HOST RESPONSES IN DOUGLAS-FIR, WESTERN HEMLOCK

AND WESTERN REDCEDAR TO INFECTION BY

ARMILLARIA OSTOYAE AND *ARMILLARIA SINAPINA*

by

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HBES Forest Conservation, Lakehead University, 2001

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

(Forest Sciences)

THE UNIVERSITY OF BRITISH COLUMBIA

April 2007

ii

ABSTRACT

Necrophylactic periderm (NP) formation and compartmentalization of infected tissue were examined in roots of 20-30 year-old western redcedar (*Thuja plicata*), western hemlock (*Tsuga heterophylla*) and Douglas-fir (*Pseudotsuga menziesii*) trees infected by *Armillaria ostoyae*. Microscopic investigation of abiotically wounded roots, as well as roots naturally infected and inoculated with *A. ostoyae* revealed distinct differences in the types and frequency of host responses between cedar and the other two conifers.

Following invasion by *A. ostoyae*, a higher frequency of successful resistance reactions was induced in western redcedar compared to Douglas-fir and western hemlock. Breaching of non-suberized impervious tissue (NIT) and NP was common in Douglas-fir and western hemlock trees. The barrier zone in cedar formed by the uninjured cambium was comprised of axial parenchyma with pigmented deposits and provided a permanent barrier to spread by the fungus. Unique resistance mechanisms in cedar involving induced rhytidome formation impart increased resistance to the spread of *A. ostoyae* in host tissue.

In three inoculation trials, penetration of living bark on host roots by *A. sinapina* did not differ from *A. ostoyae*. However, the frequency of successful resistance reactions induced following invasion by *A. sinapina* in Douglas-fir and western hemlock was significantly higher than the same species infected with *A. ostoyae*. Inoculum potential and host-pathogen interactions were key determinants of pathogenicity of *A. sinapina* on all hosts.

In a survey of twenty juvenile mixed species plantations throughout the southern Interior of B.C., cumulative mortality in Douglas-fir trees was significantly higher than in western redcedar trees (p < 0.001). The incidence of mortality decreased with increasing tree size for both species, however the rate of decrease was markedly greater among cedar compared to Douglas-fir trees. The proportion of trees that showed compartmentalization and callusing at the root collar increased with increasing tree size, but the increase was markedly greater for cedar than Douglas-fir and occurred much earlier even when the trees were relatively small. Results indicate that the higher degree of resistance against *A. ostoyae* in western redcedar may help alleviate long-term impacts of root disease when regenerated on sites infested with Armillaria root disease.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Acronyms and labels for macro- and micrographs:
APh, adjacent phloem;
BPh, browned phloem;
BF, viewed in bright field;
BL, viewed under blue light fluorescence;
BZ, barrier zone
CT, callus tissue
HPh, healthy phloem;
IT infected tissue;
My, mycelium;
NIT, non-suberized impervious tissue;
NP, necrophylactic periderm;
OCT, optimum cutting temperature compound;
P, original periderm;
Pg, phellogen zone;
Pe, thin-walled phellem;
Rh, rhizomorph;
Rhyt, rhytidome;
TD, traumatic resin ducts
TPRD, traumatic phloem resin ducts;
RZ, redifferentiated zone;
Scl, sclerieds;
SP, stone phellem;
VC, vascular cambium

ACKNOWLEDGEMENTS

I would like to thank many of my colleagues who have assisted me with this work, extended support in the development of this thesis, and provided a stimulating environment in which to learn and grow over the past four years: Chef Terry Holmes, Shawn Cleary, Jason Holland, Sue Askew, Rona Sturrock, Fred Peet, Lesley Manning, Hadrian Merler, Liyousa Shafei, Bob Johnson, Tom Johnston, Trevor Ryan, and David Jackson. I also wish to thank Ed Setliff at Lakehead University for exposing me to the field of forest pathology and the fascinating fungus which has become the focus of my study.

I wish to thank my long-time friends Tania, Sarah and Laura who have remained extremely supportive despite my abandoning the circle in London, and particularly in the midst of the many other important events which unfolded this past year. Thanks for all the emotional support, camaraderie, and caring.

I wish to thank my family and extended family for providing a loving environment for me. My sister and brother-in-law were particularly supportive and understanding of the pressures of academia and I'm extremely grateful for all the 'perspective' pep-talks.

Lastly, and most importantly, I wish to thank my parents, Ruth and Alf Cleary whose foresight and values paved the way for an advanced education. They have supported and loved me and have never doubted my ability to succeed. And to my husband Jason, who witnessed first hand the hardships and successes that this academic journey has brought to both our lives. I thank him for his patience, particularly during the several months of separation each year while carrying out this research, his assistance with several aspects of the field work, his continuous encouragement, understanding, support, and love.

CHAPTER ONE:
INTRODUCTION AND GENERAL LITERATURE REVIEW

1.1   GENERAL INTRODUCTION

Species of *Armillaria* causing root diseases of trees, shrubs and some non-woody perennials occur in forested areas worldwide. *Armillaria ostoyae* (Romangnesi) Herink, is the primary pathogen causing root disease of conifers in the northern hemisphere (Morrison 1981, Guillaumin *et al.* 1993, Klein-Gebbinck *et al.* 1993, Berube and Dessureault 1998, McLaughlin 2001). In British Columbia (B.C.), *A. ostoyae* is a natural component of undisturbed forests and is most abundant and frequent in the mid-elevation forests in the Interior-Cedar-Hemlock (ICH) biogeoclimatic zone (Lloyd *et al.* 1990) in the southern Interior of B.C. Within this zone, *A. ostoyae* causes considerable losses in immature stands by killing natural and planted coniferous trees, causing growth loss on trees sustaining non-lethal infection, and predisposing trees to attack by other pathogens and insects. It can also act as a secondary parasite and the immediate cause of death of trees infected by root diseases such as *Phellinus sulphurascens* Pilat (syn. *P. weirii*) and *Inonotus tomentosus* (Fr:Fr) S. Teng. or weakened by other pathogens or stress factors. Annual losses in timber are estimated to be more than half a million cubic metres as a result of mortality, growth repression and regeneration delay (Taylor 1986). Armillaria root disease is particularly problematic in new plantations where the fungus is carried over to the next rotation in colonized stump and root systems. Cumulative mortality in juvenile stands can be as much as 20% by age 20 years (Morrison & Pellow 1994) and numerous small disease centres may coalesce to form unstocked or understocked openings in the stand.

Few options are available to mitigate potential losses due to Armillaria root disease. Mechanical removal of stumps is a very effective means of reducing the amount of woody inoculum that would otherwise be available to the fungus and minimizing the extent of Armillaria-caused mortality in the regenerating stand. Another less intrusive

option is to plant coniferous species that have a low susceptibility to killing by *A. ostoyae*. The use of resistant species in Armillaria-infested stands is economically practicable so long as the resistant hosts are a high-value merchantable species and well adapted to the particular site conditions. There are no woody hosts that show complete immunity to Armillaria. It is generally accepted that all conifers less than 15-years of age are highly susceptible to killing by Armillaria root disease (Morrison *et al*. 1992). However, with the exception of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and western larch (*Larix occidentalis* Nutt.) (Robinson and Morrison 2001), very little is known about the relative susceptibility of conifers in B.C.

Western redcedar (*Thuja plicata* Donn ex D. Don) and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) are two conifers that regenerate naturally in the ICH zone, often comprising a significant component as seral stands develop and becoming the climax species in the mature overstory (Lloyd *et al*. 1990). However, the mechanisms by which both species are able to tolerate *Armillaria* and survive in the presence of inoculum are not well understood. Traditionally, western hemlock and western redcedar are not planted, with the possible exception of the latter in wetter microsites within a cutblock. Both species hold high value in terms of usage in the forest industry. Western redcedar accounts for approximately 7% of the total lumber production in B.C., while hemlock/*Abies* spp. account for more than 12% (Council of B.C. Forest Industries 2000). Western redcedar heartwood in particular contains several potent, antimicrobial toxins and is well known for its natural durability and decay resistance for exterior uses of timber which make it a desirable species for high-yield forest management. Other species also contain various toxins in the heartwood. However, such chemical barriers in the heartwood would not likely influence infection and spread by *Armillaria* in the bark, vascular cambium and outer sapwood tissue. There is a paucity of information concerning the ability of certain conifers, including western redcedar and western hemlock, to activate defense mechanisms in those tissues that would enable them to respond to and halt infection by *A. ostoyae*. Knowledge of precisely which mechanisms are effective against the fungus and other host factors that might influence the expression

of resistance would provide the necessary information to support the use of either species for regeneration on sites where *Armillaria* may be of concern.

The aim of this study was to compare the host response to infection by *A. ostoyae* in the roots of western redcedar and western hemlock, at the macroscopic and microscopic level, specifically targeting the anatomical changes in cells and tissues that lead to necrophylactic periderm formation in the inner bark and barrier zone formation associated with the compartmentalization of infected woody tissue. Douglas-fir was included in this study as a susceptible control, providing a benchmark to which to compare host reactions of the other species. It was also the intent of this study to determine whether the pattern of basal lesion development and mortality in mixed stands of the three species was consistent with the efficiency of natural resistance mechanisms operating in the roots of these species.

1.2   *ARMILLARIA* TAXOMONY AND SPECIES IDENTIFICATION

In early literature, there was much confusion surrounding the nature of host-Armillaria interactions because the pathogen used to be considered a single variable or polymorphic species, *Armillaria mellea* (Vahl ex Fr.) Kummer (Watling 1982). Hintikka (1973) demonstrated that Armillaria has a bifactorial, heterothallic mating system in which two haploid monokaryons with compatible mating-type alleles would anastomose to form a secondary diploid mycelium. His work enabled subsequent studies to be undertaken that conclusively differentiated "biological species" of *Armillaria* in both Europe and North America (Korhonen 1978, Anderson and Ullrich 1979). Anderson *et al.* (1980) subsequently observed that certain species from Europe were partially compatible with those from North America. Since the division of *Armillaria mellea sensu lato* into a number of distinct biological species, a number of studies of pathogenicity and virulence on species of Armillaria have been carried out which have helped explain much of the variation in host-pathogen interactions and damage described for different host species

worldwide. Watling *et al.* (1991) suggests the genus *Armillaria* contains about 40 species.

Somatic incompatibility mating tests are commonly performed using haploid tester strains (monospore isolates). Single spore cultures from *Armillaria* mushrooms typically have white or light brown aerial mycelium whereas cultures from basidiocarps, rhizomorphs and mycelial fans generally appear to be dark, flat, crustose and lacking aerial mycelium (Hintikka 1973). The pairing reaction of single spore isolates follow a tetrapolar pattern which suggests nuclei of crustose mycelia are diploid rather than dikaryotic (Hintikka 1973). Two diploid genets of the same species paired in a Petri dish will form a nearly hyphae-free gap between opposing mycelia, whereas the same genotypes will show complete intermingling or fusion of opposing mycelia to form a single homogenous colony (compatible reaction). Failure of mycelia to grow together indicates dissimilar genotypes (incompatible reaction) and a dark demarcation line will develop in the growth medium between the two opposing mycelial colonies (Korhonen 1978, Anderson and Ullrich 1979, Mallet *et al.* 1989). Vegetative mycelium of *Armillaria* species can sometimes discolour the media dark brown which makes judging reactions of opposing mycelia difficult. Furthermore, *Armillaria* isolated from lesions is frequently cultured on malt extract agar amended with Benomyl, in order to inhibit common contaminants (Worrall 1991). However, this can sometimes induce somatic haploidization of diploid mycelia, resulting in fluffy segregants of mycelial cultures and this can further increase difficulties with species identification (Guillaumin *et al.* 1991).

More recently, molecular techniques using polymerase chain reaction (PCR) focussed on restriction site differences within the first and second ribosomal intergenic spacer region have proven to be a fast, accurate, and relatively inexpensive technique for confirming species identification (Harrington and Wingfield 1995; White *et al.* 1998). Random amplified polymorphic DNA (RAPD) analysis have been used to describe the population structure of *Armillaria* species in the Interior forests of British Columbia (Dettman and van der Kamp 2001a; Dettman and van der Kamp 2001b).

1.3   *ARMILLARIA* SPECIES IN BRITISH COLUMBIA

Using interfertility tests of vegetative mycelia, Morrison *et al.* (1985) identified six intersterile groups of *Armillaria* in British Columbia originally designated A-F and later converted to the Roman numeral system for classifying intersterility groups as North American Biological Species (NABS) according to the system used by Anderson and Ullrich (1979). *Armillaria ostoyae* (NABS I), and *Armillaria sinapina* Bérubé & Dessureault (NABS V) have widespread distribution throughout the coastal and interior forest regions of B.C. The range of *A. ostoyae* is from 49° to 53°N latitude and it is found primarily attacking conifer species but can also occur on hardwoods (Morrison *et al.* 1985; Morrison *et al.* 1992). The range of *A. sinapina* is similar to that of *A. ostoyae* but extends farther north up to about 57°N latitude, and is primarily found on both dead conifer and hardwood trees and stumps (Morrison *et al.* 1985, Dettman and van der Kamp 2001a).

Pathogenicity tests have shown that *A. ostoyae* is highly pathogenic (Rishbeth 1982, (Morrison & Pellow 2002). *A. ostoyae* rhizomorphs possess a dichotomous branching habit whereas those of *A. sinapina* exhibit monopodial-type branching. Morrison (2004) suggested that *Armillaria* species possessing dichotomous branching tend to be more pathogenic than those producing monopodial branching.

Armillaria gallica Marxmüller & Romagn. (NABS VII) and *Armillaria nabsnona* Volk & Burdsall (NABS IX) have been found on living and dead hardwoods in the Coastal-Douglas-Fir (CDF) biogeoclimatic zone in southwestern B.C. *Armillaria cepistipes* Velenovsky (NABS XI) has been collected only twice from broadleaved hosts from two geographically very distant sites, Hope and Stewart, B.C. Another species, as yet unnamed and referred to as NABS X, was collected from a relatively small area in southeastern B.C. on coniferous and hardwood hosts (Morrison *et al.* 1985).

1.4   INOCULUM AND RHIZOMORPHS

Armillaria spp. have a very wide host range, attacking many conifer and broad-leaved trees, shrubs, and herb species (Raabe 1962). Armillaria may survive for decades in colonized stump and root systems, depending on the size of the substrate. Although tree stumps occasionally become infected by basidiospores produced by the fruiting bodies of some *Armillaria* species (Rishbeth 1985, Legrand *et al.* 1996, Hood *et. al* 2002), spore infection by *A. ostoyae* is thought to be a very rare event because genets tend to occupy large areas (Anderson *et al.* 1979, Ferguson *et al.* 2003). For this reason, the natural infection pathway(s) of *A. ostoyae* have not been identified.

Vegetative growth is the primary mode of spread at root contacts or through the soil via rhizomorphs. Rhizomorphs are highly differentiated vegetative organs that enable the fungus to grow out of a suitable nutrition base and through the soil in search of new substrates to colonize, thereby increasing the infective potential of the pathogen (Garraway *et al.* 1991). Inoculum potential is defined as "the energy of growth of a parasite available for infection at the surface of the host organ to be infected" (Garrett 1970), and is influenced by the size of the inoculum source and species, time since colonization, the distance between the inoculum and the host and any environmental factors affecting growth of the fungus (Redfern and Filip 1991). Inoculum potential is maximized where healthy roots and inoculum (stumps) are in contact. Where infection occurs primarily at root contact, the distance from the inoculum source becomes less important in estimating inoculum potential than the size of the inoculum itself. Conversely, when infection occurs primarily by rhizomorphs, the inoculum potential of the fungus decreases with increasing distance between the inoculum source and the host (Redfern and Filip 1991). Hence, when large gaps are bridged by rhizomorphs, such great distances may affect the infective capacity of the fungus and consequently the host response to infection at the point of penetration on the root.

Although rhizomorphs are largely dependent on a supply of nutrients translocated from the food base to their growing tips, they are also able to absorb ions and oxygen through

the unpigmented, apical region of the growing tip. Morrison (1975) found that ammonium ion uptake was not translocated to the food source suggesting that nutrient supplies available to rhizomorphs from a food base may also be supplemented by uptake from the soil.

The histology of rhizomorphs was studied in detail by Schmid and Liese (1970) and Motta (1969). Their structure consists of well-defined cellular regions that include a melanized and densely packed outer cortex that surrounds a subcortical layer of fungal cells and the medulla which consists of a loose mesh of wide-diameter hyphae that eventually form a central canal (Garraway et al. 1991). In older rhizomorphs, hyphae with thicker cell walls fill in the central canal of the medulla. Schmid and Liese (1970) suggest that the central canal of the medulla is not hollow, but rather comprised of fungal hyphae that are loosely arranged. The primary meristem is located in the apical region of the rhizomorph which is well-endowed with dense cytoplasm, protein and nucleic acids (Motta 1969). Secondary meristems associated with lateral growth are located in the subcortical layer distal to the apical center (Motta 1969). The medulla region is responsible for the transport of water and nutrients (Jennings 1984) and oxygen (Smith and Griffin 1971). A gelatinous sheet and a mucilaginous layer surrounds the rhizomorph at its apex and its melanized rind helps protect the fungus against dessication or damaging external agents in the soil, or both (Garraway et al. 1991).

Early mycologists in the mid-nineteenth century like Schmitz (1848), as cited in Garraway et al. (1991), did not associate rhizomorphs found beneath the bark and in the soil with Armillaria root disease causing mortality on coniferous and broadleaved hosts. First described as *Rhizomorpha fragilis* Roth., the fungus was later divided into two subforms, *R. subterranean* to describe the melanized strands of hyphae growing through the soil and *R. subcorticalis* to describe the form that produces white mycelial fans in the bark and cambial tissue (Garraway et al. 1991). Hartig (1874) later resolved this issue by confirming the association of rhizomorphs in the soil with that of the Honey Fungus *Agaricus melleus* L., now known as *Armillaria*.

1.5   INFECTION BIOLOGY

The external tissues of woody plants, comprised of periderm and rhytidome, provide an important protection barrier to underlying tissues from microbial invasion. However, it offers little resistance to penetration by *Armillaria* species. The infection process by rhizomorphs has been described in some detail for both conifers and hardwoods (Day 1927, Thomas 1934, Woeste 1956, Rykowski 1975, 1980). Rhizomorphs may become firmly attached to the outer bark at several points along the surface of the root by hardening of the mucilaginous substance surrounding the growing tip (Day 1927, Thomas 1934). Next, a lateral branch or mycelial wedge, still connected to the rhizomorph, penetrates the rhytidome as a single unit rather than individual hyphae (Thomas 1934). The ability of rhizomorphs to penetrate the rhytidome and inner bark depends on the enzymatic capacity of the fungus (Rykowski 1975). The fungus is capable of producing suberinase, a suberin-degrading enzyme, to facilitate the destruction and dissolution of suberized walls in the outer phellem layers (Swift 1965, Zimmermann and Seemüller 1984). Penetration of the outer bark is similar among susceptible and resistant hosts and involves both mechanical and enzymatic processes (Rykowski 1975, 1980). However, the ability of some *Armillaria* species to penetrate host tissue, including those that are typically 'weak parasites', may also depend on the inoculum potential of the fungus.

Once the fungus has penetrated the periderm, it causes necrosis of the living bark and cambial tissue. Enzymatic degradation of cells is caused by extracellular, phenol-oxidizing enzymes produced by the fungus such as peroxidase, tyrosinase and laccase (Garraway *et al.* 1991). Browning of phloem parenchyma cells occurs ahead of mycelial colonization. Such tissue typically contains high levels of phenol oxidases compared with adjacent healthy tissue (Wargo 1984).

Cell hypertrophy was observed in phloem tissue located immediately underneath a penetrating rhizomorph of *A. mellea sensu lato* (Day 1927). In almost all vigorous hosts, one or more periderms were formed in an attempt to contain the infection in the bark. However, at times no periderms were formed and the fungus appeared to be advancing freely in the host tissue without any host reaction (Day 1927). Thomas (1934) reported

that in black walnut (*Juglans nigra* L.), which he considered to be moderately resistant to attack by *Armillaria*, a high proportion of infections was apparently halted and walled off by periderm tissue. Day (1927) and Thomas (1934) noted that in susceptible hosts, the fungus spreads rapidly in the bark and cambial invasion often resulted in girdling of the root and the advance of the fungus towards the root collar. In resistant roots, cankers often formed around sites of penetration and the spread of the fungus was checked by the formation of callus at the edge of the lesion (Thomas 1934, Rykowski 1975).

1.6   HOST RESPONSE TO INFECTION

Rishbeth (1985) stated that the most important host responses occur after the bark has been infected by *Armillaria mellea sensu stricto*. On plum rootstocks, penetration of *A. mellea* (*sensu stricto*) was similar for both susceptible and resistant rootstocks and resistance was mainly due to post-infection reactions (Guillaumin *et al.* 1989). Day (1927) observed *A. mellea sensu lato* to be contained by a new cork layer that was formed deeper in the cortex following invasion in the roots of a Corsican pine (*Pinus nigra ssp. laricio* Maire) tree. Mullick (1977) reported that host reactions in the living bark induced in response to initial penetration by a pathogen will determine the outcome of a particular reaction as being either susceptible or resistant. Host responses in tissues affected by Armillaria root disease fall into four categories: exudate production, meristematic activity, biochemical interaction (Morrison *et al.* 1991), and compartmentalization (Shigo and Tippett 1981).

1.6.1   EXUDATE PRODUCTION

Conifer hosts will exude resin in response to mechanical injury, pathogenic invasion, or both (Buckland 1953, Rishbeth 1972, Redfern 1978). Increased resin synthesis in the roots of Corsican pine was considered to be an important factor in resistance of pines against further ingress of *Heterobasidion annosum* (Fr.) Bref (Prior 1975, 1976). Rishbeth (1972) reported that the volatile turpentine component of resin produced by Scots pine (*Pinus sylvestris* L.) partially inhibited growth of *A. mellea sensu lato* in

culture. Rykowski (1975) also observed that resin produced by conifers in wood and callus limited further spread of *A. mellea* (= *A. ostoyae*).

Vigorous resin flow in roots may become toxic to the fungus and/or play some role in terms of forcing infected tissue away from the root wood (Rishbeth 1982, 1985). Prior (1975) suggested that the main effect of oleoresin on the fungus is by physical blockage of the tracheids, rather than direct toxicity. Robinson (1997) observed that copious resin production formed in the roots of Douglas-fir and western larch help facilitate sloughing of infected tissue away from the root surface.

Broadleaved hosts infected with *Armillaria* do not produce resin but instead, exude a sticky plant exudate called gum which is deposited in xylem vessels (Thomas 1934, Rishbeth 1985). Eucalyptus trees showing resistance against *Armillaria luteobubalina* Watling & Kile often exhibited kino exudation and callusing around the base of infected trees (Podger *et al.* 1978, Kile 1980, Shearer and Tippett 1988).

1.6.2. MERISTEMATIC ACTIVITY

Meristematic activity involves the renewal of tissues affected (killed) by the fungus. At least three categories can be distinguished, namely phellogen renewal and the formation of a secondary periderm in the bark (Day 1927, Thomas 1934, Mullick and Jensen 1973), the formation of callus tissue and new vascular cambium (Sharples and Gunnery 1933, Shigo and Tippett 1981, Dujesiefken *et al.* 2001), and adventitious rooting (Rishbeth 1972, 1985). The regeneration of these tissues following invasion by the fungus serves to renew and replace lateral meristems, prevent desiccation and maintain gas exchange, and restrict the ingress of plant pathogens.

Early investigations by Thomas (1934) give complete and detailed descriptions of resistance reactions in the bark following penetration by *A. mellea sensu lato*. In resistant hosts, a layer of cork was often formed which restricted further spread of the fungus in the root while in susceptible hosts, the fungus often breached new cork layers (Thomas 1934).

Mullick (1977) conducted a series of developmental studies on wound healing in bark and was the first to term this new periderm barrier as 'necrophylactic periderm' (Mullick and Jensen 1973). He considered the process of necrophylactic periderm formation to be non-specific, induced in response to either biotic or abiotic injury and/or simply age, and triggered whenever the phellogen is rendered non-functional (Mullick 1977). Mullick's (1977) model for non-specific wound healing in bark is presented in Figure 1.1.

NP - NECROPHYLACTIC PERIDERM

ep - EXOPHYLACTIC PERIDERM

VC(n) - POSITION OF VASCULAR CAMBIUM AT TIME OF PHELLOGEN RESTORATION

VC(i) - POSITION OF VASCULAR CAMBIUM AT TIME OF INJURY

- CONDUCTING SAPWOOD FORMED AFTER INJURY

- CONDUCTING SAPWOOD EXTANT AT TIME OF INJURY

- TRANSFORMED CAMBIAL-PHLOIC ZONE

- ZONE OF NEWLY RESTORED VC

- ZONE OF NON-CONDUCTING SAPWOOD
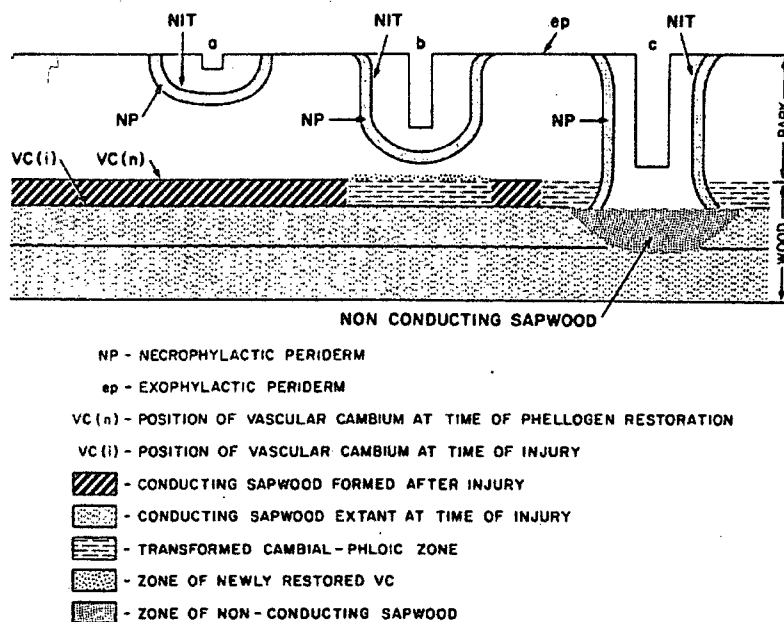
Fig. 1.1. Diagrammatic views of the anatomical model for non-specific defense mechanisms that follow (a) penetration of the bark surface, (b) penetration of the vascular cambium, and (c) penetration of the sapwood (From Mullick 1977).

The first process shown in Figure 1.1 (a) is that of phellogen restoration which is triggered whenever the phellogen becomes non-functional (Mullick 1977). The necrophylactic periderm (NP) that establishes is preceded by a layer of non-suberized impervious tissue (NIT). Periderm formation in response to injury or infection and various stages of development involved in phellogen restoration is discussed further in Chapter 2. Shallow injury to the preexisting periderm and the first few layers of living bark cells trigger only the process of phellogen restoration.

In Figure 1.1 (b), Mullick (1977) illustrates vascular cambium restoration when penetration or injury reaches a certain depth of the bark. Thus, deep injuries to the bark may cause a disruption in the normal cambial activity without any direct injury to the cambium itself. The vascular cambium directly underneath the injury stops producing normal xylem derivatives while xylary and phloic derivatives continue to be produced on either side of the injured tissue (Mullick 1977). Mullick (1977) describes the deranged vascular cambium as a modified cambial-phloic zone which is characterized by enlarged cells. Presumably, this modified cambial-phloic zone is callus (isodiametric, undifferentiated tissue). Subsequently, xylem-like cells appear sporadically at the external boundary of the transformed cambial-phloic zone and a new vascular cambium develops between the sporadic xylem-like cells and the enlarge phloic cells. The newly restored vascular cambium will continue to produce normal xylem and phloem derivatives (Mullick 1977). NIT and NP are essential to completion of the second process and a new vascular cambium is restored (Mullick 1977).

Figure 1.1 (c) illustrates blockage of sapwood conduction following even deeper injuries to the bark which Mullick (1977) characterizes as non-conductive discoloured wood. Air is prevented from spreading through the xylem proximal and distal to the point of injury because of tracheid occlusion. This blockage or barrier that forms is analgous with Wall 1 of the CODIT model (Shigo and Marx 1977). In addition to NP formation in the bark, the living vascular cambium at the periphery of the wound forms callus. A new phellogen differentiates at the outer part of the callus tissue and meristematic tissue forms as a replacement of the vascular cambium in the inner part of the callus (Mullick 1977).

Several studies have since shown necrophylactic periderm to be involved in resistant reactions in woody plants in response to mechanical injury or pathogenic invasion (Blanchette and Biggs 1992, Wahlström and Johansson 1992, Robinson and Morrison 2001).

The formation of new roots, including adventitious roots, is important in the process of wound healing largely because the survival of a tree depends, to some extent, upon the

balance between killing and regeneration of roots (Rishbeth 1985). Adventitious rooting occurs primarily in situations where a root has been girdled to restore the critical functions of absorption through the initiation of new root tips.

The production of callus tissue and the regeneration of a new vascular cambium within the callus have been described for several hosts following direct injury to the cambium or the xylem (Sharples and Gunnery 1933, Warren-Wilson and Warren-Wilson 1960, Oven and Torelli 1999, Dujesiefken *et al.* 2001). Many of the tissues involved in callus formation are also involved in barrier zone formation during the process of compartmentalization and will be discussed in more detail in the following section. The contribution of different tissues to the formation of callus differs between gymnosperms and angiosperms. In angiosperms, callus originates mainly by the living vascular ray cells in addition to the parenchyma of the phloem and xylem (Sharples and Gunnery 1933). Novitskaya (1998) showed that in European white birch (*Betula pendula* Roth) callus originated from proliferation of xylem mother cells on the exposed surface of the wood. Dujesiefken *et al.* (2001) also demonstrated that in wounded hardwoods callus can form from tissue within the wound area, but only when the xylem remains undamaged. Oven and Torelli (1999) described a similar sequence of histological changes in the cambial zone of several different conifer hosts following wounding. However, the authors noted callus formation to occur as a result of progressive hypertrophy and hyperplasia of cambial cells as well as phloem parenchyma cells. Increased frequency of transverse divisions was observed tangentially in the fusiform cells of the cambial zone (Oven and Torelli 1999).

