. This difference in recovered fungi may be attributable to several factors. Increased endophyte recovery from ponderosa pine may reflect a more hospitable environment within roots of this host, or an increased opportunity for colonization. Intensity and season of the treatments and the differing tolerance of the host to the treatments could also contribute to different rates of fungal isolation. Another influencing factor upon differing rates of fungal isolation is that more ponderosa pine hosts were sampled than Douglas-fir.

The burn-only and the combined thin and burn treatments were conducted in the spring of 2004. During the spring months forest plants are actively growing and are rapidly utilizing available water sources before the dry summer months begin. Actively growing tissues have higher moisture content than tissues during dry summer months when plant growth slows. Plant tissues with high moisture content are more sensitive to heating and cambial tissues may suffer serious damage during high-intensity fires or low-intensity fires with extended duration (BROWN et al. 2000). The cambial tissue of mature Douglas-fir and ponderosa pine is protected by thick insulating bark layers, but may be

damaged by low-intensity fires of long-duration burning in litter layers (BROWN et al. 2000). Douglas-fir is less resistant to burning than ponderosa pine, but more resistant than spruces, true firs, lodgepole pine, western hemlock, western redcedar, and western white pine (HARRINGTON 1991). Damage and mortality of Douglas-fir can occur from heating of cambial stem tissue, roots and canopy foliage (HARRINGTON 1991), which occurs with higher frequency during spring burns than those conducted in the late summer and fall (PETERSON and ARBAUGH 1986). Since Douglas-fir is more sensitive to fire damage, and the prescribed burns were conducted when Douglas-fir tissues may sustain the most damage, the fungal endophytes in Douglas-fir might be indirectly negatively affected by damage sustained by the above ground host tissues.

Fungi isolated by culture media

MEA is a commonly used medium for the isolation and identification of hyphomycete and yeast genera. MEA was refined and used by THOM and CHURCH (1926) following the work of REDDISH (1919). THOM and CHURCH used MEA for isolating and identifying hyphomycetes in their 1926 treatment of the aspergilli. PITT (2000) uses 2% MEA as a standard medium for the identification of *Penicillium* species. Both 2% MEA and BDS were used in a previous endophyte survey by HOFF et al. (2004a). However, HOFF et al. (2004a) initiated all cultures on BDS only and later transferred any fungal growth to 2% MEA, whereas I initiated 50% of the total cultures on 2% MEA and 50% on BDS.

Given that MEA is commonly used by experts isolating and examining hyphomycetes, such as *Penicillium* and *Aspergillus*, it was expected that a higher proportion of hyphomycetes would be recovered from those root-cores plated on MEA than on BDS during this study.

HOFF et al. (2004a) most frequently isolated representatives of the Ascomycota and Zygomycota when root-cores where plated on BDS, but these data were not statistically analyzed. BDS is a selective media used by WORRALL and HARRINGTON (1993) for the isolation of the Basidiomycota and was used during this study to facilitate the isolation of Basidiomycetes and Zygomycetes. There were no significant differences between the two media regarding the recovery of the Basidiomycota; however, more Zygomycetes were recovered from those cores plated on BDS.

The use of culture media for fungal isolation has an inherent bias that favors isolation of fast growing fungi that respond to our selected culture conditions and slower growing fungi will be under-represented. Also, those organisms that are currently unculturable will not be represented during a study of this nature. One approach to address this issue is a combined approach using both 2% MEA as the only culture media and a complimentary environmental PCR sampling to quantify slower growing and currently unculturable fungi (MENKIS et al. 2006). However, fungal identifications based solely on environmental PCR products are prone to errors based on PCR artifacts and

provide no cultures to verify identifications or describe potentially novel species (WINTZINGERODE et al. 1997).

Endophytic fungi isolated from thin-only units

The maintenance of fungal endophytes within the host plant following the thinning treatment might have important ecological implications. Thinning of forested areas release trees from competition, increases tree vigor, and increases the amount of available resources such as water, soil minerals, sunlight and growing space (OLIVER and LARSON 1996). Given the increase of available resources following thinning and the reduced competition for those resources, it follows changes in host physiology may influence fungal endophytes of roots. The host, when released from competition, is generally more efficient at growth and development and this might decrease fungal endophytes that contribute to host competitiveness. Alternatively, increased resources and decreased competition might increase fungal endophyte colonization by providing more water and nutrients to the endophyte. Other indirect effects of thinning, such as wounding, might influence fungal endophytes by providing a route of ingress. These assumptions are hypothetical and unproven.

It was unexpected that only minor changes were observed in the recovery of fungi from the control units and the thin-only prescription. A possible reason for the maintenance of fungal endophytes post thinning is that the time between treatment and unit sampling was insufficient (several months as opposed to one growing season) for any detectable changes to occur. To examine this

hypothesis, a re-sampling of the treatment units is required. This response may also reflect complex interactions among host physiology, fungal endophytes and other environmental components that are not well understood.

In domestic grass species, some endophytic fungi are thought to be beneficial to their hosts by defending them against herbivores and pathogens in certain environments (FAETH 2002). It has been shown that the bird-cherry aphid (*Rhopalosiphum padi*) is able to survive at higher levels on meadow grass (*Lolium pretense*) infected by *Neotyphodium*, a common grass endophyte that produces multiple mycotoxins, growing in soil with low nutrient composition compared to soils with elevated nutrient availability (LEHTONEN et al. 2005). Thus, increasing soil nutrients increases the ability of the fungus to produce inhibitory mycotoxins and consequently increases host fitness. Furthermore, LEHTONEN et al. (2005) grew endophyte-free and manipulatively endophyte-free plants under similar conditions of high and low nutrient availability and found that aphid numbers increased on manipulatively endophyte-free plants grown under conditions of high nutrient availability. The authors hypothesize that endophyte infection is of significant importance to the survival of the host and may have resulted from a coevoluntionary relationship.

Fungal endophyte colonization is a likely function of many interacting factors, including competition and available resources. Many fungal endophytes were retained by the host following the thin only treatment. This retention of the fungal endophyte assemblages in thin only units suggests that the infection of

conifers by fungal endophytes might begin at the seedling stage, or earlier, and perhaps some conifers and fungal endophytes have coevolved relationships that are maintained though environmental and developmental changes.

Endophytic fungi isolated from burn-only units

The direct effects of fire on vegetation depends largely upon the intensity of the fire (OLIVER and LARSON 1996), and the season during which the fire occurs, but effects are generally unpredictable because of many interacting site factors (BORCHERS and PERRY 1990). For the most part, those units selected for a burn-only treatment experienced a low-intensity surface fire conducted in the spring of 2004. However, some areas of the study units burned with high intensity, with individual tree torching, occasional group torching and mortality observed. Following a fire, many soil nutrients are readily available for uptake; some can be volatilized (i.e. nitrogen), and some are leached to groundwater (OLIVER and LARSON 1996). Fires typically remove forest floor litter and foliage on lower tree branches, which decreases shade and leads to increased rates of erosion, a temporary hydrophobicity of the mineral soil surface and increased soil temperatures. Elevated soil temperature is a result of increased sunlight reaching the forest floor. Fire will heat the mineral soil below the surface under specific conditions, such as concentrated fuels, dry soils, or where fire burns within tree roots (OLIVER and LARSON 1996). Increasing soil temperature also increases the amount of microbial activity. It was expected that fungi exhibiting

heat tolerance would survive the fire and be favored in the post-burn environment.

For all treatment units, the burn-only units yielded the lowest number of fungi. The prescribed fire reduced the representatives of every fungal group recovered, but this reduction was not statistically significant. Within the identified fungi from the current study several species have been classified as heat tolerant. These heat-tolerant fungi are *Byssochalmys nivea* (BEUCHAT AND PITT 2002), *Umbelopsis isabellina* (BOLLEN AND VAN DER POL-LUITEN 1975) and *Paecilomyces variotii* (WANG et al. 1990). In a previous study, HOFF et al. (2004a) recovered *Byssochlamys* sp. and *Umbelopsis* spp., identified by DNA sequence analysis, as the two most dominant fungal endophytes, being 20.4% and 10.4% of total recovered endophytes, respectively.

Because of their heat tolerance, it was expected that the recovery of *B. nivea*, *U. isabellina*, and *P. variotii* would increase post burn; however, the presence of these species was reduced following the burn treatment. The reduction of heat-tolerant fungi may be an effect of elapsed time between treatment and sampling, the season of treatment, an effect of smoke or an indirect effect from damaging the host. A resurvey of these units would clarify long-term effects of the burn treatment.

A previous study that is perhaps relevant here was conducted on fire scars of giant sequoia (*Sequoia gigantea*) and showed that the time elapsed from fire disturbance influences fungal communities recovered from fire scars (PIIRTO

et al. 1998). PIIRTO et al. (1998) sampled fire scars of *S. gigantea* 1-year post burn, 5-year post burn and unburned (50 years from last fire event). *Byssochlamys fulva* was recovered frequently (85%) from fire scars 5-year post burn, moderately (29%) from unburned fire scars and infrequently (8%) from fire scars 1-year post burn. Other fungi isolated showed a similar pattern of increased incidence with increasing time post disturbance.

The season during which the disturbance occurs will likely influence post-burn plant and fungal communities (BORCHERS and PERRY 1990). As previously mentioned, the burn units received a low-intensity surface fire during the spring of 2004. The burns were conducted during the active growing time of year, when many herbaceous plants were emerging from the soil and some areas of these units contained high amounts of moisture in the litter layer (personal observation). Consequently, the burn treatments were low-intensity surface fires that produced high volumes of smoke. An obscure effect of fire is that smoke has a detrimental effect upon the growth of fungi as observed by PARMETER and UHRENHOLDT (1976). The detrimental effects of smoke may have had an effect on the fungi of the burn-only units but strong effects seem unlikely given that these fungi occupy a protected environment within their host plant.

Because the burn treatments were of low-intensity, it seems unlikely that the fire caused any detectable heating or alteration to the roots at the point where they were sampled. In fact, during sample collection each root was excavated and investigated to a distance of 1 m from the base of the tree and all roots

appeared healthy. In many cases, excavating a selected root to 1 m proved difficult, as the roots were protected by a thick soil layer. However, fire might have affected soil organisms or the tree cambium. Fire often kills organisms within the litter layers and upper soil surfaces (BORCHERS and PERRY 1990). Fire may also kill or injure the plants upon which many other organisms depend (BORCHERS and PERRY 1990). The indirect effects of fire upon the organisms associated with killed or injured hosts may be another contributing factor to changes in the endophyte communities found in the woody roots of host trees.

Indirect influences, such as damage sustained by the host and changes in the microorganisms of the litter layer, might affect fungal endophytes. The exact mechanisms that might facilitate observable differences are undetermined at this time. However, it appears that prescribed fire has a direct or indirect deleterious effect upon fungal endophytes of ponderosa pine and Douglas-fir roots.

Endophytic fungi isolated from the thin-and-burn treatment

It was determined that fuel treatments had an overall effect upon fungal endophyte recovery which revealed that the recovered fungi were in greater frequency from the thin and burn units. A thin and burn treatment is commonly used in forest harvest systems and it has been shown that thin and burn treatments significantly reduce the number of active mycorrhizae compared to thin only treatments or undisturbed sites (HARVEY et al. 1980). It is not known how this reduction of mycorrhizae relates to fungal endophytes; however, it does demonstrate fire effects on root-associated fungi.

From the thin-and-burn units the hyphomycetes were the most dramatically affected taxonomic group. Specifically, *Penicillium* spp. was reduced to eight representatives compared to 35 representatives isolated from the controls. All other fungal groups were relatively unchanged, and no changes were statistically significant when compared to controls.

Because endophyte occurrence was reduced by the burn treatment environment and relatively unaffected by the thin-only treatment when compared to control units, a net loss of endophytic fungi was expected in the thin-and-burn units. However, the reduced competition for available resources and/or increases in available soil nutrients that occur following fire might have contributed to the enhancement of fungal endophytes upon the thin-and-burn units. Again, resampling these units will prove to be useful for comparing the changes in endophyte assemblages over time and might help explain the increase in fungal endophytes following the combined thin-and-burn treatment.

Previous and Present Investigation Comparison

Comparisons with other endophyte surveys are difficult but perhaps the most relevant survey to the present investigation was conducted by HOFF et al. (2004). HOFF et al. (2004) conducted a survey on the same units prior to the burn treatments as the present investigation; however, different trees were sampled during the present investigation. Their fungal identifications were based largely on molecular data where the fungi isolated during the present investigation were identified more on morphological characterizations. Another marked difference

is the absence of dematiaceous hyphomycetes identified by HOFF et al. (2004); however, they were probably present. Dematiaceous hyphomycetes might have been included in the unidentified isolates of Hoff et al. (2004) as melanized hyphae and might not have been successfully amplified by PCR (personal observation). Another noteworthy difference is that *Byssochlamys* sp. and *Umbelopsis* spp. were isolated with higher frequency during HOFF et al. (2004) than the present investigation.

