AN ABSTRACT OF THE THESIS OF

V. Eleanor Vandegrift for the degree of Master of Science in Forest Science presented on Date May 1, 2002.
Title: Fungal Diversity within Decomposing Woody Conifer Roots in Oregon.

Abstract approved: Signature redacted for privacy.
Mark E. Harmon

Signature redacted for privacy.
Hua Chen

Previous studies have indicated that roots from five tree species (*Picea sitchensis, Tsuga heterophylla, Pseudotsuga menziesii, Pinus ponderosa,* and *Pinus contorta*) decompose at different rates across an environmental gradient in Oregon. Measurements of wood chemistry from each tree species as well as moisture and temperature from each location do not explain the differences in decomposition rates. Molecular techniques were employed to generate Internal Transcribed Spacer - Restriction Fragment Length Polymorphism (ITS-RFLP) patterns to examine saprotrophic fungi in roots of these tree species and to see if differences in the fungal communities might explain observed differences in decomposition rates. However, due to a large number of ITS-RFLP patterns recovered and low levels of similarity in ITS-RFLP patterns across samples, we were unable to explain root decomposition based upon the fungal community information. Consequently, the analysis focused on comparing levels of ITS-RFLP similarity at each sampling level, determining the extent to which the sampling methods captured the total fungal biodiversity, and examining samples with microscopy and gene sequencing

techniques to identify fungi. Root samples were retrieved from trees cut seven to fifteen years prior to sampling. Two tree species were sampled at each of three locations across Oregon. DNA was extracted from hyphae samples collected directly from roots, and fungi cultured from root chips. DNA was also extracted from fungal herbarium specimens, field collected samples, and cultures of saprotrophic fungi. To analyze genetic diversity of the samples, they were amplified using polymerase chain reaction (PCR) techniques, digested with endonucleases (*Hinf* I, *Dpn* II, and *Hae* III), and ITS-RFLP patterns were evaluated. Nei and Li similarity index analyses were used to compare differences in fungal composition based upon ITS-RFLP patterns between tree species, sites, and harvest stands. Over two hundred distinct ITS-RFLP patterns were recognized from fungal samples. Similarities in ITS-RFLP patterns of hyphal and cultured samples ranged 0 to 1, where 0 indicated no overlap and 1 indicated 100% matching of ITS-RFLPs. When all ITS-RFLPs obtained from each stump were combined, similarities in patterns between sites ranged from 0 to 0.07, from 0 to 0.13 between tree species, from 0 to 0.11 between harvest stands, and from 0 to 0.67 between individual stumps. Linearly increasing ITS-RFLP sampling intensity curves indicate a large diversity of fungi. Using microscopy, cultured samples were examined for hyphae and reproductive structures. In culture, zygomycetous structures were prevalent. DNA gene sequences of the nuclear large and small subunits were used to place unknown ITS-RFLP patterns into family and generic groups. Twenty-three common and five uncommon ITS-RFLP patterns were sequenced; most matched with the Mortierellaceae and Mucoraceae families of the zygomycetes. The large diversity of ITS-RFLP patterns indicates the coarse roots provided habitat to many fungi at the stage of decomposition when samples were collected.

Fungal Diversity within Decomposing Woody Conifer Roots in Oregon

by

V. Eleanor Vandegrift

A THESIS

submitted to

Oregon State University

in partial fulfillment of

the requirements for the

degree of

Master of Science

Presented May 1, 2002

Commencement June 2002

ACKNOWLEDGEMENTS

I would like to thank my advisors Mark Harmon and Hua Chen and committee members Tom Horton and Joey Spatafora for all of their help, never-ending ideas, and encouragement during my time at OSU. Thank you to John Bliss for serving as the graduate school representative to my committee.

I have been fortunate to have received advice, help with fieldwork, instruction on lab techniques, and lab space to carry out my research from a large number of people. Thanks specifically to Kermit Cromack, Jr., Joyce Eberhardt, Becky Fasth, Camille Frietag, Admir Giachini, Bob Griffiths, Amy Grotta, Jill Hoff, Kathy Jacobs, Michelle Jeffers, Kentaro Hosaka, Dan Luoma, Doni McKay, Jay Sexton, Jane Smith, Pearce Smithwick, Gi-Ho Sung, Wendy Sutton, Kirk Waterstripe, Travis Wooley, and Yuriko Yano for all of your help in steering my project in the right direction. Kerry O'Donnell provided primers for sequencing.

Thanks to the Departments of Forest Science and Botany and Plant Pathology at Oregon State University and the Pacific Northwest Research Station of the US Forest Service for use of their facilities. Field research was conducted on the Siuslaw National Forest, Cascade Head Experimental Forest, H.J. Andrews Experimental Forest (Willamette National Forest), and Deschutes National Forest. I am grateful for access to field sites. This research was funded by USDA grant # 99-35107-7783.

I wish to thank my extended family and friends for trying to understand the reasons, methods, and results of my research. Thanks to all of my Forest Science cohorts for great discussions in the computer labs—sometimes related to our theses. Finally, a big thank you to Inky and Deven Holmgren for providing support and lots of study breaks and stress relievers from school and thesis writing.

TABLE OF CONTENTS

Page

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF APPENDICES

FUNGAL DIVERSITY WITHIN DECOMPOSING WOODY CONIFER ROOTS IN OREGON

CHAPTER 1 INTRODUCTION

V. Eleanor Vandegrift

Fungi play important ecological roles in ecosystems as saprotrophs, parasites, and symbionts with other organisms. It is estimated that worldwide there may be three million fungal species of which only a fraction have been identified (Hawksworth 1991). To date, 8,500 species of saprotrophs have been identified that are known to decompose a wide variety of substrates (Hibbett et al. 2000). Saprotrophic fungi have a principal role in the decomposition of organic matter and hence the cycles of carbon and nutrients throughout the planet (Gilbertson 1980). The activity of fungi and other saprotrophs are often easily witnessed and even measured within decaying coarse and fine woody debris aboveground. The community of fungi within decomposing wood plays a part in the decay rate of lignin, cellulose, and hemicellulose and which in turn changes the structure of decomposed wood. Several studies have measured aboveground saprotrophic fungal activity and diversity and microfungi diversity in soil (Christensen 1969, Polishook et al. 1996, Edmonds and Lebo 1998, Allen et al. 2000, Peter et al. 2001). However, few studies have concentrated on the belowground saprotrophic fungal communities within coarse woody roots.

Chen and others (2001 and 2002) studied multiple aspects of root decomposition among several conifer tree species including rates of decay, wood chemistry, and respiration. They measured the decay rates of fine and coarse roots from stumps of five tree species at three sites across an environmental gradient from a wet and warm maritime climate along the Oregon coast to a dry and cool climate in eastern Oregon. *Picea sitchensis, Tsuga heterophylla, Pseudotsuga menziesii, Pinus ponderosa,* and *Pinus contorta* were studied with two tree species at each of three locations with *Tsuga heterophylla* appearing at two locations. There were differences in decay rates among species with *Pinus ponderosa,* from the eastern Oregon, having the fastest rate of decomposition. However, the differences in the rates of decay are not completely explained by moisture or temperature at each location or wood chemistry as measured from each of the tree species. These differences might be caused by the difference in decomposer

communities. Wood from the same tree species may decompose at different rates depending upon the fungal species present, which could be influenced by site location (Ritschkoff 1996). The communities of saprotrophic fungi may also vary between tree species and affect root decomposition. To test for potential site differences in decomposition, Chen collected root samples from four tree species and buried them in different locations (Chen et al. 2002). Roots will be measured for long-term differences in decomposition when location is not a confounding factor.

There has been limited research into the saprotrophic fungal communities associated with coarse roots. Increased knowledge of the root fungal communities from different tree species could help to increase our understanding of the process of root decomposition and the role that fungi play. The first goal of this study was to explore the genetic diversity as determined by internal transcriber spacer region-restriction fragment length polymorphisms (ITS-RFLP) and gene sequences of fungi found in five species of roots seven to fifteen years after tree harvest. The objectives of this study were to use molecular techniques to examine potential similarities or differences in fungal communities on roots of each tree species that might explain differences in decomposition.

Chapter 2 contains a review of literature on wood decomposition, saprotrophic fungi diversity, mycodiversity, Zygomycota, molecular techniques used to identify fungi, and fungal succession. Chapter 3 has methods and results from this study. Chapter 4 has conclusions from the research.

LITERATURE CITED

Allen, R.B., P.K. Buchanan, P.W. Clinton, and A.J Cone. 2000. Composition and diversity of fungi on decaying logs in a New Zealand temperate beech (Nothofagus) forests. Canadian Journal of Forest Research 30:1025-1033.

Chen, H., M.E. Harmon, and R.P. Griffiths. 2001. Decomposition and nitrogen release from decomposing woody roots in coniferous forests of the Pacific Northwest: A chronosequence approach. Canadian Journal of Forest Research 31:246-260.

Chen, H. M.E. Harmon, J. Sexton, and B. Fasth. 2002. Fine-root decomposition and N dynamics in coniferous forests of the Pacific Northwest, U.S.A. Canadian Journal of Forest Research 32:320-331.

Christensen, M. 1969. Soil microfungi of dry to mesic conifer-hardwood forest in northern Wisconsin. Ecology 50:9-27.

Edmonds R.L. and D.S. Lebo. 1998. Diversity, production, and nutrient dynamics of fungal sporocarps on logs in an old-growth temperate rain forest, Olympic National Park, Washington. Canadian Journal of Forest Research 28:665-673.

Gilbertson, R.L. 1980. Wood-rotting fungi of North America. Mycologia 72:1-49.

Hawksworth, D.L. 1991. The Fungal Dimension of Biodiversity Magnitude Significance and Conservation. Mycological Research 95:641-655.

Hibbett, D.S., L.-B. Gilbert, and M.J. Donoghue. 2000. Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. Nature (London) 407:506-508.

Peter, M., F. Ayer, and S. Egli. 2001. Nitrogen addition in a Norway spruce stand altered macromycete sporocarp production and below-ground ectomycorrhizal species composition. New Phytologist 149:311-325.

Polishook, J.D., G.F. Bills, and D.J. Lodge. 1996. Microfungi from decaying leaves of two rain forest trees in Puerto Rico. Journal of Industrial Microbiology. 17:284-294.

Ritschkoff, A.-C. 1996. Decay mechanisms of brown rot fungi. Technical Research Centre of Finland, Espo. VTT Publications 268.

CHAPTER 2 SAPROTROPHIC FUNGAL DIVERSITY

V. Eleanor Vandegrift

DECOMPOSITION OF COARSE ROOTS, LOGS, AND STUMPS

Coarse woody debris is an essential component of productive forest ecosystems. Decomposing wood provides a seedbed for trees. Invertebrates, mammals, birds, and plants utilize habitat provided by coarse woody debris as snags, logs, stumps, and belowground roots (Renvall 1985, Harmon and Franklin 1989, Harmon et al. 1986). Decomposing roots may also aid in the formation of mycorrhizae and tree seedling establishment (Harvey et al. 1976). Forest soils are enhanced with the by-products of fungal metabolism from woody material decomposition. These by-products increase soil water holding capacity, nutritional stores, and atmospheric cation exchange while providing a site for nitrogen fixation and mycorrhizal root development (Gilbertson 1980, Gilbertson 1981). The decomposition of wood adds stable organic matter and a rich nutrient base to forest soils that may remain for up to five hundred years (Gilbertson 1980, Gilbertson 1981).

Decomposition of coarse woody debris is influenced by the woody substrate, activities of saprotrophic fungi and microorganisms, and external abiotic factors. The number of fallen logs and the rate of decomposition of coarse woody debris can vary depending upon the forest type and location on the landscape (Maser and Trappe 1984). Areas within a stump, log, or snag may decompose at different rates due to variation in the levels of carbon, nitrogen, minerals, and organic molecules. As fungi have specific nutritional needs, the initial nutrient base of wood can influence the ability of fungi to acquire the carbohydrates necessary for continued metabolism and decomposition (Gilbertson 1980, Rayner and Boddy 1988, Boddy 1992).

Bacteria, yeasts, saprotrophic and sugar fungi, and invertebrates facilitate wood decomposition. The initial colonization of wood by bacteria and other microorganisms enables saprotrophic fungi to invade the wood and utilize available resources (Rayner and Boddy 1988, Boddy 1992). Colonization and breakdown of

wood components, by macro- and microorganisms may release particular resources needed by future decomposers allowing wood decomposition to continue (Rayner and Boddy 1988). As substrate quality alters, the community composition of these organisms may change with a lower diversity of fungi found in later stages of decay (Allen et al. 2000). When most of the largest molecules have been broken down, zygomycetes, bacteria, yeasts, and other microfungi may be the only organisms readily cultured from wood (Rayner and Boddy 1988).

The interaction of environmental factors (temperature, moisture, pH, and light levels) influence hyphal growth more than the individual components alone which in turn affects the activity of decomposers and the rate of decomposition (Shea 1960, Hulme and Shields 1970, Gilbertson 1980, Illman et al. 1988, Rayner and Boddy 1988, Boddy 1992). Depending upon individual species requirements, fungal growth is optimized at temperatures between twenty and forty degrees Celsius. Excessive light tends to hinder growth and decreases hyphal wood colonization, while manganese, iron, and other elements are necessary for metabolism (Shea 1960, Illman et al. 1988, Boddy 1992, Ritschkoff 1996). Wood moisture content between thirty to eighty percent provides a favorable habitat for fungal growth and higher levels may inhibit hyphae growth (Ritschkoff 1996). Depending upon the species requirements, relative humidity between ninety-five to ninety-eight percent creates conditions optimal for decomposition (Ritschkoff 1996). Localized variation in the most advantageous conditions for fungal growth may affect the decomposition rate of woody debris (Allen et al. 2000).

SAPTROTROPHIC FUNGI

Through wood decomposition, fungi help to recycle carbon and nutrients from dead material into sources accessible for other living material (Gilbertson 1980). Many primary decomposers are basidiomycete and ascomycete fungi.

However, other microorganisms including zygomycetes are important in wood decomposition (Rayner and Boddy 1988, Boddy 1992). Fungal communities expand in a decay column along the length of a tree or down log rather than radially from the heartwood to the bark or vice versa as this is the path of least resistance (Boddy 1992, Frankland 1992). The expanse of hyphae throughout, however, is always affected by resource availability (Cooke and Rayner 1984).

Carbon is a limiting resource for fungal growth, and depending upon their nutrient specialization, saprotrophic fungi require cellulose, hemicellulose, and lignin to varying degrees (Boddy 1992). As major components of vascular plants, lignin and cellulose are some of the most abundant organic carbon molecules on earth (Tien and Kirk 1983). Lignin has been discovered in limited percentages in the earliest known vascular plants, evidence of its ancestral nature as a major component of plants (Robinson 1990). The conditions in the environment and microclimate may determine which type of fungi will grow most favorably; yet, many fungi are well designed to invade wood (Rayner and Boddy 1988, Boddy 1992, Winandy and Morrell 1993).

Wood-rotting fungi are ubiquitous in North America and most known species appear to be endemic with the majority of research focused on aboveground saprotrophic fungi (Gilbertson 1980, Gilbertson 1981, Peter et al. 2001). Some of the common species in the Pacific Northwest include *Ischnoderma resinosum, Pleurocybella porrigens, Naematoloma fasciculare, Naematoloma capnoides,* and *Mycena maculata* (Edmonds and Lebo 1998). Some fungi such as *Botryobasidium ponderosum* may be restricted to a narrow ecological niche due to resource availability or competition (Larsen et al. 1996). Others such, as *Fomitopsis pinicola,* may be found on a wider variety of woody substrates and are able to capture resources from more diverse niches. Based on major morphological remains of decomposed wood, white-, brown- and soft-rot fungi are identified as the three major forms of saprotrophic fungi on wood (Otjen and Blanchette 1986, Ritschkoff 1996). The major wood decayers, white- and brown-rots, are

characterized by different enzymatic processes and preferentially decompose different carbon components in wood (Green and Clausen 1999). Zygomycetes are also commonly found in wood throughout the decomposition process and are another component of the fungal community on coarse woody debris.

White-rot basidiomycetes

White-rot fungi break down lignin and cellulose leaving behind stringy, white colored wood consisting of the remaining carbon-rich cellulose support structure that can provide a carbon source for other organisms (Ritschkoff 1996). Lignin makes up eighteen to thirty-five percent of the dry weight in wood (Boddy 1992). Generally, white-rots decompose wood more quickly than brown-rots (Tanesaka et al. 1993). White-rot fungi include both ascomycetes and basidiomycetes (Rayner and Boddy 1988, Boddy 1992). Lignin from hardwood trees, (i.e. Angiosperms), is more quickly degraded than the lignin found in conifers, (i.e. Gymnosperms) (Otjen et al. 1987). Due to variation in fungal metabolic activity, lignin degradation can occur evenly throughout woody debris or be restricted to localized decay pockets (Blanchette et al. 1988).

Species of white-rot fungi demonstrate different forms of degradation in wood. In one recognized form, the fungus simultaneously attacks lignin, cellulose, and hemicellulose, and in another form, lignin is attacked first with hemicellulose and cellulose breakdown occurring later (Blanchette 1984, Otjen et al. 1987, Yoshizawa et al. 1992, Worrall et al 1997). Some white-rot fungi favor early decomposing wood and others prefer later decomposing wood (Blanchette 1984). Some species of white-rot fungi may attack the same piece of wood in multiple ways, causing a speckled appearance in the remaining wood (Blanchette et al. 1988). At least twenty-four enzymes are part of the white-rot degradation process with dominant enzymes varying between species of basidiomycetes (Yoshizawa et

al. 1992, Green and Clausen 1999, Loenowicz et al. 1999). These enzymes are split into three functional groups which can act individually or in cooperation with each other (Loenowicz et al. 1999). The first includes enzymes that can breakdown lignin, hemicellulose, and cellulose directly. The second group consists of those that aid the first group, but never directly attack components in the wood. The third group of enzymes helps to create feed back loops throughout the process to continue decomposition.

Because of their lignin degradation properties, white-rot fungi have been used for industrial delignification in biopulping, bleaching, wood chip production, sugar-cane bagasse, and straw production (Ander and Eriksson 1977, Blanchette et al. 1988). Combinations of species of white-rot fungi on various tree species influence lignin breakdown and the end wood product (Blanchette et al. 1988). Hence, fungi and substrates can be combined to tailor lignin degradation to create specific wood products (Ander and Eriksson 1977).

Brown-rot basidiomycetes

Through consumption of the rich carbohydrates available, brown-rot fungi remove cellulose and hemicellulose from wood, leaving lignin. In each tree species, these molecules are broken down at different rates (Ritschkoff 1996). Most brown-rots are associated with dead conifer trees and all appear to be basidiomycetes (Gilbertson 1981, Rayner and Boddy 1988, Boddy 1992, Winandy and Morrell 1993). Cellulose may constitute forty to fifty percent of a plant cell wall and hemicellulose twenty-five to forty percent (Boddy 1992, Ritschkoff 1996). The removal of cellulose and hemicellulose gives wood a brown, crumbly appearance and correlates with a loss of strength and density in the wood (Green et al. 1991, Winandy and Morrell 1993, Ritschkoff 1996, Allen et al. 2000). One of the by-products of cellulose decomposition is sugar which in turn provides

nutrients for other wood inhabitants such as zygomycetes, bacteria, and invertebrates.

Early indicators of brown-rot wood decay are relatively large hyphae compared to bore holes in the wood and hyphae penetrating throughout the wood (Wilcox 1993, Ritschkoff 1996). Wood with early incipient decay may still look sound, but has often already lost considerable strength (Winandy and Morrell 1993). The bore holes remain in later stages of brown-rot decay, but over time they increase in size. As the holes increase, birefringence is lost creating the characteristic brown, crumbly, unstable wood. The widening holes create an opportunity for increased and diversified fungal colonization throughout the wood (Frankland 1992). Hemicellulose may form an external layer around cellulose within wood and hence must be broken down prior to cellulose (Winandy and Morrell 1993). In culture, cellulose is broken down by oxidation and acid hydrolysis (Green et al. 1991, Ritschkoff 1996). Areas of wood decomposed by brown-rot fungi tend to have low pH indicating the presence of acid in the wood. It appears that the acid and consequential low pH may also play a role in oxidizing iron from wood; and hence, the Fenton reaction (requiring iron) is thought to be important in brown-rot decay. Oxalic acid production is important for fungi to initiate decomposition for various combinations of tree and fungal species (Green et al. 1991). However, it is not the only factor involved. Acid production in the early stages of decomposition helps to initiate depolymerization of hemicellulose and cellulose. As brown-rot decomposition continues, fungal hyphae spread throughout wood while weight and strength decrease. In addition to proper atmospheric conditions and acid levels, brown-rot fungi require minerals such as nitrogen, iron, zinc, manganese, molybdenum, and nickel and vitamins such as thiamine to continue colonization and wood decomposition (Ritschkoff 1996).

Zygomycota

Species from the phylum of fungi, Zygomycota, are ubiquitous throughout the landscape. Zygomycota includes two classes, zygomycetes and trichomycetes. Trichomycetes are often found within the digestive tract of arthropods (Hawksworth et al. 1995). Eight hundred sixty-seven known species of zygomycetes have been identified, many of which are commonly found in air, soil, dung, tree seedlings at various ages, cultures in greenhouses, and the dark regions of caves in India (Christensen 1969, Parkinson and Crouch 1969, Kendrick et al. 1994, Koilraj et al. 1999, O'Donnell et al. 2001, Watanabe et al. 2001). Along with other microfungi, zygomycetes are often found in the early and late stages of wood decay or at interactions zones between saprotrophic fungi (Boddy 1992, Frankland 1992). Zygomycetes are found in many regions, can grow quickly, and obtain nutrients from a variety of simple carbohydrates substrates (O'Donnell et al. 2001).

As well as having a wide geographic diversity, zygomycetes play a variety of ecological roles. Zygomycetes have been recorded as parasites, endo- and ectomycorrhizae, obligate symbionts of aquatic larvae, entomopathogens, decay agents of produce, biocatalysts, facultative or obligate parasites, fermenting agents of soybean products, immunocompromised infections in animals, and saprobes in both wood and bark of roots (Kuhlman 1969, Benny 1995, O'Donnell et al. 2001, Voigt and Wöstemeyer 2001). One order of zygomycetes, Mucorales, is called "sugar fungi", and they are found in the soil where rich carbon sources are available (Kuhlman 1969). Many zygomycetes are easily cultured as they grow well with easy access to simple sugars (Kuhlman 1969, Parkinson and Crouch 1969, Watanabe et al. 2001).

Historically, taxonomy and phylogenies of fungi within the zygomycetes have been based upon the branching pattern of aerial hyphae, asexual reproductive structures, and septa when present (Benny 1995, Alexopoulos et al. 1996). However, many of the ten orders of zygomycetes have been artificially grouped

based on physical characters rather than true phylogenies (O'Donnell et al. 2001). Increasingly, molecular techniques are employed to help determine phylogentic relationships among groups, and consequently, new relationships among species of zygomycetes continue to be discovered creating complexities for species identification and help to explain ecological variation among species (O'Donnell et al. 2001, Voigt and Wöstemeyer 2001).

MYCODIVERSITY

Estimates suggest only a fraction of the existing species of fungi worldwide have been identified with the total number of species possibly topping three million (Hawksworth 1991). There are approximately 8,500 identified species of saprotrophic fungi and their number may continue to increase with new methods of isolation and identification (Hibbett et al. 2000, Bridge and Spooner 2001). In addition to identification of new taxa, investigations into genetics and ecosystem function of fungi continue. Fungi occupy a wide variety of niches, obtain their nutrients from many sources as pathogens, parasites, or mutualists, and consequently are important in many ecosystems (Hawksworth 1991, Zak and Rabatin 1997, Bridge and Spooner 2001). Robert Hartig, in 1878, discovered that fungi act as both decomposers and pathogens within forest ecosystems (Otjen and Blanchette 1986). Much of the research on belowground fungi research has focused on mycorrhizal, hypogeous species, and root pathogens (Cooke and Rayner 1984, Horton and Bruns 2001). This has included studies of distribution, the nature of symbiotic relationships, phylogeny, population dynamics, and community diversity (Horton and Bruns 2001, O'Donnell et al. 2001). However, researchers have also examined the overall diversity of soil fungi (Christensen 1969, Bridge and Spooner 2001).

