Ecology of fungal endophytes in Douglas-fir and ponderosa pine roots in eastern Washington

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This is to certify that I have examined this copy of a master's thesis by

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and have found that it is complete and satisfactory in all respects,
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CHAPTER 1

INTRODUCTION

The term 'endophyte' was originally coined by DeBary (1866) to describe microorganisms, including fungi and bacteria, in living plant tissues. Carroll (1988) restricted the term to fungi inhabiting, but not inciting disease within leaves and stems of healthy hosts. I use the description given by Redlin and Carris (1996), "Endophytic fungi are normally located in healthy plants, in leaves, needles, twigs, stems, and roots and have been shown to provide beneficial, mutualistic, pathogenic or neutral influence to their hosts." The contradictory terms used to explain the ways endophytes influence their hosts illustrate the lack of understanding scientists have concerning the ecology and function of endophytes.

Researchers have been investigating fungal endophytes for over twenty years (Clay and Schardl 2002). They have been found in every plant species and tissue examined, including leaves and twigs of woody and herbaceous tropical plant species (Bayman *et al*. 1997, Gamboa *et al*. 2002, Arnold *et al*. 2003), needles of woody plant species (Carroll 1988), and fine and coarse roots of herbaceous plants (Hadacek *et al*. 2002, Midgley *et al*. 2002, Vandenkoornhuyse *et al*. 2002) and woody plants (Grunig *et al*. 2002, Hoff 2002).

Studies of endophytic fungi have resulted in the isolation of extraordinary numbers of morphospecies, a term commonly used in endophytic fungi literature to identify

morphologically distinct individuals that are difficult to assign to known species because teleomorphic structures are commonly lacking. They include Ascomycetes, Basidiomycetes, and Deuteromycetes, as well as Oomycetes (water molds) (Sinclair and Cerkauskas 1996, Arnold *et al*. 2000). Fungal endophytes have been found in every plant examined for their presence (Gamboa *et al*. 2002), but few studies have closely examined the spatial distribution of different fungal endophytic taxa. Midgley *et al*. (2002) examined the fungal endophyte assemblage in *Woollsia pungens* (Cav.) F. Muell, an Australian heath shrub, and found that one fungal endophyte genotype occupied a wide distribution within the root system, suggesting limited functional diversity. The dominance of one endophytic species of fungus in one or multiple hosts within the same geographic area may have considerable influence on host fitness. However, few studies have examined the spatial distribution of specific fungal endophytes and their influence on the plant host remains unclear.

Many examples of fungi entering plant roots are illustrated in the mycorrhizal literature, but mycorrhizal fungi differ from endophytic fungi in that they are located in and/or on root tips (Saikkonen *et al*. 1998). Endophytic fungi have been isolated from the woody roots, not the root tips, of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and ponderosa pine (*Pinus ponderosa* Dougl. ex Loud) (Hoff *et al*. 2004). However, the mechanisms and entry modes used by these endophytes are unknown. Hessburg and Hansen (2000) suggest through the investigation of the pathogenic fungus causing black stain root disease, *Leptographium wageneri* var. *pseudotsugae* Harrington and Cobb, that wounds and natural openings may be important entrance courts for fungi, especially those

that lack pectolytic or cellulolytic enzymes. Carroll (1988) points out that taxonomically, endophytes are frequently closely related to pathogenic fungi, which may aid in elucidating of the function and ecological roles that endophytic fungi play as these organisms are studied further.

Endophytic fungi have been widely studied in grasses where they produce secondary compounds that may reduce plant herbivory. For example, Gange *et al*. (2002) showed an inverse correlation between endophytic species presence and gall insect presence in Canada thistle (*Cirsium arvense* (L.) Scop.). Relationships between endophytic presence in plant tissues and reduced herbivory on plants suggests a related hypothesis: Endophytes may cross-protect against fungal pathogens (Redman *et al*. 2001). Taechowisan *et al*. (2003) illustrated that fungal endophytes isolated from herbaceous plants in the Zingiberaceae inhibited the *in vitro* growth of two fungal pathogenic species. Arnold *et al*. (2003) found that when tropical cacao leaves were colonized with endophytic fungi and a dominant pathogen, leaf death and necrotic lesions were significantly less and were smaller than on leaves not colonized with endophytic fungi. It is unknown, however, if the endophytes found in the woody roots of Douglas-fir and ponderosa pine inhibit the growth of fungal pathogens normally associated with these two tree species.

Although we have some information on endophytes we still do not know exactly why these organisms are present in host plant tissues. To date, hypotheses concerning the functional and ecological roles of endophytic fungi have largely dealt with interactions

with their host plants. Most of the current hypotheses about the functional and ecological roles of endophytic fungi are very specific and it is unknown whether observed relationships and interactions can be generally applied to a broader range of herbaceous or woody plants. The functional and ecological roles of endophytic fungi warrant further investigation because of the lack of clear established relationships. Endophytic fungi may be protecting plants against pathogens and other biotic or abiotic agents that threaten host fitness.

We also know little about the basic biology of endophytic fungi, especially forest endophytes. Like most fungi their growth is likely influenced by light, pH, moisture, nutrients, competition, and temperature. Temperature changes in forest environments may occur as a result of fire, reduced canopy cover via harvesting, changes in understory plant cover, and climate change. These changes undoubtedly influence both hosts and endophytes. The temperature requirements (minima, maxima, optima) for growth of many species of endophytic fungi are unknown, especially for fungal endophytes in woody roots that are the subject of this study. My study was initiated to examine the biology and ecology of two common endophytic fungal genera (*Byssochlamys* and *Umbelopsis*), previously found in the woody roots of Douglas-fir and ponderosa pine in the dry, fire prone, forests of eastern Washington.

The objectives were to determine, with respect to Douglas-fir and ponderosa pine:
(1) the frequency of occurrence of the fungal endophytes *Byssochlamys nivea* Westling and *Umbelopsis* spp. in woody roots,

(2) the relationship between host size (tree diameter at breast height, DBH) and occurrence of these two endophytes,

(3) the distribution of the fungal endophytes along the roots and in the rhizosphere around woody roots,

(4) whether the fungal endophytes in woody roots are horizontally or vertically transmitted,

(5) the *in vitro* minimum, maximum, and optimum growth temperatures of the fungal endophytes and,

(6) whether *Byssochlamys nivea* is antagonistic *in vitro* against the pathogenic species *Armillaria ostoyae* Romagn., a common root pathogen.

The research was guided by the following null hypotheses:

Ho1 – *Byssochlamys* and *Umbelopsis* will have the same frequency of occurrence in woody roots and there will be no tree species difference.

Ho2 – There is no relationship between endophyte frequency in roots and host size (tree diameter at breast height, DBH).

Ho3 – The endophytes are evenly distributed along the roots.

Ho4 – The endophytes are not found in the rhizosphere around woody roots.

Ho5 – The endophytes will be transmitted horizontally not vertically.

Ho6 – There is no difference in the *in vitro* growth rates at different temperatures.

Ho7 – *Byssochlamys nivea* will not be antagonistic towards *Armillaria ostoyae* in culture.

CHAPTER 2

LITERATURE REVIEW

Douglas-fir and ponderosa pine are common early seral tree species in the interior dry forests of Washington. There is a great deal known about the ecology of these species, including their fungal pathogens, however, there is little known about the non-pathogenic fungi that grow in their living tissues. These fungi are known as endophytes. Endophytes in needles of Douglas-fir have been studied since the 1980s (Carroll 1988), but endophytes in woody roots have only been recently observed (Hoff 2002, Hoff *et al*. 2004). In this chapter, I review the ecological characteristics of Douglas-fir and ponderosa pine, fungal endophytes of herbaceous and woody plants, and what is known about endophytes in Pacific Northwest forests.

Characteristics of Douglas-fir

Douglas-fir can be found growing on a variety of aspects, slopes, land forms, soil textures and parent materials (Steele 1981, DeVelice 1986, Atzet 1990, Cooper *et al*. 1991). In the dry forests of the eastern Cascades, Douglas-fir can be found at elevations ranging from 460-1,800 m (1,500-6,000 ft). The inland variant (*Pseudotsuga menziesii* (Mirb) Franco var. *glauca* (Beissn.) Franco) is typically shade-tolerant at low and middle elevation ranges (Hermann 1990, Cooper 1991, Uchytil 1991) and is much more cold tolerant than the coastal variant (*Pseudotsuga menziesii* (Mirb) Franco var. *menziesii*) (Krajina *et al.* 1982, Burns and Honkala 1990). In dry forests, Douglas-fir tends to grow in relatively pure stands or in mixture with ponderosa pine (Steinberg 2002).

Douglas-fir occupies forests with varied fire regimes (Uchytil 1991). Fire resistance and tree survival generally increase with tree size (Harrington 1991, Agee 1996). Mature Douglas-fir, because of its thick bark, is more fire resistant than true firs, spruces, western red cedar, western hemlock, lodgepole pine, and western white pine, but are less fire resistant than ponderosa pine (Wellner 1970, Harrington 1991). Sapling and pole Douglas-fir are less fire resistant during these stages of growth (Weaver 1968, Ryker and Losensky 1983, Kalabokidis and Wakimoto 1992).

Estimates of historical fire return intervals in the eastern Cascades Douglas-fir and ponderosa pine forests range from 7 to 18 years (Agee 1996). The relatively high fire frequencies historically maintained ponderosa pine forests, instead of Douglas-fir, because Douglas-fir was unable to reach a fire resistant size before the next fire (Arno and Gruell 1983). Ponderosa pine stands now support small to moderate sized Douglas-fir because of fire suppression and extensive selective cutting (Agee and Maruoka 1994).

Characteristics of Ponderosa Pine

Ponderosa pine can be found growing on a variety of soil textures and parent materials, but does well on deep, wet, sandy gravel and clay loams with a pH between 6.0 and 7.0 (Atzet and Wheeler 1984, Van Hooser and Keegan 1988). The species typically occupies the grassland to forest transition zone (Habeck 1992).The climate best suited for ponderosa pine growth and reproduction is characterized by a relatively long growing season and minimal summer precipitation (Franklin and Dyrness 1973). Ponderosa pine sites in Oregon and Washington exhibit an average annual precipitation of 35-75 cm (14-

30 in); over half is in the form of snow (Franklin and Dyrness 1973). Ponderosa pine is shade intolerant and is typically found on dry, warm sites (Franklin and Dyrness 1973, Atzet and Wheeler 1984, Hooser and Keegan 1988).

Ponderosa pine has an open crown structure and thick bark which promotes fire resistance (Flint 1925, Saveland 1982, Fischer and Clayton 1983). Seedling establishment is greatest on sites recently burned with exposed mineral layers (Lotan *et al*. 1981). Ponderosa pine stands, with well-stocked overstories and few understory trees, were historically maintained by pre-settlement high frequency and low severity surface fires (Lampi 1960, Henderson 1967, Arno 1988, Habeck 1990). Fire suppression, however, has resulted in ponderosa pine stands being replaced by shade-tolerant and moisture demanding climax stands (Lampi 1960, Henderson 1967, Arno 1988, Habeck 1990).

Fungal Endophytes

Endophytic fungi are found in leaves, needles, twigs, stems and roots of healthy plants (Redlin and Carris 1996) and they apparently provide beneficial, mutualistic, neutral and sometimes pathogenic influences on their hosts. Generally though, they are not thought to be pathogens, but saprophytes, using non-living organic matter as a food source.

Herbaceous Plant Hosts

Fungal endophytes located within herbaceous plant tissues are taxonomically diverse and some have been shown to form symbiotic relationships with their hosts (Clay and Schardl 2002). Most studies investigating the taxonomy and ecological significance of endophytic fungi in herbaceous plant tissues have focused on grasses and grass-like plant families. In the mid-20th century 'Bermuda grass tremors,' a nervousness-like disorder affecting cattle grazing on common and coastal Bermuda grass, became a problem in the southern United States. The cause was related to alkaloids produced by the fungus *Claviceps* spp. (Porter *et al*. 1974, Clay and Schardl 2002). This represents one of the first fungal endophyte studies. Since 1974, studies of endophytic fungi inhabiting herbaceous plants have included investigations into leaf and root inhabiting fungal endophytes of grasses and forbs. Many are agriculturally important and tropical species, for example *Festuca* L., *Lolium perenne* L., *Lepanthes rupestris* Stimson, and *Peucedanum* L. (Clay and Holah 1999, Malinowski *et al*. 1999, Rillig *et al*. 1999, Bayman *et al*. 2002, Bordallo *et al*. 2002, Gange *et al*. 2002, Hadacek and Kraus 2002, Cheplick and Cho 2003, Narisawa *et al*. 2004).

The greatest amount of research on the functional and ecological roles of fungal endophytes has been conducted with grass and grass-like species and the fungal genera *Neotyphodium* and *Acremonium*. These studies suggest that fungal endophytic presence reduces herbivory, influences host population dynamics and plant diversity, and contributes to plant drought tolerance and nutrient utilization (Clement *et al*. 1996, Clay

and Holah 1999, Malinowski *et al*. 1999, Hadacek and Kraus 2002, Morse *et al*. 2002, Cheplick and Cho 2003).

Woody Plant Hosts

Endophytic fungi in woody plants have not been studied as widely as those in herbaceous plants. Both above and below-ground plant tissues have been examined for the presence of endophytic fungi in a limited number of woody hosts. Investigations into the ecological relationships among endophytic fungi and their woody hosts, as well as the influence of different environments on these relationships have not been extensively examined.

Leaf and Needle Fungal Endophytes

Fungal endophytes contribute substantially to fungal diversity based on the extensive numbers of morphospecies isolated. Arnold *et al*. (2001) sampled tropical woody plant genera representing nine plant families and found endophytes in every leaf sampled. They found a total of 418 morphospecies of endophytic fungi in their survey, but only 140 were found in more than one leaf. Arnold *et al*. (2003) studied another tropical plant species, the woody cacao (*Theobroma cacao* L.), and identified 344 morphospecies from 126 leaves of different ages. Gamboa *et al*. (2002) found an average of 13 different morphospecies in Arabian coffee (*Coffea arabica* L.) leaves. Fungal endophytes have also been isolated from temperate species, including the needles of coastal redwood (*Sequoia sempervirens* (Lamb. ex D. Don) Endl.), Douglas-fir, Norway spruce (*Picea abies* (L.) Karst), and Scots pine (*Pinus sylvestris* L.), as well as other evergreen and

deciduous woody plant species (Bernstein and Carroll 1977, Espinosa-Garcia *et al*. 1996, Bayman *et al*. 1998, Deckert and Peterson 2000, Arnold *et al*. 2000, Muller *et al*. 2001, Ahlholm *et al*. 2002).

Root Fungal Endophytes

The below-ground plant tissues of woody plants have not been surveyed for the presence of endophytic fungal colonization as extensively as above-ground plant tissues, but generally fewer morphospecies are found in roots than have been identified in leaves and needles. Grunig *et al*. (2002) identified 21 morphospecies from 160 Norway spruce roots. Fungal endophytes also have been isolated from Scots pine, Himalayan fir (*Abies spectabilis* (D. Don) Spach.), and other evergreen and deciduous roots (Ahlich and Sieber 1996, Sen *et al*. 1999). Ahlich and Sieber (1996) isolated putative fungal endophytes from the fine roots of different woody plant species, but it remains unclear whether the endophytic species isolated were actually types of mycorrhizal fungi.

Ecology and Function

There have been few studies investigating the ecology and roles of fungal endophytes in woody plants. Hypothesized ecological roles of fungal endophytes in woody plants have ranged from roles in the decomposition of needles (Muller *et al*. 2001), reductions of herbivory (Ahlholm *et al*. 2002), and antagonistic relationships with pathogens (Arnold *et al*. 2003).

Muller *et al*. (2001) were unable to prove that fungal endophytes have direct roles in the decomposition of tree needles, and suggested that the endophytes are most likely outcompeted during the decomposition process. Ahlholm *et al*. (2002) suggested that fungal endophyte infections in plants may negatively affect herbivory, but could not establish causality. Arnold *et al*. (2003) found that when the tropical cacao leaves were colonized with endophytic fungi and a dominant pathogen, leaf death was significantly reduced and necrotic lesions were significantly smaller than when the fungal endophytes were absent.

Pacific Northwest Fungal Endophytes

There are relatively few studies of fungal endophytes in the Pacific Northwest. As with the previous endophyte studies discussed, much of the work in the Pacific Northwest has remained in the realm of survey and identification of species. Few studies have delved into the function, evolutionary relationships, and effects of disturbance on these organisms. However, studies looking further into the endophyte-host relationship have reiterated that observation scale can be important.

Of Pacific Northwest tree species, Douglas-fir and its associated needle and root endophytes have been most widely studied. George Carroll was the pioneer in the fungal endophyte field in the Pacific Northwest with studies on Douglas-fir needle endophytes, insect/endophyte interactions, and spatial patterns of fungal endophytes (Carroll 1988, 1992, 1995, 1997, Wilson and Carroll 1997). Jeff Stone also has done a great deal of work on fungal endophytes in the Pacific Northwest, focusing on anatomical and

physiological aspects of endophytic fungi (Stone 1986, 1987, 1988, Stone *et al*. 1994, 1996).

Relatively recent studies have expanded beyond Douglas-fir needles to needles of western white pine (*Pinus monticola* Dougl. ex D. Don) (Ganley *et al*. 2004) and woody roots of Douglas-fir and ponderosa pine (Hoff 2002, Hoff *et al*. 2004). The fungal endophytes found in western white pine needles were cultured and shown to be non-pathogenic (Ganley *et al*. 2004).

Hoff *et al*. (2004) identified 27 fungal genera living in the woody roots of Douglas-fir and ponderosa pine. This particular habitat of these host species was one previously unexplored for the presence of endophytes. Two dominant fungi were identified; *Umbelopsis* spp. and *Byssochlamys nivea*. They were isolated 20.4 and 10.4 percent of the time, respectively (Hoff *et al*. 2004).