The differences in these endophyte surveys of the same treatment units demonstrate that fuel treatments affect fungal endophyte recovery and suggest that a multitude of complex interactions exist between host plants, endophyte assemblages and the environment within which they persist.

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Appendix A

Pacific Northwest Fungi

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Alternaria malorum: A Mini-Review with New Records for Hosts and Pathogenicity

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Abstract: Modern host-fungus indices and databases contain deceptively few entries for *Alternaria malorum* or its synonym, *Cladosporium malorum*. Close inspection of literature from the 1930s through the1960s indicates more hosts and wider prevalence than more modern indices and databases indicate. Reports from 2002 to the present document diverse additional hosts in the Pacific Northwest, including this report from roots of *Pinus ponderosa* and *Pseudotsuga menziesii*, the first reports from gymnosperms. Cherry tomato and grape tomato (*Lycopersicon esculentum*) were found to be hosts for *A. malorum* by artificial inoculation. Rarely documented in synoptic indices or databases in the last 20 years, the Cladosporium-like *C. malorum* is neither rare nor a true *Cladosporium*.

Key words: *Alternaria malorum*, *Cladosporium*, cherry tomato, grape tomato, *Lycopersicon esculentum*, *Pinus ponderosa*, *Pseudotsuga menzeisii*.

Introduction

For good reason, *Alternaria malorum* (Ruehle) U. Braun, Crous & Dugan spent most of its nomenclatural history as *Cladosporium malorum* Ruehle. Ruehle, then F.D. Heald's graduate student at the State College of Washington (now Washington State University, WSU) was the first person to receive a doctoral degree from WSU (Bruehl n.d.). Ruehle isolated the species from cold-stored apple fruit, illustrated and described the fungus and, with Heald, illustrated and described the rot it caused (Heald and Ruehle 1930, 1931; Ruehle 1930, 1931). The species fit well within the broadly applied concept for the genus *Cladosporium* during Ruehle's time, and much of the time thereafter. Long, occasionally branched chains of olive-brown, mostly 0-septate, blastic conidia arise from conidiophores of varying lengths (Figs. 1-2). Later, other mycologists isolated this species and applied different names. Matsushima (1975) isolated a fungus from radish seed in Japan, and applied the name *Cladosporium porophorum* Matsushima. In South Africa, Marasas and Bredell (1974) isolated a fungus from lucerne seed, plus oat and wheat straw, and named their isolates *Phaeoramularia kellermaniana* Marasas & Bredell. Their fungus was transferred first to *Cladophialophora*, then to *Pseudocladosporium* (Braun and Feiler 1995; Braun 1998). *C. malorum* was transferred to *Alternaria* by Braun et al. (2003). A comprehensive nomenclatural synopsis can be found in Dugan et al. (2004). In addition to the works cited above, illustrations were provided in Ho et al. (1999), and in Dugan et al. (1995), who included a drawing of material deposited at Centraalbureau voor Schimmelcultures (CBS) by F.D. Heald in 1931. The fungus illustrated under the name *Cladosporium malorum* by Zhang et al. (2000, Fig. 78) does not closely resemble *Alternaria malorum*, but appears to be a species of *Cladosporium* sensu stricto.

Shaw (1973) reported *C. malorum* on seed of *Beta vulgaris* and *Daucus carota* from British Columbia (citing Conners 1967), and on fruit of *Malus sylvestris* (citing Ruehle 1931). Conners (1967), citing an unpublished manuscript by J.W. Groves, also reported *C. malorum* on seed of *Agropyron cristatum*, *Bromis inermis*, *Linum usitatissimum*, *Medicago sativa*, *Pastinacia sativa*, *Pisum sativum*, *Spinacia oleracea* and *Zea mays*. Farr et al. (1989) listed only apple fruit (*Malus sylvestris*) as a host, citing Ruehle (1931). Ginns (1986) did not document any reports from Canada. Presently Farr et al. (n.d.) list few hosts or substrata: *Malus* and *Prunus* species (from China, citing Zhang et al. 2000, and from Washington State), smooth brome (*Bromus inermis*, Canada), grass litter (Canada), wheat (South Africa) and soil (Lebanon). Thus, recently published host-fungus indices and databases may give the impression that the fungus, although cosmopolitan, is rare.

Reports of *C. malorum* from barley straw and stored grain (Pakistan and Turkey), and *Persica vulgaris* (Libya) are mentioned by Braun and Feiler (1995). Our laboratories in the Pacific Northwest (PNW) have in a relatively short period of time isolated the fungus from dormant buds of *Vitis vinifera* (Dugan et al. 2002), from culm nodes of *Aegilops cylindrica*, *Festuca idahoensis*, and *Leymus cinereus* (Dugan and Lupien 2002), from seed of *Aegilops cylindrica*, *Bromus tectorum*, *Pseudoroengeria spicata*, and *Triticum aestivum* (Dugan and Lupien 2002), and from chickpea (*Cicer arietinum*) debris (Dugan et al. 2005). Most recently, one of us (Goetz) has repeatedly isolated *A. malorum* from conifer roots. We augment this review by formally reporting the isolation from conifer roots and describing pathogenicity tests on miscellaneous fruits.

Materials and methods

***New report*:** A. *malorum* isolates CROW3 C10-4 OUT and CROW6 G14-2 IN were recovered from root cores of *Pinus ponderosa* collected 7 July and 14 July 2004 respectively at Mission Creek, Okanogan National Forest, Washington state in habitat described by Agee et al. (2001) and Hoff et al. (2004). Isolate TRIPP Q9-1 OUT was recovered from a root core of *Pseudotsuga menzeisii* on 31 August at the same location. Cores were extracted from asymptomatic roots with a sterile increment borer and stored in plastic straws on dry ice. Xylem tissues were excised from each core, divided into paired samples from each core, dipped in 70% ethanol, flame-disinfested, transferred to benomyl-dichloran-streptomycin agar (Worrall and Harrington 1993) or 2% malt-extract agar (15 g agar, 20 g malt extract, and 100 mg streptomycin sulfate per L) and incubated in the dark at 22° C for $\geq$ 8 weeks. Isolates are stored on agar media at 4° C at the USDA Rocky Mountain Research Station, Moscow, ID. Identification on morphological criteria was according to Ho et al. (1999). Mycelium from young, actively growing cultures was used to extract template DNA for amplification of ITS1, ITS2 and 5.8S sequences of nuclear ribosomal DNA (rDNA) as described previously (Hoff et al. 2004). Amplified DNA was sent to the University of Wisconsin Biotechnology Center (Madison, WI) for sequencing. Sequences were edited using BioEdit (Hall 1997-2005). BLAST searches were conducted to compare ca. 600 bp (partial ITS1, 5.8S and partial ITS2) with sequences in GenBank.

Pathogenicity tests with isolates of* A. malorum*: A single-spore isolate of V5#19 (CBS 112048, ex-type for *A. malorum* var. *polymorpha*) from dormant bud of grape, *Vitis vinifera* (Braun et al. 2003; Dugan et al. 2002), was grown on half-strength V8 agar (Stevens 1981) under 12 hr / 12 hr fluorescent + near ultraviolet lights / darkness at 22-25° C for 14 days. Conidia were harvested into sterile distilled water, quantified with a hemacytometer, and the resultant suspension adjusted to 10^6 condia/ml. Fruits of seedless green table grape (*V. vinifera*) and cherry tomato (*Lycopersicon esculentum* var. *cerasiforme*), purchased at local retail outlets, were disinfested by immersion for 5 min in 0.5% NaOCl, rinsed in sterile water and individually wounded by penetration to a depth of 7 mm with a flame-disinfested needle. Five wounded fruits of each host species were inoculated by placing a drop of the conidial suspension directly onto the wound, and five corresponding control fruit of each species received only drops of sterile distilled water. The procedure was twice repeated, once using isolate SB99-28 (Dugan and Lupien 2002) from seed of wheat (*Triticum aestivum*) and once with isolate CP-96A (Dugan et al. 2005) from stem of chickpea (*Cicer arietinum*). Fruits were incubated at ambient laboratory conditions and lesion diameters were measured at 30 days post-inoculation.

The above trial was followed by an additional trial using CROW6 G14-2 IN (from root of ponderosa pine), with SB99-28 as a positive control, to inoculate fruits of grape tomato (*L. esculentum*). Twenty fruits were used for each treatment (CROW6, positive control, negative control inoculated with distilled water only) with growth of isolates, disinfestation and inoculation protocols as described above. Fruits were incubated 24 days under ambient lab conditions. In each trial, *A. malorum* was recovered into pure culture on half-strength V8 by transfer of lesions excised from inoculated fruits. Statistical analysis via ANOVA was with SYSTAT 9 (SPSS Science, Chicago, Illinois).

Results

***New report*:** On the basis of morphology and ITS sequence homology (99%), isolates from conifer roots were identified as *Alternaria malorum*. We here note that the GenBank sequences with which our results were compared were derived from research of Braun et al. (2003) or research performed on authentic or representative material at American Type Culture Collection. To our knowledge, these are the first reports of isolation of *A. malorum* from gymnosperms.

***Pathogenicity tests*:** All three isolates of *A. malorum* used in the first trial proved pathogenic to cherry tomato, creating sunken, brown lesions. Diameters of lesions induced by V5#19 ranged from 12 to 20 mm; those for CP96A from 5 to 25 mm; and those for SB99-28 from 3 to 18 mm. Lesions were absent on two of five fruits inoculated with V5#19 or CP96A, and from one fruit inoculated with SB99-2B. No lesions developed on controls. Overall differences (LSD) were significant at P = 0.035. P values for each isolate relative to its control were 0.059 for CP96A, 0.035 for SB99-28, and 0.029 for V5#19B. When controls were combined (since lesion diameters were zero in all instances), overall P value was 0.006, and P values of each isolate relative to controls were 0.018 for CP96A, 0.008 for SB99-28, and 0.006 for V5#19B. No lesions developed on inoculated grapes or the control grapes. In no instances did isolates significantly differ from each other.

In the second trial, CROW6 and the positive control SB99-28 differed from the non-inoculated controls at P = 0.00, and CROW6 and the positive control did not differ from each other. Except for one fruit on which grew a colony of dictyoseptate *Alternaria* sp. (not *A. malorum*), no non-inoculated controls developed lesions, whereas lesions for CROW6 ranged from 1.8 to 6 mm, and those from SB99-28 from 1.3 to 10.1 mm. Two fruits in the CROW6 treatment and 5 fruits in the SB99-28 treatment did not develop lesions. In both trials, *A. malorum* was recovered back into culture from tissues of symptomatic fruits.

Discussion

We have presented in the introduction evidence that past reports of *Cladosporium malorum* were more common than modern compendia and current databases indicate. We use our discussion of this species to emphasize its high prevalence in the PNW, its proper classification in the genus *Alternaria*, and the range of hosts on which it is pathogenic.

Imposing but overlooked documentation has long been available for the potential importance of *A. malorum* in the PNW. Schnellhardt and Heald (1936) washed samples of market wheat from Washington State, and plated the wash water to agar media. They reported, "The prevalence of *Cladosporium malorum*, found on twenty of the twenty-four samples washed, is the outstanding feature of this study." The incidence of *A. malorum* in the twenty samples ranged from 8.3% to 100%, with fourteen samples showing an incidence of 70% or above. The authors noted that the pathogenicity of the fungus on wheat was not investigated. Although Sprague (1950) did not index *C. malorum*, his monographic work on fungal diseases of cereals and grasses briefly referenced Schnellhardt and Heald (1936) and mentioned the fungus. Unknown is the extent to which the reported dominance of *A. malorum* on market wheat represented an aberration. Even if highly exceptional, the extreme prevalence of the species in the survey of Schnellhardt and Heald (1936) argues that *A. malorum* must exist in well-established reservoirs in PNW regional ecology. There

is also circumstantial evidence that the fungus was known on wheat by 1930. In a copy of Ruehle (1930) at Washington State University Libraries, there are miscellaneous marginal notes, in pencil, representing suggestions or minor corrections to the dissertation. One of these notes, immediately after '*Cladosporium malorum* n. sp.' (p. 99) reads "(also on wheat)".

Given the extreme prevalence of *A. malorum* in their results, it is somewhat surprising that neither Schnellhardt nor Heald pursued their findings. Heald, however, had many duties including department Chair (Bruehl n.d.), and Schnellhardt, like Ruehle, was a graduate student focused on apple decay (Schnellhardt 1935). After graduation, Ruehle worked on plant disease problems in Florida.