When studying fungi, collecting and identifying species is challenging. Most taxonomic identification of species is based upon morphological characteristics of sporocarps collected during fruiting seasons (Ingold and Hudsons 1993). It can be difficult to sample fungi over both space and time, and results are often influenced by the chosen technique (Boddy 1992, Ingold and Hudson 1993, Zak and Rabatin 1997). In wood, culturing does not allow for examination of spatial distribution of fungi within the wood (Boddy 1992). Collecting saprotrophic fungi directly from zone lines where the interactions between species can be noted, and then culturing for identification can increase understanding of the ecological interactions between species. Also increasing the difficulty of studying fungi, only seventeen percent of known fungi grow well in culture (Hawksworth 1991). Fungi respond to nutrients provided by different media which can also influence the fungi that can be separated with culture techniques. Parkinson and Crouch (1969) found that the number of culture isolates that they could obtain from root samples changed with each successive washing (up to twenty). For example, after multiple washings they were able to decrease the species of *Penicillium* in culture, but it also changed the number and species of zygomycetes and basidiomycetes that were recovered.

Within coarse woody debris, fruiting bodies and visible hyphae may account for only a small component of the total mass of fungi (Maser and Trappe 1984). The resources available to fungi may vary by stump or log, and different fungi may use different resource extraction methods which can influence the appearance of species over time (Harmon et al. 1994). Hintakka (1993) collected edible fungi (macromycetes) from stumps over a sixteen-year period. Some species were persistent on the same stumps for multiple years, while collections on other stumps were not as regular. Soon after tree harvest, some stumps were quickly colonized by macromycetes, but other stumps remained sporocarp free for more than four years. In another study of fungal fruiting on coarse woody debris, the largest number of fungal sporocarps was found on decay class three logs with

limited moss cover (Edmonds and Lebo 1998). Given the wide availability of fungal spores and vegetative material, this indicates that the wood may not be immediately available for fungal colonization and resource extraction by all species. Initial breakdown properties from other wood inhabiting microbes may be required to create an optimal environment for fungi (Cooke and Rayner 1984).

The largest proportion of fungal biomass is located belowground as fungi have wide ranging belowground hyphal networks. However, material sampled directly from soil may contain live, resting, and dead fungal material (Bridge and Spooner 2001). As a result, measurements of total numbers of species may not represent true belowground fungal activity (Peter et al. 2001). Species require certain environmental factors including temperature and moisture levels to produce reproductive sporocarps (Alexopoulos et al. 1996). Therefore, conditions may not be optimal each year for every species to reproduce sexually (Vainio and Hantula 2000). Consequently, an aboveground sampling of an area during only one season may miss a large portion of the overall fungal diversity that could be found belowground. However, wet regions with high rainfall may have more consistent yearly sporocarp production (Edmonds and Lebo 1998). Although, in some Pacific Northwest old-growth forest stands, there may be some species present which rarely fruit making them difficult to quantify (Smith et al. 2002). For a good representation of sporocarp diversity in one location, sampling may need to be repeated for three to eight years (Gardes and Bruns 1996b). Both the number of times and the size of the area sampled can influence measures of sporocarp diversity (Smith et al. 2002). In mycorrhizal studies, the most common sporocarps can often be the least common on root tips, thus demonstrating the unevenness of fungal distribution above- and belowground (Gardes and Bruns 1996a, Horton and Bruns 2001).

Communities of microfungi tend to be particularly diverse over relatively small geographic areas. Research in Puerto Rico and Costa Rica, on saprophytic microfungi of leaf litter, has demonstrated how diverse fungal communities may be

(Bills and Polishook 1994, Polishook et al. 1996). Overlap in fungal communities was low between different tree species that were close together geographically and between individuals of the same tree species at varying distances from each other. Moreover, there were few dominant fungal species, with many different species isolated from culture. In a study of Wisconsin forest and grassland microfungi communities, Christensen (1969) found similar results, with low overlap of fungi between and within different forest stands revealing a lack evidence for "discrete communities" of fungi. Christensen found highly variable similarity index values in fungal communities between stands ranging from a low of 5.6% to a high of 78%. However, fungi were found along narrow distributions and were plentiful within those ranges. By having limited sampling locations, the true fungal diversity of an area could be misrepresented. This creates a challenge for mycologists to measure diversity as accurately as possible with limited time and budgets.

MOLECULAR TECHNIQUES

With the advent of molecular techniques, the potential for studying belowground fungal communities has increased. Many studies have concentrated on molecular methods for identification using the ribosomal RNA. On either side of the 5.8s rRNA gene there are two non-coding internal transcribed spacer regions (ITS). One of the ITS regions is bound by the small subunit gene (SSU) and the other by the large subunit gene (LSU). The 5.8s rRNA gene is located between the two ITS regions and is highly conserved and nearly identical in all fungi. The SSU gene is slightly less conserved than the 5.8s gene. The ITS region of 600 to 800 base pair length is characterized by indels and has more variability than either the 5.8s gene or SSU gene (Horton and Bruns 2001). Portions of the LSU are the less

conserved than the other two genes with the 5' end near the D1 and D2 domains being quite variable (O'Donnell et al. 2001).

Due to the variable nature of the ITS region and because it is flanked by regions that are conserved enough to create primers, it is frequently used for restriction fragment length polymorphism comparisons (RFLP). Distinct RFLP patterns can result from insertions, translocations, additions, deletions, or single nucleotide changes in the genome (Michelmore and Hulbert 1987). To utilize RFLP techniques, the fungal ITS region is amplified using PCR protocols, and the DNA is then digested with two or three restriction enzymes (endonucleases) (White et al. 1990, Bruns et al. 1991, Gardes and Bruns 1993, Gardes and Bruns 1996b, Horton and Bruns 2001). Variability in the ITS region allows for species comparisons based upon the resulting DNA fragments. A unique RFLP pattern is one in which there are distinct RFLPs for two or three endonucleases (Horton and Bruns 2001).

Samples can be digested with multiple enzymes to decrease the likelihood of finding false positive species matches. RFLP matching has been a useful technique for studying mycorrhizal communities because the procedures are relatively simple to learn, there is great possibility of species identification, and it allows for many samples to be processed in a short period of time (Horton and Bruns 2001). Only a small fungal sample is needed to amplify PCR product and carry out RFLPs making this technique ideal for small sample amounts (Gardes and Bruns 1996a, Gardes and Bruns 1996b). However, these techniques do not discriminate between DNA from living versus dead material, and results, therefore, may not represent the present living community of fungi (Bridge and Spooner 2001).

RFLP patterns can give a researcher a good idea of the genetic diversity present within a large study area (Horton and Bruns 2001). However, relying on RFLPs may make it more difficult to positively distinguish species, and not all RFLPs will match with known species (Bridge and Spooner 2001, Lilleskov et al.

2002). As the number of samples and enzymes increases, it becomes more difficult to compare samples and differentiate true RFLP matches because distinguishing small band pattern differences between two samples is challenging (Bruns et al. 1991, Carder and Barbara 1991). The evolutionary age of a species group may also influence the ease of separating species within the group (Kårén et al. 1997). For example, some closely related species within *Cortinarius* are not readily distinguishable with ITS-RFLPs (Peintner et al. 2001). The enzymes chosen for RFLPs may also influence the ease of comparison among samples as different enzymes may favor the production of smaller or larger fragments (Gardes and Bruns 1996a).

Particularly at a local scale, most variations within the ITS region correspond well with observed phenotypic variation found in morphological characteristics of species, and in nearly all cases two species will not have the same RFLP pattern for three endonucleases (Horton and Bruns 2001, Horton 2002). However, gene flow between spatially distinct populations of the same species could influence the RFLP patterns especially in a reputed non-coding region such as the ITS. Because this region is so variable, individuals in one geographic location may have genetic mutations not appearing in others (Kårén et al. 1997). This could lead to overestimation of fungal species diversity based on genotypic variation when species are morphologically and phenotypically the same (Bruns et al. 1991, Vainio and Hantula 2000). However, in side-by-side electrophoresis gel runs of ITS-RFLP patterns of spatially separate species, this appears to be a relatively rare occurrence for most species (Kårén et al. 1997, Horton 2002). Consequently, RFLP matching works best when the geographic scope of the study is limited; however, sometimes distinguishing between species can be challenging (Horton and Bruns 2001, Horton 2002).

Polymorphic RFLP patterns for one species have been reported in between fourteen to sixteen percent of samples species with most species having only one distinct RFLP pattern (Kårén et al. 1997, Horton 2002). RFLP patterns were used

to separate six morphologically distinct species of *Verticillium* (Carder and Barbara 1991). In some cases, a species of *Verticillium* had only one monomorphic and distinct RFLP pattern for all isolates. In others, a known species was polymorphic with two consistently distinct RFLP patterns. In a few measured species, most of the isolates would match within the species with one or two isolates identically matching a different species. Variation of RFLPs is a direct consequence of nucleotide variations and can influence the differences that can be detected between and within species.

Often RFLP-type comparisons are followed up with direct gene sequencing (Bruns et al. 1991, Gardes and Bruns 1996b, Horton and Bruns 2001). Sequencing allows for greater identification power and access to a wide range of published and online databases, including GenBank, thus making it easier for many different groups to compare samples with one another (Bruns et al. 1991). Sequence comparisons with a high percentage of base pairs aligning, low error in alignment, and a high score (bits) are often considered species matches (Altschul et al. 1997). In comparing sequences, small base pair changes can be noted and help to explain RFLP results. Sequencing not only allows for comparison between samples from different geographic regions, but also provides a method for looking at phylogenetics and relations between groups (Horton and Bruns 2001).

FUNGAL COMMUNITIES AND SUCCESSION

Fungal communities are dynamic through time. Cooke and Rayner (1984) define a community as an "assemblage of different species interacting together within any more or less self contained system." The spatial limit of a community varies from an individual log to an ecosystem depending upon the system of interest. The community of wood decomposer fungi may be influenced by changes in plant communities, nutrient changes in decomposing substrate and resources

over time, spatial limitations, or competition that develops as succession continues given that each species of fungi has its own resource and spatial requirements (Frankland 1992). As wood decomposes, the proportions of cellulose, hemicellulose, and lignin throughout the wood change and by-products such as carbohydrates remain. Consequently, the fungal communities expand into regions with fresh and plentiful resources and abandon areas with depleted resources (Rayner and Boddy 1988). Additionally, the ideal habitat for a fungus may change as it ages and the external conditions alter (Schipper 1973). Many species of fungi are found in decomposing wood over long periods, but the dominant species change as conditions are modified (Frankland 1992).

There are three phases of saprotrophic fungal succession. The first is arrival, where dormant spores or physiologically active hyphae enter and colonize a new area. The second phase is establishment, exploitation, and consolidation, where the spores germinate and hyphae extend throughout the wood using different strategies to exploit available resource niches. Finally, when they have depleted the resources fungi exit and move to a new location (Boddy 1992, Frankland 1992). Individual fungal colonization may be affected by the quality of available resources including organic materials, temperature, pH, moisture content, physical structure of the wood, and the interactions with other microorganisms (Rayner and Boddy 1988). One of the early colonizers of wood may use up the nutrients that it needs to live, but this often creates a habitat suitable to another species that can then begin its process of arrival (Frankland 1992). The species that come later in time may be more competitive and perhaps have less specific nutritional requirements than those that are found in the early stages of succession.

The successional dynamics within fungal communities may progress differently than in plant communities (Frankland 1992). Often over time, directional or seral succession is noted in plant communities where there is a continuous change to a more complex and varied ecosystem ultimately reaching a climax community (Cooke and Rayner 1984). However, sometimes succession in

plant and fungal communities is more similar with complex communities early and less diverse communities in later stages. Smith et al. (2002) found as a conifer forest closes the diversity of fungal sporocarps remained fairly stable across early and later stages of succession. Saprotrophic fungal communities do not always move in one direction beginning with fungi that require simple sugars, next onto brown- and white-rot fungi that break down cellulose, hemicellulose, and lignin, and then back to simple sugar fungi (Frankland 1992). This chain of events can describe some fungal and plant communities; however, succession is also defined by changes in substratum, resource availability, and species success (Cooke and Rayner 1984, Frankland 1992). Substratum succession is driven by resource availability. The quantities of lignin and cellulose in a freshly fallen log are quite different than in a log which is fifty or one hundred years old (Allen et al. 2000). Trees of each age provide resource requirements for fungi with different species having subtle competitive advantages over each other.

Grime (1979) developed the Competitive-Stress Tolerant-Ruderal (C-S-R) strategies to explain strategies used by plants for resource capture and the resulting community dynamics and interactions (Cooke and Rayner 1984, Rayner and Boddy 1988, Boddy 1992, Frankland 1992). It is also applicable in describing fungal communities. Over extended lengths of time, saprotrophic fungi with competitive characteristics can successfully compete against other fungi for resources. Many competitive species are saprotrophic basidiomycetes. They arrive after the primary colonization of wood, and are able to out compete ruderal and stress tolerant species for resources by exploiting more complex and varied carbohydrate sources. Ruderals are often primary colonizers. As such, they characteristically utilize readily available resources such as simple sugars and rapidly reproduce during their short life spans. Many zygomycetous Mucorales, some basidiomycetes, and some ascomycetes are ruderal primary colonizers of dead wood. However, when easily accessible resources are depleted, they are quickly replaced by more competitive species (Frankland 1992). Stress tolerant species are able to survive in specialized

and limited resources environments. However, due to specific resource requirements, they may not be very competitive with other fungi. For fungi, a living tree is an example of a high stress environment, but there are many fungi well suited to colonizing and capturing resources from living tissue (Boddy 1992). Subsequently, many areas of heart rot in living trees may provide habitat for other decomposers either ruderals in search of simple carbohydrates or competitive fungi requiring more complex carbohydrates (Rayner and Boddy 1988). With the specialization of resources and requirements and under the right conditions, fungi utilizing each strategy may have a competitive advantage over other strategies.

There are numerous complex interactions among decomposer fungi. Those that are the most effective decomposers may not stay in one area for a long time as they use up the resources that they need. However, the fungi that are not very effective at breaking down wood, may be found for longer periods of time as their nutritional requirements are not as great as some of the better decomposers (Frankland 1992). Zone lines in wood indicate an interaction area between different fungi (Rayner and Boddy 1988, Frankland 1992). These interactions have been described as combative as fungal hyphae compete for limited resources (Cooke and Rayner 1984, Rayner and Boddy 1988). During periods of combativeness and replacement there may be a deadlock or intermingling. Deadlock occurs when interacting hyphae are restricted from crossing paths and die when they attempt to do so (Rayner and Boddy 1988). Intermingling occurs when two fungi have slightly different resource needs and can meet those needs by exploiting different niches within the same space (Frankland 1992).

LITERATURE CITED

Alexopolous, C.J., C.W. Mims, and M. Blackwell. 1996. Introductory Mycology 4th edition. Wiley, New York.

Allen, R.B., P.K. Buchanan, P.W. Clinton, and A.J Cone. 2000. Composition and diversity of fungi on decaying logs in a New Zealand temperate beech (Nothofagus) forests. Canadian Journal of Forest Research 30:1025-1033.

Altschul, S.F., T.L. Madden, A.A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D.J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucliec Acids Research 25:3389-3402.

Ander, P., and K.-E. Ericksson. 1977. Selective degradation of wood components by white-rot fungi. Physiologia plantarum 41:239-248.

Benny, G.L. 1995. Classical morphology in zygomycete taxonomy. Canadian Journal of Botany 73:S725-S730.

Bills, G.F., J.D. Polishook. 1994. Microfungi from decaying leaves of *Heliconia mariae* (Helioconiaceae). Brenesia 41-42:27-43.

Blanchette, R.A. 1984. Screening wood decayed by white rot fungi for preferential lignin degradation. Applied and Environmental Microbiology. 48:647-653.

Blanchette, R.A., T.A. Burnes, G.F. Leatham, and M.J. Effland. 1988. Selection of white-rot fungi for biopulping. Biomass 15:93-101.

Boddy, L. 1992. Development and function of fungal communities in decomposing wood. Pages 749-782 *in* G. C. C. a. D. T. Wicklow, editor. The Fungal Community. M. Dekker, New York.

Bridge, P. and B. Spooner. 2001. Soil fungi: diversity and detection. Plant and Soil 232:147-154.

Bruns, T.D., T.J. White, and J.W. Taylor. 1991. Fungal molecular systematics. Annual Review of Ecological Systematics 22:525-564.

Carder, J.H. and D.J. Barbara. 1991. Molecular variation and restriction fragment length polymorphisms (RFLPs) within and between six species of *Verticillium*. Mycologial Research 95:935-942.

Christensen, M. 1969. Soil microfungi of dry to mesic conifer-hardwood forest in northern Wisconsin. Ecology 50:9-27.

Cooke, R.C. and A.D.M. Rayner. 1984. Ecology of saprotrophic fungi. Longman, London.

Edmonds R.L. and D.S. Lebo. 1998. Diversity, production, and nutrient dynamics of fungal sporocarps on logs in an old-growth temperate rain forest, Olympic National Park, Washington. Canadian Journal of Forest Research 28:665-673.

Frankland, J.C. 1992. Mechanisms in fungal succession. Pages 383-401 *in* G. C. C. a. D. T. Wicklow, editor. The Fungal Community. M. Dekker, New York.

Gardes, M. and T.D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes--application to the identification of mycorrhizae and rusts. Molecular Ecology 2:113-118.

Gardes, M. and T.D. Bruns. 1996a. Community structure of ectomycorrhizal fungi in a Pinus muricata forest: above- and below-ground views. Canadian Journal of Botany 74:1572-1583.

Gardes, M. and T.D. Bruns. 1996b. ITS-RFLP matching for identification of fungi. Pages 177-186 *in* J. P. Clapp, editor. Methods in Molecular Biology. Humana Press Inc, Totowa, NJ.

Gilbertson, R.L. 1980. Wood-rotting fungi of North America. Mycologia 72:1-49.

Gilbertson, R.L. 1981. North American wood-rotting fungi that cause brown rots. Mycotaxon 7:372-416.

Green, III., F., M.J. Larsen, J.E. Winandy, and T.L. Highley. 1991. Role of oxalic acid in incipient brown-rot decay. Material un Organismen 26:191-213.

Green III, F. and C.A. Clausen. 1999. Production of polygalacturonase and increase of longitudinal gas permeability in southern pine by brown-rot and white-rot fungi. Holzforschung 53:563-568.

Harmon, M.E., J.F. Franklin, F.J. Swanson, P. Sollins, S.V. Gregory, J.D. Lattink, N.H. Anderson, S.P. Cline, N.G. Aumen, J.R. Sedell, G.W. Lienkaemper, K. Cromack, Jr., and K.W. Cummins. 1986. Ecology of coarse woody debris in temperate ecosystems. Advances in Ecological Research. 15:133-302.

Harmon, M.E. and J.F. Franklin. 1989. Tree seedlings on logs in *Picea-Tsuga* forests of Oregon and Washington. Ecology 70:48-59.

Harmon, M.E., J. Sexton, B.A. Caldwell, and S.E. Carpenter. 1994. Fungal sporocarp mediated losses of Ca, Fe, K, Mg, Mn, N, P, and Zn from conifer logs in the early stages of decomposition. Canadian Journal of Forest Research 24:1883-1893.

Harvey, A.E., M.J. Larsen, and M.F. Jurgensen. 1976. Distribution of ectomycorrhizae in a mature Douglas-fir/Larch forest soil in western Montana. Forest Science 22:393-398.

Hawksworth, D.L. 1991. The Fungal Dimension of Biodiversity Magnitude Significance and Conservation. Mycological Research 95:641-655.

Hawksworth D.L., P.M. Kirk, B.C. Sutton, and D.N. Pegler. 1995. Ainsworth and Bisby's Dictionary of the Fungi, 8th Ed. CAB International.

Hibbett, D.S., L.-B. Gilbert, and M.J. Donoghue. 2000. Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. Nature (London) 407:506-508.

Hintikka, V. 1993. Occurrence of edible fungi and other macromycetes on tree stumps over a sixteen-year period. Acta Botanica Fennica 149:11-17.

Horton, T.R., T.D. Bruns, and V.T. Parker. 1999. Ectomycorrhizal fungi associated with *Arctostaphylos* contribute to *Pseudotsuga menziesii* establishment. Canadian Journal of Botany 77:93-102.

Horton, T., and T.D. Bruns. 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. Molecular Ecology 10:1855-1871.

Horton, T. 2002 (in press). Molecular approaches to ectomycorrhizal diversity studies: variation in ITS at a local scale. Plant and Soil.

Hulme, M.A., and J.K Shields. 1970. Biological control of decay fungi in woody by competition for non-structural carbohydrates. Nature 227:300-301.

Illman, B. L., D.C. Meinholtz, and T.L. Highley. 1988. An electron spin resonance study of manganese changes in wood decayed by the brown-rot fungus, Postia placenta. *in* The International research group on wood preservation, Madrid Spain.

Ingold, C.T. and H.J. Hudson. 1993. The biology of fungi, 6th edition. Chapman Hall, London.

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

Koilraj, A.J., G. Marimuthu, K. Nataranjan, S. Saravanan, P. Maran, and M.J. Hsu. 1999. Fungal diversity inside caves in Southern India. Current Science (Bangalore) 70:1081-1084.

Kuhlman, E.G. 1969. Mucorales isolated from pine rot bark and wood. Canadian Journal of Botany 47:1719-1723.

Larsen, M.J., A.E. Harvey, R. Powers, and M.R. Jurgensen. 1996. A new species of Botryobasidium (Corticiaceae, Aphyllophorales) inhabiting the interiors of brown rotted logs of ponderosa pine. Mycotaxon 60:429-432.

Leonowicz, A., A. Matuszewska, J. Luterek, D. Ziegenhagen, M. Wjotas-Wasilewska, N.-S. Cho, M. Hofrichter, and J. Rogalski. 1999. Biodegration of lignin by white rot fungi. Fungal Genetics and Biology 27:175-185.

Lilleskov, E., T.J. Fahey, T.R. Horton, and G.M Lovett. 2002. Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. Ecology 83:104-115.

Maser, C. and J.M. Trappe. 1984. The seen and unseen world of the fallen tree. USDA Forest Service General Technical Report PNW-164:1-56.

Michelmore R.W. and S.H. Hulbert. 1987. Molecular markers for genetic analysis of phytopathogenic fungi. Annual Review of Phytopathology 25:383-404.

O'Donnell, K., F.M. Lutzoni, T.J. Ward, and G.L. Benny. 2001. Evolutionary relationships among mucoralean fungi (Zygomycota): Evidence for family polyphyly on a large scale. Mycologia 93:286-296.

Otjen, L. and R.A. Blanchette. 1986. A discussion of microstructural changes in wood during decomposition by white rot basidiomycetes. Canadian Journal of Botany 64:905-911.

Otjen, L., R.A. Blanchette, M. Effland, and G. Leatham. 1987. Assessment of 30 white rot basidiomycetes for selective lignin degradation. Holzforschung 41:343-349.

Parkinson, D. and R. Crouch. 1969. Studies on fungi in pinewood soil. V.—Root mycofloras of seedlings of *Pinus nigra* var. *laricio*. Rev. Ecol. Biol. Sol. 6: 263-275.

Peintner, U., N.L. Bougher, M.A. Castellano, J.-M. Moncalvo, M.M. Moser, J.M. Trappe, and R. Vilgalys. 2001. Multiple origins of sequestrate fungi related to *Cortinarius* (Cortniariaceae). American Journal of Botany 88:2168-2179.

Peter, M., F. Ayer, and S. Egli. 2001. Nitrogen addition in a Norway spruce stand altered macromycete sporocarp production and below-ground ectomycorrhizal species composition. New Phytologist 149:311-325.

Polishook, J.D., G.F. Bills, and D.J. Lodge. 1996. Microfungi from decaying leaves of two rain forest trees in Puerto Rico. Journal of Industrial Microbiology. 17:284-294.

Rayner, A.D.M. and L. Boddy. 1988. Fungal communities in the decay of wood. Advances in Microbial Ecology 10:115-166.

Renvall, P. 1995. Community structure and dynamics of wood-rotting Basidiomycetes on decomposing conifer trunks in northern Finland. Karstenia 35:1-51.

Ritschkoff, A.-C. 1996. Decay mechanisms of brown rot fungi. Technical Research Centre of Finland, Espo. VTT Publications 268.

Robinson, J. 1990. Lignin, land plants, and fungi: biological evolution affecting Phanerozoic oxygen balance. Geology 15:607-610.

Schipper, M.A.A. 1973. A study on variability in *Mucor hiemalis* and related species. Studies in Mycology 4:1-40.

Shea, K.R. 1960. Fungus succession and the significance of environment in the deterioration of logs. *in* 8th Western International Forest Disease Work Conference. Weyerhaeuser Company Forestry Research Note, Centralia, WA.