The rate of callus production following wounding varies by tree species (Gallagher and Sydnor 1983, Oven and Torelli 1999), but may also vary depending on the size of the wound, the season of wounding, the location of the wound on the tree (Wensley 1966), and tree vigour (Buckland 1953). van der Kamp and Hood (2002) showed that *Armillaria* species can invade and girdle trees near the root collar, killing the vascular cambium and phloem in a single dormant season when the tree is unable to compartmentalize the infection. Similarly, Robinson *et al.* (2004a) showed that roots

were incapable of initiating such defense responses immediately following wounding during the winter. Buckland (1953) observed Douglas-fir trees exhibiting good vigor frequently checked the advance of *A. mellea* (= *A. ostoyae*) by laying down a resin barrier and callus around the infected tissue, forming a latent or dormant canker. Johnson *et al.* (1972) suggested that the ability of Douglas-fir infected by *A. mellea* (= *A. ostoyae*) to form callus at lesions increases between the age of 5 and 20 years. The ability of trees to form callus will prevent mortality of the host tree (Johnson *et al.* 1972). Vigorous trees typically show more resinosus and callus formation around lesions than less vigorous trees. Kile (1981) reported that inverted V-shaped lesions with callus ingrowth at the margin on several Eucalypts hosts prevented the girdling of stems by *A. luteobubalina*.

1.6.3. BIOCHEMICAL DEFENSE

In as much as the physical, structural barriers may slow the infection process on roots, the underlying mechanisms must also involve chemical changes in host tissue in the form of mobilized constituents in response to invasion by the fungus (Garraway *et al.* 1991). In his early investigations of the infection biology of *A. mellea*, Thomas (1934) suggested that newly formed periderms confine the fungus to a lesion in the bark only after other means of resistance, presumably chemical, halted the fungus.

Many trees produce a variety of inhibitory and toxic compounds in the bark and in the wood which endow them with various degrees of resistance against an invading pathogen (Asiegbu *et al.* 1998, Biggs *et al.* 1984, Lindberg 1991, Wargo 1984, Woodward and Pearce 1988). Phenolic compounds play an important role in both constitutive defenses, those that occur prior to fungal invasion, and inducible defenses following initial invasion by the fungus. Some host enzymes can detoxify pathogen toxins enabling the host to ward off potential infections. Moreover, some compounds which individually do not offer much disease resistance may act synergistically to provide resistance against an invading pathogen (Kozlowski 1969, Lindberg *et al.* 1992). Vance and Garraway (1973) found that phenols that accumulate in *Armillaria* thalli can inhibit mycelial growth and rhizomorph development *in vitro*. Phenols may also inhibit enzymes which could be

important in mycelial growth and rhizomorph morphogenesis (Vance and Garraway 1973).

Phlobaphenes are pigmented phenolics that typically accumulate in the phellem of periderm tissue and consist of mixtures of several distinct compounds (Mullick 1977). Using thin-layer chromatography, Mullick (1969a) concluded that the pigments belonged to a new class of non-anthocyanic reddish-purple compounds, although a minor portion of the pigments consisted of anthocyanidin occurring as such in nature (Mullick 1969b, 1969c, 1969d). *Thuja plicata* was reported to have at least eight distinct pigments and *Tsuga heterophylla* at least nine; the nature of pigments varied between species (Mullick 1969a), although cyanidin and pelargonidin were common to both (Mullick 1969b). Guillaumin *et al*. (1989) reported growth of *A. mellea* in the bark and sapwood of *Prunus* species was considerably less in resistant rootstocks. This slow growth was associated with pink or purple discoloration of the tissue surrounding the lesion. The authors attributed this resistance to *de novo* synthesis of anthocyanic compounds (Guillaumin *et al*. 1989). This type of active defense mechanism may inhibit the spread of *Armillaria* long enough so that necrophylactic periderm formation can be completed.

Phellem cells of *Picea abies* (L.) Karst. root bark infected with *Heterobasidion annosum*, contained granular material (most likely phenols) that varied from sparse to dense masses filling the whole cell (Heneen *et al*. 1994b). Hyphae penetrating phellem cells were sometimes seen embedded in dense granular phenolic compounds that entrapped and immobilized penetrating hyphae suggesting a possible toxic effect on the fungus (Heneen *et al*. 1994a, 1994b). Similar observations were recorded for Scots pine infected with *A. ostoyae*, where termination and dissolution of single hyphae caused by fungitoxic compounds or lytic enzymes occurred as a post-infection host response (Wahlström and Johansson 1992). The inoculation technique described by Wahlström and Johansson (1992) involved wounding of the phellogen followed by inoculation with a small strip of infected bark directly into the wound which would certainly have facilitated penetration in which single hyphae may been observed in tissue samples. However, observations of natural infection by *A. ostoyae* on conifers in British Columbia suggests the phenomenon

of penetration by single hyphae does not occur. Instead, the fungus penetrates as a larger unit (i.e. rhizomorph) similar to that described by Day (1927), Thomas (1934), and Rykowski (1980).

Western redcedar bark contains many complex substances including tannins, phlobaphenes, vanillin, catechin and fatty acids (Barton and MacDonald 1971). Chemical analysis of the bark showed that it contains 31% lignin, not all that different from the lignin content of the wood, and the extracts are quite acidic (Gonzalez 1997). Lignins are generally resistant to microbial attack. Increased lignification of cells may increase resistance to compressive forces because cell walls are more resistant to mechanical penetration (Asigbu et al. 1998). Lignification may also restrict diffusion of enzymes and toxins from pathogen to host and can equally restrict availability of water and nutrients from host to fungus (Asigbu et al. 1998).

Defense reactions involving increased synthesis of proteins and enzymes may disrupt fungal growth by causing cell lysis of hyphal tips. Wargo (1975) found chitinase and β-1,3-glucanase in the phloem of Acer saccharum Marsh. and several Quercus spp. and suggested that these enzymes could lyse hyphal walls of A. mellea. Robinson et al. (2000) also found higher concentrations of an endochitinase-like protein in the phloem of Douglas-fir trees infected with A. ostoyae and P. weirii (= P. sulphurascens). The presence of such enzymes, induced following pathogenic invasion, may act to inhibit fungal growth and may be part of a multi-stage system of defense. Pathogens, after triggering the process of phellogen renewal, interact not only with chemicals present in the normal bark with also with new chemicals produced during the course of cellular dedifferentiation (Mullick 1977).

1.6.4. COMPARTMENTALIZATION OF DECAY IN TREES (CODIT)

The CODIT model describes the defense response of trees following injury to cambium or sapwood, or both, that results in the formation of boundaries which resist the spread of decay within the xylem. Hence, compartmentalization, which occurs in both stems and roots, will conserve the mechanical support system of the tree (Shigo and Marx 1977).

The CODIT model has two parts. Part I consists of 3 walls that surround and compartmentalize infected tissue in all planes. The formation of each boundary (wall) involves chemical changes in the living parenchyma cells of the sapwood. Wall 1 forms the upper and lower transverse boundaries of the compartment and serves to protect the water-conducting tracheids above and below the injured area from invading air (Shigo 1984). This is achieved by tracheid occlusion with deposits of polyphenols and border pit aspiration. Wall 2 lies in a tangential plane and resists inward (radial) spread by utilizing the last cells of each annual growth ring. These cells are usually thick-walled and heavily lignified. Wall 3 resists tangential spread and utilizes the xylem ray cells. Walls 1 to 3 represent the path of least resistance, with Wall 1 being the weakest and Wall 3 being moderately strong (Shigo and Marx 1977, Shigo 1984). The strength of Wall 2 depends on the resistance of the latewood tracheids, particularly the thickness of cell walls and the degree of lignification. The relative holding strength of each wall to fungal colonization is variable and may depend to a large extent on the host species and the overall vigour of the tree.

Part II of the CODIT model consists of a fourth wall, also known as the "barrier zone" (Tippett and Shigo 1980, 1981). A barrier zone is comprised of a unique layer of cells formed by the uninjured cambium that restricts the fungus to xylem formed prior to injury (Shigo and Tippett 1981). The barrier zone in some conifers may be comprised largely of distorted tracheids, increased number of axial parenchyma containing polyphenols or traumatic resin ducts or both (Tippett and Shigo 1980, 1981). Traumatic resin ducts may be formed around a wound in response to injury in all conifers, including those that do not form resin ducts in normal xylem. Such tissues comprising the barrier zone were effective in restricting A. mellea to wood formed prior to infection (Tippett and Shigo 1981, Shigo and Tippett 1981).

The barrier zone is the strongest of all four walls and results in the most effective compartmentalization. Barrier zones may be quite large, encircling the entire stem or extending for only a limited distance tangentially and longitudinally from the wounded area. The ability of barrier zone to resist spread of a pathogen will differ among and

within a host species and depends to some extent on the physiological activity of parenchyma cells and the availability and mobilization of stored material (Liese and Dujesiefken 1996). Hence, vigorous trees may react and compartmentalize quickly and more efficiently than trees with low vigour.

The position of the barrier zone in the wood will vary depending on the time of year in which the vascular cambium was injured. Since phellogen and vascular cambium activity ceases in the winter as a result of tree dormancy, host reactions involving barrier zone formation would not be expected to take place until the tree resumes growth in the spring. Bannan (1936) showed that following injuries to stems of *Tsuga canadensis* (L.) Carr. during tree dormancy, the vascular cambium showed no response until the beginning of the next growing season and the newly formed cells produced at the beginning of the annual ring were traumatic resin canals. Shigo and Tippett (1981) also suggested that the position of the barrier zone in the earliest portion of the growth ring indicates that spread of *A. mellea* occurred during a period of dormancy or soon after the onset of growth.

Once formed, the barrier zone comprising wall 4 of the CODIT model remains stationary whereas walls 1, 2, and 3 may adjust to an expanding column of decay. Liese and Dujesiefken (1996) suggest that a barrier zone should not be restricted to the small layer of cells formed immediately after wounding. In conifer roots, the natural alignment of the wood promotes more spread of fungi in the axial direction through the cell lumina of tracheids. As a result, wall boundaries may extend as decay spreads either distal or proximal from its original point of infection. Tippett *et al.* (1982) suggested that barrier zones may also actively extend as pathogens spread from a site of initial infection on a root up towards the root collar. This was evidenced by the presence of concentric tangential bands of traumatic resin ducts produced in successive growth rings (Tippett *et al.* 1982).

1.7. HOST SUSCEPTIBILITY

The extent of damage on host species depends not only on the host (relative susceptibility, the size, age, and vigour of the tree), but also on the pathogen (pathogenicity and virulence of the species/isolate and inoculum potential), and the influence of the environment (soil moisture, stress factors such as drought, and the season of injury).

The relative resistance and susceptibility of several conifers to killing by *A. ostoyae* in British Columbia has been compared (Morrison 1981, Morrison *et al.* 1992). Inoculation experiments on juvenile conifers in the northwestern United States (Entry *et al.* 1992) and observations on naturally infected roots of saplings, juvenile and mature trees from B.C. (Robinson and Morrison 2001, Morrison 2000, Robinson 1997, Morrison *et al.* 1992) show Douglas-fir to be highly susceptible to killing by *A. ostoyae*. Reports on host susceptibility for other conifers including western redcedar and western hemlock are few (Morrison *et al.* 2000, Koenigs 1969), although Morrison *et al.* (1992) considered western redcedar and western hemlock to have similar susceptibility to killing by *A. ostoyae*. Indirect evidence from previous studies (Morrison *et al.* 2000, DeLong 1997) suggests distinct differences in host susceptibility, particularly where western redcedar is concerned. Clearly, more information is needed to elucidate the relative susceptibility of these conifers to killing by *A. ostoyae*.

In British Columbia, information pertaining to host resistance in juvenile conifers to Armillaria root disease is generally restricted to Douglas-fir and western larch (Robinson 1997, Robinson and Morrison 2001). Host response, and the type of damage resulting from infection by *A. ostoyae* varies by host species and also with tree age (Robinson and Morrison 2001). Younger trees are generally killed more quickly than older trees (Morrison *et al.* 1992). Tree mortality in the new plantations usually begins about 5-7 years after stand establishment and mortality can continue throughout a rotation with mortality rates of approximately 1-2% per annum (Morrison and Pellow 1994.) A decline in the initial flush of mortality will be observed usually starting around the age of 15 years which is usually attributed to increasing host resistance and a decline in

inoculum potential in stumps and roots left over from the previous stand. Early expression of defense responses and the ability of host species to successfully contain the infection is a characteristic feature of trees that show greater resistance against the fungus.

Rykowski (1980) characterized susceptible roots as those in which the host cells become brown and filled with a mass of grainy structures as a result of pathological changes in the living protoplasm while under the influence of a penetrating mycelium. Susceptible roots did not frequently initiate defense reactions and necrosis often advanced to the cambium and xylem tissue (Rykowski 1980). On the other hand, resistant roots generally underwent cytological changes in the affected tissue to form a demarcation zone in the form of a secondary cork layer and successfully isolated mycelium from adjacent healthy tissue (Rykowski 1980). The author also noted sloughing of infected root tissue from the surface of the root following successful periderm formation. Following invasion of the cambium, resistant roots frequently formed a resin barrier and callus around the margin of the lesion (Rykowski 1980).

The location of an infection on the root system is an important factor in host resistance and disease development (Morrison *et al.* 1991). Morrison (1972) found that on Corsican pine, inoculations with isolates of *A. mellea sensu lato* at the root collar resulted in larger lesions than inoculations on lateral roots. Two root collar inoculations resulted in larger lesions than a single inoculation (Morrison 1972). Shaw (1980) noticed that infections at the root collar and/or taproot usually killed the host more rapidly than infections at other sites. This phenomenon was also noted on young Douglas-fir (Buckland 1953). However, the type of host response induced in terms of bark reactions may also vary depending on the location of the infection on the root. Robinson and Morrison (2001) found that the most effective necrophylactic periderm barriers were those that had multiple bands of thick and thin-walled phellem and these were most frequently formed on older, larger diameter roots.

Western larch shows considerable resistance to the fungus, but only after it reaches about 20-25 years of age (Robinson and Morrison 2001). Robinson (1997) noted that western larch was several times more successful at containing infections to a lesion on the root than Douglas-fir and this resistance was due in large part to the multiple bands of thick- and thin-walled phellem of periderms formed around infected bark tissue. However, in young regenerating stands in the southern Interior, mortality in western larch can be extremely high and may exceed that observed in Douglas-fir (Morrison *et al.* 1988, H. Merler, unpublished data). Fast growing, shade intolerant hosts, such as western larch may become infected earlier due to their more extensive root system and greater probability of contacting inoculum. In contrast, shade tolerant hosts such as western redcedar and western hemlock grow much more slowly in height and diameter as they are quickly overtopped by the more competitive, shade-intolerant seral species.

Indirect evidence from several studies conducted in the southern Interior of B.C. suggests that western redcedar may be more tolerant of *A. ostoyae* than other common conifers. Woods (1994) noted that in a juvenile mixed conifer stand of Douglas-fir, lodgepole pine (*Pinus cortorta* Dougl. ex Loud.), western hemlock and western redcedar, cedar was among the conifers least affected by *A. ostoyae*. In a conifer species trial near Hidden Lake, B.C., H. Merler (unpublished data) also noted that other conifers like Douglas-fir and western larch were more severely affected than western redcedar. In a 22-year-old Douglas-fir plantation infested with *A. ostoyae*, B.J. van der Kamp (unpublished data) noted western redcedar mortality to be approximately one tenth that of Douglas-fir. Morrison *et al.* (2000) reported a lower frequency of infection on cedar roots exposed to *A. ostoyae* compared to other conifers like western hemlock and Douglas-fir. Results from a 30+ year-old stumping trial showed mortality in western redcedar to be significantly lower than other planted conifers such as Douglas-fir and western larch (D.J. Morrison, unpublished data).

Relationships have been found between host vigour and the relative susceptibility of trees or stands to pathogen attack (Rosso and Hansen 1998). Host resistance is either directly or indirectly affected by the ability of host trees to synthesize biochemical defenses and initiate structural defense responses at the point of infection. Singh (1983) reported that

the roots of vigorous seedlings generally showed more resinosus and callus formation. The incidence and severity of disease in conifer seedlings was greater in non-vigorous seedlings than in vigorous seedlings, with the former becoming infected much earlier (Singh 1983). Buckland (1953) suggested that if host vigor was maintained, *A. mellea* (= *A. ostoyae*) would not be fatal to the host. Growth Efficiency (GE) has served as a measure of host vigour by defining thresholds above which resistance to insects and disease is conferred (Mainwaring and Maguire 2004). Lieutier and Ferrell (1988) used GE to quantify an index of the last annual ring or the last five annual rings. GE has been known to have a strong correlation with the parameters associated with tree health and with the trees' ability to synthesize resources towards defense in the event of an attack by some biotic factor (Lieutier and Ferrell 1988).

Bos and Parlevliet (1995) defined susceptible hosts as the relative inability to impede attack by a parasite and resistant hosts have the ability to hinder growth and development (i.e. rates of spread) within host tissue. Resistance in any particular host can be estimated by exposing a host species to a pathogen under uniform conditions and measuring disease severity. Day (1927) noted that cankers formed on roots infected with *A. mellea sensu lato*, a characteristic typical of resistant hosts, was commonly found on *T. plicata* and *T. heterophylla* and that many of these trees showed no signs of dying. In the study reported here, resistance was described by examining the frequency of successful resistance reactions induced following inoculation with pathogenic *A. ostoyae* and by comparing symptom development and species mortality rates on naturally infected individuals across several stands throughout the ICH zone in the southern Interior of B.C.

1.8   ARMILLARIA ROOT DISEASE IN THE SOUTHERN INTERIOR OF BRITISH COLUMBIA.

Armillaria is one of the most damaging disease agents affecting coniferous and deciduous trees and shrubs in both natural and artificial forested settings worldwide. *Armillaria* species may act as either primary pathogens parasitizing healthy, uninjured trees; as secondary pathogens invading trees that are stressed or weakened by other abiotic/biotic

means; or as saprophytes colonizing moribund woody substrates and utilizing them as a nutrient source for growth. Clearly, the ability of *Armillaria* species to assume multiple roles as they encounter hosts of different species, age and vigour, and their ability to survive in stumps and roots during stand renewal events has contributed to their widespread presence and longevity in forests.

A. ostoyae and *A. sinapina* are endemic throughout the southern interior of British Columbia. The species co-exist in the same stand, generally occupy the same ecological niche and can commonly be found on the same host tree. *A. ostoyae* may assume the role of both a primary and/or secondary pathogen as well as a saprophyte, although its competitive ability as a saprophyte appears to be somewhat limited since it must colonize host substrates prior to tree death in order to survive as long-lived inoculum (Garrett 1970). In contrast, *A. sinapina* may be considered a weak pathogen competitor that at times assumes the role of a secondary parasite attacking stressed trees, but typically colonizes hosts trees only after they have been killed by *A. ostoyae* or by some other agent. *A. sinapina* is distinguishable from *A. ostoyae* in the field based on the morphology of their respective rhizomorphs. *A. ostoyae* typically has dichotomous branching while *A. sinapina* has monopodial branching (Morrison 2004). According to Morrison (2004), *A. sinapina* was better able to colonized moribund tissue than *A. ostoyae*. Both *A. sinapina* and *A. ostoyae* have a reduced capacity to colonize substrates after they have been invaded by other micro-organisms (Morrison 2004).

Although it is generally considered a weak pathogen, *A. sinapina* is highly competitive and has evolved strategies for survival such as the ability to produce an extensive rhizomorph system that enables it to take advantage of position over *A. ostoyae* during the exploitation of new substrates. Redfern and Filip (1991) suggested that persistence of the more pathogenic *A. ostoyae* in forest stands was largely due to the longevity of inoculum in stumps, whereas the persistence of weakly pathogenic species, such as *A. sinapina*, may be aided by the behaviour of its extensive rhizomorph system and more frequent spore infection because as Dettman and van der Kamp (2001a) found, *A. sinapina* genets tend to be small and numerous. However, successful rhizomorph

penetration and colonization of woody substrates by weak pathogens such as *A. sinapina* may depend to a large extent on the inoculum potential of the fungus. An extensive rhizomorph system suggests lower inoculum potential at distant tips. Based on field observations, Morrison *et al.* (1992) suggested that *A. sinapina* was generally not capable of attacking healthy conifers. However, Dettman and van der Kamp (2001a) showed that *A. sinapina* was responsible for some conifer mortality in the central interior of B.C.

There is a paucity of information concerning the behaviour of *A. sinapina*, the mechanisms involved in the infection biology of this species, and whether host reactions induced by less pathogenic species of Armillaria, such as *A. sinapina* differ from those induced following invasion by *A. ostoyae*.

In the coastal forests of B.C., *A. ostoyae* behaves quite differently compared to its range in interior forests. On the coast, *A. ostoyae* causes mortality in young plantations but mortality generally ceases after about 20-years of age (Morrison 1981). Cruickshank *et al.* (1997) found that the bole volume and frequency of callus formation at *A. ostoyae* root lesions on juvenile Douglas-fir was significantly greater for coastal than interior trees. In the interior, *A. ostoyae* may continue to kill trees throughout a rotation (Morrison 1981), however, mortality among interior Douglas-fir trees appears to be somewhat varied between the Interior Douglas-fir (IDF) biogeoclimatic zone and the Interior Cedar-Hemlock (ICH) zone depending on the extent of disturbance and/or stress factors influencing the development of disease on those sites.

The ICH biogeoclimatic zone is the most productive zone in the interior forests of B.C. and supports the greatest diversity of tree species. The ICH has warm, dry summers and cold, wet winters with mean annual precipitation levels between 500-1200 mm, with 25-50% of that precipitation occurring as snowmelt (Meidinger and Pojar 1991). The structure and composition of forests located in the ICH zone have been influenced by a number of disturbance agents including harvesting, fire, insect outbreaks and pathogens such as root disease. Hence, Armillaria root disease likely plays a very important role in these forests influencing stand structure and affecting species composition. In fact, the

impact of *Armillaria* within this zone is higher than in any other zone in B.C., although the IDF, Montane Spruce (MS), and Engelmann Spruce-Subalpine Fir (ESSF) zones may also experience significant loss.

Most plantations located in the ICH will have some level of *A. ostoyae* damage. Morrison *et al.* (2001) found that up to 80% of trees in mature, undisturbed stands had *Armillaria*-caused lesions on their root systems and lacked aboveground symptoms of disease. The absence of disease symptoms in mature stands suggests that the host and fungus have reached an equilibrium whereby infections exist primarily as discrete lesions on their roots. Occasionally, the balance between this equilibrium is disrupted because of changing environmental or biological conditions which causes a fluctuation in disease levels in favour of the pathogen. The precise triggers causing the change from quiescent to active disease centres are not well understood, but may be related to the gradual increase in the number of lesions on a root system over time. In addition, stress caused by periods of drought or insect attack may play some role by reducing the overall vigor of the tree and its ability to marshal enough energy resources to the point of attack, quickly and efficiently. Following harvest, stumps and root systems remain alive for a year or two, surviving on stored carbohydrates during which time they will become colonized by *Armillaria* emerging from latent or dormant root lesions. The ability of the fungus to quickly colonize host substrates is an important characteristic of the pathogen enabling it to survive many decades in large colonized trees and ultimately increases the inoculum potential of the site, thereby increasing the risk of infection in residual and regeneration trees.

Forestry practices that create stumps will increase the amount of inoculum on a site. Brushing of hardwoods increases the proportion of stumps that the fungus can use as a food base. Woods (1994) found that mortality incidence in an unbrushed plantation was less than in a brushed plantation. Pre-commercial or commercial thinning (partial cutting) also increases inoculum in stumps. The probability of infection by *A. ostoyae* increases with tree size (Morrison 2000). However, this increase is substantially more pronounced in stands that have been selectively cut than in undisturbed stands and is

greatest in the dry climatic region (Morrison *et al.* 2001). Mortality in the understory residuals begins about 10 years following selective cutting and in the overstory and regeneration after 15 years (Morrison *et al.* 2001). Multiple stand entries maintain high fungal energy by continuously supplying the fungus with suitable substrates to utilize as a food base. The predominance of the fungus in such stands in all probability reduces site potential and productivity.

1.9   CONCLUSIONS

Armillaria root disease is a serious forest health concern in the southern interior of British Columbia. *A. ostoyae* poses a long-term threat to forest productivity and sustainable forest management because current silviculture practices increase the amount and potential of *Armillaria* inoculum and put regeneration or residual trees at risk of becoming infected by the fungus. The problem is exacerbated when susceptible host species like Douglas-fir are used for regenerating infested sites. Trees planted near stumps are killed fairly quickly. Expanding disease centres will occur when root contact occurs between adjacent and recently killed trees that have adequate inoculum potential to cause disease. This threat can be reduced by modifying silvicultural practices to minimize exposure of susceptible host species to *Armillaria* inoculum in managed second-growth stands. There is a need for more research on host-pathogen interactions and natural resistance mechanisms operating in trees that show effective resistance against Armillaria root disease (Wiensczyk 2001). Macroscopic observations of host response have been recorded for a variety of hosts, however, less is known of these interactions at the cellular level and the types of defense responses that are effective against further ingress of the fungus.

The first objective of this project was to describe host responses to invasion of the roots of western redcedar, western hemlock and Douglas-fir by *A. ostoyae* at the macro- and microscopic level, specifically targeting the anatomical changes in the bark tissue leading to necrophylactic periderm formation, as well as barrier zone formation associated with compartmentalization of infected woody tissue. This was achieved by examining host

reactions and lesion development on inoculated and naturally infected roots and the relationship between host response and root size, distance from the root collar, tree vigor and the timing of infection.

A second objective was to develop a better understanding of the behaviour and infection process of *A. sinapina* which frequently co-exists in the same stand with *A. ostoyae* (Morrison *et al.* 1985, Morrison *et al.* 1991). *A. sinapina* is generally considered to be a weak pathogen on deciduous hosts and behaves for the most part as a saprotroph on coniferous hosts. In the central Interior of B.C., Dettman and van der Kamp (2001a), however, showed that *A. sinapina* can be more pathogenic to coniferous hosts than previously reported. Clearly, more information is needed to elucidate the nature of pathogenicity of *A. sinapina* and whether there is any variation in susceptibility or host response to infection by *A. sinapina* compared to that of *A. ostoyae*.

The final objective was to demonstrate that resistance mechanisms operating in the roots of western redcedar, western hemlock and Douglas-fir and that the frequency of resistant reactions expressed in roots following invasion by the fungus help explain the pattern of symptom development and mortality rates that are observed in juvenile stands in the ICH. Results will be used to revise the table of susceptibility ratings for conifers (Anon. 1995) support the development of a decision aid and suggest recommendations for Armillaria root disease management in the southern Interior of B.C.

CHAPTER TWO:

MACRO- AND MICROSCOPIC HOST RESPONSE TO ABIOTIC WOUNDING, INOCULATION WITH *ARMILLARIA OSTOYAE* AND NATURAL INFECTIONS

2.1 INTRODUCTION

The external tissues of the bark, comprised of both periderm and rhytidome, provide an important protective barrier to underlying tissues from microbial invasion. Like other plant pathogenic fungi, direct penetration of *Armillaria* will trigger a series of predictable and coordinated events to replace injured tissue and meristems, maintain gas exchange and prevent desiccation, and restrict the ingress of the pathogen. These non-specific host responses include periderm formation (Day 1927, Thomas 1934, Rykowski 1975, Mullick 1977), barrier zone formation and callus tissue (Johnson *et al.* 1972, Shigo and Tippett 1981) and regeneration of new vascular cambium (Sharples and Gunnery 1933, Warren-Wilson and Warren-Wilson 1960, Mullick 1977, Oven and Torelli 1999, Dujesiefken *et al.* 2001). Once pathogens induce the process of phellogen restoration, they interact not only with chemicals present in the normal bark but also with a whole suite of new chemicals produced during the cellular dedifferentiation (Puritch and Jensen 1980). Although chemical resistance plays a key role in defense responses of plants, the formation of structural barriers in tissues challenged by *Armillaria* is also an important mechanism by which further spread of the fungus may be halted (Garraway *et al.* 1991). This chapter describes the host response of western redcedar, western hemlock, and Douglas-fir to infection on roots at the macroscopic and microscopic levels, particularly targeting the structural barriers formed in the bark (necrophylactic periderm) and in the wood (barrier zone formation associated with compartmentalization of infected tissue).