In 2001, an isolate of *Cladosporium malorum* from asymptomatic *V. vinifera* tissues transiently produced a small number of conidia highly reminiscent of *Alternaria* (Braun et al. 2003). These conidia were basal or intercalary in chains of Cladosporium-like conidia. This occasioned a re-examination of conidial morphology and conidiogenesis, and generation of phylogenetic data based on ITS and small subunit rRNA gene sequences (Braun et al. 2003). Several isolates of *C. malorum* clustered with *Alternaria* species, and were well separated from *Cladosporium* sensu stricto. *Cladosporium malorum* was reassigned to *Alternaria* as *A. malorum*, and the isolate from grape was named *A. malorum* var. *polymorpha*. Braun et al. (2003, Figs.3-12) illustrated characters typical of *A. malorum*, as well as conidia diagnostic for *A. malorum* var. *polymorpha*. An isolate from Höller et al. (2002), identified by U. Braun as *A. malorum*, produced metabolites commonly associated with *Alternaria* spp. (Höller et al. 2002). This strain had been isolated from an unidentified resupinate polypore; so, *A. malorum* is apparently also capable of fungicolous habit. Readers should be aware that at least one other *Alternaria* species, *A. cetera*, produces predominantly cylindrical, aseptate conidia (Simmons 1996).

Alternaria malorum has proven pathogenic on ripe apple and cherry fruits (Dugan et al. 1995; Ruehle 1931). Cherry tomato and grape tomato are experimental hosts, as demonstrated above. Grape berries were resistant to infection under our experimental conditions. Tests with blueberry (*Vaccinum corymbosum*) were inconclusive because both inoculated and non-inoculated berries rapidly shriveled and/or molded with other fungi within a few days of inoculation (data not shown). Although *A. malorum* is now documented as a pathogen, it may not be as aggressive on its hosts as several other fungi. Lesions developed slowly on inoculated tomato fruits, and not all inoculated fruits progressed to decay, probably because the isolates failed to penetrate into the wound quickly enough to overcome host defenses.

In conclusion, we can confidently state that the seemingly rare fungus previously known as *Cladosporium malorum* is neither rare nor a *Cladosporium*. Based on isolations over the past decade (Dugan et al. 1995; Dugan and Lupien 2002; Dugan et al. 2002; Dugan et al. 2005, and the report from pine roots above), and the report of Schnellhardt and Heald (1936), we predict that mycologists and plant pathologists of the PNW will see more of this fungus in the years ahead.

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Figure 1. Conidiophores of *A. malorum* SB99-28 range from small swellings (short arrow) to longer conidiophores with multiple conidi-genous loci (large arrow). DIC. Bar = 5 µm.

Fig. 2. Detail of Fig. 1. Conidiogenous loci (arrow) are poroid. DIC. Bar = 5 µm.

Appendix B

Microwave processing for Scanning Electron Microscopy (SEM)

Processing fungi for SEM can take up to 96 hours depending upon the fixation protocol used, duration of steps during ethanol or acetone dehydration and tissue sample of interest (Figure 14). The total time involved with using the microwave for fixation and dehydration including critical point drying and sputter coating, excluding a post-fixation step, is approximately 120 minutes with no visibly detectable loss of sample structural integrity (Figures 15 and 16). This is a dramatic reduction of the time involved with processing fungi for SEM and allows for rapid examination of fungal isolates.

Microwave Fixation	Traditional Fixation
Fixation	**Fixation and Post Fixation**
2.5 minutes	48 hours
Rinse with buffer	**Rinse with buffer**
3 X 5 minutes	3 X 5 minutes
Ethanol dehydration	**Ethanol dehydration**
30, 40, 50, 60% each at 40 seconds	30, 40, 50, 60% each at 10 minutes
70, 80, 90, 100% 2X each at 40 seconds	70, 80, 90% each at 10 minutes, and 100% 3X at 10 minutes each
Critical Point Drying (CPD)	**Critical Point Drying (CPD)**
1.5 hours	1.5 hours
Total processing time	**Total processing time**
120 minutes	~ 4 days

Figure 14. Processing time for microwave mediated fixation protocol compared to time required for a traditional fixation protocol.

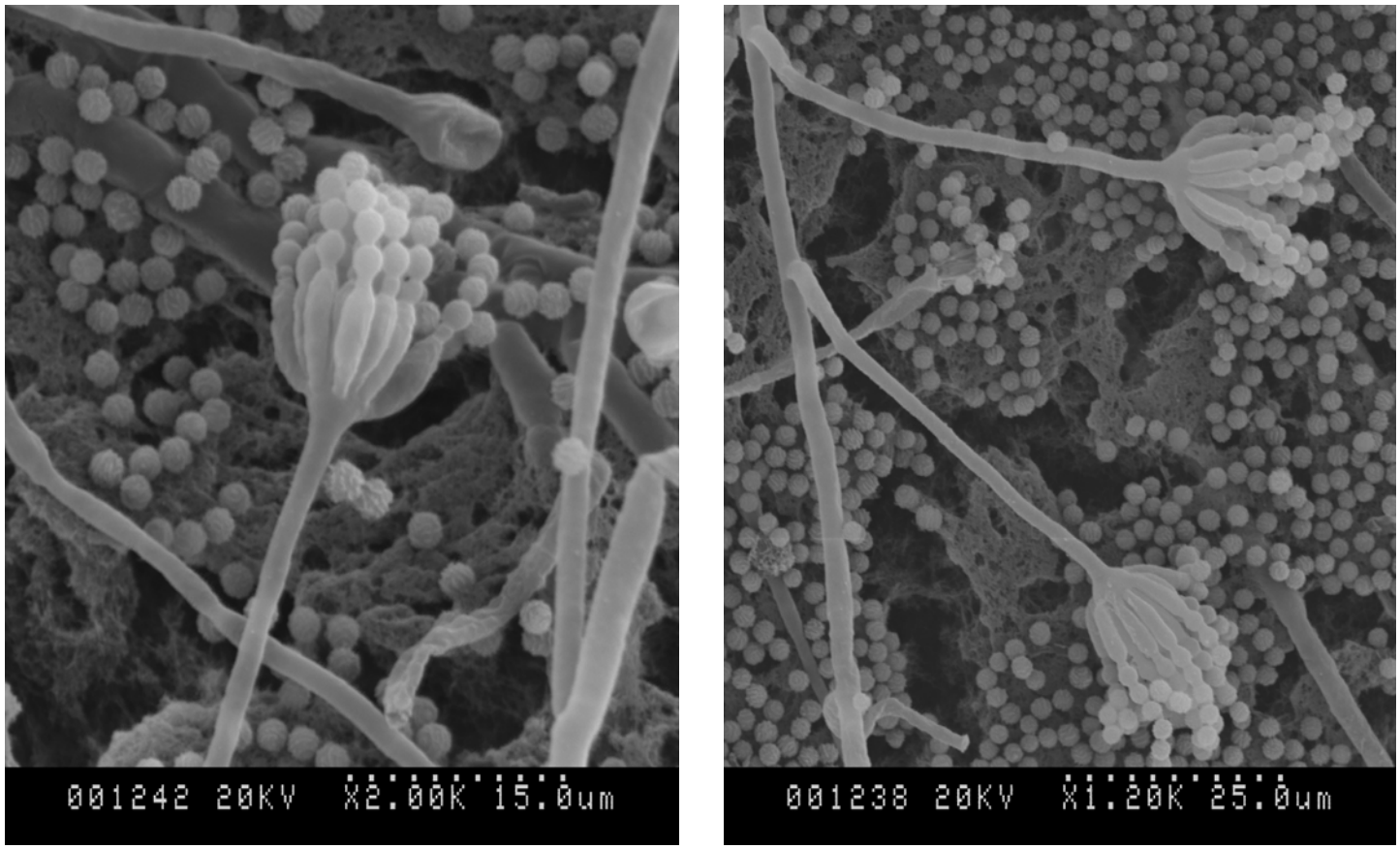

Figure 12. SEM photomicrographs of stipes, phialids and conidia of *Penicillium* processed with the microwave fixation technique.

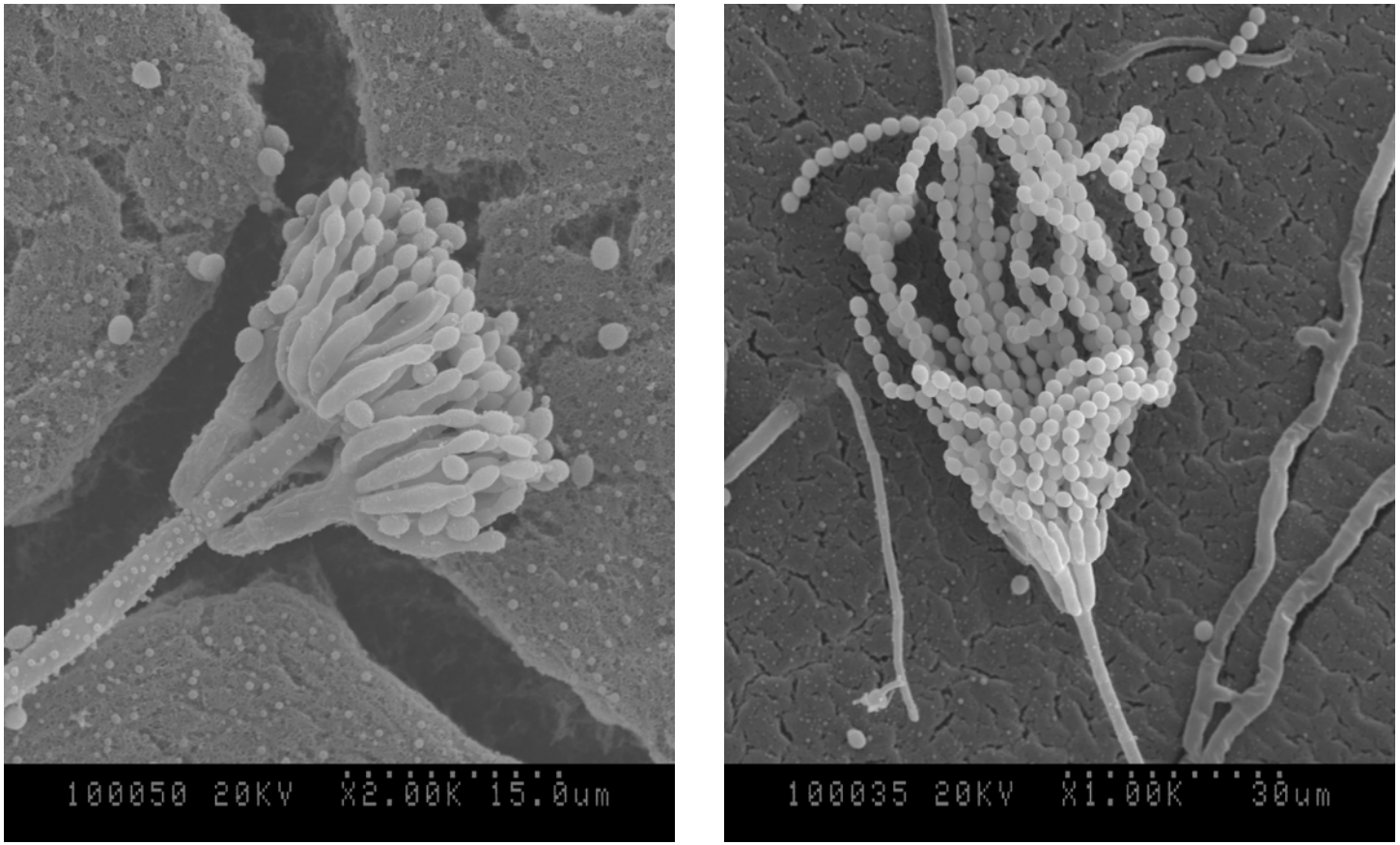

Figure 13. SEM photomicrographs of stipes, phialids and conidia of Penicillium processed with the traditional fixation technique.

Appendix C.
Inventory of root-core samples with host status, collection information and treatment.