Smith, J. E., R. Molina, M.M.P. Huso, D.L. Luoma, D. McKay, M.A. Castellano, T. Lebel, and Y. Valachovic. 2002. Species richness, abundance, and composition of hypogeous and epigeous ectomycorrhizal fungal sporocarps in young, rotation-age, and old-growth stands of Douglas-fir (*Pseudotsuga menziesii*) in the Cascade Range of Oregon, U.S.A. Canadian Journal of Botany 80:186-204.

Tanesaka, E., H. Masuda, and K. Kinugawa. 1993. Wood degrading ability of basidiomycetes that are wood decomposers, litter decomposers, or mycorrhizal symbionts. Mycologia 85:347-354.

Tien, M. and T.K. Kirk. 1983. Lignin-degrading enzyme from the Hymenomycete *Phanerochaete chrysosporium* Burds. Science 221:661-663.

Vainio, E.J. and J. Hantula. 2000. Direct analysis of wood-inhabiting fungi using denaturing gradient gel electrophoresis of amplified ribosomal DNA. Mycological Research 104:927-936.

Voigt, K. and J. Wöstemeyer. 2001. Phylogeny and origin of 82 zygomycetes from all 54 genera of the Mucorales and Mortierellales based on combined analysis of actin and translation elongation factor EF-1alpha genes. Gene 270:113-120.

Watanabe, T., Y. Watanabe, T. Fukatsu, and R. Kurane. 2001. Notes from two *Umbelopsis* species: *U. nana* and *U. vinacea* from Japan. Mycoscience 42:235-237.

White, T.J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal rna genes for phylogenetics. Pages 315-322 *in* M. A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White, editor. PCR Protocols: A guide to methods and applications. Academic Press, Inc, San Diego.

Wilcox, W.W. 1993. Comparative morphology of early stages of brown-rot wood decay. IAWA Journal 14:127-138.

Winandy, J.E. and J.J. Morrell. 1993. Relationship between incipient decay, strength, and chemical composition of Douglas-fir heartwood. Wood and Fiber Science 25:278-288.

Worrall, J.J., S.E. Anagnost, and R.A. Zabel. 1997. Comparison of wood decay among diverse lignicolous fungi. Mycologia 89:199-219.

Yoshizawa, N., N. Itoh, M. Sunagawa, S. Yokota, and T. Idei. 1992. Comparative histochemistry and enzyme activities during wood decay by white-rot fungi with selective lignin-degrading abilities. Bulletin Utsunomiya University Forestry 28:97-110.

Zak, J.C. and S.C. Rabatin. 1997. The mycota: A comprehensive treatise on fungi as experimental systems for basic and applied research. *in* K. Esser, P.A. Lemke. D.T. Wicklow, and B.E. Söderström, editors. Vol. IV: Environmental and microbial relationships. Springer, New York.

CHAPTER 3 FUNGAL DIVERSITY WITHIN DECOMPOSING WOODY CONIFER ROOTS IN OREGON

V. Eleanor Vandegrift, Hua Chen, and Mark E. Harmon

ABSTRACT

Previous studies have indicated that roots from five tree species (*Picea sitchensis, Tsuga heterophylla, Pseudotsuga menziesii, Pinus ponderosa,* and *Pinus contorta*) decompose at different rates across an environmental gradient in Oregon. Measurements of wood chemistry from each tree species as well as moisture and temperature from each location do not explain the differences in decomposition rates. Molecular techniques were employed to generate Internal Transcribed Spacer - Restriction Fragment Length Polymorphism (ITS-RFLP) patterns to examine saprotrophic fungi in roots of these tree species and to see if differences in the fungal communities might explain observed differences in decomposition rates. However, due to a large number of ITS-RFLP patterns recovered and low levels of similarity in ITS-RFLP patterns across samples, we were unable to explain root decomposition based upon the fungal community information. Consequently, the analysis focused on comparing levels of ITS-RFLP similarity at each sampling level, determining the extent to which the sampling methods captured the total fungal biodiversity, and examining samples with microscopy and gene sequencing techniques to identify fungi. Root samples were retrieved from trees cut seven to fifteen years prior to sampling. Two tree species were sampled at each of three locations across Oregon. DNA was extracted from hyphae samples collected directly from roots, and fungi cultured from root chips. DNA was also extracted from fungal herbarium specimens, field collected samples, and cultures of saprotrophic fungi. To analyze genetic diversity of the samples, they were amplified using polymerase chain reaction (PCR) techniques, digested with endonucleases (*Hinf* I, *Dpn* II, and *Hae* III), and ITS-RFLP patterns were evaluated. Nei and Li similarity index analyses were used to compare differences in fungal composition based upon ITS-RFLP patterns between tree species, sites, and harvest stands. Over two hundred distinct ITS-RFLP patterns were recognized from fungal samples. Similarities in ITS-RFLP patterns of hyphal and cultured

samples ranged 0 to 1, where 0 indicated no overlap and 1 indicated 100% matching of ITS-RFLPs. When all ITS-RFLPs obtained from each stump were combined, similarities in patterns between sites ranged from 0 to 0.07, from 0 to 0.13 between tree species, from 0 to 0.11 between harvest stands, and from 0 to 0.67 between individual stumps. Linearly increasing ITS-RFLP sampling intensity curves indicate a large diversity of fungi. Using microscopy, cultured samples were examined for hyphae and reproductive structures. In culture, zygomycetous structures were prevalent. DNA gene sequences of the nuclear large and small subunits were used to place unknown ITS-RFLP patterns into family and generic groups. Twenty-three common and five uncommon ITS-RFLP patterns were sequenced; most matched with the Mortierellaceae and Mucoraceae families of the zygomycetes. The large diversity of ITS-RFLP patterns indicates the coarse roots provided habitat to many fungi at the stage of decomposition when samples were collected.

INTRODUCTION

As components of forests, saprotrophic fungi play a key role in the decomposition of woody debris. Saprotrophs aid in the breakdown of lignin, cellulose, and hemicellulose into smaller and simpler molecules (Gilbertson 1981, Blanchette 1984, Otjen et al. 1987, Yoshizawa et al. 1992, Tanesaka et al. 1993, Winandy and Morrell 1993, Worrall et al 1997, Green and Clausen 1999, Loenowicz et al. 1999, Allen et al. 2000). The by-products of wood decomposition provide structure and nutrients to the forest ecosystem for centuries (Gilbertson 1980, Gilbertson 1981). While aboveground woody debris has often been the focus of research, belowground woody debris in the form of roots has been studied less frequently (Harmon et al. 1986, Harmon et al. 1994, Edmonds and Lebo 1998, Chen et al. 2001, Chen et al. 2002).

In a study of root decomposition, the decay rates of roots from five conifer species (*Picea sitchensis, Tsuga heterophylla, Pseudotsuga menziesii, Pinus ponderosa,* and *Pinus contorta*) were measured (Chen et al. 2001, Chen et al. 2002). *Pinus ponderosa* roots decomposed much more quickly than the other conifer species found in Oregon. Trees were sampled from three climatic areas of the state including the coast range, foothills of the Cascade Mountains, and the high desert of eastern Oregon. The environmental differences between the sites including moisture and temperature and wood chemistry of the tree species do not explain the differences in decomposition rates among the species. However, the roots of *Pinus ponderosa* were observed to have more evidence of white-rot in their roots than did the other species. We hypothesized that the basidiomycete and ascomycete saprotrophic fungal communities on each tree species may influence root decomposition. We also hypothesized that there would be a core community of fungi with dominant ITS-RFLP types found at each site and specific RFLP patterns for each tree species. To test for differences that location might have in determining the decomposer community, roots from four tree species were harvested and reciprocally transplanted to other sites in 1995. Molecular techniques were used to generate Internal Transcribed Spacer - Restriction Fragment Length Polymorphism (ITS-RFLP) patterns to sample the diversity of fungi from five tree species at three locations in Oregon and on the reciprocal transplant roots after five years. We hoped to increase our understanding of the differences in decomposition rates among roots from different tree species and the roles that saprotrophic fungal communities play in facilitating decomposition in a variety of tree species. Subsequently after examining initial data, we recovered higher than expected levels of fungal diversity. Consequently, the analysis focused on examining the ITS-RFLP sampling intensity curves, comparing similarities in RFLPs across sites, tree species, harvest stands, and individual stumps, and identification of samples.

The first objective of this study was to examine communities of saprotrophic fungi on coarse roots. Similarities were to be measured on five tree species across three sites in Oregon seven to fifteen years following harvest and on reciprocal transplant roots after five years. Identification of fungal groups was based upon fungal internal transcribed spacer region-restriction fragment length polymorphism (ITS-RFLP) patterns. The second objective was to examine the ITS-RFLP biodiversity of samples as a function of the number of samples collected. Finally through ITS-RFLP, microscopic, and gene sequencing techniques, the third objective was to match samples with known fungi.

METHODS

Study sites and trees

Roots were sampled from stumps at three locations in Oregon. The sites, Cascade Head Experimental Forest/Siuslaw National Forest (CHE), H.J. Andrews Experimental Forest (HJA), and Deschutes National Forest (DNF), follow an environmental gradient in Oregon from west to east. Three harvest stands of different clear cut or thinning harvest ages were sampled at each site. Stands were chosen based upon their ages of harvest and their inclusion in previous root decomposition studies (Chen et al. 2001, Chen et al. 2002).

CHE is located near the town of Otis, Oregon along U.S. Highway 101 where the maritime climate has a mean annual precipitation of 3420 mm and mean annual temperature of 10°C. The experimental forest extends from the edge of the Pacific Ocean inland through the Oregon Coast Range. The soils are well-drained silt and clay silt loams derived from marine silt stones with high concentrations of nitrogen and organic matter (Chen et al. 2001). *Picea sitchensis* (Bong.) Carrière

(Sitka spruce) and *Tsuga heterophylla* (Raf.) Sarg (western hemlock) were sampled here. At CHE the roots were harvested from one stand that had been harvested in 1989 and two in 1986. Each site had been clear-cut and burned. The 1989 and one of the 1986 sites were replanted with conifers including *Picea sitchensis, Tsuga heterophylla,* and *Pseudotsuga menziesii.* The second stand harvested in 1986 (Hibbs) was replanted with *Alnus rubra* and *Pseudotsuga menziesii* as part of an *Alnus/Pseudotsuga* competition study led by D. Hibbs and S. Radosevich. Also growing in this stand were *Picea sitchensis, Pseudotsuga menziesii,* and *Tsuga heterophylla.*

The HJA, 80 km miles east of Eugene, Oregon, is located in the Blue River Ranger District on the Willamette National Forest. The forest is on the west slope of the Cascade Mountain Range with a maritime climate. The area has a mean annual temperature of 8.5°C and annual precipitation of 2300 mm. The soils are deep, well-drained Dystrochrepts (Chen et al. 2001). Stumps sampled at HJA were *Tsuga heterophylla* and *Pseudotsuga menziesii* (Mirb.) Franco (Douglas-fir). The HJA location had stands that were clear-cut in 1986 (unit L116) and 1989 (unit L351), and a stand that was heavily thinned with approximately ten percent leave trees in 1993. The stand cut in 1986 and was replanted with *Pseudotsuga menziesii* and *Alnus rubra* as part of an interspecies competition study. The other stands were replanted with *Pseudotsuga menziesii* and *Abies* spp. All stands were burned at the time of harvest.

Deschutes National Forest is located 57 km south of Bend, Oregon near the town of Sun River, Oregon, east of the Cascade Range. Climate is a modified continental climate of the central Oregon high desert plateau. The mean annual temperature is 5.7°C with mean annual precipitation of 525 mm. The coarse loamy soils of the area are derived from aerial deposited dacite pumice (Chen et al. 2001). Roots from *Pinus ponderosa* P. Laws. ex C. Laws. (ponderosa pine) and *Pinus contorta* Dougl. ex Loud. (lodgepole pine) were collected on the Deschutes National Forest. Roots from DNF were collected from sites that were harvested in

1985, 1988, and 1991. The 1985 site was clear-cut, and the other two sites were thinned with approximately thirty percent trees remaining. All sites were burned.

Year 2000 root selection and collection

Roots at all sites were collected during the summer of 2000 to test if there were similarities in the root fungal communities on different tree species. At each stand, three stumps of each species were identified and selected for root harvesting (Figure 1). The first three stumps with intact roots of each species encountered while walking in from the road were selected for harvest. Stumps were identified by remaining bark and growth characteristics such as presence of buttresses and thickness of the bark. Roots were located at the base of each stump through preliminary digging. Each stump was subsampled with two root samples. A total of one hundred eight sample roots were collected. Each root was excavated along its entire length until a suitable sample with a one to twenty cm diameter was located. Samples were removed from the ground, labeled, and placed into plastic bags.

The diameter and lengths of roots were measured before they were washed with deionized water to remove adhering soil particles. Evidence of white- and brown-rot fungal activity was noted for every root. Each root sample was further subsampled into two groups. One group was kept at room temperature for two to

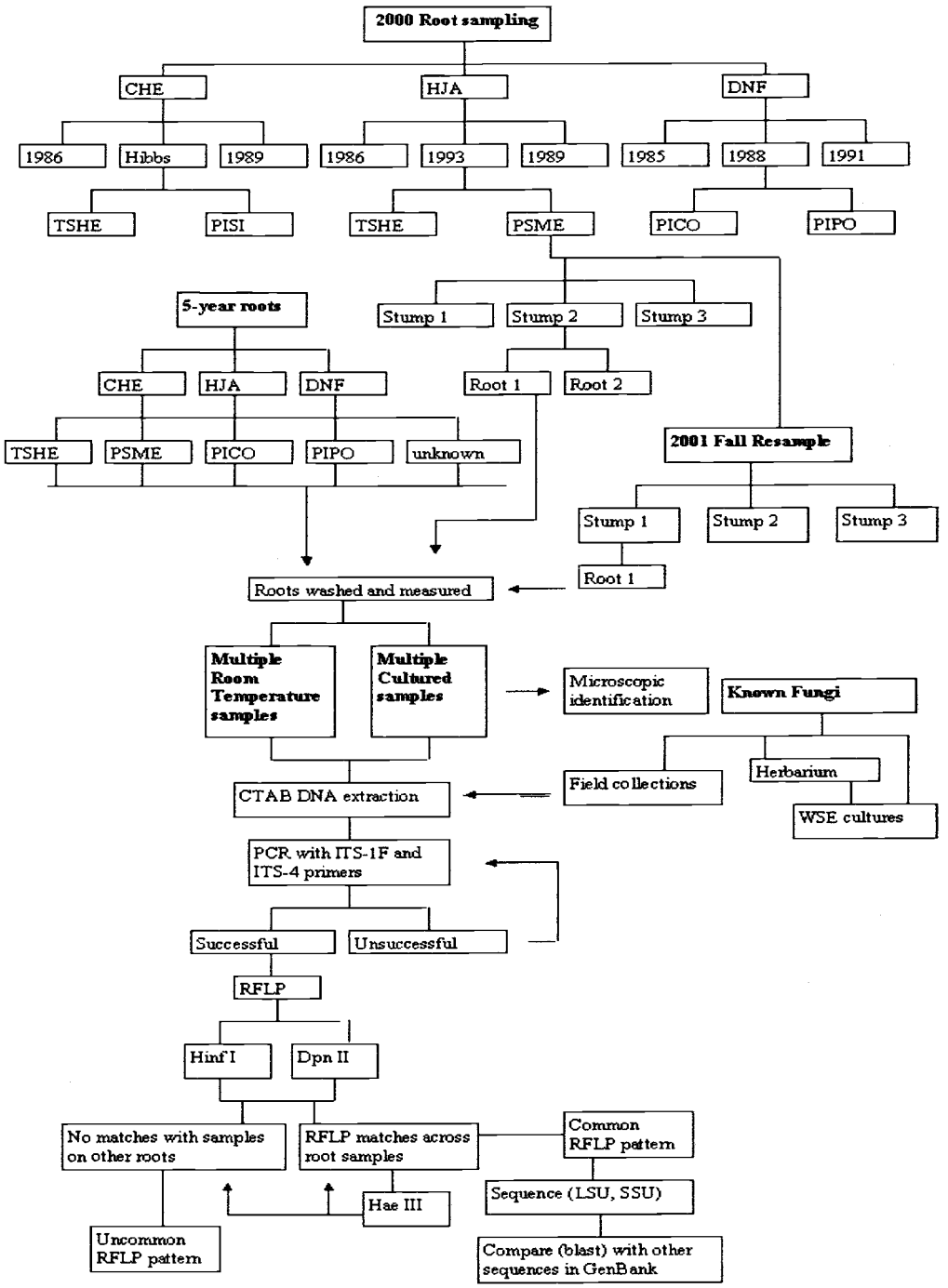

Figure 1. Flow chart of methods for root collection, culturing, hyphae collection, and molecular techniques.

four weeks until there was visible growth of fungal hyphae. Hyphae were then removed from each of these root samples using sterile forceps, stored in microcentrifuge tubes, and freeze-dried for later DNA extractions. Three hundred nine hyphae samples grown at room temperature were collected. The second sample from each root was stored at 4°C for one to two weeks until samples could be cultured to promote fungus growth.

Culturing

For the year 2000 samples, five wood pieces from each root sample were removed with a flame sterilized chisel and forceps. They were plated together onto a malt-agar petri plate (10g agar, 15g malt extract, 10ml benlate (10ppm), 10 ml streptomycin (1mg/1ml), 1ml lactic in 1 L deionized water sterilized) in a laminar flow hood. This media is commonly used to sample ascomycetes and basidiomycetes in aboveground log decomposition studies (C. Frietag personal communication). Samples were checked every week for six weeks for fungal growth. Individually recognizable fungi were then transferred to new petri plates of the same agar medium for pure isolate culture growth. Lactic acid, benlate, and streptomycin were added to prevent the growth of common Ascomycota (including *Penicillium* and *Tricoderma*) and bacteria. Fungal hyphae were harvested from petri plates, and samples were freeze-dried for DNA extraction. Four hundred forty-nine culture samples were collected. Pure culture samples were transferred to malt-agar in sealed test tubes and kept at 10°C for future reference. Each sample that amplified with PCR was then checked under a microscope for distinguishing features such as septae, sporangioles, zygosporangium, spores, and clamps.

Reciprocal transplant roots (5-year)

As part of a long-term decomposition research project, Chen and others (2002) are testing species versus environmental effects in root decomposition. Roots of *Tsuga heterophylla* (TSHE), *Pseudotsuga menziesii* (PSME), *Pinus contorta* (PICO), *Pinus ponderosa* (PIPO), and other species were collected from freshly cut stumps and buried both at the native habitat and the other two study sites using a litter bag approach in 1995. In the summer of 2000 five years after initial placement, samples from these roots were collected for this study (Table 1). Each root was split into the same two subgroups and treated as described above. Sixty-one culture samples and thirty-four room temperature samples were collected from the reciprocal transplant roots (5-year roots).

Table 1. Reciprocal transplant root samples from each species and location.

	CHE*	HJA	DNF	Total
PICO#	2	2	1	**5**
PIPO	2	2	2	**6**
TSHE	2	1	2	**5**
PSME	2	2	1	**5**
unknown	0	1	2	**3**
Total	**8**	**8**	**8**	**24**

*CHE=Cascade Head Experimental Forest/Siuslaw National Forest; HJA=H.J. Andrews Experimental Forest; DNF=Deschutes National Forest

#PICO=*Pinus contorta*; PIPO=*Pinus ponderosa*; TSHE=*Tsuga heterophylla*; PSME=*Pseudotsuga menziesii*

Known fungi

To compare ITS-RFLP patterns of known saprotrophic and native fungi to the cultured, room temperature, and reciprocal 5-year samples, two hundred twenty-four identified sporocarps, herbarium samples, and pure culture isolates were sampled (Appendix 1). One hundred twenty-four sporocarps were collected for comparison from each site with emphasis on collecting fungi from stumps and downed logs during November 2000 and April 2001. DNA from forty-nine herbarium samples of known ascomycete and basidiomycete wood rotters from the Oregon State University herbarium were sampled. Fifty-one pure cultures of basidiomycete saprotrophs were obtained from Camille Freitag (Oregon State University Wood Science and Engineering Department).

Year 2001 root resampling and culture

In October 2001, to test for RFLP similarity among samples from a single root, three *Pseudotsuga menziesii* roots were collected from the stand at the HJA harvested in 1993. Each root was washed and divided into two sections. Wood chips from the first subsample were removed with a sterilized chisel and pieces were flame sterilized and plated onto malt-agar media. Chips from the second subsample were plated on Goldfarb's media which limits the growth of ascomycetes and zygomycetes (30g malt, 20g agar, 2ml benlate (0.2g/100ml water), 2ml prochloraz (0.1g/100ml water), 2ml thiobendazole (0.0835g/100ml water), 0.1g streptomycin, 0.02g rose bengal in 1L sterile water) with the same sterile technique. When hyphal growth was present, fungi were removed and single culture isolates were plated on cellophane in their respective media for ease in hyphae collection. Samples were viewed under a light microscope for identifying characteristics such as septae, sporangioles, zygosporangium, spores,

and clamps. After two to four weeks of growth, seventy-four samples were collected and stored in 2X CTAB (100mM Tris-HCl, 1.4M NaCl, 20mM EDTA) for future DNA extractions.

DNA extraction, PCR, and RFLPs

Samples were extracted using the CTAB extraction method (Gardes and Bruns 1993, Bruns *et al.* 1998) except that a few grains of Proteinase K (Amersco) were placed directly into each sample as a substitute for ß-mercaptoethanol. With the CTAB extraction, samples were frozen with dry ice and heated to break cell walls, which were then further broken down by grinding the samples with a micropestle. DNA was separated from proteins by chloroform and then precipitated with isoproponal and washed with 70% ethanol. Samples were stored in 50 µl TE buffer (1mM Tris-HCl, 0.1M EDTA) in a -20°F freezer until PCR amplification. The internal transcriber spacer (ITS) region between the nuclear small rDNA and nuclear large rDNA was amplified using ITS-1F and ITS-4 fungal specific primers (Operon) and PCR set up protocols (Mullis and Faloona 1987, White et al. 1990, Gardes and Bruns 1996b). The year 2000 culture, room temperature, reciprocal 5-year samples, and known fungi were diluted in a 1:100 mixture with sterile water before being mixed with PCR cocktail (50 µM ITS-1F and 50 µM ITS-4 primers, *Taq*, 10x dNTPs, 10x PCR buffer, and sterilized water). The year 2001 resampled cultures were added directly to the PCR cocktail with a final dilution concentration of 1:100. An annealing temperature of 52°C was used for optimal amplification. Each sample was loaded on a 2.5% agarose gel to check for amplification of the 600-800 bp ITS region. Images of ethidium bromide stained gels were captured using Gel Analyzer.

Samples were then compared to one another using internal transcriber spacer-restriction fragment length polymorphisms (ITS-RFLP). Each sample that

successfully amplified was digested with *Hinf* I (Promega) and *Dpn* II (Promega) enzymes. Samples were run on 1% agarose/2% NuSieve (GT Technologies) electrophoresis gels. Images of ethidium bromide stained gels were captured using Gel Analyzer and lengths of bands identified using Scanalytics 4.0 software. The RFLP fragments from all samples were visually and numerically compared. Each gel was scored to obtain fragment base pair lengths for each sample. The largest, strongest band was counted as the largest fragment for an individual sample. Larger fainter bands were not included. Weak bands were evaluated on an individual basis for inclusion or exclusion from the sample. If a gel and sample fragments could not be interpreted due to smeary fragments, curves in the gel due to inconsistent agarose, or other problems, the samples were rerun on a new gel. Samples were grouped based on potential matches in which RFLP bands from the two different endonucleases matched. Samples that matched with the *Hinf* I and *Dpn* II enzymes were digested with a third enzyme, *Hae* III (Promega), and run side by side on an 1% agarose/2% NuSieve gel. Samples that matched in their RFLP patterns for three enzymes were grouped as distinct fungal ITS-RFLP types.

All known samples (harvested fruiting bodies, herbarium samples, and known cultures) were extracted, amplified, and digested to obtain RFLP patterns with the same procedure as unknown samples. RFLP patterns of known fungi were compared with unknown room temperature, culture, and reciprocal 5-year samples.

