

**SPREAD OF *PELLINUS WEIRII* INOCULATED ON THE ROOTS
OF STANDING AND RECENTLY CUT JUVENILE DOUGLAS-FIR TREES**

by

Jeffrey G. Fournier

B.S.F., University of British Columbia, 1984

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF PEST MANAGEMENT

in the Department
of
Biological Sciences

© Jeffrey G. Fournier 1997

SIMON FRASER UNIVERSITY

April 1997

All rights reserved. This work may not be
reproduced in whole or in part , by photocopy or
other means, without permission of the author.



National Library
of Canada

Acquisitions and
Bibliographic Services

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque nationale
du Canada

Acquisitions et
services bibliographiques

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file *Votre référence*

Our file *Notre référence*

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur conserve la propriété du droit d'auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-24261-7

Canada

APPROVAL

Name: JEFFREY GARY FOURNIER

Degree: Master of Pest Management

Title of Thesis:

**SPREAD OF *PHELLINUS WEIRII* INOCULATED ON THE ROOTS OF
STANDING AND RECENTLY CUT JUVENILE DOUGLAS-FIR TREES.**

Examining Committee:

Chair: Dr. M. Winston, Professor

Dr. Z. Punja, Professor, Senior Supervisor
Department of Biological Sciences, SFU

Dr. J. Borden, Professor
Department of Biological Sciences, SFU

Mr. Hadrian Merler, Msc., Forest Pathologist
B.C. Ministry of Forests, Kamloops Forest Region
Public Examiner

Date Approved: April 3, 1997

Abstract

The ectotrophic spread of *Phellinus weirii* inoculated on the roots of juvenile Douglas-fir was compared on trees that were left standing, cut-down immediately after inoculation or cut-down 6 months after inoculation. Measurements were taken at 6, 12 and 18 months post inoculation. A new “combined” inoculation technique provided significantly higher ($P > 0.005$) transfer success than the standard “block” inoculation method. Only data from the combined method was determined to be reliable enough to assess spread-related relationships. The use of linear spread distance to characterize pathogen spread was as effective as the more exacting measurement of area of spread. Differences in mean spread in proximal and distal directions were not significant. At 6 months, spread of *P. weirii* was significantly higher in both linear ($P < 0.01$) and area ($P < 0.02$) measurements in trees cut immediately after inoculation compared to those left standing. At 18 months, pathogen spread in these cut trees was lower than in trees left standing and was significantly lower ($P < 0.01$ for area and $P < 0.02$ for linear spread) than in trees cut 6 months after inoculation. Linear and area pathogen spread did not differ between 6, 12 and 18 months in cut trees, but were significantly less at 6 months ($P < 0.001$) than at 12 and 18 months in standing trees. The results show that felling a host tree just prior to or during infection may induce conditions that enhance pathogen spread for periods up to 12 months and discourage spread in the longer term. Cutting of hosts with well established infections may directly enhance pathogen spread for periods up to 12 months post treatment. Pathogen spread in pre-commercially thinned stands could be minimized by pre-treatment identification of areas of *P. weirii* infection and proper application of cutting and host retention treatments. Felling in the spring season may increase short term pathogen spread, with the opposite being true for cutting in the fall season.

Acknowledgments

I thank Drs. Z. Punja and J. H. Borden for their support and guidance throughout this project; Rona Sturrock, George Reynolds and Dan Hall of Natural Resources Canada - Pacific Forestry Center, for insight, inoculation materials and many hours of assistance in the inoculation and sampling phases of this experiment; Robert Balshaw of Simon Fraser University for statistical analysis services; and Steffan Zeglan and Don Heppner of the Ministry of Forests, Lorraine Rugg, Mike Cruickshank and Tei Sik Kyung of Simon Fraser University, and Brenda Callan of the Pacific Forestry Center for their advice and assistance. I acknowledge the funding and cooperation provided by the Ministry of Forests - Vancouver Forest Region and Squamish Forest District. The experimental site was provided by the Malcolm Knapp Research Forest. I especially thank my family for their support and sacrifices they made which allowed me to complete this project.

Table of Contents

Abstract	iii
Acknowledgments	iv
Table of Contents	v
List of Tables.....	vi
List of Figures	vii
CHAPTER 1 - INTRODUCTION AND LITERATURE REVIEW.....	1
1.1 History and Economic Importance.....	1
1.2 Incidence and Damage	2
1.3 Infection Process	3
1.4 Factors That Influence Pathogen Spread.....	3
1.5 Role of Environmental Factors in Pathogen Growth and Survival.....	4
1.6 Spread of The Pathogen	6
1.7 Disease Detection	6
1.8 Disease Management.....	7
1.8.1 Management in Mature Stands	8
1.8.1.1 Inoculum Reduction	8
1.8.1.2 Reforestation With Intermediate Susceptibility to Immune Tree Species.....	8
1.8.1.3 Biological Control	9
1.8.2 Management in Immature Stands	10
1.8.2.1 Bridge Tree Removal.....	11
1.9 Research Objectives	12
CHAPTER 2.0 - MATERIALS AND METHODS.....	13
2.1 Site Location and Characteristics.....	13
2.2 Experimental Treatments	13
2.3 Data Collection.....	19
2.4 Statistical Analysis.....	20
CHAPTER 3.0 - Results.....	22
3.1 Inoculation Success	22
3.2 Linear and Area Spread of Mycelium	22
3.3 Presence and Antagonism of Other Fungi.....	23
3.4 The Effect of Soil Horizon	24
CHAPTER 4.0 - DISCUSSION.....	28
4.1 Inoculation Success	28
4.2 Linear and Area Spread Analyses	29
4.3 Effects of Season of Treatment	32
4.4 Implication on management of young stands.....	33
REFERENCES	35

List of Tables

Table 1 - Attributes of pooled soil samples collected in the study area, September, 1994.....	14
Table 2 - Listing of significant general linear model relationship probabilities for rank transformed spread data - all data. Probability values ≤ 0.05 are in bold and negative correlations are in parentheses.....	27

List of Figures

- Figure 1 - Experimental design used in research to schedule activities. In parentheses are the numbers of trees treated or sampled.15
- Figure 2 - Illustration of inoculum placement for combined and block inoculation methods.18
- Figure 3 - Spread of *P. weirii* on Douglas-fir roots by time since block inoculation and treatment. Significant differences ($P \leq 0.05$) are denoted by different uppercase bar letters (within time) and by lowercase letters (within treatment - between times).....25
- Figure 4 - Spread of *P. weirii* on Douglas-fir roots by time since combined inoculation and treatment. . Significant differences at $P \leq 0.05$ are denoted by different uppercase bar letters (within time) and by lowercase letters (within treatment - between times).....26

CHAPTER 1 - INTRODUCTION AND LITERATURE REVIEW

1.1 History and Economic Importance

Phellinus weirii (Murr.) Gilbn. (Aphyllophorales: Hymenochaetaceae), the causal agent of laminated root rot, was originally described as *Poria weirii* Murr. based on a 1914 sample collected from western redcedar (*Thuja plicata* Donn. ex D. Don). This pathogen was found on Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) in 1929 on Vancouver Island (Nelson 1980) and it quickly became evident that two forms of the fungus existed (Childs 1963). In 1974, both forms of the fungus were classified under the name *Phellinus weirii* (Thies and Sturrock 1995). Only the Douglas-fir pathoform is referred to in this research.

Douglas-fir, followed by amabilis fir (*Abies amabilis* Dougl. ex Forbes), grand fir (*Abies grandis* Dougl. ex D. Don) and mountain hemlock (*Tsuga mertensiana* (Bong.) Carr.) are the most susceptible hosts of laminated root rot (Childs 1970). This disease has significantly impacted the productivity of Douglas-fir forests in the western United States and Canada (Thies 1984). In intermediate-aged and mature forests of Southern British Columbia, *P. weirii* is responsible for annual losses in excess of 800 000 m³ (Taylor 1986, 1989).

Losses in immature coastal Douglas-fir forests have yet to be quantified but are believed to be significant (Bloomberg and Reynolds 1985). Significant increases (possibly doubling) of inoculum levels can occur when sequential generations of Douglas-fir forests are subjected to traditional silviculture treatments and disease management is not applied (Childs 1963, Hadfield et. al. 1986, Thies 1984). In the absence of disease control, growing two to three rotations of Douglas-fir on an infected area can result in a 50 to 90 % reduction in harvestable volume (Thies 1984).

1.2 Incidence and Damage

In coastal British Columbia, the incidence of laminated root rot increases with proportion of Douglas-fir in a stand (Bloomberg and Beale 1985) and is highest on rich-fresh to dry sites where stands are 81 to 100 years old (Beale 1987). In Oregon, Kastner et al. (1994) found that prevalence of infection was nearly five times higher on ridge tops and upper slopes than middle and lower slopes.

The presence of *P. weirii* can be considered a site characteristic because damage in succeeding generations of trees is normally caused by the same pathogen clone (Nelson 1980, Goheen and Hansen 1994). *P. weirii* inoculum can remain viable in old root systems for over 50 years (Childs 1963, Wallis and Reynolds 1965). Physical and chemical properties of the host can affect the pathogen's ability to ward off other soil fungi and influence its persistence (Nelson 1967). A variety of site factors can also influence disease impact (Goheen and Hansen 1994).

Laminated root rot infections kill root tissues and reduce a host's ability to take up soil nutrients and water. Within 5 to 15 years of infection, immature trees will normally develop crown symptoms that range from reduced leader growth, thin and yellow foliage, to distress cone crops (Wallis 1976). Development of disease symptoms in mature trees can take longer than in immature trees (Wallis and Bloomberg 1981). Trees with outwardly healthy appearance often have weakened root systems that cause it to blow over prior to dying from infection (Thies 1984). Standing dead trees break up from the top downward or blow down in a typically random pattern. Only in advanced infections will root collar inspections reveal the grey, mauve or tan ectotrophic mycelium of the pathogen. With advanced disease, woody root tissues become stained, pitted and eventually develop characteristic laminations. Close inspection of wood with advanced decay will reveal reddish brown whisker-like structures called setal hyphae. Trees in the interior portions of laminated root rot centers typically have more advanced symptoms than those on the periphery (Wallis and Reynolds 1965, Wallis 1976, Thies 1984).