Resistance responses of woody plants to plant pathogenic fungi are classified as either 'passive' or 'active' (Blanchette and Biggs 1992). Passive resistance involves preformed morphological and/or chemical barriers to penetration or colonization by microorgansims. Examples of preformed barriers may include the suberized cork cells of the periderm or various toxic extractives contained in the outer layers of the bark. Active resistance involves induced morphological and/or chemical barriers to microbial colonization that develop when the host plant responds to injury. Two examples are the

formation of necrophylactic periderm (NP) in the bark and barrier zone formation in the wood. Both types of responses occur in stems and roots and serve to protect tissues from further desiccation or necrosis and limit the spread of the invading pathogen in healthy host tissue. The underlying mechanisms of active defense must also involve chemical changes in the host tissues so that they resist enzymatic attack or become inhibitory or toxic to pathogens. Guillaumin *et al.* (1989) reported that growth of *A. mellea* in the bark and sapwood was considerably less in resistant plum rootstocks. This slow growth was associated with a pink or purple discoloration of the tissue surrounding the lesion and was attributed to *de novo* synthesis of anthocyanic compounds involved in the resistance. This defense mechanism may inhibit the spread of *Armillaria* enough so that the host is able to complete NP formation and confine the fungus to a lesion in the bark. However, the ability of trees to develop wound periderms in response to pathogen invasion quickly and efficiently to successfully contain the fungus varies between species. Robinson *et al.* (2004b) reported that western larch was capable of successful NP development in advance of infection by *A. ostoyae* at a greater frequency and at a younger age than Douglas-fir. Resistant and susceptible hosts can be differentiated by comparing host-pathogen interactions on different species and assessing the degree of interference in the process of phellogen restoration (Struckmeyer and Riker 1951, Mullick 1977).

Tree roots respond to mechanical wounds or any factor that kills or removes bark by forming barriers in bark and wood in ways that are parallel to those observed in response to *Armillaria* invasion. However, the ability of a host to form such barriers under the influence of an aggressive pathogen may be interfered with, resulting in delays in NP formation in the bark or inhibited altogether.

Host reactions induced following invasion by pathogenic fungi may exhibit a broad range of temporal variability depending on the inherent resistance of the host species, the size and quality of the inoculum, the season of wounding, and the influence of the environment on host-pathogen interactions. Moreover, observations have shown that host response within all species depends on age, size, and vigour of the tree and the

location of the infection on the root (Morrison 1972, Morrison *et al.* 1991, Pearce *et al.* 1986, Redfern 1978, Robinson 1997, Rosso and Hansen 1998).

Morrison *et al.* (1991) reported that the location of an infection on the root system is an important factor in disease development. Robinson (1997) showed that infected Douglas-fir and western larch roots located close to the root collar produced NP's with multiple bands of thick- and thin-walled phellem, a structural characteristic that increased the trees' resistance to spread of the fungus. The frequency at which either species produced this type of NP decreased with increasing distance from the root collar (Robinson 1997). However, Morrison (1972) found that on Corsican pine, inoculations with isolates of *A. mellea* at the root collar resulted in larger lesions than inoculations on the lateral roots. Furthermore, two root collar inoculations resulted in larger lesions than a single inoculation (Morrison 1972). Shaw (1980) noticed that infections initiated at the root collar and/or taproot usually killed the host more frequently than infections initiated on distal lateral roots. This phenomenon was also noted on young Douglas-fir (Buckland 1953). Pearce *et al.* (1986) reported that more vigorous, dominant trees were killed less frequently than the less vigorous, suppressed trees. To build upon Morrison's (1972) and Robinson's (1997) observations, several host variables were measured on trees inoculated with *A. ostoyae* to investigate whether size, age, tree vigour, or distance of the infection from the root collar have any effect on the host response to abiotic wounding and/or *A. ostoyae* infection in roots of Douglas-fir, western hemlock and western redcedar.

The time of year in which trees become infected by *Armillaria*, together with the inherent growth characteristics of those trees will determine the nature and extent of injury (Kozlowski 1969). Field observations (van der Kamp, pers. comm.) indicate that in many cases when larger trees succumb to death within one or two years, much of the damage may be caused by the fungus advancing and killing tissue while the tree is dormant and unable to initiate defense responses to stop the spread of the fungus. Examination of basal lesions from trees infected with *A. ostoyae* indicates that the killed cambial surface is almost always coincident with the end of an annual ring. However, invasion and partial or total girdling of the root collar often arises from an infection that was

established perhaps several years previously. Preliminary examinations of basal stem disks under this study showed traumatic resin canals as the first cell types formed at the start of an annual ring which suggests that the fungus progressively killed host tissue while the tree was dormant. Shigo and Tippett (1981) showed that trees infected with *Armillaria* formed a barrier zone in the early portion of the growth ring and the position of that barrier zone indicated that the spread of *A. mellea* occurred during the dormancy period or soon after the onset of growth. Fahn *et al.* (1979) showed that wounding during months of no cambial activity usually resulted in traumatic ducts being produced at the onset of the new growing season. To build on these observations, inoculation trials were conducted to investigate whether *A. ostoyae* can indeed successfully penetrate and cause infection in host species during the winter months. To compare the infection process and host reactions with those that were inoculated at the beginning of the growing season, trees of Douglas-fir, western hemlock and western redcedar were used.

Woody substrates provide the only effective base from which *Armillaria* can spread and cause infection (Redfern and Filip 1991). Under experimental conditions, excised rhizomorphs of sufficient length were capable of forming new growing tips with adequate inoculum potential to infect healthy seedlings (Redfern 1973). Many reports support the view that hardwoods are generally better substrates for the production of *Armillaria* rhizomorphs than conifers (Morrison 1972, Rishbeth 1972, 1978). Garry oak (*Quercus garryana* Dougl.) branchwood is a high quality substrate for *Armillaria* inoculum to be used in field trials (Robinson 1997, Morrison, pers. comm.) and therefore was used as the substrate in the field inoculation trials in this study.

Understanding the role of wound responses and the resistance of woody plants to *Armillaria* should lead to innovative control measures for managing root disease in new plantations. The *A. ostoyae* inoculation trials were in essence time-course studies that enabled complete characterization of wound related phenomena and lesion development over time on the three conifer species studied by systematically sampling roots several weeks and months following inoculation.

2.1.1 PERIDERM ANATOMY

The periderm is a protective tissue of secondary origin that replaces the epidermis in stems and roots that have continual secondary growth (Fahn 1960, Esau 1965). Periderm formation is also an important phase in the development of protective layers at sites of leaf abscission, and injured tissues resulting from mechanical wounding, insect feeding and fungal infection (Mullick 1975, Mullick and Jensen 1973, Struckmeyer and Riker 1951). In roots, periderm arises from the pericycle after rupture of the cortex and endodermis during secondary growth (Fink 1999). In stems, the first periderm originates from anaplasia of the epidermis, of outer cortical parenchyma cells, or of phloem cells (Fink 1999). The periderm consists of three distinct tissues: the meristematic phellogen (cork cambium), the phellem (cork), and the phelloderm (Figure 2.1).
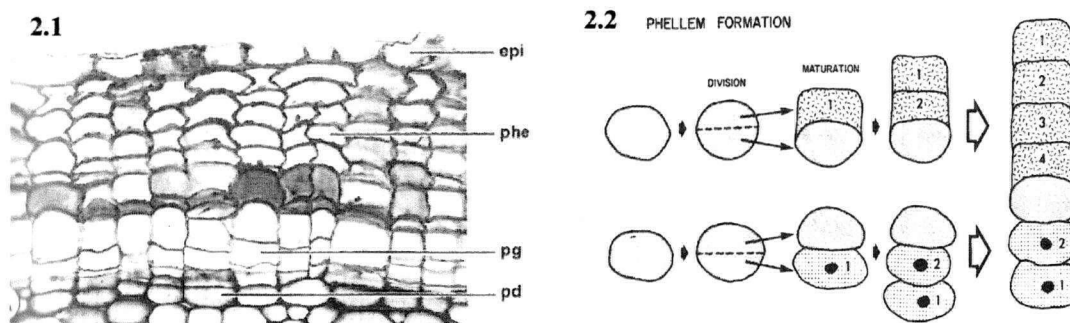
Fig. 2.1. A transverse section of stem of *Sambucus nigra* L. showing an early stage in the development of periderm with phellogen and its derivative tissues: phellem and phelloderm. Abbreviations: epi, epidermis; phe, phellem; pg, phellogen; pd, phelloderm. (courtesy of P.G. Mahlberg, cited from Dickison 2000). **Fig. 2.2.** Phellem are formed by periclinal divisions of the phellogen. The division of the phellogen cell gives either a phellem or phelloderm cell (cell 1). Over time, additional phellem and phelloderm derivatives are formed either externally or internally abutting the phellogen, respectively. The first derivatives of the phellogen are pushed outward so that they appear farthest from the phellogen (far right). Phellem production is generally greater than phelloderm (from Mullick 1977).

Phellogen cells have thin walls which are primary in nature. They are generally oblong or rectangular in transverse and radial sections, and polygonal or irregularly shaped in tangential section. The phellogen usually consists of just a single layer of cells but may also appear as a zone of meristematic tissue depending on the season of wounding or its stage of development during the process of phellogen restoration. Divisions of the

phellogen are mostly periclinal while periodic anticlinal divisions keep pace with the increase in circumference of the axis (Esau 1965). Derivatives of the phellogen usually form in a radial file that extends outward through the cork and inward through the phelloderm (Esau 1965). Each phellogen cell will divide to form two cells, the outer daughter cell matures into a phellem cell while the inner cell remains the phellogen (Figure 2.2). Occasionally, the inner cell matures into a phelloderm and the outer cell remains a phellogen cell. Typically, the phellogen produces more phellem than phelloderm (Figure 2.2). In the coastal region of B.C., phellogen renewal in the stems of conifers is usually initiated in May or June (Mullick 1977), slows down in the autumn and virtually ceases in the winter. Robinson (1997) reported similar observations on the roots of western larch in the southern interior of B.C.

Phelloderm cells are non-suberized, living parenchyma cells that resemble cortical parenchyma cells in shape and content. Their radial arrangement internally abutting the phellogen usually distinguishes them from normal cortical phloem cells. However, in some plants, phelloderm may not be produced at all or may exist as only a single layer of cells internally abutting the phellogen following initial periclinal division (Srivastava 1964, Esau 1965).

Phellem cells comprise the outer protective tissues of the bark that are formed external to the phellogen. Phellem are typically elongated with the long axis running parallel to the axis of the stem (Lier 1952). There are two main types of phellem cells: thin-walled phellem and thick-walled phellem. Thin-walled phellem are suberized which offer chemical impermeability (Esau 1965, Grozdits et al. 1982). Suberin is a complex and variable polymer, composed of both lipid and phenolic components. The presence of lipids, including long chain fatty acids and fatty alcohols, w-hydroxy fatty acids and dicarboxylic acids, render the polymer hydrophobic, a property that contributes to the relatively inert nature of suberized plant tissues (Kolattukudy 1984, Fink 1999). Thick walled phellem are heavily lignified (Esau 1965) and provide physical and mechanical protection (Grozdits et al. 1982). The occurrence and arrangement of these two different cell types in the periderm varies by species. Phellem cells are non-living at maturity

(Esau 1965). They usually appear compacted and lack intercellular spaces except at lenticels. The walls of the cork cells may be colored brown or yellow, and the lumina contain colored resinous or tanniniferous materials (Esau 1965), non-anthocyanic and anthocyanic compounds (Mullick 1977), and/or hydrolysable tannins (Fengel and Wegener 1984) which vary among species. Rhytidome consists of all tissues external to the phellem of the most recent phellogen.

In conifers, the secondary phloem which comprises the living inner bark is composed of a variety of different cell types including ray and axial parenchyma, functional and non-function sieve cells, sclerenchyma cells (fibers and sclereids) and secretory cells (i.e. resin canals). The secondary phloem of Douglas-fir is made up of primarily axial parenchyma arranged in longitudinal rows. Sieve cells are invariably interspersed throughout. Ray parenchyma cells extend radially through the phloem. Sclereids and resin cysts are sometimes found in low abundance in the mid- and outer phloem. The same cell types occur in the bark of western hemlock, although the sclereids are both larger and more abundant than in Douglas-fir. In western redcedar, the phloem tissue generally contains fibers, sieve cells, and axial and ray parenchyma cells. The fibers typically alternate with parenchyma cells as single-layered tangential bands.

2.1.2 NP FORMATION IN RESPONSE TO WOUNDING

The ability of trees to halt the ingress of *Armillaria* infections in their roots depends on intricate sequences of physiological, chemical and anatomical events in the challenged cells of the living bark. The non-specific nature of defense in the bark of conifer and hardwood species has been well described through a series of developmental studies and investigations of host-pathogen interactions (Mullick 1971; Mullick and Jensen 1973; Mullick 1975, 1977; Biggs 1984, 1985; Biggs *et al.* 1984).

Mullick (1971) observed distinct differences in the pigment composition in the periderms of different coniferous trees and utilized cryofixation techniques to examine and characterize these tissues. Mullick and Jensen (1973) classified all periderms in conifers into three types belonging to two basic categories: exophylactic and necrophylactic.

For many tree species, the first exophylactic periderm (FEP) replaces the epidermis within its first year and serves to protect underlying tissues from the external environment. FEP phellem are renewed every year so long as the FEP phellogen remains functional (Mullick 1977). At a certain age, which varies among conifers, FEP phellogen becomes non-functional and is replaced by the second category of periderm: necrophylactic periderm (Mullick 1971, Mullick and Jensen 1973). Hence, a necrophylactic periderm arises as a non-specific response whenever phellogen is rendered non-functional because of age or in response to physical or pathogenic injury to the living bark tissue.

The process of necrophylactic periderm (NP) formation, also synonymous to the term wound periderm, enables the reestablishment of an intact periderm forming in successively deeper layers of bark. Dead tissues that accumulate external to NP on the surface of stems and/or roots comprise the outer dead bark (rhytidome), and give the surface of the bark its characteristic rough appearance (Mullick 1977). Successive NP formation in the bark of some species like Douglas-fir, combined with retention of all the dead tissues, can give rise to very thick rhytidome tissue, especially on older stems, branches and roots. In western redcedar, thin bark may be attributed to thin phellem layers, NP's that cut off relatively thin layers of phloem, lower rates of phloem production and exfoliation of older bark to give a relatively smooth surface.

A sequent exophylactic periderm (SEP) may develop internally abutting NP. Mullick (1977) observed the separation of necrotic tissue following SEP formation resulting in sloughing or scaling of the outer rhytidome tissue, and leaving SEP intact at the surface of the bark (Mullick and Jensen 1973). Pigments of the phellem of NP and that of FEP and SEP in Pacific silver fir (*Abies amabilis* (Dougl.) Forbes), grand fir (*A. grandis* (Dougl.) Lindl.), western redcedar and western hemlock were chemically distinct (Mullick 1971, Mullick and Jensen 1973).

The terminology used for bark anatomy in stems first described by Mullick and Jensen (1973) may also apply to secondary tissues on roots. However, the age at which FEP

changes to a NP in roots certainly lags behind that of stems. In some conifers like western redcedar which normally does not form very thick rhytidome layers, FEP may function for a longer period of time. In other conifers, FEP may be comprised of multiple layers of phellem whereby a new layer of phellem is formed each year. However, the distinction between FEP, NP, and SEP in coniferous roots requires further scrutiny. The occurrence of NP and SEP likely varies by root age and by species. Evidence for NP and SEP are more visible on conifer roots that typically produce thick rhytidome like Douglas-fir. In contrast, the number of layers of phellem comprising the periderm on western redcedar roots is markedly lower than Douglas-fir or western hemlock. Field observations in this study suggest that sloughing of outer phellem layers on cedar roots is common. However, in this study accurate classification of periderm tissues prior to injury into either the exophylactic or necrophylactic categories was not possible without biochemical analysis of pigmentation of phellem layers. Mullick (1971) reported distinctness of pigmentation in the first (i.e. FEP) and sequent (i.e. NP) periderms in each of the 40 species representing 13 genera of conifers. Due to the variation in root age it was possible that, for all species, a NP that formed in response to abiotic injury or infection by *A. ostoyae* deeper in the bark tissue could have formed underneath the FEP or a NP that formed previously.

2.1.3 NON-SUBERIZED IMPERVIOUS TISSUE (NIT): A TISSUE ESSENTIAL FOR REGENERATION OF NP

There has been some controversy surrounding the non-suberized nature of the impervious zone of tissue formed during the initial stages of phellogen restoration. Mullick (1975) demonstrated the development of a zone of impervious tissue bordering injured bark which invariably precedes formation of a new periderm. Histochemical tests revealed that imperviousness was not due to suberization of cells. Thus Mullick coined the term 'non-suberized impervious tissue' (NIT). These observations were also confirmed by Soo (1977) when a sequential application of a ferric chloride and then a potassium ferricyanide solution (F-F test), applied to prepared samples through either the wound or cambial surface, failed to penetrate a zone of hypertrophied tissue. Histochemical tests confirmed that impervious tissue stained positively for lignin, but not suberin.

Lignin is predominantly composed of different polymers of phenyl-propanes including coniferyl alcohol (Fink 1999). Precisely how lignin inhibits attack by potential pathogens is uncertain, but several possible mechanisms have been postulated including the physical strength of the polymer which makes cell walls more resistant to mechanical or enzymatic breakdown, restriction of diffusion of toxins from infected into non-infected cells and the transport of nutrients in the opposite direction, the physical shielding of host cell wall polysaccharides from attack by fungal enzymes, and the effects of the phenolic precursors of lignin on the pathogen themselves (Fink 1999). Subsequent developmental studies revealed that NIT does not develop from meristematic activity, but instead via hypertrophic dedifferentiation of extant phloem cells around the periphery of the injured tissue. The cell hypertrophy reduces flow or diffusion of pathogens and their detrimental products by elimination of the apoplastic pathway (through cell walls and intercellular spaces). Furthermore, NIT always preceded formation of NP, and NP developed specifically from tissues internally abutting NIT (Mullick 1977). Hence, Mullick hypothesized that the development of NIT around wounded bark provides the underlying tissue the time and conditions necessary for phellogen restoration to occur.

However, subsequent studies of periderm formation in response to physical wounding or pathogenic injury failed to confirm that this zone of impervious tissue was indeed non-suberized. Biggs (1984, 1985) reported the presence of intracellular suberin lamellae associated with the layer of lignified, impervious cells. Using a more sensitive technique to detect suberin, Biggs (1984, 1985) demonstrated that suberized cells were always found internally abutting the non-suberized lignified cells. The author proposed that impermeability of this tissue was attributed to cell wall suberization and that the term NIT should no longer be used (Biggs 1985).

Woodward and Pearce (1988) also demonstrated suberization in the impervious layer formed in Sitka spruce (*Picea sitchensis* (Bong.) Carr.) trees challenged with *Phaeolus schweinitzii* (Fr.) Pat following treatment of the impervious tissue with chlorine dioxide to remove the lignin in cell walls. The authors suggest that masking of suberin staining by phenolic compounds likely caused earlier confusion by Mullick. Removal of the wall-

bound phenolics revealed the early onset of suberization and the F-F test confirmed that imperviousness was delineated by this layer of suberized tissue (Woodward and Pearce 1988).

Very few researchers have adopted the terminology proposed by Mullick (1975). Subsequent studies typically recognize this zone of impervious tissue as a ligno-suberized zone (LSZ) (Lindberg 1991, Wahlström and Johannson 1992, Oven and Torelli 1994, Oven *et al.* 1999, Simard *et al.* 2001).

However, Mullick (1977) recognized that while tissue underlying the NIT zone may be in the process of redifferentiation, sporadic immature phellem-like cells may occur around the periphery of NIT. A newly restored phellogen may form phellem as early as two days after impermeability sets in and at this time, suberin may be detected in both the phellem cell walls and the cell walls of some NIT cells immediately adjacent to the newly formed phellem (Mullick 1977). Often, there may be cells caught between the NIT and the zone of redifferentiation where the new phellogen is differentiating which develop suberized linings and take on the appearance of phellem cells. However, these cells are neither NIT nor phellem cells. Robinson (1997) reported that it was the cells caught between the NIT and the developing phellogen that become suberized, not the impervious tissue itself.

This study did not use other histochemical staining techniques for lignin other than Phloroglucinol + HCl and as such, no confirmation of suberization in the impervious zone was conducted aside from viewing a Phloroglucinol-HCl stained section under both BF (to distinguish a lignified zone of NIT) and UV fluorescence (to distinguish between suberized and non-suberized tissue). Sudan III was also used on separate sections to confirm presence of suberin in cells. Because Mullick (1977) acknowledged lignification and hypertrophic dedifferentiation of tissues leading to the formation of impervious tissue and that the suberization of cells may appear immediately following impermeability, the provisional view of this author is to continue to use the terminology proposed by Mullick (1977).

2.1.4 FACTORS AFFECTING NIT DEVELOPMENT AND NP FORMATION

NIT and NP may be triggered by a number of factors including insect (Mullick 1975) and fungal attack (Struckmeyer and Riker 1951; Biggs 1984, 1985; Wahlström and Johansson 1992; Robinson 1997; Simard *et al.* 2001; Solla *et al.* 2002), the time of year in which the tree is wounded (Mullick and Jensen 1976), and other environmental stress factors including drought (Puritch and Mullick 1975), all of which may slow the rate of NIT or NP formation in the bark.

Mullick postulated that NIT and NP formation surrounding bark occupied by the balsam woolly adelgid (*Adelges piceae* Ratz.) may be inhibited or delayed by chemicals secreted by the adelgid's stylets (Mullick 1975, 1977). Wahlström and Johansson (1992) found that *A. ostoyae* infection in the roots of *Pinus sylvesteris* delayed the formation of NP but increased the number of lignified cell layers in the bark relative to bark with non-challenged wounding. A subsequent study showed similar results whereby *P. sitchensis* roots inoculated with *A. ostoyae* or *A. mellea* delayed formation of impervious tissue and wound periderm compared to superficial wounds where the rhytidome tissues were removed or deeper wounding to the vascular cambium (Solla *et al.* 2002). Robinson *et al.* (2004b) reported that NPs formed in response to invasion by *A. ostoyae* halted further fungal advance on 68% and 45% of the roots examined from 10- and 27-year old western larch trees, respectively. The majority of Douglas-fir roots in the same relative age classes showed frequent breaching of NPs resulting in progressive lesions in the bark and cambial zone (Robinson *et al.* 2004b).

The rate of NIT formation varies at different times of the year and similar to other physiological processes, NIT formation displays a cyclical pattern (Mullick and Jensen 1976). Following mechanical wounding to conifer stems, the rate of NIT formation was fastest in June, slowed down in August/September, virtually ceased in the winter and then resumed slowly again in the spring. Trees that were wounded in November during the dormant period did not complete NIT formation until the following spring in May (Mullick and Jensen 1976). Robinson (1997) reported similar results in western larch roots following injuries made to the bark at various times throughout the year. Following

mechanical injuries to the stems of western hemlock and western redcedar in July, Mullick and Jensen (1976) reported that NIT was complete after about 30 days for both species and the authors speculated that impermeability may occur after just 2 weeks. However, these experiments were conducted in the coastal region of B.C. where the climatic conditions are generally more favourable for tree growth and vigour. Puritch and Mullick (1975) showed that water stress could significantly delay the development of NIT tissues in *A. grandis* seedlings. Adverse effects of stress factors such as drought on the susceptibility of host species to attack by pathogens is a familiar concept, however the indirect effects of drought on the pathogen are less well known. Pathogenesis depends on specific interactions between the pathogen and the initiation of defense responses in the host involving complex biochemical and structural cellular alterations such as NIT and NP formation. The outcome of this interaction results in either susceptible or resistant host reactions.

2.1.5 NIT DEVELOPMENT AND NP FORMATION: THEIR ROLE IN RESISTANCE AGAINST ARMILLARIA ROOT DISEASE.

Non-specific responses including NIT development and NP formation are induced following direct injury to the phellogen which renders the meristem non-functional. Mullick (1977) suggested that while NP *per se* may not be involved in host-pathogen interactions, the process of its restoration, including NIT formation, is, and that the development of NIT in response to wounding may be the physiological basis of host response to disease in conifers (Mullick 1975). Robinson (1997) reported that NP's with multiple bands of phellem were more frequently formed in the roots of western larch and more effective at halting further spread of *A. ostoyae* in adjacent host tissue than in Douglas-fir. Less is known about the resistance responses of other conifers commonly found in the southern interior region of B.C.

Host susceptibility occurs when NIT formation is not triggered and/or when a fungus is able to circumvent the process of phellogen renewal (Mullick 1977). In the case of *Armillaria,* penetration of the outer bark is similar in both susceptible and tolerant hosts, however host responses initiated following penetration of the fungus will determine

whether a particular reaction is susceptible or resistant. In this study, resistance or susceptibility was determined by how successfully the host was able to complete the process of phellogen restoration and NP formation while under the influence of the fungus. In situations where the fungus advanced to and killed the vascular cambium, resistance reactions were judged as those which appeared to have successfully compartmentalized the infection and showed lateral ingrowth of callus tissue over the wounded tissue. To understand the effects *Armillaria* had on the initiation and development of NIT and NP formation, an understanding of the host response in the absence of *Armillaria* was required. Thus, characterization of the process of wound healing in abiotically wounded root bark was used for comparison.

Cryofixation, fluorescence microscopy and standard histological techniques were developed by Mullick (1971) to characterize fresh samples of bark tissue and the anatomical changes in cells in response to wounding. This method preserves the chemical integrity of fresh tissue samples and permits physiological interpretation of infected host tissue by observing changes in fluorescence characteristics when compared to healthy host tissue. Similar methods were used by Robinson (1997) to describe the host response to infection by *A. ostoyae* in the roots of western larch and Douglas-fir. To build on Robinson's work, similar techniques were employed to describe the host response to abiotic wounding, inoculation with *A. ostoyae*, and roots naturally infected with *A. ostoyae* in Douglas-fir, western hemlock, and western redcedar trees.

2.2 MATERIALS AND METHODS

2.2.1 STUDY SITES

Four inoculation trials were conducted at three different sites in the Okanagan-Shuswap and Arrow-Boundary Forest Districts of the Southern Interior Forest Region (Appendix I). The three Douglas-fir sites were 20-30-years old and contained naturally regenerated western redcedar and western hemlock more than 15-years old. Table 2.1 gives details of site locations and characteristics.

2.2.2 INOCULUM BLOCK PREPARATION

The method used to prepare inoculum blocks was based on that described in Redfern (1970, 1975), Morrison (1972) and used by Robinson (1997) with some modification. Inoculum blocks were prepared using known isolates of *A. ostoyae* (provided by D. Morrison, CFS) grown on segments of Garry oak branch wood. Isolate No's. Y-4 (collected from 70-Mile House) and 87-01 (collected from Victoria) were used to prepare inocula in the Hidden Lake (2002) and Kingfisher (2003) inoculation trials, whereas only isolate 87-01 was used in the Kingfisher (2004) and Nakusp (2004) trials. Different methods for making "starter discs" were used from year-to-year, all of which resulted in successful inoculation of garry oak segments. In the first method, *A. ostoyae* was grown in Petri dishes containing 3% malt extract–1.5% agar medium. Autoclaved beech (*Fagus grandiflora* Ehrh.) and garry oak discs approximately 1 cm in diameter and 5 mm thick were added to mycelial colonies of *A. ostoyae*. Once discs were fully colonized by the fungus they were used as "starter discs" to inoculate fresh segments of Garry oak. In the second method, fully colonized inoculum blocks (approximately 2 x 8 cm) were cut into discs (approximately 5 mm thick) using a band saw and then cut into smaller pie-shaped "starter discs" to be used to inoculate fresh segments of Garry oak.

Garry oak branch wood (approximately 2-3 cm in diameter) was collected from living trees and taken back to the lab. All surface organisms such as lichen and mosses were scraped from the surface of the bark. Using a band saw, branches were then cut into 8 cm segments, surface sterilized in a 1% solution of bleach for 15 minutes and rinsed in water.

Table 2.1. Location and characteristics of three sites used in the four inoculation trials at which the host response to infection by *Armillaria ostoyae* in the roots of western hemlock, western redcedar and Douglas-fir trees was investigated.

Site	Year(s) Trial was Initiated	BEC[1] subzone/ site series	Location	Elevation (m)	Species Composition[2] Planted	Natural	Soil Type[3]	Plant Associations	
Hidden Lake	2002	ICHmw2 03	50°31′N, 118°50′W	600	Fd, Lw	Cw, Hw, Pw, Ep, At, Se	Brunisol	*Paxistima myrsinites* *Clintonia uniflora* *Frageria virginiana* *Linnaea borealis*	*Rubus parviflorus* *Pleurozium schreberi* *Viola glabella* *Gymocarpium dryopteris*
King- fisher	2003 & 2004	ICHmw2 03	50°42′N, 118°44′W	600	Fd, Pl	Cw, Hw Pw, Ep, Pl, Act, Tw, Se, W	Brunisol	*Paxistima myrsinites* *Clintonia uniflora* *Athyrium filix-femina* *Pleurozium schreberi* *Peltigera aphthosa* *Linnaea borealis*	*Rubus parviflorus* *Viola glabella* *Frageria virginiana* *Cladina* spp. *Gymocarpium dryopteris*
Naksup	2004	ICHmw2 05	50°17′N, 117°44′W	700	Fd	Cw, Hw Ep, Tw	Luvisol	*Paxistima myrsinites* *Clintonia uniflora* *Athyrium filix-femina* *Pleurozium schreberi* *Peltigera aphthosa* *Gymocarpium dryopteris* *Mahonia aquifolium* *Acer glabrum*	*Rubus parviflorus* *Viola glabella* *Frageria virginiana* *Cladina* spp. *Streptopus roseus* *Galium triflorum* *Linnaea borealis*

[1] See Meidinger and Pojar (1991). Actual soil hygrotopes and tropotopes were read from tables in Lloyd *et al.* (1990) and Braumandl and Curran (1992).