DATE	SITE	UTM CELL	TREE #	CORE ID	HOST	HOST STATUS	TREATMENT
6/24/2004	Pendleton	D10	1	D10-1	PIPO	NS ROOT CORE	CONTROL
6/22/2004	Pendleton	F10	1	F10-1	PIPO	NS ROOT CORE	CONTROL
6/22/2004	Pendleton	G9	1	G9-1	PIPO	ROOT CORE	CONTROL
6/21/2004	Pendleton	I9	1	I9-1	PIPO	NS ROOT CORE	CONTROL
6/22/2004	Pendleton	H10	1	H10-1	PSME	NS ROOT CORE	CONTROL
6/21/2004	Pendleton	J10	2	J10-2	PSME		CONTROL
6/21/2004	Pendleton	L10	2	L10-2	?	NS ROOT CORE	CONTROL
6/21/2004	Pendleton	J10	3	J10-3	PIPO	SYM ROOT CORE	CONTROL
6/22/2004	Pendleton	E11	5	E11-5	PIPO	NS ROOT CORE	CONTROL
6/21/2004	Pendleton	I10	11	I10-11	PIPO	RHIZOMORPH	CONTROL
6/21/2004	Pendleton	I10	12	I10-12	PIPO	RHIZOMORPH	CONTROL
6/21/2004	Pendleton	I19	13	I10-13	PIPO	RHIZOMORPH	CONTROL
6/21/2004	Pendleton	I10	15	I10-15	PIPO	RHIZOMORPH	CONTROL
6/24/2004	Pendleton	E9	4	E9-4	PIPO	NS ROOT COOKIE	CONTROL
6/21/2004	Pendleton	I10	10	I10-10	PIPO	ROOT COOKIE	CONTROL
6/21/2004	Pendleton	I10	11	I10-11	PIPO	ROOT COOKIE	CONTROL
6/21/2004	Pendleton	I10	12	I10-12	PIPO	ROOT COOKIE	CONTROL
6/21/2004	Pendleton	I10	13	I10-13	PIPO	ROOT COOKIE	CONTROL
6/21/2004	Pendleton	I10	14	I10-14	PIPO	ROOT COOKIE	CONTROL
6/21/2004	Pendleton	I10	15	I10-15	PIPO	ROOT COOKIE	CONTROL
6/27/2004	Pendleton	J8	5	J8-5	PIPO	SYM ROOT CORE	CONTROL
6/28/2004	Pendleton	D10	5	D10-5	PIPO	CURA	CONTROL
6/28/2004	Pendleton	D10	6	D10-6	PIPO	CURA	CONTROL
6/28/2004	Pendleton	D10	7	D10-7	PIPO	CURA	CONTROL
6/28/2004	Pendleton	D10	8	D10-8	PIPO	CURA	CONTROL
6/28/2004	Pendleton	D10	9	D10-9	PIPO	CURA	CONTROL
6/28/2004	Pendleton	H8	1	H8-1	PIPO	AS ROOT CORE	CONTROL
6/28/2004	Pendleton	J8	3	J8-3	?	RECK	CONTROL
6/28/2004	Pendleton	D10	10	D10-10	PIPO	CURA	CONTROL
6/28/2004	Pendleton	E9	4A	E9-4A	PIPO	NS ROOT CORE	CONTROL
6/29/2004	Pendleton	E7	1	E7-1	PIPO	NS ROOT CORE	CONTROL
6/29/2004	Pendleton	G8	2	G8-2	PIPO	TPKL	CONTROL
6/29/2004	Pendleton	I7	1	I7-1	PIPO	NS ROOT CORE	CONTROL
6/29/2004	Pendleton	G1	1	G1-1	?	NS ROOT CORE	CONTROL
6/29/2004	Pendleton	D8	1	D8-1	PIPO	NS ROOT CORE	CONTROL
6/29/2004	Pendleton	F8	1	F8-1	?	NS ROOT CORE	CONTROL
6/29/2004	Pendleton	C7	1	C7-1	PIPO	NS ROOT CORE	CONTROL
6/29/2004	Pendleton	G8	1	G8-1	PIPO	CURK, ROTTEN	CONTROL
6/30/2004	Pendleton	F6	2	F6-2	PIPO	CURA	CONTROL
6/30/2004	Pendleton	H6	4	H6-4	PIPO	CURA	CONTROL
6/30/2004	Pendleton	K4	1	K4-1	PIPO	NS ROOT CORE	CONTROL

DATE	SITE	UTM CELL	TREE #	CORE ID	HOST	HOST STATUS	TREATMENT
6/30/2004	Pendleton	F6	4	F6-4	PIPO	CURA	CONTROL
6/30/2004	Pendleton	H6	2	H6-2	PIPO	CURA	CONTROL
6/30/2004	Pendleton	F6	3	F6-3	PIPO	NS ROOT CORE	CONTROL
6/30/2004	Pendleton	J4	10	J4-10	PIPO	NS ROOT CORE	CONTROL
6/30/2004	Pendleton	I5	4	I5-4	PIPO	NS ROOT CORE	CONTROL
6/30/2004	Pendleton	J6	2	J6-2	PIPO	NS ROOT CORE	CONTROL
6/30/2004	Pendleton	H6	3	H6-3	PIPO	NS ROOT CORE	CONTROL
6/30/2004	Pendleton	D6	2	D6-2	PIPO	SYM ROOT CORE	CONTROL
6/30/2004	Pendleton	F6	5	F6-5	PIPO	CURA	CONTROL
6/30/2004	Pendleton	D6	1	D6-1	PIPO	NS ROOT CORE	CONTROL
6/30/2004	Pendleton	F7	3	F7-3	PIPO	CURA	CONTROL
6/30/2004	Pendleton	K7	1	K7-1	PIPO	NS ROOT CHUNK	CONTROL
7/1/2004	Pendleton	E3	2	E3-2	PIPO	NS ROOT CORE	CONTROL
7/1/2004	Pendleton	B6	2	B6-2	PIPO	SYM ROOT CORE	CONTROL
7/1/2004	Pendleton	B6	4	B6-4	PIPO	SYM ROOT CORE	CONTROL
7/1/2004	Pendleton	F5	4	F5-4	PIPO	SYM ROOT CORE	CONTROL
7/1/2004	Pendleton	G5	2	G5-2	PIPO	NS ROOT CORE	CONTROL
7/1/2004	Pendleton	B6	5	B6-5	PIPO	NS ROOT CORE	CONTROL
7/1/2004	Pendleton	E5	1	E5-1	PIPO	NS ROOT CORE	CONTROL
7/1/2004	Pendleton	C5	1	C5-1	PIPO	NS ROOT CORE	CONTROL
7/1/2004	Pendleton	F4	6	F4-6	PIPO	NS ROOT CORE	CONTROL
7/1/2004	Pendleton	D4	3	D4-3	PIPO	NS ROOT CORE	CONTROL
7/8/2004	Crow 3	C8	2	C8-2	PIPO	SYM ROOT CORE	CONTROL
7/8/2004	Crow 3	E6	1	E6-1	PIPO	NS ROOT CORE	CONTROL
7/8/2004	Crow 3	B7	1	B7-1	PSME	NS ROOT CORE	CONTROL
7/8/2004	Crow 3	E8	1	E8-1	PSME	NS ROOT CORE	CONTROL
7/8/2004	Crow 3	D7	1	D7-1	PIPO	NS ROOT CORE	CONTROL
7/8/2004	Crow 3	D8	2	D8-2	PIPO	DEAD - RHIZO	CONTROL
7/8/2004	Crow 3	C8	4	C8-4	PIPO	NS ROOT CORE	CONTROL
7/8/2004	Crow 3	F7	4	F7-4	PSME	NS ROOT CORE	CONTROL
7/8/2004	Crow 3	C6	1	C6-1	PIPO	NS ROOT CORE	CONTROL
7/8/2004	Crow 3	?	?	?	PSME	SYM ROOT CORE	CONTROL
7/12/2004	Crow 3	D1	1	D1-1	PSME	NS ROOT CORE	CONTROL
7/12/2004	Crow 3	F3	1	F3-1	PIPO	NS ROOT CORE	CONTROL
7/12/2004	Crow 3	E2	2	E2-2	PIPO	NS ROOT CORE	CONTROL
7/12/2004	Crow 3	E4	1	E4-1	PIPO	NS ROOT CORE	CONTROL
7/12/2004	Crow 3	D5	1	D5-1	PIPO	NS ROOT CORE	CONTROL
7/12/2004	Crow 3	C4	1	C4-1	PSME	NS ROOT CORE	CONTROL
7/12/2004	Crow 3	C3	1	C3-1	PSME	SYM ROOT CORE	CONTROL
7/12/2004	Crow 3	F5	4	F5-4	PSME	NS ROOT CORE	CONTROL
7/12/2004	Crow 3	D6	1	D6-1	PSME	NS ROOT CORE	CONTROL

DATE	SITE	UTM CELL	TREE #	CORE ID	HOST	HOST STATUS	TREATMENT
7/7/2004	Crow 3	C10	4	C10-4	PIPO	NS ROOT CORE	CONTROL
7/7/2004	Crow 3	C9	2	C9-2	PIPO	DEAD - RHIZO	CONTROL
7/7/2004	Crow 3	D4	7	D4-7	PIPO	SYM ROOT CORE	CONTROL
7/7/2004	Crow 3	F9	1	F9-1	PSME	NS ROOT CORE	CONTROL
7/7/2004	Crow 3	G10	1	G10-1	PIPO	NS ROOT CORE	CONTROL
7/7/2004	Crow 3	E11	1	E11-1	PIPO	NS ROOT CORE	CONTROL
7/7/2004	Crow 3	E10	1	E10-1	PIPO	NS ROOT CORE	CONTROL
7/7/2004	Crow 3	C9	2	C9-2	PIPO	RHIZO	CONTROL
7/7/2004	Crow 3	G8	2	G8-2	PIPO	NS ROOT CORE	CONTROL
7/7/2004	Crow 3	F11	1	F11-1	PIPO	NS ROOT CORE	CONTROL
7/6/2004	Crow 3	E11	1	E11-1	PIPO	SYM ROOT CORE	CONTROL
7/6/2004	Crow 3	D13	1	D13-1	PIPO	NS ROOT CORE	CONTROL
7/6/2004	Crow 3	E12	1	E12-1	PIPO	NS ROOT CORE	CONTROL
7/6/2004	Crow 3	F13	3	F13-3	PIPO	CURA	CONTROL
7/6/2004	Crow 3	F15	1	F15-1	PIPO	NS ROOT CORE	CONTROL
7/6/2004	Crow 3	G12	1	G12-1	PIPO	NS ROOT CORE	CONTROL
7/6/2004	Crow 3	G16	9	G16-9	PIPO	CURA	CONTROL
7/6/2004	Crow 3	F17	1	F17-1	PIPO	NS ROOT CORE	CONTROL
7/6/2004	Crow 3	H13	3	H13-3	PSME	NS ROOT CORE	CONTROL
7/6/2004	Crow 3	G14	1	G14-1	PIPO	NS ROOT CORE	CONTROL
7/6/2004	Crow 3	G16	2	G16-2	PIPO	CURA	CONTROL
7/6/2004	Crow 3	G16	8	G16-8	PIPO	NS ROOT CORE	CONTROL
7/6/2004	Crow 3	F13	4	F13-4	PIPO	NS ROOT CORE	CONTROL
7/20/2004	Crow 1	H6	1	H6-1	PSME	NS ROOT CORE	THIN ONLY
7/20/2004	Crow 1	D8	1	D8-1	PIPO	NS ROOT CORE	THIN ONLY
7/20/2004	Crow 1	F10	1	F10-1	PIPO	NS ROOT CORE	THIN ONLY
7/20/2004	Crow 1	D10	1	D10-1	PIPO	NS ROOT CORE	THIN ONLY
7/20/2004	Crow 1	E5	1	E5-1	PIPO	NS ROOT CORE	THIN ONLY
7/20/2004	Crow 1	G9	3	G9-3	PIPO	NS ROOT CORE	THIN ONLY
7/20/2004	Crow 1	G7	1	G7-1	PIPO	NS ROOT CORE	THIN ONLY
7/20/2004	Crow 1	E7	4	E7-4	PIPO	NS ROOT CORE	THIN ONLY
7/20/2004	Crow 1	F6	1	F6-1	PSME	NS ROOT CORE	THIN ONLY
7/20/2004	Crow 1	F12	1	F12-1	PIPO	NS ROOT CORE	THIN ONLY
7/20/2004	Crow 1	E9	1	E9-1	PIPO	NS ROOT CORE	THIN ONLY
7/20/2004	Crow 1	F8	1	F8-1	PSME	NS ROOT CORE	THIN ONLY
7/20/2004	Crow 1	G5	5	G5-5	PIPO	NS ROOT CORE	THIN ONLY
7/20/2004	Crow 1	G11	1	G11-1	PIPO	NS ROOT CORE	THIN ONLY
7/20/2004	Crow 1	D11	1	D11-1	PIPO	NS ROOT CORE	THIN ONLY
7/20/2004	Crow 1	E11	1	E11-1	PIPO	NS ROOT CORE	THIN ONLY
7/20/2004	Crow 1	D6	3	D6-3	PIPO	NS ROOT CORE	THIN ONLY
7/21/2004	Crow 1	I9	1	I9-1	PIPO	NS ROOT CORE	THIN ONLY
7/21/2004	Crow 1	K11	1	K11-1	PIPO	NS ROOT CORE	THIN ONLY
7/21/2004	Crow 1	K9	3	K9-3	PIPO	NS ROOT CORE	THIN ONLY
7/21/2004	Crow 1	I5	1	I5-1	PIPO	NS ROOT CORE	THIN ONLY