The genus *Byssochlamys* (Ascomycetes/Eumycota) is the teleomorphic stage of the anamorphic genus *Paecilomyces. Byssochlamys nivea* and *Byssochlamys fulva* Olliver et Smith ascospores have been shown to persist for thirty minutes in temperatures of 86-88° C and some can survive at 90°C (Brown and Smith 1957). Literature from the late 19th century to the mid 20th century identified the genus *Spicaria*, now known to be the form genus *Paecilomyces*, as occurring in or on paper and leather products, in various food products, cotton textiles, insects, nematodes and other fungi, and soils (Ellis and Everhart 1883, Saccardo 1886, Massee 1898, Sopp 1912, See 1919, Marchal and Marchal 1921,

Moesz 1921, Saito 1921, Bakhtin 1928, Kennelly and Grimes 1930, Macy *et al*. 1931, Thom and Raper 1932, Bisby *et al*. 1933, Naumoff and Kiryalova 1935, Petch 1936, von Szilvinyi 1936, Sakaguchi *et al*. 1939, Hausam and Liebscher 1940, Mascera 1940, Charles 1941, Drechsler 1941, Conant *et al*. 1945, Macmillan and Basu 1947, Basu 1948, Raper and Thom 1949).

Discovery of the production of toxins and/or secondary metabolites by endophytic fungi has resulted in a small amount of research investigating the possibility that endophytes may reduce host attack or infection by pathogenic fungi. Several studies have isolated metabolites and putative toxins from fungal endophytes and some have been shown to inhibit host attack by pathogenic fungi (Schulz *et al*. 2002, Wang *et al*. 2002, Taechowisan *et al*. 2003, Thines *et al*. 2004).

Umbelopsis (Zygomycete/Eumycota) is a genus of fungi that has not been widely studied. Species in the genus have been found in soils (Kwasna 2004), perennial and woody plant roots (Fisher *et al*. 1991, Holdenrieder and Sieber 1992, Vandegrift 2002, Halmschlager and Kowalski 2004), and decaying plant matter (Sugiyama *et al*. 2003).

CHAPTER 3

MATERIALS AND METHODS

Study Area

The Mission Creek study area, one of thirteen Fire and Fire Surrogate (FFS) study areas in a national study, is located in the Wenatchee River Valley Ranger District of the Wenatchee National Forest, approximately 15 km southwest of Wenatchee, Washington (Figure 1) (Agee *et al*. 2001). The study sites were within the Mission Creek watershed, a tributary of the Wenatchee River, which is located on the east side of the Cascade Mountain range, in central Washington state, USA (47°25'N, 120°50'W) (Dolan 2002). My study included three control experimental sites (Crow 3, Sand 19, and Pendleton 30) of the twelve total experimental sites in the study area. Experimental sites ranged in size from 10 to 20 ha (Figure 2); site characteristics are shown in Table 1 and Appendix A. The two southeastern sites, Crow 3 and Pendleton 30, were dominated by ponderosa pine and generally have southerly aspects with relatively gentle slopes (average 35 percent). The other site, Sand 19, was dominated by Douglas-fir, had a southwesterly aspect and relatively steeper slopes (average 61 percent) than Crow 3 and Pendleton 30 (Dolan 2002). Elevations ranged from 735 to 960 m.

The Wenatchee National Forest climate is strongly influenced by the Pacific Ocean and geography provided by the Cascade Mountains and prevailing westerly winds, with most of the precipitation occurring in late fall and winter (USDA 1990). Annual precipitation ranges from 25-100 cm, with the majority occurring between October and April as snow

(USDA 1990, Lillybridge *et al*. 1995). Leavenworth, Washington, approximately 23 km northwest of the study area, receives on average 65 cm of annual precipitation, with only 4.3 cm between June and August (WRCC 2004). Hot, dry summers are typical of the eastern Cascades, with average maximum temperatures of 29.5º C occurring between June and August (WRCC 2004). Average annual temperature is 9º C (WRCC 2004).

The vegetation of the area is typical of eastern Cascade dry forests, dominated by Douglas-fir and ponderosa pine. While the plant communities at each study site show definite variability, most fall within the dry Douglas-fir plant association group, with various shrubs, grasses, and herbaceous species (Lillybridge *et al*. 1995). The dry forests of the Wenatchee National Forest displayed a natural fire return interval of ten to twenty years (Everett *et al*. 2000) and this strongly affects the vegetation communities. All study units are in the Douglas-fir/ pinegrass (*Calamagrostis rubescens* Buckl.) vegetation community, but elk sedge (*Carex geyeri* Boott) and common snowberry (*Symphoricarpos albus* (L.) Blake) also comprise the community at Sand 19 (Lolley 2005). Fire suppression and selective logging has influenced the current vegetation on the sites (Agee *et al*. 2001).

Soils are sandy and well-drained, developing on non-glaciated Tyee sandstone with limited amounts of shale and conglomerate arising from the Swauk and Chumstick formations (Tabor *et al*. 1982, Soil Survey Staff 1995). Ash deposits from fire and volcanic eruptions also are present, but are scattered and difficult to trace. Soil types include Haploxerepts, Haploxerolls, Argizerolls, and Haploxeralfs throughout the study

area (Dolan 2002), with Ultic Haploxeralf, Lithic Haploxeroll, and Lithic Argixeroll subgroups found on Pendleton, Crow 3, and Sand 19, respectively.

Field Sampling

Sample Area Establishment and Tree Sampling

All field samples were collected in July, August and September 2004. The initial sampling plot was chosen based on Hoff *et al*. (2004). The trees that were positively identified as having *Byssochlamys* or *Umbelopsis* spp. present were mapped (US Forest Service, Wenatchee, WA). One tree from each site was used to establish the plot for this investigation. Forty, 39 and 39 trees were randomly selected in the Crow 3, Pendleton 30, and Sand 19 sites, respectively, for root core and rhizosphere samples within the specific 75 m² study plot at each site. Douglas-fir and ponderosa pine were equally sampled over the three sites. Diameter at breast height (DBH, 1.37 m above the forest floor on the uphill side of the tree, in cm) and species were recorded for each tree except for the saplings, which were denoted as Douglas-fir saplings (Table 2). Tree locations, except those which were sampled for cones, were mapped using a Trimble ProXRS GPS unit.

Root Core and Rhizosphere Sampling

Root cores were collected by excavating a major lateral tree root from the bole out to 35 cm. An increment borer, typically used for tree stem coring, was used to take two increment cores (ca. 5 mm diameter) from roots. Cores were taken at 10 cm and 30 cm from the bole. Each root increment core was placed into a plastic soda straw and sealed at each end with masking tape. When the saplings were sampled, the entire sapling was

collected and no root increment cores were taken. A total of 220 root cores and eight saplings were collected. All root cores and saplings were stored on ice in a plastic cooler and transported to the Forest Pathology and Soil Microbiology Lab, at the University of Washington, College of Forest Resources, Seattle, Washington. They were stored in a refrigerator at 3º C until processed.

Soil samples were collected at every location where a woody root increment core was obtained. Approximately 10 g of soil were collected from the rhizosphere within 3 cm of the root increment core extraction location. The soil samples were placed in plastic baggies, sealed, and labeled the same as the collected increment cores. A total of 232 soils samples were collected. All soil samples were stored on ice in a plastic cooler and transported to the Forest Pathology and Soil Microbiology Lab, at the University of Washington, College of Forest Resources, Seattle, Washington. They were stored in a refrigerator at 3º C until processed.

Seed Sampling

Eighteen recently fallen Douglas-fir cones were collected from six different parent trees (three cones from each tree). Two parent trees were sampled from, or on the trails leading to, the three Crow 3, Sand 19, and Pendleton 30 sites. Ponderosa pine seeds were not collected because of the infrequency of recently fallen cones during field sampling. Each set of cones from differing host trees were sealed in separate plastic baggies. All cone samples were stored on ice in a plastic cooler and transported to the Forest Pathology and

Soil Microbiology Lab, at the University of Washington, College of Forest Resources, Seattle, Washington. They were stored in a refrigerator at 3º C until processed.

Lab Analyses

Fungi in Root Cores

Woody root cores were initially split into smaller pieces (ca. 2.0 mm in length) and surface sterilized in a Clorox bleach solution (2 mL bleach:1 mL sterilized water) followed by two sterilized water rinses at 60 seconds each. This reduced the growth of surface contaminating organisms present on root cores. After surface sterilization, all root core pieces were placed into 60 x 15 mm petri plates containing a selective medium (2% Malt Extract Agar [MEA] and 100 mg streptomycin sulfate per L). The plates were incubated in the dark at 25° C for one month. After incubation of the root core pieces, fungi were examined for morphological features using brightfield and stereo microscopy. Fungal keys (Brown and Smith 1957, Amos and Barnett 1966, Kendrick *et al*. 1994) were used to determine the presence of the genera *Umbelopsis* and *Byssochlamys*. *Byssochlamys* was also identified to species based on mycelial colony color and conidial structure and size. Stolk and Samson (1971) described the mycelial colony color of *Byssochlamys nivea* as ranging from olive-buff to deep olive-buff. *B. nivea* has conidiophores 15-300 µm long and 2-3 µm in diameter. Phialides have a swollen base and taper into a thin tube at the end and are often variable in length (18-45 x 2.7-5.0 µm). Conidia are borne in chains, 3.8-6 x 2.5-5 µm in size, typically smooth, broadly ellipsoidal, and often have one end that is slightly flattened (Brown and Smith 1957). *Byssochlmays fulva* Olliver and Smith has phialides borne in groups of two to three and

conidia 4-8.7 x 1.5-5 µm (Stolk and Samson 1971). *Byssochlamys verrucosa* Samson and Tansey and *Byssochlamys striata* (Raper and Fennell) Arx have ornamented ascospores, and *Byssochlamys zollerniae* Ram has black, echinulate chlamydospores (Ram 1968, Stolk and Samson 1971, Samson and Tansey 1975).

Presence or absence of *B. nivea* and *Umbelopsis* spp. was recorded for each root core. The results were then reported as percent frequency of occurrence of each fungal endophyte.

Fungi in Rhizosphere Soil

Byssochlamys nivea presence in the rhizosphere soil was determined by placing 2-3 g of non-dried soil into a sterilized 50 mL screw top glass tube. Twenty mL of sterilized de-ionized water were added to each tube, shaken for 1 min, then heated in a 75º C water bath for 30 min (Beuchat and Pitt 2002). After heating, each sample was shaken for 1 min, then equally distributed into three 100 x 15 mm petri plates, and swirled with 2% MEA. All samples were then incubated at 25º C in the dark for one month. Plates were then examined under a stereo microscope for the presence of *B. nivea*. Identifications were based on morphological characters, as described above.

Umbelopsis spp. presence in the rhizosphere soil was determined using a soil dilution plate method. One g of non-dried soil was placed in a 20 mL sterilized test tube and 9 mL of de-ionized, sterilized water were added to the test tube. The soil and water solution was then thoroughly shaken. One mL of the solution was then added to a second test

tube containing 9 mL of sterilized water. This was done five times, for a final soil dilution of 1g:100,000 mL, soil to water. One mL of the final dilution was equally divided and plated onto two 100 x 15 mm petri plates with 2% MEA then swirled. Ten samples from each study site were randomly selected, for a total of 30 samples across the three study sites. They were processed using the methods described above. The 60 total plates were then turned upside down, and incubated at 25º C, in the dark, for one month. Plates were then examined under a stereo microscope for the presence of *Umbelopsis* spp. Identifications were based on morphological characters.

Presence or absence of *B. nivea* and *Umbelopsis* spp. was initially recorded for each individual soil plate. Results were then combined for each soil sample at the 10 cm and 30 cm sampling distance from each tree and reported as the frequency of fungal endophyte occurrence.

Fungi in Douglas-fir Seeds

Byssochlamys and *Umbelopsis* presence in Douglas-fir seeds was determined using cultural methods. Three seeds were removed from the 18 cones collected and surface sterilized. The same methods used to surface sterilize the root cores were used to surface sterilize the 54 Douglas-fir seeds. The seeds were then placed into 60 x 15 mm petri plates with 2% MEA and incubated in the dark at 25º C for one month. The fungi growing on the plates were then examined for *Byssochlamys* and *Umbelopsis* using brightfield and stereo microscopy. Presence or absence of *B. nivea* and *Umbelopsis* spp. was recorded for each seed, then reported as percent frequency of occurrence.

Influence of Temperature on Growth of Byssochlamys and Umbelopsis

The minimum, maximum, and optimal temperatures for growth of the *Byssochlamys* and *Umbelopsis* spp. isolated by Hoff *et al*. (2004) were determined. Isolates for this portion of the study were also obtained from the USDA Forest Service, Forestry Sciences Laboratory in Moscow, ID. One *Byssochlamys* isolate and three *Umbelopsis* isolates (one *Umbelopsis versiformis* Amos & HL Barnett and two *Umbelopsis vinacea* (Dixon-Stew.) Arx) were plated. All isolates were incubated in the dark at 25° C and grown for one to several weeks.

After the establishment of each isolate, ten 2 x 2 mm subcultures of each isolate were placed on ten 60 x 15 mm petri plates with 2% MEA, but without streptomycin sulfate. Each of the ten subcultures was grown in the dark at 3° C. The radial growth, in mm, of each subcultured isolate was recorded after ten days. Each isolate was prepared, grown, and measured in the same way at 10, 15, 20, 25, 30, and 35º C. Growth rates were expressed as mm/day.

Influence of Byssochlamys nivea on Armillaria ostoyae Growth in Culture

Fungal antagonism was investigated using one *Byssochlamys nivea* isolate and three *Armillaria ostoyae* isolates. The *Byssochlamys* isolate was collected from the study area during a previous fungal endophyte survey (Hoff *et al*. 2004) and the *A. ostoyae* isolates were previously collected from eastern Washington (See Appendix B for isolate information). No *Armillaria* was observed in the actual study area. All isolates were

obtained from the USDA Forest Service, Forestry Sciences Laboratory in Moscow, ID in April, 2004.

Fungal isolates were initially grown in 60 x 15 mm petri plates on 2% MEA, without streptomycin sulfate, for one to several weeks in an incubator at 25° C. After each isolate became established, five replicates of each of the three different *Byssochlamys-Armillaria* growth pairings were made, as well as five *Armillaria* controls. A 2 x 2 mm section of *A. ostoyae* was aseptically cut from the original established isolate cultures and transferred to the middle half of a 100 x 15 mm petri dish, then were placed in a dark, 25º C incubator for three weeks. After three weeks, 2 x 2 mm *Byssochlamys* sections were placed in the middle of the other half of the 100 x 15 mm petri dishes. Control plates were prepared by placing only the 2 x 2 mm fungal isolate plugs into the middle of the 100 x 15 mm petri plates.

Hyphal interactions among *B. nivea* and *A. ostoyae* isolates were examined using a glass microscope slide method. Standard microscope slides were sterilized using an autoclave. Then two to three drops of 2% MEA were placed onto the center of the slide using a glass rod. A glass cover slip was then placed on top of the agar. One 2 x 2 mm section of *Byssochlamys* was then placed onto one side of the cover slip (right side), ensuring the section had contact with the existing agar on the slide, and one 2 x 2 mm section of *Armillaria* was placed on the other side of the cover slip (left side). Each slide was then placed into an empty petri dish and incubated at 25º C in the dark for 14 days. The same three *A. ostoyae* and one *Byssochlamys* isolates were used in the microscope slide

pairings. All fungal growth pairings were examined for morphological characters, with close attention directed towards any changes on hyphal morphology, at magnification ranging from 3X for the petri plates and 400X for the microscope slides.

Statistical Analyses

Chi-square tests (Zar 1999) were used to determine whether or not there were differences in the incidence of *B. nivea* and *Umbelopsis* spp. among the study sites, between Douglas-fir and ponderosa pine roots, and if the incidence differed with distance along the root (10 and 30 cm from the bole). Paired, two tailed T-tests ($p \leq 0.05$) (Zar 1999) were used to determine whether or not *B. nivea* was inhibiting the growth of *A. ostoyae* in the growth pairings. Linear regression analyses were used to relate *B. nivea* and *Umbelopsis* spp. frequency to host DBH (SPSS 2004).

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Radial growth rates of each isolate at each temperature were averaged and plotted against incubation temperature to determine optimum growth temperatures. Minimum and maximum temperatures for growth were also determined. One-way ANOVA was used to determine if radial growth rates for each genus were different ($p \leq 0.05$) at each temperature (Zar 1999, SPSS 2004).

Figure 1. Mission Creek Watershed in Wenatchee National Forest (Indicated with red star).

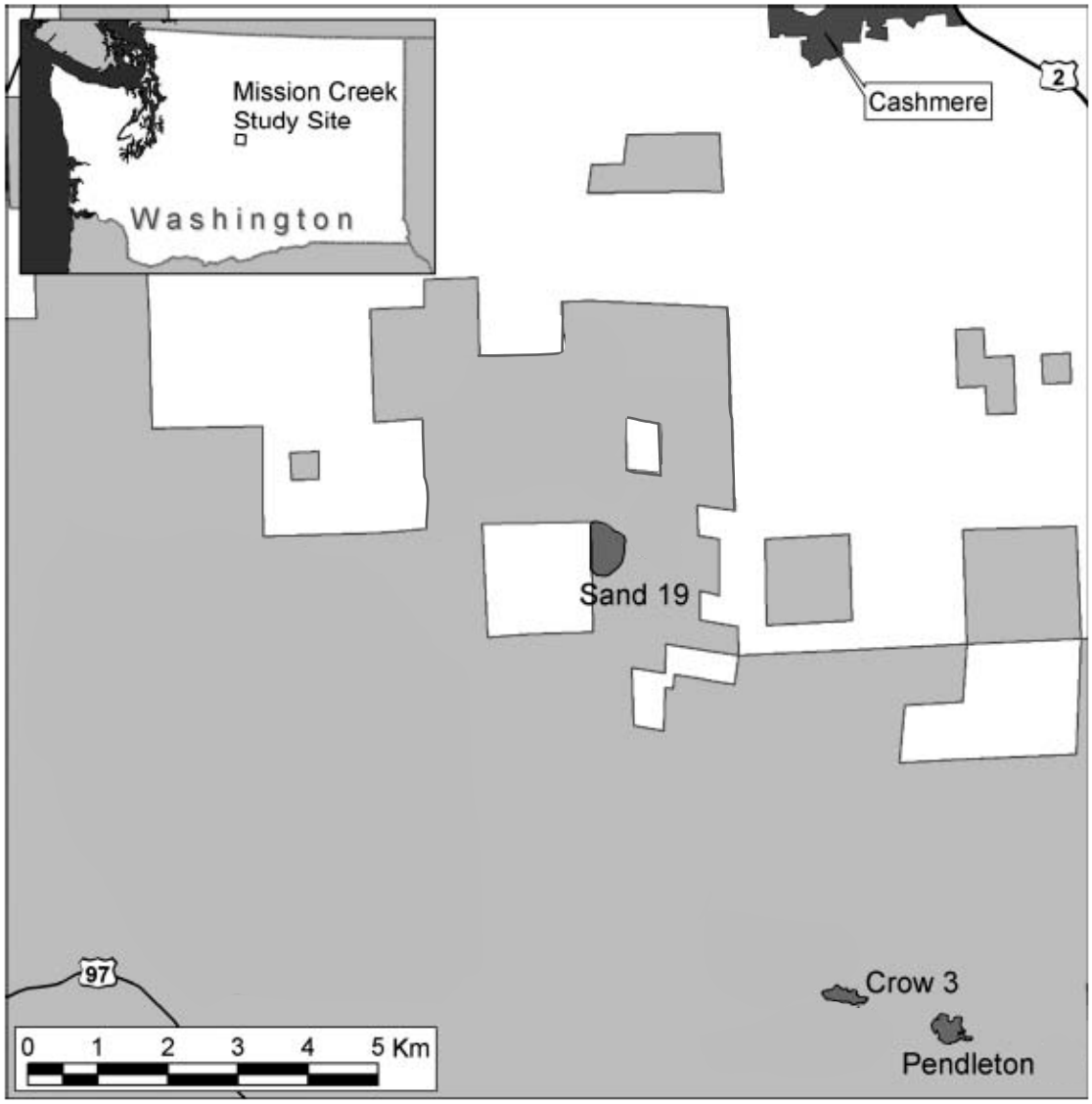


Figure 2. Locations of the three sampling sites at the Wenatchee National Forest, Mission Creek Study Area. Shaded areas: Wenatchee National Forest ownership; Top of map is north. Map modified from Hoff *et al*. (2004).