Sequencing

Samples from twenty common RFLP patterns, defined as those found in more than one stump, and three uncommon RFLP patterns were sequenced for further identification. Samples were amplified using NL1 and NL4 primers from domains one and two on the nuclear large subunit (K. O'Donnell) or NS1 and NS2 primers for the small subunit with an annealing temperature of 52°C. An agarose

gel was run to ensure amplification. Each sample was purified with glass milk (Bio 101) or purification columns (Qiagen). After purification, samples were again run on an agarose gel to quantify DNA. A sequence reaction was set up (terminator reaction mix, purified DNA product, 5X sequence buffer) with ZNL2 or ZNL3 internal primers for the nuclear large subunit (O'Donnell) or NS1 or NS2 primers for the nuclear small subunit. After the sequence reaction, samples were precipitated with ethanol and sodium acetate before sequence loading buffer was added. Sequences were run on Applied Biosystems 373A DNA Sequencer machine. Completed sequences were edited with Sequence Editor and Sequencing Analysis 3.41, aligned with each other in PAUP*. Sequences were entered into a BLAST program which is used to compare DNA sequences potential alignment matches with other fungi sequences in GenBank. (National Center for Biotechnology Information http://www.ncbi.nlm.nih.gov/). The sequence that aligned the best with a known sequence in the GenBank database at a high percentage accuracy, a low error ($E<0.05$) value, and a large score (bits sizes) was considered to be strong match (Table 17) (Altschul et al. 1997).

Analysis

To calculate how similar the groups of fungal RFLPs were between sites, species, stands, and stumps, a similarity index was calculated using RAPDPLOT 3.0 (William Black, 1997, Colorado State University, Fort Collins). This program employs the Nei and Li (1979) similarity index:

$$S=2C/(A + B)$$

where A equals the number of ITS-RFLP types in the first sample, and B equals the number of ITS-RFLP types in the second sample. C is the number of bands that A and B share in common. A and B represented RFLP patterns from a site, species, stand, or stump. The ranges between zero and one where one is equal to one

hundred percent similarity between groups and zero equals zero percent similarity. Sites, species, stands, or stumps which did not yield any RFLP patterns were not included in the analysis. The analysis was broken into seven components 1) year 2000 room temperature (RT) and year 2000 culture samples combined, 2) year 2000 culture samples alone, 3) year 2000 room temperature samples alone, 4) year 2000 culture and year 2000 room temperature samples compared separately, 5) 5-year roots harvested in 2000, 6) year 2000 common RFLP patterns, and 7) year 2001 resampled cultured samples in Goldfarb's and MEA media. For each of these, similarity analysis of RFLP patterns was conducted at the level of sites, species, stands, stumps, or roots if appropriate.

The similarity indices attached to each set of comparisons were then run on Phylip 3.6 to compute a neighbor-joining tree (Joseph Felsenstein, 1991-2000, University of Washington, Seattle). This creates an unrooted tree and distances of relatedness in terms of similarities of the fungal communities between samples based upon the ITS-RFLP types. Trees were created with Treeview (1.6.1) (Page 1996).

RESULTS

Brown-and white-rot on roots

Many roots had evidence of stringy white wood and crumbly brown wood indicating the present or past activity of white- and brown-rot fungi (Table 2). CHE and HJA had equal numbers of stumps with both white- and brown-rot and brown-rot alone. Many samples from HJA did not have any evidence of decomposition through wood discoloration. The large majority of samples from DNF had white-rot alone and none from DNF had brown-rot alone. The

characteristics of white- and brown-rot decomposition do not necessarily correlate with the fungi that are active in the wood at the time of collection.

Table 2. Number samples with evidence of white- and brown-rot in roots sampled from each stump. Numbers in parenthesis are percentages of the total fifty-four samples.

	CHE*	HJA	DNF	Total for all sites
Both	5 (9.2%)	5 (9.2%)	1 (1.9%)	**11 (20.4%)**
Brown-rot	4 (7.4%)	4 (7.4%)	0	**8 (14.8%)**
White-rot	7 (13.0%)	2 (3.7%)	15 (27.8%)	**24 (44.4%)**
None	2 (3.7%)	7 (13.0%)	2 (3.7%)	**11 (20.4%)**
Total samples	**18**	**18**	**18**	**54**

*CHE=Cascade Head Experimental Forest/Siuslaw National Forest; HJA=H.J. Andrews Experimental Forest; DNF=Deschutes National Forest

Decay classes of stump

The decay classes were recorded on a scale of 1 to 5 for each of the three stumps of each species at each stand. Decay class 1 represents relatively intact and undecomposed wood, and decay 5 is highly decomposed and quite old. CHE generally had more stumps in later stages of decay, especially class 3, than stumps of the same harvest age at DNF and HJA (Table 3).

Microscopic evidence of zygomycetes in culture

Ninety percent of the cultures from year 2000 RFLP groups, year 2000 common RFLP groups, reciprocal 5-year samples, and year 2001 resampled fungi showed evidence of common zygomycete characteristics including aseptate hyphae

Table 3. Number of stumps out of a possible total of three per stand of each decay class for each species and stand age.

Stand	Year stand harvested	Stand age	Species	Decay class 1	Decay class 2	Decay class 3
DNF*	1985	15	PICO#		3	
DNF	1985	15	PIPO		3	
CHE	1986	14	PISI		1	2
CHE	1986	14	TSHE			3
CHE	HIBBS (1986)	14	PISI			3
CHE	HIBBS (1986)	14	TSHE			3
HJA	1986	14	TSHE			3
HJA	1986	14	PSME			3
DNF	1988	12	PICO		3	
DNF	1988	12	PIPO		3	
CHE	1989	11	PISI		3	
CHE	1989	11	TSHE			3
HJA	1989	11	TSHE		3	
HJA	1989	11	PSME	2	1	
DNF	1991	9	PICO	3		
DNF	1991	9	PIPO	3		
HJA	1993	7	TSHE	3		
HJA	1993	7	PSME	3		

*CHE=Cascade Head Experimental Forest/Siuslaw National Forest; HJA=H.J. Andrews Experimental Forest; DNF=Deschutes National Forest

#PICO=*Pinus contorta*; PIPO=*Pinus ponderosa*; TSHE=*Tsuga heterophylla*; PSME=*Pseudotsuga menziesii*; PISI=*Picea stichensis*

or irregularly septate hyphae and reproductive structures (Table 4). Most samples were aseptate and many of those had asexual sporangium or sexual zygosporangium structures. Several samples were irregularly septate which can be a frequent appearance in older zygomycetes (Benjamin 1979). There were some dark septate fungi, commonly found in soil, and very few fungi that were strictly septate. There was no evidence of clamp connections (a general basidiomycete characteristic) in these samples.

Table 4. Occurrence of fungal hyphal structures in year 2000 cultures, common RFLP groups from year 2000 cultures, reciprocal 5-year samples, and year 2001 resampled cultures. Numbers in parenthesis are the percentage occurrence of each structure for each type of sample.

	Culture	Common	5-year	Resampled	**Total number with each structure**
Aseptate with zygomycete reproductive structures	67 (47%)	11 (46%)	3 (15%)	14 (21%)	**95 (38%)**
Aseptate	58 (41%)	11 (46%)	11 (55%)	46 (71%)	**126 (50%)**
Irregularly septate with zygomycete reproductive structures	3 (2%)	1 (4%)	1 (5%)	0	**5 (2%)**
Irregularly septate	5 (3%)	0	5 (25%)	2 (3%)	**12 (5%)**
Dark septate	4 (3%)	0	0	2 (3%)	**6 (2%)**
Septate	6 (4%)	1 (4%)	0	1 (2%)	**8 (3%)**
Total samples from each sampling source	**143**	**24**	**20**	**65**	**252**

RFLPs

One thousand one hundred sixty-eight samples were collected for RFLP analysis (Table 5). Overall, there was a forty-six percent success ratio of PCR amplification

with these samples using ITS-1F and ITS-4 primers. The PCR amplification rate varied by sample type. Of the four hundred forty-nine culture samples, fifty-five percent amplified. Twenty-six of the three hundred twenty-six room temperature samples amplified. Of the ninety-five reciprocal 5-year culture and room temperature samples, thirty-seven percent amplified. One hundred twenty-four sporocarps were collected in the field and fifty-three percent of these amplified. Fifty-seven percent of the forty-nine herbarium samples amplified. Of the fifty-one known Wood Science and Engineering cultures, ninety-two percent amplified.

Two hundred distinct ITS-RFLP fungal types were found for year 2000 unknown culture, room temperature, and reciprocal 5-year root samples. One hundred forty-two of these patterns were found in cultured samples. Forty-four RFLP patterns were found in room temperature samples. Nineteen RFLP patterns were found in reciprocal 5-year roots. Twenty-eight common RFLP patterns from 5-year, room temperature, and culture samples were found in more than one stump. Two of the common patterns were only found in room temperature samples. One pattern was shared between room temperature and culture samples, and three patterns were shared between culture and reciprocal 5-year root samples. In addition, RFLP patterns were obtained for forty-seven known Wood Science and Engineering Department culture collections, sixty-six field collected sporocarps, and twenty herbarium specimens. One culture RFLP matched with the RFLP pattern for *Phanerochaete sordida* from the Wood Science and Engineering Department.

Table 5. Number of samples collected and examined by RFLP analysis. Percentage success is the number of samples that could be sampled for RFLP patterns divided by the number of samples collected for each category. Totals for each category are in bold. 2000 cultures, 2000 room temperature, and reciprocal 5-year samples are split into subcategories for site and collection methods shown above each total in regular type. Unknown totals are based on 2000 cultures, 2000 room temperature, reciprocal 5-year roots, and 2001 resampled roots. Known totals are based on field collected, herbarium, and WSE cultures.

	Samples collected	Samples for RFLPs	Percentage amplification success
CHE	126	71	56%
HJA	162	96	59%
DNF	161	78	48%
2001 Culture	**449**	**245**	**55%**
CHE	110	24	22%
HJA	108	16	15%
DNF	108	14	13%
2001 Room Temperature	**326**	**84**	**26%**
Culture	61	33	54%
Room Temperature	34	2	6%
Reciprocal 5-year	**95**	**35**	**37%**
Resampled root cultures	**74**	**57**	**77%**
Root collection of unknowns Totals	**944**	**421**	**45%**
Field collected	**124**	**66**	**53%**
Herbarium	**49**	**28**	**57%**
Wood Science and Engineering (WSE) cultures	**51**	**47**	**92%**
Known fungi Totals	**224**	**141**	**63%**
Totals	**1168**	**562**	**46%**

Biodiversity results for year 2000 culture, room temperature, and reciprocal transplant roots

An ITS-RFLP sampling intensity curve was created to see if the total potential fungal biodiversity in the study sites was captured in 2000. When the number of stumps sampled was compared with the number of new RFLP patterns obtained for each stump, an RFLP sampling intensity curve indicated a continual linear increase in RFLP pattern groups for each new stump sampled (Figure 2). This trend occurred for cultured samples and room temperature samples alone and combined. A similar pattern was observed in the 5-year roots. (Figure 3).

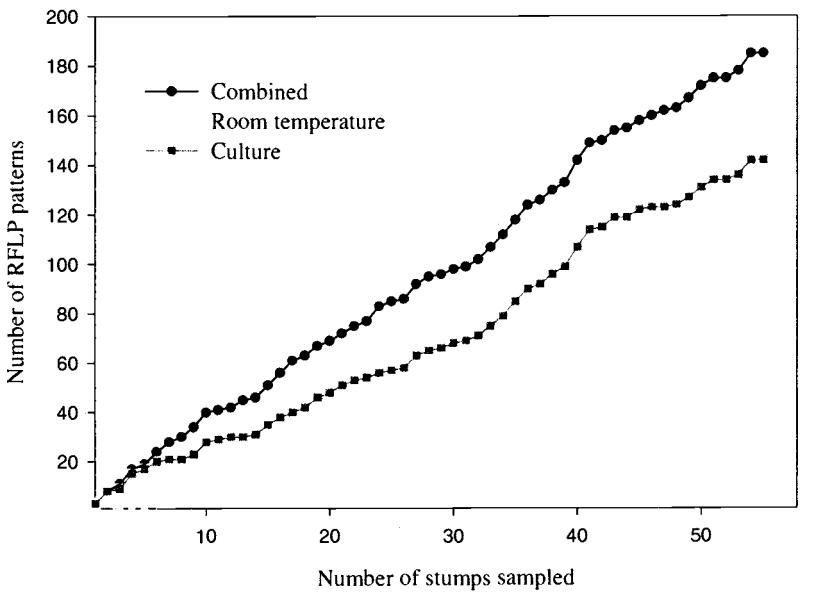

Figure 2. ITS-RFLP sampling intensity curve. Represents the number of new RFLP patterns gained for each new stump sampled. Linear curve indicates an underestimation of biodiversity.

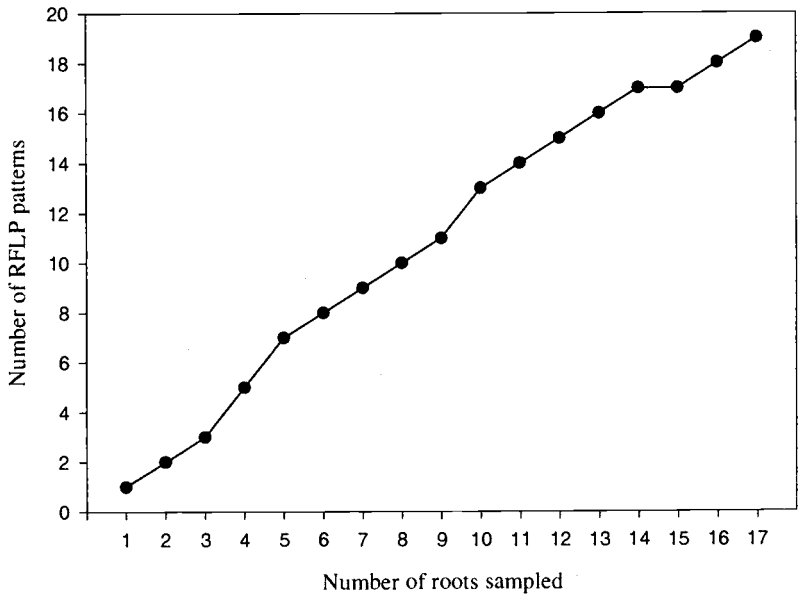

Figure 3. ITS-RFLP sampling intensity curve for reciprocal transplant roots (5-year).

RFLP similarity from year 2000 cultured and room temperature samples combined

Similarity of the cultured and room temperature samples between sites, stands, species, and stumps was low. CHE and HJA RFLP patterns had a similarity of 0.048. HJA and DNF had a similarity in RFLP patterns of 0.062. CHE and DNF had a similarity in RFLP patterns of 0.031.

When the RFLP patterns of the culture and room temperature samples were combined at the tree species level, the levels of similarity ranged from 0 to 0.20. *Picea sitchensis* (PISI) and *Tsuga heterophylla* (TSHE) from CHE had a similarity of 0.032. *Tsuga heterophylla* from CHE and *Tsuga heterophylla* from HJA had an RFLP pattern similarity of 0.029. *Tsuga heterophylla* and *Pseudotsuga menziesii* (PSME) from HJA had an RFLP pattern similarity of 0.20. *Pinus ponderosa*

(PIPO) and *Pinus contorta* (PICO) from the DNF had an RFLP similarity index of 0.11 (Table 6).

Table 6. Similarity matrix of room temperature and culture samples combined for each species combination.

	CHE-PISI	CHE-TSHE	HJA-PSME	HJA-TSHE	DNF-PICO	DNF-PIPO
CHE*-PISI#	1					
CHE-TSHE	0.032	1				
HJA-PSME	0.031	0.068	1			
HJA-TSHE	0	0.029	0.20	1		
DNF-PICO	0	0.061	0.060	0.053	1	
DNF-PIPO	0	0.064	0.094	0.082	0.11	1

*CHE=Cascade Head Experimental Forest/Siuslaw National Forest; HJA=H.J. Andrews Experimental Forest; DNF=Deschutes National Forest
#PICO=*Pinus contorta*; PIPO=*Pinus ponderosa*; TSHE=*Tsuga heterophylla*; PSME=*Pseudotsuga menziesii*; PISI=*Picea stichensis*

The similarity of RFLP patterns when compared across stands ranged from 0 to 0.28 with the largest similarity between the HJA site cut in 1993 and the HJA site cut in 1988 (Table 7). The CHE site cut in 1989, DNF sites cut in 1988 and 1991 each had 0 similarity in all but one site comparison.

For individual stumps the similarity of fungal RFLP patterns ranged from 0 to 0.67. The 0.67 level of similarity was found between a *Tsuga heterophylla* stump sampled in the stand cut in 1989 at HJA and a *Pseudotsuga menziesii* stump from the 1986 cut from the HJA (Figure 4). The RFLPs from CHE *Picea sitchensis* stumps had no level of similarity with stumps from the other two sites. The only stump comparison among the *Tsuga heterophylla* stumps from CHE and HJA that was similar was a CHE stump from Hibbs cut and a HJA stump from the 1993 cut (0.18). All other comparisons between this species at the two sites were zero. Within the *Pinus ponderosa* stumps from DNF there was no similarity.

Table 7. Similarity matrix of room temperature and culture samples combined for each stand combination.

	CH-1986	CH-1989	CH-Hibbs	HJ-1993	HJ-1989	HJ-1986	DF-1991	DF-1985	DF-1988
CH*-1986	1								
CH-1989	0.049	1							
CH-Hibbs	0	0	1						
HJ-1993	0	0	0.048	1					
HJ-1989	0.049	0	0	0.28	1				
HJ-1986	0	0	0.080	0.083	0.039	1			
DF-1991	0.061	0	0	0	0	0	1		
DF-1985	0	0	0.036	0.15	0.035	0.032	0	1	
DF-1988	0	0	0	0	0	0	0.059	0	1

*CH=Cascade Head Experimental Forest/Siuslaw National Forest; HJ=H.J. Andrews Experimental Forest; DF=Deschutes National Forest

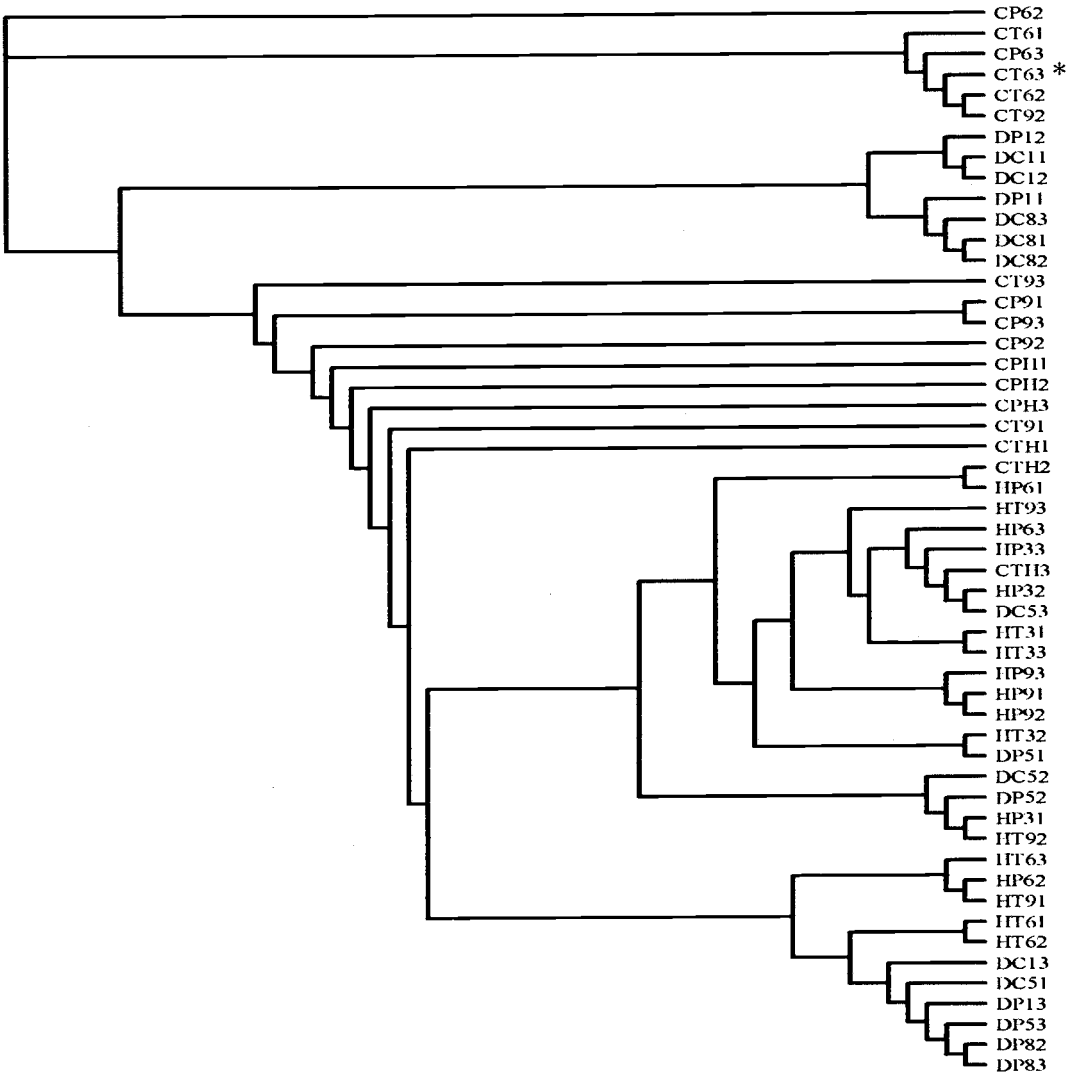

Figure 4. Unrooted neighbor joining tree for similarity for stumps from combined room temperature and culture results. Branches on the tree that are closer together represent stumps with greater similarity in RFLP patterns than stumps on branches that are further apart. The first letter in the code represents the site, the second letter indicates the species, the third number is the year the stand was harvested, and the last digit in code refers to stump 1, 2, or 3 from each species at each stand.

*(CT6=CHE, TSHE, 1986; CT8=CHE, TSHE, 1988; CTH=CHE, TSHE, HIBBS; CP6=CHE, PISI, 1986; CP9=CHE, PISI, 1989; CPH=CHE, PISI, HIBBS; HP6=HJA, PSME 1986; HP9=HJA, PSME, 1989; HP3=HJA, PSME, 1993; HT6=HJA, TSHE, 1986; HT9=HJA, TSHE, 1989; HT3=HJA, TSHE, 1993; DC5=DNF, PICO, 1985; DC8=DNF, PICO, 1988; DC1=DNF, PICO, 1991; DP5=DNF, PIPO, 1985; DP8=DNF, PIPO, 1988; DP3=DNF, PIPO, 1993)

RFLP similarities for year 2000 culture samples

Overall, the levels of similarity are higher when the culture results were compared alone versus the year 2000 culture and room temperature combined results. Not all fungi could be recovered from the wood by growing them in culture; therefore, the total possible species would be less than with the room temperature samples and allow for a greater level of RFLP overlap. There was greater similarity among the site level comparisons than when the year 2000 culture and room temperature samples were combined. CHE and HJA had an RFLP pattern similarity of 0.065. DNF and HJA had a similarity of 0.074. CHE and DNF had a similarity of 0.061.

In comparisons of each tree species, the similarities ranged from 0 to 0.24 with the greatest similarity between HJA *Pseudotsuga menziesii* and HJA *Tsuga heterophylla*. HJA *Tsuga heterophylla* and CHE *Tsuga heterophylla* had a similarity in RFLP patterns of 0.037 (Table 8).

In a site level comparison, the similarities ranged from 0 to 0.16 (Table 9). Culture samples compared at the individual stump level ranged in similarity from 0 to 0.67. As with the combined results, *Picea sitchensis* stumps from CHE did not have RFLP patterns that overlapped with HJA *Tsuga heterophylla,* or either species from DNF (Figure 5). DNF *Pinus contorta* and DNF *Pinus ponderosa* stumps did not demonstrate any similarity to each other. The greatest number of RFLP similarities above zero occurred in the comparisons of *Picea sitchensis* and *Tsuga heterophylla* from CHE in the 1986 site and between HJA *Pseudotsuga menziesii* and *Tsuga heterophylla* stumps at all sites.