DATE	SITE	UTM CELL	TREE #	CORE ID	HOST	HOST STATUS	TREATMENT
7/21/2004	Crow 1	H10	1	H10-1	PSME	NS ROOT CORE	THIN ONLY
7/21/2004	Crow 1	K7	1	K7-1	PIPO	NS ROOT CORE	THIN ONLY
7/21/2004	Crow 1	J6	2	J6-2	PSME	NS ROOT CORE	THIN ONLY
7/21/2004	Crow 1	K6	3	K6-3	PSME	SYM ROOT CORE	THIN ONLY
7/21/2004	Crow 1	K6	2	K6-2	PSME	SYM ROOT CORE	THIN ONLY
7/21/2004	Crow 1	I6	2	I6-2	PSME	SYM ROOT CORE	THIN ONLY
7/21/2004	Crow 1	K5	8	K5-8	PIPO	NS ROOT CORE	THIN ONLY
7/21/2004	Crow 1				PIPO		THIN ONLY
7/21/2004	Crow 1	I7	1	I7-1	PIPO	NS ROOT CORE	THIN ONLY
7/21/2004	Crow 1	J8	5	J8-5	PIPO	NS ROOT CORE	THIN ONLY
7/21/2004	Crow 1	H8	3	H8-3	PIPO	NS ROOT CORE	THIN ONLY
7/21/2004	Crow 1	J10	1	J10-1	PIPO	NS ROOT CORE	THIN ONLY
7/21/2004	Crow 1	J12	1	J12-1	PSME	NS ROOT CORE	THIN ONLY
7/21/2004	Crow 1	K12	2	K12-2		SYM ROOT CORE	THIN ONLY
7/21/2004	Crow 1	I11	3	I11-3	PSME	NS ROOT CORE	THIN ONLY
7/13/2004	Crow 6	L9	1	L9-1	PIPO	NS ROOT CORE	THIN ONLY
7/13/2004	Crow 6	I6	1	I6-1	PIPO	NS ROOT CORE	THIN ONLY
7/13/2004	Crow 6	K12	1	K12-1	PIPO	NS ROOT CORE	THIN ONLY
7/13/2004	Crow 6	L7	2	L7-2	PIPO	NS ROOT CORE	THIN ONLY
7/13/2004	Crow 6	K8	1	K8-1	PIPO	NS ROOT CORE	THIN ONLY
7/13/2004	Crow 6	I8	1	I8-1	PIPO	NS ROOT CORE	THIN ONLY
7/13/2004	Crow 6	K6	3	K6-3	PIPO	NS ROOT CORE	THIN ONLY
7/13/2004	Crow 6	J7	1	J7-1	PIPO	NS ROOT CORE	THIN ONLY
7/13/2004	Crow 6	L11	1	L11-1	PIPO	NS ROOT CORE	THIN ONLY
7/13/2004	Crow 6	H11	3	H11-3	PSME	NS ROOT CORE	THIN ONLY
7/13/2004	Crow 6	J11	2	J11-2	PIPO	NS ROOT CORE	THIN ONLY
7/13/2004	Crow 6	H9	1	H9-1	PIPO	NS ROOT CORE	THIN ONLY
7/13/2004	Crow 6	J9	1	J9-1	PIPO	NS ROOT CORE	THIN ONLY
7/13/2004	Crow 6	I10	1	I10-1	PIPO	NS ROOT CORE	THIN ONLY
7/13/2004	Crow 6	K10	5	K10-5	PIPO	NS ROOT CORE	THIN ONLY
7/14/2004	Crow 6	E14	3	E14-3	PIPO	NS ROOT CORE	THIN ONLY
7/14/2004	Crow 6	E16	1	E16-1	PIPO	NS ROOT CORE	THIN ONLY
7/14/2004	Crow 6	F13	5	F13-5	PIPO	NS ROOT CORE	THIN ONLY
7/14/2004	Crow 6	H15	2	H15-2	PIPO	TPKL	THIN ONLY
7/14/2004	Crow 6	G16	1	G16-1	PIPO	NS ROOT CORE	THIN ONLY
7/14/2004	Crow 6	C14	3	C14-3	PIPO	NS ROOT CORE	THIN ONLY
7/14/2004	Crow 6	I16	2	I16-2	PIPO	NS ROOT CORE	THIN ONLY
7/14/2004	Crow 6	G14	1	G14-1	PIPO	NS ROOT CORE	THIN ONLY
7/14/2004	Crow 6	E12	1	E12-1	PIPO	NS ROOT CORE	THIN ONLY
7/14/2004	Crow 6	D15	1	D15-1	PIPO	NS ROOT CORE	THIN ONLY
7/14/2004	Crow 6	H15	3	H15-3	PIPO	NS ROOT CORE	THIN ONLY
7/14/2004	Crow 6	I12	1	I12-1	PIPO	NS ROOT CORE	THIN ONLY
7/14/2004	Crow 6	H13	1	H13-1	PIPO	NS ROOT CORE	THIN ONLY

DATE	SITE	UTM CELL	TREE #	CORE ID	HOST	HOST STATUS	TREATMENT
7/14/2004	Crow 6	F15	1	F15-1	PIPO	NS ROOT CORE	THIN ONLY
7/14/2004	Crow 6	G14	2	G14-2	PIPO	NS ROOT CORE	THIN ONLY
7/14/2004	Crow 6	J13	1	J13-1	PIPO	NS ROOT CORE	THIN ONLY
7/14/2004	Crow 6	H12	1	H12-1	PIPO	SYM ROOT CORE	THIN ONLY
7/15/2004	Slawson	C15	1	C15-1	PSME	NS ROOT CORE	THIN ONLY
7/15/2004	Slawson	B14	1	B14-1	PSME	NS ROOT CORE	THIN ONLY
7/15/2004	Slawson	D12	1	D12-1	PSME	NS ROOT CORE	THIN ONLY
7/15/2004	Slawson	F10	1	F10-1	PSME	NS ROOT CORE	THIN ONLY
7/15/2004	Slawson	E13	1	E13-1	PIPO	NS ROOT CORE	THIN ONLY
7/15/2004	Slawson	E11	1	E11-1	PIPO	SYM ROOT CORE	THIN ONLY
7/15/2004	Slawson	B10	1	B10-1	PIPO	NS ROOT CORE	THIN ONLY
7/15/2004	Slawson	F12	1	F12-1	PSME	NS ROOT CORE	THIN ONLY
7/15/2004	Slawson	E11	2	E11-2	PIPO	NS ROOT CORE	THIN ONLY
7/15/2004	Slawson	F14	1	F14-1	PIPO	NS ROOT CORE	THIN ONLY
7/15/2004	Slawson	A7	2	A 7-2	PIPO	NS ROOT CORE	THIN ONLY
7/15/2004	Slawson	D10	1	D10-1	PSME	NS ROOT CORE	THIN ONLY
7/15/2004	Slawson	E9	1	E9-1	PIPO	NS ROOT CORE	THIN ONLY
7/22/2004	Slawson	D14	2	D14-2	PSME	NS ROOT CORE	THIN ONLY
7/22/2004	Slawson	B12	1	B12-1	PSME	NS ROOT CORE	THIN ONLY
7/22/2004	Slawson	C13	1	C13-1	PSME	NS ROOT CORE	THIN ONLY
7/22/2004	Slawson	B8	1	B8-1	PSME	NS ROOT CORE	THIN ONLY
7/22/2004	Slawson	C11	2	C11-2	PSME	NS ROOT CORE	THIN ONLY
7/22/2004	Slawson	C9	1	C9-1	PSME	NS ROOT CORE	THIN ONLY
7/22/2004	Slawson	E15	1	E15-1	PSME	NS ROOT CORE	THIN ONLY
7/26/2004	Slawson	B3	1	B3-1	PIPO	NS ROOT CORE	THIN ONLY
7/26/2004	Slawson	D4	1	D4-1	PIPO	NS ROOT CORE	THIN ONLY
7/26/2004	Slawson	A5	1	A 5-1	PSME	NS ROOT CORE	THIN ONLY
7/26/2004	Slawson	B5	2	B5-2	PSME	NS ROOT CORE	THIN ONLY
7/26/2004	Slawson	E5	1	E5-1	PSME	NS ROOT CORE	THIN ONLY
7/26/2004	Slawson	C3	2	C3-2	PIPO	NS ROOT CORE	THIN ONLY
7/26/2004	Slawson	D6	1	D6-1	PSME	NS ROOT CORE	THIN ONLY
7/26/2004	Slawson	A8	1	A 8-1	PIPO	SYM ROOT CORE	THIN ONLY
7/26/2004	Slawson	B4	1	B4-1	PSME	NS ROOT CORE	THIN ONLY
7/26/2004	Slawson	C5	1	C5-1	PSME	NS ROOT CORE	THIN ONLY
7/26/2004	Slawson	F8	1	F8-1	PSME	NS ROOT CORE	THIN ONLY
7/26/2004	Slawson	D8	1	D8-1	PSME	NS ROOT CORE	THIN ONLY
7/26/2004	Slawson	B6	1	B6-1	PSME	NS ROOT CORE	THIN ONLY
7/26/2004	Slawson	C7	1	C7-1	PSME	NS ROOT CORE	THIN ONLY
7/29/2004	Poison	G5	7	G5-7	PIPO	SYM ROOT CORE	BURN ONLY
7/29/2004	Poison	F6	11	F6-11	PIPO	SYM ROOT CORE	BURN ONLY
7/29/2004	Poison	G5	10	G5-10	PIPO	NS ROOT CORE	BURN ONLY
7/29/2004	Poison	G7	2	G7-2	PSME	NS ROOT CORE	BURN ONLY
7/29/2004	Poison	H5	5	H5-5	PIPO	SYM ROOT CORE	BURN ONLY
7/29/2004	Poison	G5	9	G5-9	PIPO	SYM ROOT CORE	BURN ONLY

DATE	SITE	UTM CELL	TREE #	CORE ID	HOST	HOST STATUS	TREATMENT
7/29/2004	Poison	G5	1	G5-1	PIPO	SYM ROOT CORE	BURN ONLY
7/29/2004	Poison	G6	2	G6-2	PIPO	SYM ROOT CORE	BURN ONLY
7/29/2004	Poison	F6	3	F6-3	PIPO	SYM ROOT CORE	BURN ONLY
7/29/2004	Poison	G5	8	G5-8	PIPO	SYM ROOT CORE	BURN ONLY
7/29/2004	Poison	F6	1	F6-1	PIPO	NS ROOT CORE	BURN ONLY
7/27/2004	Poison	K13	1	K13-1	PIPO	NS ROOT CORE	BURN ONLY
7/27/2004	Poison	G10	5	G10-5	PIPO	CURA	BURN ONLY
7/27/2004	Poison	G13	1	G13-1	PIPO	NS ROOT CORE	BURN ONLY
7/27/2004	Poison	G13	7	G13-7	PIPO	CURA	BURN ONLY
7/27/2004	Poison	G10	6	G10-6	PIPO	CURA	BURN ONLY
7/27/2004	Poison	G12	1	G12-1	PIPO	NS ROOT CORE	BURN ONLY
7/27/2004	Poison	I12	1	I12-1	PIPO	NS ROOT CORE	BURN ONLY
7/27/2004	Poison	H9	1	H9-1	PIPO	CURA	BURN ONLY
7/27/2004	Poison	K14	1	K14-1	PSME	SYM ROOT CORE	BURN ONLY
7/27/2004	Poison	J10	1	J10-1	PIPO	NS ROOT CORE	BURN ONLY
7/27/2004	Poison	H13	1	H13-1	PIPO	NS ROOT CORE	BURN ONLY
7/27/2004	Poison	G10	7	G10-7	PSME	NS ROOT CORE	BURN ONLY
7/27/2004	Poison	I10	1	I10-1	PIPO	NS ROOT CORE	BURN ONLY
7/27/2004	Poison	H11	1	H11-1	PIPO	NS ROOT CORE	BURN ONLY
7/27/2004	Poison	G11	1	G11-1	PIPO	CURA	BURN ONLY
7/27/2004	Poison	G10	1	G10-1	PIPO	CURA	BURN ONLY
7/27/2004	Poison	K13	3	K13-3	PIPO	SYM ROOT CORE	BURN ONLY
7/27/2004	Poison	I14	1	I14-1	PIPO	NS ROOT CORE	BURN ONLY
7/27/2004	Poison	G13	2	G13-2	PIPO	CURA	BURN ONLY
7/27/2004	Poison	G13	3	G13-3	PIPO	CURA	BURN ONLY
7/27/2004	Poison	G9	1	G9-1	PSME	NS ROOT CORE	BURN ONLY
7/27/2004	Poison	K13	2	K13-2	PSME	SYM ROOT CORE	BURN ONLY
7/27/2004	Poison	H10	1	H10-1	PIPO	SYM ROOT CORE	BURN ONLY
7/27/2004	Poison	G10	2	G10-2	PIPO	CURA	BURN ONLY
7/28/2004	Poison	K9	1	K9-1	PSME	SYM ROOT CORE	BURN ONLY
7/28/2004	Poison	J12	1	J12-1	PIPO	NS ROOT CORE	BURN ONLY
7/28/2004	Poison	I7	1	I7-1	PSME	NS ROOT CORE	BURN ONLY
7/28/2004	Poison	J8	1	J8-1	PSME	NS ROOT CORE	BURN ONLY
7/28/2004	Poison	K8	1	K8-1	PSME	NS ROOT CORE	BURN ONLY
7/28/2004	Poison	H8	1	H8-1	PSME	NS ROOT CORE	BURN ONLY
7/28/2004	Poison	K9	4	K9-4	PIPO	CURA	BURN ONLY
7/28/2004	Poison	J9	1	J9-1	PSME	NS ROOT CORE	BURN ONLY
7/28/2004	Poison	K7	2	K7-2	PSME	NS ROOT CORE	BURN ONLY
7/28/2004	Poison	J11	1	J11-1	PIPO	NS ROOT CORE	BURN ONLY
7/28/2004	Poison	K9	3	K9-3	PIPO	CURA	BURN ONLY
7/28/2004	Poison	J9	3	J9-3	PSME	SYM ROOT CORE	BURN ONLY
7/28/2004	Poison	J9	2	J9-2	PIPO	SYM ROOT CORE	BURN ONLY
7/28/2004	Poison	F7	6	F7-6	PIPO	ROOT SAMPLE	BURN ONLY