1.3 Infection Process

Phellinus weirii transmission occurs when the roots of living host trees make contact with viable inoculum on the surface of infected host material (Thies 1984, Wallis and Reynolds 1965). The pathogen can spread 5 to 8 cm directly through the soil and up to 15 cm over the surface of rocks, non-host roots and dead host material (Wallis and Reynolds 1965, Wallis 1976). This type of spread is not believed to be important in pathogen transmission because living host material is required for infection and further spread to take place (Wallis and Reynolds 1965, Wallis 1976, Hadfield et. al 1986). Although the fungus does produce viable fruiting bodies, there is no concrete evidence that spread by spores occurs in the field situation (Nelson 1971).

Upon infection, the pathogen spreads primarily by ectotrophic mycelial growth (Wallis and Reynolds 1962). Advancing ectotrophic mycelium intermittently penetrate the bark of the host root and initiate endotrophic infections in the cambium, sapwood and eventually the heartwood (Wallis and Reynolds 1965, Bloomberg and Reynolds 1981). Ectotrophic mycelial spread can be as much as 2 m in advance of any endotrophic mycelium, but this distance decreases with increasing root diameter (Wallis and Reynolds 1965). Inward penetration of the pathogen is slowed by increased bark thickness associated with larger roots (Wallis and Reynolds 1962). Endotrophic spread can occur in the sapwood but is predominantly achieved in the heartwood. Endotrophic spread increases with time after infection and plays the greatest role in large old roots with thick bark (Wallis and Reynolds 1962). The pathogen will spread both proximally and distally from an infection point (Wallis and Reynolds 1965).

1.4 Factors That Influence Pathogen Spread

Hansen et al. (1983) indicated that spread on a root system is a function of the pathogen, host characteristics and environmental factors, but that rate of spread within a stand is largely determined by root contact frequency. Similarly, Bloomberg (1990)

found that fungal genetics, host physiology, ecological factors, site characteristics, stand attributes and interactions between all these factors played an important role in pathogen spread. In particular, he noted that the spatial distribution of host tree, root contacts, stocking levels and mean tree diameter were of particular relevance to pathogen spread within a stand. Bloomberg and Reynolds (1981) reported that root contact transmission usually involved ectotrophic mycelium and that transfer success increased with root diameter and decreased with depth of contacts in the soil profile.

Host vigor class has no effect on pathogen growth (Hansen et al. 1983, Goheen and Hansen 94). Hansen et al. (1983) attributed significant differences in spread capabilities to different genetic origins of the fungus. McCauley and Cook (1980) reported that clonal differences caused average rate of pathogen spread to range between 25.3 to 48.5 cm/yr.

1.5 Role of Environmental Factors in Pathogen Growth and Survival

Phellinus weirii can survive *in vitro* at -5° to 30° C but maximal growth occurs between 15° and 25° C (Angwin 1985, Li et al. 1967, Nelson and Fay 1975). In the field, pathogen survival in buried inoculum could not be maintained above 15° C due to the increased activity of antagonistic soil microflora (Nelson and Fay 1974).

The optimal pH range for pathogen growth in culture is 5.0 to 6.0 and growth is suppressed at pH > 6.0 (Li et al. 1967). In the field, the effects of soil pH are not easily defined. Angwin (1985) found that pathogen growth in unamended forest soils (pH 5.6) was similar to that of acid-amended soils (pH 4.3) but was significantly better than lime-amended soils (pH 6.4). Fungal antagonists of *P. weirii* were strongly favored by acidic conditions and the availability of ammonium as a nitrogen source for the pathogen was drastically reduced at pH < 5.5. Nelson (1969) felt that fungal populations were not linked to pH.

Optimal growth of *P. weirii* *in vitro* occurred at 15% soil moisture (Hansen et al. 1983). In the forest environment, the direct effect of soil moisture on pathogen growth is unclear but moisture can be significantly and positively correlated with population levels of *Trichoderma* spp. that are antagonistic to the pathogen (Nelson 1972).

P. weirii can utilize ammonium and urea forms of nitrogen only after nitrate reduction. Antagonistic soil fungi on the other hand are able to utilize these forms of nitrogen directly and thereby have a competitive advantage over the pathogen in nitrogen-rich soil (Nelson 1968, 1972; Angwin 1985). *P. weirii* inoculum was found to have a lower survival rate in nitrogen-rich alder soils and those amended with nitrate, ammonium or urea (Nelson 1968, 1970, 1976). However, Hansen (1979a) found that the survival of *P. weirii* in Douglas-fir stumps was not reduced due to the presence of red alder regeneration.

Activity of soil micro-organisms can decrease root pathogen spread and survival (Nelson 1967). Studies have shown that bacteria, aerobic actinomycetes and especially fungi can have a significant effect on the spread and persistence of *P. weirii* (Nelson 1967, 1969, 1970; Hutchins and Li 1981). *Trichoderma* spp. have by far the greatest success in reducing *P. weirii* inoculum longevity (Nelson 1969, 1970; Nelson et al. 1978, Nelson and Thies 1985, 1986). Declining *P. weirii* populations were inversely correlated ($P < 0.05$) with soil *Trichoderma* spp. levels (Nelson 1976). Populations of antagonist are inversely related to soil temperature and are highest in the winter and early spring and lowest in the summer (Nelson 1969). *Trichoderma* spp. levels are highest in the upper regions of the soil profile where organic matter is most prevalent (Nelson 1967, 1982). The relative ability of *Trichoderma* spp. and other soil inhabiting fungi to antagonize *P. weirii* in culture correlated well with that seen in the field (Nelson 1967).

1.6 Spread of The Pathogen

Centers of *P. weirii* can expand 30 to 35 cm/yr (Childs 1963, 1970; Nelson and Hartman 1975, McCauley and Cook 1980, Bloomberg 1990) but information on the extent of mycelium spread on living roots is limited. In natural infections, the time of initial infection cannot be accurately determined. *In vivo* tracking of pathogen spread on naturally infected root systems over time is virtually impossible because the requisite inspections usually destroy or seriously impact any ectotrophic mycelium that is exposed.

Wallis and Reynolds (1965) inoculated 50 yr old trees that were living, recently felled and felled 3, 6 and 12 months prior. Ectotrophic spread of *P. weirii* was comparable between living roots and all felled trees. Success in penetrating through the bark and into the wood decreased with time since felling (80% at 3 months, 45% at 6 months and 17% at 12 months)(Wallis and Reynolds 1965). Thus, successful infection decreased rapidly with time after felling, suggesting that cutting trees immediately adjacent to a disease center could help contain the pathogen.

1.7 Disease Detection

Effective disease management requires that the majority of infected areas are identified and delineated for treatment. Considering that *P. weirii* is only visible upon extensive and costly soil excavations, surveyors need to rely heavily on the observation of above-ground disease symptoms that may not become evident until 5 to 15 years after infection in immature stands (Wallis 1976) or substantially longer in mature stands. In very young stands, symptoms usually reflect only a small portion of the area infected, because the roots have not contacted all residual inocula (Hansen 1975). The earliest and most important above ground symptoms in young stands are reduced leader growth and chlorotic foliage; unfortunately, these symptoms are only detectable a few years prior to tree death. Root collar excavations have limited utility in juvenile trees since above

ground symptoms are usually advanced by the time mycelium reaches this area (Hansen 1975).

Even as stands age, the full extent of an infected area remains difficult to delineate (Hadfield 1985). Wallis and Bloomberg (1981) found that in 25 to 30 yr old stands of coastal Douglas-fir, only half of the infected trees and two-thirds of the infected area could be detected based on above-ground symptoms. These above-ground indicators provide a consistent but inaccurate estimate of total disease area and specific stems infected (Wallis and Bloomberg 1981). The use of predictive tools to estimate the extent and direction of *P. weirii* spread has little operational utility. Even from known infection points, asymmetric growth of root systems and the presence of physical obstructions hamper the prediction of when and where hosts will contact inoculum sources. The prediction of secondary spread is complicated by the frequent intermingling of host and non-host roots and the presence of treeless gaps in a stand (Eis 1974, Reynolds and Bloomberg 1982). To compensate for inadequate detection of recently infected roots, operational disease detection techniques usually employ buffer zones around visibly infected areas to include adjacent asymptomatic areas of infection (Wallis and Bloomberg 1981). Delineation of infected areas is more difficult in the interior portion of British Columbia because the defined disease centers, commonly found in the coastal situation, are found less frequently (H. Merler, Ministry of Forests, Kamloops Forest Region, Kamloops B.C., pers comm.).

1.8 Disease Management

Laminated root rot control is most effectively and efficiently implemented in mature stands (Thies 1984, Morrison et al. 1992). At this stage, disease centers are generally well defined and several management options exist. Failure to manage disease in mature stands usually results in increased damage in the subsequent regenerating forest, or requires the application of costly and inefficient mid-rotation control

techniques. In mature stands, the disease management emphasizes reforestation with immune or tolerant species and inoculum reduction. In young and intermediate aged stands, the most promising control method involves the selective cutting of susceptible trees while retaining less tolerant and immune host species (Hadfield 1985, Hadfield et al. 1986, Morrison et al. 1991, Thies 1994). To date, there is little evidence to support the use of genetically based host resistance in controlling laminated root rot (Hansen et al. 1983, Hadfield 1985).

1.8.1 Management in Mature Stands

1.8.1.1 Inoculum Reduction

Push-falling or post-harvest stump removal using an excavator have proven to be cost-effective methods for reducing inoculum at harvest (Thies 1984, Morrison et al. 1992, Thies and Sturrock 1995). However, high costs as well as soil and terrain constraints have limited the operational application of these techniques (Thies and Sturrock 1995).