Table 8. Similarity matrix of culture samples for each species combination

	CH-PISI	CH-TSHE	HJ-PSME	HJ-TSHE	DF-PICO	DF-PIPO
CH*-PISI#	1					
CH-TSHE	0.047	1				
HJ-PSME	0.043	0.091	1			
HJ-TSHE	0	0.037	0.24	1		
DF-PICO	0	0.074	0.069	0.059	1	
DF-PIPO	0	0.13	0.12	0.098	0.13	1

*CH=Cascade Head Experimental Forest/Siuslaw National Forest; HJ=H.J. Andrews Experimental Forest; DF=Deschutes National Forest

#PICO=*Pinus contorta*; PIPO=*Pinus ponderosa*; TSHE=*Tsuga heterophylla*; PSME=*Pseudotsuga menziesii*; PISI=*Picea stichensis*

Table 9. Similarity matrix for culture samples for each stand combination

	CH-1986	CH-1989	CH-Hibbs	CH-1993	CH-1989	CH-1986	CH-1991	CH-1985	CH-1988
CH*-1986	1								
CH-1989	0.061	1							
CH-Hibbs	0	0	1						
CH-1993	0	0	0.069	1					
CH-1989	0.061	0	0	0.33	1				
CH-1986	0	0	0.12	0.093	0.049	1			
CH-1991	0.071	0	0	0	0	0	1		
CH-1985	0.044	0	0.050	0.16	0.043	0.037	0	1	
CH-1988	0	0	0	0	0	0	0.071	0	1

*CH=Cascade Head Experimental Forest/Siuslaw National Forest; HJ=H.J. Andrews Experimental Forest; DF=Deschutes National Forest

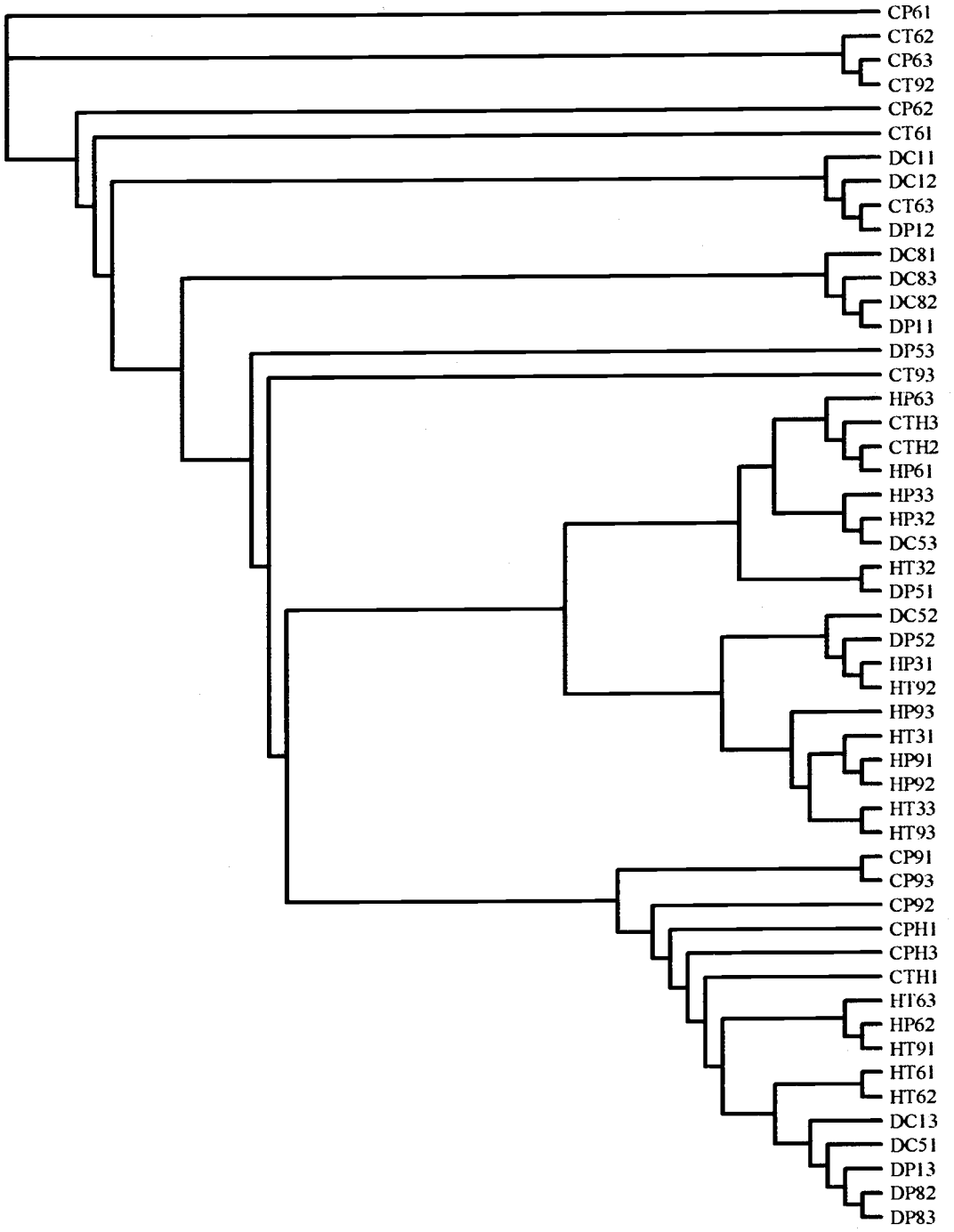

Figure 5. Unrooted neighbor joining tree for similarity in RFLP groups for cultured samples by stump. See Figure 4 for explanation of codes

RFLP similarity of year 2000 room temperature samples

In similarity analysis of the room temperature samples there was no similarity in RFLP patterns for sites, species, or stands. With this sampling technique even less of the potential biodiversity was sampled than with culturing methods which may account for the lack of similarity among samples. Stump comparisons ranged from 0 to 1. The similarities of level 1 occurred between all three stumps of room temperature HJA *Pseudotsuga menziesii* from the 1989 cut. These three had a unique RFLP type only found in these samples. The same phenomenon occurred with room temperature samples from two *Tsuga heterophylla* stumps in the 1993 cut at HJA

RFLP similarity of year 2000 room temperature and culture samples compared separately

The similarity of RFLPs from room temperature and cultured samples compared separately was also low even when the same sites, species, stands, and stumps were compared. This is influenced by the room temperature and cultured methods of fungal collection yield different fungal RFLP patterns. The similarity of RFLP patterns at the site level ranged from 0 to 0.074 (Table 10).

Pinus contorta was the only tree species to have any level of similarity between room temperature and culture sample RFLP patterns at a level of 0.053 (Table 11). A similar pattern of dissimilarity was found when stands were compared. All stand comparisons of room temperature to culture samples had a similarity of 0 except for the DNF 1988 cut with a similarity of 0.11 between room temperature and culture samples (Table 12). Stump comparisons ranged from 0 to 1 (Figure 6). The similarities equal to 1 were found in the comparison listed above in the room temperature analysis.

Table 10. Similarity of room temperature and culture samples separately for each site combination

	Cult-CHE	RT-CHE	Cult-HJA	RT-HJA	Cult-DNF	RT-DNF
Cult#-CHE*	1					
RT-CHE	0	1				
Cult-HJA	0.065	0	1			
RT-HJA	0	0	0	1		
Cult-DNF	0.061	0	0.074	0	1	
RT-DNF	0	0	0	0	0.030	1

*CHE=Cascade Head Experimental Forest/Siuslaw National Forest; HJA=H.J. Andrews Experimental Forest; DNF=Deschutes National Forest

#Cult=Cultured samples; RT=hyphae samples collected from room temperature roots

Table 11. Similarity matrix of room temperature and culture samples separately for each species combination

	CH-PISI	CH-RT-PISI	CH-TSHE	CH-RT-TSHE	HJ-PSME	HJ-RT-PSME	HJ-TSHE	HJ-RT-TSHE	DF-PICO	DF-RT-PICO	DF-PIPO	DF-RT-PIPO
CH*-PISI#	1											
CH-RT-PISI	0	1										
CH-TSHE	0.047	0	1									
CH-RT-TSHE	0	0	0	1								
HJ-PSME	0.043	0	0.091	0	1							
HJ-RT-PSME	0	0	0	0	0	1						
HJ-TSHE	0	0	0.037	0	0.24	0	1					
HJ-RT-TSHE	0	0	0	0	0	0	0	1				
DF-PICO	0	0	0.074	0	0.069	0	0.059	0	1			
DF-RT-PICO	0	0	0	0	0	0	0	0	0.053	1		
DF-PIPO	0	0	0.13	0	0.12	0	0.098	0	0.13	0	1	
DF-RT-PIPO	0	0	0	0	0	0	0	0	0	0	0	1

*CH=Cascade Head Experimental Forest/Siuslaw National Forest; HJ=H.J. Andrews Experimental Forest; DF=Deschutes National Forest

#PICO=*Pinus contorta*; PIPO=*Pinus ponderosa*; TSHE=*Tsuga heterophylla*; PSME=*Pseudotsuga menziesii*; PISI=*Picea stichensis*

Table 12. Similarity matrix of room temperature and culture samples separately for each site combination

	CH-1986	CH-RT-1986	CH-1989	CH-RT-1989	CH-Hibbs	CH-RT-Hibbs	HJ-1993	HJ-RT-1993	HJ-1989	HJ-RT-1989	HJ-1986	HJ-RT-1983	DF-1991	DF-RT-1991	DF-1985	DF-RT-1985	DF-1988	DF-RT-1988
CH*-1986	1																	
CH-RT-1986	0	1																
CH-1989	0.061	0	1															
CH-RT-1989	0	0	0	1														
CH-Hibbs	0	0	0	0	1													
CH-RT-Hibbs	0	0	0	0	0	1												
HJ-1993	0	0	0	0	0.069	0	1											
HJ-RT-1993	0	0	0	0	0	0	0	1										
HJ-1989	0.061	0	0	0	0	0	0.33	0	1									
HJ-RT-1989	0	0	0	0	0	0	0	0	0	1								
HJ-1986	0	0	0	0	0.12	0	0.093	0	0.049	0	1							
HJ-RT-1983	0	0	0	0	0	0	0	0	0	0	0	1						
DF-1991	0.071	0	0	0	0	0	0	0	0	0	0	0	1					
DF-RT-1991	0	0	0	0	0	0	0	0	0	0	0	0	0	1				
DF-1985	0.044	0	0	0	0.05	0	0.16	0	0.043	0	0.037	0	0	0	1			
DF-RT-1985	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1		
DF-1988	0	0	0	0	0	0	0	0	0	0	0	0	0.071	0	0	0	1	
DF-RT-1988	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.11	1

*CH=Cascade Head Experimental Forest/Siuslaw National Forest; HJ=H.J. Andrews Experimental Forest; DF=Deschutes National Forest

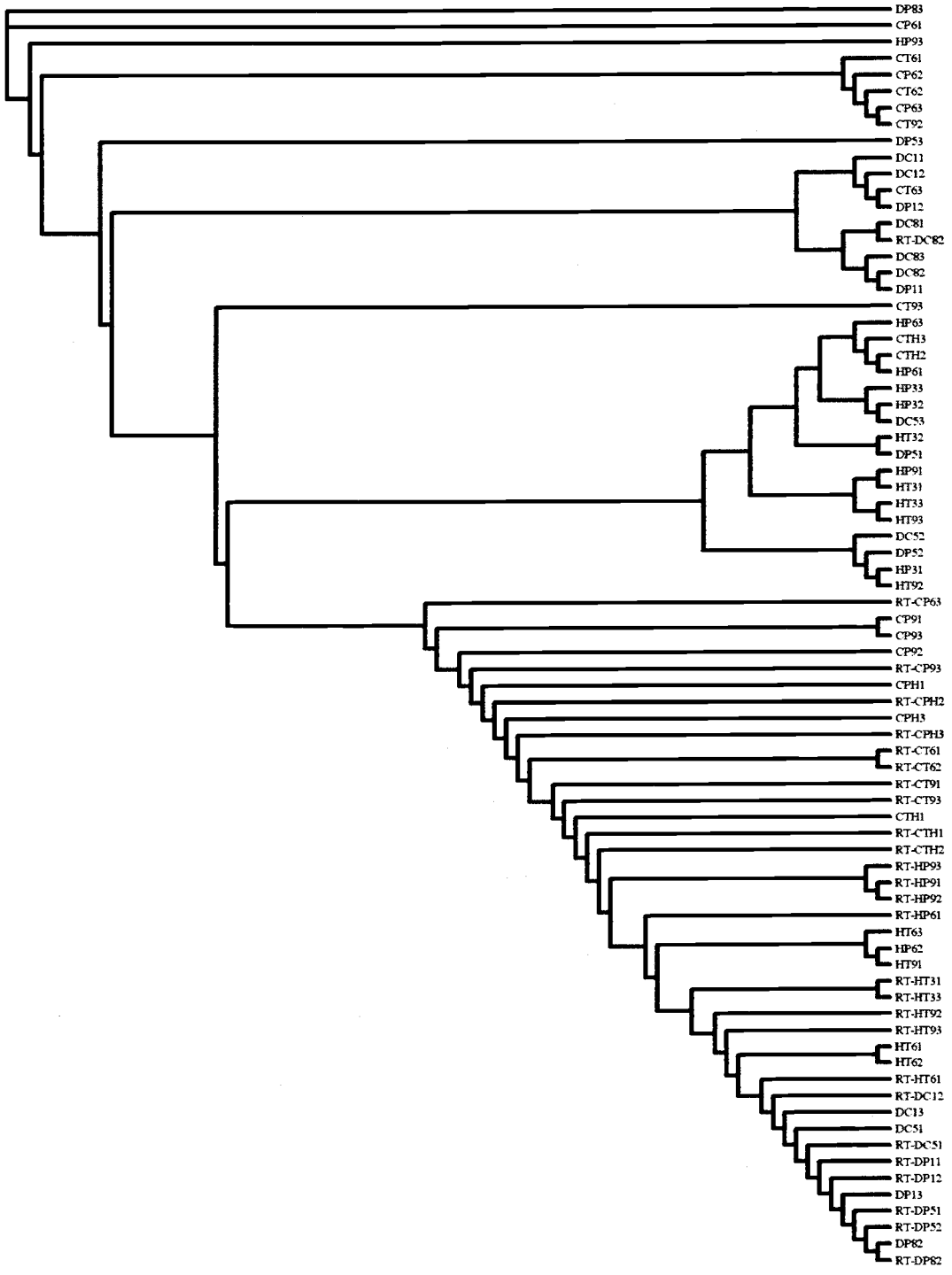

Figure 6. Unrooted neighbor joining tree for similarity in RFLP groups for room temperature and cultured samples separately by stump. See Figure 4 for explanation of codes.

RFLP similarity of reciprocal transplant roots (5-year)

There were few comparisons among the 5-year old reciprocal transplant root RFLPs with levels of similarity above zero. Of the thirty-five samples from reciprocal transplant roots, only two were from room temperature samples. Because there were only two room temperature samples from reciprocal transplant roots that amplified, all methods of fungi collection from 5-year roots have been combined for this analysis. The RFLP types at CHE and HJA had a similarity of 0.14 and there was no similarity between the other two site comparisons. The comparisons of individual root RFLP similarities yielded only two pairs where the RFLP patterns were similar. A *Pseudotsuga menziesii* root from CHE and a *Pinus ponderosa* root from DNF had a similarity of 0.67. The same level of similarity occurred between two *Pinus ponderosa* roots from DNF. The *Pseudotsuga menziesii* and *Pinus contorta* roots from all three sites had a similarity of 0.15. There was no similarity of ITS-RFLP types between any of the other tree species.

RFLP similarity of common patterns from year 2000 culture, room temperature, and reciprocal transplant roots

There were twenty-eight common RFLP patterns where samples with a particular RFLP pattern were found in more than one stump or root from either the culture, room temperature, or 5-year roots harvested in 2000. There was a similarity of 0.24 between the CHE and HJA common RFLP types. HJA and DNF had a similarity of 0.31. CHE and DNF had a similarity of 0.29. The similarity among tree species for common RFLP types ranged from 0 to 0.64 with the greatest similarity in the comparison of HJA *Pseudotsuga menziesii* and HJA *Tsuga heterophylla* (Table 13). HJA *Tsuga heterophylla* and CHE *Tsuga heterophylla* had a similarity of 0.12. At CHE, *Picea sitchensis* and *Tsuga heterophylla* had a

similarity of 0.18. At DNF, *Pinus ponderosa* and *Pinus contorta* had a similarity in RFLP types of 0.53. The similarity among stands ranged from 0 to 0.57 (Table 14).

Table 13. Similarity matrix of common RFLP patterns for each species combination

	CH-PISI	CH-TSHE	HJ-PSME	HJ-TSHE	DF-PICO	DF-PIPO
CH*-PISI#	1					
CH-TSHE	0.18	1				
HJ-PSME	0.13	0.21	1			
HJ-TSHE	0	0.12	0.64	1		
DF-PICO	0	0.27	0.20	0.22	1	
DF-PIPO	0	0.43	0.32	0.35	0.53	1

*CH=Cascade Head Experimental Forest/Siuslaw National Forest; HJ=H.J. Andrews Experimental Forest; DF=Deschutes National Forest
#PICO=*Pinus contorta*; PIPO=*Pinus ponderosa*; TSHE=*Tsuga heterophylla*; PSME=*Pseudotsuga menziesii*; PISI=*Picea stichensis*

Table 14. Similarity matrix of common RFLP patterns for each stand combination.

	CH-1986	CH-1989	CH-Hibbs	HJ-1993	HJ-1989	HJ-1986	DF-1991	DF-1985	DF-1988
CH*-1986	1								
CH-1989	0.22	1							
CH-Hibbs	0	0	1						
HJ-1993	0	0	0.17	1					
HJ-1989	0.15	0	0	0.56	1				
HJ-1986	0	0	0.57	0.27	0.15	1			
DF-1991	0.22	0	0	0	0	0	1		
DF-1985	0.20	0	0.29	0.53	0.15	0.20	0	1	
DF-1988	0	0	0	0	0	0	0.29	0	1

*CH=Cascade Head Experimental Forest/Siuslaw National Forest; HJ=H.J. Andrews Experimental Forest; DF=Deschutes National Forest

Biodiversity results for year 2001 resampled roots

In the resampling of three *Pseudotsuga menziesii* stumps, seventy-seven percent of the seventy-four resampled culture isolates amplified. There were twenty-five fungal ITS-RFLP types. Six of these matched with RFLP types found in the original culturing experiments. There were three matches of RFLP patterns between the Goldfarb's media and malt-agar media with Goldfarb's showing thirteen RFLP types and malt-agar having eighteen. As with the year 2000 samples, the RFLP sampling intensity curves demonstrate a continual linear increase in biodiversity with each new cultured sample and with each new harvested root sample (Figure 7 and Figure 8).

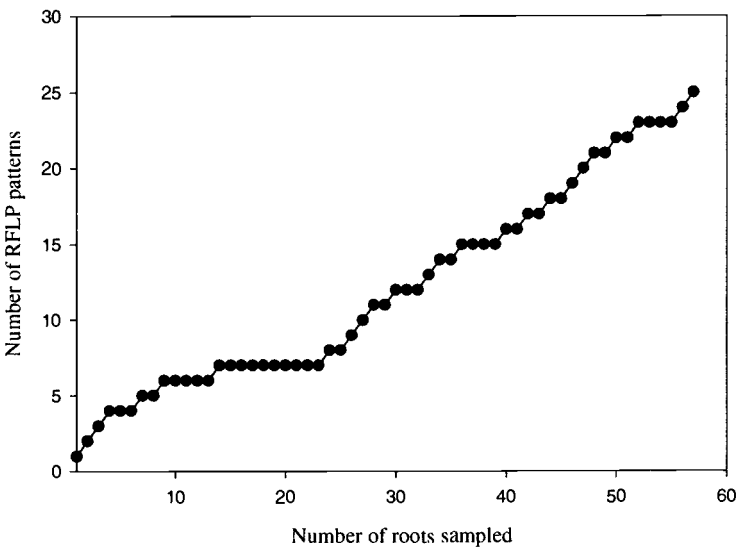

Figure 7. ITS-RFLP sampling intensity curve for year 2001 resampled roots

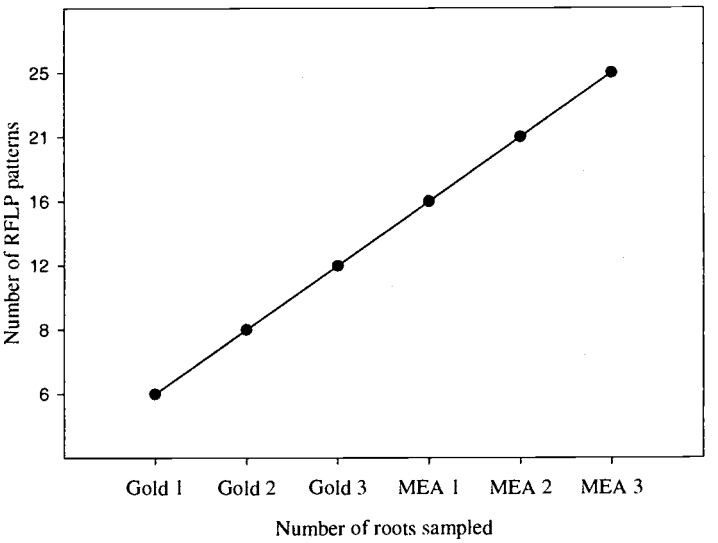

Figure 8. ITS-RFLP sampling intensity curve for resampled roots from each root and media type

RFLP similarity from year 2001 resampled roots

The year 2001 resampled roots were comparably similar to each other as with the year 2000 culture samples. When the results of the two media were combined, the first and second roots sampled had a similarity index of 0.30. The second and third roots had a similarity of 0.20. The first and third roots had a similarity of 0.10. The samples from the first root cultured with Goldfarb's and malt-agar had a similarity to each other of 0.33. In the second root, the cultures with the two medias had a similarity of 0.18. The similarities between the two media in the third root were 0.31. Root one cultured with Goldfarb's had similarities of 0.40 and 0.18 with roots two and three respectively (Table 15). Goldfarb's roots two and three had a similarity of 0.22. Root one cultured with malt-agar had a similarity of 0.15 and 0.14 with roots two and three respectively. Malt-agar roots two and three had a similarity of 0.27.

Table 15. Similarity matrix for resampled roots (1, 2, 3) with Goldfarb's (GF) and Malt-agar (MA) media

	GF-1	GF-2	GF-3	MA-1	MA-2	MA-3
GF-1	1					
GF-2	0.40	1				
GF-3	0.18	0.22	1			
MA-1	0.33	0.20	0.18	1		
MA-2	0.31	0.18	0	0.15	1	
MA-3	0.14	0	0.31	0.14	0.27	1

Sequences

Sequences were run for the twenty-three of the common RFLPs and five uncommon RFLPs in either the small unit or large subunit rRNA. Eleven RFLP patterns were sequenced for both the LSU and SSU genes representing all of the sampled tree species (Table 16 and Table 17). Sequences from two room temperature samples and one 5-year root matched with basidiomycete fungi. All other culture sequences fell out within the zygomycete class (Sequences Appendix 2). Many samples matched with fungi in the Mortierellaceae family in the genera of *Umbelopsis, Mortierella,* and *Micromucor*. These genera are closely related to each other (O'Donnell et al. 2001). This corroborates the microscopic evidence suggesting that zygomycetes were common fungi in these saprotrophic root communities. When sequences were compared with both the LSU and SSU for one sample, the LSU was able to detect greater variability and species variation than could be seen with the more conserved SSU.

Table 16. Sequence matches for RFLP groups in the large subunit and small subunit based upon sequence blasts with GenBank. Information includes GenBank accession number of potential matches, number of base pairs compared, percent matched basepairs (higher number indicates better match), error rating (lower number indicates better match), and score in bits (higher number indicates better match).