Table 1. Environmental, topographic, and vegetation characteristics of study sites. Crow 3, Pendleton 30, and Sand 19 study sites represent three control, out of twelve total, study sites in the Fire and Fire Surrogates Mission Creek Study. Standard deviations in parenthesis.

Site	Vegetation Communities*	Slope (%)	Aspect	Elevation (m)	Forest Floor Depth (cm)**	Canopy Cover (%)	Basal Area (m^2 ha^{-1})	Trees (conifers, ha^{-1})
Crow 3	PSME/CARU	20-70	S	735-925	5.0 (2.7)	41	24 (11)	340 (254)
Pendlton 30	PSME/CARU	5-55	S	775-820	6.5 (3.1)	36	22 (8)	431 (389)
Sand 19	PSME/CAGE-CARU-SYAL	35-70	S W	655-960	4.4 (2.7)	47	28 (17)	363 (414)

*Vegetation communities key: CACA = pinegrass (*Calamagrostis rebescens*), CAGE = elk sedge (*Carex geyeri*), PSME = Douglas-fir (*Pseudotsuga menziesii*), SYAL = common snowberry (*Symphoricarpos albus*) **Forest Floor Depth includes litter and duff layers

Table 2. Douglas-fir and ponderosa pine trees sampled from three study sites.

Study Site	Tree Species	DBH (cm)	Study Site	Tree Species	DBH (cm)	Study Site	Tree Species	DBH (cm)
Crow 3	PIPO	3	Pendelton 30	PIPO	13	Sand 19	PIPO	8
Crow 3	PIPO	6	Pendelton 30	PIPO	13	Sand 19	PIPO	8
Crow 3	PIPO	12	Pendelton 30	PIPO	18	Sand 19	PIPO	14
Crow 3	PIPO	15	Pendelton 30	PIPO	22	Sand 19	PIPO	16
Crow 3	PIPO	16	Pendelton 30	PIPO	22	Sand 19	PIPO	17
Crow 3	PIPO	18	Pendelton 30	PIPO	22	Sand 19	PIPO	20
Crow 3	PIPO	24	Pendelton 30	PIPO	23	Sand 19	PIPO	22
Crow 3	PIPO	25	Pendelton 30	PIPO	25	Sand 19	PIPO	22
Crow 3	PIPO	25	Pendelton 30	PIPO	28	Sand 19	PIPO	29
Crow 3	PIPO	29	Pendelton 30	PIPO	29	Sand 19	PIPO	29
Crow 3	PIPO	29	Pendelton 30	PIPO	29	Sand 19	PIPO	38
Crow 3	PIPO	30	Pendelton 30	PIPO	30	Sand 19	PIPO	42
Crow 3	PIPO	34	Pendelton 30	PIPO	30	Sand 19	PIPO	47
Crow 3	PIPO	35	Pendelton 30	PIPO	30	Sand 19	PIPO	51
Crow 3	PIPO	35	Pendelton 30	PIPO	33	Sand 19	PIPO	52
Crow 3	PIPO	40	Pendelton 30	PIPO	56	Sand 19	PIPO	71
Crow 3	PIPO	48	Pendelton 30	PIPO	66	Sand 19	PIPO	79
Crow 3	PIPO	50	Pendelton 30	PIPO	71	Sand 19	PIPO	80
Crow 3	PIPO	56	Pendelton 30	PIPO	73	Sand 19	PIPO	102
Crow 3	PIPO	58	Pendelton 30	PIPO	74	Sand 19	PSME	sapling
Crow 3	PSME	sapling	Pendelton 30	PSME	sapling	Sand 19	PSME	7
Crow 3	PSME	sapling	Pendelton 30	PSME	sapling	Sand 19	PSME	10
Crow 3	PSME	sapling	Pendelton 30	PSME	sapling	Sand 19	PSME	10
Crow 3	PSME	5	Pendelton 30	PSME	sapling	Sand 19	PSME	11
Crow 3	PSME	20	Pendelton 30	PSME	5	Sand 19	PSME	13
Crow 3	PSME	20	Pendelton 30	PSME	6	Sand 19	PSME	15
Crow 3	PSME	22	Pendelton 30	PSME	9	Sand 19	PSME	16
Crow 3	PSME	23	Pendelton 30	PSME	10	Sand 19	PSME	17
Crow 3	PSME	24	Pendelton 30	PSME	10	Sand 19	PSME	42
Crow 3	PSME	25	Pendelton 30	PSME	31	Sand 19	PSME	42
Crow 3	PSME	31	Pendelton 30	PSME	32	Sand 19	PSME	44
Crow 3	PSME	31	Pendelton 30	PSME	36	Sand 19	PSME	45
Crow 3	PSME	32	Pendelton 30	PSME	41	Sand 19	PSME	45
Crow 3	PSME	32	Pendelton 30	PSME	45	Sand 19	PSME	45
Crow 3	PSME	32	Pendelton 30	PSME	50	Sand 19	PSME	46
Crow 3	PSME	32	Pendelton 30	PSME	50	Sand 19	PSME	46
Crow 3	PSME	36	Pendelton 30	PSME	59	Sand 19	PSME	54
Crow 3	PSME	37	Pendelton 30	PSME	67	Sand 19	PSME	58
Crow 3	PSME	39	Pendelton 30	PSME	90	Sand 19	PSME	63
Crow 3	PSME	48						

PSME = Douglas-fir (*Pseudotsuga menziesii*)
PIPO = ponderosa pine (*Pinus ponderosa*)

CHAPTER 4

RESULTS

Presence of *Byssochlamys* and *Umbelopsis* in Roots

Byssochlamys nivea and *Umbelopsis* spp. were found at all three sites (Crow 3, Pendleton 30, and Sand 19) with an average frequency of 14.5 and 26 percent, respectively (Figure 3, Appendix C). The *B. nivea* and *Umbelopsis* spp. isolates cultured had morphological similarities with reference isolates of *B. nivea*, *Umbelopsis versiformis*, and *Umbelopsis vinacea* (all provided by N. Klopfenstein from Hoff *et al*. 2004). *B. nivea* isolates were a tannish brown color in culture, with tapering phialides and conidia borne on chains. Chlamydospore (4-7 x 2.5-5 µm) and conidia (3-5.7 x 2.2-4 µm) sizes matched those descriptions given by Stolk and Samson (1971). *Umbelopsis* spp. were irregularly septate, with red to pink colored globose sporangia (7-12.5 µm in diameter) observed in most cultures, which matched the description of the *Umbelopsis* spp. cultures isolated by Hoff (2002).

Crow 3, Pendleton 30, and Sand 19 sites were previously examined by Hoff (2002) for the presence of fungal endophytes and she found *B. nivea* and *Umbelopsis* spp. occurence frequencies of 20.4 and 10.4 percent, respectively (Figure 3). I found a lower occurrence of *B. nivea* and a higher occurrence of *Umbelopsis* spp. than Hoff (2002). The difference in percentage of *Umbelopsis* spp. was significant ($\chi^2 = 6.635$; $p = 0.010$; d.f. = 1). The difference in percentage of *B. nivea* was not significant ($\chi^2 = 0.998$; $p = 0.318$; d.f. = 1).

Fungal endophyte occurrence as a percentage of isolation is shown relative to tree DBH classes for combined ponderosa pine and Douglas-fir data in Figure 4. Data from Hoff (2002) also are shown in Figure 4. Fungal endophyte occurrence did not differ by host ($p \geq 0.05$), so results by study site were pooled. Fungal endophyte occurrence across seven diameter classes was determined (< 15.0, 15.1-25, 25.1-35, 35.1-45, 45.1-55, 55.1-65, and > 65.1 cm). Using linear regression the occurrence of *Umbelopsis* spp. was shown to be positively related to DBH class ($R^2 = 0.796$; $p = 0.007$) (Figure 5); however, there was no relationship between occurrence of *B. nivea* and tree size ($R^2 = 0.047$; $p = 0.642$).

Combining data with those of Hoff (2002) (Figure 4) increased the number of samples and the power of analyses. However, tree DBH was not significantly related to the occurrence of *B. nivea* or *Umbelopsis* spp. when using Hoff's data (Figures 4 and 5).

The occurrence (number of samples with fungus out of total number of samples) of *B. nivea* was significantly higher at the Pendleton 30 than at other two sites ($\chi^2 = 13.816$; $p = 0.001$; d.f. = 2) (Figure 6). Statistical differences between hosts (Figure 7) and distances along the root (Figure 8) were not examined because the frequency of occurrence of *B. nivea* could not be pooled across sites due to significant differences.

The occurrence of *Umbelopsis* spp. was similar among the three study sites ($\chi^2 = 4.326$; $p = 0.115$; d.f. = 2) (Figure 9). Host species significantly influenced the occurrence of *Umbelopsis* spp. ($\chi^2 = 4.546$; $p = 0.033$; d.f. = 1), with *Umbelopsis* spp. recovered more

frequently from ponderosa pine than Douglas-fir (Figure 10). However, occurrence did not vary significantly along the root ($\chi^2 = 1.466$; $p = 0.226$; d.f. = 1) (Figure 11).

In summary, there were differences in the occurrence of the two fungal endophytes at the three different sites. *B. nivea* was found most frequently on the Pendleton 30 site (Figure 6, 20 total isolates), while there was a trend for *Umbelopsis* spp. to be found most frequently on the Sand 19 site (Figure 9, 26 total isolates). The average occurrence of *Umbelopsis* spp. in ponderosa pine was nearly double those found in Douglas-fir (12:6.7), but the difference was not significant ($\chi^2 = 1.504$; $p = 0.220$; d.f. = 1). There was no difference in the occurrence of *B. nivea* and *Umbelopsis* spp. in roots 10 cm and 30 cm from the bole.

Presence of *Byssochlamys* and *Umbelopsis* in the Rhizosphere and Seeds

Byssochlamys nivea was isolated from the rhizosphere from 52 percent of the samples, but *Umbelopsis* spp. was not detected. There were no statistically significant differences among study sites, tree species, or root sampling locations for *B. nivea* occurrence in the rhizosphere. Neither *Umbelopsis* spp. nor *B. nivea* were found in Douglas-fir seeds.

Growth of *Byssochlamys* and *Umbelopsis* at Different Temperatures

The optimum growth of *B. nivea*, *U. versiformis*, and *U. vinacea* all occurred at 25º C (Figure 12). Radial growth of endophytic species differed significantly as a function of temperature ($F(2,28) = 11.374$; $p = 0.000$), however, post-hoc Tukey's HSD tests showed that *U. versiformis* grew significantly more at 25º C than *U. vinacea* and *B. nivea*

($p \leq 0.05$). The minimum temperature for growth of all isolates was 3º C. The radial growth of endophytic species at this temperature differed significantly ($F(2,37) = 34.166$; $p = 0.000$), with post-hoc Tukey's HSD tests showing *B. nivea* growing significantly less than *Umbelopsis* spp. ($p \leq 0.05$).

Fungal growth at 10º C, 15º C, and 20º C was significantly different among species ($F(2,32) = 371.075$; $p = 0.000$, $F(2,34) = 221.195$; $p = 0.000$, and $F(2,34) = 16.487$; $p = 0.000$, respectively), with post-hoc Tukey's HSD tests showing *Umbelopsis* spp. growing at a significantly higher rate than *B. nivea* at 10º C and 15º C ($p \leq 0.05$). At 20º C, *U. versiformis* grew significantly faster than *U. vinacea* and *B. nivea* isolates ($p \leq 0.05$). However, at 30º C *B. nivea* grew significantly faster than *Umbelopsis* spp. ($F(2,37) = 690.415$; $p = 0.000$). At 30º C *Umbelopsis* spp. growth had nearly ceased while the *B. nivea* growth did not cease growth until 35º C.

Influence of *Byssochlamys nivea* on *Armillaria ostoyae* Growth

When *Armillaria ostoyae* and *B. nivea* were grown together at 25º C there was no evidence of *B. nivea* inhibiting the growth of *A. ostoyae* (Figure 13). Only in one case was there a significant difference in growth (*A. ostoyae* 286 and *B. nivea*; $t = 7.790$; $p = 0.001$; d.f. = 4; two tailed paired t-test) and in this case *A. ostoyae* growth was enhanced relative to controls (Figure 13). No other differences were significant (*A. ostoyae* 957; $t = 0.333$; $p = 0.761$; d.f. = 3; two tailed paired t-test; *A. ostoyae* 284; $t = 0.727$; $p = 0.507$; d.f. = 4; two tailed paired t-test). There was a yellow pigmented interaction zone formed between *A. ostoyae* and *B. nivea* in some of the pairings, but this zone formation did not

appear to be related to growth trends (Figure 14). There were also no observable changes in hyphal morphology as a result of the pairings either in the petri dishes or on the microscope slides.

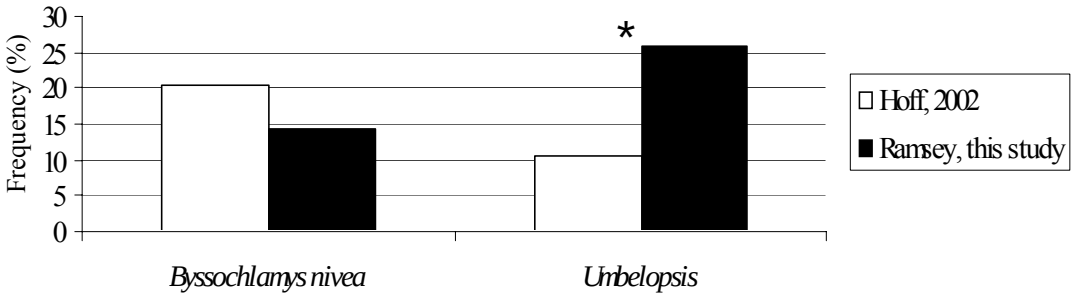


Figure 3. Percentage of sampled roots with fungal endophytes across the three study sites. *Difference in the percentage of *Umbelopsis* spp. was significant ($\chi^2 = 6.635$; $p = 0.010$; d.f. = 1).

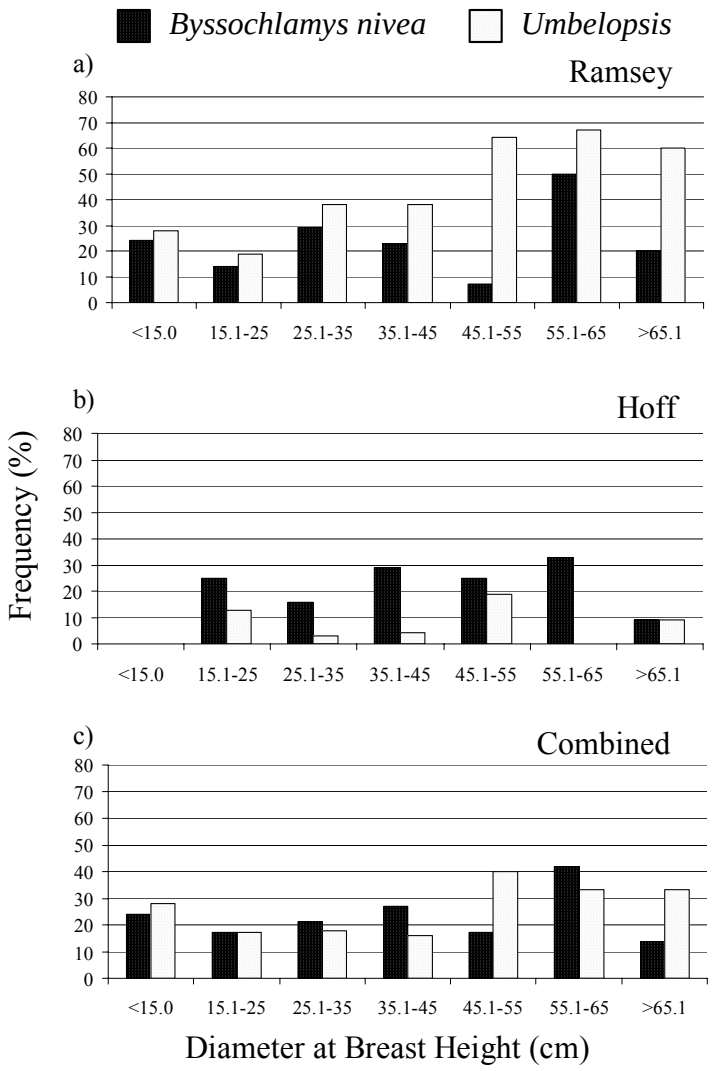


Figure 4. Occurrence of *Byssochlamys nivea* and *Umbelopsis* spp. as a percentage of isolations from combined Douglas-fir and ponderosa pine roots at three study sites by Diameter at Breast Height class. a) Ramsey, this study; b) Hoff (2002); c) Combined, data from both studies combined.