1.8.1.2 Reforestation With Intermediate Susceptibility to Immune Tree Species

Interplanting of desired, but susceptible, species with resistant or immune species may reduce disease impact in some situations but not in others (Hansen 1975, McCauley and Cook 1980, Thies and Sturrock 1985). Reforesting exclusively with intermediate susceptible species such as western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), resistant species like western redcedar, or immune species, such as red alder (*Alnus rubra* Bong.), is believed to reduce losses due to laminated root rot (Thies 1984, Hadfield et al 1986, Morrison et al. 1992, Thies and Sturrock 1995). Reforesting with these alternate species is particularly effective when mechanical inoculum reduction techniques are not feasible as long as they are ecologically suited to the growing site. On sites best suited to

Douglas-fir where mechanical control is possible, use of alternative species is undesirable because their growth rates and associated economic returns fall below acceptable levels.

Reforestation with red alder has been advocated because of its rapid growth rate and theorized ability to enhance the demise of *P. weirii* inoculum on a site (Thies 1984, Hadfield et al. 1986, Morrison et al. 1992, Thies and Sturrock 1995). The ability of red alder to produce phenolic and lipid compounds may explain its immunity to *P. weirii* infection (Nelson et al. 1978). The growth of red alder on a site is believed to have an indirect affect on the pathogen by creating soil chemical and microbial conditions that are detrimental to its survival (Nelson et al. 1978, Hutchins and Li 1981). However, there is evidence that this effect may not be as pronounced as anticipated (Hansen 1978, 1979a). Nonetheless, occupying the site with a non-host will allow the inoculum to naturally decline to a level at which susceptible tree species could be safely re-established (Thies 1984, Hadfield et al. 1986, Morrison et al. 1992, Thies and Sturrock 1995).

1.8.1.3 Biological Control

There are a number of soil micro-organisms that can decrease the survival and disease-causing capabilities of *P. weirii*; however, long periods of time are required for their effects to be significant. Applications of these "biological control" agents have not yet been able to reduce *P. weirii* inoculum to levels at which regeneration of highly susceptible hosts would not be significantly affected (Nelson 1967,1971; Nelson and Thies 1986b, Goldfarb et al. 1989). To be operationally effective, pathogen inoculum levels must be significantly reduced within 5 to 10 years (Nelson and Thies 1985). Fungal antagonists and especially *Trichoderma* spp., appear to offer the most potential for operational disease control agents.

Many species of *Trichoderma* impact *P. weirii*-infected materials (Nelson 1982, Nelson and Thies 1986, Goldfarb et al. 1989). An understanding of differences in the

hyperparasitic traits of fungal isolates may hold the key to successful biological control of laminated root rot (Nelson 1982, Goldfarb et al. 1989). Modifications of soil pH, moisture, temperature and other conditions may give *Trichoderma* a competitive advantage over *P. weirii* (Hutchins and Li 1981, Nelson 1982, Nelson and Thies 1985, 1986; Goldfarb et al. 1989). However, applications of *Trichoderma* and other biological control agents are greatly limited by the inability to get them deep into the roots where they can be effective (Nelson and Thies 1985).

1.8.2 Management in Immature Stands

Past practices of harvesting infected stands and reforesting them with dense plantations of Douglas-fir have resulted in a build-up of *P. weirii* inoculum (Thies 1984). In these plantations, remedial treatments are limited and often cost-prohibitive. Applications of nitrogen-based fertilizers have had mixed success in reducing mycelial growth and have not been proven to be effective in controlling disease (Nelson 1970, 1976; Hansen et al. 1983, Nelson et al. 1994). These compounds may possibly only be effective in reducing the saprophytic survival of *P. weirii* (Nelson et al. 1994).

The full effect of pre-commercial thinning on laminated root rot damage or spread is not fully understood (Morrison et al. 1991). Enhanced crop tree vigor associated with pre-commercial thinning does not appear to impart an ability to defend against infection by the pathogen (Wallis and Reynolds 1965, Goheen and Hansen 1994). Conventional pre-commercial thinning can inadvertently reduce disease spread by the random removal of hosts that would otherwise link healthy and infected host trees (Wallis and Reynolds 1965). Conversely, they can also make disease impact worse if Douglas-fir or other highly susceptible hosts are preferentially retained as crop trees. Pre-commercial thinning that targets the cutting of potential “bridge trees” around disease centers may be a cost-effective method for mitigating disease spread in young Douglas-fir stands (Hadfield et al. 1986, Morrison et al. 1992).

1.8.2.1 Bridge Tree Removal

Bridge tree removal is currently used operationally, on a limited basis, to lessen *P. weirii* impact in coastal stands dominated by Douglas-fir. This technique is thought not to be operationally effective in the interior of British Columbia (H. Merler, pers. com.). Bridge trees are defined as susceptible hosts within a set distance “buffer zone” (usually 5 m for coastal British Columbia) of the outer most visible edges of each disease center. Douglas-fir and other highly susceptible bridge trees are preferentially cut down in the buffer zones. Moderately susceptible to immune tree species are retained within the center and buffer zone to maintain site occupancy and productivity (Hadfield 1985, Hadfield et al. 1986, Morrison et al. 1992, Sturrock and Garbutt 1994).

Three key principles provide the basis for the concept of bridge tree removal. First, felling is thought to hasten the death of healthy root tissues in both infected and uninfected trees, reducing their suitability for *P. weirii* colonization and secondary pathogen spread. Second, the cutting-related decline and death of host roots may encourage the rapid colonization by fungal antagonists that may slow, halt or even reverse pathogen spread (Wallis and Reynolds 1965). Third, the roots of infected juvenile trees are small in diameter and will rapidly lose their inoculum potential (Hansen 1979a). It is believed that most pathogen inoculum will have died before the roots of healthy peripheral crop trees grow through the bridge tree zone and contact previously infected roots in the disease center.

The retention of tree species that have low susceptibility to *P. weirii* in the buffer zone provides a risk of maintaining viable spread routes. The pathogen can spread up to a total of 15 cm on western redcedar (Wallis and Reynolds 1965) but this extent of spread is also possible on the roots of salal or other brush species (Wallis 1976). Hemlock, on the other hand, is a greater risk than western redcedar because the pathogen is able to infect and initiate secondary spread from its roots. Hemlock can also appear disease-free even though the majority of its roots are infected or dead (Wallis and Reynolds 1965).

Nonetheless, it is believed that from the standpoint of site occupancy and short term timber production, the benefits of retaining these species appear to outweigh the risks.

1.9 Research Objectives

To better understand how disease spreads in young spaced stands, this study was initiated to observe the spread of inoculated *P. weirii* in living and recently cut trees. The effects of host and site conditions and inoculation methods were also investigated.

The specific objectives were to:

- 1) document and contrast the spread of inoculated *P. weirii* on the roots of juvenile Douglas-fir trees that were left standing, cut-down at the time of inoculation or cut-down 6 months after inoculation;
- 2) compare the effectiveness of two inoculation techniques;
- 3) document and compare pathogen growth through an assessment of linear spread distance (cm) and area spread (cm^2);
- 4) document presence of visible rhizoplane soil fungi, investigate their effect on *P. weirii* spread and assess potential treatment effects;
- 5) elucidate the effects of soil variations, sample tree attributes, inoculation site characteristics or interactions between these factors, on the spread of *P. weirii* ; and
- 6) use the results to make inferences on the utility of the operational bridge tree removal technique to mitigate *P. weirii* spread in juvenile stands.

CHAPTER 2.0 - MATERIALS AND METHODS

2.1 Site Location and Characteristics

A 0.5 ha portion of a 15 year old Douglas-fir stand was utilized at the University of British Columbia's Malcolm Knapp Research Forest, Maple Ridge, B. C. (one hour drive east of Vancouver B.C.). The stand has an elevation of 205 m, is situated in the Coastal Western Hemlock Biogeoclimatic Zone - dry maritime variant, receives an average annual precipitation of 220 cm and has average daily mean temperatures that range from 17 to 0° C (Feller 1974). The site has a rich nutrient regime and a fresh moisture regime that is typically dominated by Douglas-fir and western redcedar tree species with sword fern, salmonberry, step moss and Oregon beaked moss as common ground vegetation (Green and Klinka 1994). The dominant soil profile of the area is a mini humo-ferric podzol of a morainal blanket origin (Feller 1974) but, the study area had been harshly land cleared and smoothed by crawler tractors in 1978. Six evenly distributed pits found the current upper soil profile to consist of an Ap horizon (35 cm average depth and 5 to 60 cm range) overlying a Bf horizon (30 cm average depth and 15 to 40 cm range) and physical and chemical profiles each were markedly different (Table 1). The existing stand was planted in 1978 and 1979 on a 2.5 to 3 m grid with Douglas-fir stock. The site had been brushed in 1985 and lightly pre-commercially thinned in 1987. In 1994, destructive sampling of 14 dominant and co-dominant plantation trees indicated average site index is 41 m (at 50 years). Crown observations and root surface examinations have confirmed the area to be essentially free of root disease.