				Large subunit								Small subunit							
Site	Species	Stand	RFLP group	Genus	Species	Family	GenBank Accession	Number of Basepairs	%	Error	Score (bits)	Genus	species	Family	GenBank Accession	Number of Basepairs	%	Error	Score (bits)
Cascade Head	PISI#	1986	10									*Mucor*	*hiemalis*	Mortierellaceae	AF113428	505	98	0	959
	PISI	1989	11	*Micromucor*	*ramannianus*	Mortierellaceae	AF113463	205	98	3.00E-99	387	*Mucor*	*hiemalis*	Mucorales	AF113428	437	99	0	858
	PISI	1989	39	*Umbelopsis*	*isabellina*	Mortierellaceae	AF157220	306	99	E-147	525								
	PISI	1986*	5	*Umbelopsis*	*nana*	Mortierellaceae	AF157221	301	98	E-137	494								
	TSHE	1986	8									*Mucor*	*hiemalis*	Mucorales	AF113428	416	99	0	806
	TSHE	1986	26	*Umbelopsis*	*nana*	Mortierellaceae	AF157221	302	100	E-157	541	*Umbelopsis*	*isabellina*	Mortierellaceae	AF157166	391	95	E-151	541
	TSHE	1989	3									*Umbelopsis*	*isabellina*	Mortierellaceae	AF157166	514	98	0	876
	TSHE	1986*	5									*Umbelopsis*	*isabellina*	Mortierellaceae	AF157166	517	99	0	995
	TSHE	Hibbs**	7									*Mucor*	*hiemalis*	Mucorales	AF113428	412	98	0	771
	RT-TSHE$	1986	18	*Resinicium*	*bicolor*	Corticiaceae [B]	AF141635	273	98	E-142	509								
	PSME	5-yr	22									*Mortierella*	*chlamydospora*	Mortierellaceae	AF157143	491	99	0	942
HJ Andrews	PSME	1986	35	*Umbelopsis*	*nana*	Mortierellaceae	AF157221	124	97	3.00E-51	206								
	PSME	1988	6	*Ellisomyces*	*anomalus*	Thamnidiaceae	AF157188	211	98	E-103	381								
	PSME	1988	12									*Umbelopsis*	*isabellina*	Mortierellaceae	AF157166	503	99	0	989
	PSME	1988	17									*Umbelopsis*	*isabellina*	Mortierellaceae	AF157166	517	99	0	1009
	PSME	1988	21									*Umbelopsis*	*isabellina*	Mortierellaceae	AF157166	507	99	0	997
	PSME	1989	41	*Mortierrella*	*verticillata*	Mortierellaceae	AF157199	249	100	E-119	432								
	PSME	1986**	7	*Micromucor*	*ramannianus*	Mortierellaceae	AF113463	301	99	E-146	523								
	PSME	1993***	14									*Umbelopsis*	*isabellina*	Mortierellaceae	AF157166	491	100	0	973
	PSME	5-yr	29									*Umbelopsis*	*isabellina*	Mortierellaceae	AF157166	508	99	0	989
	TSHE	1986	2	*Umbelopsis*	*isabellina*	Mortierellaceae	AF157220	278	94	E-100	371	*Umbelopsis*	*isabellina*	Mortierellaceae	AF157166	491	99	0	930
	TSHE	1986	9	*Umbelopsis*	*nana*	Mortierellaceae	AF157221	300	98	E-137	494	*Umbelopsis*	*isabellina*	Mortierellaceae	AF157166	416	100	0	823
	TSHE	1993	16	*Umbelopsis*	*isabellina*	Mortierellaceae	AF157220	297	99	E-157	525	*Umbelopsis*	*isabellina*	Mortierellaceae	AF157166	517	100	0	1017
	TSHE	1993	23	*Micromucor*	*ramannianus*	Mortierellaceae	AF113463	257	97	E-122	442	*Umbelopsis*	*isabellina*	Mortierellaceae	AF157166	437	99	0	858
	TSHE	1988***	14	*Umbelopsis*	*isabellina*	Mortierellaceae	AF157221	301	100	E-151	539								
Deschutes NF	PICO	1988	20	*Umbelopsis*	*nana*	Mortierellaceae	AF157221	231	97	4.00E-83	313	*Umbelopsis*	*isabellina*	Mortierellaceae	AF157166	518	99	0	987
	PICO	1988	43	*Zygohynchus*	*heterogamus*	Mucoraceae	AF157224	190	95	5.00E-82	309								
	PICO	1991	27	*Micromucor*	*ramannianus*	Mortierellaceae	AF113463	219	98	E-110	404	*Umbelopsis*	*isabellina*	Mortierellaceae	AF157166	491	98	0	916
	PIPO	1985	13									*Zygorhynchus*	*heterogamus*	Mucoraceae	AF157170	481	98	0	874
	RT-PICO	1988	19	*Resinicium*	*bicolor*	Corticiaceae [B]	AF141635	265	98	E-129	468	*Ossicaulis*	*lignatilis*	Coriolaceae [b]	AF334923	91	97	8.00E-39	165
	unk	5-yr	30	*Multiclavula*	*vernalis*	Clavariaceae[B]	MVU66439	293	97	E-143	513	*Hydnum*	*repandum*	Hydnaceae [B]	AF026641	431	96	E-150	537

*, **, and *** represent samples from the same RFLP group
[B] represents basidiomycetes, all others are zygomycetes
$5-year=Reciprocal transplant; RT=Room temperature samples
#PICO=*Pinus contorta*; PIPO=*Pinus ponderosa*; TSHE=*Tsuga heterophylla*; PSME=*Pseudotsuga menziesii*; PISI=*Picea sitchensis*

Table 17. RFLP groups where one sample was sequenced and others within the group were found on additional tree species

Site	Species	Stand	RFLP group	CHE-TSHE	PISI	HJA-TSHE	PSME	PICO	PIPO	5-YEAR	RT
Cascade Head	PISI	1986	10		**XX**						
	PISI	1989	11		X						
	PISI	1989	39		X						
	PISI	1986*	5	X	X						
	TSHE	1986	8	**XX**							
	TSHE	1986	26	X				X			
	TSHE	1989	3	X							
	TSHE	1986*	5	X	X						
	TSHE	Hibbs**	7					X		X	
	RT-TSHE$	1986	18								X
	PSME	5-yr	22							**XX**	
HJ Andrews	PSME	1986	35	X		X	X	X	X	X	
	PSME	1988	6			X	X				
	PSME	1988	12			X	X				
	PSME	1988	17				X		X		
	PSME	1988	21			X	X				
	PSME	1989	41		X		X				
	PSME	1986**	7	X			X			X	
	PSME	1993***	14			X	X	X			
	PSME	5-yr	29							**XX**	
	TSHE	1986	2			X	X				
	TSHE	1986	9			X					
	TSHE	1993	16				X				
	TSHE	1993	23			X					
	TSHE	1988***	14			X	X	X			
Deschutes NF	PICO	1988	20					X	X		
	PICO	1988	43					X			
	PICO	1991	27					X			
	PIPO	1985	13						**XX**		
	RT-PICO	1988	19					X			X
	Unknown	5-yr	30					X		X	

*, **, and *** represent samples from the same RFLP group
X are common RFLP groups
XX are noncommon RFLP groups
$5-year=Reciprocal transplant; RT=Room temperature samples
#PICO=*Pinus contorta*; PIPO=*Pinus ponderosa*; TSHE=*Tsuga heterophylla*; PSME=*Pseudotsuga menziesii*; PISI=*Picea sitchensis*

DISCUSSION

The fungi sampled in this study represented many RFLP patterns signify a diversity of fungi on coarse roots. Based on ITS-RFLP patterns, there is little overlap between individual sites, tree species, harvest stands, or stumps and roots. The pattern of low similarity in RFLP types is consistent throughout the analysis from all of the various methods used to collect fungal hyphae from roots. Samples from HJA had the greatest levels of similarity to each other. The 1989 stand at CHE, 1991 stand at DNF, and 1988 stand at DNF had the lowest levels of similarity to all other stands. The RFLP patterns from *Tsuga heterophylla* from CHE and HJA were not similar to each other at the site, stand, or stump level. Results are parallel to those others have found for microfungi diversity (Christensen 1969, Bills and Polishook 1994, Polishook et al. 1996). The diversity of fungi on these roots was much greater than plant diversity at the same locations (personal observation). Studies have indicated that there are a finite number of described tree, shrub, and herb plant species yet many fungi are still to be described (Christensen 1969). Plant communities tend to be much more similar between distant stands than fungal communities are in stands the same distance apart (Christensen 1969, Gauch 1982).

The ITS-RFLP sampling intensity curves for each of the sampling methods demonstrate that with each new sample taken, the number of RFLP patterns continues to increase. These low levels of overlap in fungal RFLP patterns and the increasing of the RFLP sampling intensity curves, points to an underestimation of the total fungal diversity in these roots and the need for greater sampling for more accurate biodiversity estimates (Polishook et al. 1996). This is exacerbated by the fact that the methods currently available for isolation and identification of these fungi may be inadequate to measure the true fungal diversity (Bridge and Spooner 2001). Only seventeen percent of known fungi can be grown in culture (Hawksworth 1991). The room temperature samples had disappointingly low

amplification rates, and this might be due to the difficulties in separating fungal hyphae from solid wood. These two factors combined create a challenge in sampling all fungi from the roots. As there is practically no overlap in fungal communities between the cultured, room temperature, and 5-year root samples, it is important to investigate new techniques for more successfully collecting and amplifying fungal DNA directly from wood. This could also increase the accuracy of fungal biodiversity measurements.

One way to increase the potential to sample all fungi would be to systematically collect samples along the entire length of a root. In the resampled roots from this study, each root was cultured into at least twenty isolates. However, the RFLP area curves indicate that this was still not enough samples to measure the complete community diversity of the system. Due to the incompleteness of sampling simply through culturing, it is impossible to estimate how many fungi were missed by this sampling method. Other alternative sampling methods to address the biodiversity could include more concentrated sampling of greater numbers of stumps and roots from one stand over time and increased numbers of replicates at the site or stand level. To get the most accurate estimate of fungal diversity possible, several methods for collecting fungi from roots should be established to discover at what point the species area curve will level off. It would be ideal to develop long-term studies to follow the change in fungal communities as the resources from the wood alter throughout time and how that affects the changing community. Future studies should continue to focus on identification of saprotrophic fungi and their roles in decomposition to explain tree species differences in root decay rates.

Due to the ubiquitous nature of zygomycetes, it is not surprising that they were prominent species within this study. They could have been part of the active wood decomposer community or taking advantage of the simple carbohydrates created through decomposition on these roots. Washing the roots in deionized water as opposed to direct collection of hyphae from the roots could have changed

the community of fungi that was extracted (Parkinson and Crouch 1969). However, even after several washings of roots, zygomycetes are often still present as they are common soil microfungi (Christensen 1969, Kuhlman 1969, Parkinson and Crouch 1969, O'Donnell et al. 2001). Zygomycetes are found in the earliest and latest stages of decomposition where simple carbohydrates are readily available (Rayner and Boddy 1988). We hypothesized that communities of white- and brown-rot basidiomycetes would be prominent in the roots. While some roots showed evidence of white- and brown-rot, others did not, possibly indicating that the sampled roots could represent both early and later stages of decomposition. The sequences of room temperature samples indicate that there are basidiomycetes in the roots. However, basidiomycetes were not recovered in the cultures. Zygomycetes are relatively easy to culture compared to other orders of fungi and that may account for their prominence. Because the aboveground field collected fungi were ascomycete and basidiomycete species and not the same as the zygomycetes cultured from roots in the same areas, it indicates that basidiomycete and ascomycete hyphae could potentially be recovered from belowground coarse roots using different techniques.

Based on the sequences of the common RFLP types and microscopic structures, we conclude that zygomycetes rather than basidiomycetes grew best in the culture media selected for this study. Whatever media a researcher chooses will influence the fungal isolates that will grow, although few known fungi can be successfully grown in culture (Parkinson and Crouch 1969,Hawksworth 1991). These two factors create the likelihood that even with multiple media recipes and different methods for removing hyphae from wood not all fungi will be sampled with culturing methods. Because the potential number of species which can be cultured is potentially lower than the number of actual species in the wood, this may explain the higher levels of similarity found in culture samples compared to room temperature samples. In the initial sampling, a malt-agar media was selected that is often used to promote growth of basidiomycetes from wood (Camille

Freitag, personal communication); however, zygomycetes also grow well in a malt-agar media (Kuhlman 1969). In the resampled roots, Goldfarb's media was used as it is designed to limit the growth of zygomycetes and ascomycetes and hence encourage the growth of basidiomycetes. Even with this media, zygomycetes still grew well with no evidence of basidiomycete growth.

Sequences provide a tool to look more closely at the genetic diversity of samples. Some samples had limited base pair changes in the large subunit (LSU) and many samples looked identical when sequences from the small subunit (SSU) were compared. The SSU is conserved and hence quite similar within the kingdom Fungi. However, this gene can still be used to group closely related fungi together. When identified herbarium samples and known cultures were sequenced in the SSU, the resulting sequences matched with the correct species when compared with the GenBank database. The large subunit is often used to distinguish greater variation between samples (O'Donnell et al. 2001). However, even among the samples that matched with *Umbelopsis isabellina* for the LSU there was individual base pair variation between samples (Appendix 2). *Umbelopsis* have recently been collected and sequenced from *Pseudotsuga menziesii* roots in Idaho and have been discovered in the Duke Forest in North Carolina indicating that this genus is common and widespread (Jill Hoff personal communication).

While RFLPs provide a powerful tool for assessing the potential genetic diversity of saprotrophic fungi on coarse roots, the data must be interpreted with caution. Several conditions must be met to reach optimal potential of species sampling of saprotrophic fungi. The fungi must first be recovered from the wood and distinctions between primary saprobes and wood inhabitants made. Once the hyphae are collected, the DNA must be cleanly extracted and amplified. Finally, the amplified gene must be variable enough to distinguish species. In this study, difficulties in assessing the true species diversity of saprotrophic and wood inhabiting fungi came in three forms. The first was due to the inability to collect all fungi from the roots based on difficulties with culturing techniques and

mechanically collecting hyphae from roots. Secondly, only forty-five percent of all samples from wood amplified (Table 4). Finally, there were no RFLPs of known zygomycetes for comparison. Limited sequence analysis was carried out on the common RFLPs, but that leaves approximately one hundred seventy RFLPs still unidentified. While the vast quantities of RFLP patterns collected from this study provide an interesting glimpse into the genetic diversity of fungi in Oregon coarse conifer roots, we still do not know the identification of the majority of the samples. The problems in data collection and comparison create bottlenecks in collecting the data and makes drawing conclusions about the complete community composition of species present in the wood impossible.

In many ectomycorrhizal studies that employ ITS-RFLP techniques, samples are identified with a combination of matches to sporocarps, morphology, RFLP databases, and gene sequencing (Horton and Bruns 2001). To enhance the study of saprotrophic fungal diversity, it would be worthwhile to obtain RFLP patterns for common identified zygomycetes and check for potential matches with zygomycetes sampled in culture. ITS-RFLP techniques combined with longer-term successional studies, comparison with a wider variety of fungi including zygomycetes, and gene sequencing could help in gaining a better picture of which species of fungi are actively involved in decomposition of woody conifer roots.

We began this study with the hypothesis that there would be a core community of fungi with dominant RFLP types for each site and tree species. We hoped that this information might give us insights to the decomposition rates of roots. Because there is not a consistent pattern to the RFLPs, we are unable to explain the differences in decomposition of roots based upon RFLP similarities. However, these results provide a glimpse into the complexities of the microfungi communities that may be found throughout the decomposition process of coarse roots.

LITERATURE CITED

Allen, R.B., P.K. Buchanan, P.W. Clinton, and A.J Cone. 2000. Composition and diversity of fungi on decaying logs in a New Zealand temperate beech (Nothofagus) forests. Canadian Journal of Forest Research 30:1025-1033.

Altschul, S.F., T.L. Madden, A.A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D.J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucliec Acids Research 25:3389-3402.

Benjamin, R.K. 1979. Zygomycetes and their spores. pages 573-622 *in* B. Kendrick, editor. The Whole Fungus. National Museum of Canada Ottawa, Canada

Bills, G.F. and J.D. Polishook. 1994. Microfungi from decaying leaves of *Heliconia mariae* (Helioconiaceae). Brenesia 41-42:27-43.

Blanchette, R.A. 1984. Screening wood decayed by white rot fungi for preferential lignin degradation. Applied and Environmental Microbiology. 48:647-653.

Bridge, P. and B. Spooner. 2001. Soil fungi: diversity and detection. Plant and Soil 232:147-154.

Bruns, T.D., T.M. Szaro, M. Gardes, K.W. Cullings, J.J. Pann, D.L. Taylor, T.R. Horton, A. Kretzer, M. Garbelotto, and Y. Liss. 1998. A sequence database for the identification of ectomycorrhizal basidiomycetes by phylogenetic analysis. Molecular Ecology 7:257-272.

Chen, H., M. E. Harmon, and R. P. Griffiths. 2001. Decomposition and nitrogen release from decomposing woody roots in coniferous forests of the Pacific Northwest: A chronosequence approach. Canadian Journal of Forest Research 31:246-260.

Chen, H. M.E. Harmon, J. Sexton, and B. Fasth. 2002. Fine-root decomposition and N dynamics in coniferous forests of the Pacific Northwest, U.S.A. Canadian Journal of Forest Research 32:320-331.

Christensen, M. 1969. Soil microfungi of dry to mesic conifer-hardwood forest in northern Wisconsin. Ecology 50:9-27.

Edmonds R.L. and D.S. Lebo. 1998. Diversity, production, and nutrient dynamics of fungal sporocarps on logs in an old-growth temperate rain forest, Olympic National Park, Washington. Canadian Journal of Forest Research 28:665-673.

Gardes, M. and T.D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes--application to the identification of mycorrhizae and rusts. Molecular Ecology 2:113-118.

Gardes, M. and T.D. Bruns. 1996b. ITS-RFLP matching for identification of fungi. Pages 177-186 *in* J. P. Clapp, editor. Methods in Molecular Biology. Humana Press Inc, Totowa, NJ.

Gauch, Jr., H.G. 1982. Multivariate analysis in community ecology. Cambridge University Press, Cambridge. pg 109-172.

Gilbertson, R.L. 1980. Wood-rotting fungi of North America. Mycologia 72:1-49.

Gilbertson, R.L. 1981. North American wood-rotting fungi that cause brown rots. Mycotaxon 7:372-416.

Green III, F., and C.A. Clausen. 1999. Production of polygalacturonase and increase of longitudinal gas permeability in southern pine by brown-rot and white-rot fungi. Holzforschung 53:563-568.

Harmon, M.E., J.F. Franklin, F.J. Swanson, P. Sollins, S.V. Gregory, J.D. Lattink, N.H. Anderson, S.P. Cline, N.G. Aumen, J.R. Sedell, G.W. Lienkaemper, K. Cromack, Jr., and K.W. Cummins. 1986. Ecology of coarse woody debris in temperate ecosystems. Advances in Ecological Research. 15:133-302.

Harmon, M.E., J. Sexton, B.A. Caldwell, and S.E. Carpenter. 1994. Fungal sporocarp mediated losses of Ca, Fe, K, Mg, Mn, N, P, and Zn from conifer logs in the early stages of decomposition. Canadian Journal of Forest Research 24:1883-1893.

Harmon, M.E. 2001. Carbon sequestration in forests. Journal of Forestry 99:24-29.

Hawksworth, D. L. 1991. The Fungal Dimension of Biodiversity Magnitude Significance and Conservation. Mycological Research 95:641-655.

Horton, T. 2002 (in press). Molecular approaches to ectomycorrhizal diversity studies: variation in ITS at a local scale. Plant and Soil.

Kuhlman, E.G. 1969. Mucorales isolated from pine rot bark and wood. Canadian Journal of Botany 47:1719-1723.

Leonowicz, A., A. Matuszewska, J. Luterek, D. Ziegenhagen, M. Wjotas-Wasilewska, N.-S. Cho, M. Hofrichter, and J. Rogalski. 1999. Biodegration of lignin by white rot fungi. Fungal Genetics and Biology 27:175-185.

Mullis, K.B. and F.A. Falonna. 1987. Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. Methods in Enzymology. 155: 335-350.

Nei, M. and W.-H. Li. 1979. Mathematical Model for Studying Genetic Variation in Terms of Restriction Endonucleases. Proceedings of the National Academy of Sciences of the United States of America 76:5269-5273.

O'Donnell, K., F.M. Lutzoni, T.J. Ward, and G.L. Benny. 2001. Evolutionary relationships among mucoralean fungi (Zygomycota): Evidence for family polyphyly on a large scale. Mycologia 93:286-296.

Otjen, L., R.A. Blanchette, M. Effland, and G. Leatham. 1987. Assessment of 30 white rot basidiomycetes for selective lignin degradation. Holzforschung 41:343-349.

Page, R.D.M. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences 12: 357-358.

Parkinson, D. and R. Crouch. 1969. Studies on fungi in pinewood soil. V.—Root mycofloras of seedlings of *Pinus nigra* var. *laricio.* Rev. Ecol. Biol. Sol. 6: 263-275.

Polishook, J.D., G.F. Bills, and D.J. Lodge. 1996. Microfungi from decaying leaves of two rain forest trees in Puerto Rico. Journal of Industrial Microbiology. 17: 284-294.

Rayner, A.D.M. and L. Boddy. 1988. Fungal communities in the decay of wood. Advances in Microbial Ecology 10:115-166.

Tanesaka, E., H. Masuda, and K. Kinugawa. 1993. Wood degrading ability of basidiomycetes that are wood decomposers, litter decomposers, or mycorrhizal symbionts. Mycologia 85:347-354.

White, T.J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal rna genes for phylogenetics. Pages 315-322 *in* M. A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White, editor. PCR Protocols: A guide to methods and applications. Academic Press, Inc, San Diego.

Winandy, J.E. and J.J. Morrell. 1993. Relationship between incipient decay, strength, and chemical composition of Douglas-fir heartwood. Wood and Fiber Science 25:278-288.

Worrall, J.J., S.E. Anagnost, and R.A. Zabel. 1997. Comparison of wood decay among diverse lignicolous fungi. Mycologia 89:199-219.

Yoshizawa, N., N. Itoh, M. Sunagawa, S. Yokota, and T. Idei. 1992. Comparative histochemistry and enzyme activities during wood decay by white-rot fungi with selective lignin-degrading abilities. Bulletin Utsunomiya University Forestry 28:97-110.

CHAPTER 4 CONCLUSIONS

V. Eleanor Vandegrift

This research demonstrates the high diversity ITS-RFLP patterns in fungi on coarse conifer roots (*Picea sitchensis, Tsuga heterophylla, Pseudotsuga menziesii, Pinus ponderosa,* and *Pinus contorta*) in Oregon. There was little similarity in ITS-RFLPs from hyphae collected directly and cultured from roots of stumps or fungi from reciprocal transplant roots buried for five years. The low levels of overlap were found when comparisons were made of sites, tree species, harvest stands, and individual stumps for all types of fungi collection combinations. ITS-RFLP sampling intensity curves demonstrated a linear increase in the number of RFLPs with each increasing sample indicating that the full measure of ITS-RFLP diversity among these samples was not measured. This increasing trend in ITS-RFLP diversity also held true for samples where forty culture samples were taken from each root. Sequences of most samples placed them within the zygomycetes class of kingdom Fungi. Zygomycetes are typically found in the earliest and latest stages of wood decomposition.

At one time, two-thirds of land on earth was forested (Cooke and Rayner 1984). With increasing awareness of causes and potential alleviants for global warming, carbon sequestration in forests has gained increased attention. Management plans must include both human desires for wood products and habitat for a variety of species while locating potential sources and sinks of carbon for the present and the future. As management for global carbon budgets becomes more important, one factor managers, politicians, and scientists will have to account for is the level of respiration and carbon dioxide released from forests over time (Harmon 2001). The activity of microbes, including fungi, is important in the decomposition of woody debris. One piece of global carbon budgets is the level of respiration accounted for in the decomposition of above- and belowground woody debris. However, there has been limited research on belowground coarse root decomposition and associated fungal communities that could potentially be important components of global carbon budgets. The moisture, temperature, and wood chemistry data for these trees combined with the knowledge that there are

highly diverse groups of fungi in roots creates complexities for studying the decomposition of these conifer roots. Further studies focusing on the short-term and long-term activities and community succession of saprotrophic fungal communities may aid in determining the role that fungi play in the decomposition of roots over time. The community of fungi found in coarse wood and roots provides one piece of the puzzle. Increased understanding of all aspects of decomposition is important in developing forest management plans that account for long-term sources of decomposing wood and the essential elements provided to the environment.

BIBLIOGRAPHY

Alexopolous, C.J., C.W. Mims, and M. Blackwell. 1996. Introductory Mycology 4th edition. Wiley, New York.

Allen, R.B., P.K. Buchanan, P.W. Clinton, and A.J Cone. 2000. Composition and diversity of fungi on decaying logs in a New Zealand temperate beech (Nothofagus) forests. Canadian Journal of Forest Research 30:1025-1033.

Altschul, S.F., T.L. Madden, A.A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D.J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucliec Acids Research 25:3389-3402.

Ander, P. and K.-E. Ericksson. 1977. Selective degradation of wood components by white-rot fungi. Physiologia plantarum 41:239-248.

Benjamin, R.K. 1979. Zygomycetes and their spores. pages 573-622 *in* B. Kendrick, editor. The Whole Fungus. National Museum of Canada Ottawa, Canada

Benny, G.L. 1995. Classical morphology in zygomycete taxonomy. Canadian Journal of Botany 73:S725-S730.