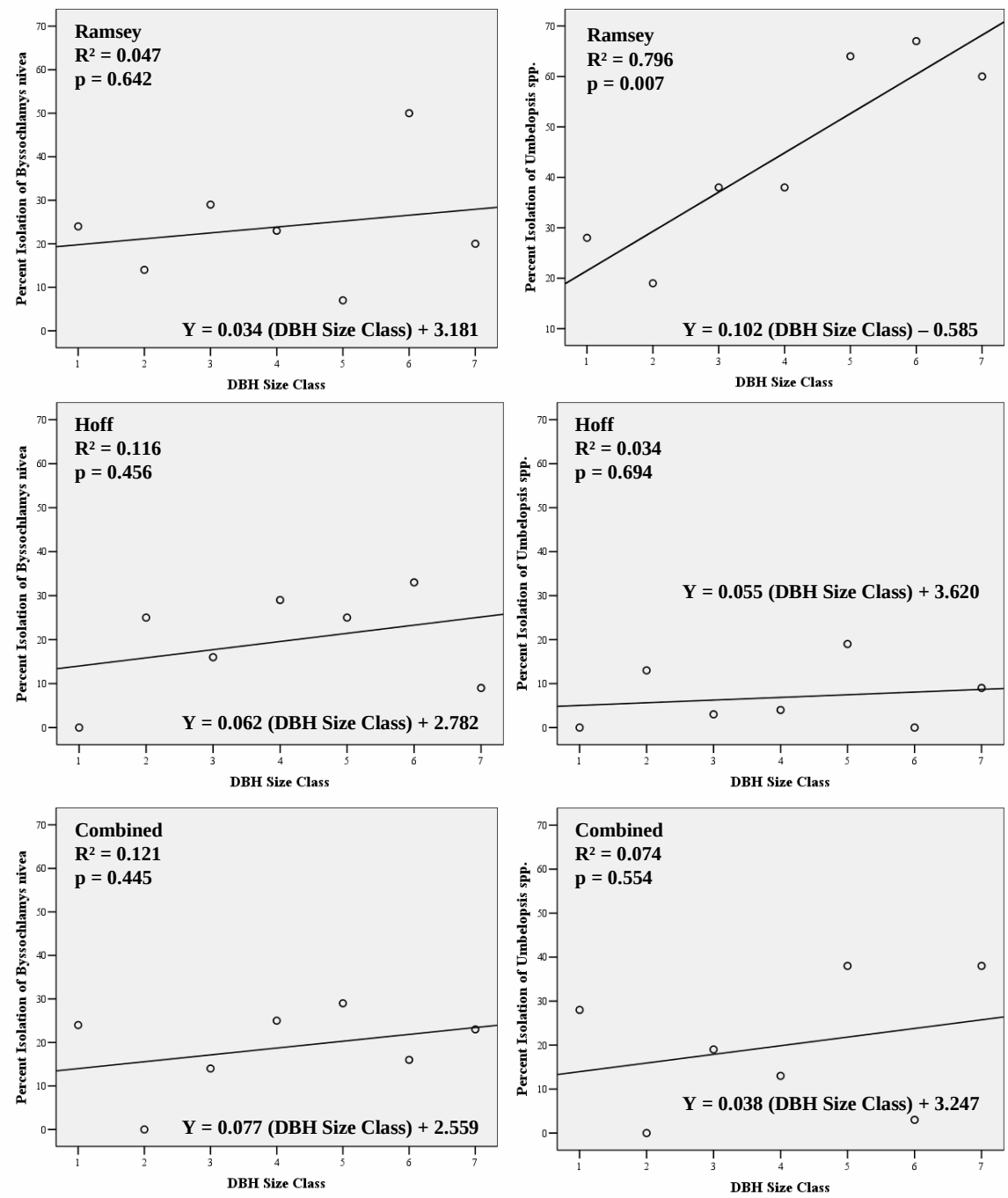


Figure 5. Linear regressions showing percent occurrence of fungal endophytes by tree diameter classes. DBH (diameter at breast height) size classes are in cm; 1 = < 15.0, 2 = 15.1 – 25.0, 3 = 25.1 – 35.0, 4 = 35.1 – 45.0, 5 = 45.1 – 55.0, 6 = 55.1 – 65.0, 7 = > 65.1. Ramsey, this study; Hoff (2002); Combined, data from both studies combined.

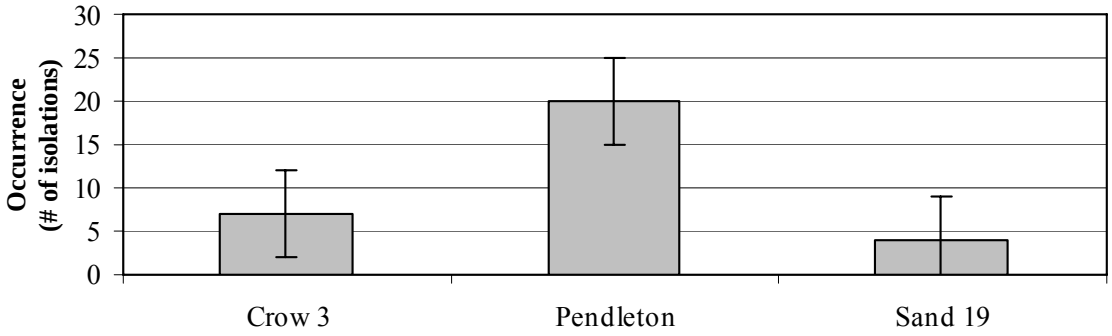


Figure 6. *Byssochlamys nivea* occurrence among study sites in both Douglas-fir and ponderosa pine. Occurrence indicates raw data numbers out of 214 samples and is not statistically equal across sites ($\chi^2 = 13.816$; $p = 0.001$; d.f. = 2). Standard error bars are shown.

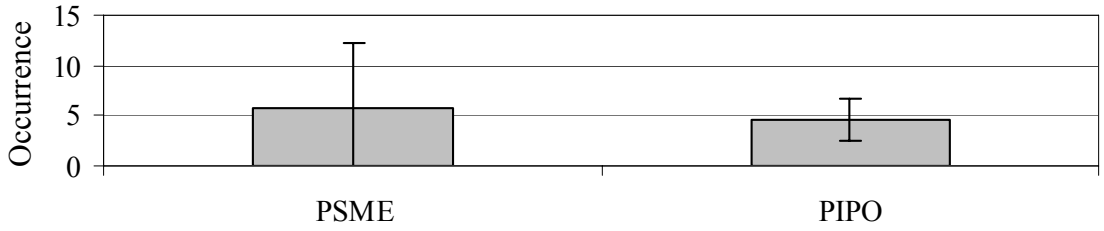


Figure 7. *Byssochlamys nivea* occurrence in different hosts. Occurrence indicates raw data numbers out of 214 samples. Standard deviation bars are shown. PSME = Douglas-fir, PIPO = ponderosa pine

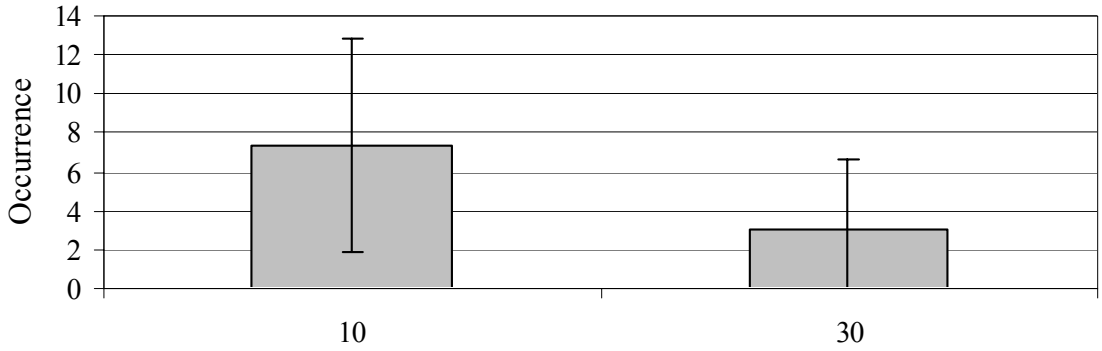


Figure 8. *Byssochlamys nivea* occurrence in different root locations. Root locations are lateral cm distances from the base of the bole. Occurrence indicates raw data numbers out of 214 total samples. Standard deviation bars are shown.

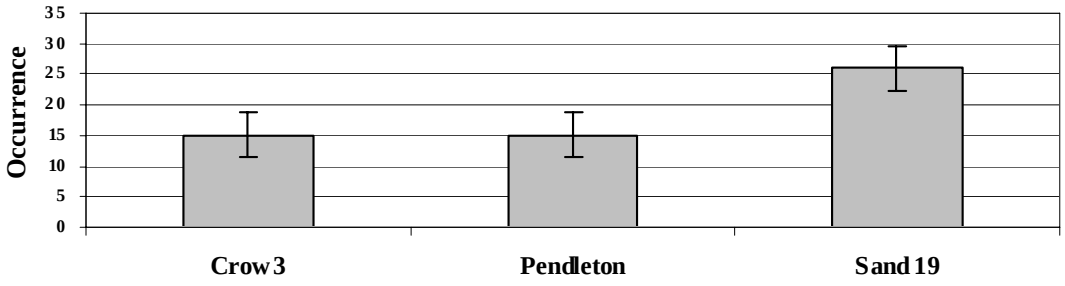


Figure 9. *Umbelopsis* spp. occurrence among study sites in Douglas-fir and ponderosa pine. Occurrence indicates raw data numbers out of 215 samples. No statistical differences among sites ($\chi^2 = 4.326$; $p = 0.115$; d.f. = 2). Standard error bars are shown.

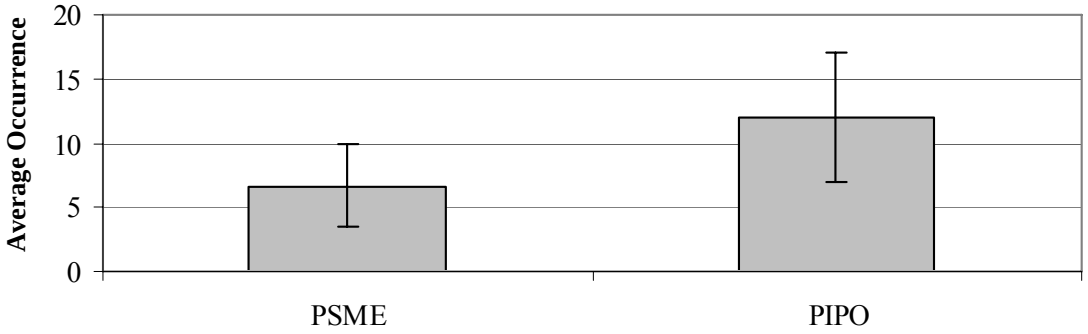


Figure 10. *Umbelopsis* spp. occurrence in different hosts. Frequency indicates raw data numbers out of 215 samples. Standard deviation bars are shown. Host species significantly influenced the presence of *Umbelopsis* spp. ($\chi^2 = 4.546$; $p = 0.033$; d.f. = 1). PSME = Douglas-fir, PIPO = ponderosa pine

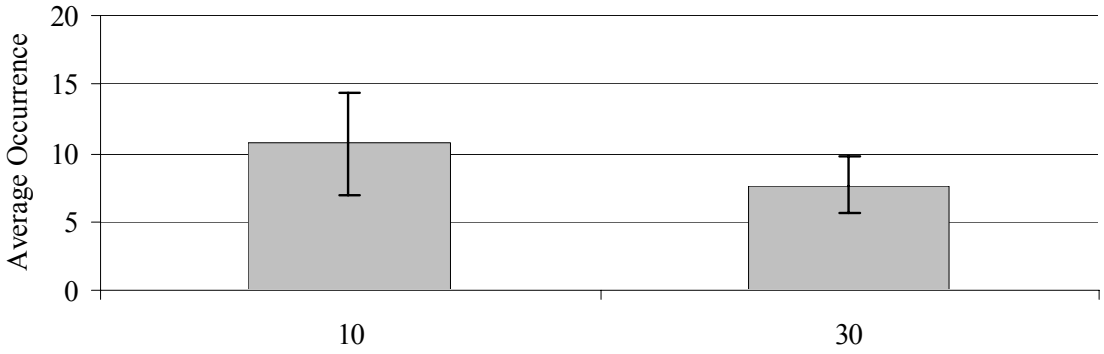


Figure 11. *Umbelopsis* spp. occurrence in different root locations. Root locations are distances (cm) from the base of the bole. Frequency indicates raw data numbers out of 215 samples. Standard deviation bars are shown. No statistical difference between root locations ($\chi^2 = 1.466$; $p = 0.226$; d.f. = 1).

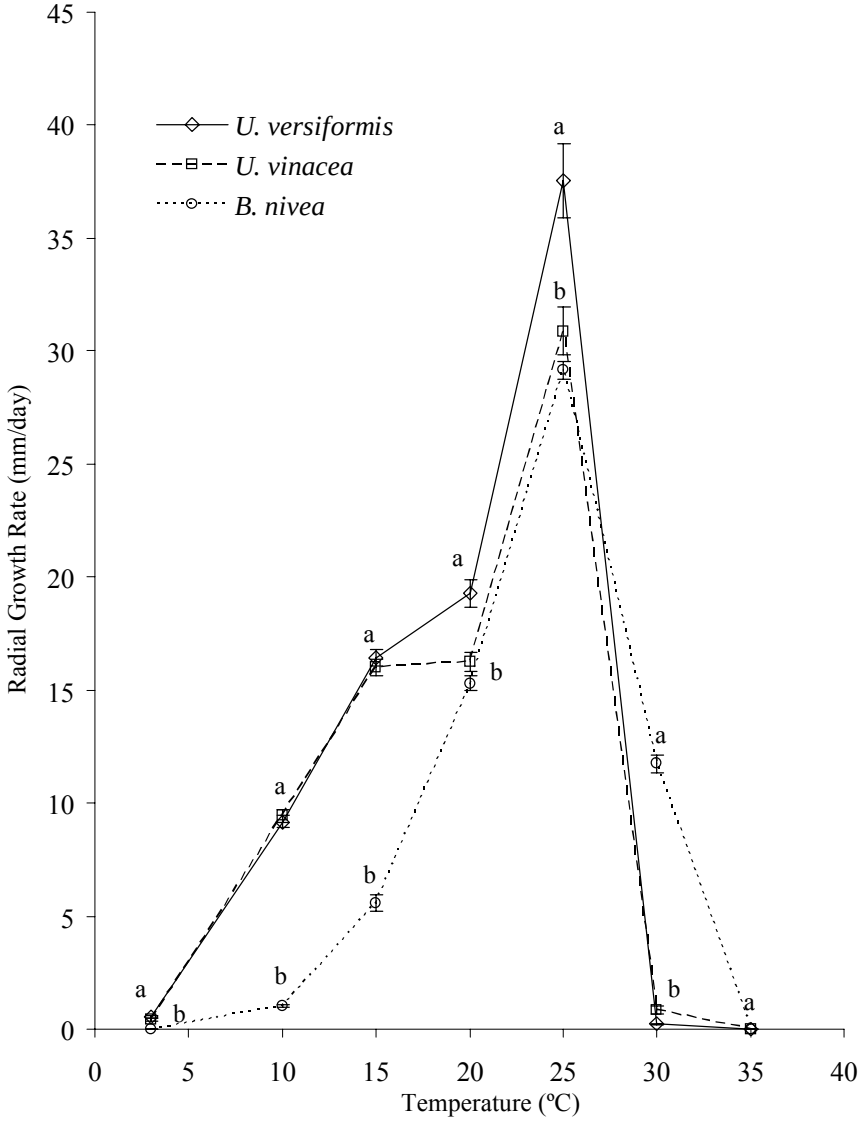


Figure 12. Growth rates of *Umbelopsis* and *Byssochlamys* isolates at differing temperatures. Different letters at the same temperature show statistically different growth rates (ANOVA; $p \leq 0.05$). Standard deviation bars are shown.

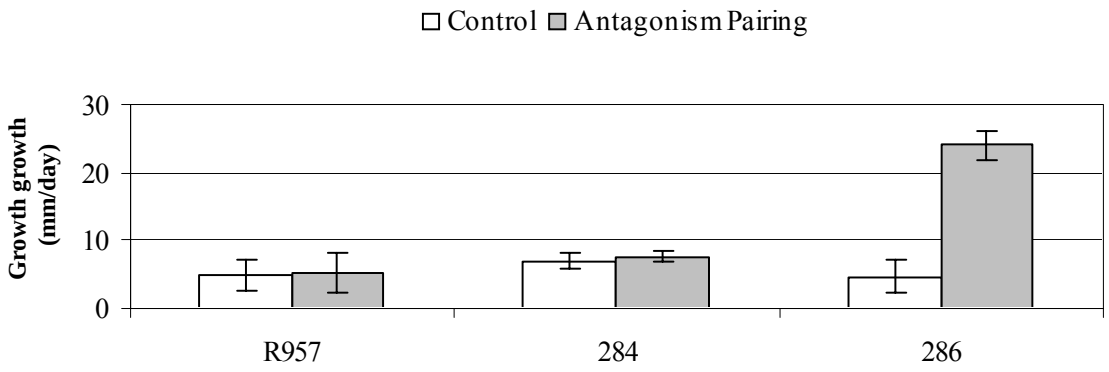


Figure 13. Radial growth rates of *Armillaria ostoyae* alone (control) and *A. ostoyae* growing with a single isolate of *Byssochlamys nivea* (antagonism pairing) at 25ºC. There were no statistical differences among *A. ostoyae* 957 and *B. nivea* or among *A. ostoyae* 284. Growth of *A. ostoyae* 286 was enhanced nearly four-fold when grown with *B. nivea* ($t = 7.790$; $p = 0.001$; d.f. = 4; two tailed paired t-test). Standard deviation bars are shown.