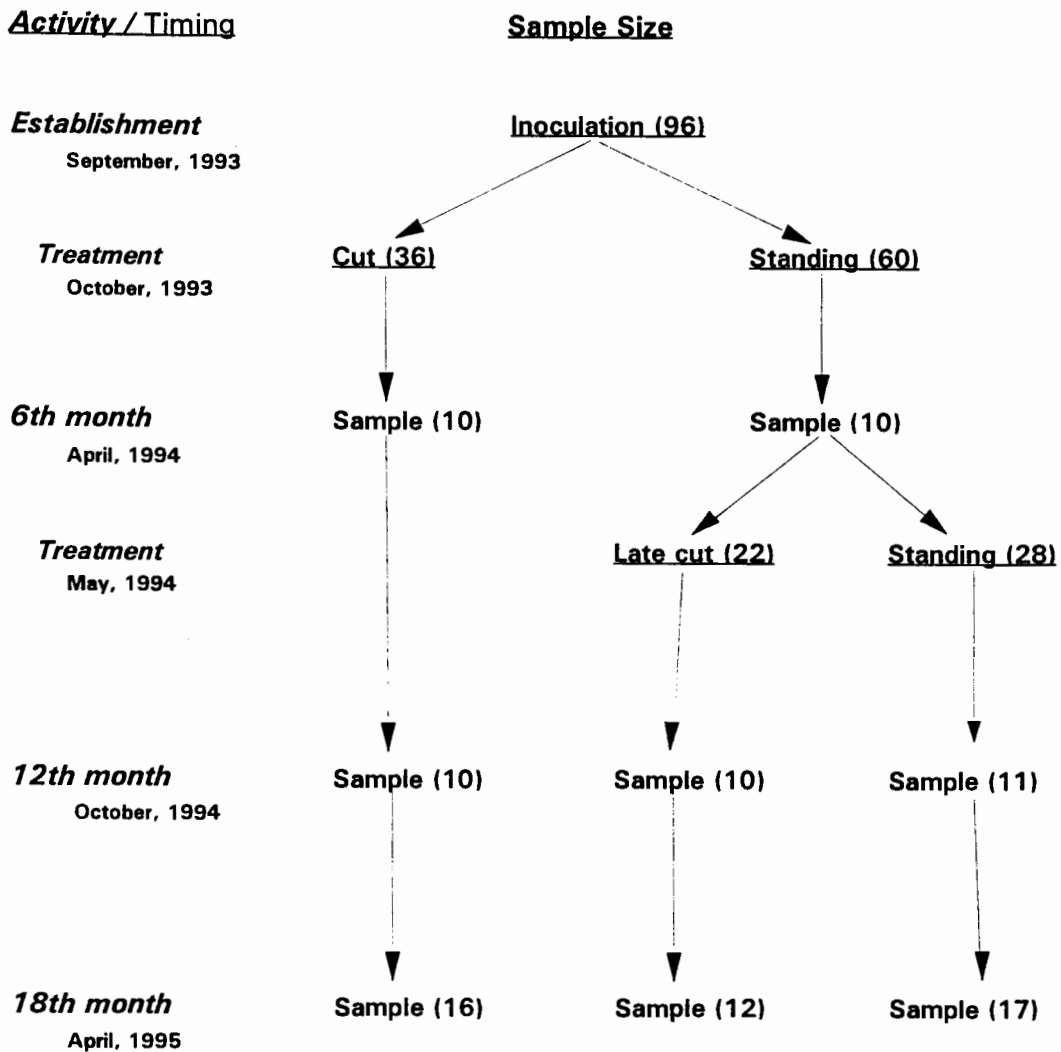
2.2 Experimental Treatments

The rate of *P. weirii* ectotrophic and endotrophic mycelial spread on Douglas-fir root systems was compared on 96 trees that were artificially inoculated and according to the protocol in Figure 1, were either: 1) cut down immediately after inoculation, 2) cut

**Table 1 - Attributes of pooled soil samples collected in the study area,
September, 1994.**

Attribute	Ap Horizon	Bf Horizon
Soil pH	4.8	5.3
% Coarse content (>2 mm)	20.0	40.0
Organic matter (%)	24.8	7.1
Carbon / nitrogen Ratio	31.0	26.0
Mineralizable nitrogen (ppm)	135.0	29.0
Total nitrogen (%)	0.47	0.16
Available phosphorus (ppm)	18.0	4.5
Available potassium (ppm)	60.0	13.0
Available calcium (ppm)	650.0	150.0
Available magnesium (ppm)	100.0	16.0

**Figure 1 -Experimental design used in research to schedule activities.
In parentheses are the numbers of trees treated or sampled.**



down 6 months after inoculation; or 3) left standing. Sample trees were selected so as to minimize variations in tree height, diameter and vigor as well as local soil characteristics.

Two inoculation methods were used to infect each sample tree with *P. weirii*: a “combined” method (whereby a transfer stick links inoculum source block to the target root) and a “block” method (whereby the inoculum source block is put in direct contact with the target root). Both methods utilized the same type of inoculum source blocks that had been prepared in advance by Rona Sturrock and George Reynolds (Natural Resources Canada, Pacific Forestry Center, Victoria, B.C.) in the following fashion. Alder stems (4 to 6.5 cm wide) were cut into 6 cm-long sections and drilled longitudinally through the center with a 0.6 cm bit. Groups of 25 sections were sealed in a double layer of heat-tolerant plastic bags, autoclaved overnight, left to cool and autoclaved for an additional 90 min. 40 ml of liquid *P. weirii* inoculum (Forestry Canada isolate # 583 - Shawnigan Lake in a 2% malt solution) was added to each cooled bags, which were then resealed and incubated at 22° C in the dark for 3 months.

The two separate root systems of each sample tree were selected for inoculation so that: 1) inoculation would occur in the mineral soil; 2) there was at least 1 m between the inoculation site and the bole of the tree or any portion of the root that protruded above the soil surface; 3) roots at the site of inoculation were 2 to 5 cm in diameter; and 4) the inoculation site on the root system was not scarred nor was the surrounding soil severely disturbed.

To ensure that root systems were exposed at only the inoculation site, a narrow pick or small hand trowel was used to probe the soil and locate candidate roots. A small pit was excavated around each suitable root site and the upper root surface was brushed off to provide a clean inoculation point. Soil depth, depth of initiation point, distance from bole of tree to initiation point and target root diameter were measured for each inoculation site and recorded along with diameter at breast height (dbh = 1.3 m) of each inoculated tree.

For the block inoculation method, soil excavations were extensive enough to allow access to both the top and bottom of the root at the inoculation site. Inoculum source blocks were individually removed from the autoclaved bags and placed lengthwise into 15 x 30 cm separate polyethylene freezer bags. Masking tape was used to secure the bag tightly around the circumference of the block. Two longitudinal cuts were made on opposite sides of the bag from the open end down to the proximal cross section of the inoculum block. The cross sectional surface was scraped with a clean knife (to expose fresh mycelium) and placed directly against the upper surface of the target root. The flaps created when the bag was longitudinally cut were then used to “tie” the block to the root surface. This effectively sealed off root/block interface from the soil environment (Figure 2).

With the combined method (Figure 2), only the upper half of the root surface was exposed during inoculation. A spike (1 cm diameter) was used to create a hole adjacent to the root that would accommodate the transfer stick’s snug placement against the target. As with the block technique, each source block was removed from the autoclaved bag and individually wrapped in a freezer bag. A 30 x 1 cm transfer stick (a recently cut piece of Douglas-fir branch with needles removed) was then pushed 5 cm deep into the central hole of the inoculum block. The mid-section of bag was then twisted around the transfer stick at the base of the block, folded back and taped tightly around the block. The exposed stick portion of the inoculation unit was slotted into the hole made by the spike until the middle portion of the transfer stick was in firm contact with the target root (Figure 2). Soil was carefully replaced in the excavation pit to restore the original soil profile.

Figure 2 - Illustration of inoculum placement for combined and block inoculation methods.



A



B



C



D

A- "Block" inoculation unit - inoculum source block wrapped in freezer bag with "tie-down" flaps.

B- "Combined" inoculation unit - source block wrapped in freezer bag with transfer stick.

C - "Block" inoculation unit "tied" into place against target root in excavated inoculation pit.

D - "Combined" inoculation unit with transfer stick placed against target root in excavated pit.

2.3 Data Collection

Roots were unearthed and sampled at the predetermined periods (Figure 1). The surface of each root system was exposed 15 cm proximal and distal to the furthest visible extent of the pathogen. Where a combined inoculum had been used, a small push pin was used to mark the point where the root was contacted by the transfer stick. For the block inoculations, the block unit was left secured in place. Each root system was sheared or sawed off at a point about 10 cm beyond the furthest point of visible fungal advancement to ensure that the leading mycelium edge was included. The cut surfaces of the removed root sections and the inoculum pieces were labeled with indelible pencil, immediately wrapped in clean newspaper and transported to the laboratory. Samples were stored at 4° C until they could be assessed and measured.

A dissecting microscope was used to observe the physical characteristics of pathogen mycelium, presence of other fungi, host reactions and interactions between these parameters. To measure the extent of the ectotrophic and endotrophic colonization, different approaches were taken. For ectotrophic mycelium, separate measurements were taken from the initiation point to the respective proximal or distal leading mycelial edges. Pathogen initiation points were defined as the push pin marker (combined method) or the point where the mycelium emerged from the protective plastic root wrapping (block method). Linear spread was measured as the linear distance between the initiation point and the leading mycelial front. To determine the area spread for completely colonized root sections, the infected area was calculated using a simplified equation for a cone frustum. For partially colonized root segments, graph paper was used to measure infected area until it was determined that careful visual estimates provided roughly equal accuracy in a fraction of the time. For partially colonized root segments, a visually-based adjustment factor was applied to account for portions of cone frustum that had not been colonized.

Each root section was then sub-sectioned and split longitudinally to examine for the presence of necrotic cambial tissue or wood staining. Stain columns and necrotic tissues were excised and aseptically cultured on a malt extract agar with 2 mg/l (a.i.) benomyl and botran (Worral 1991) to determine if the pathogen or other fungi were present. The presence and extent of root beetles or other insects were also noted.

Representative samples of mycelium from inoculum blocks, transfer sticks and infected root materials were tested in culture for compatibility to confirm that they were the same organism and clone (Nelson 1967, Hansen 1979b). All fungi found on the roots or in inoculum blocks were recorded and cultured for identification. All fungi were cultured against the pathogen to verify *in vitro* antagonism (Nelson 1969).

2.4 Statistical Analysis

Simple descriptive statistics were derived using SYSTAT ver. 5.05 (SPSS Inc., Evanston IL.) but detailed analyses were conducted using the SAS statistical analysis package (SAS Institute., Cary, N.C.). In all cases, results were considered significant when $P \leq 0.05$. All roots with dead inocula at time of excavation were removed from the analysis. Both linear and area spread were assessed on the basis of their proximal, distal and total components. As parametric data transformations could not adequately resolve problems with frequent zero or near zero measurement values, as well as questionable data and residual normality, Kuskal-Wallis rank transformations (Zar 1984) were applied to all spread data.