Bills, G.F. and J.D. Polishook. 1994. Microfungi from decaying leaves of *Heliconia mariae* (Helioconiaceae). Brenesia 41-42:27-43.

Blanchette, R.A. 1984. Screening wood decayed by white rot fungi for preferential lignin degradation. Applied and Environmental Microbiology. 48:647-653.

Blanchette, R.A., T.A. Burnes, G.F. Leatham, and M.J. Effland. 1988. Selection of white-rot fungi for biopulping. Biomass 15:93-101.

Boddy, L. 1992. Development and function of fungal communities in decomposing wood. Pages 749-782 *in* G. C. C. a. D. T. Wicklow, editor. The Fungal Community. M. Dekker, New York.

Bridge, P. and B. Spooner. 2001. Soil fungi: diversity and detection. Plant and Soil 232:147-154.

Bruns, T.D., T.J. White, and J.W. Taylor. 1991. Fungal molecular systematics. Annual Review of Ecological Systematics 22:525-564.

Bruns, T.D., T.M. Szaro, M. Gardes, K.W. Cullings, J.J. Pann, D.L. Taylor, T.R. Horton, A. Kretzer, M. Garbelotto, and Y. Liss. 1998. A sequence database for the identification of ectomycorrhizal basidiomycetes by phylogenetic analysis. Molecular Ecology 7:257-272.

Carder, J.H. and D.J. Barbara. 1991. Molecular variation and restriction fragment length polymorphisms (RFLPs) within and between six species of *Verticillium*. Mycological Research 95: 935-942.

Chen, H., M. E. Harmon, and R. P. Griffiths. 2001. Decomposition and nitrogen release from decomposing woody roots in coniferous forests of the Pacific Northwest: A chronosequence approach. Canadian Journal of Forest Research 31:246-260.

Chen, H. M.E. Harmon, J. Sexton, and B. Fasth. 2002. Fine-root decomposition and N dynamics in coniferous forests of the Pacific Northwest, U.S.A. Canadian Journal of Forest Research 32:320-331.

Christensen, M. 1969. Soil microfungi of dry to mesic conifer-hardwood forest in northern Wisconsin. Ecology 50:9-27.

Cooke, R.C. and A.D.M. Rayner. 1984. Ecology of saprotrophic fungi. Longman, London.

Edmonds R.L. and D.S. Lebo. 1998. Diversity, production, and nutrient dynamics of fungal sporocarps on logs in an old-growth temperate rain forest, Olympic National Park, Washington. Canadian Journal of Forest Research 28: 665-673.

Frankland, J.C. 1992. Mechanisms in fungal succession. Pages 383-401 *in* G. C. C. a. D. T. Wicklow, editor. The Fungal Community. M. Dekker, New York.

Gardes, M. and T.D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes--application to the identification of mycorrhizae and rusts. Molecular Ecology 2:113-118.

Gardes, M. and T.D. Bruns. 1996a. Community structure of ectomycorrhizal fungi in a Pinus muricata forest: above- and below-ground views. Canadian Journal of Botany 74:1572-1583.

Gardes, M. and T.D. Bruns. 1996b. ITS-RFLP matching for identification of fungi. Pages 177-186 *in* J. P. Clapp, editor. Methods in Molecular Biology. Humana Press Inc, Totowa, NJ.

Gauch, Jr., H.G. 1982. Multivariate analysis in community ecology. Cambridge University Press, Cambridge. pg 109-172.

Gilbertson, R.L. 1980. Wood-rotting fungi of North America. Mycologia 72:1-49.

Gilbertson, R.L. 1981. North American wood-rotting fungi that cause brown rots. Mycotaxon 7:372-416.

Green, III., F., M.J. Larsen, J.E. Winandy, and T.L. Highley. 1991. Role of oxalic acid in incipient brown-rot decay. Material un Organismen 26:191-213.

Green III, F. and C.A. Clausen. 1999. Production of polygalacturonase and increase of longitudinal gas permeability in southern pine by brown-rot and white-rot fungi. Holzforschung 53:563-568.

Harmon, M.E., J.F. Franklin, F.J. Swanson, P. Sollins, S.V. Gregory, J.D. Lattink, N.H. Anderson, S.P. Cline, N.G. Aumen, J.R. Sedell, G.W. Lienkaemper, K. Cromack, Jr., and K.W. Cummins. 1986. Ecology of coarse woody debris in temperate ecosystems. Advances in Ecological Research. 15:133-302.

Harmon, M.E. and J.F. Franklin. 1989. Tree seedlings on logs in *Picea-Tsuga* forests of Oregon and Washington. Ecology 70:48-59.

Harmon, M.E., J. Sexton, B.A. Caldwell, and S.E. Carpenter. 1994. Fungal sporocarp mediated losses of Ca, Fe, K, Mg, Mn, N, P, and Zn from conifer logs in the early stages of decomposition. Canadian Journal of Forest Research 24:1883-1893.

Harmon, M.E. 2001. Carbon sequestration in forests. Journal of Forestry 99:24-29.

Harvey, A.E., M.J. Larsen, and M.F. Jurgensen. 1976. Distribution of ectomycorrhizae in a mature Douglas-fir/Larch forest soil in western Montana. Forest Science 22:393-398.

Hawksworth, D. L. 1991. The Fungal Dimension of Biodiversity Magnitude Significance and Conservation. Mycological Research 95:641-655.

Hawksworth D.L., P.M. Kirk, B.C. Sutton, and D.N. Pegler. 1995. Ainsworth and Bisby's Dictionary of the Fungi, 8th Ed. CAB International.

Hibbett, D.S., L.-B. Gilbert, and M.J. Donoghue. 2000. Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. Nature (London) 407:506-508.

Hintikka, V. 1993. Occurrence of edible fungi and other macromycetes on tree stumps over a sixteen-year period. Acta Botanica Fennica 149:11-17.

Horton, T.R., T.D. Bruns, and V.T. Parker. 1999. Ectomycorrhizal fungi associated with *Arctostaphylos* contribute to *Pseudotsuga menziesii* establishment. Canadian Journal of Botany 77:93-102.

Horton, T. and T.D. Bruns. 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. Molecular Ecology 10:1855-1871.

Horton, T. 2002 (in press). Molecular approaches to ectomycorrhizal diversity studies: variation in ITS at a local scale. Plant and Soil.

Hulme, M. A. and J.K Shields. 1970. Biological control of decay fungi in woody by competition for non-structural carbohydrates. Nature 227:300-301.

Illman, B.L., D.C. Meinholtz, and T.L. Highley. 1988. An electron spin resonance study of manganese changes in wood decayed by the brown-rot fungus, Postia placenta. *in* The International research group on wood preservation, Madrid Spain.

Ingold, C.T. and H.J. Hudson. 1993. The biology of fungi, 6th edition. Chapman Hall, London.

Kårén, O., N. Högberg, A. Dahlberb, L. Jonsson, and J.-E. Nylund. 1997. Inter- and intraspecific variation in the ITS region of rDNA of ectomycorrhizal fungi in Fennoscandia as detected by endonucleases analysis. New Phytologist 136:313-325.

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

Koilraj, A.J., G. Marimuthu, K. Nataranjan, S. Saravanan, P. Maran, and M.J. Hsu. 1999. Fungal diversity inside caves in Southern India. Current Science (Bangalore) 70:1081-1084.

Kuhlman, E.G. 1969. Mucorales isolated from pine rot bark and wood. Canadian Journal of Botany 47:1719-1723.

Larsen, M.J., A.E. Harvey, R. Powers, and M.R. Jurgensen. 1996. A new species of Botryobasidium (Corticiaceae, Aphyllophorales) inhabiting the interiors of brown rotted logs of ponderosa pine. Mycotaxon 60:429-432.

Leonowicz, A., A. Matuszewska, J. Luterek, D. Ziegenhagen, M. Wjotas-Wasilewska, N.-S. Cho, M. Hofrichter, and J. Rogalski. 1999. Biodegration of lignin by white rot fungi. Fungal Genetics and Biology 27:175-185.

Lilleskov, E., T.J. Fahey, T.R. Horton, and G.M Lovett. 2002. Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. Ecology 83:104-115.

Maser, C. and J.M. Trappe. 1984. The seen and unseen world of the fallen tree. USDA Forest Service General Technical Report. PNW-164:1-56.

Michelmore R.W. and S.H. Hulbert. 1987. Molecular markers for genetic analysis of phytopathogenic fungi. Annual Review of Phytopathology 25: 383-404.

Mullis, K.B. and F.A. Falonna. 1987. Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. Methods in Enzymology 155: 335-350.

Nei, M. and W.-H. Li. 1979. Mathematical Model for Studying Genetic Variation in Terms of Restriction Endonucleases. Proceedings of the National Academy of Sciences of the United States of America 76:5269-5273.

O'Donnell, K., F.M. Lutzoni, T.J. Ward, and G.L. Benny. 2001. Evolutionary relationships among mucoralean fungi (Zygomycota): Evidence for family polyphyly on a large scale. Mycologia 93:286-296.

Otjen, L. and R.A. Blanchette. 1986. A discussion of microstructural changes in wood during decomposition by white rot basidiomycetes. Canadian Journal of Botany 64:905-911.

Otjen, L., R.A. Blanchette, M. Effland, and G. Leatham. 1987. Assessment of 30 white rot basidiomycetes for selective lignin degradation. Holzforschung 41:343-349.

Page, R.D.M. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences 12: 357-358.

Parkinson, D. and R. Crouch. 1969. Studies on fungi in pinewood soil. V.—Root mycofloras of seedlings of *Pinus nigra* var. *laricio*. Rev. Ecol. Biol. Sol. 6: 263-275.

Peintner, U., N.L. Bougher, M.A. Castellano, J.-M. Moncalvo, M.M. Moser, J.M. Trappe, and R. Vilgalys. 2001. Multiple origins of sequestrate fungi related to *Cortinarius* (Cortniariaceae). American Journal of Botany 88:2168-2179.

Peter, M., F. Ayer, and S. Egli. 2001. Nitrogen addition in a Norway spruce stand altered macromycete sporocarp production and below-ground ectomycorrhizal species composition. New Phytologist 149:311-325.

Polishook, J.D., G.F. Bills, and D.J. Lodge. 1996. Microfungi from decaying leaves of two rain forest trees in Puerto Rico. Journal of Industrial Microbiology 17:284-294.

Rayner, A.D.M. and L. Boddy. 1988. Fungal communities in the decay of wood. Advances in Microbial Ecology 10:115-166.

Renvall, P. 1995. Community structure and dynamics of wood-rotting Basidiomycetes on decomposing conifer trunks in northern Finland. Karstenia 35:1-51.

Ritschkoff, A.-C. 1996. Decay mechanisms of brown rot fungi. Technical Research Centre of Finland, Espo. VTT Publications 268.

Robinson, J. 1990. Lignin, land plants, and fungi: biological evolution affecting Phanerozoic oxygen balance. Geology 15:607-610.

Schipper, M.A.A. 1973. A study on variability in *Mucor hiemalis* and related species. Studies in Mycology 4:1-40.

Shea, K.R. 1960. Fungus succession and the significance of environment in the deterioration of logs. *in* 8th Western International Forest Disease Work Conference. Weyerhaeuser Company Forestry Research Note, Centralia, WA.

Smith, J. E., R. Molina, M.M.P. Huso, D.L. Luoma, D. McKay, M.A. Castellano, T. Lebel, and Y. Valachovic. 2002. Species richness, abundance, and composition of hypogeous and epigeous ectomycorrhizal fungal sporocarps in young, rotation-age, and old-growth stands of Douglas-fir (*Pseudotsuga menziesii*) in the Cascade Range of Oregon, U.S.A. Canadian Journal of Botany 80:186-204.

Tanesaka, E., H. Masuda, and K. Kinugawa. 1993. Wood degrading ability of basidiomycetes that are wood decomposers, litter decomposers, or mycorrhizal symbionts. Mycologia 85:347-354.

Tien, M. and T.K. Kirk. 1983. Lignin-degrading enzyme from the Hymenomycete *Phanerochaete chrysosporium* Burds. Science 221:661-663.

Vainio, E.J. and J. Hantula. 2000. Direct analysis of wood-inhabiting fungi using denaturing gradient gel electrophoresis of amplified ribosomal DNA. Mycological Research [print] 104:927-936.

Voigt, K. and J. Wöstemeyer. 2001. Phylogeny and origin of 82 zygomycetes from all 54 genera of the Mucorales and Mortierellales based on combined analysis of actin and translation elongation factor EF-1alpha genes. Gene 270:113-120.

Watanabe, T., Y. Watanabe, T. Fukatsu, and R. Kurane. 2001. Notes from two *Umbelopsis* species: *U. nana* and *U. vinacea* from Japan. Mycoscience 42:235-237.

White, T.J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal rna genes for phylogenetics. Pages 315-322 *in* M. A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White, editor. PCR Protocols: A guide to methods and applications. Academic Press, Inc, San Diego.

Wilcox, W.W. 1993. Comparative morphology of early stages of brown-rot wood decay. IAWA Journal 14:127-138.

Winandy, J.E. and J.J. Morrell. 1993. Relationship between incipient decay, strength, and chemical composition of Douglas-fir heartwood. Wood and Fiber Science 25:278-288.

Worrall, J.J., S.E. Anagnost, and R.A. Zabel. 1997. Comparison of wood decay among diverse lignicolous fungi. Mycologia 89:199-219.

Yoshizawa, N., N. Itoh, M. Sunagawa, S. Yokota, and T. Idei. 1992. Comparative histochemistry and enzyme activities during wood decay by white-rot fungi with selective lignin-degrading abilities. Bulletin Utsunomiya University Forestry 28:97-110.

Zak, J.C. and S.C. Rabatin. 1997. The mycota: A comprehensive treatise on fungi as experimental systems for basic and applied research. *in* K. Esser, P.A. Lemke D.T. Wicklow, and B.E. Söderström, editors. Vol. IV: Environmental and microbial relationships. Springer, New York.

APPENDICES

Appendix 1. Number of samples of each species of known fungi including field collected samples (Cascade Head Experimental Forest (CHE); H.J. Andrews Experimental Forest (HJA); and, Deschutes National Forest (DNF)), Oregon State University (OSU) herbarium, and Wood Science and Engineering (WSE) cultured samples.

Genus	***species***	**WSE cultures**	**OSU herbarium**	**CHE**	**HJA**	**DNF**
Acanthophysium	*lividocoeruleum*	1				
Agaricus	sp.				2	
Agaricus	*subrutilescens*			1		
Amanita	sp.				3	
Antrodia	*carbonica*	2				
Antrodia	*serialis monok.*	1				
Antrodia	*xantha*	1				
Armillaria	*mellea*		1			
Armillaria	*ostoye*		1			
Auricularia	*auricula*		1			
Bjerkandera	*adusta*	1				
Bondarzewia	*montana*	1				
Calocera	*cornea*		1			
Calocera	*viscosa*		1			
Ceriporiopsis	*rivulosa*	1				
Ceriporiopsis	*subvermispora*	1				
Collybia	*oregonensis*		1			
Coniphora	*puteana*	1				
Cortinarius	sp.				3	
Crustoderma	*dryinium*	1				
Crystostereum	*pini-canadense*	1				
Dacrymyces	*chrysospermus*		1			
Exidia	*glandulosa*		1			
Fomitopsis	*pinicola*	1				
Fomitopsis	sp.			1		
Fomitopsis	*cajanderi*	2				
Fomitopsis	*officialis*	1				
Fomitopsis	*rosea*	1				
Fomitopsis	*pinicola*			1	2	

Genus	*species*	WSE cultures	OSU Herbarium	CHE	HJA	DNF
Gloeophyllum	sp.				2	
Gloeophyllum	*saepiarium*	1				
Gymnopilus	*aeruginosus*		1			
Gyromitra	*esculenta*				1	
Heterobasidion	*annosum*	1				
Hyphoderma	*puberum*	1				
Irpex	*lacteus*	1				
Ischnoderma	*resinosum*		1			
Laetiporus	*cincinnatus*			1		
Laetiporus	*sulphureus*	1				
Lentinus	*ponderosus*		1			
Lenzites	*subferrugenia*	1				
Lycoperdon	*pyriforme*		1			
Mycena	sp.			1		
Mycoacia	*uda*	1				
Naematoloma	*subochraceum*		1			
Neolentinus	*lepideus*	1				
Oligoporus	*leucospongia*		1			
Oligoporus	*undosus*		1			
Panellus	*serotinus*		1			
Panus	*conchatus*		1			
Peniophora	*tenius*	1				
Perenniporia	*subacida*	1				
Phaeolus	*schweinitzi*	1				
Phanerochaete	*sordida*	1				
Phanerochaete	*gigantea*	2				
Phellinus	*ferruginosus*		1			
Phlebia	*rufa*	1				
Phlebia	*albida*	1				
Phlebia	*subserialis*	1				
Phlebia	*merismoides*	1				
Phlebia	*tremellosa*	1				
Phlebia	*tremulosus*		1			
Pholiota	*mutabilis*		1			

Genus	*species*	WSE cultures	OSU Herbarium	CHE	HJA	DNF
Pleurotus	*ostreatus var. salignus*		1			
Pleurotus	sp.			1		
Polyporus	*badisus*				1	
Postia	*placenta*	1				
Pycnoporellus	*alboluteus*		1			
Rhodophyllus	sp.		1			
Schizophyllum	*commune*	1				
Sistotrema	*brinkmanii*	2				
Sphaerobolus	*stellatus*		1			
Stereum	*hirsutum*	2	1			
Trametes	*hirsutus*	1				
Trametes	*versicolor*	1		1	1	
Tremella	*mesenterica*		1	1		
Trichaptum	*abietinus*		1			
Tricholomopsis	*decora*		1			
Wolfiporia	*cocos*	1				
Xylobolus	*frustulatus*	1				
unidentified polypore				1	2	
unidentified puffball						1
unidentiried resupinate					3	
unidentified other				15	18	3
Totals		**46**	**28**	**24**	**38**	**4**

Appendix 2. Gene sequences of common and uncommon RFLP patterns in the nuclear large and nuclear small subunits. Sequences are listed by RFLP group with information on the site, tree species, and stand harvest year.

Large Subunit

RFLP group 11, CHE, PISI, 1989

GAGCTCAAATTTAAAATCTGGTTTGCTTGCAAGCCCGAGTTGTAGTCTG
AAAAACGTTTTCCATAAGCACTGAGGCAAAAGTCCCTTGGAACAGGGC
ATCATAGAGGGTGAGAATCCCGTCCATGGTCTCAAGTTGTTTATTTTGT
GATACGTTTTCAAAGAGTCAGGTTGTTTGGGAATGCAGCCTAAAATGGG
TGGTATTTCT

RFLP group 39, CHE, PISI, 1989

TCTCTCGCCAATATTTAGCTTTAGATGAAATTTACCACCCATTTTAGGCT
GCATTCCCAAACAACCTGACTCTTAGAAAACGTATCACAAAATGAAAG
ACTTGAGACCATGGACGGGATTCTCACCCTCTATGATGCCCTGTTCCAA
AGGACTTGTGCCTCAGCTTTCATGGAAAACGTTTCTTCAGACTACAACT
CGAGTTTGCAAGCAAACTAGATTTTAAATTTGAGCTCTTCCCGCTTCACT
CGCCGTTACTAGGGGAATCCTTGTTAGTTTCTTTTCCTCCGCTTATTGAT
ATGCA

RFLP group 5, CHE, PISI, 1986

TCTCTCGCCAATATTTAGCTTTAGATGAAATTTACCACCCATTTTAGGCT
GCATTCCCAAACAACCTGACTCTTAGAAAACGTATCACAAAATGAAAG
ACTTGAGACCATGGACGGGATTCTCACCCTCTATGATGCCCTGTTCCAA
AGGACTTGTGCCTCAGCTTTCATGGAAAACGTTTCTTCAGACTACAACT
CGAGTTTGCAAGCAAACTAGATTTTAAATTTGAGCTCTTCCCGCTTCACT
CGCCGTTACTAGGGGAATCCTTGTTAGTTTCTTTTCCTCCGCTTATTGAT
ATGCA

RFLP group 26, CHE, TSHE, 1986

TCTCTCGCCAATATTTAGCTTTAGATGAAATTTACCACCCATTTTAGGCT
GCATTCCCAAACAACCTGACTCTTAGAAAACGTATCACAAAATGAAAG
GCTTGAGACCATGGACGGGATTCTCACCCTCTATGATGCCCTGTTCCAA
AGGACTTGTGCCTCAGCTTTCATGGAAAACGTTTCTTCAGACTACAACT
CGGATTTGCAAAGCAAACCAGATTTTAAATTTGAGCTCTTCCCGCTTCA
CTCGCCGTTACTAGGGGAATCCTTGTTAGTTTCTTTTCCTCCGCTTATTG
ATATGCA

RFLP group 18, CHE, RT-TSHE, 1986
CAAGGATTCCCCTAGTAACTGCGAGTGAAGAGGGAAAAGCTCAAATTT
AAAATCTGGCAGCTTTGGCTGTCCGAGTTGTAATCTGGAGAAGTGTTTT
CAGTGCAGGACCGGTGTCCAAGTCTCTTGGAATGGAGCATCATAGAGG
GTGAGAATCCCGTCTTTGACACGGACTACCTGTGCTTTGTGATACACTC
TCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAAATGGGTGGTAAATT
CCATCTAAAGCTAAATATTGGCGAGAGACC

RFLP group 35, HJA, PSME, 1986
TAGGCCACAAGTCCTTTGGAACAGGGCATCATAGAGGGTAGAATCCCG
TCCATGGTCTCAAGTCTTTCATTTTGTGATACGTTTTCTAAGAGTCAGGT
TGTTTGGGAATGCAACCTAAAATGGG

RFLP group 6, HJA, PSME, 1989
ACTGTTTGGCTTAGCTAAACCGTATTGTAAACTGTAGAACATTTTCCTG
GCACACCAGATTAATAAGTCCTTTGGAACAAGGCATCATGGAGGGTGA
GAATCCCGTCTTTGATCTGAGTAGTTGTCTTTTGTGATATGTTTTCAAAG
AGTCAGGTTGTTTGGGAATGCAACCTAAATTGGGTGGTAAATCCTCACC
TAAAGCTAAATATTT

RFLP group 41, HJA, PSME, 1989
TGCATACAATAAGCGGAGGAAAAGAAACTACCAAGGATTCCCCTAGTA
ACGGCGAGTGAAGCGGGATGAGCTCAAATTTGAAATCTGGCTGGTTTA
CTAGTCCGAGTTGTAATTTAAAGAAGCGTTTTCGGTGGCAGCCTGGGTA
TAAATCCTTTGGAATAGGGTATCATAGAGGGTGAGAATCCCGTCTTTGA
CCTAGTGCATGCTACTATGTGATACGTTTTCAAAGAGTCGAGTTGTTTG
GGAATG

RFLP group 7, HJA, PSME, 1986
TCTCTCGCCAATATTTAGCTTTAGATGAAATTTACCACCCATTTTAGGCT
GCATTCCCAAACAACCTGACTCTTTGAAAACGTATCACAAAATAAACAA
CTTGAGACCATGGACGGGATTCTCACCCTCTATGATGCCCTGTTCCAAG
GGACTTTTGCCTCAGTGCTTATGGAAAACGTTTCTTCAGACTACAACTC
GGGCTTGCAAGCAAACCAGATTTTAAATTTGAGCTCTTCCCGCTTCACT
CGCCGTTACTGAGGGAATCCTTGTTAGTTTCTTTTCCTCCGCTTATTGAT
ATGCA

RFLP group 2, HJA, TSHE, 1986
TGCATTCATTAAGCGAGAAAAGAACCTACCAAGAATTCCCCTAGTACCG
CCGAGTAACGGAAAAGCCAAATTTAAATCTGGCTTGCTTGCAAGTCCGA
GTTGTAGTCTGAAGAAACGTTTTCCATAAAGCTAGGCACCAGTCCTTTG
GAACAGGGCATCATAGAGGGTGAGAATCCCGTCCATGGTCTCATGCATT
TCATTTTGTGATACGTTTTCTAAGAGTCAGGTTGTTTGGGAATGCAGCCT
AAAATGGGTGGTAAATTTCATCTAAAGC