[2] At, trembling aspen; Act, black cottonwood; Cw, western redcedar; Fd, Douglas-fir; Ep, paper birch; Hw, western hemlock; Lw, western larch; Pl, lodgepole pine; Pw, western white pine; Se, Engelmann spruce; Tw, pacific yew; W, willow

[3] Soil orders were determined by examining soil profiles and determining soil texture from two soil pits dug at each site and using the Canadian System of Soil Classification, 3rd Ed. Ottawa. Agriculture and Agri-Food Canada and http://www.environment.ualberta.ca/SoilsERM/class.html

A colonized disc was pinned over the cambium at the end of a fresh garry oak segment using small brass pins. Intimate contact between the starter disc and the fresh cambial surface of the garry oak segment is essential for colonization to take place (Morrison 1972). Inoculum blocks were then placed in buckets of pre-washed, non-sterile coarse sand. After 8-12 weeks, the fungus fully colonized bark and cambial tissue and actively growing rhizomorphs emerged from the cambial edge at the opposite end from the starter disc and from cracks in the bark along the length of the segment. Inoculum blocks showing abundant rhizomorph production and/or mycelium in the cambial region were used to inoculate healthy trees in the field. Blocks partially colonized by other fungi such as *Xylaria* sp. were still used for inoculum so long as the *Armillaria* mycelium was still present as emerging rhizomorphs.

2.2.3 INOCULATION AND SAMPLING TECHNIQUE

In the field, root systems of western redcedar, western hemlock and Douglas-fir trees which did not exhibit any aboveground signs or symptoms of root disease were carefully excavated up to a meter away from the root collar. At least ten trees of each species were inoculated in each trial. Existing rhizomorphs were removed from inoculum blocks in order to stimulate new rhizomorph growth and blocks were then placed either directly below or alongside the root without injuring the root itself. When possible, all inoculations on a single tree (up to four) occurred on separate primary roots. Flagging tape was tied around the root distal to the point of inoculation. The root diameter and the distance from the root collar to the point of inoculation were then accurately measured and then the root and the block were covered with mineral soil. In the first inoculation trial at Hidden Lake in 2002, the selection criterion for root diameter and distance from the root collar was usually in the range of 2-3 cm and between 30-60 cm, respectively. Secondary roots were utilized when primary roots were unable to fulfill the selection criteria. In subsequent trials, a range of root diameters and distances were obtained to determine what, if any, difference these host variables may have on the type and success of the host response. Trees were tagged and measured for diameter at 1.3 m (DBH).

As similar techniques were used for producing inoculum in the lab and the same technique was used for inoculating roots in the field, experimental conditions for *Armillaria* inoculum with respect to the viability and inoculum potential were more or less homogenous from year to year. Figure 2.3 shows the inoculation technique *in situ* with a garry oak segment colonized by *A. ostoyae* placed alongside a healthy root without injuring the root itself. Figure 2.4 shows profuse rhizomorphs emerging from the inoculum block and adhering to the outer surface of the root.

Fig 2.3. Inoculation technique shown *in situ*. *A. ostoyae* inoculum block placed alongside a healthy western hemlock root. **Fig. 2.4.** An inoculum block as it was placed against a western redcedar root prior to harvesting from the ground. Clusters of *A. ostoyae* rhizomorphs (arrows) are shown emerging from the inoculum block and adhering to the outer surface of the root.

In all but the first inoculation trial, abiotic wounding to the roots was used as an additional treatment to allow comparison between it and host reactions to *Armillaria* invasion. Abiotic wounding was performed on healthy roots separate from those roots used for inoculations. Wounding was carried out without physically disrupting the outer bark by soaking a small circular polyurethane sponge (approximate 8-10 mm in diameter) in liquefied SHUR/Freeze™ cryogen spray (Triangle Biomedical Sciences, Durham, NC, U.S.A.) which has temperatures of -55 to -60°C. Using tweezers, the soaked sponge was applied to the exterior surface of the root for a given length of time depending on the thickness of the bark (usually between 10-15 seconds). This method achieved a freezing injury in the phloem tissue to at least half the bark thickness and thereby permitted characterization of phellogen restoration and host response in the absence of *Armillaria*.

Flagging tape was tied around the root approximately 3 cm distal to the point of injury so that the wound could be easily located at some later sampling date. Both treatments (abiotic wounding and inoculations) covered the same range of root diameters and distances from the root collar.

In the last two field inoculation trials, a further control treatment was added, namely placement of uninfected fresh segments of garry oak next to uninfected roots. This control treatment was applied to the same trees but on separate roots from those used for abiotic wounding and inoculations.

Trees were inoculated on different dates depending on the field trial. In the Hidden Lake field trial, inoculations occurred on two separate dates: July 8, 2002 and August 22, 2002. Roots were harvested at 11 weeks and approximately 1 year following each inoculation date. In all subsequent field trials, trees were inoculated in early May and roots were harvested at two or three of the following intervals: 5 weeks, 8-9 weeks, 11 weeks, 5 months, and 1 year after inoculation depending on the specific trial.

At each harvesting date, the number of samples collected was relatively even among all species. Roots were carefully excavated and examined *in situ* to ensure that the placement of the inoculum block against the root had not shifted. In situations where there was clearly no contact between the cambial surface of the inoculum block and the root, the root was omitted from any further analysis. Also, the viability of inoculum at the time of harvesting was assessed by removing the bark from the inoculum unit. If mycelia were absent from the inoculum block, it was considered a non-viable unit. Roots associated with inoculum blocks with non-viable inoculum (lacking rhizomorphs) were excluded from the analysis. Roots that escaped disease because the fungus was not present at the root surface were also excluded from the analysis.

Roots were marked with paint to define the point of contact with the inoculum block after the root had been harvested from the ground. Where evidence of infection was present on the root (i.e. resinosus, or clumps of rhizomorphs adhering to the surface), sections of

the root up to 60 cm in length were removed from the ground. Root sections were washed with water to remove soil from the root surface and dissected for examination of host response to infection.

Macroscopic observations of host response (i.e. the type of host reaction in the bark, bark hypertrophy, resin exudation, compartmentalization and callusing) were noted. In addition to root diameter and the distance from the root collar, the following measurements were also recorded: root age, inner bark thickness, and lesion length. Tree vigour was determined by taking cores at four cardinal directions at the root collar area from each tree showing successful inoculations. Tree vigour index was calculated as the most recent average annual increment divided by the 5-year average. The previous years' annual average annual increment was used for samples harvested during the growing season while the current average annual increment was used for samples harvested at the end of the growing season. Tree age was also determined from the core sample.

Abiotic wounds were sampled concurrently with inoculations. Bark samples from roots with control blocks were collected in the same manner as that for abiotic wounding and inoculations, described below. Healthy bark samples were collected from treated roots at least 30 cm proximal or distal to the point of injury.

2.2.3.1 WINTER INOCULATIONS

Three additional inoculation trials were implemented to investigate whether *A. ostoyae* can successfully penetrate and cause infection in Douglas-fir, western hemlock, and western redcedar in the winter months during tree dormancy, and what, if any host response is initiated during that time.

The inoculation trials were conducted at the Hidden Lake and Kingfisher sites in 2002-2004 (refer to Table 2.1 for site locations and characteristics). Inoculation and sampling techniques followed the same methodology outlined in Section 2.2.2, Inoculum block

Preparation, and Section 2.2.3, Inoculation and sampling technique. Neither abiotic wounding nor controls were used in any of the winter inoculation trials.

Trees were inoculated on different dates in each field trial. Inoculations occurred in early November in 2002, in mid-October in 2003 and in mid-September in 2004. Roots were harvested the following spring at the onset of growth (late April/early May). In 2003, additional roots of each species were left in the ground and subsequently harvested at different intervals over the course of the growing season to observe how infection developed on inoculated hosts over time. The viability of the inoculum block was observed at the time of collection by removing the bark from the inoculum unit and assessing the presence of mycelial fans. Sample treatment was carried out only on roots showing visible evidence of infection (browning of the phloem at inoculum contact or rhizomorph penetration) according to the methods described in Section 2.2.4.

2.2.3.2 EXAMINATION OF ROOTS NATURALLY INFECTED WITH *A. OSTOYAE*

In the summer of 2002, roots naturally infected with *A. ostoyae* were examined to determine the host reactions involved in lesion development in Douglas-fir, western hemlock and western redcedar trees, enabling a comparison with artificial inoculations of those species. Two Douglas-fir plantations that had naturally regenerated hemlock and cedar and that had *Armillaria*-caused mortality were selected for this component of the study. The sites were located near Hidden Lake, 30 km east of Enderby in the ICH mw2 (Lloyd *et al.* 1990) in the Okanagan Shuswap Forest District.

In the field, root systems of trees showing no above-ground evidence of Armillaria root disease and growing in close proximity to a recently killed tree were carefully excavated to reveal root contacts (Figure 2.5). Roots were examined for symptoms of infection caused by *A. ostoyae* (i.e. resinosus on the surface of the root or irregularities on the surface of the bark) at root contact as well as proximal and distal regions for rhizomorph-initiated lesions. Root samples were collected and examined in the same manner as that outlined in Section 2.2.4, Sample Treatment.

Fig. 2.5. Excavation of roots systems between a recently killed Douglas-fir and healthy western redcedar. Root contacts are flagged for examination of *Armillaria*-caused lesions at root contact.

2.2.4 SAMPLE TREATMENT

In the field, tissue sections were collected by taking a 2 x 1 cm sample of bark either at the point where the inoculum block contacted the root or from the proximal and distal infection fronts at the margin of *A. ostoyae*-caused lesions. Infection fronts were recognized as either a necrophylactic periderm or necrotic host tissue separating apparently healthy (uncolonized) tissue from infected (colonized) tissue either proximal or distal to the point of invasion. When infection had spread to the vascular cambium, samples were collected from the infection front in the inner bark immediately below the original outer periderm as well as from the infection front at the vascular cambium. The radial face of the sample contained all the details of interest. Samples were trimmed to have the infection front centred on the radial face. Abiotically wounded samples were also collected with the margin of the lesion centred on the radial face. In order to minimize reactions to cutting and trimming during tissue harvest, samples were immediately coated in OCT® (Optimum Cutting Temperature compound; Thermo Shandon, Pittsburgh, PA, USA) and frozen in liquid nitrogen.

For histological studies of woody tissue, samples from root lesions showing evidence of cambial invasion and/or compartmentalization and callusing were trimmed to approximately 2 x 1 cm and fixed in FAA (70% ethanol, formalin, glacial acetic acid, glycerin). In the lab, the sample blocks were dehydrated through a gradual series of alcohols, embedded in paraffin wax, and the radial face was mounted and sectioned on a rotary microtome. Sections 16 μm thick were transferred to a glass microslide (Fisher Finest *Premium* Superfrost, Fisher Scientific, Pittsburgh, PA, USA), with approximately 6 sections per slide. In preparation for staining, the paraffin wax was removed from the slides using Hemo-De (Fisher Scientific, Pittsburgh, PA, USA) clearing solution and then hydrated through a gradual series of alcohols. Slides were stained with 0.2% Fast Green in 95% ethanol and 1% Safranin in H_2O (Jensen 1962), a common stain used to delineate between cellulosic cell walls of plant tissue, and then mounted with Permount™ (Fisher Scientific, Pittsburgh, PA, USA). Samples were examined under the light microscope to describe the host response following cambial invasion and barrier zone anatomy associated with compartmentalization for each species.

In the lab, healthy, abiotically wounded, and infected bark samples were transferred from liquid nitrogen to a cryostat chamber at -20°C housing a microtome. Samples were mounted and dissected to reveal the radial (longitudinal) face of the sample. Host reactions including necrophylactic periderm formation, compartmentalization and callusing were examined macroscopically while still mounted in the cryostat at x6 to x25 magnification using a WILD M5 stereo zoom microscope (WILD Heersbrugg, Switzerland) and photographed using Kodak Elitechrome 100 color slide film (Eastman Kodak Co., Chicago, IL, USA) using a 35-mm camera mounted to a phototube. Sections 14 μm thick were picked up off the microtome knife using pre-chilled slides, mounted in pre-chilled cryostat oil (Cryo-cut Microtome Lubricant, American Optical Corporation) and covered with a pre-chilled 22 x 22 mm No.1. glass coverslip (Fisher Finest™ *Premium* cover glass, Fisher Scientific, USA). Usually two sections were mounted per slide.

The frozen sections mounted on slides were examined microscopically on a Carl Zeiss Photomicroscope II (Carl Zeiss, Oberkochen, Germany) equipped with a freezing stage maintained at approximately $-35°C$. Slides were examined under tungsten-illuminated bright field (BF) and incident mercury-illuminated fluorescence. Fluorescence was achieved using an OSRAM HBO® 50 W mercury lamp and fluorescence filter combinations for blue light (BL) excitation in the 390-440 nm range and ultra-violet (UV) excitation at 365/366 nm. The microscope was equipped with a Carl Zeiss epi-fluorescence condenser and with filter sets 48 77 05 (comprising a BP 390-440 exciter filter, FT 460 chromatic beam splitter and an LP 475 barrier filter for viewing in BL) and 48 77 01 (comprising a BP 365/10 exciter filter, FT 390 Chromatic beam splitter and an LP 530 barrier filter) for viewing in UV. Carl Zeiss Neofluar objectives were also fitted to the microscope.

Additional cryostat sections were air-dried and stained with a saturated aqueous solution of phloroglucinol in 20% HCl (Ruzin 1999) to detect lignin in cell walls. Phloroglucinol-HCl will stain lignin red. One to two drops of phloroglucinol-HCl solution was added to air-dried sections, covered with a glass coverslip and examined immediately under BF. Separate individual sections were also stained with 0.1% toludine blue in H_2O (Jensen 1962) to visualize fungal hyphae in host tissue. Sudan III is a highly specific stain for cutinized and suberized cell walls (Jensen 1962). A few drops of Sudan III in ethylene glycol were added to air-dried sections and placed in a desiccator. After 12-24 hours, sections were generously rinsed with 85% ethylene glycol, covered with a glass coverslip and examined on the microscope. In BF, suberized cell walls appear orange-red. Suberized cell walls fluorescence bright orange in BL. Suberin was also detected in sections following staining with phloroglucinol-HCl. Phloroglucinol-HCl stains the aldehyde groups of both lignin and suberin but autofluoresence of lignin is quenched whereas suberin is unaffected and suberized cells fluoresce bright blue-violet under UV. Although phloroglucinol-HCl is highly specific for detecting lignin (Jensen 1962, Ruzin 1999), and suberin (Biggs 1984) in plant cells, Pérez-de-Luque et al. (2006) suggest that its specificity might also be expanded to include polyphenols. Safrannin-Fast green was used for staining serial cryostat sections of cedar root bark. Photomicrographs were taken

of both frozen and stained cryostat sections, as well as paraffin-embedded sections with Kodak Ekrachrome 160 Tungsten film (Eastman Kodak Co., Chicago, IL, USA).

2.2.5 RE-ISOLATION OF *A. OSTOYAE* FROM LESIONS

Re-isolation of the fungus was attempted from a sample of roots that showed distinct *Armillaria*-caused lesions. Mycelial fans or rhizomorphs surface-sterilized with 95% ethanol were transferred to Petri-dishes or tubes containing 3% MEA amended with BDS solution (Worrall 1991), sealed with Parafilm® M (Pechiney Plastic Packaging, Menasha,WI, USA), and stored in the dark at room temperature. For inoculations, the isolate collected from the inoculated root was challenged in a diploid-diploid pairing with the corresponding isolate of *A. ostoyae* from the inoculum block used to inoculate the root to confirm that the resulting infection was not caused by on-site inoculum. To demonstrate incompatibility reactions in culture, the same isolate was paired against different known isolates of *A. ostoyae* collected elsewhere in British Columbia (BC-9 collected from Campbell River, provided by D. Morrison, CFS) and Britain (Ag1 and Sp6 collected from Elveden and Lynford, respectively, provided by D. Morrison, CFS), and known British Columbia isolates of *A. sinapina*: one from Merritt (provided by D. Morrison) and one from Mica Creek (Isolate #29-2-8C provided by M. Cruickshank, CFS). For naturally infected roots, the isolate collected from *Armillaria*-caused lesions was paired with known isolates of *A. ostoyae* (Canadian Centre for the Culture of Microorganisms (CCCM) No. 63) and *A. sinapina* (CCCM No. 64) to examine intra- and inter-specific reactions and confirm which *Armillaria* species caused infection on the trees sampled. All pairings were done in triplicate on 3% MEA and incubated at room temperature in the dark. Pairings were carefully monitored and scored as either compatible or incompatible after 6-8 weeks depending on the distance between the two opposing mycelial colonies and the rapidity of mycelial growth. Compatible-type matings display complete intermingling of opposing mycelia. Incompatible mating as a result of intraspecific crosses (two isolates of the same species) display incomplete intermingling of mycelia at the opposing edges, sometimes appearing as a hyphal-free zone between the two opposing mycelia. Incompatible matings as a result of interspecific crosses (two isolates of different species) develop a dark pigmented demarcation line in

the agar at the margin where the two opposing colonies meet (Korhonen 1978). Photographs were taken to demonstrate interspecific and intraspecific reactions between isolates.

2.2.6 STATISTICAL ANALYSIS

Chi-square tests were used to analyze whether there were any differences in (i) the total number of root inoculations that resulted in successful penetration of the outer bark between species at each harvest date within a given field trial, (ii) the frequency of infection between the different harvest times for each species within a given field trial, and (iii) the frequency of infection between field trials at a given harvest date (i.e. 1 year) within a given species.

The number of sites or years or both, of field data represent a good random sample of all sites in the moist-warm subzone of the ICH zone over time whereby host reactions induced following invasion by the fungus are visible at all harvest dates. Data were pooled from all field trials across sites, years and time since inoculation and Chi-square tests were used to determine whether (iv) there was any difference in the frequency of successful resistance reactions induced following invasion by *A. ostoyae* between species.

Frequency data for all field trials showing the different stages of host response induced in each species following invasion by *A. ostoyae* were analyzed using Chi-square tests to determine whether there was any significant variation between species in the frequency of (v) NIT formation, (vi) NIT breached, (vii) NP formation, (viii) NP breached, and (ix) compartmentalization and callusing.

Data for host variables (i.e. root diameter, distance from the root collar, and tree vigor) collected from all infected trees in each field inoculation trial were pooled. Successful resistance reactions as a proportion of the total number of roots penetrated by *A. ostoyae* were plotted against each of the host variables to determine whether there was any relationship between host response and a particular host variable.

2.3 RESULTS AND DISCUSSION

2.3.1 INOCULATION TRIAL: FREQUENCY OF INFECTION

The number of roots sampled at each harvest date was generally divided evenly for all species. However, uneven sample sizes among species resulted from the exclusion of individual host roots that were not considered to be challenged by the fungus as described below. Table 2.2 shows the frequency of infection caused by *A. ostoyae* in the roots of Douglas-fir, western hemlock and western redcedar at each harvest date for each trial.

All inoculum blocks produced rhizomorphs. However, the amount of rhizomorphs produced was quite variable. Some inoculum blocks produced abundant rhizomorphs at the opposite end from where the starter disc was pinned to the cambial edge of the Garry oak segment. It was projected that the fungus would grow out of a starter disc into the bark and cambial region of a fresh Garry oak segment, and its progressive colonization strategy would result in the fungus emerging at the opposite end of the block where it would produce tufts of mycelium or rhizomorphs or both (D. Morrison, pers. commun.). This end was then placed directly alongside the root so that fresh, newly emerging mycelium or rhizomorphs would be in direct contact with the host. However, there was a number of situations where the fungus produced significantly more rhizomorphs around the starter disc and very little from the cambial edge of the block situated against the root.

Furthermore, there were some situations in which considerably more rhizomorphs emerged from the cracks in the bark along the length of the block and had not yet grown to attach themselves onto the host root at the time of sampling. In addition, because of the diversity of and interactions among microbial communities in the soil, inoculum blocks were often colonized by common cord-forming fungi like *Xylaria sp.* Blocks that had surface colonization of *Xylaria* sp. were nevertheless still considered to be viable inoculum because other fungi were strictly superficial on the bark yet the inner bark and cambium were fully colonized by *A. ostoyae* and blocks produced rhizomorphs. Indeed, many of these blocks resulted in successful penetration by the fungus. However, cases where competing fungi occupied the cambial zone of the block and inhibited *A. ostoyae*

Table 2.2. Number of root inoculations on Douglas-fir, western hemlock and western redcedar and the number and proportion producing infection by *A. ostoyae* at various harvest times in four field trials.

Species	Field Trial	Harvest Time	No. of root inoculations	No. of infections	Frequency of infection
Douglas-fir	Hidden Lake (2002)	11 weeks	11	9	0.82
		1 year	10	10	1.00
	Kingfisher (2003)	5 weeks	2	0	0.00
		9 weeks	4	0	0.00
		1 year	30	10	0.33
	Kingfisher (2004)	9 weeks	5	0	0.00
		5 months	14	9	0.64
		1 year	13	12	0.92
	Nakusp (2004)	8 weeks	2	0	0.00
		5 months	2	2	1.00
		1 year	2	2	1.00
Western hemlock	Hidden Lake (2002)	11 weeks	19	14	0.74
		1 year	6	5	0.83
	Kingfisher (2003)	5 weeks	7	1	0.14
		9 weeks	8	3	0.38
		1 year	30	23	0.77
	Kingfisher (2004)	9 weeks	7	1	0.14
		5 months	14	11	0.79
		1 year	17	12	0.71
	Nakusp (2004)	8 weeks	3	2	0.67
		5 months	7	6	0.86
		1 year	3	2	0.67
Western redcedar	Hidden Lake (2002)	11 weeks	19	6	0.32
		1 year	10	4	0.40
	Kingfisher (2003)	5 weeks	10	0	0.00
		9 weeks	5	2	0.40
		1 year	24	8	0.33
	Kingfisher (2004)	9 weeks	4	1	0.25
		5 months	20	16	0.80
		1 year	12	10	0.83
	Nakusp (2004)	8 weeks	2	1	0.50
		5 months	7	7	1.00
		1 year	2	2	1.00

rhizomorphs from emerging from the end of the block, were omitted from further analysis. Also, where the inoculum blocks failed to produce rhizomorphs at surface contact with the root, the host was not considered to be 'challenged' by the fungus and those samples were excluded from the data set.

As fungal infection is necessary for subsequent analysis of defense mechanisms induced in the roots of the different conifer species, the success of fungal inoculation was a critical and a fundamental aspect of this study. Assessment of host response to infection could only be done on roots showing successful penetration of the outer root bark. Chi-square tests among and between species for all field trials is given in Appendix II.

There was no difference in the frequency of infection between the two isolates of *A. ostoyae* at either the 2002 Hidden Lake trial or the 2003 Kingfisher trial. Both isolates colonized the Garry oak branch segments and rhizomorphs emerged from slits in the bark and from the cambial zone of the block. The fungus penetrated intact (non-wounded) root bark on each species. However, there was variation in the frequency of infection among species on the different harvest dates for the first two field trials. In the Hidden Lake trial, Douglas-fir and western hemlock had a higher proportion of infected roots than western redcedar at 11 weeks (χ^2, p < 0.01) and 1-year (χ^2, p < 0. 1) following inoculation, whereas in all subsequent trials infection on cedar was as frequent as the other conifers.

The low frequency of infection in cedar in the first inoculation trial may have been due to microsite differences or variations in microbial root surface communities among species, or both. Cedar trees selected for inoculations were limited to those within a 20-30-m-wide strip bordering a separate study site and many individual trees within that area had a tendency to be clumped particularly in wetter microsites such as depressions. Excavation of roots on cedar trees growing in these areas revealed soils rich in organic matter and it was not uncommon for roots to be growing through large pieces of decaying wood. Although roots selected for inoculation were buried with mineral soil obtained from adjacent areas, the difference in soil type and microbial root surface communities may

have inhibited infection of *Armillaria* at surface contact with the root, particularly with respect to cedar. Morrison (1972) found that rhizomorph production (measured as the cumulative dry weight) was positively correlated with the amount of organic matter in the soil. In fact, in this study it was not uncommon to unearth a profuse network of *A. sinapina* rhizomorphs inhabiting the organic matter and the large pieces of decaying wood. General observations suggest that rhizomorphs production from blocks buried in organic soils was as good as from those in mineral soil.

In the 2003 trial, infection rates were lower on Douglas-fir and western redcedar trees than on western hemlock trees. Very little infection occurred during the 5 weeks following inoculation, except for 1 hemlock root. In subsequent weeks, more infection occurred, particularly on western hemlock and western redcedar, however, there was no statistically significant difference in the frequency of infection among species at either 5 weeks or 9 weeks. Douglas-fir roots examined at 9 weeks showed an abundance of rhizomorphs at root contact and rhizomorphs adhering to and running underneath outer bark scales. However because Douglas-fir has relatively thick rhytidome tissue, more time was required for the fungus to degrade the outer rhytidome layers and penetrate the inner bark.

The summer of 2003 was particularly dry which contributed to the high incidence and severity of forest fires throughout the province. In the study area, the last moderate precipitation event occurred in mid-June, approximately 2 weeks before the 9 week sampling date. Subsequently, soil conditions became especially arid and remained as such until the next decent rainfall in October. Little infection occurred on roots sampled at 9 weeks. *In-situ* examinations of a few inoculated trees in the weeks following revealed very little evidence of infection (i.e. lack of resinosus on the surface of the root at inoculum contact) and further sampling of roots was postponed until the following year. At 1-year post inoculation, hemlock showed significantly more infection than Douglas-fir (χ^2, $p < 0.001$) or western redcedar (χ^2, $p < 0.01$). The difference in infection rates between hemlock and the other conifers is not well understood, however the high

frequency of infection observed in hemlock after 1 year in the 2003 trial parallels infection rates for that species in the other field trials.

The above results are interpreted to mean that the arid soil conditions in 2003 delayed or inhibited infection on host roots. Adverse effects of seasonal drying on rhizomorph growth in upper soil layers has been reported elsewhere. Morrison (1976) suggests the spread of rhizomorphs in the soil depends in large part on soil moisture and that periods of dry weather may affect growth of *A. mellea* rhizomorphs in the upper 5 cm of the soil. Pearce and Malajczuk (1990) found that no rhizomorphs grew in dry soil and that periods of dry weather may account for the paucity of rhizomorphs in forest soils of western Australia. Cruickshank *et al.* (1997) also confirmed this by reporting higher incidence of *Armillaria* rhizomorphs on wetter sites in the drier hygrotopes.

In this study, although *A. ostoyae* was viable in inoculum blocks after 1 year, seasonal drying the year prior may have reduced its infective capability and overall incidence of infection. Smith and Griffin (1971) suggested the growing tips of rhizomorphs tend to melanize in the absence of a film of water. Similarly, the low soil moisture content of the soil in mid-late summer in 2003 probably inhibited rhizomorph growth and development on host roots where they remained latent until more favourable conditions became available for renewed growth.

In 2004, two trials located at two geographically separate sites showed similar results with respect to infection frequency on the three conifer species. No difference in the rate of infection occurred among species at 8-9 weeks, 5 months or 1 year following inoculation at both sites. These results suggest that infection does not appear to occur sooner on some species than others. On all species, infection frequency was related to the amount of time since inoculation. Inoculum blocks were primed in the field before they were buried by disturbing the already melanized rhizomorphs to stimulate new rhizomorph growth. It generally takes several weeks for rhizomorphs to grow and adhere to the root surface before they can penetrate the living root. All species showed more

infection at 5 months than at 9 weeks. No more infection was observed at 1 year following inoculation than what was observed at 5 months.

In future inoculation studies, harvesting roots 4-5 months following inoculation in the spring would be more desirable since adequate time would have passed to allow the fungus to grow from the inoculum block to the root and for the host to initiate defense mechanisms in an attempt to contain the fungus. However, during a period of summer drought the frequency of infection may be significantly less.

2.3.2 CHARACTERIZATION OF HEALTHY ROOT BARK TISSUES

The macro- and micrograph sample features in Figures 2.6-2.166 are radially sectioned and oriented so that the rhytidome is at or above the top of the figure and the vascular cambium is at or below the bottom of the figure, unless otherwise stated. The magnification of all macrographs is x 15, and the magnification of cryofixed sections viewed with bright field (BF), blue light excitation (BL), and ultra violet excitation (UV) is x 35 unless otherwise stated. Other features of interest which are made reference to in the text are indicated with an arrow. Acronyms describing the specific tissues of interest are defined on page xxviii.

Macroscopically, fresh samples of the living phloem tissue of western redcedar roots were white in colour, the phellem of the outer periderm was a deep red, and all dead tissue external to the last formed periderm was brown (Figure 2.6). In western hemlock, the living phloem appeared pink and the phellem of the outer periderm was a dark red-purple colour (Figure 2.7). In Douglas-fir roots, the phloem tissue was pale yellow/orange and the phellem of the outer periderm was dark brown (Figure 2.8).

Typical frozen sections of healthy root bark were viewed with BF, BL excitation, and UV excitation for western redcedar (Figures 2.9-2.11), western hemlock (Figures 2.12-2.14), and Douglas-fir (Figures 2.15-2.17), respectively. The walls of healthy phellogen cells

did not fluoresce. In the late spring or early summer when the phellogen was actively dividing, a zone of non-fluorescent meristematic cells was evident and it was sometimes difficult to distinguish between phellogen and immature phellem because early stages of differentiation of phellem are nonsuberized and do not yet have pigmented contents.

A typical periderm in healthy bark tissue of western redcedar consisted of 1-2 rows of thin-walled phellem, a single row of phellogen and 1-2 rows of phelloderm cells. The bark surface was invariably smooth. In most coniferous trees, thin-walled phellem cells are typically suberized whereas thick-walled (stone) phellem cells are heavily lignified. However in western redcedar the phellem cells are both lignified and suberized (Figures 2.18 and 2.19).