Frequency data for inoculation success with respect to inoculation method, treatment type and sample period were analyzed by Chi-square tests. Separate ANOVAs, General Linear Models (GLM) procedure, were conducted on block and combined inoculation data to assess significance of sample period, treatment type, dbh, root diameter at inoculation site, inoculation site depth and distance from base of the tree to root inoculation site. Paired difference tests were applied to pathogen spread and area

colonized values to assess within tree differences that related to inoculation technique. The Bonferroni t-test ($P = 0.05$) was used for multiple comparisons. The original split-plot analysis approach had to be discarded because: 1) chi-square tests found that inoculum failures were significantly higher with the block technique than the combined technique (thus they appeared to be distinct from distinct populations); and 2) variability of spread within-trees exceeded that seen between trees, thus the inoculation types could be assessed independently.

Logistic regression analysis was used to test for significant effects of treatment and period on the presence of soil fungi on sampled roots. Multidimensional Chi-square tests could not be utilized due to low and zero frequency values (Zar 1984).

CHAPTER 3.0 - Results

3.1 Inoculation Success

Inoculation success with the combined inoculation method (97%) was significantly better ($P < 0.005$) than with the block method (81%). Success rates on trees sampled at 6 (93%), 12 (90 %) and 18 months (84 %) were not different ($P < 0.25$). Inoculum success for cut (75%), standing (72%) and late-cut (85%) treatments were also not different ($P < 0.85$).

Isolations confirmed that all *P. weirii* samples taken from the roots were vegetatively compatible (Hansen 1979b) and to be of the same clone as the original isolate. Sample cultures were not vegetative compatible with different isolates that originated from two locations in the Chilliwack valley.

3.2 Linear and Area Spread of Mycelium

Observations and culturing assessments determined that endotrophic spread was infrequent (<5% of cultured samples) and was time consuming to investigate; thus, after the 12 month sample, only ectotrophic spread was monitored.

ANOVA of combined inoculations showed at 6 months, spread of *P. weirii* was significantly higher for both linear ($P < 0.01$) and area ($P < 0.02$) spread in trees cut immediately after inoculation than those left standing. ANOVA showed that linear and area spread at 6 months post inoculation were significantly less ($P < 0.001$) than at 12 and 18 months. Linear and area pathogen spread did not differ between 6, 12 and 18 months in cut trees but were significantly less at 6 months ($P < 0.001$) than at 12 and 18 months in standing trees. Spread means recorded at 12 and 18 months were not significantly different but separate analyses were warranted due to conflicting data trends (Figures 3, 4). Differences in proximal and distal directions of linear or area mean spread were not significant.

General linear modeling (GLM) of block inoculation spread and host or site variables revealed only a significant relationship between total area spread and inoculum distance at 12 months post inoculation (Table 2, Figure 3). For combined inoculation data, similar GLM revealed: 1) at 6 months, all spread parameters showed spread to be significantly higher in cut than standing trees; and 2) at 18 months, total linear, total area and distal area spread were significantly higher in late-cut than in cut trees (Table 2, Figure 4). GLM also disclosed a few other significant effects for the combined inoculation data, specifically: dbh and root diameter at 6 months, inoculum depth at 12 months and dbh and root diameter at 18 months (Table 2).

3.3 Presence and Antagonism of Other Fungi

The overall frequency of fungi on the roots of inoculated trees was not enough to allow investigations into the effects of individual antagonist species on pathogen spread. However, pooling of different fungal types did allow the assessment of this parameter as a treatment effect. Presence of root surface fungi at the 6 month sample appeared to be substantially lower than in subsequent periods; unfortunately, due to different sampling intensities, the significance of this difference could not be tested. These differences in sampling intensities were due to having root surface area examined for fungal presence equal to that examined for pathogen colonization. Pure cultures of potential fungal antagonists isolated from rhizoplane fungal samples were identified to be *Trichoderma* spp., *Peniophora subacida*, *Armillaria sinapina* and an unidentified basidiomycete. Cultures were commonly contaminated with *Trichoderma* spp., *Candida* spp., *Verticillium* spp., *Mucor* spp., *Epicoccum nigura* and an unknown phycmycete. Using the methodology of Nelson (1969), isolates of *Trichoderma* spp., *Peniophora subacida*,

Verticillium spp., *Mucor spp.*, *Epicoccum nigra* and the unidentified basidiomycete were found to be moderately to highly antagonistic to *P. weirii* on cultures of malt extract agar. Contamination problems experienced with 6 and 12 month samples (linked to use of a different original selective media (as per Hunt and Cobb 1971) prevented frequency and compatibility correlations between pure isolates and recorded rhizoplane fungi.

3.4 The Effect of Soil Horizon

The effect of soil horizon on linear and area spread was found to be insignificant using T-tests. However, it should be noted that sampled roots frequently passed through both the Ap and Bf soil horizons (Table 1) likely negating the detection of any effects.

FIGURE 3 - Spread of *P. weirii* on Douglas-fir roots by time since BLOCK INOCULATION and treatment. Significant differences ($P < 0.05$) are denoted by different uppercase bar letters (within time) and by lowercase letters (within treatment-between times).

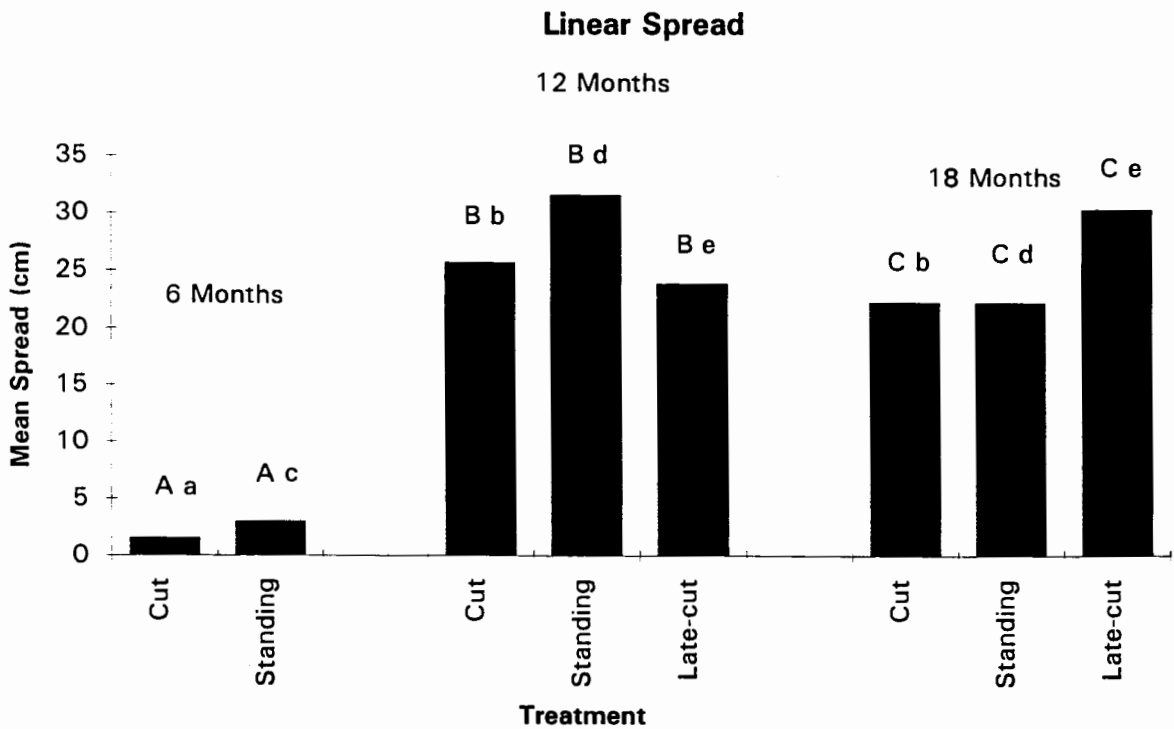
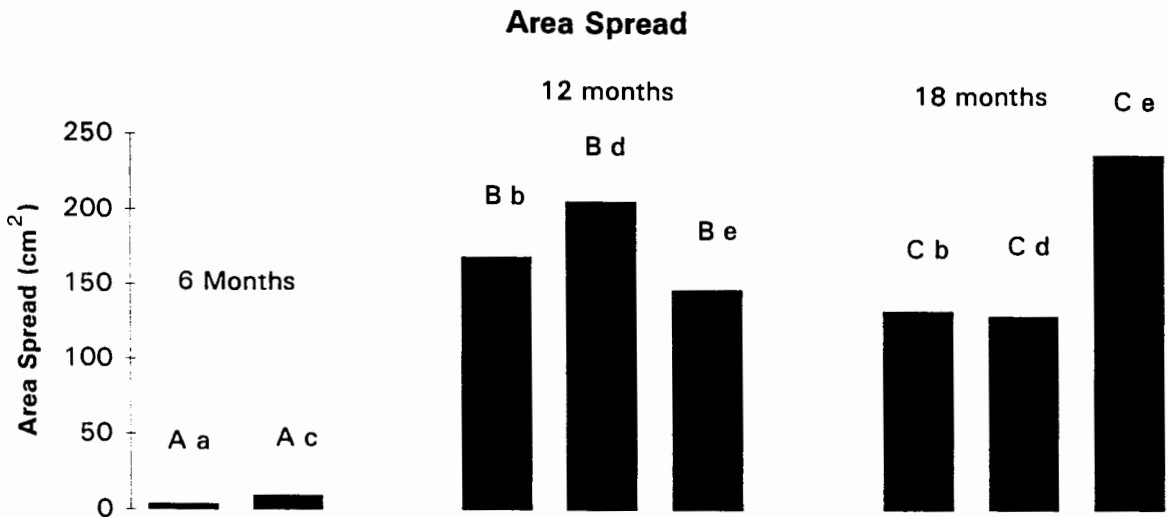


FIGURE 4 - Spread of *P. weirii* on Douglas-fir roots by time since COMBINED INOCULATION and treatment. Significant differences ($P < 0.05$) are denoted by different uppercase bar letters (within time) and lowercase letters (within treatment-between times).