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7/28/2004	Poison	G7	11	G7-11	?	ROOT SAMPLE	BURN ONLY
7/28/2004	Poison	F6	10	F6-10	PIPO	SYM ROOT CORE	BURN ONLY
8/4/2004	Spromberg	M7	1	M7-1	PIPO	CURA	BURN ONLY
8/4/2004	Spromberg	L6	1	L6-1	PIPO	CURA	BURN ONLY
8/4/2004	Spromberg	P8	1	P8-1	PIPO	NS ROOT CORE	BURN ONLY
8/4/2004	Spromberg	N9	1	N9-1	PIPO	NS ROOT CORE	BURN ONLY
8/4/2004	Spromberg	N8	1	N8-1	PIPO	NS ROOT CORE	BURN ONLY
8/4/2004	Spromberg	K9	2	K9-2	PIPO	CURA	BURN ONLY
8/4/2004	Spromberg	K9	1	K9-1	PIPO	CURA	BURN ONLY
8/4/2004	Spromberg	G6	1	G6-1	PIPO	CURA	BURN ONLY
8/4/2004	Spromberg	J7	2	J7-2	PIPO	CURA	BURN ONLY
8/4/2004	Spromberg	J7	1	J7-1	PIPO	CURA	BURN ONLY
8/4/2004	Spromberg	E6	1	E6-1	PSME	NS ROOT CORE	BURN ONLY
8/4/2004	Spromberg	L8	2	L8-2	PIPO	NS ROOT CORE	BURN ONLY
8/4/2004	Spromberg	H9	2	H9-2	PSME	NS ROOT CORE	BURN ONLY
8/4/2004	Spromberg	J6	1	J6-1	PIPO	NS ROOT CORE	BURN ONLY
8/4/2004	Spromberg	I6	2	I6-2	PIPO	CURA	BURN ONLY
8/4/2004	Spromberg	K6	2	K6-2	PIPO	CURA	BURN ONLY
8/4/2004	Spromberg	H11	1	H11-1	PSME	NS ROOT CORE	BURN ONLY
8/4/2004	Spromberg	J9	1	J9-1	PIPO	CURA	BURN ONLY
8/4/2004	Spromberg	I8	2	I8-2	PSME	NS ROOT CORE	BURN ONLY
8/4/2004	Spromberg	L9	1	L9-1	PSME	NS ROOT CORE	BURN ONLY
8/4/2004	Spromberg	H9	1	H9-1	PIPO	CURA	BURN ONLY
8/4/2004	Spromberg	F6	2	F6-2	PIPO	CURA	BURN ONLY
8/4/2004	Spromberg	H9	8	H9-8	PIPO	CURA	BURN ONLY
8/4/2004	Spromberg	J9	3	J9-3	PIPO	SYM ROOT CORE	BURN ONLY
8/4/2004	Spromberg	Q6	1	Q6-1	PSME	NS ROOT CORE	BURN ONLY
8/4/2004	Spromberg	I6	1	I6-1	PSME	NS ROOT CORE	BURN ONLY
8/4/2004	Spromberg	O6	1	O6-1	PIPO	NS ROOT CORE	BURN ONLY
8/4/2004	Spromberg	L8	1	L8-1	PIPO	SYM ROOT CORE	BURN ONLY
8/4/2004	Spromberg	J9	5	J9-5	PIPO	NS ROOT CORE	BURN ONLY
8/4/2004	Spromberg	I10	1	I10-1	PSME	NS ROOT CORE	BURN ONLY
8/4/2004	Spromberg	M6	1	M6-1	PSME	NS ROOT CORE	BURN ONLY
8/4/2004	Spromberg	L7	1	L7-1	PSME	NS ROOT CORE	BURN ONLY
8/4/2004	Spromberg	M6	4	M6-4	PIPO	CURA	BURN ONLY
8/4/2004	Spromberg	G6	2	G6-2	PSME	NS ROOT CORE	BURN ONLY
8/4/2004	Spromberg	K7	1	K7-1	PSME	NS ROOT CORE	BURN ONLY
8/4/2004	Spromberg	G10	1	G10-1	PIPO	CURK, ROTTEN	BURN ONLY
8/4/2004	Spromberg	G10	13	G10-13	PIPO	CURA	BURN ONLY
8/4/2004	Spromberg	N7	1	N7-1	PSME	NS ROOT CORE	BURN ONLY
8/4/2004	Spromberg	O8	3	O8-3	PIPO	NS ROOT CORE	BURN ONLY
8/9/2004	Spromberg	D8	1	D8-1	PIPO	SYM ROOT CORE	BURN ONLY
8/9/2004	Spromberg	E7	1	E7-1	PIPO	CURA	BURN ONLY
8/9/2004	Spromberg	E7	2	E7-2	PSME	CURA	BURN ONLY

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8/9/2004	Spromberg	D10	14	D10-14	PSME	NS ROOT CORE	BURN ONLY
8/9/2004	Spromberg	G9	1	G9-1	PSME	SYM ROOT CORE	BURN ONLY
8/9/2004	Spromberg	D9	1	D9-1	PIPO	NS ROOT CORE	BURN ONLY
8/9/2004	Spromberg	G6	3	G6-3	PSME	SYM ROOT CORE	BURN ONLY
8/9/2004	Spromberg	D8	2	D8-2	PIPO	SYM ROOT CORE	BURN ONLY
8/9/2004	Spromberg	E9	1	E9-1	PIPO	CURA	BURN ONLY
8/9/2004	Spromberg	E8	1	E8-1	PSME	?	BURN ONLY
8/9/2004	Spromberg	D7	1	D7-1	PSME	NS ROOT CORE	BURN ONLY
8/9/2004	Spromberg	F9	1	F9-1	PIPO	NS ROOT CORE	BURN ONLY
8/9/2004	Spromberg	E7	3	E7-3	PSME	CURA	BURN ONLY
8/9/2004	Spromberg	F7	1	F7-1	PSME	NS ROOT CORE	BURN ONLY
8/9/2004	Spromberg	E7	4	E7-4	PIPO	CURA	BURN ONLY
8/9/2004	Spromberg	E7	5	E7-5	PSME	NS ROOT CORE	BURN ONLY
8/9/2004	Spromberg	F11	1	F11-1	PSME	NS ROOT CORE	BURN ONLY
8/9/2004	Spromberg	G8	1	G8-1	PSME	NS ROOT CORE	BURN ONLY
8/9/2004	Spromberg	D10	1	D10-1	PIPO	CURA	BURN ONLY
8/9/2004	Spromberg	C7	3	C7-3	PIPO	NS ROOT CORE	BURN ONLY
8/9/2004	Spromberg	C7	2	C7-2	PSME	TPKL	BURN ONLY
8/9/2004	Spromberg	E10	1	E10-1	PSME	NS ROOT CORE	BURN ONLY
8/9/2004	Spromberg	E8	2	E8-2	PSME	NS ROOT CORE	BURN ONLY
8/11/2004	Sand 2	K15	5	K15-5	PSME	CURA	CONTROL
8/11/2004	Sand 2	K16	1	K16-1	PSME	NS ROOT CORE	CONTROL
8/11/2004	Sand 2	K15	7	K15-7	PSME	DEAD - RHIZO	CONTROL
8/11/2004	Sand 2	M16	7	M16-7	PSME	NS ROOT CORE	CONTROL
8/11/2004	Sand 2	M20	1	M20-1	PSME	NS ROOT CORE	CONTROL
8/11/2004	Sand 2	L16	4	L16-4	PSME	DEAD - RHIZO	CONTROL
8/11/2004	Sand 2	K15	7	K15-7	PSME	DEAD - RHIZO	CONTROL
8/11/2004	Sand 2	L19	1	L19-1	PSME	NS ROOT CORE	CONTROL
8/11/2004	Sand 2	K15	6	K15-6	PSME	CURA	CONTROL
8/11/2004	Sand 2	M22	1	M22-1	PIPO	NS ROOT CORE	CONTROL
8/11/2004	Sand 2	M16	1	M16-1	PSME	NS ROOT CORE	CONTROL
8/11/2004	Sand 2	M15	1	M15-1	PSME	NS ROOT CORE	CONTROL
8/11/2004	Sand 2	N15	1	N15-1	PSME	NS ROOT CORE	CONTROL
8/11/2004	Sand 2	N19	1	N19-1	PSME	NS ROOT CORE	CONTROL
8/11/2004	Sand 2	N18	1	N18-1	PSME	NS ROOT CORE	CONTROL
8/11/2004	Sand 2	M21	1	M21-1	PSME	NS ROOT CORE	CONTROL
8/11/2004	Sand 2	N17	1	N17-1	PIPO	NS ROOT CORE	CONTROL
8/12/2004	Ruby	K16	6	K16-6	PSME	TPKL	THIN ONLY
8/12/2004	Ruby	J15	2	J15-2	PSME	DEAD - RHIZO	THIN ONLY
8/12/2004	Ruby	L16	3	L16-3	PSME	DEAD - RHIZO	THIN ONLY
8/12/2004	Ruby	K16	2	K16-2	PSME	NS ROOT CORE	THIN ONLY
8/12/2004	Ruby	I16	1	I16-1	PSME	NS ROOT CORE	THIN ONLY

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8/12/2004	Ruby	J15	1	J15-1	PSME	NS ROOT CORE	THIN ONLY
8/12/2004	Ruby	K16	5	K16-5	PIPO	CURA	THIN ONLY
8/12/2004	Ruby	G16	1	G16-1	PSME	NS ROOT CORE	THIN ONLY
8/12/2004	Ruby	K16	3	K16-3	PIPO	CURA	THIN ONLY
8/12/2004	Ruby	G15	1	G15-1	PSME	NS ROOT CORE	THIN ONLY
8/12/2004	Ruby	F15	1	F15-1	PIPO	NS ROOT CORE	THIN ONLY
8/12/2004	Ruby	K14	1	K14-1	PSME	NS ROOT CORE	THIN ONLY
8/12/2004	Ruby	H16	1	H16-1	PIPO	NS ROOT CORE	THIN ONLY
8/12/2004	Ruby	H15	1	H15-1	PSME	NS ROOT CORE	THIN ONLY
8/12/2004	Ruby	F16	1	F16-1	PIPO	NS ROOT CORE	THIN ONLY
8/19/2004	Ruby	H13	1	H13-1	PSME	NS ROOT CORE	THIN ONLY
8/19/2004	Ruby	I12	1	I12-1	PSME	NS ROOT CORE	THIN ONLY
8/19/2004	Ruby	I14	1	I14-1	PIPO	NS ROOT CORE	THIN ONLY
8/19/2004	Ruby	G13	3	G13-3	PIPO	CURA	THIN ONLY
8/19/2004	Ruby	G12	1	G12-1	PSME	NS ROOT CORE	THIN ONLY
8/19/2004	Ruby	K12	2	K12-2	PSME	NS ROOT CORE	THIN ONLY
8/19/2004	Ruby	J13	1	J13-1	PIPO	NS ROOT CORE	THIN ONLY
8/19/2004	Ruby	G13	5	G13-5	PIPO	NS ROOT CORE	THIN ONLY
8/19/2004	Ruby	G13	5	G13-5	PIPO	RHIZO	THIN ONLY
8/19/2004	Ruby	H14	1	H14-1	PSME	DEAD - RHIZO	THIN ONLY
8/19/2004	Ruby	G15	3	G15-3	PIPO	CURK, ROTTEN	THIN ONLY
8/19/2004	Ruby	G13	1	G13-1	PIPO	DEAD	THIN ONLY
8/17/2004	Sand 2	J15	1	J15-1	PSME	NS ROOT CORE	CONTROL
8/17/2004	Sand 2	J14	1	J14-1	PSME	NS ROOT CORE	CONTROL
8/17/2004	Sand 2	K14	1	K14-1	PSME	NS ROOT CORE	CONTROL
8/17/2004	Sand 2	J18	1	J18-1	PIPO	NS ROOT CORE	CONTROL
8/18/2004	Sand 2	H19	1	H19-1	PSME	NS ROOT CORE	CONTROL
8/17/2004	Sand 2	I18	1	I18-1	PSME	NS ROOT CORE	CONTROL
8/17/2004	Sand 2	J16	1	J16-1	PSME	NS ROOT CORE	CONTROL
8/17/2004	Sand 2	G20	4	G20-4	PSME	NS ROOT CORE	CONTROL
8/16/2004	Sand 2	K21	1	K21-1	PSME	NS ROOT CORE	CONTROL
8/17/2004	Sand 2	K17	1	K17-1	PSME	NS ROOT CORE	CONTROL
8/17/2004	Sand 2	I20	1	I20-1	PIPO	NS ROOT CORE	CONTROL
8/18/2004	Sand 2	G18	1	G18-1	PSME	NS ROOT CORE	CONTROL
8/16/2004	Sand 2	K20	2	K20-2	PSME	NS ROOT CORE	CONTROL
8/17/2004	Sand 2	I22	1	I22-1	PSME	NS ROOT CORE	CONTROL
8/16/2004	Sand 2	K18	3	K18-3	PSME	NS ROOT CORE	CONTROL
8/16/2004	Sand 2	K22	1	K22-1	PSME	NS ROOT CORE	CONTROL
8/16/2004	Sand 2	J19	1	J19-1	PSME	NS ROOT CORE	CONTROL
8/17/2004	Sand 2	I21	1	I21-1	PIPO	NS ROOT CORE	CONTROL
	Sand 2	G19	1	G19-1	PSME		CONTROL
8/16/2004	Sand 2	K21	6	K21-6	PSME	SYM ROOT CORE	CONTROL
8/16/2004	Sand 2	J22	4	J22-4	PIPO	DEAD	CONTROL
8/17/2004	Sand 2	K17	3	K17-3	PSME	FAN	CONTROL