The periderm of western hemlock roots usually had 2-4 rows of thin-walled phellem, a single row of phellogen and 1-2 rows of phelloderm cells. 1-3 rows of stone phellem were observed on older larger diameter roots external to the thin-walled phellem. Mature thin-walled phellem were suberized and had dark brown or reddish-purple pigmented contents. Irregularities in the bark surface existed as small bumps on the surface of the root (Figure 2.7).
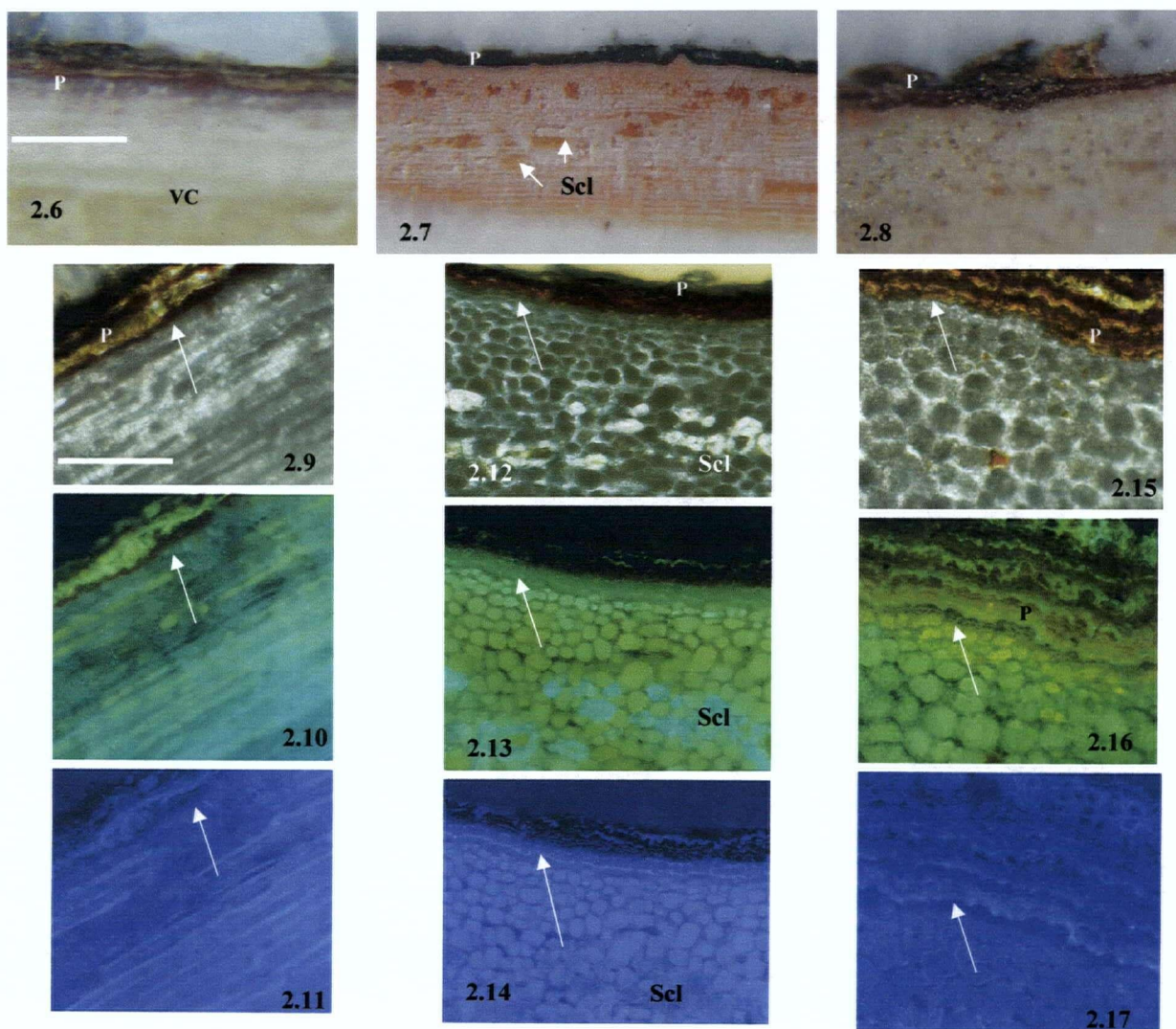
Fig. 2.6. A sample of root bark from a healthy 19-year-old western redcedar tree. **Fig. 2.7.** A sample of a root bark from a healthy 19-year-old western hemlock tree. **Fig. 2.8.** A sample of root bark from a healthy 31-year-old Douglas-fir tree. **Fig. 2.9.** A cryofixed section of healthy western redcedar root bark, BF. **Fig. 2.10.** Same section shown in Fig. 2.9., BL. **Fig. 2.11.** Same section shown in Fig. 2.9, UV. **Fig. 2.12** A cryofixed section of healthy western hemlock root bark, BF. **Fig. 2.13.** Same section shown in Fig. 2.12., BL. Note clusters of sclereids visible in Fig. 2.12 and 2.13. **Fig. 2.14.** Same section shown in Fig. 2.12, UV. **Fig. 2.15.** A cryofixed section of healthy Douglas-fir root bark, BF. **Fig. 2.16** Same section shown in Fig. 2.15, BL. Note thick-walled stone phellem in Figures 2.15 and 2.16. **Fig. 2.17.** Same section shown in Fig. 2.15, UV. Arrows in Figures 2.9-2.17 point to the phellogen. Bar on photomacrographs and photomicrographs are 1 mm and 25 µm, respectively, and are applicable to successive photomacrographs and photomicrographs in this Chapter unless stated otherwise.

Periderms in Douglas-fir usually had 2-4 rows of thin-walled phellem, 1-4 rows of stone phellem, a single row of phellogen and 1-3 rows of phelloderm cells. Mature thin-walled phellem were suberized and had orange-pigmented contents. Sometimes, Douglas-fir periderms had a band of stone phellem on the outside and several rows of thin-walled phellem externally abutting the phellogen. The rhytidome was comprised of dead phellem layers as well as any dead phloem tissue isolated by the last formed periderm (Figure 2.20).
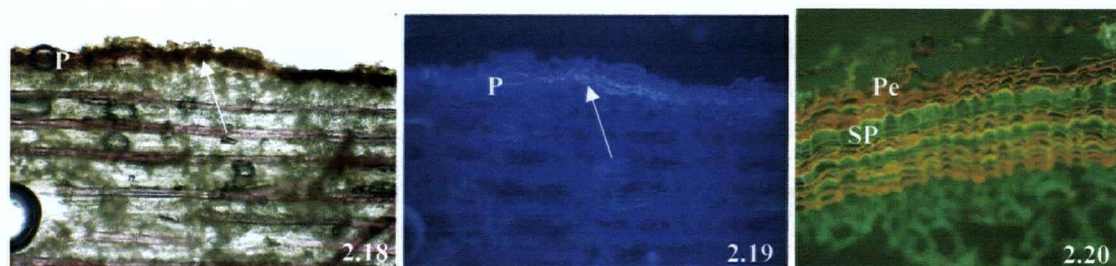
Fig. 2.18. A phloroglucinol-HCl-treated section of a healthy western redcedar root. Both the thin-walled phellem (arrow) and phloem fibres stain positively for lignin, viewed under BF. **Fig. 2.19**. The same section viewed under UV which shows the same phellem cells fluorescence brightly for suberin. **Fig. 2.20**. A Sudan III stained section of a healthy Douglas-fir root bark showing alternating layers of thin-walled (suberized) phellem shown here as bright orange, and thick-walled (stone) phellem, BL, x 45.

2.3.3 CHARACTERIZATION OF ABIOTICALLY WOUNDED ROOT BARK TISSUES

Tissue samples were collected at various dates to determine the host response to abiotic wounding in the bark and the length of time required for wound healing. Wounding was conducted in three of the four inoculation trials: Kingfisher 2003, 2004, and Nakusp 2004. Table 2.3 gives the total number of roots examined following abiotic wounding in Douglas-fir, western hemlock and western redcedar trees in the different field trials.

Table 2.3. The total number of trees sampled and the total number of roots examined following abiotic wounding of Douglas-fir, western hemlock and western redcedar roots at the different harvest dates for each field trial. Roots were initially wounded in early May of each year.

Species/Field Trial	Date of sample collection	No. of trees sampled	No. of roots examined	Lesion length (mm)
Douglas-fir				
Kingfisher (2003)	5 weeks	3	4	8-20
	9 weeks	2	4	11-26
	1 year	17	27	10-21
Kingfisher (2004)	9 weeks	2	4	12-22
	5 months	8	13	12-28
	1 year	3	3	14-26
Nakusp (2004)	8 weeks	2	3	15-20
	5 months	2	5	14-25
	1 year	1	2	20-21
Western hemlock				
Kingfisher (2003)	5 weeks	4	4	10-20
	9 weeks	2	8	9-18
	1 year	17	25	11-22
Kingfisher (2004)	9 weeks	2	4	11-23
	5 months	4	8	14-22
	1 year	2	5	16-25
Nakusp (2004)	8 weeks	2	2	16-25
	5 months	2	3	20-22
	1 year	1	1	23
Western redcedar				
Kingfisher (2003)	5 weeks	8	9	16-25
	9 weeks	3	8	12-25
	1 year	14	23	10-61
Kingfisher (2004)	9 weeks	2	3	11-20
	5 months	9	10	15-25
	1 year	4	6	13-24
Nakusp (2004)	8 weeks	1	2	13-34
	5 months	3	3	20-25
	1 year	1	1	20

Douglas-fir

After 5 weeks, roots showed a distinct zone of necrotic tissue that extended to a depth of approximately half the bark thickness (Figure 2.21). The freeze-killed tissue appeared as a mass of necrotic tissue that fluoresced yellow-green when viewed under BL (Figure 2.22). Some of these cells were situated along the outer boundary of a NIT zone. During the early stages of dedifferentiation, a distinct zone of irregularly-shaped hypertrophied cells with reticulated contents developed internally abutting necrotic tissue. At the time of sampling (5 weeks post-wounding), a well developed, but narrow zone of NIT was seen under the necrotic zone. Cell walls fluoresced bright yellow and all tissue external to NIT, including the freeze-killed phellogen and phelloderm of the original periderm and underlying phloem, also fluoresced bright yellow (Figure 2.22). Staining with phloroglucinol-HCl clearly distinguished the NIT zone from surrounding host tissue because cell walls comprising the NIT zone stain more intensely for lignin.

Macroscopically, a clear-white zone of modified living tissue appeared internal to the zone of brown hypertrophied cells (Figure 2.23). This zone of modified tissue represented an active, meristematic zone of phellogen 3-5 cells wide, but narrower at the junction of the original NP. Cells had thin walls and were non-fluorescent when viewed under BL or UV, indicating a zone of active cell division. Newly formed derivatives were produced in radial files. Single rows of phellem cells were continuous along the newly restored phellogen and eventually merged with the phellogen cells of the original periderm. Cell walls internal to the NIT zone stained positively for suberin following treatment with Sudan III.

After 9 weeks, a NP was fully differentiated and usually comprised of 1-2 rows of stone phellem and 1-2 rows of thin-walled phellem. A newly restored phellogen produced stone phellem cells first which were positioned in the central region underneath the NIT zone. These cells typically had thicker walls than the adjacent NIT cells. They were rectangular or square shaped in radial section, were aligned in radial files with thin-walled phellem, and stained very strongly with phloroglucinol-HCl (Figure 2.24). The appearance of the freezing lesion was no different at 5 months than what was observed at

9 weeks with the exception of the additional layers of thin-walled phellem cell and narrowing of the phellogen zone which usually appeared as a single layer of cells. At 5 months, coincident with the end of the growing season, the NP had about 3-4 rows of stone phellem and 4-6 of thin-walled phellem (Figure 2.25). There were no more rows of phellem observed on wounded roots at 1 year than were present at 5 months. Apparently phellogen activity typically ceased during tree dormancy.
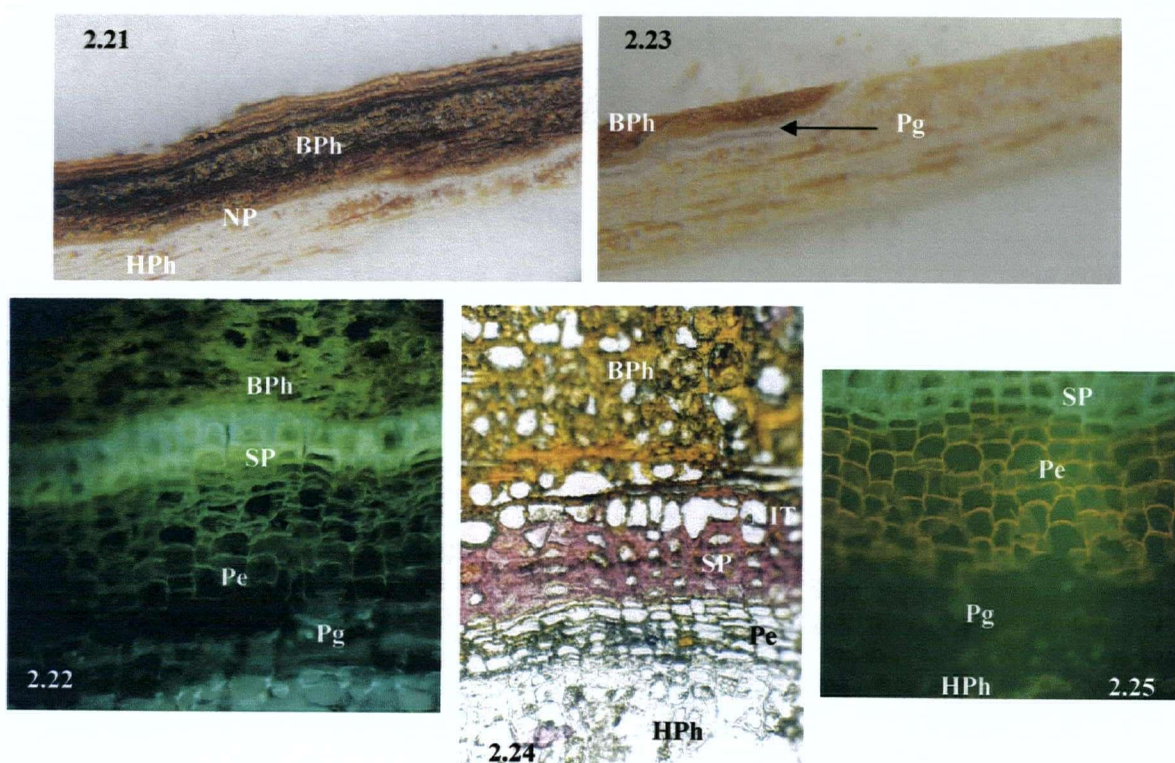
Fig. 2.21. A sample of Douglas-fir root bark 5 weeks after wounding, x 12. **Fig. 2.22.** A cryofixed section of abiotically wounded Douglas-fir root bark. Freeze-killed tissue and outer boundary of NIT zone fluoresce bright-yellow green with BL, x 45. **Fig. 2.23.** A sample of Douglas-fir root bark, 9 weeks after wounding showing distinct zone of clear-white tissue underlying browned tissue, x 12. Note: the original periderm sloughed off during the process of cryofixation. **Fig. 2.24.** A phloroglucinol-HCl treated section of abiotically wounded Douglas-fir root bark, sampled 5 months after wounding showing dark staining of sieve cells comprising the NIT zone and positive staining for lignin in the thick-walled stone phellem overlying radially compressed thin-walled phellem. **Fig. 2.25.** A Sudan III treated section of abiotically wounded Douglas-fir root bark, sampled 5 months after wounding showing suberized thin-walled phellem internally abutting two layers of stone phellem, x 45.

In larger diameter roots of Douglas-fir, freezing injuries did not extend past the rhytidome layer and therefore did not permit characterization of the host response to abiotic wounding. On roots where injury did occur, NP formation was complete at 5 weeks in all 4 (100%) of the root samples examined. Only 1 of the 8 (12%) Douglas-fir samples that were examined at 9 weeks showed necrosis extending beyond the zone of incomplete redifferentiation. The sample had initially formed a NIT zone. Phellogen restoration occurred in mid-phloem in the central area of the lesion but a zone of redifferentiation was still developing at the junction of the original periderm on either side of the lesion. Incomplete phellogen restoration in these areas permitted extension of necrosis into the adjacent phloem. Beyond this zone of necrosis a new zone of dedifferentiating tissue had not yet developed at the time the root was sampled. However, it is conceivable that NIT and NP formation would be complete in approximately 3-4 weeks because according to Mullick (1977) the rate of phellogen restoration is typically greatest during the summer months.

All samples collected at 5 months (n=18) and 1 year (n=32) showed NPs to be complete and at the latter harvesting date, many roots displayed *en masse* sloughing of the freeze-killed tissue external to the new periderm. Only 1 of the 18 (5%) roots sampled at 5 months showed necrosis extending down to the vascular cambium. Compartmentaliz-ation and callusing was evident around the margin of the wounded cambium.

Western hemlock

Similar to Douglas-fir roots, western hemlock roots that were abiotically wounded showed distinct zones of necrotic tissue to at least half the thickness of the bark when sampled after 5 weeks (Figure 2.26). Necrotic tissue fluoresced yellow-green with BL. Tissue sections treated with phloroglucinol-HCl showed a well developed NIT zone comprised of hypertrophied and heavily lignified cells internally abutting necrotic phloem (Figure 2.27). All roots sampled at 5 weeks (n = 4) had formed a NP with approximately 1-3 layers of thin-walled phellem cells (Figure 2.28). However, NP formation was not always continuous along the periphery of the necrotic zone. NIT and NP typically form last at the junction with the original periderm and discontinuities in

periderm development occurred around clusters of sclereids. Sclereids are abundant in the phloem and were readily visible at the macroscopic level (Figure 2.26 and 2.29).

At 9 weeks, a clear zone of modified tissue was visible underlying the necrotic zone (Figure 2.29). Microscopically, this modified tissue showed a well defined meristematic phellogen zone, approximately 2-3 cells wide which did not fluoresce under BL or UV. Newly formed phellem cells accumulated dark red-purple contents and were organized in radial files. After 9 weeks, fully differentiated NPs consisted of up to 5 rows of thin-walled phellem. One to two rows of stone phellem plus 2-4 rows of thin-walled phellem were noticed only on two roots, both of which were more than 7 cm diameter (Figure 2.30).

The newly restored phellogen producing single rows of phellem cells was discontinuous around clusters of sclereids. Often a phellogen was fully restored adjacent to a cluster of sclereids while a zone of redifferentiation was still developing directly underneath a cluster of sclereids. Phellem production on either side of the cluster of sclereids was far more advanced than immediately underneath (Figure 2.31). Eventually, a new phellogen became established around the cluster of sclereids, however the timing of phellogen renewal around this cell type lagged behind that which occurs in phloem parenchyma.

Sclereids essentially became part of the NIT zone as indicated by more intense staining for lignin following treatment with phloroglucinol-HCl. A zone of dedifferentiated tissue that becomes hypertrophied, impermeable, and heavily lignified serves to seal off the affected region and provide the underlying cells the time and conditions necessary for phellogen restoration to occur. However, because sclereids lack protoplasm (Esau 1965) they also lack the ability to hypertrophy. The cell walls of sclereids are moderately thick and typically contain numerous pits. These anatomical characteristics suggest that in hemlock roots which normally contain an abundance of sclereids in the phloem, periderm formation may be delayed around the periphery of the wound, particularly in the region internally abutting clusters of sclereids.

At 5 months, the phellogen produced organized rows of phellem in neat radial files. Up to 9 rows of thin-walled phellem were observed at this sampling date (Figure 2.32). Sclereids no longer presented an obstacle to NP formation because tissue had sufficient time to redifferentiate underneath a cluster of sclereids. However, the delay in phellogen restoration caused by the presence of sclereids is evidenced by the higher number of phellem cells that occurred on either side of the cluster of sclereids than those which occurred directly underneath. The phellogen zone typically became much narrower at the end of the growing season, usually appearing as a single layer of cells. A number of lesions on abiotically wounded roots collected at 5 months and 1 year showed *en masse* sloughing of the freeze-killed tissue with an intact periderm established underneath. When the sampling of roots occurred at the 1 year harvest date in late May or early June, the phellem produced around the necrotic tissue in the year of injury appeared radially compressed and the current year's phellogen produced up to 3-5 rows of thin-walled phellem (Figure 2.33).

A higher proportion of roots with killed cambium following rapid freezing was observed in western hemlock roots than in Douglas-fir roots. Freeze-killed tissue extended down to the vascular cambium on 2 of 4 (50%) of the roots examined at 9 weeks and 6 of 11 (55%) of the roots examined 5 months following wounding in the Kingfisher trial. In all but one sample, compartmentalization and callusing were evident. The one root that lacked evidence of compartmentalization and callusing was sampled at 5 months. NP formation was initiated but incomplete around clusters of sclereids and necrosis breached through the sclereids and extended down to the vascular cambium. The lack of host response of the cambial tissue after 5 months suggests that perhaps the extension of necrosis occurred later in the growing season, coinciding with the onset of tree dormancy at the time the root was sampled. In this situation, a host response involving compartmentalization would not be expected to take place until growth resumes the following spring.
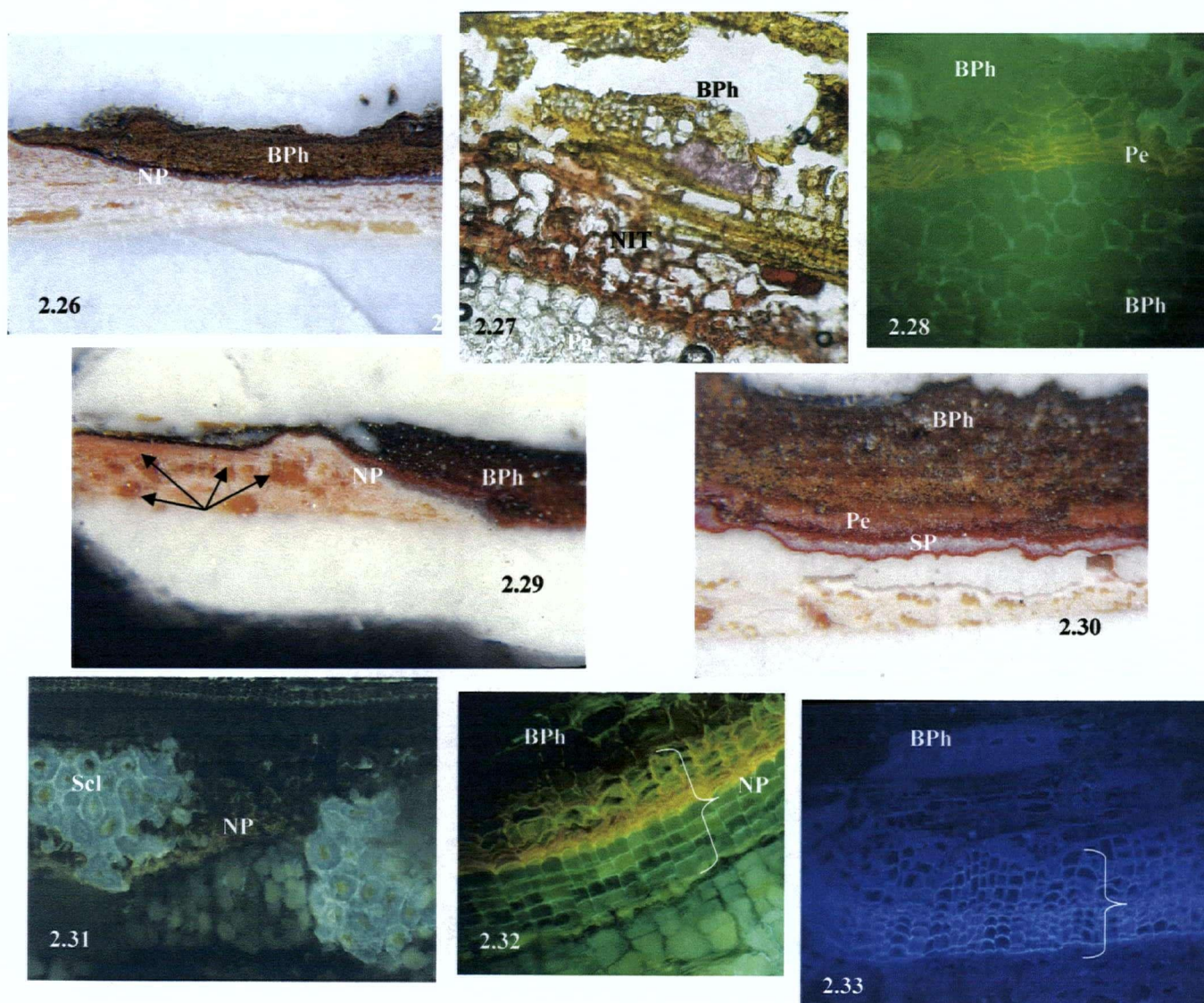
Fig. 2.26 A sample of western hemlock root bark, 5 weeks after wounding, x 12. Note clusters of sclereids. **Fig. 2.27.** A phloroglucinol-HCl treated section of abiotically wounded western hemlock root bark, sampled 5 months following wounding, BF. NIT zone is distinct as the zone of hypertrophied tissue that stains very strongly for lignin. **Fig. 2.28**. A Sudan III treated section of abiotically wounded western hemlock root bark, sampled 5 weeks after wounding, BL. **Fig. 2.29.** A sample of a western hemlock root bark, 5 months following abiotic wounding showing a colorless zone of modified tissue internally abutting the necrotic zone indicative of a newly restored zone of phellogen, x 12. Note clusters of sclereids (arrows). **Fig. 2.30**. A sample of a western hemlock root, 7 cm in diameter, sampled 5 months following abiotic injury. A single band of phellem (stone phellem) can be seen as the clear zone of tissue internally abutting the necrotic zone and externally abutting a zone of thin-walled phellem (dark purple pigmented line). This clear zone comprised a band of thick-walled phellem. In this image the phellogen was separated from the necrotic tissue since OCT (shown in white) occupied the space between the necrotic tissue and underlying phloem, x 25. **Fig. 2.31.** A cryofixed section of abiotically wounded western hemlock showing incomplete NP formation around clusters of sclereids, BL. **Fig. 2.32.** A cryofixed section of wounded root bark sampled 5 months following injury showing a well organized NP with up to 9 thin-walled phellem in neat radial files, UV. **Fig. 2.33** A cryofixed section of wounded root bark sampled 1 year following injury, showing at least 4 rows of thin-walled phellem internally abutting the radially compressed phellem produced the year prior, BF, x 45.

Western redcedar

Abiotically wounded roots of western redcedar sampled at 5 weeks showed necrosis extending to approximately half the thickness of the bark (Figure 2.34). The freeze-killed tissue fluoresced yellow-green when viewed microscopically under BL (Figure 2.35). One to two rows of thin-walled phellem were formed immediately abutting the necrotic zone (Fig. 2.36)

In contrast to Douglas-fir and western hemlock roots, a zone of white modified tissue was not visible in cedar roots on any of the harvest dates. This was primarily due to the fact that the zone of white-clear modified tissue underlying necrotic tissue usually represents a wide zone of re-differentiating tissue (a new meristematic phellogen zone) that could expand up to 4 cell layers wide during the late spring or early summer. The phellogen in cedar roots usually appeared as a single layer of cells that was non-fluorescent when viewed under BL or UV. In cedar, the phellem of the original periderm was typically 1-2 cells wide and unlike the other species studied, the periderm did not accumulate additional layers of phellem throughout the growing season.

NP formation was complete in all samples examined at 5 weeks, as well as at subsequent harvest dates. Killed cambium was found only on 2 of 13 (15%) and 2 of 30 (7%) of the roots sampled at 5 months and 1 year, respectively. In all cases, compartmentalization had occurred, callus tissue was evident around the margin of the lesion and NP formation was complete in the bark.

The chemical nature of cell wall constituents associated with thin-walled phellem in cedar roots differed from Douglas-fir and western hemlock roots. Whereas thin-walled phellem in hemlock and Douglas-fir appear to be suberized, the same cell type in cedar was both suberized and lignified. Following treatment of cedar sections with phloroglucinol-HCl, the thin-walled phellem of a newly formed NP stained very strongly for lignin (Figure 2.37). These phellem cells were not considered to be stone phellem. Stone phellem typically have thick cell walls and are rectangular- or square-shaped in radial section. Examination of the same sections treated with phloroglucinol-HCl under

UV revealed that the same lignified, thin-walled phellem were also suberized (Figure 2.38). Suberization in walls of phellem cells was also confirmed following staining of the sections from the same samples with Sudan III.

The NIT zone in wounded cedar bark tissue was very difficult to discern in most of the sections examined following staining with phloroglucinol-HCl. There is no evidence suggesting NIT formation does not precede NP formation as many studies documenting the early stages of development in NP formation were carried out on western redcedar trees (Mullick and Jensen 1976). However in this study, NIT zones were not well defined. It is possible that NIT formation comprised just a single layer of phloem parenchyma cells and/or fibres, the latter cell types being heavily lignified already. In addition, cell hypertrophy in a single layer of cells that is radially oriented in alternating rows of phloem parenchyma and fibers may be more difficult to distinguish from cases where a larger number of cell layers become hypertrophied throughout the inner phloem. In cedar, new immature phellem cells may be produced around the periphery of a single layer of impervious tissue and lignin may be detected in both the phellem cell walls and the cell walls of the parenchyma comprising the single layer of NIT externally abutting the newly formed phellem. Further developmental studies are warranted to confirm that western redcedar does indeed follow the classic model of non-specific defense in the bark of conifers as defined by Mullick (1977), particularly with respect to NIT formation, but also because many unique and never-before described defense mechanisms are involved in the process of wound healing and resistance against the ingress of pathogens in cedar (see below).