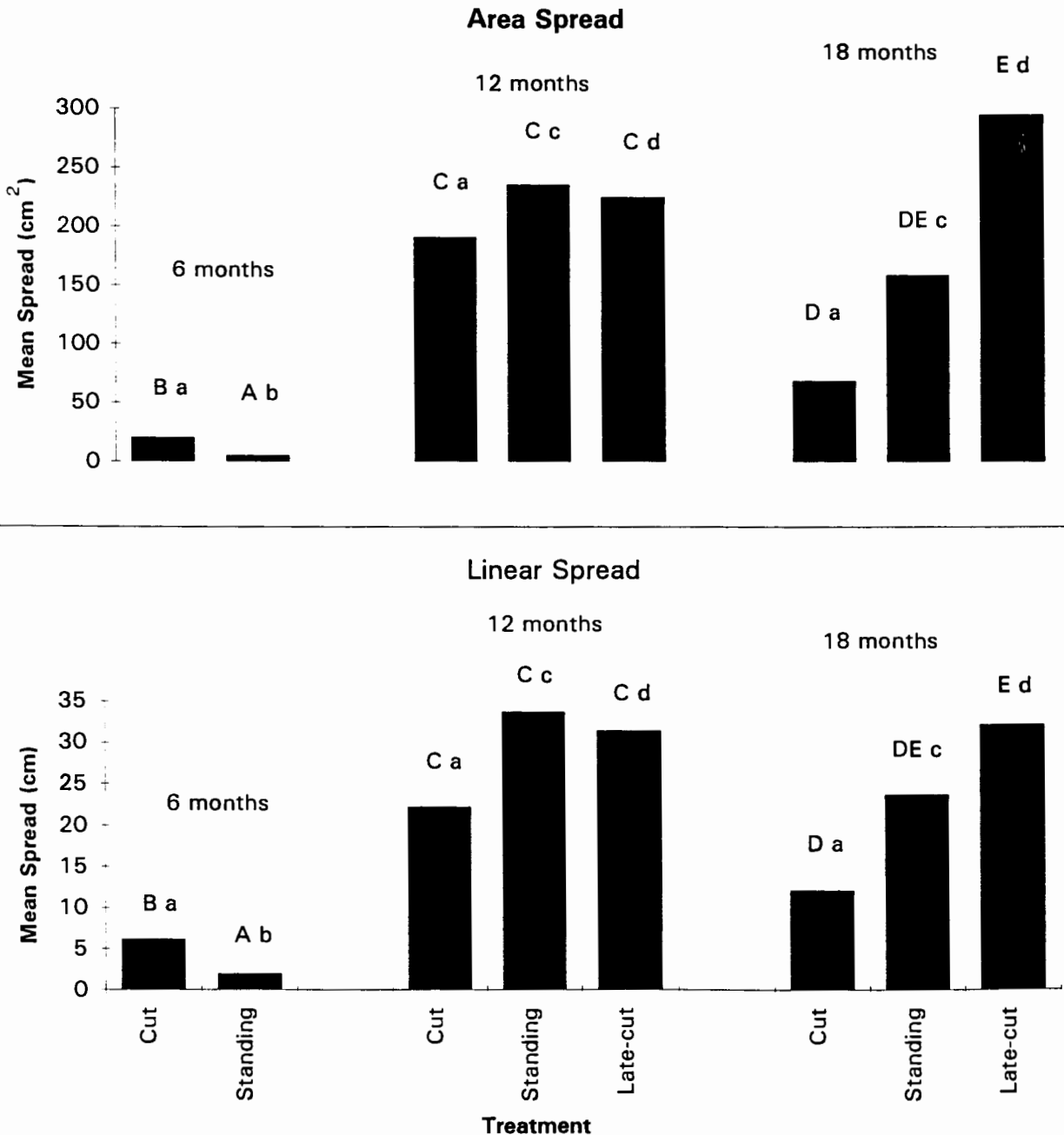


Table 2- Listing of significant general linear model relationship probabilities for rank transformed spread data - all data. Probability values ≤ 0.05 are in bold and negative correlations are in parentheses.

Inoculation Method	Sample Time	Spread Measurement	DBH	Root Diameter	Initiation Point Distance	Initiation Point Depth	Treatment
BLOCK	12 months	total area	0.24	0.71	(0.02)	0.21	0.88
COMBINED	6 months	total linear	0.22	0.09	0.57	0.35	0.01
		proximal linear	0.43	(0.05)	0.42	0.97	0.02
		distal linear	0.31	0.21	0.48	0.17	0.02
		total area	(0.03)	0.14	0.79	0.51	0.00
		proximal area	0.36	0.30	0.82	0.60	0.03
		distal area	0.09	0.25	0.51	0.13	0.01
	12 months	proximal linear	0.60	0.69	0.55	0.03	0.27
		total area	0.50	0.23	0.89	0.02	0.76
		proximal area	0.97	0.57	0.62	0.02	0.24
	18 months	total linear	0.22	(0.05)	0.27	0.48	0.02
		proximal linear	0.03	0.10	0.95	0.88	0.11
		total area	0.10	0.06	0.28	0.71	0.01
		proximal area	0.03	0.09	0.75	0.96	0.07
		distal area	0.89	0.22	0.30	0.55	0.04

CHAPTER 4.0 - DISCUSSION

4.1 Inoculation Success

Two inoculation methods were tested in this study to evaluate success of pathogen transfer. Historically, block type inocula have been used with a reasonable frequency of success to initiate and sustain *P. weirii* infections (Wallis and Reynolds 1962, Hansen et al. 1983, Goheen and Hansen 1994). This method uses plastic wrappings to secure the inoculum in place and to shelter the inoculation site from the soil environment. The greater the level of inoculum block protection (wrapping), the greater the proportion of successful inoculations (Wallis and Reynolds 1962, Hansen et al. 1983, Goheen and Hansen 1994).

The combined inoculation method was designed to mimic the natural infection process in seedlings and was relatively untested in older trees. The transfer stick was designed to allow pathogen transfer to occur at a single root contact point in the open soil environment without exposing the inoculum source (G. Reynolds pers. com.). My results show for the first time that the combined inoculation method can be superior to the block method for infecting juvenile trees. I believe that this difference is attributable to the combined inoculation method having only the transfer stick exposed to the soil while the block method has the base of an inoculum block directly exposed to the rhizoplane environment and to potentially antagonistic soil microorganisms that exist there. However, it is also possible that the added inoculum potential of the transfer stick may also explain the greater success rate of the combined technique.

The effect of inoculum technique on pathogen transfer success may only be important in soils that are unfavourable to *P. weirii* growth. Unfortunately, most studies do not collect or provide enough information to fully investigate this hypothesis. Inoculum success in excess of 97% was achieved in the nearby Chilliwack Valley using only the block inoculation method (R. N. Sturrock pers. com.). The pathogen clone, inoculum materials, and season of inoculation were the same for both experiments but

soils in my study site appeared to be substantially richer, finer textured and apparently less favourable (or more antagonistic as per Hansen et al. 1983) to *P. weirii* growth. Respective differences in fungal diversity (five visibly distinct non- *P. weirii* fungal types compared to one) and fungal populations (25% of the roots colonized compared to > 5%) supports the hypothesis that pathogen antagonism was greater at my site than the Chilliwack site. It should be noted that the perceived differences in antagonism may simply be a reflection of one soil environment being closer to mycostasis than the other.

Overall, the combined method has the advantage of being quicker and easier to apply and creates less disturbance of the rhizosphere soil conditions and micro-organism populations than the block method. Whether it or its block counterpart accurately mimic the spread of naturally-initiated infection still needs to be resolved.

4.2 Linear and Area Spread Analyses

Historically, experiments on *P. weirii* have measured ectotrophic spread only in linear terms. In this study, area of spread was used to help offset the potential effects of highly variable root diameters. Based on the similarity of results seen between the area and linear spread parameters (Figure 3, 4; Table 2), the substantially greater effort required to collect information based on area may not be warranted for this type of study.

With the block inoculation treatment, spread in the plastic-protected zone was not included in the spread measurements because the plastic shielding could have provided localized atypical environmental conditions that could bias initial spread values (Wallis and Reynolds 1962, Hansen et al. 1983). Direct spread comparisons between the inoculation types at 6 months could not be pursued because much of the block initiated infection was confined to the plastic protected zone. Variability in the size of the plastic

protected zones and pathogen spread rates made it impossible to estimate the amount of time required to colonize these areas. At 12 and 18 months, the effects of the protected were negligible due to substantially higher linear and area spread rates.

Spread data generated from block inoculations was considered to be not as reliable as that from combined inoculations for two key reasons other than the plastic wrapping issue . First, block inoculations showed a significantly lower transfer success rate than the combined method and these failures were seen as an indicator of a potentially larger underlying problem with these data (V. Lemay; Biometrician, Faculty of Forestry, University of British Columbia, Vancouver, B.C. pers. comm.). Second, general linear modeling of the block data consistently failed to indicate significant treatment effects while analysis of combined inoculum data and visual observations indicated this was not the case (Table 2). Although discussion points focus on combined inoculation data for the above reasons, it should be noted that block data trends closely followed those associated with combined inoculations (Figures 3,4).

Linear and area spread at six months were significantly higher in cut than standing trees. It appears that the pathogen was able to benefit from the cutting-related decline of the host, while soil inhabiting antagonists were still unable to take immediate advantage of this host material. The positive effect of host cutting on pathogen spread was short-lived and by 12 months, death of the host caused *P. weirii* to lose its competitive advantage over soil inhabiting saprophytes. The differences seen between cut and standing trees at 18 months is expected to represent the long term trend.

Inoculum depletion over time and associated mycelium die-back are believed to be the reasons why spread means for standing trees decreased between 12 and 18 months (Figure 4). Effects similar to this were noted by Wallis and Reynolds (1962), Hansen et al. (1983) and Angwin (1985). Inoculum source depletion and mycelium die-back as related to spread reductions were greater in cut than standing treated trees due to the influence of soil inhabiting saprophytes. Late-cut trees appear to have overcome the depletion die-back problems by establishing the host roots as an alternate or new primary food source. It is hypothesized that spread in late-cut trees will eventually follow a similar pattern as the cut trees except that the decline will be delayed by at least the time differential between the treatments. The rate of decline in total spread in the late-cut trees will not be as rapid due to better establishment of the pathogen on the host at the time of the treatment.