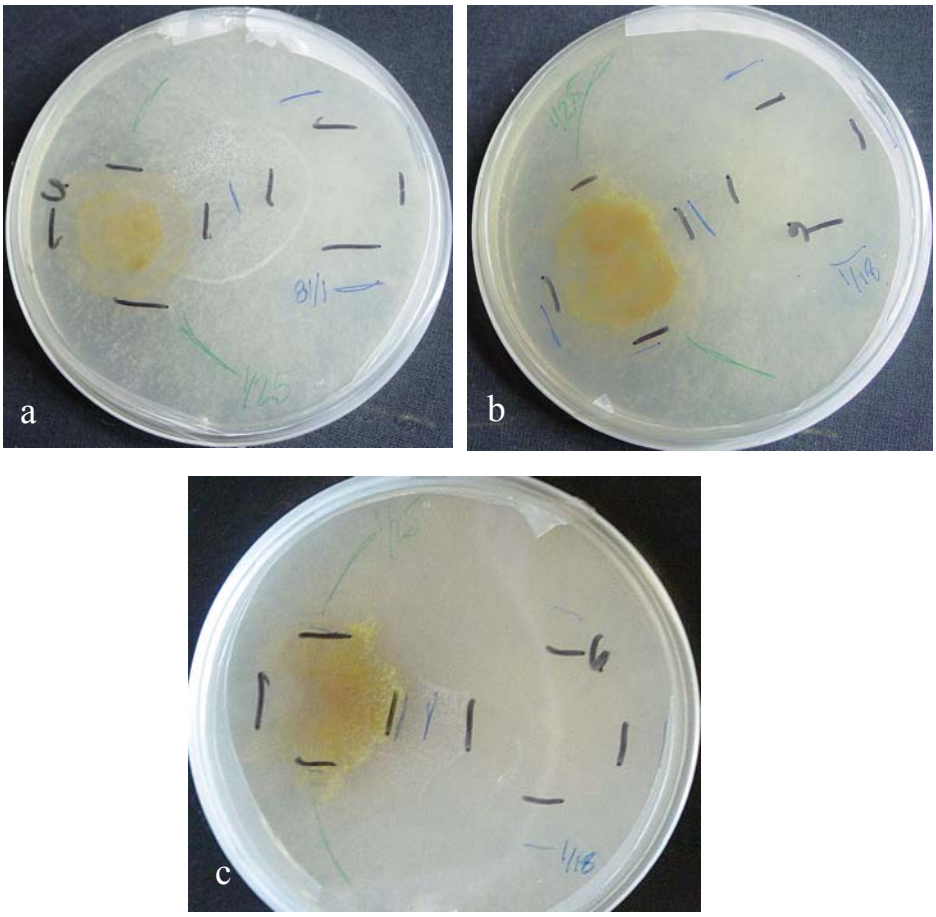


Figure 14. Examples of yellow pigmented interaction zones forming between *Armillaria ostoyae* (left) and *Byssochlamys nivea* (right) isolates. a = no pigment, b = narrow line of pigment, c = thick line of pigment

CHAPTER 5

DISCUSSION

Hoff (2002) and Hoff *et al*. (2004) found that *Byssochlamys nivea* and *Umbelopsis* spp. were the most common fungal endophytes in woody roots of Douglas-fir and ponderosa pine in the same area I conducted this study (the Mission Creek watershed in eastern Washington). In the present study, these fungi were found in the roots of both tree species, but *Byssochlamys nivea* and *Umbelopsis* spp. were not isolated from all roots. *B. nivea* and *Umbelopsis* spp. occurred at frequencies of 14.5 and 26 percent, respectively. In contrast, Hoff *et al*. (2004) found more *B. nivea* (20.4 percent) than *Umbelopsis* spp. (10.4 percent). However, only the difference in the occurrence of *Umbelopsis* spp. was significant. This suggests that the year of sampling may influence the occurrence. However, the trees I sampled were not the same trees sampled by Hoff *et al*. (2004), although they were nearby. In fact, spatial variability in the fungal endophyte population more likely explains differences between our results.

I hypothesized that there would be no difference in endophyte occurrence by host species, but I found significant differences in the occurrence of *Umbelopsis* spp. There were no significant differences in the frequency of *Umbelopsis* spp. across the three study sites, but the frequency of *B. nivea* did have significant differences. There was a much higher occurrence of *B. nivea* at Pendleton 30 than the other two sites, which were similar. This could have been due to differences in moisture, temperature or other environmental variables at the sites.

My null hypothesis was that there was no relationship between endophyte occurrence in woody roots and tree size. My results showed that *Umbelopsis* spp. occurrence increased with tree diameter, but *B. nivea* did not. There was no relationship with endophyte occurrence and tree diameter using Hoff's (2002) data alone or in combination with my data.

My null hypothesis concerning endophyte occurrence along the roots was that sampling distance from the bole would not significantly influence the occurrence of the fungal endophytes. My results confirmed this hypothesis.

Byssochlamys nivea was found in the soil rhizosphere in each study site, but *Umbelopsis* spp. was not detected using the soil dilution plate method. My null hypothesis was that neither endophyte would be found in the rhizosphere. *Umbelopsis* spp. has been found in soils in other studies in Washington and Idaho (Raini Rippi, USFS Moscow, ID, personal communication). The soil dilution plating method I used may not be the best method for detecting this genus from the soil. An alternative method for identifying *Umbelopsis* spp. presence in the soil may include molecular identification, such as those methods outlined in Meyer and Gams (2003).

I had hypothesized that the endophytes are transmitted horizontally not vertically. The lack of *B. nivea* and *Umbelopsis* spp. in the seeds of Douglas-fir is an indication that these fungi may not be vertically transmitted in their hosts, but rather horizontally

transmitted from the soil and inside roots. The lack of the isolation of these organisms from all root samples further supports the horizontal transmission concept. The fungi are most likely entering the root from the soil environment since they are present in the soil rhizosphere.

The optimum growth temperatures for *B. nivea* and the *Umbelopsis* spp. *in vitro* were 25º C. However, *B. nivea* is known as a heat-tolerant fungus and was able to grow at higher temperatures than *Umbelopsis* spp. *Umbelopsis* spp. also grew better at lower temperatures than *B. nivea*. The eastern Washington study area has a climate that is dominated by high temperatures and little moisture, which may explain the prevalence of *B. nivea* in the roots and in the soil.

The *Armillaria ostoyae* and *B. nivea* growth pairings were done to elucidate the function of fungal endophytes in woody roots. *Byssochlamys nivea* has previously been shown to inhibit the growth *in vitro* of several plant pathogenic species (Park *et al*. 2001). The species range from ascomycetous fungi to oomycetous water molds. Other natural and potential biological control studies have been conducted with such genera as *Trichoderma* and *Gliocadium*, and there is evidence to support the concept of natural biological control of *Armillaria* by *Trichoderma* (Bliss 1951, Ohr *et al*. 1973, Ayers and Adams 1981, Cook and Baker 1983, Hubbard *et al*. 1983).

My null hypothesis was that *B. nivea* is not antagonistic against the root pathogen *A. ostoyae* I saw no evidence of antagonism in pairings and *A. ostoyae* growth in the

controls was the same as that in pairings. An unexpected result also occurred: The five replicates of the *A. ostoyae* isolate 286, when paired with *B. nivea*, grew significantly faster than the controls. This suggests growth enhancement of *A. ostoyae* in the presence of *B. nivea*. When the hyphae of *A. ostoyae* and *B. nivea* in the pairings were examined microscopically, no changes in hyphal morphology were observed.

This study examined many different aspects of two fungal endophytes that were discovered in a previous study (Hoff *et al*. 2004) to be dominant in woody roots of Douglas-fir and ponderosa pine. The goal of this study was to provide some basic information about the biology and ecology of these organisms, in hopes of laying a ground work for future investigation into the function of these organisms. The field of fungal endophytes, especially in roots of woody species, is very new. The spatial information, as well as information about multiple habitats of these fungal endophytes, the horizontal transmission concept, and the evidence showing that these endophytes are not antagonistic towards the common root-rotting fungus, *A. ostoyae*, provides a jumping off point for future research about these organisms.

Conclusions

(1) The fungal endophytes, *B. nivea* and *Umbelopsis* spp. were found in the roots of Douglas-fir and ponderosa pine among the three study sites at frequencies of 20.4 and 10.4 percent, respectively.

(2) There was a statistical difference in *Umbelopsis* spp. occurrence by tree species, with this fungal endophyte occurring more frequently in ponderosa pine than Douglas-fir.

(3) The frequency of *B. nivea* was higher at the Pendleton site than the other two sites, but there was no difference for *Umbelopsis* spp. among sites.

(4) There was no significant difference in endophyte occurrence along roots.

(5) *Byssochlamys nivea* was commonly found in the soil rhizosphere.

(6) Both fungi are assumed to be horizontally transmitted, through infection of roots from the soil and growth in the roots, rather than vertically. Neither fungus was detected in Douglas-fir seeds and ponderosa pine seeds were not examined.

(7) The optimum temperature for growth of both fungi is 25^{o} C, but *B. nivea* grew somewhat better at temperatures warmer than 25^{o} C and *Umbelopsis* spp. grew somewhat better at cooler temperatures.

(8) *Byssochlamys nivea* did not inhibit the growth of *Armillaria ostoyae* in culture. In fact, it appeared to stimulate the growth of this pathogen in one sample.

Future Studies

The occurrence of *B. nivea* and *Umbelopsis* spp. in the woody roots of Douglas-fir and ponderosa pine, the occurrence *B. nivea* in the rhizosphere soils, and the assumed occurrence of *Umbelopsis* spp. in the rhizosphere soils suggests that these fungal endophytes are distributed across the dry landscape of the eastern Washington Cascades. Further investigations should be conducted to determine if these endophytes are located in hosts other than Douglas-fir and ponderosa pine and if there are geographic, topographic, and elevational factors contributing to the distribution of these fungi.

Results suggest that these fungal endophytes are horizontally, not vertically, transmitted and the fungi were found or are assumed to be found in the soils, suggesting a soil to root host infection mechanism. Hessburg and Hansen (2000) suggested through the investigation of the pathogenic fungus, *Leptographium wageneri* that natural root wounding, through new root formation and root growth, may be an important entry court for fungi, which could explain the presence of endophytic fungi within the secondary tissues of tree roots. The mechanisms of host infection by *B. nivea* and *Umbelopsis* spp. into Douglas-fir and ponderosa pine roots should be investigated.

Byssochlamys nivea and *Umbelopsis* spp. were not found in Douglas-fir seeds, but the sample size was small and ponderosa pine seeds were not examined. Douglas-fir and ponderosa pine seeds should be examined in greater number than were examined in this study to confirm whether or not these fungal endophytes are horizontally or vertically transmitted.

Fungal endophytes, in general, are difficult organisms to work with. Sample collection, fungal isolation for identification and other species manipulation and experimental purposes can be a lengthy process. Determining function of a specific fungal endophyte within a host in which the entire life cycle cannot be observed within the time frame of a researchers life adds another factor into the difficulty level of working with these fungi. A proposed method for overcoming the difficulties in working with and identifying the function of fungal endophytes in tree species would be to identify and work with a model tree species with a relatively short life cycle. This could involve identifying the fungal

endophytic species inhabiting a *Trichocarpa* sp., which grows relatively quickly and can be vegetatively propagated, then setting up field plots and plant cultures in the lab to assess the various host physiological responses to the presence and absence of the endophytic fungi.

REFERENCES

Agee, J. 1996. Fire in the Blue Mountains: a history, ecology, and research agenda. In: Jaindl, R. G., Quigley, T. M., eds. Search for a solution: sustaining the land, people and economy of the Blue Mountains. Washington, DC: American Forests: 119-145.

Agee, J. K., Edmonds, R. L., Gaines, W. L., Harrod, R. J., Hessburg, P. F., Lehmkuhl, J. F., Zabowski, D. 2001. Fire and fire surrogates national study: Mission Creek site: Okanogan and Wenatchee National Forests. Final Study Plan. Vegetation and Fuels Protocols.

Agee, J., Maruoka, K. 1994. Historical fire regimes of the Blue Mountains. BMNRI-TN-1. La Grande, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station, Blue Mountains Natural Resources Institute, 4 p.

Ahlholm, J., Helander, M., Elamo, P., Saloniemi, I., Neuvonen, S., Hanhimaki, S., Saikkonen, K. 2002. Micro-fungi and invertebrate herbivores on birch trees: fungal mediated plant-herbivore interactions or responses to host quality? Ecology Letters 5: 648.

Ahlich, K., Sieber, T. N. 1996. The profusion of dark septate endophytic fungi in non-mycorrhizal fine roots of forest trees and shrubs. New Phytologist 132: 259-270.

Amos, J.K., Barnett, H.L. 1966. *Umbelopsis versiformis*, a new genus and species of the Imperfects. Mycologia 58: 805-808.

Arno, S. 1988. Fire ecology and its management implications in ponderosa pine forests. In: Baumgartner, David M., Lotan, James E., compilers. Ponderosa pine: The species and its management. Symposium proceedings, 1987 September 29 - October 1; Spokane, WA. Pullman, WA: Washington State University, Cooperative Extension: 133-139.

Arno, S. Gruell, G. 1983. Fire history at the forest-grassland ecotone in southwestern Montana. Journal of Range Management 36: 332-336.

Arnold, A. E., Maynard, Z., Gilbert, G. S. 2001. Fungal endophytes in dicotyledonous neotropical trees: patterns of abundance and diversity. Mycological Research 105: 1502-1507.

Arnold, A. E., Maynard, Z., Gilbert, G. S., Coley, P. D., Kursar, T. A. 2000. Are tropical fungal endophytes hyperdiverse? Ecology Letters 3: 267-274.

Arnold, A. E., Mejia, L. C., Kyllo, D., Rojas, E. I., Maynard, Z., Robbins, N., Herre, E. A. 2003. Fungal endophytes limit pathogen damage in a tropical tree. Proceedings of the National Academy of Sciences 100: 15649-15654.

Atzet, T., McCrimmon, L. 1990. Preliminary plant associations of the southern Oregon Cascade Mountain Province. Grants Pass, OR: U.S. Department of Agriculture, Forest Service, Siskiyou National Forest, 330 p.

Atzet, T., Wheeler, D. 1984. Preliminary plant associations of the Siskiyou Mountain Province. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Region: 278 p.

Ayers, W. D., Adams, P. B. 1981. Mycoparasitism and its application to biological control of plant diseases. In Papavizas, G. C. ed. Biological control in plant production: 91-103.

Bakhtin, V. S. 1928. Fungi which damage books. [Trans. Title]. Proc. Pan-Soviet Congr. Bot. Leningrad: 169-170.

Basu, S. N. 1948. Fungal decomposition of jute fibre and cellulose. Part I. A preliminary survey of commonly occurring species. J. Text. Inst 39: 232-237. Part II. The effect of some environmental factors. Ibid: 237-248.

Bayman, P., Angulo-Sandoval, P., Baez-Ortiz, Z., Lodge, J. 1998. Distribution and dispersal of *Xylaria* endophytes in two tree species in Puerto Rico. Mycological Research 102: 944-948.

Bayman, P., Gonzalez, E. J., Fumero, J. J., Tremblay, R. L. 2002. Are fungi necessary? How fungicides affect growth and survival of the orchid *Lepanthes rupestris* in the field. Journal of Ecology 90: 1002-1008.

Bayman P. L., Lebrón R. L., Tremblay J. L. 1997. Variation in endophytic fungi from roots and leaves of *Lepanthes* (Orchidaceae). New Phytologist 135: 143-149.

Beuchat, L. R., Pitt, J. I. 2002. Detection and enumeration of heat-resistance molds. In: Compendium of Methods for Microbiological Examination of Foods, 4th edn. Ed. by Downes, F. P.; Ito, K. Weimar, Texas: Culinary and Hospitality Industry Publications Services: 217–221.

Bernstein, M. E., Carroll, G. C. 1977. Internal fungi in old growth Douglas-fir foliage. Canadian Journal of Botany 55: 644-653.

Bisby, G. R., Jamieson, M. C., Tomonin, N. 1933. The fungi in butter. Canadian Journal of Research 9: 97-107.

Bliss, D. E. 1951. The destruction of *Armillaria mellea* in citrus soils. Phytopathology 41: 665-683.

Bordallo, J. J., Lopez-Llorca, L. V., Jansson, H. B., Salinas, J., Persmark, L., Asensio, L. 2002. Colonization of plant roots by egg-parasitic and nematode-trapping fungi. New Phytologist 154: 491-499.

Brown, A. H. S., Smith, G. 1957. The genus *Paecilomyces* Bainier and its perfect stage *Byssochlamys* Westling. Transactions of the British Mycological Society 40: 17-89.

Burns, R., Honkala, B., tech. coords. 1990. Silvics of North America. Volume 1. Conifers. Agric. Handb. 654. Washington, DC: U.S. Department of Agriculture, Forest Service, 675 p.

Carroll, G.C. 1988. Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. Ecology 69: 2-9.

Carroll, G.C. 1992. Fungal Mutualism. In Carroll, G. C., Wicklow, D. T., eds. The Fungal Community. New York: Marcel Denker, Inc.: 327-254.

Carroll, G.C. 1995. Forest endophytes: pattern and process. Canadian Journal of Botany 73 (Suppl. 1): S1316-S1324.

Carroll, G.C. 1997. Fungus/Plant Interactions - An Overview. In Carroll, G. C., Tudzynski, P., eds. The Mycota, Vol 5A, Springer Verlag, Berlin: 1-8.

Charles, V. K. 1941. A preliminary check list of the entomogenous fungi of North America. U.S. Bur. Ent. Pl. Quar. Insect Pest Survey 21 (Supp. 9): 759-760.

Cheplick, G. P., Cho, R. 2003. Interactive effects of fungal endophyte infection and host genotype on growth and storage in *Lolium perenne*. New Phytologist 158: 183-191.

Clay, K., Holah, J. 1999. Fungal endophyte symbiosis and plant diversity in successional fields. Science 285: 1742-1744.

Clay, K., Schardl, C. 2002. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. The American Naturalist 160: S99-S127.

Clement, S. L., Lester, D. G., Wilson, A. D., Johnson, R. C., Bouton, J. H. 1996. Expression of Russian wheat aphid (Homoptera: Aphididae) resistance in genotypes of tall fescue harboring different isolates of *Acremonium* endophyte. Journal of Economic Entomology 89: 766-770.

Conant, N. F., Martin, D. S., Smith, D. T., Baker, R. D., Callaway, J. L. 1945. Manual of clinical mycology. London: Saunders.