Unlike Douglas-fir and western hemlock, cedar did not produce additional phellem layers throughout the growing season after a NP was formed around injured tissue. No more phellem was observed around root lesions at 1 year than was detected at 9 weeks and 5 months, whereas phellem production on western hemlock and Douglas-fir increased with time since injury. One obstruction to phellogen restoration in the phloem of cedar bark may be heavily lignified cells, such as fibers. During the process of phellogen restoration, the cell hypertrophy and dedifferentiation of tissue caused the phloem fibers,

which are normally longitudinally aligned in radial sections, to become displaced and this served as an obstacle to continuous phellogen renewal around these displaced cells at the periphery of the wound. The result was a plethora of phellem wrapped around individual fibers (Figure 2.38). In contrast to hemlock roots in which clusters of sclereids presented a temporary barrier to phellogen restoration, fibers in the phloem of cedar did not affect the rate of phellogen renewal since NP formation was always complete at each sampling date.

In addition to NP formation in the bark, a unique phenomenon involving a type of localized response was induced in some roots. This response involved the activation of a cell death program, similar to the process of apoptosis defined by Fink (1999), in tissue adjacent to freeze-killed phloem. This host reaction was not an immediate response to injury and was predictably induced at or following completion of NP formation in the bark. The induced response was indicative of rhytidome formation resulting in the formation of a successive periderm, one that is structurally and biochemically the same as a NP; however, the cellular alterations used to derive this new periderm were quite different. The resulting lesion showed a NP bordering injured tissue and additional periderms initiated in the mid-phloem area that extended proximally and distally for some distance (up to 2 cm) from the primary freezing injury. Eventually, the new periderm merged with the original periderm, forming one continuous periderm layer that separated necrotic from adjacent living tissue (Figure 2.39). The author is unaware of any literature documenting this type of host reaction and in this study the term "induced rhytidome" will be used to describe the host reaction which involves induced apoptosis and successive periderm formation.

Various stages of induced rhytidome formation were observed in 4 of 21 (19%), 2 of 13 (15%), and 10 of 30 (30%) of the cedar roots examined at 8-9 weeks, 5 months and 1 year following wounding, respectively. No evidence of induced rhytidome formation was observed on roots sampled at 5 weeks.

The earliest stage of induced rhytidome formation appeared as an amorphous mass of tissue that underwent hypertrophy and hyperplasia. The hypertrophy, in particular, was excessive and significantly more distended than that normally observed during NIT development at an abiotic wound (Figure 2.40). However, this zone of tissue was non-lignified. During the early stages of development, the zone of hypertrophy and hyperplasia appeared non-fluorescent when viewed under BL or UV indicating a zone of dedifferentiation or an active zone of meristematic activity. Many of these cells were elongated and had reticulated contents, some forming cross-walls. Over time, cell wall fluorescence developed and a narrow zone of re-differentiating tissue occurred internally abutting the excessively hypertrophied phloem. The presence of a single radial row of cells (phellem) indicated that a new phellogen had developed (Figure 2.40). Lignification and suberization of phellem cells preceded the accumulation of pigmented contents. The single row of phellem was continuous along the restored phellogen, extending for some distance, usually up to 2 cm, beyond the margin of the NP and eventually merged with the phellem of the original periderm and the new NP (Figure 2.41 and 2.42).

Another unique response in cedar involving traumatic resin duct formation in the phloem was observed in only 2 of 30 (7%) of the roots sampled at 1 year. This response was not detected in any roots on any other date. Traumatic phloem resin duct (TPRD) formation in cedar was detected in a higher number of roots following inoculation with *A. ostoyae* than in roots with abiotic wounding. In many cases, induced rhytidome and TPRD's were induced simultaneously immediately following NP formation. As the intensity of the responses involving induced rhytidome and TPRD's was significantly higher in roots inoculated with *A. ostoyae* than in roots with abiotic wounding, a thorough description of these host responses including the early developmental stages and lesion development are presented in the Section 2.3.5 documenting host response to inoculation with *A. ostoyae*.
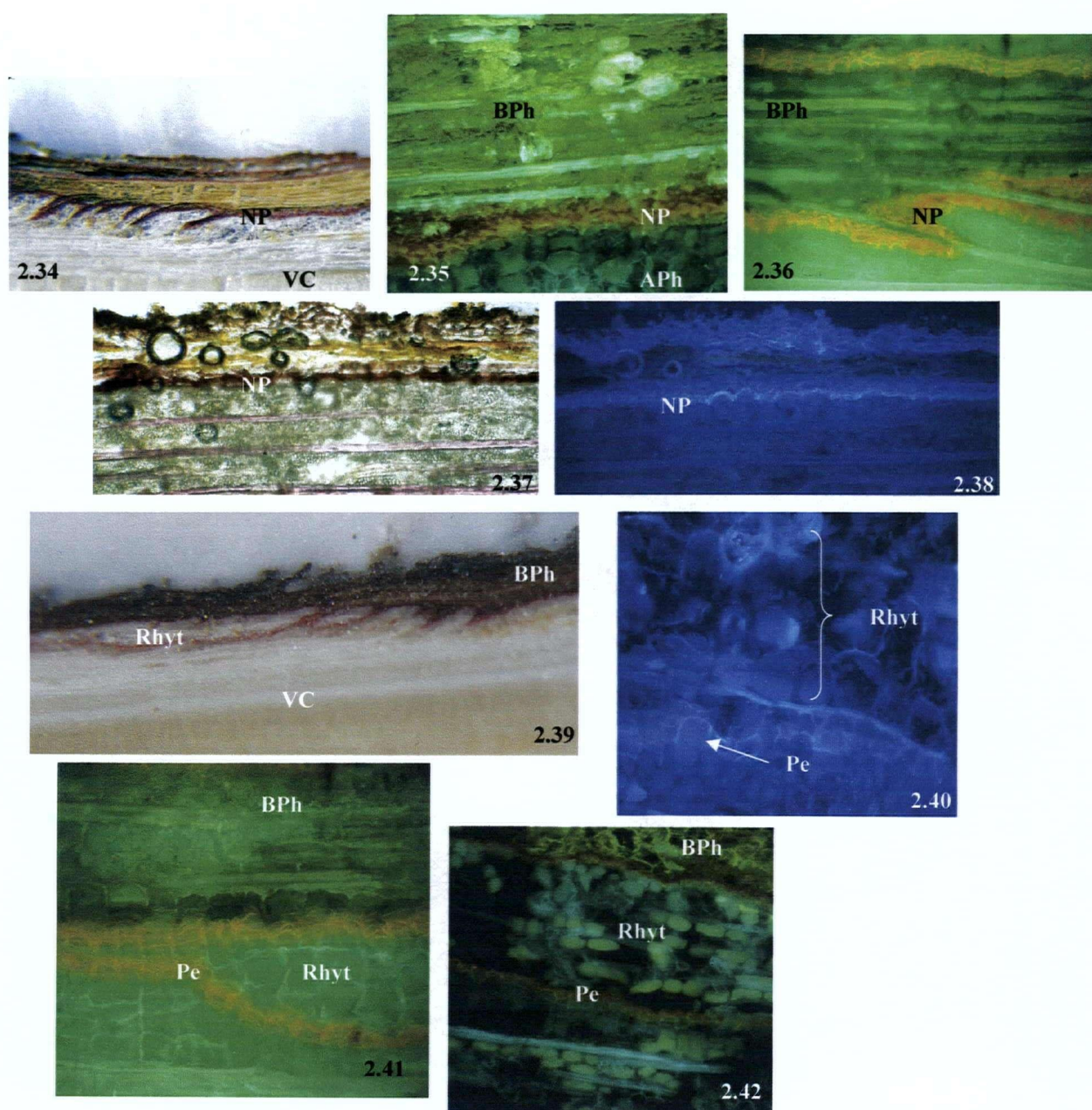
Fig. 2.34. A sample of western redcedar root bark showing freeze-killed tissue to approximately half the bark thickness and a NP bordering injured tissue. Note pigmented phellem wrapped around phloem fibers, x12. **Fig. 2.35.** A cryofixed section of abiotically wounded western redcedar root showing bright yellow-green fluoresced necrotic tissue and a NP with 2 layers of thin-walled phellem. **Fig. 2.36.** A Sudan III treated section of abiotically wounded western redcedar root bark, sampled 9 weeks after wounding, BL. Significantly more phellem production around individual fibers than adjacent areas is seen. **Fig. 2.37.** A phloro-glucinol-HCl treated section of wounded root bark, sampled 5 months following wounding, BF. NIT zone is inconspicuous and masked by lignification in the walls of the thin-walled phellem. **Fig. 2.38.** The same section as in 2.37 but viewed under UV. Lignified, thin-walled phellem now fluoresce bright blue-violet indicating suberization of cell walls. **Fig. 2.39.** A sample of western redcedar root bark showing NP formation around necrotic tissue and successive periderm formation (induced rhytidome formation) in adjacent phloem tissue. The new NP will extend proximally and distally away from the primary lesion to become continuous with the original periderm. **Fig. 2.40.** A cryofixed section of abiotically wounded western redcedar showing a distinct zone of hypertrophy and hyperplasia associated with the induced rhytidome response. Internal to this zone, a meristematic layer of cells produced a single layer of thin-walled phellem,x45, UV. **Fig.2.41.** A Sudan III treated section of tissue showing the suberized phellem formed internal to the induced rhytidome merging with the original periderm at the distal end of the lesion, x 45, BL. **Fig. 2.42.** A cryofixed section of Fig. 2.40 showing a second periderm resulting from induced rhytidome formation deeper in the bark tissue. Eventually all cells external to the last formed periderm become moribund and fluoresce bright yellow-green, BL.

2.3.4. CONTROL BLOCKS

A total of 46 control blocks (uncolonized segments of Garry oak branchwood) were used in the Kingfisher trial and a total of 24 control blocks were used in the Nakusp trial. Control blocks were placed in early May and sampled at 8-9 weeks, 5 months and 1-year later. Table 2.4 shows the number of control blocks used in both inoculation trials for each species, the number of control blocks that became colonized by on-site inoculum, the percentage of roots that showed lesions at root surface in contact with the control block, their corresponding lesion length and host response to injury

Table 2.4. The total number of control blocks in both the Kingfisher and Nakusp field inoculation trials by species, the number of control blocks colonized by on-site inoculum, and the number of roots that resulted in a lesion in the bark at surface contact with the control block.

Host Species	No. Control blocks	No. control blocks colonized by *Armillaria*	No. of roots with lesion at root contact	Lesion length (mm)	Host response
Douglas-fir	27	1	1	10	NP
Western hemlock	18	0	3	5-18	NP
Western redcedar	25	0	1	5	NP

Only one control block was found to be fully colonized by an *Armillaria* species. At the time of sampling (1 year) this block produced fresh rhizomorphs at the cambial edges and through cracks in the bark along the length of the unit. The fungus isolated in culture showed prolific monopodial branched rhizomorphs (Figure 2.43). This isolate was somatically incompatible with isolate 87-01 (*A. ostoyae*) and produced a typical demarcation line in the agar when tested in dual culture (Figure 2.44). The control block

was apparently colonized by on-site *A. sinapina* inoculum. No infection was observed in the host root associated with the control block colonized by *A. sinapina*.

In field inoculation trials involving western larch and Douglas-fir, Robinson (1997) reported similar results whereby control blocks became colonized by naturally occurring *A. sinapina* which subsequently failed to infect the host root. Rhizomorphs of the fungus were mostly superficial on the surface of the host roots (Robinson 1997).

Lesions in the bark at contact with a control block were found only on only 1 of 27 (4%) and 1 of 25 (4%) of the Douglas-fir and western redcedar roots respectively. In both cases, the lesion length was usually less than 10 mm. Three of the 18 (16.7%) western hemlock roots treated with the control had shallow lesions, approximately one-third the thickness of the bark and lesion length varied between 5-18 mm. In all cases, the outer periderm did not appear to be ruptured and any evidence of physical injury to the root was lacking. However, slight browning of the inner phloem tissue was evident and when examined microscopically, phloem tissue appeared to be slightly hypertrophied and fluoresced bright yellow-green under BL. Further examination of a western hemlock root sampled at 9 weeks that showed slight browning of the inner phloem tissue at surface contact with the control block revealed a distinct zone of NIT and 3-4 layers of thin-walled phellem (Figure 2.45). All roots that showed abnormal discoloration in the phloem (i.e. browning) formed a NP in response to injury.
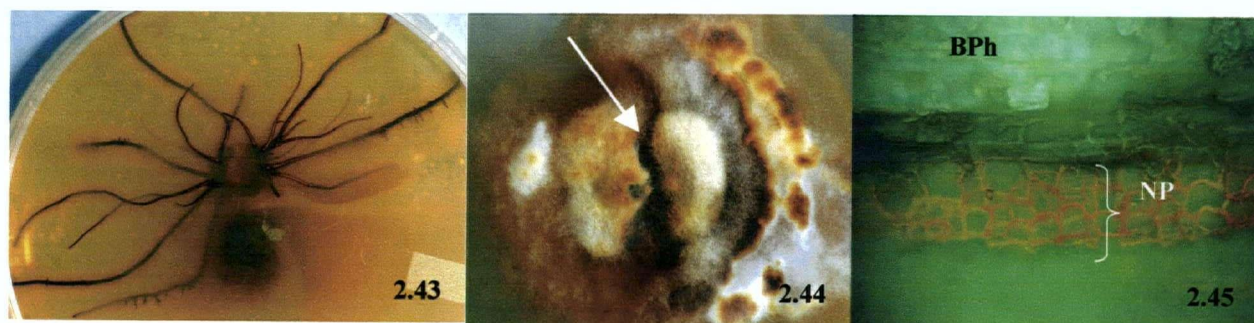
Fig. 2.43. Monopodially branched rhizomorphs typical of *A. sinapina* produced in culture from an isolate from the control block. **Fig. 2.44.** Incompatibility reaction in dual culture between the isolate obtained from the control block and isolate 87-01 used in the field trials. A dark pigmented zone developed in the medium between the two opposing mycelia (white arrow) and radial growth became flattened in the zone of inhibition. **Fig. 2.45.** A Sudan III treated section of a western hemlock root showing slight browning of phloem tissue at surface contact with a control block after 9 weeks. Phellogen restoration was complete and at least 3-4 layers of thin-walled phellem were formed. BL, x 45.

Only 5 of 70 (7%) of the control blocks caused lesions in the phloem underlying the block. Since the large majority of roots treated with controls showed no evidence of lesions in the underlying phloem and no blocks showed evidence of physical disruption of the outer bark tissues, the probability is low that inoculations with *A. ostoyae* were facilitated by initial injury to the root. Fungal infection usually takes longer than 2 months to occur given the time required for rhizomorphs to grow and adhere to the outer surface of the root. If a root was injured at the time of inoculation, prior to infection by the fungus, it is more likely that a NP would be formed within the first 5 weeks following block placement and the NP would be evident upon examination. It is possible that the mere pressure of a block placed against a root may cause slight injury to a few layer of cells underlying the phellogen, particularly during times of elevated cambial activity (i.e. at the onset of the annual period of tree growth) when tissues are loosely arranged and conceivably more susceptible to damage.

2.3.5. HOST RESPONSE TO INOCULATION WITH *A. OSTOYAE* IN ROOTS

During this study, infected lesions on several roots of each of the species studied were harvested at various intervals following inoculation with *A. ostoyae* in order to permit characterization over time of the host responses to infection and lesion development on roots on the three conifer species (Table 2.2).

Infected roots of Douglas-fir and western hemlock commonly exuded resin on the surface of the root (Figure 2.46 and 2.47). Discoloration on the external surface of the bark was sometimes noticeable, delineating the zone of necrotic tissue underlying the periderm (Figure 2.47). Western redcedar roots did not produce surface resin in response to infection and discolouration of the outer bark was difficult to detect. However, infected cedar roots sometimes exhibited irregular swellings usually associated with the induced rhytidome response following invasion by the fungus (Figure 2.48).

The mode of penetration by *A. ostoyae* rhizomorphs in all hosts appeared to follow the generally accepted pattern described by Thomas (1934). Rhizomorphs adhered to the

outer surface of the root and formed a lateral branch that penetrated the phellem as a larger unit (Figure 2.49). Typically, the phellogen remained unharmed (i.e. still functional) until the penetrating mycelia degraded the most recent layers of phellem and exposed the living meristem. However, at times there was evidence of suppression of phellogen activity in the area underlying a penetrating rhizomorph and/or stimulation of phellogen activity immediately adjacent to the penetrating rhizomorph (Fig. 2.50). This reaction was observed more often on Douglas-fir than in other conifers. The phloem tissue underlying a penetrating rhizomorph appeared deformed indicative of mechanical pressure exerted on those cells during the initial penetration phase.

Infection also resulted without direct penetration by a rhizomorph. These reactions were typically seen as small necrotic lesions in the bark and always occurred directly underneath the cambial end of the inoculum block (Fig. 2.51). Infections of this type were also associated with resin. Both mechanisms of entry (direct and indirect) resulted in the collapse of underlying host cells. Approximately 36% (n=143) of the total number of roots sampled in the 2003 and 2004 field trials showed indirect penetration of the fungus whereas the majority of lesions on roots were rhizomorph initiated. However, considerably more western hemlock and western redcedar roots showed evidence of indirect penetration by *A. ostoyae* than did Douglas-fir. Whitney *et al.* (1989) also reported non-rhizomorph infections in conifers following inoculation with *A. ostoyae*.

The above results are interpreted to indicate that the healthy bark of roots will react to toxic substances produced by the fungus (i.e. extracellular cell-wall degrading enzymes or toxic metabolites, or both) exuding from the cambial end of the inoculum block as it situated against a root. Similarly, Wargo (1983) suggested that extracellular secretions of laccase and peroxidase causes the browning of tissue in advance of a penetrating mycelia.

Once the fungus penetrated the periderm, the phellogen and underlying phloem were killed. Lateral and tangential proliferation of mycelial fans or wedges of mycelium were seen colonizing inner bark tissue and often advanced to the vascular cambium (Figure 2.52). The fungus always killed tissue in advance of the mycelium and lateral spread of mycelia in the bark always preceded cambial colonization (Figure 2.53 and 2.54).
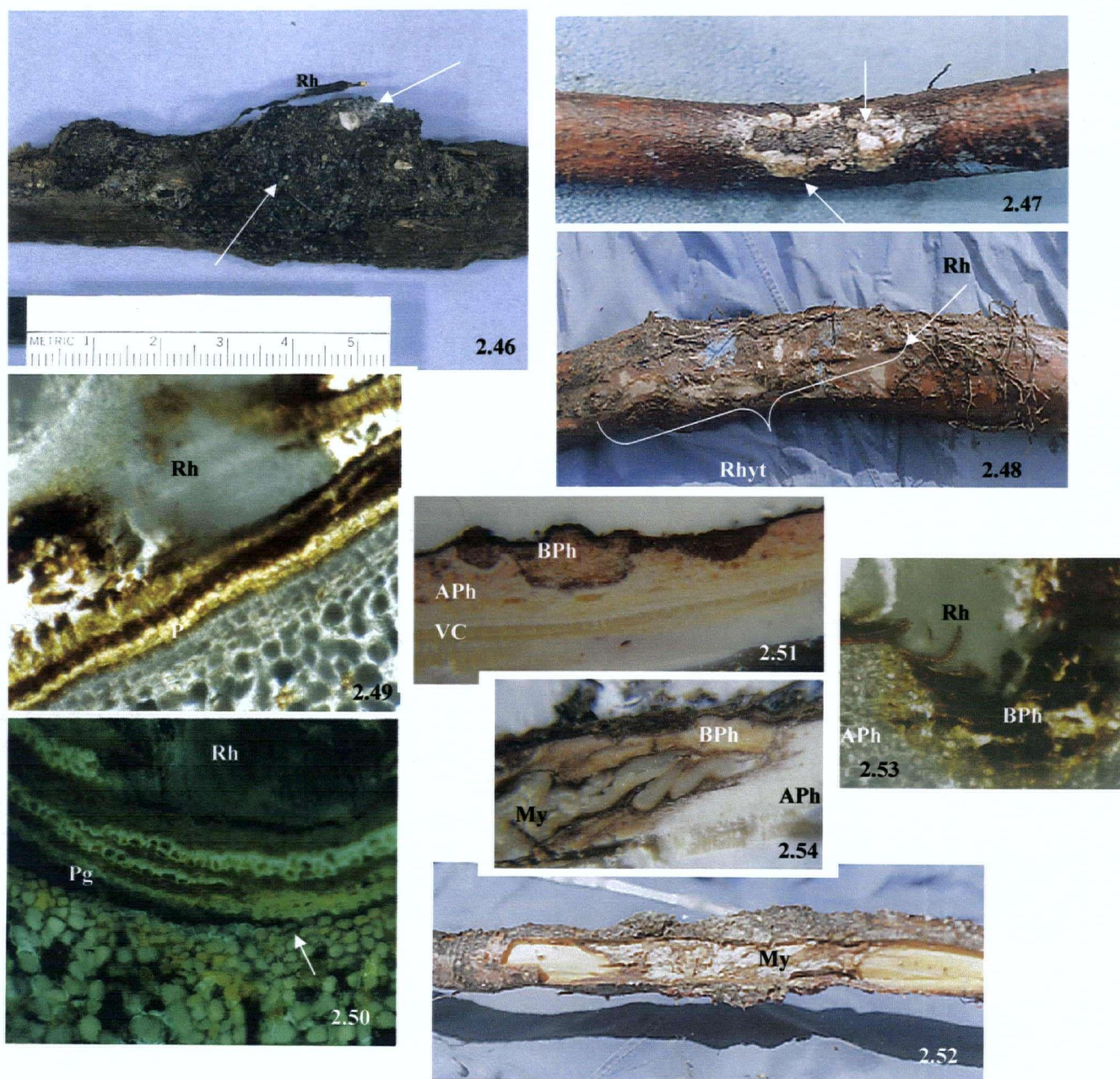
Fig. 2.46. A photomacrograph of an infected Douglas-fir root showing resin exudation (arrows) on the surface of the root 1 year following inoculation with *A. ostoyae*. **Fig. 2.47.** A photomacrograph of an infected western hemlock root showing resinosus (arrows) on the surface of the root 5 months following inoculation with *A. ostoyae*. Note darker discoloration of the bark showing the extent of necrosis in the underlying phloem. **Fig. 2.48.** A photomacrograph of a western redcedar root showing uneven irregularities on the surface of the roots. Note rhizomorph adhering to the outer surface. **Fig. 2.49**. A cryofixed section of a Douglas-fir root showing a lateral branch of a rhizomorph penetrating the outer cork layer, BF. **Fig. 2.50.** A cryofixed section of a Douglas-fir root showing a rhizomorph adhering to the outer bark surface. A narrow zone of phellogen activity (typically 1 cell layer wide) was seen in the area immediately underlying the rhizomorph (arrow) compared to a more active phellogen zone (2-4 cells wide) on either side of the rhizomorph, BL. **Fig 2.51**. A western hemlock root showing a distinct zone of necrotic tissue resulting from inoculation with *A. ostoyae*. Fungal mycelia are absent and necrosis likely resulted from secretions of fungal enzymes/toxins from the cambial surface of the inoculum block. **Fig. 2.52.** A photomacrograph of an *Armillaria*-lesion on a Douglas-fir root showing mycelial fans and the extent of cambial necrosis in the root. **Fig. 2.53.** A mycelial fan invading the phloem of a western hemlock root. Distinct browning of tissue occurs ahead of mycelial colonization. **Fig. 2.54**. A Douglas-fir root showing progressive browning of host tissue ahead of the penetrating mycelial fan.

2.3.5.1. CHARACTERIZATION OF THE DIFFERENT STAGES OF HOST RESPONSE TO INFECTION

Following invasion of *A. ostoyae* in host roots, trees attempt to contain the fungus by initiating defense response involving NP formation in the bark and/or barrier zone formation in the wood. The ability of trees to successfully contain the fungus will depend on the speed of formation and efficacy of these barriers and the degree of interference by the fungus. This process, to some degree, depends on the relative susceptibility of the host, host genetics, the inoculum potential of the fungus, and environmental factors such as low temperature and drought stress. Susceptibility occurs when the pathogen is able to overcome non-specific defense responses associated with wound healing.

A key event in the development of a new periderm barrier in the bark is the early formation of an impervious zone in the vicinity of infected tissue. Deposition of lignins and cell wall thickening occur concomitantly in a zone of dedifferentiating tissue, called NIT in this thesis.   If the host fails to trigger the development of NIT, the fungus is commonly seen to be advancing in the inner bark tissue with no visible host response. The classification of reaction as 'no or ineffective host response' in this thesis was done on the basis of tissues either lacking cell hypertrophy altogether or appearing as irregularly hypertrophied but ineffective lacking distinctive microscopic characteristics indicative of a zone of NIT including strong lignification in cells walls following staining with phloroglucinol + HCl and cell wall fluorescence in the zone of hypertrophy.

A zone of NIT can be formed initially, but then the fungus can breach the barrier either directly through mature NIT or by circumventing the zone of NIT where dedifferentiation of tissue is incomplete (i.e. through clusters of sclereids, at the junction of the original periderm, or at the junction with the vascular cambium). The same can be said for NP formation. In most cases, NIT and NP do not form synchronously along the whole necrotic boundary zone. The cells and subsequent tissues form first in the mid-phloem between the wound edge and the vascular cambium and last in the region of the original phellogen. Therefore, within the same histological section, one can often view wound tissues ranging from no visible host reaction to those exhibiting NIT regeneration and NP

formation. In addition, any breaching of barriers detected in samples harvested on different dates may not be the final outcome in terms of lesion development, particularly for those samples harvested within the first 5-11 weeks following inoculation which presumably would be the early stages of infection on the host roots. It is possible that continual formation and breaching of barriers may occur before the outcome of the infection event is conclusively determined. Nevertheless, roots harvested at 5 months, coincident with the end of the growing season, and 1-year following inoculation should give a good indication of the probable progression of the fungus in host tissue in the early stages of infection and its ability to contain the fungus to a lesion on the root.

Cambial invasion occurs in one of three ways: (1) when the fungus progresses through the inner bark and precludes the development of NIT or NP, eventually reaching the cambium; (2) when NIT is initiated, then breached before a NP develops and the fungus advances to the vascular cambium; (3) when NIT and NP are formed, then breached either directly through the newly formed barrier or where NIT and NP become discontinuous around clusters of sclereids or at the junction of the vascular cambium. Cambial invasion takes time. In this study, *A. ostoyae* killed the vascular cambium on 11 of 40 (27%) of infected roots harvested at 5-11-weeks and on 70 of 151 (46%) of roots harvested at 5-months or later.

2.3.5.2. MODEL OF HOST-PATHOGEN INTERACTIONS

Figure 2.55 illustrates a schematic representation of the non-specific responses associated with wounding or pathogenic invasion of the bark and cambial tissue and the variation in the type of host response induced with the depth of injury on the species examined in this study. This model of non-specific host response is a modified and expanded version of that presented by Mullick (1977) in Figure 1.1.

Fig. 2.55.1

a)

b)

c)

Fig. 2.55.2

a)

b)

c)
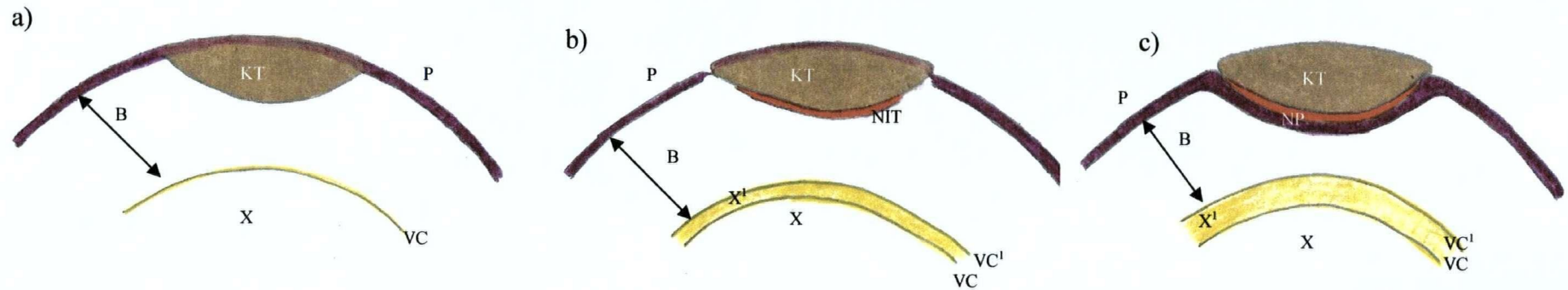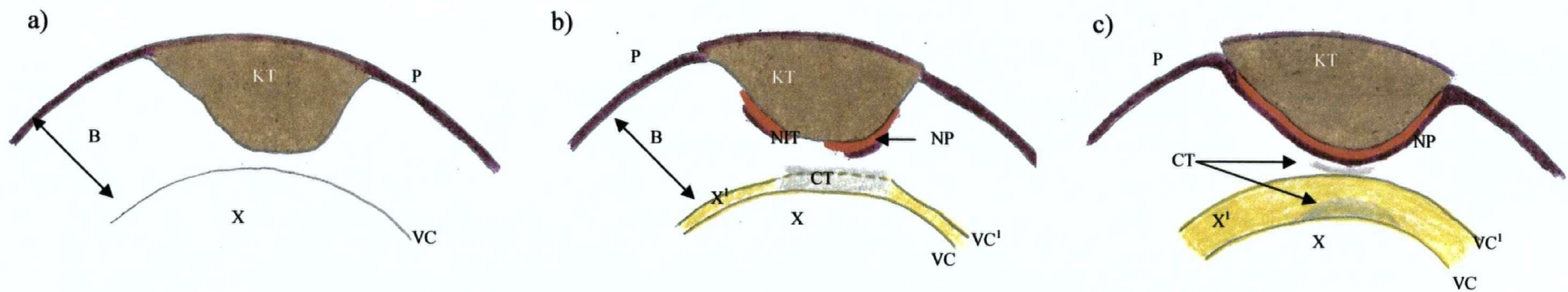
Fig. 2.55. Schematic diagram showing the anatomical model for non-specific defense mechanisms induced following (Fig. 2.55.1) shallow injury to the living bark where the injury is limited to the vicinity of the living phellogen and underlying phloem, (Fig. 2.55.2) deeper injury to the living bark where necrosis extends to tissues in close vicinity, but not directly affecting the vascular cambium, showing modified vascular cambium within the zone of callus and (Fig. 2.55.3.) injury to living phloem, vascular cambium and functional sapwood. Successive letters denote a sequence of time consisting of several weeks. Abbreviations: **B**, bark tissue; **CT**, callus tissue; **KT**, killed tissue; **NP**, necrophylactic periderm; **P**, original periderm; **VC**, original vascular cambium; **VC^1**, new position of vascular cambium; **X**, original xylem; **X^1**, xylem formed since injury
D. decayed wood; **E**, empty space; **TC**, xylem tissue derived from callus; **RD**, traumatic resin ducts.
Note: NIT formation was indiscernable in western redcedar.