Analysis of host and inoculation site variables indicated there were a number of significant effects on pathogen spread (Table 2). However, considering that the probabilities for each variable fluctuated so much within and between sample times, it would be misleading to perceive these relationships to be individually meaningful. My findings support Wallis and Reynolds' (1981) observation that root diameter does not influence ectotrophic spread but appear to conflict with Bloomberg and Reynolds' (1981) findings that ectotrophic spread between root contacts is strongly influenced by root diameter and possibly by depth in the soil. Inoculum success could not be linked to tree dbh or diameter of inoculated root as was previously reported by Hansen et al (1983). Similarities in significant probabilities seen at 6 and 18 months and their contrast to those

seen at 12 months, indicated the existence of seasonal spread trends. Such trends may be a reflection of annual changes in the biological condition of the pathogen, host, soil environment or interactions between these factors (Hansen et al. 1983 and Bloomberg 1990).

4.3 Effects of Season of Treatment

Pathogen spread may be influenced by biological condition of the pathogen and host, physical site factors, annual changes in soil environment or interactions between these factors (Hansen et al. 1983, Bloomberg 1990). There are several reasons why having a fall cut and spring late-cut treatment added potentially significant variability to this experiment. First, conifers reduce or cease many biological functions in the fall and reactivate them in the spring. Second, the fall cutting coincided with the time when the activity of the pathogen was decreasing from seasonal peaks and fungal antagonist activity was increasing from seasonal lows (Nelson 1969, Kellas 1984). Conversely, the late-cut application (spring) occurred after a time when pathogen activity would have been at its lowest and fungal antagonist activity at its highest (Nelson 1969, Kellas 1984). Evidence of the later pathogen-antagonist interaction was manifested by abundant zone lines at the pathogen's mycelial margins during the spring (6 and 18 month) samples. Third, because *P. weirii* is a facultative saprophyte, it is likely that the effect of the felling on recently inoculated trees (cut treatment) was different from the felling of trees that had been inoculated 6 months prior (late-cut). In the cut trees, *P. weirii* would at best have been on equal terms as far as competing for host materials yet; in late-cut trees, the

pathogen would have been established on the root system and had a competitive advantage over other soil fungi. Finally, any one of these factors or combinations thereof could have significantly influenced the differences seen between the cut and late-cut treatments (Figures 3, 4).

4.4 Implication on management of young stands

With ever increasing pressures to maximize the timber productivity of forest land, pre-commercial thinning and other stand-tending treatments are now common place. Beale (1987) and others have found that Douglas-fir stands have a high probability of containing laminated root rot, suggesting that disease mitigation should be undertaken as part of any silvicultural regime. My results indicate that cutting juvenile trees in these stands can have an impact on the spread of inoculated *P. weirii* and that the timing of cutting relative to season or time since infection may be important. Depending on the circumstances, there is the potential for pre-commercial thinning to either have a very positive or negative influence on disease management. Specifically, it appears that cutting previously infected trees will promote short term *P. weirii* spread and possibly increase disease impact, but cutting uninfected hosts can reduce the pathogen's spread rate and likely, disease impact. These results support the use of bridge tree removal during pre-commercial thinning to mitigate *P. weirii* spread and point out the risks of applying this treatment without consideration of the pathogen. It is recommended that further research in this area focus on clarifying: the long term effects that cutting infected trees have on pathogen spread, the effects of season of pathogen spread in felled infected trees and the role that site related differences in soil fungal antagonists have on the rate of pathogen spread in standing and felled trees.

Until more conclusive research can be completed, it is recommended that operational activities in juvenile coastal Douglas-fir stands be adjusted in several ways to account for the findings of my research. First, a general assessment of disease incidence

levels should be conducted concurrent with a preliminary evaluation of the suitability of a stand for pre-commercial thinning. This disease assessment should be used to determine if: 1) stand disease levels are low enough that pre-commercial thinning can proceed without the need for disease mitigation strategies; 2) disease levels are so high and widespread that pre-commercial thinning should not be undertaken; 3) disease levels are within a range that could be successfully managed if bridge tree removal and alternate tree species selection were applied during pre-commercial thinning activities; or 4) a more detailed disease survey is required so that the stand can be stratified into units that can be handled as per the aforementioned approaches.

In stands or strata suitable for pre-commercial thinning-related disease management, potential bridge trees around each laminated root rot disease center must be delineated prior to commencement of the felling activities. In coastal Douglas-fir stands, highly susceptible trees (Douglas-fir and *Abies* spp.) within 5 m of the outermost symptomatic trees in a disease center are deemed to be bridge trees. Bridge tree zones are flagged so that pre-commercial thinning crews can identify areas where highly susceptible tree species are to be removed and alternate (less impacted or immune) tree species are retained as crop trees. Within the actual disease centers, thinning crews are to leave all infected trees standing to prevent the potential for the felling-related promotion of pathogen spread.

Pre-commercial thinning should occur in the fall to take advantage of conditions that are apparently least favorable for the pathogen and most favorable for soil fungal antagonists. However, disease survey and bridge tree delineation work needs to be done in the spring time prior to trees breaking dormancy and when disease symptoms are most evident.

REFERENCES

- Angwin, P. A. 1985. Environmental factors influencing the rate of spread of *Phellinus weirii* in young growth Douglas-fir. M. Sc. thesis Oregon State University, Corvallis, OR.
- Beale, J. D. 1987. A survey of the incidence and severity of root disease and their implications for management in the Vancouver Forest Region. For. Serv. Int. Rep. PM-V-10, B.C. Min. of For., Burnaby, B.C.
- Bloomberg, W. J. 1990. Effect of stand conditions on advance of *Phellinus weirii* in Douglas-fir plantations. *Phytopathology* 80:553-559.
- Bloomberg, W. J., and Beale, J. D. 1985. Relationship of ecosystems to *Phellinus weirii* root rot on Southern Vancouver Island. pp. 20-28 *In* Proceedings of the 33rd Western International Forest Disease Work Conference: Olympia, WA.
- Bloomberg, W. J., and Reynolds, G. 1981. Factors affecting transfer and spread of *Phellinus weirii* mycelium in roots of second growth Douglas-fir. *Can. J. For. Res.* 12:424-427.
- Bloomberg, W. J., and Reynolds, G. 1985. Growth loss and mortality in laminated root rot infection centers in second-growth Douglas-fir on Vancouver Island. *For. Sci.* 31:497-508.
- Bloomberg, W. J., and Wallis, G. W. 1979. Comparison of indicator variables for estimating growth reduction associated with *Phellinus weirii* root rot in Douglas-fir plantations. *Can. J. For. Res.* 9:76-81.
- Childs, T. W. 1955. Synopsis of present information concerning *Poria weirii* root rot in Douglas Fir. USDA For. Serv. Res. Note PNW-116.
- Childs, T. W. 1963. *Poria weirii* root rot. *Phytopathology* 53:1124-1127.
- Childs, T. W. 1970. Laminated root rot of Douglas-Fir in Western Oregon and Washington. USDA For. Serv. Res. Note PNW-102.

- Eis, S. 1974. Root system morphology of western hemlock, western red cedar and Douglas-fir. *Can. J. For. Res.* 4: 28-38.
- Entry, J. A., Vance, N. C., and Copes, D. L. 1993. Resistance of graft-compatible and graft-incompatible *Pseudotsuga menziesii* rooted cuttings to *Phellinus weirii*. *Can. J. For. Res.* 24: 878-881.
- Etheridge, D. E., and Craig, H. M. 1973. A bilayer plate technique to detect broad-spectrum antagonism in microorganisms and its application to wood-inhabiting fungi. *Can. J. Microbiol.* 19:1455-1458.
- Feller, M. C. 1974. Initial effects of clearcutting on the flow of chemicals through a forest watershed ecosystem in southwestern British Columbia. Ph.D. dissertation University of British Columbia, Vancouver B.C.
- Goheen, E. M., and Hansen, E. M. 1994. Tree vigor and susceptibility to infection by *Phellinus weirii* : results of field inoculations. pp. 45-51 *In* Proceedings of the 8th IFURO conference on root and butt rots of forest trees: August 9-16 ,Wik, Sweden and Haikko, Finland. IFURO working party S2.06.01 Uppsala, Sweden.
- Goldfarb, B., Nelson, E. E., and Hansen E. M. 1989. *Trichoderma* species from Douglas-fir stumps and roots infested with *Phellinus weirii* in the western Cascade Mountains of Oregon. *Mycologia* 81:134-138.
- Green, R. N., and Klinka, K. 1994. A field guide to site identification and interpretation for the Vancouver Forest Region. Land Management Handbook no. 28. Research Branch, B.C. Min. For., Victoria, B.C.
- Hadfield, J. S. 1985. Laminated root rot: a guide for reducing and preventing losses in Oregon and Washington Forests. USDA For. Serv., PNW Region.
- Hadfield, J. S., Goheen, D. J., Filip, J. M., Schmitt, C. L., and Harvey, R. D. 1986. Root diseases in Oregon and Washington conifers. Pacific Northwest Region, USDA For. Serv. FPM-250-86.