Cook, R. J., Baker, K. F. 1983. The nature and practice of biological control of plant pathogens. St. Paul, MN: American Phytopathological Society, 539 p.

Cooper, S., Neiman, K., Roberts, D. 1991 [Revised]. Forest habitat types of northern Idaho: a second approximation. Gen. Tech. Rep. INT-236. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Research Station, 143 p.

De Bary, A. 1866. Morphologie und Physiologie del Pilze: Flechten und Myxomyceten. Engelmann, Leipzig, Germany.

Deckert, R. J., Peterson, R. L. 2000. Distribution of foliar fungal endophytes of *Pinus strobus* between and within host trees. Canadian Journal of Forest Research 30: 1436-1442.

DeVelice, R., Ludwig, J., Moir, W., Ronco, F., Jr. 1986. A classification of forest habitat types of northern New Mexico and southern Colorado. Gen. Tech. Rep. RM-131. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station, 59 p.

Dolan, E. 2002. Soil and site variability in the northeast Wenatchee mountains, Washington. MS Thesis, University of Washington, Seattle, WA, 103 p.

Drechsler, C. 1941. Some Hyphomycetes parasitic on free-living terricolous nematodes. Phytopathology 31: 773-802.

Ellis, J. B., Everhart, B. M. 1883. New species of fungi. Bull. Torrey bot. Cl. 10: 97-98.

Espinosa-Garcia, F. J., Rollinger, J. L., Langenheim, J. H. 1996. Coastal redwood leaf endophytes: their occurrence, interactions and response to host volatile terpenoids. In Redlin, S. C., Carris, L. M., eds. Endophytic fungi in grasses and woody plants. St. Paul: APS Press: 101-120.

Everett, R. L., Schellhaas, R., Keenum, D., Spurbeck, D., Ohlson, P. 2000. Fire history in the ponderosa pine/Douglas-fir foresst on the east slope of the Washington Cascades. Forest Ecology and Management 129: 207-225.

Fischer, W., Clayton, B. 1983. Fire ecology of Montana forest habitat types east of the Continental Divide. Gen. Tech. Rep. INT-141. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station, 83 p.

Fisher, P., Petrini, O., Petrini, L. 1991. Endophytic ascomycetes and deuteromycetes in roots of *Pinus sylvestris*. Nova Hedwigia 52: 11-15.

Flint, H. 1925. Fire resistance of northern Rocky Mountain conifers. Idaho Forester 7: 7-10, 41-43.

Franklin, J., Dyrness, C. T. 1973. Natural vegetation of Oregon and Washington. Gen. Tech. Rep. PNW-8. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Forest and Range Experiment Station, 417 p.

Gamboa, M. A., Laureano, S., Bayman, P. 2002. Measuring diversity of endophytic fungi in leaf fragments: Does size matter? Mycopathologia 156: 41-45.

Gange, A. C., Croft, R., Wu, W. 2002. Gall insect and endophytic fungal co-occurrence in a xeric and mesic site. Ecological Entomology 27: 362-265.

Ganley, R., Brunsfeld, S., Newcombe, G. 2004. A community of unknown, endophytic fungi in western white pine. PNAS 101: 10107-10112.

Grunig, C. R., Sieber, T. N., Rogers, S. O., Holdenrieder, O. 2002. Spatial distribution of dark septate endophytes in a confined forest plot. Mycological Research 106: 832-840.

Habeck, J. 1990. Old-growth ponderosa pine-western larch forests in western Montana: ecology and management. Northwest Environmental Journal 6: 271-292.

Habeck, R. J. 1992. *Pinus ponderosa* var. *ponderosa*. In: Fire Effects Information System, [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). Available: http://www.fs.fed.us/database/feis/ [2005, April 29].

Hadacek, F., Kraus, G. F. 2002. Plant root carbohydrates affect growth behaviour of endophytic microfungi. FEMS Microbiology Ecology 41: 161-170.

Halmschlager, E., Kowalski, T. 2004. The mycobiota in nonmycorrhizal roots of healthy and declining oaks. Canadian Journal and Botany 82: 1446-1458.

Harrington, M. 1991. Fire management in interior Douglas-fir forests. In: Baumgartner, David M., Lotan, James E., compilers. Interior Douglas-fir: The species and its management: Symposium proceedings; 1991 February 27 - March 1; Spokane, WA. Pullman, WA: Washington State University, Department of Natural Resource Sciences, Cooperative: 209-214.

Hausam, W., Liebscher, E. 1940. Uber fleckenbildungen auf lohgarem schafleder durch mikroorganismen. Collegium, HaltingenL 506-508.

Henderson, R. 1967. Thinning ponderosa pine in western Montana with prescribed fire. MS Thesis, University of Montana, Missoula, MT, 108 p.

Hermann, R., Lavender, D. 1990. *Pseudotsuga menziesii* (Mirb.) Franco Douglas-fir. In: Burns, Russell M.; Honkala, Barbara H., technical coordinators. Silvics of North America. Volume 1. Conifers. Agric. Handb. 654. Washington, DC: U.S. Department of Agriculture, Forest Service: 527-540.

Hessburg, P. F., Hansen, E. M. 2000. Infection of Douglas-fir by *Leptographium wageneri*. Canadian Journal of Botany 78: 1254-1261.

Hoff, J. A. 2002. Fungal diversity in woody roots of east-slope Cascade ponderosa pine (*Pinus ponderosa*) and Douglas-fir (*Pseudotsuga menziesii*). MS Thesis, Washington State University, Pullman, WA, 76 p.

Hoff, J., Klopfenstein, N., McDonald, G., Tonn, J., Kim, M., Zambina, P., Hessburg, P., Rogers, J., Peever, T., Carris, L. 2004. Fungal endophytes in woody roots of Douglas-fir (*Pseudotsuga menziesii*) and ponderosa pine (*Pinus ponderosa*). Forest Pathology 34: 255-271.

Holdenrieder, O., Sieber, T. 1992: Fungal associations of serially washed healthy non-mycorrhizal roots of *Picea abies*. Mycological Research 96: 151-156.

Hubbard, J. P., Harman, G. E., Hadar, Y. 1983. Effect of soilborne *Pseudomonas* spp. on the biological control agent, *Trichoderma hamatum*, on pea seeds. Phytopathology 73: 655-659.

Kalabokidis, K., Wakimoto, R 1992. Prescribed burning in uneven-aged stand management of ponderosa pine/ Douglas-fir forests. Journal of Environmental Management. 34: 221-235.

Kendrick, B., Smith, J.E., Weber, N.S. 1994. A study of morphological variability, character analysis, and taxonomic significance in the zygomycetous anamorph-like genus *Umbelopsis*. Mycotaxon 51: 15-26.

Kennelly, V. C. E., Grimes, M. 1930. *Paecilomyces hibernicus* new species. Sci. Proc. R. Dublin Soc. 19: 513-515.

Krajina, V. J., Klinka, K., Worrall, J. 1982. Distribution and ecological characteristics of trees and shrubs of British Columbia. Vancouver, BC: University of British Columbia, Department of Botany and Faculty of Forestry, 131 p.

Kwasna, H. 2004. Natural shifts in communities of rhizosphere fungi of common oak after felling. Plant and Soil 264: 209-218.

Lampi, A. 1960. The use of fire in ponderosa pine management. MS Thesis, Montana State University, Missoula, MT, 76 p.

Lillybridge, T.R., Kovalchik, B.L., Williams, C.K., Smith, B.G. 1995. Field guide for forested plant associations of the Wenatchee National Forest. USDA Forest Service General Technical Report PNW-GTR-359. Pacific Northwest Research Station, Portland, OR.

Lolley, M.R. 2005. Wildland fuel conditions and effects of modeled fuel treatments on wildland fire behavior and severity in dry forests of the Wenatchee Mountains. MS Thesis, University of Washington, Seattle, WA.

Lotan, J., Alexander, M., Arno, S., [and others]. 1981. Effects of fire on flora: A state-of-knowledge review. National fire effects workshop; 1978 April 10-14; Denver, CO. Gen. Tech. Rep. WO-16. Washington, DC: U.S. Department of Agriculture, Forest Service, 71 p.

Macmillan, W. C., Basu, S. N. 1947. The detection and estimation of damage in jute fibre. Part I. A new microscopical test and the implication of certain chemical tests. J. Text. Inst. 38: 350-367.

Macy, H., Combs, W. B., Morrison, H. B. 1931. The churn as a source of moulds in butter. Journal Dairy Science 14: 398-403.

Malinowski, D. P., Brauer, D. K., Belesky, D. P. 1999. The endophyte *Neotyphodium coenophialum*. Journal of Agronomy and Crop Science 183: 53-60.

Marchal, El., Marchal, Em. 1921. Contribution des champignons fructicoles de Belgique. Bull. Soc. Bot. Belg. 54: 108-129.

Mascera, E. 1940. Miceti patogeni al *Bombyx mori* L. Riv. Parassit. 4: 51-60.

Massee, G. 1898. British mycology. Transactions of the British Mycological Society 1: 24.

Meyer, W., Gams, W. 2003. Delimitation of *Umbelopsis* (Mucorales, Umbelopsidaceae fam. nov.) based on ITS sequence and RFLP data. Mycological Research 107: 339-350.

Midgley, D. J., Chambers, S. M., Cairney, J. W. G. 2002. Spatial distribution of fungal endophyte genotypes in a *Woollsia pungens* (Ericaceae) root system. Australian Journal of Botany 50: 559-565.

Moesz, G. 1921. Spicaria fimetaria. Bot. Kozl. 19: 58-59.

Morse, L. J., Day, T. A., Faeth, S. H. 2002. Effect of *Neotyphodium* endophyte infection on growth and leaf gas exchange of Arizona fescue under contrasting water availability regimes. Environmental Experimental Botany 48: 257-268.

Muller, M. M., Valjakka, R., Suokko, A., Hantula, J. 2001. Diversity of endophytic fungi of single Norway spruce needles and their role as pioneer decomposers. Molecular Ecology 10: 1801-1810.

Narisawa, K., Kawamata, H., Currah, R. S., Hashiba, T. 2004. Suppression of *Verticillium* wilt in eggplant by some fungal root endophytes. European Journal of Plant Pathology 108: 103-109.

Naumoff, N. A., Kiryalova, D. N. 1935. On two new mould fungi, Byssochlamys musticola and Spicaria taurica. [Trans. title]. Acta Institute Botanical Academy of Science URSS, Ser. II (Plant. crypt.) (1934) 2: 361-364.

Ohr, H. D., Munnecke, D. E., Bricker, J. L. 1973. The interaction of *Armillaria mellea* and *Trichoderma* spp. as modified by methyl bromide. Phytopathology 63: 965-973.

Park, J. O., Hargreaves, J. R., Cole, A. L. J., Ghisalberti, E. L., Gams, W., Sivasithamparam, K. 2001. Cuticular disruption and mortality of *Caenorhabditis elegans* exposed to culture filtrate of *Byssochlamys nivea* Westling. Nematology 4: 355-363.

Petch, T. 1936. New and rare Yorkshire fungi. Naturalist, Lond.: 59-60.

Porter, J. K., Bacon, C. W., Robbins, J. D. 1974. Major alkaloids of a *Claviceps* isolated from toxic Bermuda grass. Journal of Agricultural Food Chemistry 22: 838-841.

Ram, C. 1968. Timber-attacking fungi from the state of Maranhao, Brazil. Some new species of *Paecilomyces* with its perfect stage *Byssochlamys* Westl VIII. Nova Hedwigia 16: 305-314.

Raper, K. B., Thom, C. 1949. A manual of the Penicillia. London: Bailliere, Tindall and Cox.

Redlin, S. C., Carris, L. M. 1996. Endophytic fungi in grasses and woody plants. St. Paul: American Phytopathological Society Press: 223 p.

Redman, R. S., Dunigan, D., Rodriguez, R. 2001. Fungal symbiosis from mutualism to parasitism: who controls the outcome, host or invader? New Phytologist 151: 705-716.

Rillig, M. C., Field, C. B., Allen, M. F. 1999. Fungal root colonization responses in natural grasslands after long-term exposure to elevated atmospheric CO2. Global Change Biology 5: 577-585.

Rippi, R. 2005. U. S. Forest Service, Moscow, ID. Personal communication.

Ryker, R., Losensky, J. 1983. Ponderosa pine and Rocky Mountain Douglas-fir. In: Burns, Russell M., technical compiler. Silvicultural systems for the major forest types of the United States. Agric. Handb. 445. Washington, DC: U.S. Department of Agriculture, Forest Service: 53-55.

Saccardo, P. A. 1886. Sylloge fungorum 4: 166.

Saikkonen, K., Faeth, S. H., Helander, M., Sullivan, T. J. 1998. Fungal endophytes: A continuum of interactions with host plants. Annual Review of Ecological Systems 29: 319-343.

Saito, K. 1921. Investigations of sorghum brandy, kaoliangchim. [Trans. title]. Rep. S. Manchur. Rly. Co. cent. Lab. no. 6.

Sakaguchi, K., Inoue, T., Tada, S. 1939. On the production of ethyleneoxide-α, β-dicarboxylic acid by moulds (I). Zbl. Bakt. (Abt. II) 100: 302-307.

Samson, R.A., Tansey, M. R. 1975. *Byssochlamys verrucosa* sp. nov. Transactions of the British Mycological Society 65: 512-514.

Saveland, J. 1982. Predicting mortality and scorch height in ponderosa pine from understory prescribed burning. MS Thesis, University of Idaho, Moscow, ID, 39 p.

Schulz, B., Boyle, C., Draeger, S., Rommert, A., Krohn, K. 2002. Endophytic fungi: a source of novel biologically active secondary metabolites. Mycological Research 106: 996-1004.

See, P. 1919. Les maladies du papier pique. Paris: O. Doin et Fils.

Sen, R., Hietala, A. M., Zelmer, C. D. 1999. Common anastomosis and internal transcribed spacer RFLP groupings in binucleate *Rhizoctonia* isolates representing

root endophytes of *Pinus sylvestris*, *Ceratorhiza* spp. from orchid mycorrhizas and a phytopathogenic anastomosis group. New Phytologist 144: 331-341.

Sinclair, J. B., Cerkauskas, R. F. 1996. Latent infection vs. endophytic colonization by fungi. In Endophytic fungi in grasses and woody plants. Eds. Redlin, S. C., Carris, L. M. St. Paul: APS Press: 3-29.

Soil Survey Staff. 1995. Cashmere mountain soil survey: Leavenworth Ranger District soil handbook: non-wilderness. U. S. Department of Agriculture, Natural Resource Conservation Service. [Internal Report]: 374 p.

Sopp, O. 1912. Monographie der Pilzgruppe Penicillium mit besonderer Berucksichtigung der in Norwegan gefundenen Arten. Vidensk. (Skrift. I, Mat. Naturv. Kl.) no. II: 98-103.

SPSS for Windows. 2004. Version 13.0. SPSS Inc.

Steele, R., Pfister, R., Ryker, R., Kittams, J. 1981. Forest habitat types of central Idaho. Gen. Tech. Rep. INT-114. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station: 138 p.

Steinberg, Peter D. 2002. *Pseudotsuga menziesii* var. *glauca*. In: Fire Effects Information System, [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer).

Stolk, A.C., Samson, R.A. 1971. Studies on *Talaromyces* and related genera. I. *Hamigera* gen. nov. and *Byssochlamys*. Persoonia 6: 341-357.

Stone, J.K. 1986. Foliar endophytes of *Pseudotsuga menziesii* (Mirb.) Franco: cytology and physiology of the host-endophyte relationship. PhD Dissertation, University of Oregon, Eugene, OR.

Stone, J.K. 1987. Initiation and development of latent infections by *Rhabdocline parkeri* on Douglas-fir. Canadian Journal of Botany 65: 2614-2621.

Stone, J.K. 1988. Fine structure of latent infections by Rhabdocline parkeri on Douglas-fir, with observations on uninfected epidermal cells. Canadian Journal of Botany 66: 45-54.

Stone, J.K., Sherwood, M.A., and G.C. Carroll. 1996. Canopy microfungi: function and diversity. Northwest Science **7**0:37-45.

Stone, J.K., Viret, O., Petrini, O., Chapela, I. 1994. Histological studies of host penetration and colonization by endophytic fungi. In Host Wall Alterations by Parasitic Fungi (O. Petrini and G.B. Ouellette, eds), American Phytopathological Society Press, St Paul, MN: 115-128.

Sugiyama, M., Tokumasu, S., Gams, W. 2003. *Umbelopsis gibberispora* sp. nov. from Japanese leaf litter and a clarification of *Micromucor ramannianus* var. *angulisporus*. Mycoscience 44: 217-226.

Tabor, R.W., Waitt Jr., R.B., Frizzell Jr., V.A., Swanson, D.A., Byerly, G.R., Bentley, R.D. 1982. Geologic Map of the Wenatchee 1:100,000 Quadrangle Central Washington. [Reston, VA]: USGS Miscellaneous Investigations Series, MI 1-311: 25 p.

Taechowisan, T., Peberdy, J. F., Lumyong, S. 2003. Isolation of endophytic actinomycetes from selected plants and their antifungal activity. World Journal of Microbiology and Biotechnology 19: 381-385.

Thines, E., Anke, H., Weber, W. S. 2004. Fungal secondary metabolites as inhibitors of infection-related morphogenesis in phytopathogenic fungi. Mycological Research 108: 14-25.

Thom, C., Raper, K. B. 1932. The arsenic fungi of Gosio. Science 76: 548-550.

Uchytil, R. 1991. *Pseudotsuga menziesii* var. *menziesii*. In: Fire Effects Information System, [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer).

U. S. Department of Agriculture. Forest Service. Pacific Northwest Region. 1990. Final environmental impact statement, land and resource management plan, Wenatchee National Forest. Wenatchee, WA.

Vandegrift, V. 2002. Fungal diversity within decomposing woody conifer roots in Oregon. MS Thesis, Oregon State University, Corvallis, OR, 102 p.

Vandenkoornhuyse, P., Baldauf, S. L., Leyval, C., Straczek, J., Young, J. P. W. 2002. Extensive fungal diversity in plant roots. Science 295: 2051.