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8/17/2004	Sand 2	H19	1	H19-1	PSME	FAN	CONTROL
8/16/2004	Sand 2	J20	1	J20-1	PSME	DEAD - FAN	CONTROL
8/18/2004	Sand 2	G19	3	G19-3	PSME	DEAD - FAN	CONTROL
8/17/2004	Sand 2	I22	6	I22-6	PSME	FAN	CONTROL
8/16/2004	Sand 2	J22	4	J22-4	PIPO	DEAD	CONTROL
8/18/2004	Sand 2	G19	2	G19-2	PSME	DEAD - FAN	CONTROL
8/16/2004	Sand 2	K19	3	K19-3	PSME	DEAD - RHIZO	CONTROL
8/16/2004	Sand 2	K20	3	K20-3	PSME	DEAD - FAN	CONTROL
8/24/2004	Sand 19	E13	1	E13-1	PIPO	NS ROOT CORE	CONTROL
8/24/2004	Sand 19	J10	1	J10-1	PSME	NS ROOT CORE	CONTROL
8/24/2004	Sand 19	J12	1	J12-1	PSME	NS ROOT CORE	CONTROL
8/24/2004	Sand 19	H10	1	H10-1	PSME	NS ROOT CORE	CONTROL
8/24/2004	Sand 19	F13	1	F13-1	PIPO	CURA	CONTROL
8/24/2004	Sand 19	I11	1	I11-1	PSME	CURA	CONTROL
8/24/2004	Sand 19	F10	2	F10-2	PIPO	NS ROOT CORE	CONTROL
8/24/2004	Sand 19	D12	1	D12-1	PSME	NS ROOT CORE	CONTROL
8/24/2004	Sand 19	D10	1	D10-1	PSME	NS ROOT CORE	CONTROL
8/24/2004	Sand 19	I13	1	I13-1	PSME	NS ROOT CORE	CONTROL
8/24/2004	Sand 19	E11	1	E11-1	PIPO	NS ROOT CORE	CONTROL
8/24/2004	Sand 19	I11	2	I11-2	PIPO	NS ROOT CORE	CONTROL
8/24/2004	Sand 19	F12	1	F12-1	PSME	NS ROOT CORE	CONTROL
8/24/2004	Sand 19	G11	2	G11-2	PSME	NS ROOT CORE	CONTROL
8/24/2004	Sand 19	G13	2	G13-2	PSME	NS ROOT CORE	CONTROL
8/24/2004	Sand 19	H12	1	H12-1	PSME	NS ROOT CORE	CONTROL
8/24/2004	Sand 19	K13	1	K13-1	PIPO	NS ROOT CORE	CONTROL
8/24/2004	Sand 19	I13	2	I13-2	PSME	DEAD - ROOT	CONTROL
8/24/2004	Sand 19	F10	3	F10-3	PSME	DEAD - ROOT	CONTROL
8/24/2004	Sand 19	G11	1	G11-1	PSME	DEAD - ROOT	CONTROL
8/23/2004	Sand 19	D8	2	D8-2	PSME	NS ROOT CORE	CONTROL
8/23/2004	Sand 19	L9	1	L9-1	PSME	CURA	CONTROL
8/23/2004	Sand 19	F8	4	F8-4	PIPO	NS ROOT CORE	CONTROL
8/23/2004	Sand 19	J6	1	J6-1	PSME	NS ROOT CORE	CONTROL
8/23/2004	Sand 19	G8	2	G8-2	PSME	NS ROOT CORE	CONTROL
8/23/2004	Sand 19	K9	1	K9-1	PSME	NS ROOT CORE	CONTROL
8/23/2004	Sand 19	E7	1	E7-1	PIPO	NS ROOT CORE	CONTROL
8/23/2004	Sand 19	I6	2	I6-2	PSME	NS ROOT CORE	CONTROL
8/23/2004	Sand 19	H8	1	H8-1	PSME	NS ROOT CORE	CONTROL
8/23/2004	Sand 19	J7	2	J7-2	PIPO	NS ROOT CORE	CONTROL
8/23/2004	Sand 19	G6	1	G6-1	PIPO	NS ROOT CORE	CONTROL
8/23/2004	Sand 19	E9	1	E9-1	PSME	NS ROOT CORE	CONTROL
8/23/2004	Sand 19	I9	1	I9-1	PSME	NS ROOT CORE	CONTROL
8/23/2004	Sand 19	I6	1	I6-1	PSME	SYM ROOT CORE	CONTROL
8/23/2004	Sand 19	K8	1	K8-1	PSME	NS ROOT CORE	CONTROL
8/23/2004	Sand 19	G8	1	G8-1	PSME	TPKL	CONTROL

DATE	SITE	UTM CELL	TREE #	CORE ID	HOST	HOST STATUS	TREATMENT
8/23/2004	Sand 19	K7	1	K7-1	PSME	NS ROOT CORE	CONTROL
8/23/2004	Sand 19	L8	1	L8-1	PSME	NS ROOT CORE	CONTROL
8/23/2004	Sand 19	I7	1	I7-1	PSME	NS ROOT CORE	CONTROL
8/23/2004	Sand 19	G9	1	G9-1	PSME	NS ROOT CORE	CONTROL
8/26/2004	Ruby	F7	1	F7-1	PSME	NS ROOT CORE	THIN ONLY
8/26/2004	Ruby	G8	2	G8-2	PSME	NS ROOT CORE	THIN ONLY
8/26/2004	Ruby	D6	6	D6-6	PSME	NS ROOT CORE	THIN ONLY
8/26/2004	Ruby	D7	2	D7-2	PIPO	NS ROOT CORE	THIN ONLY
8/26/2004	Ruby	G6	1	G6-1	PSME	NS ROOT CORE	THIN ONLY
8/26/2004	Ruby	E10	1	E10-1	PSME	NS ROOT CORE	THIN ONLY
8/26/2004	Ruby	E8	1	E8-1	PIPO	NS ROOT CORE	THIN ONLY
8/26/2004	Ruby	F5	2	F5-2	PSME	NS ROOT CORE	THIN ONLY
8/26/2004	Ruby	E6	2	E6-2	PSME	NS ROOT CORE	THIN ONLY
8/26/2004	Ruby	F11	2	F11-2	PIPO	CURA	THIN ONLY
8/26/2004	Ruby	I6	1	I6-1	PSME	NS ROOT CORE	THIN ONLY
8/26/2004	Ruby	H6	1	H6-1	PIPO	NS ROOT CORE	THIN ONLY
8/26/2004	Ruby	I4	1	I4-1	PIPO	NS ROOT CORE	THIN ONLY
8/26/2004	Ruby	D9	1	D9-1	PIPO	NS ROOT CORE	THIN ONLY
8/26/2004	Ruby	F9	1	F9-1	PSME	NS ROOT CORE	THIN ONLY
8/26/2004	Ruby	G11	1	G11-1	PIPO	NS ROOT CORE	THIN ONLY
8/26/2004	Ruby	J5	1	J5-1	PIPO	NS ROOT CORE	THIN ONLY
8/26/2004	Ruby	F11	1	F11-1	?	NS ROOT CORE	THIN ONLY
8/26/2004	Ruby	G10	2	G10-2	PIPO	NS ROOT CORE	THIN ONLY
8/26/2004	Ruby	H5	1	H5-1	PIPO	NS ROOT CORE	THIN ONLY
8/26/2004	Ruby	H7	2	H7-2	PSME	NS ROOT CORE	THIN ONLY
8/26/2004	Ruby	D7	1	D7-1	PIPO	DEAD - RHIZO	THIN ONLY
8/26/2004	Ruby	D7	1	D7-1	PIPO	DEAD - ROOT	THIN ONLY
8/26/2004	Ruby	G10	1	G10-1	PIPO	ROOT SAMPLE	THIN ONLY
8/26/2004	Ruby	I5	2	I5-2	PIPO	CURK, ROTTEN	THIN ONLY
8/26/2004	Ruby	F12	2	F12-2	PIPO	DEAD - ROOT	THIN ONLY
8/9/2004	Spromberg	D8	1	D8-1	PIPO	SYM ROOT CORE	BURN ONLY
9/2/2004	Camas 11	F10	7	F10-7	PSME	NS ROOT CORE	THIN& BURN
9/2/2004	Camas 11	D10	18	D10-18	PIPO	CURA	THIN& BURN
9/2/2004	Camas 11	F11	1	F11-1	PIPO	CURA	THIN& BURN
9/2/2004	Camas 11	E11	1	E11-1	PIPO	CURA	THIN& BURN
9/2/2004	Camas 11	B10	1	B10-1	PIPO	CURA	THIN& BURN
9/2/2004	Camas 11	F10	14	F10-14	PIPO	CURA	THIN& BURN
9/2/2004	Camas 11	F10	15	F10-15	PIPO	CURA	THIN& BURN
9/2/2004	Camas 11	D10	4	D10-4	PSME	NS ROOT CORE	THIN& BURN
9/2/2004	Camas 11	D11	11	D11-11	PIPO	CURA	THIN& BURN
9/2/2004	Camas 11	B10	3	B10-3	PSME	CURA	THIN& BURN
9/2/2004	Camas 11	D10	3	D10-3	PIPO	CURA	THIN& BURN

DATE	SITE	UTM CELL	TREE #	CORE ID	HOST	HOST STATUS	TREATMENT
9/2/2004	Camas 11	D10	7	D10-7	PSME	SYM ROOT CORE	THIN& BURN
9/2/2004	Camas 11	B10	2	B10-2	PIPO	NS ROOT CORE	THIN& BURN
9/2/2004	Camas 11	E10	5	E10-5	PSME	NS ROOT CORE	THIN& BURN
9/2/2004	Camas 11	F10	13	F10-13	PIPO	CURA	THIN& BURN
9/2/2004	Camas 11	E10	9	E10-9	PIPO	CURA	THIN& BURN
9/2/2004	Camas 11	B10	4	B10-4	PIPO	CURA	THIN& BURN
9/2/2004	Camas 11	B9	1	B9-1	PIPO	CURA	THIN& BURN
9/2/2004	Camas 11	C11	1	C11-1	PIPO	CURK, RHIZO	THIN& BURN
9/2/2004	Camas 11	F10	18	F10-18	PSME	DEAD - ROOT	THIN& BURN
9/2/2004	Camas 11	F10	18	F10-18	PSME	DEAD - ROOT	THIN& BURN
9/2/2004	Camas 11	F10	12	F10-12	PIPO	CURK, ROTTEN	THIN& BURN
9/2/2004	Camas 11	F10	16	F10-16	PSME	DEAD - ROOT	THIN& BURN
9/2/2004	Camas 11	C11	1	C11-1	PIPO	CURK, ROTTEN	THIN& BURN
9/2/2004	Camas 11	D10	5	D10-5	PSME	DEAD - ROOT	THIN& BURN
8/31/2004	Tripp 9	I8	1	I8-1	PSME	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	O8	1	O8-1	PSME	SYM ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	R10	1	R10-1	PSME	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	T9	1	T9-1	PSME	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	U8	2	U8-2	PIPO	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	Q8	1	Q8-1	PSME	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	F7	1	F7-1	PSME	SYM ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	Q9	1	Q9-1	PSME	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	O8	4	O8-4	PSME	SYM ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	O9	1	O9-1	PSME	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	P10	1	P10-1	PSME	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	N8	2	N8-2	PSME	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	L9	2	L9-2	PSME	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	K7	1	K7-1	PSME	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	H7	1	H7-1	PSME	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	R8	3	R8-3	PIPO	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	V5	1	V5-1	PSME	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	V8	1	V8-1	PSME	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	Q8	2	Q8-2	PSME	SYM ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	P8	1	P8-1	PSME	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	U5	1	U5-1	PSME	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	R10	3	R10-3	PSME	SYM ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	X6	4	X6-4	PIPO	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	Y5	2	Y5-2	PSME	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	T5	1	T5-1	PSME	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	V6	1	V6-1	PSME	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	X5	1	X5-1	PSME	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	W5	1	W5-1	PSME	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	S6	2	S6-2	PIPO	CURA	THIN& BURN