- Hansen, E. M. 1975. *Phellinus (Poria) weirii* root rot in Douglas Fir-Alder stands 10-17 years old. USDA For. Serv. Res. Note PNW-250.
- Hansen, E. M. 1978. The survival of *Phellinus weirii* in Douglas-fir stumps on sites regenerated to red alder and Douglas-fir. pp. 328-332. *In Proceedings of the 5th International Conference on problems of root and butt rots in conifers*. Aug. 7-12 Kassel, Germany, IUFRO Working Party S2.06.01, Ed. by Dimitri, L. FRTG, Kassel, Germany.
- Hansen, E. M. 1979a. Survival of *Phellinus weirii* in Douglas-fir stumps after logging. *Can. J. For. Res.* 9:484-488.
- Hansen, E. M. 1979b. Sexual and vegetative incompatibility reactions in *Phellinus weirii*. *Can. J. Bot.* 57:1573-1578.
- Hansen, E. M. 1979c. Nuclear condition and vegetative characteristics of homokaryotic and heterokaryotic isolates of *Phellinus weirii*. *Can. J. Bot.* 57:1579-1582.
- Hansen, E. M., Reynolds, K., and Angwin, P. 1983. Factors Influencing spread of *Phellinus [Inonotus] weirii* in Douglas-fir. *Phellinus [Inonotus] weirii* and *Armillaria mellea*. pp. 77-94. *In proceedings of the 6th IFURO Conference on Root and Butt rots, 25-31 August 1983, Melbourne, Australia*. IUFRO Working Party S2.06.01. Ed. by Kile, G.A.. CSIRO, Melbourne, Australia.
- Hunt, R.S., and Cobb, F.W. 1971. Selective medium for the isolation of wood-rotting basidiomycetes. *Can. J. Bot.* 49:2064-2065.
- Hutchins, A. S., and Li, C. Y. 1981. Relative capacities of filamentous and non-filamentous bacteria from two forest soils to inhibit *Phellinus weirii* in culture. *Northwest Sci.* 55:219-224.
- Kastner Jr., W. W., D. J., and Edmonds, R. L. 1994. Relationship between occurrence of laminated root rot and site characteristics in Douglas-fir forests of the northern Oregon Coast Range. *West. J. Appl. For.* 9:14-17.

- Kellas, J. D. 1984. Inoculation of Douglas-fir and western hemlock roots with naturally infected inocula of *Phellinus weirii*. *Can. J. For. Res.* 15:183-185.
- Li, C. Y., Lu, K. C., Trappe, J. M., and Bollen, W. B. 1967. Effect of pH and temperature on growth of *Poria weirii* in vitro. USDA For. Serv. Res. Note PNW-66.
- Li, C. Y., Lu, K. C., Trappe, J. M., and Bollen, W. B. 1972. *Poria weirii* - inhibiting and other phenolic compounds in roots of red alder and Douglas-fir. *Microbios* 5:65-68.
- McCauley, K. J., and Cook, S. A. 1980. *Phellinus weirii* infestation of two mountain hemlock forests in the Oregon Cascades. *For. Sci.* 26:23-29.
- Morrison, D. J., Merler, H., and Norris, D. 1992. Detection, recognition and management of *Armillaria* and *Phellinus* root diseases in the southern interior of British Columbia. Canada-British Columbia Partnership Agreement on Forest Renewal and Development: FRDA II. FRDA Rep. 179.
- Morrison, D. J., Wallis, G. W., and Weir, L. C. 1988. Control of *Armillaria* and *Phellinus* root diseases: 20-year results from the Skimikin stump removal experiment. Government of Canada, *Can. For. Ser., Inf. Rep.* BC-X-302.
- Nelson, E. E. 1967. Factors affecting survival of *Poria weirii* in small buried cubes of Douglas-fir heartwood. *For. Sci.* 13:78-84
- Nelson, E. E. 1968. Survival of *Poria weirii* in conifer, alder, and mixed conifer-alder stands. USDA For. Serv. Res. Note PNW-83.
- Nelson, E. E. 1969. Occurrence of fungi antagonistic to *Poria weirii* in a Douglas-fir forest soil in western Oregon. *For. Sci.* 15:49-54.
- Nelson, E. E. 1970. Effect of nitrogen fertilizer on survival of *Poria weirii* and populations of soil fungi and aerobic Actinomycetes. *Northwest Sci.* 44:102-106.
- Nelson, E. E. 1971. Invasion of freshly cut Douglas-Fir stumps by *Phellinus weirii*. USDA For. Serv. Res. Note PNW-144.

- Nelson, E. E. 1972. Effect of urea and wood shavings on populations of soil microfungi, especially *Trichoderma* species. *Microbios* 5:69-72.
- Nelson, E. E. 1975. Survival of *Poria weirii* in wood buried in urea-amended forest soil. *Phytopathology* 65:501-502.
- Nelson, E. E. 1976. Effect of urea on *Poria weirii* and soil microbes in an artificial system. *Soil Biol. and Biochem.* 8:51-53.
- Nelson, E. E. 1980. Laminated root rot damage in a young Douglas-fir stand. USDA For. Serv. Res. Note PNW-359.
- Nelson, E. E. 1982. Occurrence of *Trichoderma* in a Douglas-fir soil. *Mycologia* 74:280-284.
- Nelson, E. E., and Fay, H.A. 1974. Thermal tolerance of *Poria weirii*. *Can. J. For. Res.* 4:288-290.
- Nelson, E. E., and Fay, H. A. 1975. Effect of temperature on growth and survival of high and low elevation isolates of *Phellinus (Poria) weirii*. *Northwest Sci.* 49:119-121.
- Nelson, E. E., Goldfarb, B., and Thies, W. G. 1987. *Trichoderma* species from fumigated Douglas-fir roots decayed by *Phellinus weirii*. *Mycologia* 79:370-376.
- Nelson, E. E., Hansen, E. M., Li, C. Y., and Trappe, J. M. 1978. The role of red alder in reducing losses from laminated root rot. pp. 273-282. *In Utilization and management of alder.* USDA For. Serv. Gen. Tech. Rep. PNW-70.
- Nelson, E. E., and Hartman, T. 1975. Estimating spread of *Poria weirii* in a high elevation, mixed conifer stand. *J. For.* 73:141-143.
- Nelson, E. E., McWilliams, M. G., and Thies, W. G. 1994. Mortality and growth of urea-fertilized Douglas-fir on a *Phellinus weirii*-infested site in Oregon. *West. J. Appl. For.* 9:52-56.
- Nelson, E. E., and Thies, W. G. 1984. Potential of *Trichoderma* for biological control of laminated root rot. pp. 69-73. *In Proceedings of the 32nd Western International Forest Disease Work Conf.; Taos, N. M..*

- Nelson, E. E., and Thies, W. G. 1985. Colonization of *Phellinus weirii* infested stumps by *Trichoderma viride* 1. Effect of isolate and inoculum base. Eur. J. For. Path. 15:425-431.
- Nelson, E. E., and Thies, W. G. 1986. Colonization of *Phellinus weirii* infested stumps by *Trichoderma* 2. Effects of season of inoculation and stage of wood decay. Eur. J. For. Path. 16:56-60.
- Nobles, M. K. 1948. Studies in forest pathology VII. Identification of cultures of wood-rotting fungi. Can. J. Res. Sect. C. 26:281-431.
- Oren, R., Thies, W. G., and Waring, R. H. 1985. Tree vigor and stand growth of Douglas-fir as influenced by laminated root rot. Can. J. For. Res. 15: 985-988.
- Reynolds, K. M., and Bloomberg, W. J. 1982. Estimating probability of intertree root contact in second growth Douglas-fir. Can. J. For. Res. 12:493-498.
- Sturrock, R. N., and Garbutt, R. 1994. Laminated root rot of Douglas-fir. Pac. For. Cent., Natural Resources Canada, For. Pest Leaf. 3.
- Sturrock, R. N., Reynolds, G., and Nevill, R. 1994. Demonstration of a novel fungus inoculation technique to screen coastal Douglas-fir for genetic resistance to laminated root rot. R & D Update. Pac. For. Cent., Natural Resources Canada.
- Taylor, S.P. 1986. Forest insect and disease impacts in timber supply areas. British Columbia Ministry of Forests, Forest Protection Branch. Pest Management Report No. 6.
- Taylor, S. P. 1989. Forest insect and disease impacts in tree farm licences. British Columbia Ministry of Forests, Forest Protection Branch. Pest Management Report No. 11.
- Thies, W. G. 1983. Determination of growth reduction in Douglas-fir infected by *Phellinus weirii*. For. Sci. 29:305-315.
- Thies, W. G. 1984. Laminated root rot. The quest for control. J. For. 82:345-356.

- Thies, W. G. 1992. Laminated root rot. All about it and how to break the cycle. Northwest Woodlands 8:20-31.
- Thies, W. G., Nelson, E. E., and Zabowski, D. 1993. Removal of stumps from a *Phellinus weirii* infested site and fertilization affect mortality and growth of planted Douglas-fir. Can. J. For. Res. 24:234-239.
- Thies, W. G., and Russel, K. W. 1983. Controlling root rots in coniferous forests of northwestern North America. *Phellinus [Inonotus] weirii* and *Armillaria mellea*. pp. 379-398. In proceedings of the 6th IUFRO Conference on Root and Butt rots, 25-31 August 1983, Melbourne, Australia. IUFRO Working Party S2.06.01. Ed. by Kile, G.A.. CSIRO, Melbourne Australia.
- Thies, W. G., and Sturrock, R. 1995. Laminated root rot in western North America. USDA For. Serv. Gen. Tech. Rep. PNW-GTR-349.
- Wallis, G. W. 1976. Growth characteristics of *Phellinus (Poria) weirii* in soil and on root and other surfaces. Can. J. For. Res. 6:229-232.
- Wallis, G. W., and Bloomberg, W. J. 1981. Estimating the total extent of *Phellinus weirii* root rot centers using above- and below-ground disease indicators. Can. J. For. Res. 11:827-830
- Wallis, G. W., and Reynolds, G. 1962. Inoculation of Douglas fir roots with *Poria weirii*. Can. J. Bot. 40:637-645.
- Wallis, G. W., and Reynolds, G. 1965. The initiation and spread of *Poria weirii* root rot of Douglas-fir. Can. J. Bot. 43:1-9.
- Wallis, G. W., and Reynolds, G. 1975. Mass inoculation of Douglas-fir seedling roots with *Phellinus (Poria) weirii* (Murr.) Gilbertson. Can. J. For. Res. 5:741-742.
- Worral, J. J. 1991. Media for selective isolation of hymenomycetes. Mycologia, 83:296-302.
- Zar, J. H. 1984. Biostatistical Analysis - second ed. Prentice-Hall Inc. Englewood Cliffs, N. J.. pp. 70 -71, 228-231.