Fig. 2.55.3

a)

b)

c)

d)
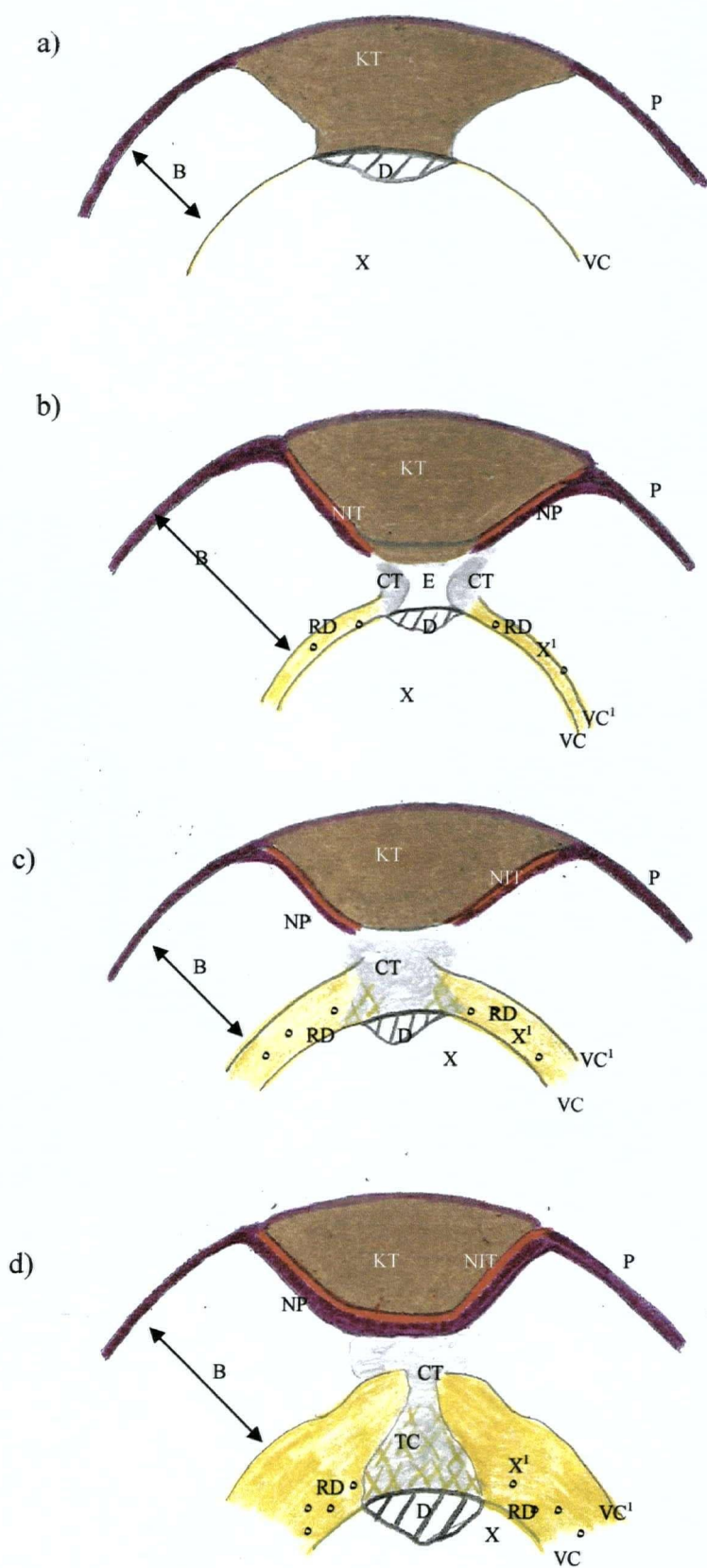
The first processes shown in Figure 2.55.1 (a) shows a shallow injury to the living bark causing a disruption of the living phellogen and is similar to that reported by Mullick (1977) in Figure 1.1 (a). The normal and simplest response following initial penetration of the first few cell layers of the living bark involves the development of NIT and then a NP around the periphery of the wound which has been described in detail above. This new barrier is usually formed first in the central region of the wound (2.55.1 (b)) and eventually becomes continuous with the original periderm and its normal function is resumed (Figure 2.55.1 (c)).

The second process shown in Figure 2.55.2 (a) arises from deeper injuries to the bark, closer to the vascular cambium. Similar to that described by Mullick (1977) in Figure 1.1 (b), deeper injuries in the bark may cause a disruption in the normal cambial activity without any direct injury to the cambium itself. In the central region, the original vascular cambium forms callus tissue instead of normal tracheids and ray parenchyma while xylem and phloem derivatives continue to be produced on either side of the injured tissue (Figure 2.55.2 (b)). Callus (undifferentiated, isodiametric parenchyma) arises from increased divisions of the cambial intials as well as recent phloem derivatives. In contrast to what Mullick (1977) describes, sporadic zones of NIT and NP may be initiated in the bark at the margin of killed tissue prior to or concurrent with vascular cambium restoration. The NP develops either uniformly or non-uniformly along the periphery of the wound. In the latter case, a NP is usually formed last in the area contiguous with the original periderm. Eventually, the altered vascular cambium resumes normal activity to produce normal xylem and phloem cells and may do so even while a new NP is still differentiating (Figure 2.55.2. (c)). Tissue derived from callus remains embedded within the xylem and over time develops secondary walls and becomes lignified. Sometimes, semi-differentiated or fully differentiated traumatic resin canals may be formed in place of callus in the modified cambial zone in species like Douglas-fir and western hemlock (not shown). These resin canals may extend further tangentially from the modified cambial zone. Hence, when the cambial and xylem surface remains intact underneath a zone of necrotic bark, callus or resin ducts will develop across the periphery of the affected tissue.

The third process shown in Figure 2.55.3 (a) involves deep injuries to the sapwood in which vascular cambium and the outer xylem (ray parenchyma) below the dead vascular cambium are also killed. The interpretation of the sequence of events involved in wound healing (e.g. callus formation and regeneration of vascular cambium) is an expanded version of that described by Mullick (1977) in Figure 1.1. In addition to NP formation in the bark, the living vascular cambium at the periphery of the wound will form callus by progressive hyperplasia of parenchyma cells in the uninjured cambial zone (Figure 2.55.3 (b)). Callus originates mainly from the uninjured vascular cambium immediately adjacent to killed cambial tissue, but sometimes phloem parenchyma cells in close proximity to the vascular cambium can also participate in the formation of callus. Dead tissue will remain between the two callus curls and sometimes as the wounded bark tissue is lifted as a result of tissue expansion over the face of the wound, an empty space will occur. Callus curls will eventually merge over the wounded cambium which can be seen as a zone of undifferentiated parenchyma external to the killed cambium (Figure 2.55.3 (c)). NIT formation followed by phellogen restoration occurs in the outer part of the callus tissue and meristematic tissue forms as a replacement of the vascular cambium in the inner part of the callus, usually starting in the callus tissue contiguous with the detached ends of the original vascular cambium and then eventually meeting in the middle (Figure 2.55.3 (d)). Soon after the formation of these meristems, derivative tissues are produced as phellem and phelloderm to the outside and inside of the phellogen, and phloem and xylem elements to the outside and inside of the vascular cambium. In some species like Douglas-fir and western hemlock, traumatic resin ducts (TD) may be produced instead of normal tracheids in xylem (Figure 2.55.3 (b-d)). In others like western red cedar, a zone of axial parenchyma is formed in the xylem immediately adjacent to the killed cambium (not shown). The tissue derived from callus in the xylem area develops secondary, lignified cell walls. At this point, this tissue no longer participates in active division, except perhaps at the outermost edge of the lesion or the callus curl where cells may still be non-lignified and actively dividing. In most lesions, the callus tissue bridges the surface of the wound and the newly regenerated vascular cambium will form a uniform meristematic layer. Some callus tissue located in the modified cambial zone will become part of the phloem tissue itself. The tissue

derived from callus which ends up in the phloem is more difficult to detect than callus tissue which remains embedded in the xylem. No specific tests were conducted to determine the extent of non-conductive sapwood as reported in Mullick (1977) although it's assumed that following direct injury to the vascular cambium, trees will undergo the process of compartmentalization according to the CODIT model proposed by Shigo and Marx (1977).

Douglas-fir

Roots of Douglas-fir typically showed an initial host reaction involving resinosus in the bark which commonly exuded to the outer surface of the rhytidome. Large wedges of mycelium were often found interspersed with clumps of resin in the necrotic tissue. However, the presence of resin did little to deter the fungus from advancing to adjacent host tissue. The fungus killed host tissue ahead of mycelial colonization and on several occasions advanced rapidly enough to preclude the initiation of any host defenses leading to NP formation.

At any one sampling date, a percentage of Douglas-fir roots infected with *A. ostoyae* showed no visible or ineffective host response as the fungus advanced in the bark. In the Hidden Lake trial, host reactions were not initiated on 2 of 9 (22%) and on 3 of 10 (30%) of infected Douglas-fir roots sampled at 11 weeks and 1 year, respectively (Appendix III). In subsequent trials, between 50-100% (n=36) of Douglas-fir roots that showed initial penetration by the fungus showed no host response to infection. The adjacent phloem appeared brown and either lacked significant hypertrophy or appeared irregularly hypertrophied. Host reactions which failed to develop NIT typically showed a distinct zone of necrotic tissue. The boundary between the necrotic zone and the apparently healthy tissue at the infection front was either abrupt (Figure 2.56) or diffuse (Figure 2.57). In more advanced stages of infection, the fungus decayed tissue in the area underlying the initial site of penetration and necrosis continued to expand in the bark and cambial zone (Figure 2.58).

When viewed under fluorescence, infected tissue appeared as an amorphous mass of cells with bright yellow-green cell walls (Figure 2.56). Progressive lesions commonly showed more advanced stages of tissue degradation. This stage was characterized by a zone of cells devoid of contents with bright yellow cell walls bordering a zone of moribund cells with sporadic hypertrophy and intercellular spaces stained yellow-brown (Figure 2.59). Despite the hypertrophy in the zone of necrosis or at the infection front, sections did not stain positively for lignin following phloroglucinol-HCl treatment which would otherwise indicate NIT development.

The occurrence of NIT in infected Douglas-fir roots was quite variable. Early stages of NIT development were recognized under BL by cell wall fluorescence along the outer perimeter of the zone of dedifferentiation. However, this was rarely observed, probably due to the timing of sampling. Distinct zones of NIT were more recognizable following staining with phloroglucinol-HCl or under fluorescence as the abrupt zone of cell hypertrophy immediately external to a zone of redifferentiation (Figure 2.60). Even after 1 year, NIT developed in only 15 of 34 (44%) of the roots sampled. Since NIT invariably precedes NP formation, more than half the infected Douglas-fir roots had not contained their lesions in the bark. The low frequency of NIT formation in roots infected with *A. ostoyae* permitted the fungus to advance to the cambium. Of the 34 roots sampled at 1 year that showed successful penetrations, bark tissues in 13 (38.2%) were killed to the vascular cambium and none of these showed any evidence of compartmentalization and callusing (Appendix III).

In Douglas-fir, sporadic lignification of adjacent phloem tissue was sometimes seen in advance of a penetrating mycelium. However, distinct zones of NIT were frequently lacking or the fungus penetrated beyond the developing NIT or both. Breaching of NIT was frequently observed in Douglas-fir roots. NIT generally developed first in the mid-phloem area underlying or adjacent to a zone of necrotic tissue and formed last at the junction of the original periderm. Breaching of NIT usually occurred in areas where zones of dedifferentiation were incomplete (e.g. at the original periderm) or deeper in the bark tissue at the junction with the vascular cambium.
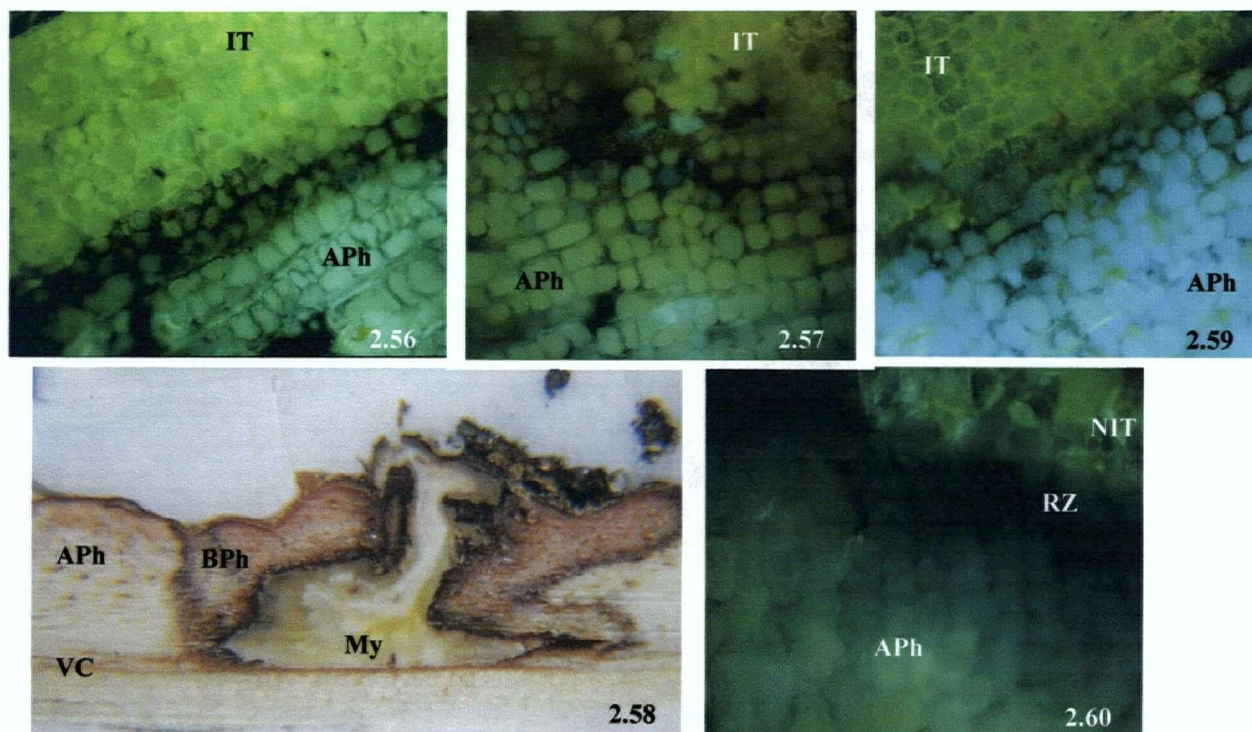
Fig. 2.56. A cryofixed section of a Douglas-fir root inoculated with *A. ostoyae* showing an abrupt demarcation between necrotic and adjacent healthy tissue at the infection front. Necrotic tissue has cell walls that fluoresce bright yellow-green under BL. **Fig. 2.57.** Another cryofixed section from an infected Douglas-fir root showing a diffuse boundary between necrotic and adjacent healthy tissue at the infection front, BL. **Fig. 2.58.** A photomacrograph of a Douglas-fir root showing a large wedge of mycelium invading the bark and cambial zone. **Fig. 2.59.** A cryofixed section of Douglas-fir root showing a progressive lesion at the infection front. Cells in the adjacent phloem appear moribund as cells show sporadic hypertrophy and intercellular spaces are stained yellow-brown, BL. **Fig. 2.60.** A cryofixed section of a Douglas-fir root showing the early stages of redifferentiation as a zone of non-fluorescence internal to a zone of hypertrophied tissue (NIT), BL; x45.

In tissue sections showing breaching of NIT, NIT typically appeared as an incomplete zone of hypertrophied, heavily lignified parenchyma and further browning of cells was observed deeper into the phloem. Interestingly, the number of cell layers involved in NIT development was noticeably higher in tissue infected with *A. ostoyae* (Figure 2.61) compared to that which develops following abiotic wounding (Figure 2.62).

The above results are interpreted to indicate that the increased frequency of lignified cell layers involved in NIT development may be a delayed response to NP formation under the influence of an advancing fungus. Wahlström and Johansson (1992) reported similar results in *Pinus sylvesteris* seedlings mechanically wounded and inoculated with *A.*

ostoyae. More cell layers were lignified in roots challenged by *A. ostoyae*, than non-challenged roots (Wahlström and Johansson 1992).

The breaching of NIT may depend on its speed of formation relative to the speed at which the fungus or the toxins produced by the fungus moves through host tissue. In this study, all species showed breaching of NIT although breaching was consistently more frequent in Douglas-fir and western hemlock than western redcedar (Appendix III).

NP formation in Douglas-fir roots occurred in only 20 of 54 (37%) of roots that had successful penetrations. A newly restored phellogen produced layers of thin-walled phellem that ranged between 2-4 cells wide at 11 weeks (Figure 2.63), 3-6 cells wide after 5 months, and 4-7 cells wide after 1 year (Figure 2.64). Stone phellem was not always associated with NP's, however when it was present, periderms had between 2-6 layers of thick-walled phellem with thin-walled phellem internally abutting it.

More than half of the NPs that initially formed on roots were breached by the fungus. The resulting lesion exhibited either an abrupt or diffuse boundary of moribund and healthy phloem. Cells internal to breached barriers displayed irregular hypertrophy and fluorescent yellow-green cell walls when viewed under BL. Following the breaching of NP's, second attempts at NIT formation were seen in only a few roots as a faint staining for lignin in the adjacent hypertrophied phloem. However, in all cases the host was unsuccessful at containing the infection within a new periderm barrier within the time frame from which it was sampled (i.e. at 1 year). The host might have attempted NP formation in the subsequent growing season, although if the fungus was rapidly advancing in the inner bark and cambial tissue, changes in inoculum potential would most certainly occur as the fungus utilizes more and more host tissue to the point where the fungus might overwhelm any type of host response to contain the infection. The fungus may colonize the root distally and infection may or may not be checked by the host when the fungus reaches the junction of a larger diameter root or the root collar (Figure 2.65).

Overall, *A. ostoyae* advanced to and killed the vascular cambium in 20 of 54 (37%) of the Douglas-fir roots that showed successful penetration by the fungus. In all situations, cambial invasion was observed only on those roots harvested at 5 months and 1 year following inoculation (Appendix III). Therefore, cambial invasion in Douglas-fir depended on time since infection. Of the number of roots with killed cambium only 2 of 20 (10%) showed evidence of compartmentalization and callusing.

Cross-sectional views of a barrier zone formed in a Douglas-fir root 5 months following inoculation revealed that the cambium was injured mid-season as evidenced by the location of the traumatic resin canals in the middle of that year's annual growth ring (Figure 2.66). Traumatic resin canals typically occurred in tangential bands. Epithelial cells surrounded the large lumen of individual resin ducts and the cells surrounding the traumatic resin ducts had pigmented deposits. Ray parenchyma adjacent to resin ducts in the vicinity of the injured cambium also had pigmented contents. Resin duct formation was disorganized in the area adjacent to where a new vascular cambium differentiated within the callus (Figure 2.67).

The tracheids formed after the tangential series of resin canals showed similar disorganization. Several rows of tracheids in the earlywood zone were slightly disoriented and some cells appeared to be occluded. The tracheids formed by the newly differentiated cambium became more organized with increasing distance from the callus edge. The xylem underlying the area of killed cambium showed slight discoloration.

A. ostoyae interfered with the initiation of active defense mechanisms involving NP formation and compartmentalization in more than half the infected Douglas-fir roots. The remaining roots responded by forming NIT and NP adjacent to the advancing fungus in the bark. However, breaching of these barriers and absence of compartmentalization in roots following cambial invasion enabled the fungus to progressively develop in the roots.
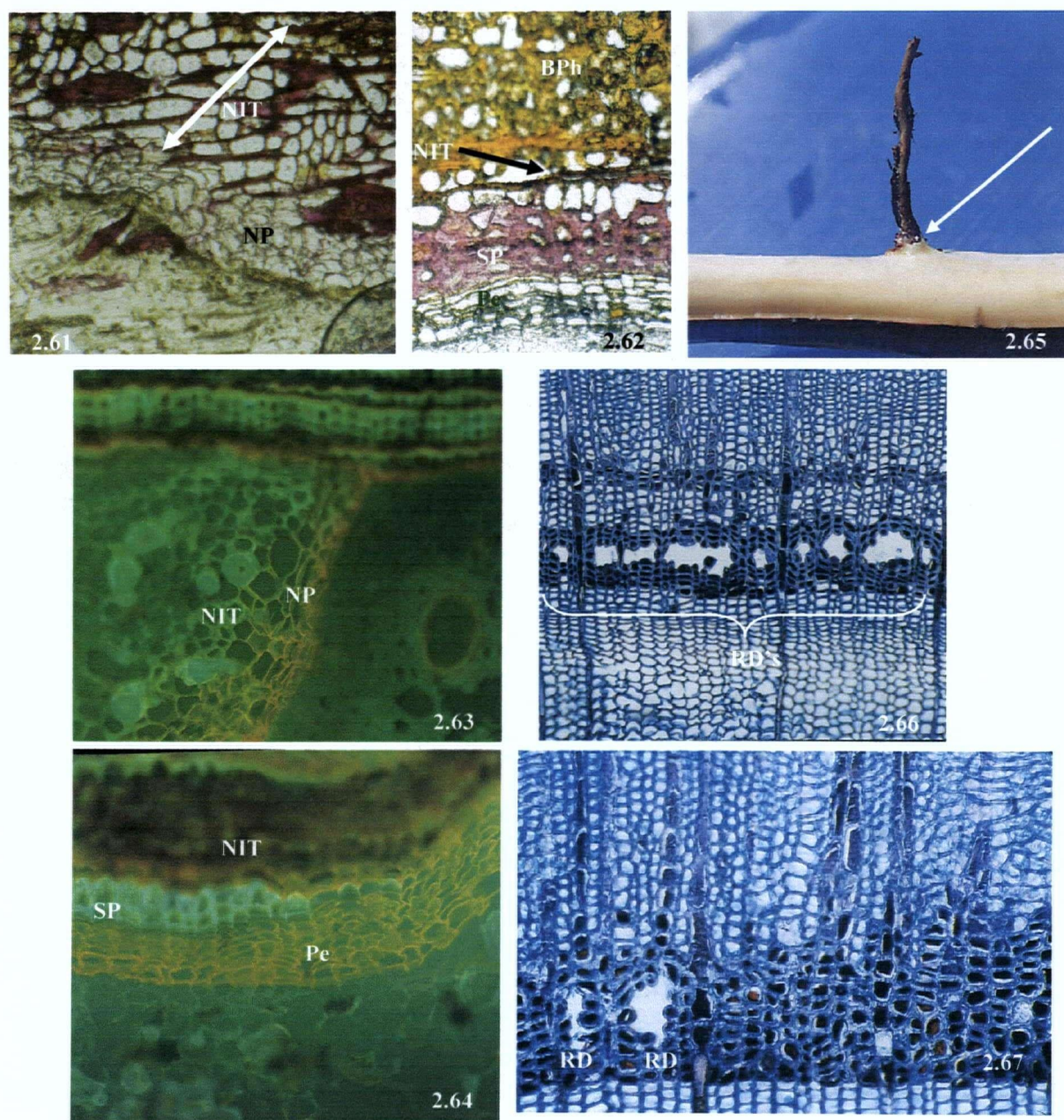
Fig. 2.61. A phloroglucinol-HCl treated section of a Douglas-fir root inoculated with *A. ostoyae* showing a distinct zone of NIT comprised of several cell layers, BF. **Fig. 2.62.** Phloroglucinol-HCl treated section of abiotically wounded Douglas-fir root showing the lignified NIT zone comprising only 1-2 cell layers followed by three layers of stone phellem, BF. **Fig. 2.63.** A Sudan III treated section of Douglas-fir root 11 weeks following inoculation with *A. ostoyae* showing a newly restored periderm with up to 4 layers of thin-walled, suberized phellem. Note resin blister in the adjacent phloem with epithelial cells also staining positively for suberin, BL. **Fig. 2.64.** 1-year following inoculation with *A. ostoyae*, a newly restored phellogen produced up to 7 layers of thin-walled phellem. Note the stone phellem externally abutting the thin-walled phellem, BL. **Fig. 2.65.** A photomacrograph of a Douglas-fir root with its bark removed showing a smaller secondary root colonized by the fungus distally and the infection checked (compartmentalized) at the junction of the larger diameter root. **Fig. 2.66.** A paraffin embedded section of a Douglas-fir root showing a barrier zone formed by the uninjured cambium comprised of a tangential series of traumatic resin ducts following invasion by *A. ostoyae*. The surrounding axial and ray parenchyma appear occluded with polyphenolic bodies. The vascular cambium (not shown) lies above the tracheids in this micrograph. **Fig. 2.67.** Immediately adjacent to the killed vascular cambium, resin duct formation appears disorganized and is comprised of polyphenolic-rich axial parenchyma; deposits also accumulate in the ray cells. The vascular cambium (not shown) lies above the tracheid and ray parenchyma in this micrograph.

Western hemlock

Resinosus on the surface of the bark was also a common symptom of an *A. ostoyae* infection in roots of western hemlock trees (Figure 2.68). Similar to Douglas-fir, resin soaked phloem apparently did little to deter advance of the fungus in the host. On several root samples, the fungus appeared to have advanced fairly rapidly as evidenced by the extent of lateral spread in bark tissue, thin mycelial fans, and a low frequency of roots that initiated defense responses leading to NP formation in the bark. Phloem necrosis always occurred ahead of mycelial colonization (Figure 2.69 and 2.70).
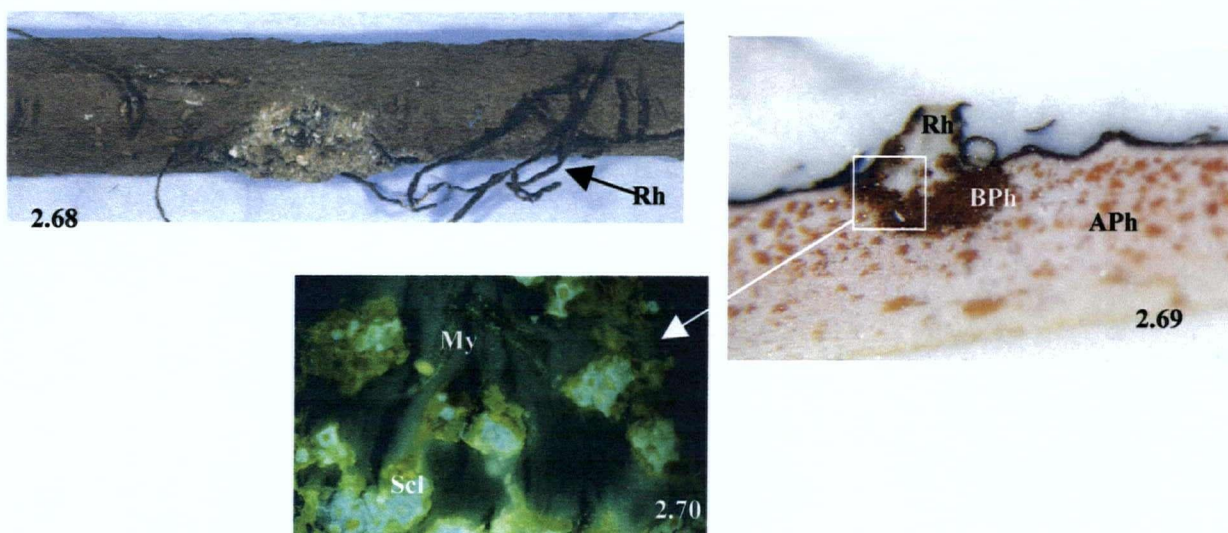
Fig. 2.68. A western hemlock root showing resin exudation and rhizomorphs on the root surface following inoculation with *A. ostoyae*. **Fig. 2.69.** A photomacrograph of western hemlock root showing rhizomorph penetration and necrosis of the inner bark. **Fig. 2.70.** A cryofixed section of Fig. 2.69 showing the large mycelial fans degrading and digesting the phloem tissue following invasion by the fungus, BL.

The proportion of hemlock roots showing no host response at the infection front varied between sampling dates and field trials (Appendix III). Overall, 29 of 80 (36%) of western hemlock roots showed no visible host response following penetration by *A. ostoyae* (refer to earlier definition of 'no host response' on page 80). Cell hypertrophy in roots lacking any visible host reaction was variable. The host either showed no cell hypertrophy at the infection front, irregular hypertrophy, or significant hypertrophy in advance of a penetrating mycelium (Figures 2.71-2.73). Roots showing massive zones of bark hypertrophy did not stain positively for lignin following treatment with

phloroglucinol-HCl, nor was there any cell wall fluorescence detected in the outer boundary of that zone which otherwise might indicate the early stages of NIT formation. The boundary between the necrotic zone and the adjacent, healthy or moribund phloem was always quite abrupt (Figure 2.74). When viewed under BL or UV, the large zone of killed tissue fluoresced bright yellow-green or blue-violet, respectively (Figures 2.75 and 2.76). In most instances where the host showed no visible host response, the fungus had advanced to and killed the vascular cambium.