DATE	SITE	UTM CELL	TREE #	CORE ID	HOST	HOST STATUS	TREATMENT
8/31/2004	Tripp 9	W7	1	W7-1	PSME	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	S6	1	S6-1	PIPO	CURA	THIN& BURN
8/31/2004	Tripp 9	Z5	1	Z5-1	PIPO	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	S6	4	S6-4	PIPO	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	S6	3	S6-3	PIPO	CURA	THIN& BURN
8/31/2004	Tripp 9	W6	3	W6-3	PSME	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	Y6	1	Y6-1	PIPO	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	V7	1	V7-1	PSME	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	AA3	1	AA3-1	PSME	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	S9	1	S9-1	PSME	NS ROOT CORE	THIN& BURN
8/31/2004	Tripp 9	Q10	1	Q10-1	PSME	SYM ROOT CORE	THIN& BURN
9/13/2004	Camas 11	A7	19	H7-19	PIPO	CURA	THIN& BURN
9/13/2004	Camas 11	G7	6	G7-6	PIPO	CURA	THIN& BURN
9/13/2004	Camas 11	F6	11	F6-11	PIPO	CURA	THIN& BURN
9/13/2004	Camas 11	J3	6	J3-6	PSME	CURA	THIN& BURN
9/13/2004	Camas 11	J3	8	J3-8	PIPO	CURA	THIN& BURN
9/13/2004	Camas 11	I5	1	I5-1	PIPO	CURA	THIN& BURN
9/13/2004	Camas 11	?	?	?	PIPO	CURA	THIN& BURN
9/13/2004	Camas 11	J3	1	J3-1	ABGR	NS ROOT CORE	THIN& BURN
9/13/2004	Camas 11	H6	2	H6-2	PSME	CURA	THIN& BURN
9/13/2004	Camas 11	J2	1	J2-1	PIPO	NS ROOT CORE	THIN& BURN
9/13/2004	Camas 11	J4	4	J4-4	ABGR	NS ROOT CORE	THIN& BURN
9/13/2004	Camas 11	G9	3	G9-3	PIPO	NS ROOT CORE	THIN& BURN
9/13/2004	Camas 11	?	1	?-1	?	TPKL	THIN& BURN
9/13/2004	Camas 11	G7	5	G7-5	PIPO	CURA	THIN& BURN
9/13/2004	Camas 11	J3	5	J3-5	PIPO	CURA	THIN& BURN
9/13/2004	Camas 11	J3	7	J3-7	PIPO	CURA	THIN& BURN
9/13/2004	Camas 11	G7	4	G7-4	PIPO	CURA	THIN& BURN
9/13/2004	Camas 11	H7	5	H7-5	PIPO	CURA	THIN& BURN
9/13/2004	Camas 11	J3	3	J3-3	PIPO	CURA	THIN& BURN
9/13/2004	Camas 11	I4	1	I4-1	PIPO	CURA	THIN& BURN
9/13/2004	Camas 11	I3	1	I3-1	PIPO	CURA	THIN& BURN
9/13/2004	Camas 11	H7	4	H7-4	PIPO	CURA	THIN& BURN
9/13/2004	Camas 11	H7	?	H7-?	PSME	NS ROOT CORE	THIN& BURN
9/13/2004	Camas 11	F6	2	F6-2	PIPO	CURA	THIN& BURN
9/13/2004	Camas 11	G6	6	G6-6	PIPO	CURA	THIN& BURN
9/13/2004	Camas 11	E8	4	E8-4	PIPO	CURA	THIN& BURN
9/13/2004	Camas 11	F7	1	F7-1	PIPO	CURA	THIN& BURN
9/14/2004	Camas 11	F8	1	F8-1	PIPO	CURA	THIN& BURN
9/14/2004	Camas 11	G6	13	G6-13	PSME	SYM ROOT CORE	THIN& BURN
9/14/2004	Camas 11	E9	14	E9-14	PIPO	CURA	THIN& BURN
9/14/2004	Camas 11	E9	15	E9-15	PIPO	CURA	THIN& BURN
9/14/2004	Camas 11	C9	2	C9-2	PIPO	NS ROOT CORE	THIN& BURN
9/14/2004	Camas 11	H5	6	H5-6	PIPO	CURA	THIN& BURN

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9/14/2004	Camas 11	D8	3	D8-3	PIPO	CURA	THIN& BURN
9/14/2004	Camas 11	E7	3	E7-3	PSME	NS ROOT CORE	THIN& BURN
9/14/2004	Camas 11	G6	4	G6-4	PIPO	SYM ROOT CORE	THIN& BURN
9/14/2004	Camas 11	D9	3	D9-3	PIPO	CURA	THIN& BURN
9/14/2004	Camas 11	E8	3	E8-3	PIPO	CURA	THIN& BURN
9/14/2004	Camas 11	E8	10	E8-10	PIPO	CURA	THIN& BURN
9/14/2004	Camas 11	F7	2	F7-2	PIPO	CURA	THIN& BURN
9/14/2004	Camas 11	G6	5	G6-5	PIPO	CURA	THIN& BURN
9/14/2004	Camas 11	F8	5	F8-5	PIPO	NS ROOT CORE	THIN& BURN
9/14/2004	Camas 11	E9	5	E9-5	PSME	NS ROOT CORE	THIN& BURN
9/14/2004	Camas 11	E8	11	E8-11	PIPO	CURA	THIN& BURN
9/14/2004	Camas 11	G6	1	G6-1	PIPO	CURA	THIN& BURN
9/14/2004	Camas 11	E9	13	E9-13	PIPO	CURA	THIN& BURN
9/9/2004	Camas 11	K5	3	K5-3	PIPO	CURA	THIN& BURN
9/9/2004	Camas 11	K3	2	K3-2	PIPO	CURA	THIN& BURN
9/9/2004	Camas 11	L4	3	L4-3	PIPO	NS ROOT CORE	THIN& BURN
9/9/2004	Camas 11	M3	1	M3-1	PIPO	NS ROOT CORE	THIN& BURN
9/9/2004	Camas 11	K3	14	K3-14	PIPO	NS ROOT CORE	THIN& BURN
9/9/2004	Camas 11	M5	2	M5-2	PIPO	CURA	THIN& BURN
9/9/2004	Camas 11	M5	1	M5-1	PSME	NS ROOT CORE	THIN& BURN
9/9/2004	Camas 11	L2	1	L2-1	PIPO	NS ROOT CORE	THIN& BURN
9/9/2004	Camas 11	K3	17	K3-17	PIPO	CURA	THIN& BURN
9/9/2004	Camas 11	K5	2	K5-2	PIPO	NS ROOT CORE	THIN& BURN
9/9/2004	Camas 11	L4	1	L4-1	PSME	SYM ROOT CORE	THIN& BURN
9/9/2004	Camas 11	L4	6	L4-6	PSME	SYM ROOT CORE	THIN& BURN
9/9/2004	Camas 11	L4	4	L4-4	PIPO	CURA	THIN& BURN
9/7/2004	Camas 11	F9	9	F9-9	PIPO	CURA	THIN& BURN
9/7/2004	Camas 11	J7	1	J7-1	PSME	NS ROOT CORE	THIN& BURN
9/7/2004	Camas 11	I8	2	I8-2	PIPO	CURA	THIN& BURN
9/7/2004	Camas 11	G9	8	G9-8	PIPO	NS ROOT CORE	THIN& BURN
9/7/2004	Camas 11	H8	4	H8-4	PIPO	NS ROOT CORE	THIN& BURN
9/7/2004	Camas 11	G9	7	G9-7	PIPO	CURA	THIN& BURN
9/7/2004	Camas 11	F9	5	F9-5	PIPO	CURA	THIN& BURN
9/7/2004	Camas 11	I8	5	I8-5	PIPO	CURA	THIN& BURN
9/7/2004	Camas 11	G9	6	G9-6	PIPO	CURK	THIN& BURN
9/7/2004	Camas 11	I8	1	I8-1	PSME	SYM ROOT CORE	THIN& BURN
9/8/2004	Camas 11	L6	1	L6-1	PIPO	CURA	THIN& BURN
9/8/2004	Camas 11	I6	4	I6-4	PIPO	CURA	THIN& BURN
9/8/2004	Camas 11	O4	3	O4-3	PIPO	CURA	THIN& BURN
9/8/2004	Camas 11	N4	2	N4-2	PSME	CURA	THIN& BURN
9/8/2004	Camas 11	L6	4	L6-4	PIPO	CURA	THIN& BURN
9/8/2004	Camas 11	J6	4	J6-4	PIPO	CURA	THIN& BURN
9/8/2004	Camas 11	N4	1	N4-1	PIPO	CURA	THIN& BURN

DATE	SITE	UTM CELL	TREE #	CORE ID	HOST	HOST STATUS	TREATMENT
9/8/2004	Camas 11	L6	2	L6-2	PIPO	CURA	THIN& BURN
9/8/2004	Camas 11	J6	3	J6-3	PIPO	CURA	THIN& BURN
9/8/2004	Camas 11	I6	7	I6-7	PIPO	CURA	THIN& BURN
9/8/2004	Camas 11	I6	5	I6-5	PSME	SYM ROOT CORE	THIN& BURN
9/8/2004	Camas 11	L6	7	L6-7	PIPO	CURA	THIN& BURN
9/8/2004	Camas 11	J6	8	J6-8	PIPO	NS ROOT CORE	THIN& BURN
9/8/2004	Camas 11	J6	1	J6-1	PIPO	CURA	THIN& BURN
9/8/2004	Camas 11	I7	10	I7-10	PSME	NS ROOT CORE	THIN& BURN
9/8/2004	Camas 11	N4	3	N4-3	PIPO	NS ROOT CORE	THIN& BURN
9/8/2004	Camas 11	L6	3	L6-3	PIPO	NS ROOT CORE	THIN& BURN
9/8/2004	Camas 11	M7	2	M7-2	PSME	NS ROOT CORE	THIN& BURN
9/8/2004	Camas 11	O5	1	O5-1	PSME	NS ROOT CORE	THIN& BURN
9/8/2004	Camas 11	L7	2	L7-2	PIPO	CURA	THIN& BURN
9/2/2004	Camas 11	F10	18	F10-18	PSME	SNAG	THIN& BURN
9/7/2004	Camas 11	G9	6	G9-6	PIPO	CURK	THIN& BURN
9/2/2004	Camas 11	F10	12	F10-12	PIPO	CURA	THIN& BURN
9/7/2004	Camas 11	F9	8	F9-8	PIPO	SNAG	THIN& BURN
9/2/2004	Camas 11	F10	16	F10-16	PSME	SNAG	THIN& BURN

Appendix D.

Table 15.
Summary of treatment units and plant associations

Treatment Unit	Treatment	Plant Association[1]
Sand 19	Control	PSME
Pendleton 30	Control	PIPO
Crow 3	Control	PIPO & PSME
Sand 2	Control	PSME
Crow 1	Thin only	PIPO & PSME
Crow 6	Thin only	PIPO & PSME
Slawson 8	Thin only	PSME & PIPO
Ruby	Thin only	PSME & PIPO
Poison 6	Burn only	PSME & PIPO
Spromberg 4	Burn only	PSME & PIPO
Camas 11	Thin and Burn	PIPO & PSME
Tripp 9	Thin and Burn	PSME & PIPO

1PIPO typically included PIPO/PUTR, PIPO/CARU, and PIPO/AGSP plant associations; PSME includes PSME/SPBEL, PSME/PUTR, PSME/CARU, PSME/SYAL, and PSME/AGSP plant associations (HOFF et al. 2004). AGSP, *Agropyron spicatum*; CARU, *Calamagrostis rubescens*; PIPO, *Pinus ponderosa*; PSME, *Psuedotsuga menziesii*; PUTR, *Purshia tridentata*; SPBEL, *Spiraea betulifolia*; SYAL, *Symphoicarpos albus*.