RFLP group 9, HJA, TSHE, 1986
TCTCTCGCCAATATTTAGCTTTAGATGAAATTTACCACCCATTTTAGGCT
GCATTCCCAAACAACCTGACTCTTAGAAAACGTATCACAAAATGAAAG
ACTTGAGACCATGGACGGGATTCTCACCCTCTATGATGCCCTGTTCCAA
AGGACTTGTGCCTCAGCTTTCATGGAAAACGTTTCTTCAGACTACAACT
CGAGTTTGCAAGCAAACTAGATTTTAAATTTGAGCTCTTCCCGCTTCACT
CGCCGTTACTAGGGGAATCCTTGTTAGTTTCTTTTCCTCCGCTTATTGAT
ATGC

RFLP group 16, HJA, TSHE, 1993
CAATAAGCGGAGGAAAAGAAACTAACAAGGATTCCCCTAGTAACGGCG
AGTGAAGCGGAAGAGCTCAAATTTAAAATCTGGCTTGCTTGCAAGTCCG
AGTTGTAGTCTGAAGAAGCGTTTTCCATGAAAGCTGAGGCACAAGTCCT
TTGGAACAGGGCATCATAGAGGGTGAGAATCCCGTCCATGGTCTCATGC
ATTTCATTTTGTGATACGTTTTCTAAGAGTCAGGTTGTTTGGGAATGCAG
CCTAAAATGGGTGGTAAATTTCATCTAAAGCTAAATATTGGCGAAGACC
GA

RFLP group 23, HJA, TSHE, 1993
TTTAGCTTTAGATGAAATTTACCACCCATTTTAGGCTGCATTCCCAAACA
ACCTGACTCTTTGAAAACGTATCACAAAATAAACAACTTGAGACCATGG
ACGGGATTCTCACCCTCTATGATGCCCTGTTCCAAGGGACTTTTGCCTCA
GTGCTTATGGAAAACGTTTCTTcAGACTACAACTCGGGCTTGCAAGCAA
ACCAGATTTTAAATTGAGCTCTTCCCGTTTACTCGCCGTACTGAGGGAA
TCCTGGTAG

RFLP group 14, HJA, TSHE 1988
TCTCTCGCCAATATTTAGCTTTAGATGAAATTTACCACCCATTTTAGGCT
GCATTCCCAAACAACCTGACTCTTAGAAAACGTATCACAAAATGAAAT
GCATGAGACCATGGACGGGATTCTCACCCTCTATGATGCCCTGTTCCAA
AGGACTTGTGCCTCAGCTTTCATGGAAAACGCTTCTTCAGACTACAACT
CGGACTTGCAAGCAAGCCAGATTTTAAATTTGAGCTCTTCCCGCTTCAC
TCGCCGTTACTAGGGGAATCCTTGTTAGTTTCTTTTCCTCCGCTTATTGA
TATGCA

RFLP group 20, DNF, PICO, 1988
ACCGCCAATAAACCGGAAGAGCTCCAATTTTAAATCTAGTTGCTTGCAA
CTCGAGTTGTAGTCTGAAGAAACGTTTTCCATAAAGCTAGGCCAAGTCC
TTTGGAACAGGGCATCATAGAGGGTGAGAATCCCGTCCATGGTCTCAA
GTCTTTCATTTTGTGATACGTTTTCTAAGAGTCAGGTTGTTTGGGAATGC
AGCCTAAAATGGGTGGTAAATTTCATCTAAAGC

RFLP group 43, DNF, PICO, 1988
CTGTAGAAGTGTTTTCCAGGCACCCAGGTAAAAAAGTCCTTTGGAACAG
GGCATCATAGAGGGTGAGAATCCCGTCTGGCCTACTGTTGCCTTTTGTG
ATACTTTTCAAAGAGTCAGGTTGTTTGGGAATGCAGCCCTAAATTGGGT
GGTAAATCTCACCTAAAGCTAAATATTGCGAGAGA

RFLP group 27, DNF, PICO 1991
ATCTGGTTTGCTTGCAAGCCGAGTTGTAGTCTGAAGAAACGTTTTCCAT
AAGCACTGAGGCAAAAGTCCCTTGGAACAGGGCATCATAGAGGGTGAG
AATCCCGTCCATGGTCTCAAGTTGTTTATTTTGTGATACGTTTTCAAAGA
GTCAGGTTGTTTGGGAATGCAGCCTAAAATGGGTGGTAAATTTCATCTA
AAGCTAAATATTGGCGAGAGACC

RFLP group 19, DNF RT-PICO 1988
GAGGAAAAGAAACTAACAAGGATTCCCCTAGTAACTGCGAGTGAAGAG
GGAAAAGCTCAAATTTAAAATCTGCAGCTTTGGCTGTCCGAGTTGTAAT
CTGGAGAAGTGTTTTCAGTGCAGGCCGGTGTACAAGTCTCTTGGAATGG
AGCATCATAGAGGGTGAGAATCCCGTCTTTGACACGGACTACCTGTGCT
TTGTGATACACTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAAA
TGGGTGGTAAATTCCCATCTA

RFLP group 30, DNF, 5yr unknown species
AATAAGCGGAGGAAAAGAAACTACAAGGATTCCCCTAGTAACGGCGAG
TAAGCGGGATGAGCTCAAATTTAAAATCTGGCAGTCTTCGATTGTCCGA
GTTGTAATCTAGAGAAGCGTTTTCCGCGCTGGACTGTGTACAAGTTTCC
TGGATACGGAACATCATAGAGGGTGAGAATCCCGTCCTTGACACAGAC
TCCCAGTGCTCTGCGATGCGCTCTCGACGAGTCGAGTTGTTTGGGATTG
CAGCTCAAAATGGGAGGTAAATTCCTTCTAAAGCTAAATATTGGCGAG
A

Small Subunit

RFLP group 10, CHE, PISI, 1986

TAAATAAATTTATATTGTGAAACTGCGAATGGCTCATTAAATCAGTTAT
GATCTACGTGACATATTCTTTACTACTTGGATAACCGTGGTAATTCTAG
AGCTAATACATGCAAAAAAACCCTGACTTCGGAAGGGGTGCACTTATT
AGATAAAGCCAACGCGGGGTAAAACCTGTTTCCCTTGGTGATTCATAAT
AATTAAGCGGATCGCATGGCCTTGTGCTAGCGACGGTCCACTCGATTTT
CTGCCCTATCATGGTTGAGATTGTAAGATAGAGGCTTACAATGCCTACA
ACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAA
ACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATC
CCGACACGGGGAGGTAGTGACAATAAATAACAATGCAGGGCCTTTAAG
GTCTTGCAATTGGAATGAGTACAATTTAAATCCCTTAACGAGGATCAAT
TGGAGGGCAAGTCTGGTGC

RFLP group 11, CHE, PISI, 1989

CTTACTACTTGGATAACCGTGGTAATTCTAGAGCTAATACATGCGTACA
TAACCTCGACTTCTGGAAGAGGTGCACTTATTAGATAAAAAGCCAACGC
GGGCAACCGCTTGTATGGTGATTCATAATAATTAAGCGAATCGCATGGC
CTTGTGCCGGCGATGATGCATTCAAATTTCTGCCCTATCAAGTTTCGATG
GTAGGTTAGTGGCCTACCATGCTTTTAACGGGTAACGGGGAATTAGGGT
TCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAG
GCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACA
ATAAATAACAATACAGGGCCTTTACGGGTCTTGTAATTGGAATGAGTAC
AATTTAAATCCCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGC

RFLP group 8, CHE, TSHE, 1986

TAATTCTAGAGCTAATACATGCGTACATAACCTCGACTTCTGGAAGAGG
TGCACTTATTAGATAAAAAGCCAACGCGGGCAACCGCTTGTATGGTGAT
TCATAATAATTAATCGAATCGCATGGCCTTGTGCCGGCGATGATGCATT
CAAATTTCTGCCCTATCAAGTTTCGATGGTAGGTTAGTGGCCTACCATG
CTTTTAACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCC
TGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTAC
CCAATCCCGACACGGGGAGGGTAGTGACAATAAATAACAATACAGGGC
CTTTACGGGTCTTGTAATTGGAATGAGTACAATTTAAATCCCTTAACGA
GGAACAATTGGAGGGCAAGTCTGGTGC

RFLP group 26, CHE, TSHE, 1986

TGCTGTTAATATCAAAAATTATACTTTAAACTGCAAGGTTTTTAAACAC
CTTCATATGGGTATTACTTACTACTTGGATAACCGTGGTAATCCTAGAG
CTAAACATGCGTACATAACCTCGACTTCTGGAAGAGGTGCACTTATTAG
ATAAAAAGCCAACGCGGGCAACCGCTTGTATGGTGATTCATAATATTAA
TCGAATCGCATGGCCTTGTGCCGGCGATGATCATCAAATTTCTGCCCTA
TCAAGTTTCGATGGTGGTTAGTGGCCTACCATGCTTCAACGGGTAACGG
GAATCAGGGCTCGATTCCGACAGGGAGCCGAGAAACGGCTACCACATC
CAAGGAAGGCAGCAGCGCGCAAATTACCCAATCCC

RFLP group 3, CHE, TSHE, 1989

TGCTTGTCTCAGATTAAGATGCATGTCTAAGAAACCAATTTATACAGTG
AAACTGCGAATGGCTCATTAAATCAGTTATAATTTATTTGATATTCACTT
ACTACTTGGATAACCGTGGTAATTCTAGAGCTAATACATGCGTACATAA
CCTCGACTTCTGGAAGAGGTGCACTTATTAGATAAAAAGCCAACGCGG
GCAACCGCTTGTATGGTGATTCATAATAATTAAATCGAATCGCATGGCC
TTGTGCCGGCGATGATGCATTCAAATTTCTGCCCTATCAAGTTTCGATG
GTAGGTTAGTGGCCTACCATGCTTTTAACGGGTAACGGGAATTAAGGGT
TCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAG
GCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGTAGTGACAA
TAAATAACAATACAGGGCCCTTTACGGGTCTTGTAATTGGAATGAGTAC
AATTTAAATCCCTTAACAGGAAC

RFLP group 5, CHE, THSE, 1986

TGCTGTCTAAGTATAAACAAATTTATACAGTGAAACTGCGAATGGCTCA
TTAAATCAGTTATAATTTATTTGATATTCACTTACTACTTGGATAACCGT
GGGTAATTCTAGAGCTAATACATGCGTACATAACCTCGACTTCTGGAAG
AGGTGCACTTATTAGATAAAAAGCCAACGCGGGCAACCGCTTGTCTGGT
GATTCATAATAATTAATCGAATCGCATGGCCTTGTGCCGGCGATGATGC
ATTCAAATTTCTGCCCTATCAAGTTTCGATGGTAGGTTAGTGGCCTACC
ATGCTTTTAACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGA
GCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAAT
TACCCAATCCCGACACGGGGAGGTAGTGACAATAAATAACAATACAGG
GCCTTTACGGGTCTTGTAATTGGAATGAGTACAATTTAAATCCCTTAAC
GAGGAACAATTGGAGGGCAAGTCTGGTGC

RFLP group 7, CHE, TSHE, Hibbs

TTCTAGAGCTAATACATGCAAAAAAACCCTGACTTCGGAAGGGGTGCA
CTTATTAGATAAAGCCAACGCGGGGTAAAACCTGTTTCCCTTGGTGATT
CATAATAATTAAGCGGATCGCATGGCCTTGTGCTAGCGACGGTCCACTC
GATTTTCTGCCCTATCATGGTTGAGATTGTAAGATAGAGGCTTACAATG
CCTACAACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCC
TGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTAC
CCAATCCCGACACGGGGAGGTAGTGACAATAAATAACAATGCAGGGCC
TTTAAGGTCTTGCAATTGGAATGAGTACAATTTAAATCCCTTAACGAGG
ATCAATTGGAGGGCAAGTCTGGGC

RFLP group 22, CHE, 5-year PSME

TTTATACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATAGTTTATTT
GATTATACTTTACTACTTGGATAACCGTGGTAATTCTAGAGCTAATACA
TGCTAAAAATCCCGACTTCTGGAAGGGATGTATTTATTAGATAAAAAAC
CAATGCGGGCAACCGCTTCTTTGGTGATTCATAATAACTTTTCGAATCG
CATGGCCTTGTGCTGGCGATGTTTCATTCAAATTTCTGCCCTATCAACTT
TCGATGGTAGGATAGAGGCCTACCATGGTTTTAACGGGTAACGGGGAA
TTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCC
AAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGT
AGTGACAATAAATAACAATACAGGGCTTTCTAGTCTTGTAATTGGAATG
AGTACAATTTAAATCCCTTAACGAGGAACAATTGGAGGGCAAGTCTGG
TGC

RFLP group 12, HJA, PSME, 1989

TAAACAAATTTATACAGTGAAACTGCGAATGGCTCATTAAATCAGTTAT
AATTTATTTGATATTCACTTACTACTTGGATAACCGTGGTAATTCTAGAG
CTAATACATGCGTACATAACCTCGACTTCTGGAAGAGGTGCACTTATTA
GATAAAAAGCCAACGCGGGCAACCGCTTGTCTGGTGATTCATAATAATT
AATCGAATCGCATGGCCTTGTGCCGGCGATGATGCATTCAAATTTCTGC
CCTATCAAGTTTCGATGGTAGGTTAGTGGCCTACCATGCTTTTAACGGG
TAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGC
TACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGAC
ACGGGGAGGTAGTGACAATAAATAACAATACAGGGCCTTTACGGGTCT
TGTAATTGGAATGAGTACAATTTAAATCCCTTAACGAGGAACAATTGGA
GGGCAAGTCTGGTGC

RFLP group 17, HJA, PSME 1989

CTGCTGTCTAAGTATAAACAAATTTATACAGTGAAACTGCGAATGGCTC
ATTAAATCAGTTATAATTTATTTGATATTCACTTACTACTTGGATAACCG
TGGTAATTCTAGAGCTAATACATGCGTACATAACCTCGACTTCTGGAAG
AGGTGCACTTATTAGATAAAAAGCCAACGCGGGCAACCGCTTGTATGG
TGATTCATAATAATTAAGCGAATCGCATGGCCTTGTGCCGGCGATGATG
CATTCAAATTTCTGCCCTATCAAGTTTCGATGGTAGGTTAGTGGCCTACC
ATGCTTTTAACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGA
GCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAAT
TACCCAATCCCGACACGGGGAGGTAGTGACAATAAATAACAATACAGG
GCCTTTACGGGTCTTGTAATTGGAATGAGTACAATTTAAATCCCTTAAC
GAGGAACAATTGGAGGGCAAGTCTGGTGC

RFLP group 21, HJA, PSME, 1989

AGTATAAACAAATTTATACAGTGAAACTGCGAATGGCTCATTAAATCAG
TTATAATTTATTTGATATTCACTTACTACTTGGATAACCGTGGTAATTCT
AGAGCTAATACATGCGTACATAACCTCGACTTCTGGAAGAGGTGCACTT
ATTAGATAAAAAGCCAACGCGGGCAACCGCTTGTATGGTGATTCATAAT
AATTAAGCGAATCGCATGGCCTTGTGCCGGCGATGATGCATTCAAATTT
CTGCCCTATCAAGTTTCGATGGTAGGTTAGTGGCCTACCATGCTTTTAAC
GGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAAC
GGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCC
GACACGGGGAGGTAGTGACAATAAATAACAATACAGGGCCTTTACGGG
TCTTGTAATTGGAATGAGTACAATTTAAATCCCTTAACGAGGAACAATT
GGAGGGCAAGTCTGGTGC

RFLP group 14, HJA, PSME 1993

TACAGTGAAACTGCGAATGGCTCATTAAATCAGTTATAATTTATTTGAT
ATTCACTTACTACTTGGATAACCGTGGTAATTCTAGAGCTAATACATGC
GTACATAACCTCGACTTCTGGAAGAGGTGCACTTATTAGATAAAAAGCC
AACGCGGGCAACCGCTTGTATGGTGATTCATAATAATTAATCGAATCGC
ATGGCCTTGTGCCGGCGATGATGCATTCAAATTTCTGCCCTATCAAGTTT
CGATGGTAGGTTAGTGGCCTACCATGCTTTTAACGGGTAACGGGGAATT
AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAA
GGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAG
TGACAATAAATAACAATACAGGGCCTTTACGGGTCTTGTAATTGGAATG
AGTACAATTTAAATCCCTTAACGAGGAACAATTGGAGGGCAAGTCTGG
TGC

RFLP group 29, HJA, 5-yr PSME

TAAGTTCAACAAATTTATACAGTGAAACTGCGAATGGCTCATTAAATCA
GTTATAATTTATTTGATATTCACTTACTACTTGGATAACCGTGGTAATTC
TAGAGCTAATACATGCGTACATAACCTCGACTTCTGGAAGAGGTGCACT
TATTAGATAAAAAGCCAACGCGGGCAACCGCTTGTATGGTGATTCATAA
TAATTAAGCGAATCGCATGGCCTTGTGCCGGCGATGATGCATTCAAATT
TCTGCCCTATCAAGTTTCGATGGTAGGTTAGTGGCCTACCATGCTTTTAA
CGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAA
CGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCC
CGACACGGGGAGGTAGTGACAATAAATAACAATACAGGGCCTTTACGG
GTCTTGTAATTGGAATGAGTACAATTTAAATCCCTTAACGAGGAACAAT
TGGAGGGCAAGTCTGGTGC

RFLP group 2, HJA, TSHE, 1986

GTACTCATTCCAATTACAAGACCCGTAAAGGCCCTGTATTGTTATTTATT
GTCACTACCTCCCCGTGTCGGGATTGGGTAATTTGCGCGCCTGCTGCCTT
CCTTGGATGTGGTAGCCGTTTCTCAGGCTCCCTCTCCGGAATCGAACCC
TAATTCCCCGTTACCCGTTAAAAGCATGGTAGGCCACTAACCTACCATC
GAAACTTGATAGGGCAGAAATTTGAATGCATCATCGCCGGCACAAGGC
CATGCGATTCGATTAATTATTATGAATCACCATACAAGCGGTTGCCCGC
GTTGGCTTTTTATCTAATAAGTGCACCTCTTCAGAAGTCGAGGTTATGTA
CGCATGTATTAGCTCTAGAATTACCACGGTTATCCAAGTAGTAAGTGAA
TATCAAATAAATTATAACTGATTTAATGAGCCATTCGCAGTTTCACTGT
ATAAATTGTTTATACTTAGACATGCATGGCTTAATCTTTGAGACAAGC

RFLP group 9, HJA, TSHE, 1986

TAATTCTAGAGCTAATACATGCGTACATAACCTCGACTTCTGGAAGAGG
TGCACTTATTAGATAAAAAGCCAACGCGGGCAACCGCTTGTATGGTGAT
TCATAATAATTAATCGAATCGCATGGCCTTGTGCCGGCGATGATGCATT
CAAATTTCTGCCCTATCAAGTTTCGATGGTAGGTTAGTGGCCTACCATG
CTTTTAACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCC
TGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTAC
CCAATCCCGACACGGGGAGGTAGTGACAATAAATAACAATACAGGGCC
TTTACGGGTCTTGTAATTGGAATGAGTACAATTTAAATCCCTTAACGAG
GAACAATTGGAGGGCAAGTCTGGTGCG

RFLP group 16, HJA, TSHE, 1993

CTGCTGTCTAAGTATAAACAAATTTATACAGTGAAACTGCGAATGGCTC
ATTAAATCAGTTATAATTTATTTGATATTCACTTACTACTTGGATAACCG
TGGTAATTCTAGAGCTAATACATGCGTACATAACCTCGACTTCTGGAAG
AGGTGCACTTATTAGATAAAAAGCCAACGCGGGCAACCGCTTGTATGG
TGATTCATAATAATTAATCGAATCGCATGGCCTTGTGCCGGCGATGATG
CATTCAAATTTCTGCCCTATCAAGTTTCGATGGTAGGTTAGTGGCCTACC
ATGCTTTTAACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGA
GCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAAT
TACCCAATCCCGACACGGGGAGGTAGTGACAATAAATAACAATACAGG
GCCTTTACGGGTCTTGTAATTGGAATGAGTACAATTTAAATCCCTTAAC
GAGGAACAATTGGAGGGCAAGTCTGGTGC

RFLP group 23, HJA, TSHE, 1993

CTTACTACTTGGATAACCGTGGTAATTCTAGAGCTAATACATGCGTACA
TAACCTCGACTTCTGGAAGAGGTGCACTTATTAGATAAAAAGCCAACGC
GGGCAACCGCTTGTATGGTGATTCATAATAATTAAGCGAATCGCATGGC
CTTGTGCCGGCGATGATGCATTCAAATTTCTGCCCTATCAAGTTTCGATG
GTAGGTTAGTGGCCTACCATGCTTTTAACGGGTAACGGGGAATTAGGGT
TCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAG
GCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACA
ATAAATAACAATACAGGGCCTTTACGGGTCTTGTAATTGGAATGAGTAC
AATTTAAATCCCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGC

RFLP group 20, DNF, PICO, 1988

CTGCTGTCTAAGTATAAACAAATTTATACAGTGAAACTGCGAATGGCTC
ATTAAATCAGTTATAATTTATTTGATATTCACTTACTACTTGGATAACCG
TGGTAATTCTAGAGCTAATACATGCGTACATAACCTCGACTTCTGGAAG
AGGTGCACTTATTAGATAAAAAGCCAACGCGGGCAACCGCTTCTATGGT
GATTCATAATAATTAAGCGAATCGCATGGCCTTGTGCCGGCGATGATGC
ATTCAAATTTCTGCCCTATCAAGTTTCGATGGTAGGTTAGTGGCCTACC
ATGCTTTTAACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGA
GCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAAT
TACCCAATCCCGACACGGGGAGGGTAGTGACAATAAATAACAATACAG
GGCCTTTACGGGTCTTGTAATTGGAATGAGTACAATTTAAATCCCTTAA
CGAGGAACAATTGGAGGGCAAGTCTGGTGC

RFLP group 27, DNF, PICO, 1991

ATACAGTGAAATGCGAATGGTTTATTAAATCAGTTATAATTTATGGGAT
ATTCACTTACTACTTGGATAACCGTGGTAATTCTAGAGCTAATACATGC
GTACATAACCTCGACTTCTGGAAGAGGTGCACTTATTAGATAAAAAGCC
AACGCGGGCAACCGCTTGTATGGTGATTCATAATAATTAACGAATCGCA
TGGCCTTGTGCCGGCGATGATGCATTCAAATTTCTGCCCTATCAAGTTTC
GATGGTAGGTTAGTGGCCTACCATGCTTTTAACGGGTAACGGGGAATTA
GGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAG
GAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGT
GACAATAAATAACAATACAGGGCCTTTACGGGTCTTGTAATTGAATGAG
TACAATTTAAATCCCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGC

RFLP group 13, DNF, PIPO, 1985

CGAATGGTTTATTAAATCAGTTATGATCTACGTGACATATTCTTTACTAC
TTGGGATAACCGTGGTAATTCTAGAGCTAATACATGCAAAAAAGCCCTG
ACTCACGAAGGGGTGCACTTATTAGATAAAGCCAACGCGGGGTAAAAC
CTGTACTTCTTGGTGATTCATAATAATTAAGCGGATCGCATGGCCTTGT
GCTAGCGACGGTCCACTCGATTTTCTGCCCTATCATGGTTGAGATTGTA
AGATAGAGGCTTACAATGCCTACAACGGGTAACGGGGAATTAGGGTTC
GATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGC
AGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAAT
AAATAACAATGCAGGGCCTTTAAGGTCTTGCAATTGGAATGAGTACAAT
TTAAATCCCTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGC

RFLP group 19, DNF-RT-PICO, 1988

ACAAACAAATTGTACTGTGAAACTGCGAATGGCTCATTAAATCAGTTAT
AGTTTATTTGATGGTATCTGCTACATGGATAACTGTGGTA

RFLP group 30, DNF 5-yr unknown species

GCTGTTAAGTATCAACAATTTATACAGTGAAACTGCGAATGGTTATTAA
ATCAGTTATAGTTTATGGATGGTACCTTGCTACATGGATAACTGTGGTA
ATTCTAGAGCTAATACATGCTACAAAGCCCCGACTTCTGGAAGGGGTGT
ATTTATTAGATAAAAAACCAACGCGGTTCAGCCCTCCTTGGTGATCATA
ATAACTTCTCGAATCGCATGGCCTTGTGCCGGCGATGCTTCATTCAAAT
ATCTGCCCTATCAACTTTCGATGGTAGGATAGAGGCCTACCATGGTTTC
AACGGGTAACGGGGAATAAGGGTTTGATTCCGGAGAGGGAGCCTCGGC
TCTTTCCTTCCCCCGCTAATGCATTTCGGGACGAGTCGATACGACACGG
TCCAAATCCATTCATGCGACTGTAACATCACA