Van Hooser, D., Keegan, C., III. 1988. Distribution and volumes of ponderosa pine forests. In: Baumgartner, David M.; Lotan, James E., compilers. Ponderosa pine: The species and its management: Symposium proceedings; 1987 September 29 - October 1; Spokane, WA. Pullman, WA: Washington State University, Cooperative Extension: 1-6.

von Szilvinyi, A. 1936. Zur mikrobiologischen Kenntnis des Tobakeide auf Sumatra. Arch. Hydrobiol. (Supp., Band XIV, Tropische Binnengewasser) 6: 512-553.

Wang, J., Huang, Y., Fang, M., Zhang, Y., Zheng, Z., Zhao, Y., Su, W. 2002. Brefeldin A, a cytotoxin produced by *Paecilomyces* sp. and *Aspergillus clavatus* isolated from *Taxus mairei* and *Torreya grandis*. FEMS Immunology and Medical Microbiology 34: 51-57.

Weaver, H. 1968. Fire and its relationship to ponderosa pine. In: Proceedings, California Tall Timbers fire ecology conference; 1967 November 9-10; Hoberg, CA. Number 7. Tallahassee, FL: Tall Timbers Research Station: 127-149.

Wellner, C. 1970. Fire history in the northern Rocky Mountains. In: The role of fire in the Intermountain West: Symposium proceedings; 1970 October 27-29; Missoula, MT. Missoula, MT: Intermountain Fire Research Council. In cooperation with: University of Montana, School of Forestry: 42-64.

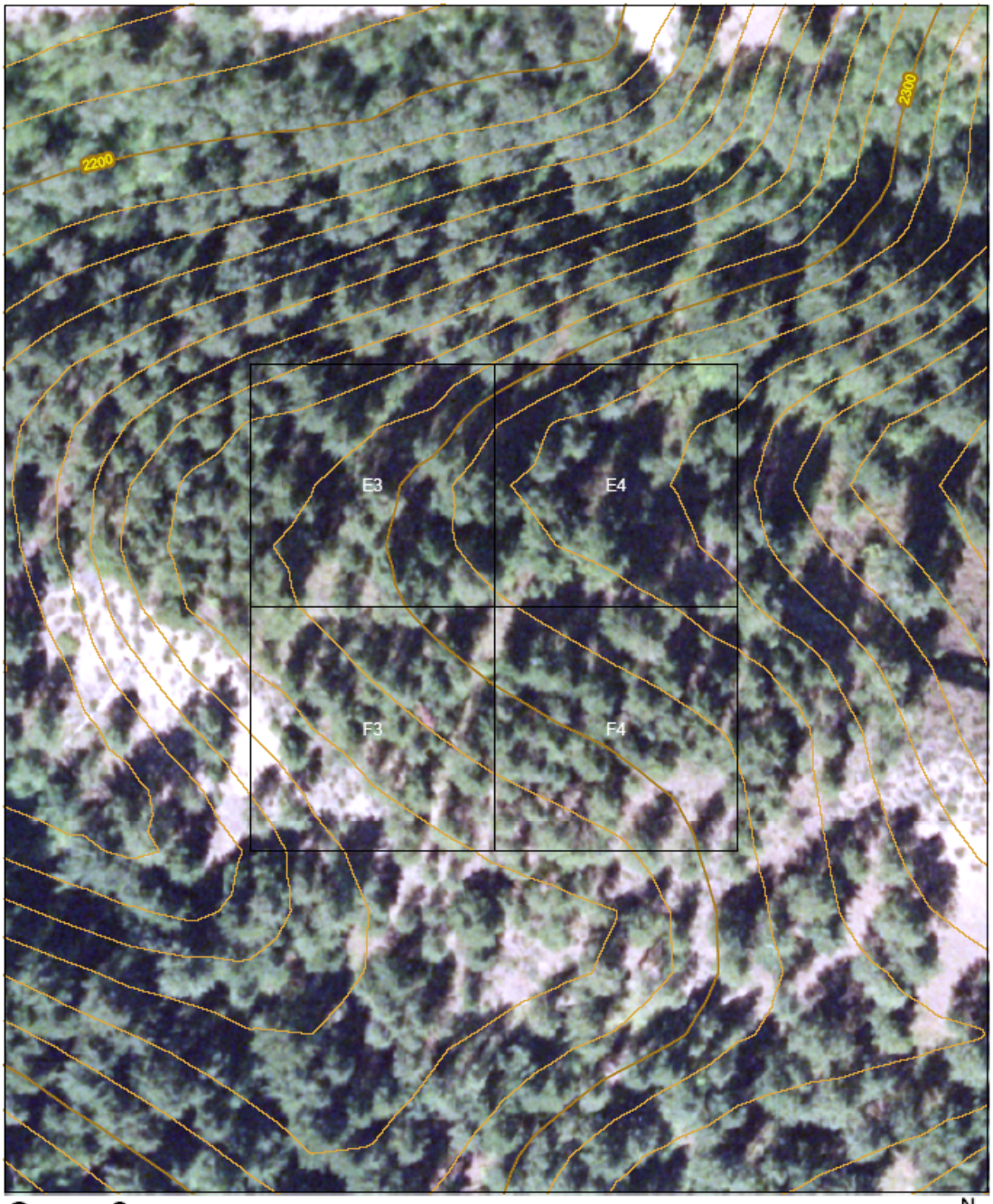
Western Regional Climate Center (WRCC). 2004. Climate summary for Leavenworth 3S, Washington (454572). [http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?waleav].

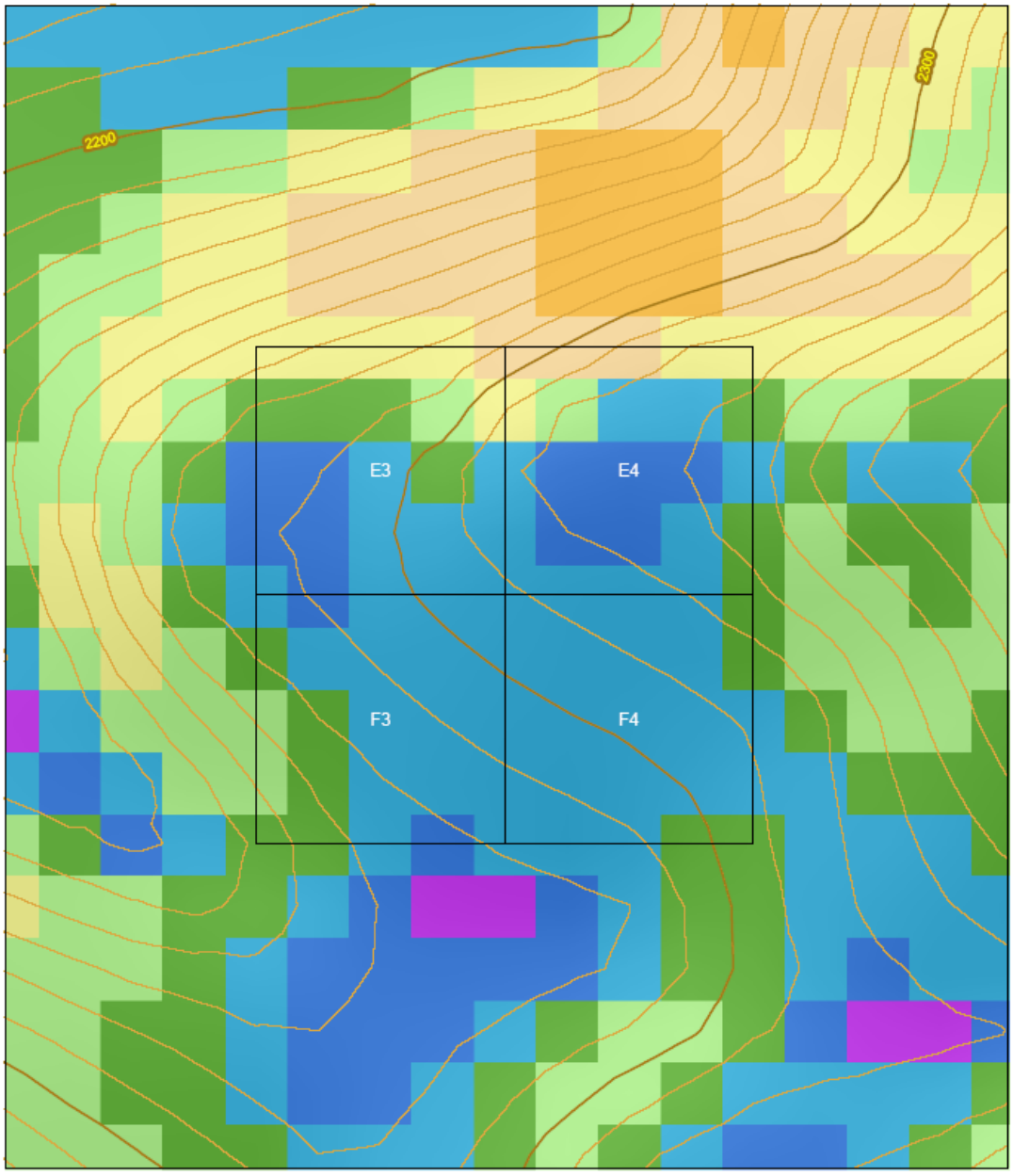
Wilson, D., Carroll, G.C. 1997 Avoidance of high-endophyte space by gall-forming insects. Ecology **7**8: 2153-2163.

Zar, J. H. 1999. Biostatistical Analysis, 4th Ed. Prentice Hall: New Jersey: 1-663.

APPENDIX A

Orthophoto, Slope, and Aspect Maps of the Three Study Sites





Crow 3

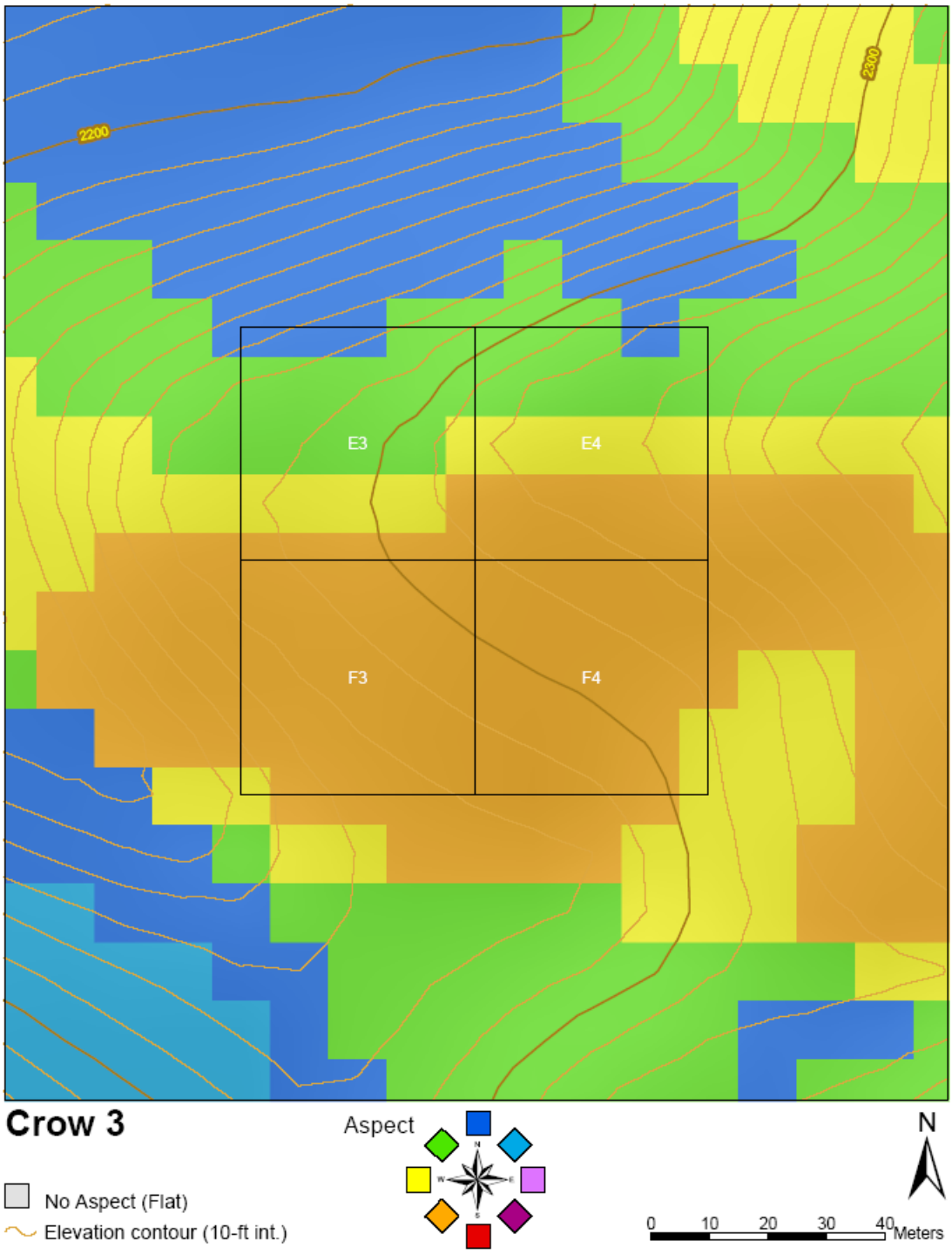
Slope (Percent)

0 to 10 | 10 to 20 | 20 to 30 | 30 to 40 | 40 to 50 | 50 to 60 | 60 to 70 | 70 to 80 | 80 to 90 | > 90

Elevation contour (10-ft int.)

0 10 20 30 40 Meters

N

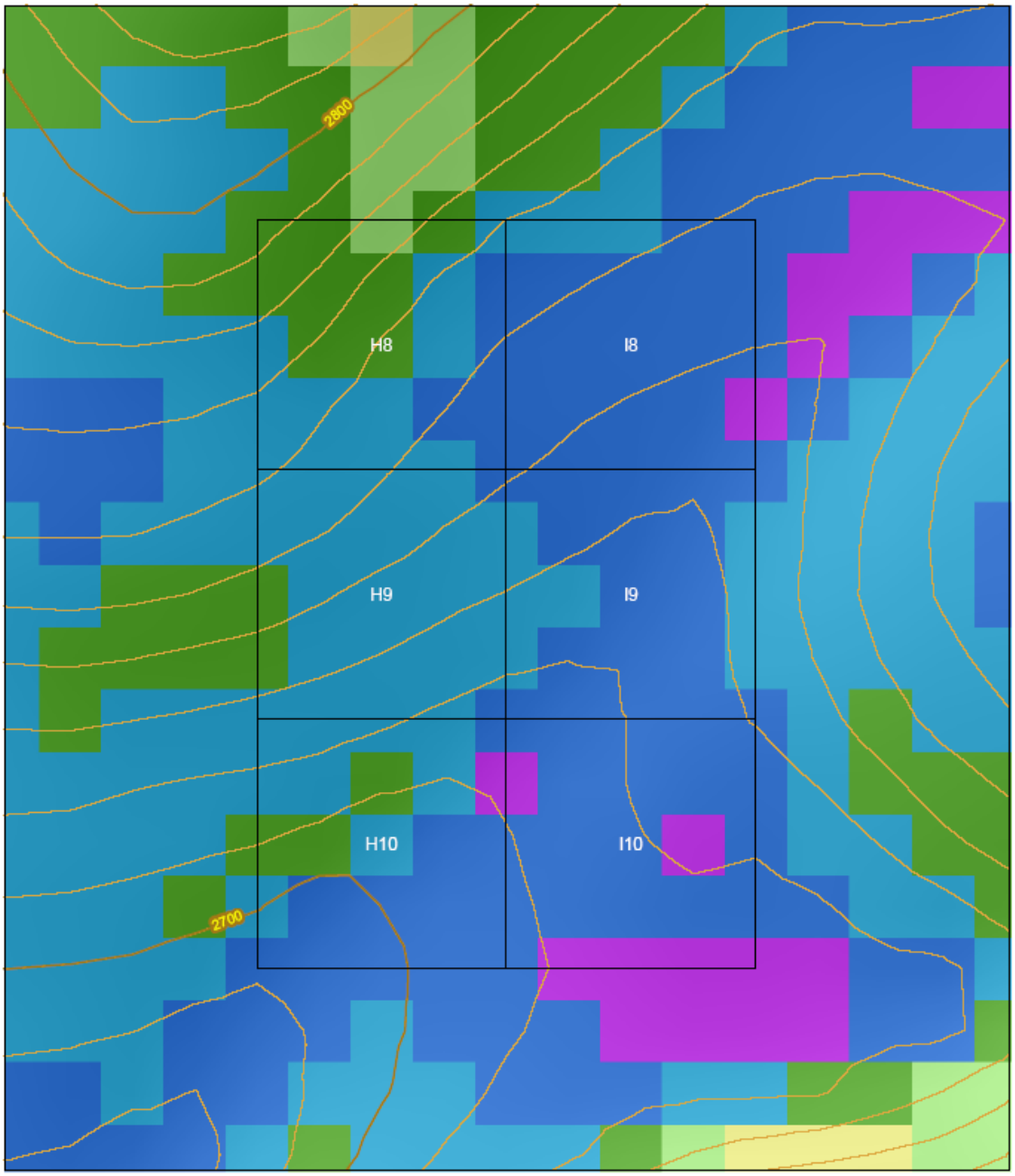

2200
2200
E3
E4
F3
F4
Crow 3
Aspect
N
No Aspect (Flat)
Elevation contour (10-ft int.)
0 10 20 30 40 Meters

Pendleton 30

N

Elevation contour (10-ft int.)

0 10 20 30 40 Meters



Pendleton 30

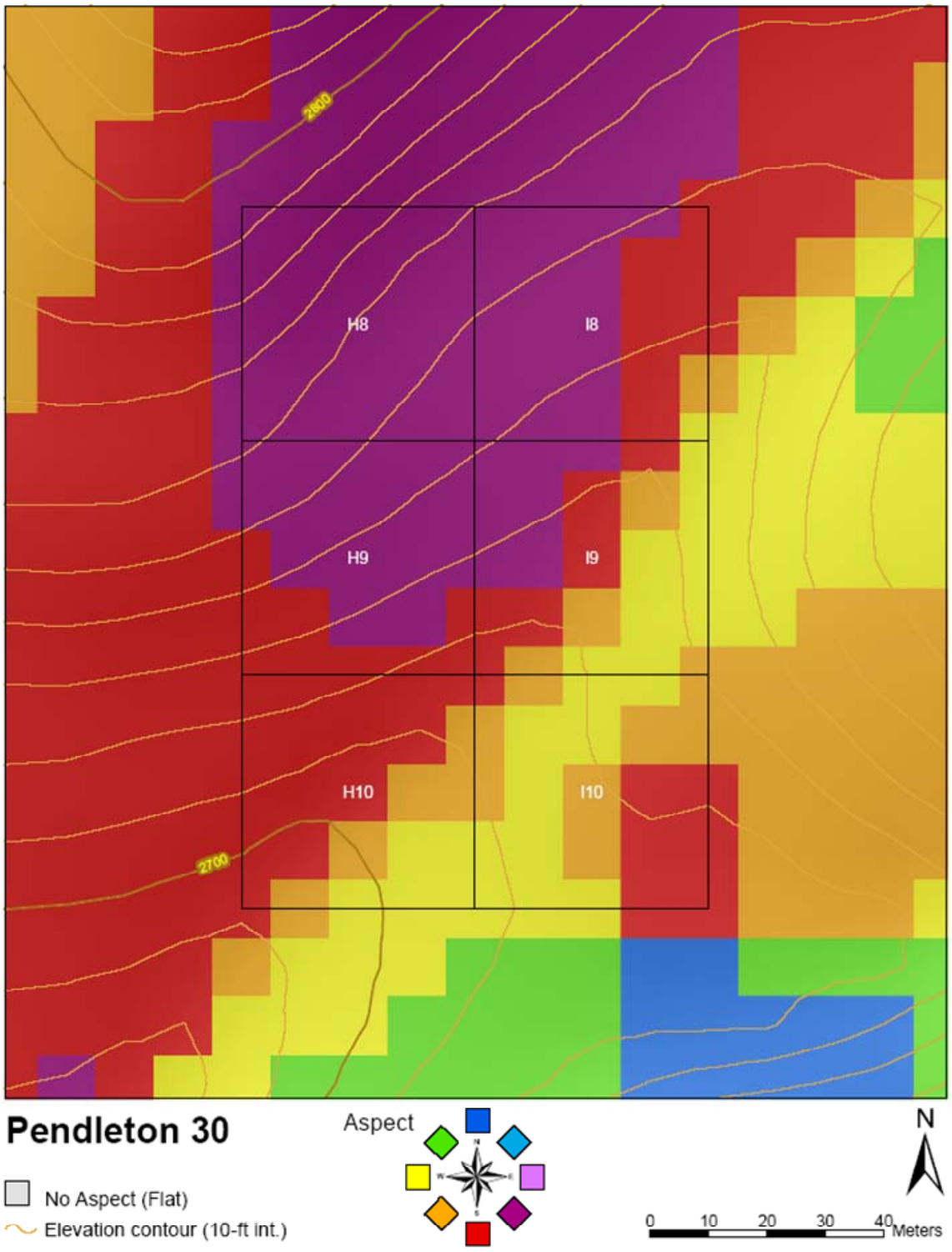
Slope (Percent)

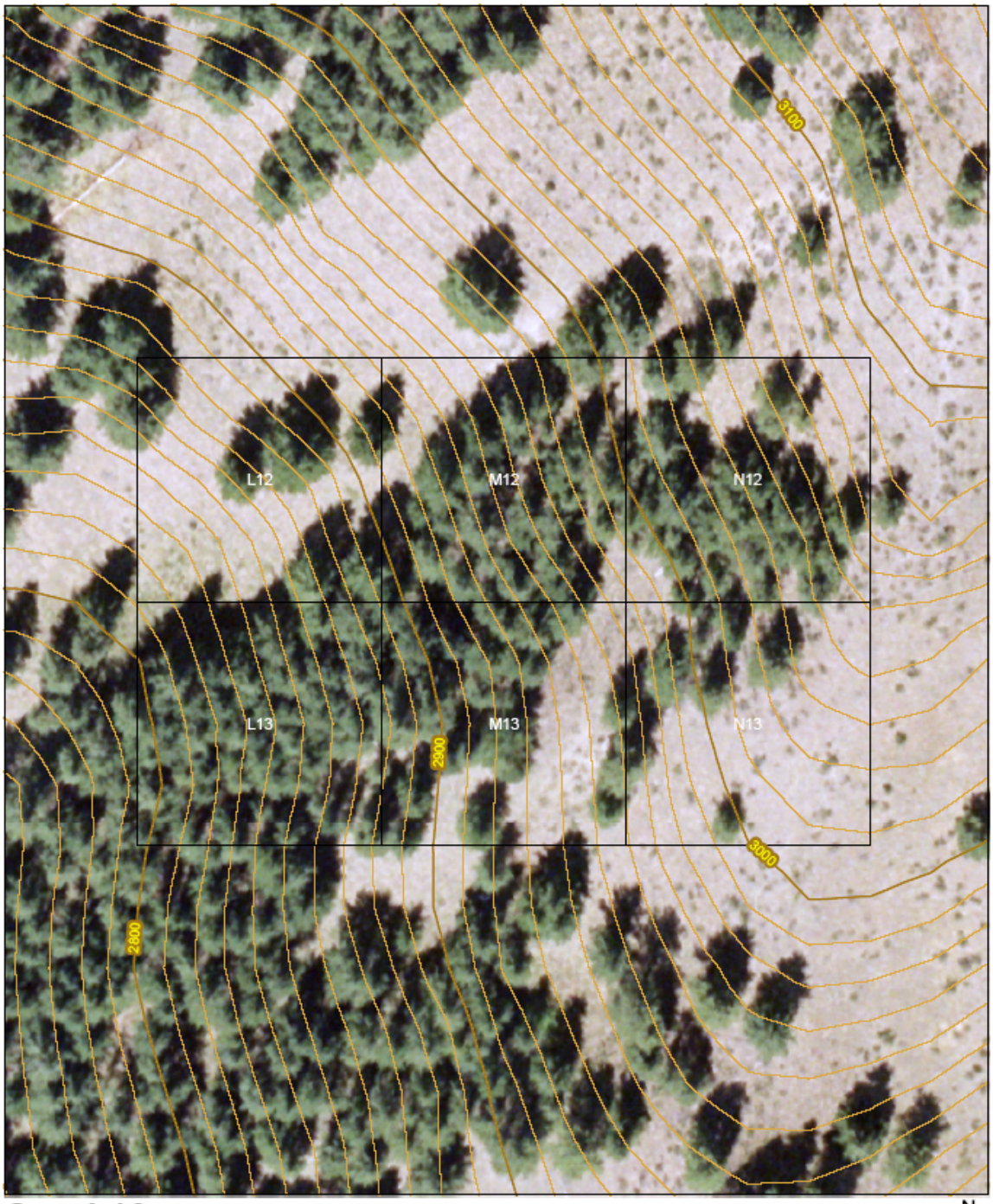
0 to 10 | 10 to 20 | 20 to 30 | 30 to 40 | 40 to 50 | 50 to 60 | 60 to 70 | 70 to 80 | 80 to 90 | > 90

Elevation contour (10-ft int.)

0 10 20 30 40 Meters

N


2800
H8
I8
H9
I9
H10
I10
2700
Pendleton 30
Aspect
N
No Aspect (Flat)
Elevation contour (10-ft int.)
0 10 20 30 40 Meters

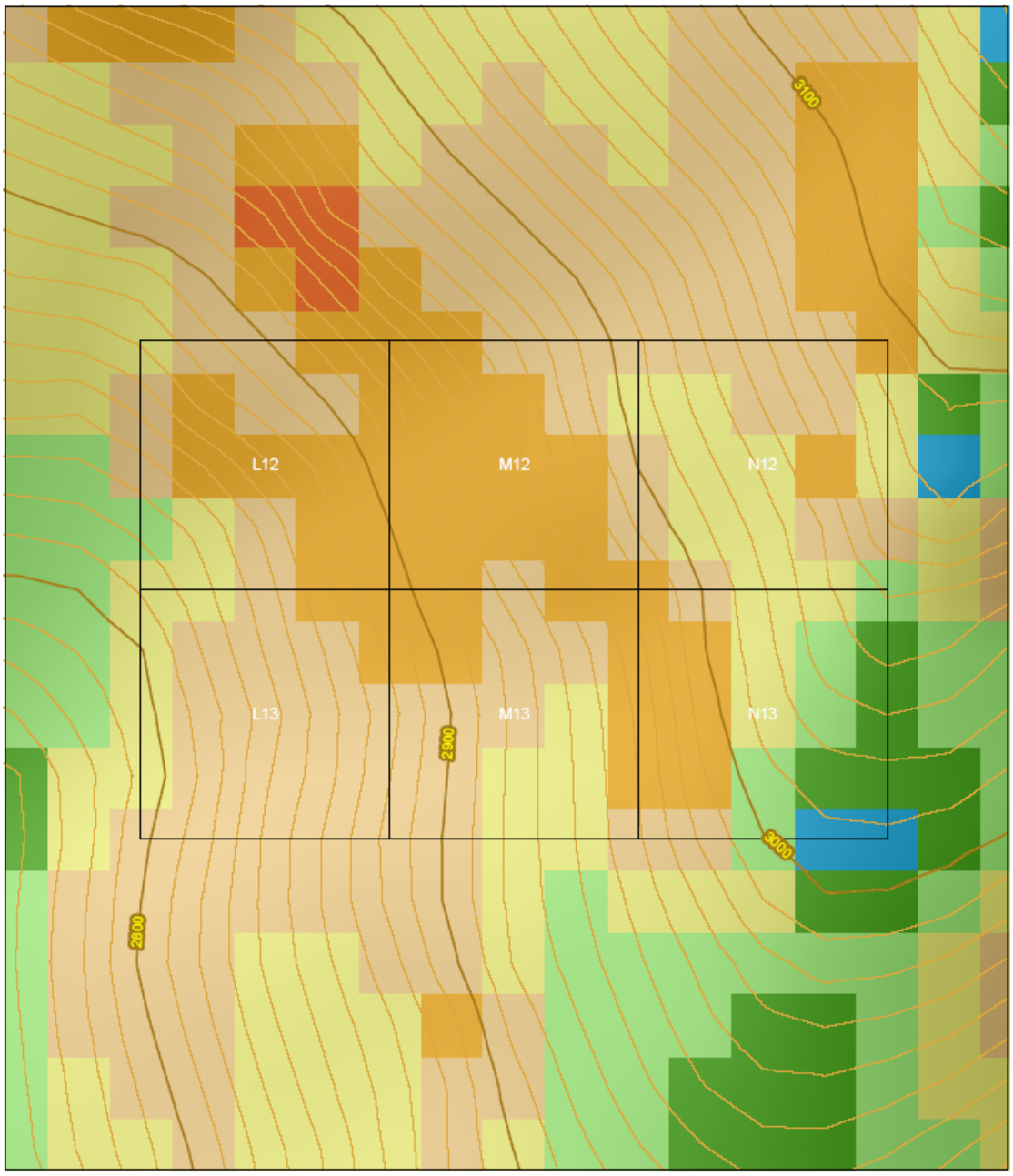


Sand 19

Elevation contour (10-ft int.)

N

0 10 20 30 40 Meters



Sand 19

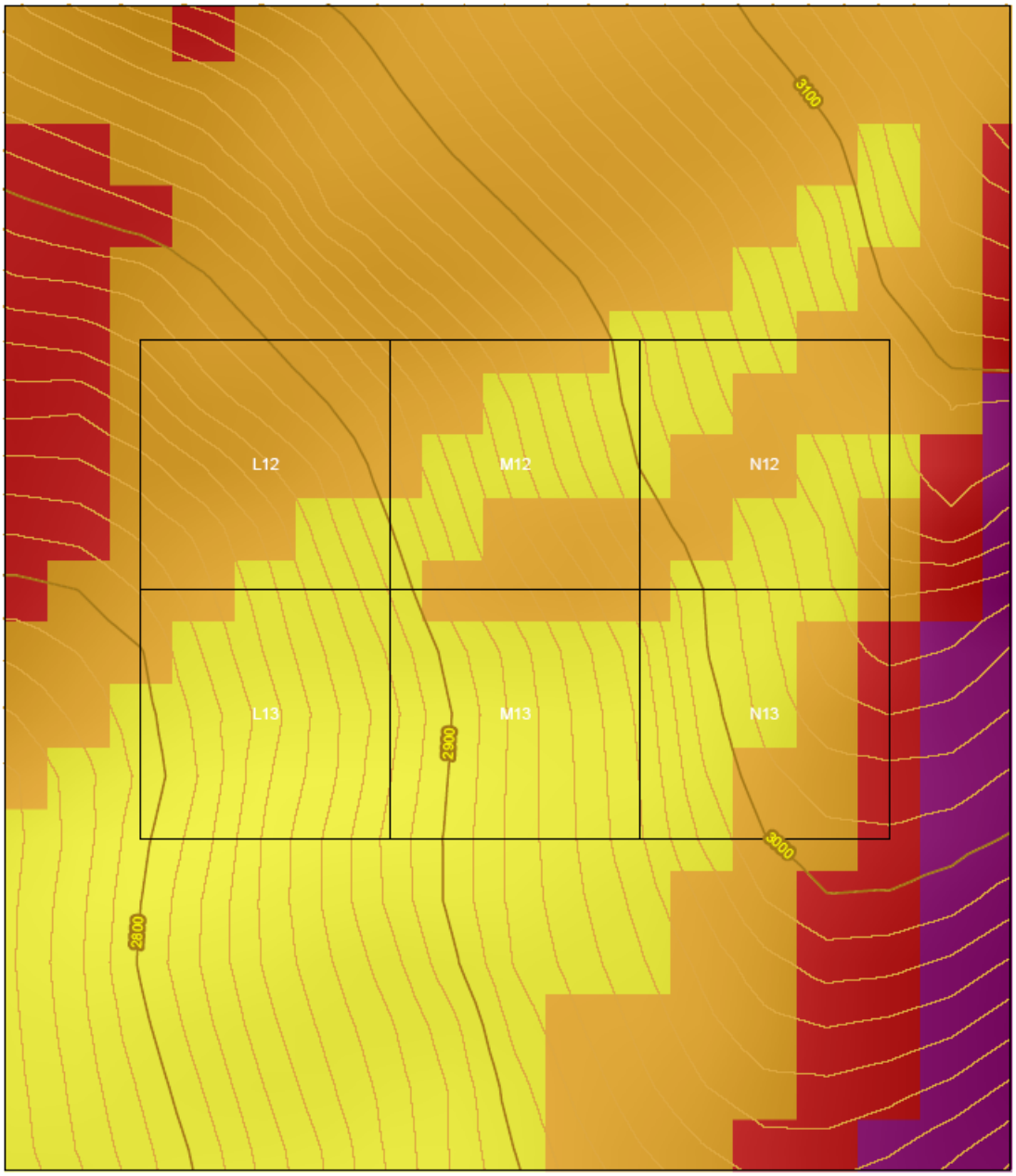
Slope (Percent)

0 to 10 | 10 to 20 | 20 to 30 | 30 to 40 | 40 to 50 | 50 to 60 | 60 to 70 | 70 to 80 | 80 to 90 | > 90

Elevation contour (10-ft int.)

0 10 20 30 40 Meters

N


3100
L12
M12
N12
L13
M13
N13
2900
3000
2800
Sand 19
Aspect
N
No Aspect (Flat)
Elevation contour (10-ft int.)
0 10 20 30 40 Meters

APPENDIX B

Fungal Isolates from USDA Forest Service, Forestry Sciences Laboratory in Moscow, ID: Number, Identification, Location, Host, and Plant Association

Isolate Identification	Isolate #	Original Location	Host	Plant Association
Armillaria ostoyae Romagn.	BC-286F	Curlew	PSME	PSME/PHMA
Armillaria ostoyae Romagn.	BC-284F	Chewelah	PIPO	ABGR
Armillaria ostoyae Romagn.	R957	Colville	ABLA	TSHE/CLUN
Byssochlamys nivea Westling	Tripp P9-2-1	Wenatchee	PSME	PSME/CARU
Umbelopsis versiformis Amos & HL Barnett	Crow 3-D7-1	Wenatchee	PIPO	PSME/AGSP
Umbelopsis vinacea (Dixon-Stew.) Arx	Crow 1-J11-1	Wenatchee	PIPO	PIPO/SYAL
Umbelopsis vinacea (Dixon-Stew.) Arx	Crow 3-E11-2	Wenatchee	PSME	PSME/SYAL

ABGR = *Abies grandis* (Dougl. ex D. Don) Lindl., ABLA = *Abies lasiocarpa* (Hook.) Nutt., AGSP = *Agropyron spicatum* (Pursh) Scribn. & J. G. Sm., CARU = *Calamagrostis rubescens* Buckl., CLUN = *Clintonia uniflora* (Menzies ex J. A. and J. H. Schultes), PHMA = *Physocarpus malvaceus* (Greene) Kuntze, PIPO = *Pinus ponderosa* P. & C. Lawson, PSME = *Pseudotsuga menziesii* (Mirbel) Franco, SYAL = *Symphoricarpos albus* (L.) Blake, TSHE = *Tsuga heterophylla* (Raf.) Sarg.

APPENDIX C

Frequency of *Byssochlamys nivea* and *Umbelopsis* spp. by site and root location

Frequency of *Byssochlamys nivea*

Total Samples Examined for *Byssochlamys nivea* : 214
B. nivea present: 31
Percent *B. nivea* present: 14.5

Site	Species Sampled	Root Location (cm)	Frequency of Occurrence
Crow 3	PIPO	10	4
	PIPO	30	0
	PSME	10	3
	PSME	30	0
Pendleton	PIPO	10	10
	PIPO	30	3
	PSME	10	3
	PSME	30	4
Sand 19	PIPO	10	0
	PIPO	30	0
	PSME	10	2
	PSME	30	2

Frequency of *Umbelopsis* spp.

Total Samples Examined for *Umbelopsis* spp.: 215
Umbelopsis spp. present: 55
Percent *Umbelopsis* spp. present: 26

Site	Species Sampled	Root Location (cm)	Frequency of Occurrence
Crow 3	PIPO	10	7
	PIPO	30	5
	PSME	10	1
	PSME	30	2
Pendleton	PIPO	10	4
	PIPO	30	3
	PSME	10	5
	PSME	30	3
Sand 19	PIPO	10	10
	PIPO	30	6
	PSME	10	5
	PSME	30	4