Fifty-one of 80 (63%) of western hemlock roots examined throughout this study developed NIT in response to invasion by *A. ostoyae*. Figures 2.77 and 2.78 show a typical zone of NIT in hemlock as an amorphous mass of hypertrophied cells underlying necrotic (browned) tissue. After a zone of dedifferentiation became established, cells internally abutting NIT redifferentiated to form a meristematic phellogen which appeared non-fluorescent when viewed under BL (Figure 2.79).

Like Douglas-fir, the NIT comprised several layers of phloem cells that stained positively for lignin following treatment with phloroglucinol-HCl (Figure 2.80), considerably more layers than were detected in roots with abiotic wounds.

Breaching of NIT was common and was observed on several sampling dates. It occurred at higher frequency than in Douglas-fir (Appendix III). A number of infected hemlock roots showed large zones of induced lignification in phloem tissue and at times, lignification extended for some distance along the tissue sample (Figure 2.81). These observations suggest that the host triggered NIT development and as mycelium advanced, phloem tissue increasingly underwent dedifferentiation in an attempt to form an impervious zone of tissue.
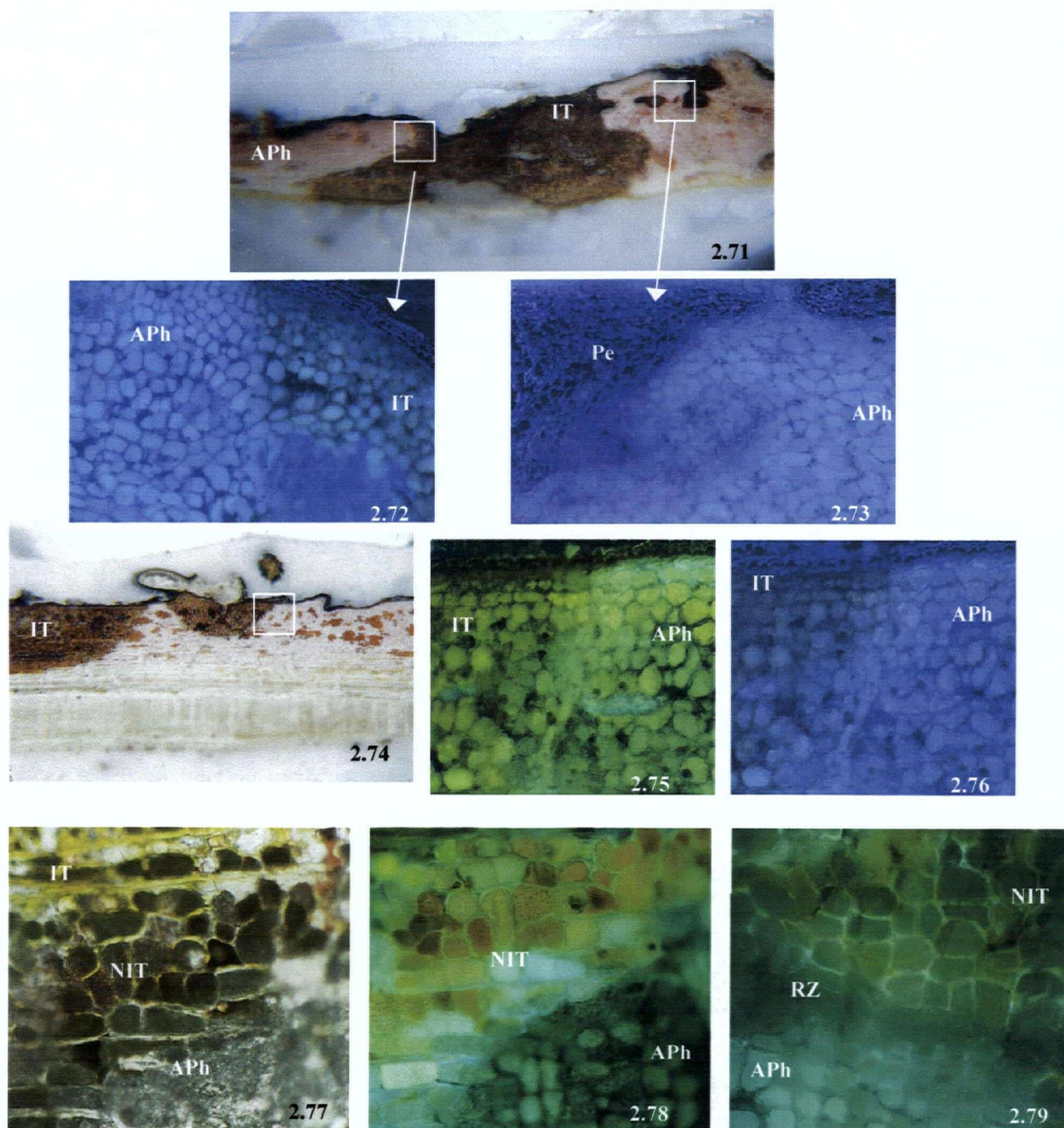
Fig. 2.71. A photomacrograph of a western hemlock root following inoculation with *A. ostoyae* showing a lack of hypertrophy on the proximal infection front and significant hypertrophy at the distal infection front. Note distinct zone of necrosis extended to the depth of the vascular cambium. Mycelial fans and large wedges of resin can be seen throughout. **Fig. 2.72.** A cryofixed section of the proximal infection front shown in Fig. 2.70 showing a lack of hypertrophy at the infection front (no host response) in advance of a penetrating mycelium, UV. **Fig. 2.73.** A cryofixed section of the distal infection front shown in Fig. 2.70 showing significant hypertrophy in the adjacent phloem and small zones of redifferentiated tissue as clusters of phellem embedded within the phloem, UV. **Fig. 2.74.** A photomacrograph of a western hemlock root showing rhizomorph penetration of the inner phloem. The boundary between the necrotic tissue and adjacent healthy tissue is quite abrupt. **Fig. 2.75.** A cryofixed section of Fig. 2.74 showing no visible host response at the infection front (lack of cell hypertrophy in the adjacent phloem), BL. **Fig. 2.76.** The same cryofixed section shown in Fig. 2.75 but view under UV. **Fig. 2.77.** A cryofixed section of a western hemlock root following inoculation with *A. ostoyae* showing a distinct zone of dedifferentiated tissue (NIT) internally abutting a zone of necrosis, BF. **Fig. 2.78.** The same section shown in Fig. 2.77 but viewed under BL. Note cell wall hypertrophy in the hypertrophied phloem and polyphenolic deposits occurring in phloem parenchyma cells. The zone of redifferentiation is not obvious here. **Fig. 2.79.** Another cryofixed section of a western hemlock root showing a distinct zone of redifferentiation internally abutting a zone of NIT. The newly restored phellogen zone appears non-fluorescent in BL.

The above results are consistent with those reported for Scots pine seedlings infected with *A. ostoyae* (Wahlström and Johansson 1992). In this study, the lack of adjoining NIT in the mid- and outer-phloem region allowed the fungus to advance in the bark. Sporadic lignification in hypertrophied phloem tissue was seen underlying infected phloem tissue since this is the area in which NIT is usually initiated. However, distinct zones of NIT were absent at the junction of the original periderm, which enabled the fungus to circumvent the developing NIT and continue to advance in the inner bark. In addition, NIT was regularly incomplete in areas occupied by large clusters of sclereids (Figure 2.82) and necrosis commonly extended deeper into the phloem immediately underlying clusters of sclereids (Figure 2.83).

Of the total number of hemlock roots sampled, more than half formed at least a partial NP. After 11 weeks, the newly restored phellogen had produced 1-3 layers of thin-walled phellem (Figure 2.84). Mature phellem commonly developed pigmented contents and cell walls stained positive for suberin after treatment with Sudan III (Figure 2.85). The number of phellem cells produced by the phellogen ranged between 2-6 and 3-9 after 5 months and 1 year (Figure 2.86), respectively. The wide range of phellem cells produced at the latter harvesting dates was likely a reflection of the timing of infection and the ability of the host to trigger defense reactions leading to NIT and NP formation while under the influence of the fungus.

Stone phellem was observed in only 3 roots that formed a NP. In all three cases, these roots were harvested 1-year following inoculations and root diameters ranged between 3.5-5.5 cm. NP's showed a single band of stone phellem (1-2 cells wide) followed by 1-3 rows of thin-walled phellem (Figure 2.87).

Robinson (1997) suggested that NP's with multiple bands of thick- and thin-walled phellem were a structural characteristic that helped impart increased resistance to the spread of *A. ostoyae* in roots of western larch. In this study, lesion sizes on hemlock were noticeably smaller on roots with a band of thick- and thin-walled phellem compared to roots with NP's consisting of only thin-walled phellem.

Field observations showed small necrotic lesions underlying the cambial edge of the inoculum block where it was situated against the root. It is assumed that there is no penetration by single hyphae and that penetration occurs primarily by mechanical and enzymatic degradation by rhizomorphs. However, in several cases, a penetrating rhizomorph was absent. The necrosis likely resulted from fungal toxins or exudates affecting tissue in the area immediately underlying the inoculum block. Lesions on such roots were commonly contained within a new NP barrier and lesion lengths were considerably smaller compared to lesions where the fungus was present as a large wedge of mycelium or advancing mycelial fan in the bark or cambial zone.

The above results suggest that the lack of the physical presence of the fungus in the host may reduce the inoculum potential of the fungus because fungal toxins may be acting upon the host's defense mechanisms to a lesser degree, enabling the host to have a higher probability of forming a NP than it would under the influence of an advancing mycelium. Rykowski (1975) reported that further stages of penetration of the phloem parenchyma depend not only on host reactions induced in response to infection but also on the morphological form of the infecting mycelium. For example, if after penetration, the mycelium does not keep the rhizomorphous structure, and instead invades the phloem in the form of single hyphae, the fungus may encounter a lysigenous zone that inhibits further ingress of the pathogen and allows host defenses to wall out the pathogen (Kusano 1911 as cited in Rykowski 1980). In this study, visible mycelium occurred as larger units (i.e. mycelial fans/wedges or rhizomorphs) and no further investigation was conducted to determine whether individual hyphae were observed in lesions resulting from indirect penetration.

Fifty-one percent (n=43) of hemlock NPs that initially formed in roots were breached by the fungus (Figure 2.88 and 2.89). Internal to breached periderms a rather diffuse boundary between the necrotic tissue and the adjacent phloem was evident. Adjacent to an expanding zone of necrosis, cells appeared hypertrophied and walls fluoresced bright yellow-green under BL. Again, two possible areas of weakness where the fungus was

able to circumvent the developing or newly formed periderm were identified: at the junction with the vascular cambium and around clusters of sclereids (Figure 2.83).

Oven *et al.* (1999) suggested that neither phloem rays nor groups of sclereids in the bark of European beech lessened the ability of the trees to form a zone of impervious tissue and NP following injury to the bark. Histological evidence in this study revealed that sclereids become part of the NIT zone, as indicated by more intense staining for lignin compared to sclereids found in adjacent healthy tissue. However, staining of secondary phloem parenchyma cells internal to groups of sclereids was not always evident. Impermeability testing of these sclereids comprising the NIT zone was not conducted in this study. Despite the enhanced lignification in sclereids within the zone of NIT, it appeared that fungal toxins were able to pass presumably through the apoplastic pathway or numerous pits found in the cell walls causing necrosis deeper in the bark. These results suggest that the presence of sclereids in the phloem of hemlock delays or results in discontinuities in NIT development and NP formation, allowing the fungus to grow through the bark before these boundaries are complete. A second attempt at NP formation was not observed on any roots examined on the different harvest dates.

A. ostoyae advanced to and killed the vascular cambium in 37 of 80 (46%) of western hemlock roots showing initial penetration by the fungus. Of those roots with killed cambium, only 3 of 37 (8%) showed successful compartmentalization and callusing from the margin of the lesion.

The formation of callus tissue is a normal reaction to repair injured cambia. The cataplasmic structure of callus (i.e. undifferentiated, isodiametric parenchyma) originates as a primary reaction following wounding of the vascular cambium. In the uninjured cambium, elongated fusiform initials are usually the first cells that segment transversely and then segregate into isodiametric parenchyma cells (Fink 1999). However, since most living cells are totipotent (i.e. have the ability to reset their developmental program and differentiate into new cell types), a variety of cell types in the xylem and phloem may actively contribute to the formation of callus.
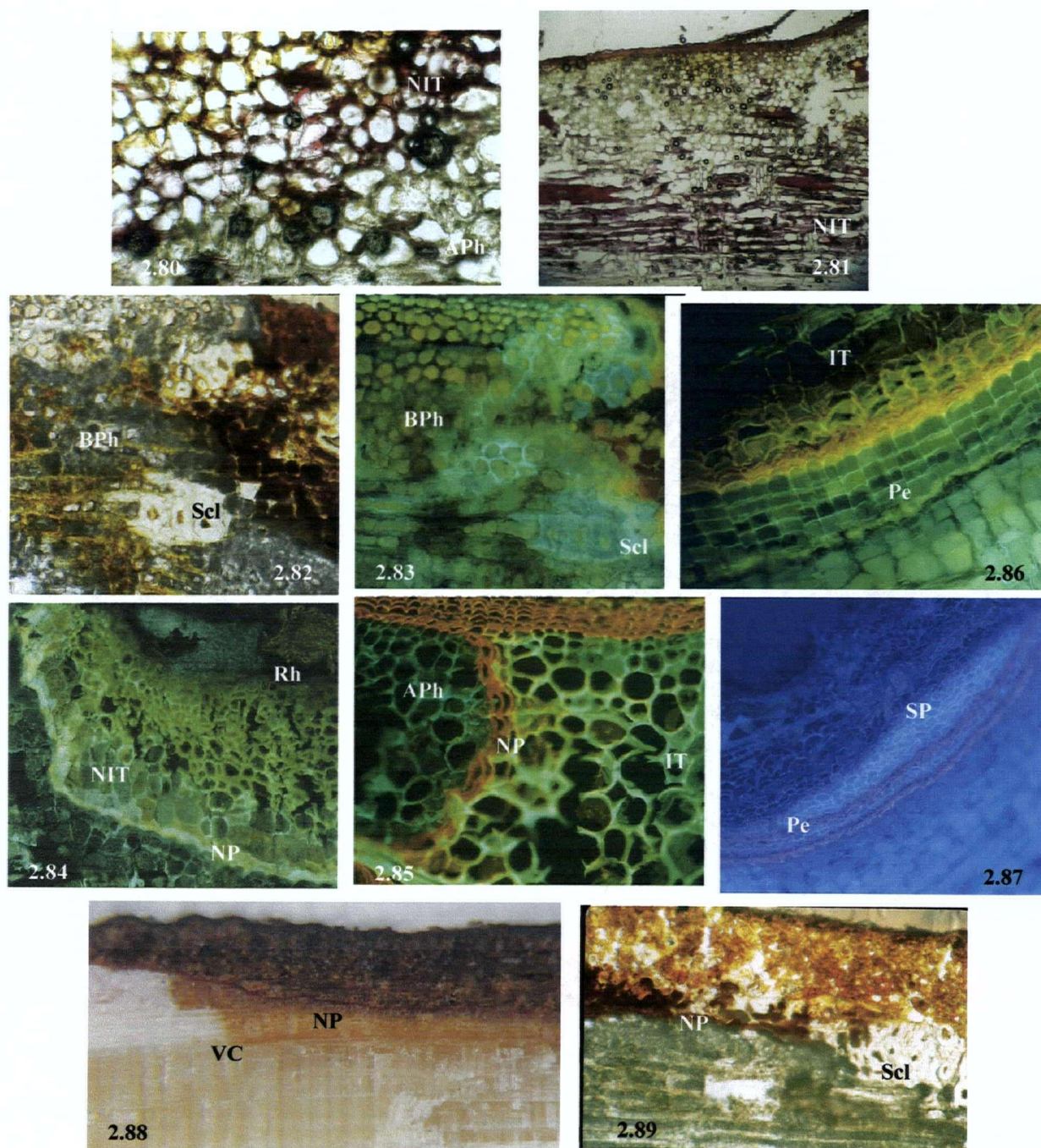
Fig. 2.80. A phloroglucinol-HCl treated section of a western hemlock root inoculated with *A. ostoyae* showing lignification of phloem parenchyma underlying necrotic tissue, BF. **Fig. 2.81.** A phloroglucinol-HCl treated section of an infected hemlock root showing the lignified NIT comprising several cell layers and extending for some distance along the length of the sample close to the vascular cambium, BF. **Fig. 2.82.** A cryofixed section of a western hemlock root inoculated with *A. ostoyae* showing incomplete differentiation of NIT around clusters of sclereids in the bark, BF. **Fig. 2.83.** The same section shown in Fig. 2.82 viewed in BL. Note erratic hypertrophy and cell wall fluorescence in moribund tissue and adjacent phloem in the areas of incomplete dedifferentiation of NIT. **Fig. 2.84.** A cryofixed section of a western hemlock root 11 weeks following inoculation with *A. ostoyae* showing a typical resistant reaction involving the complete formation of a NP around infected, necrotic tissue, BL. **Fig. 2.85.** A Sudan III treated section of a hemlock root 11 weeks following inoculation with *A. ostoyae* showing the suberized phellem of the new NP becoming continuous with the original periderm, BL. **Fig. 2.86.** A cryofixed section of a western hemlock root sampled approximately 1 year following inoculation with *A. ostoyae*. The resulting lesion was bound by a NP in which the thin-walled phellem appear to be radially compressed. At the time of sampling in the late spring, the current year's phellogen activity had already produced up to 5 layers of phellem. **Fig. 2.87.** A cryofixed section of a larger diameter hemlock root showing a newly formed NP comprised of thick and thin-walled phellem, UV. **Fig. 2.88.** A photomacrograph of a hemlock root showing initial NP formation in the bark and breaching of the newly formed periderm and necrosis extending down to the vascular cambium. **Fig. 2.89.** A cryofixed section of a hemlock root showing incomplete differentiation of NP around clusters of sclereids, BF.

On injured hemlock roots, both the living xylem ray cells and the cambial initials of the uninjured cambium contributed to the formation of callus tissue (Figures 2.90-2.93). Progressive hyperplasia of cells created a large zone of callus tissue that expanded over the surface of the wound.

Callus that formed in areas suffering from temporary disruption in normal cambial activity eventually developed secondary cell walls and became lignified and the resulting lesion appeared as a zone of hypertrophied cells in the middle of an annual ring (Figure 2.94).

The barrier zone in western hemlock was similar to that in Douglas-fir. The uninjured cambium formed a series of traumatic resin ducts that extended for some distance tangentially away from the primary infected tissue (Figure 2.95). Some of the first resin ducts that differentiated adjacent to the area of killed cambium sometimes showed fusion of individual canals to form larger cavities. The longitudinal extent of resin canal formation in such lesions were not measured. However, Cruickshank *et al.* (2006) reported that traumatic resin canals could be traced up to 110 cm proximally along an infected Douglas-fir root from a more distal lesion.

In red pine (*Pinus resinosa* Ait.) roots showing compartmentalization, polyphenolic parenchyma cells formed first immediately adjacent to the killed cambium and resin ducts were seen extending tangentially away from the killed cambium (Tippett and Shigo 1980). Thus, physiologically active parenchyma that accumulate secondary metabolites that are associated with traumatic resin ducts in the barrier zone of some conifers may be more important in resisting spread of the fungus along the vascular cambium than traumatic resin ducts by themselves.

In this study, parenchyma and tracheids surrounding the resin ducts appeared occluded and somewhat distorted in shape. Ray parenchyma formed between individual resin ducts also showed some occlusions (Figure 2.96), but became less occluded with increasing distance from the killed cambium. Tracheids laid down immediately following vascular
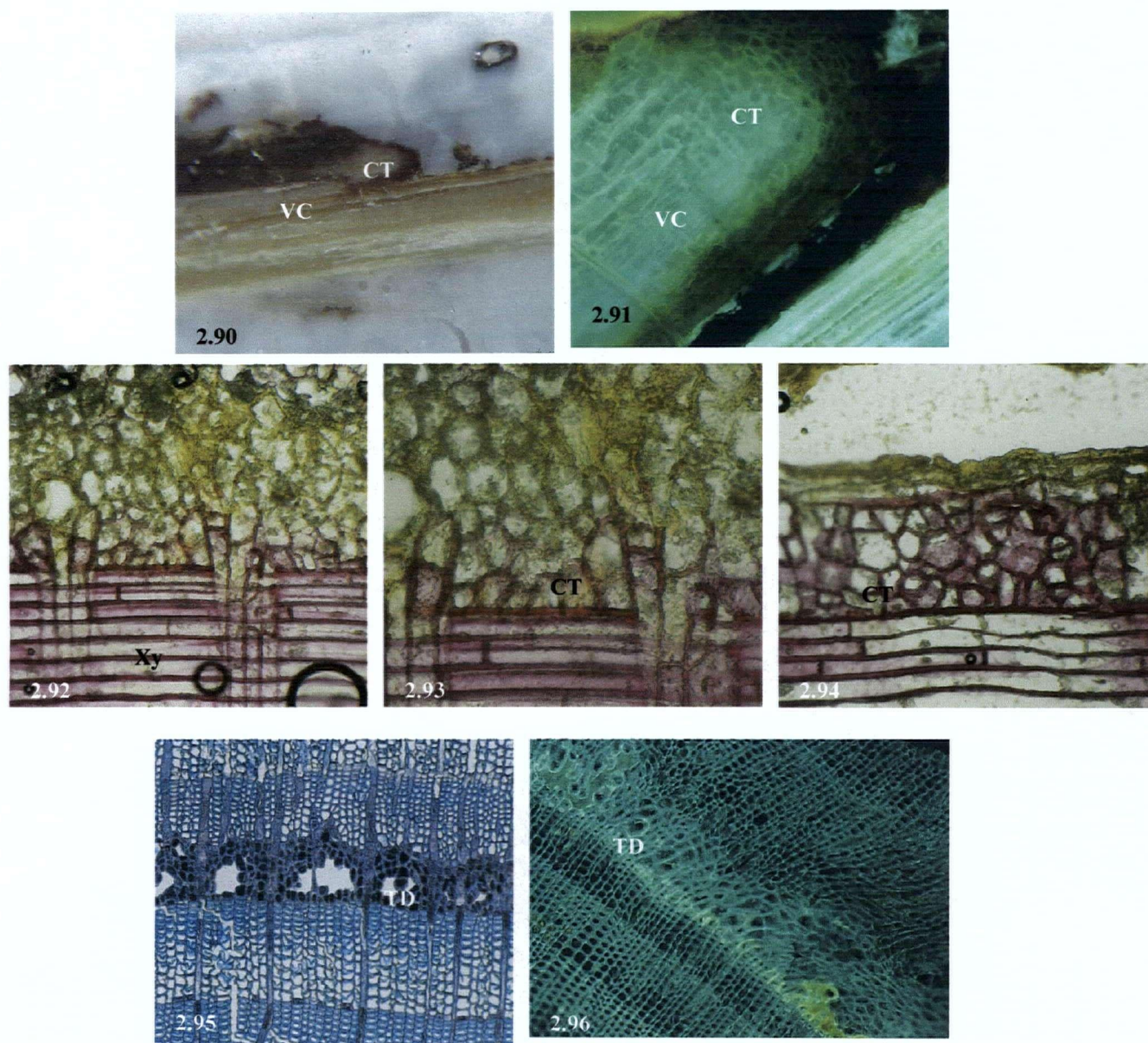
Fig. 2.90. A photomacrograph of a western hemlock root showing lateral ingrowth of callus following cambial invasion by *A. ostoyae*. Note newly differentiated NP in the outer periphery of the callus. **Fig. 2.91**. A cryofixed section of western hemlock sample shown in Fig. 2.90 but viewed in BL. A NP became established in the outer periphery of the callus and a new vascular cambium was restored within the callus. Derivatives of the cambial initials are disoriented in radial section. **Fig. 2.92**. Callus formation originates via progressive hypertrophy and hyperplasia of cambial initials from the uninjured cambium as well as proliferation of the living xylem ray cells; section stained in phloroglucinol-HCl. **Fig. 2.93**. The same section viewed in Fig. 2.92 but under 45X magnification showing more clearly the proliferation of xylem rays. **Fig. 2.94**. A phloroglucinol-HCl treated section of a western hemlock root that showed temporary disruption of the normal cambial activity resulting in callus formation. The tissue derived from callus (CT) eventually develops secondary walls, become lignified, and remain embedded between tracheids in the annual growth ring. **Fig. 2.95**. A western hemlock root sampled approximately 1-year following inoculation with *A. ostoyae* showed a series of traumatic resin canals following injury to the vascular cambium. Cells surrounding the resin ducts appear occluded. **Fig. 2.96**. A cryofixed section of a western hemlock root showing callus formation at the edge of the killed cambium. Early differentiation of resin ducts show an oblique orientation but their structure becomes increasingly normal with increased distance from the area of killed cambium, BL.

cambium regeneration, particularly in the area where the callus curl appeared to be growing over the lesion, were often obliquely or horizontally oriented but as growth continued, normal alignment of cells was restored. A new periderm was formed in the outer periphery of the callus.

Traumatic resin ducts were also induced in the xylem in the absence of cambial invasion, primarily where the fungus was superficial in the bark. Even when the cambium was not directly affected (i.e. killed), stimuli triggered host defense mechanisms which caused a disruption to the normal cambial activity and formation of traumatic resin ducts.

Incomplete closure of the wound is common in trees that have sustained injuries to the vascular cambium as a result of either mechanical wounding or pathogenic invasion. If the vascular cambium turns inward during the process of wound healing, continued growth over a long period of time will enable the host to form thick rhytidome tissue which may prevent physically co-mingling of vascular cambium and a "bark inclusion" may be visible as a fine crack between converged callus ribs (Fink 1999). Such reactions on roots infected with *A. ostoyae* may more likely result in species that have a slower rate of healing or individual trees with low tree vigor. At the time of sampling, no injuries to the cambium were completely healed over by callus.

A. ostoyae interfered with the initiation of host defense mechanisms. Although a higher percentage of hemlock roots triggered the formation of NIT and NP than Douglas-fir, weaknesses in the development of these barriers allowed the fungus to breach or circumvent them in the bark. A low frequency of compartmentalization and callusing was observed on roots following cambial invasion. This frequency was not all that different from Douglas-fir. Thus, the low frequency of resistance reactions observed in roots following inoculation with *A. ostoyae* indicates that western hemlock and Douglas-fir are equally susceptible to infection by the fungus.

Western redcedar

Following infection by *A. ostoyae*, western redcedar roots showed different and less frequent external symptoms of infections on roots. Unlike Douglas-fir and western hemlock, cedar roots did not show resinosus at lesions. Rhizomorphs were observed on the surface of the root and at times irregularity in the otherwise smooth bark surface was indicative of *A. ostoyae* lesions (Figure 2.97).

As cedar has relatively thin, fibrous bark, the outer periderm was readily breached. Several roots showed a lateral branch of a rhizomorph penetrating the outer phellem via mechanical and enzymatic degradation (Figure 2.98). As a result, the underlying phloem cells collapsed giving the appearance of compressed tissue (Figure 2.99). Penetration of the living bark rendered the phellogen non-functional and necrosis expanded into the underlying phloem (Figure 2.100).

Only 1 of 57 (2%) cedar roots examined in this study showed no visible or ineffective host response. This percentage is in strong contrast to the 36% (n=80) and 54% (n=54) of roots showing no host response in hemlock and Douglas-fir, respectively (Appendix III). On this single root harvested at 5 months, sporadic hypertrophy was observed in phloem cells, however neither NIT nor NP was observed following staining of sections with either phloroglucinol-HCl or Sudan III.

NIT was presumably initiated in nearly all samples examined on each harvesting date. However distinct zones of NIT were difficult to discern. Whereas the NIT in Douglas-fir and hemlock comprised several layers of lignified phloem underlying necrotic tissue or at an advancing infection front, the NIT formed in cedar never comprised more than 1 cell layer; however in most cases, a distinct zone of NIT was lacking. It is customary during NIT development for secondary lignification to occur in phloem tissue. This was most easily noticed when phloem fibers which normally stain positive for lignin following treatment with phloroglucinol-HCl showed more intense staining for lignin within the vicinity where NIT would be expected to lie (i.e. externally abutting newly differentiated phellem) (Figure 2.101). However, lignification in a zone of NIT may have also be

masked by the fact that thin-walled phellem cells were not only suberized, but also lignified (Figure 2.102).

The above results are interpreted to indicate that the more intense staining of phloem fibers may in fact be caused by the thin-walled phellem which typically wrap around phloem fibers (Figure 2.103 and 2.104) rather than the fibers themselves assuming a greater role in the formation of NIT. Fibers, like sclereids, have thick secondary walls, but are considerably more elongated and usually occur in tangential rows in the secondary phloem (Esau 1965). It may be that lignification in the cell walls of a single row of parenchyma occurs only on the side of the wall that borders a newly differentiated phellogen. When phellem cells are mature they develop deep reddish-purple contents, and their walls become suberized and lignified. Given the fact that cedar produces only 1-2 layers of phellem compared to several layers of thin-walled and possibly thick-walled phellem like that found in Douglas-fir and western hemlock, it is not unreasonable to suspect that the NIT zone itself would also be minimal.

NP formation occurred in 56 of 57 (98%) of cedar roots inoculated with *A. ostoyae* (Figure 2.105). A newly restored phellogen produced a layer of phellem that was typically 1-2 cells wide after 8-9 weeks (Figure 2.106). Surprisingly, the number of phellem layers observed in a NP was essentially the same at 5 months or 1 year following inoculation than was observed at 8-9 weeks (Figure 2.107). This differed considerably from Douglas-fir and hemlock, where the number of phellem in a NP depended on time since phellogen renewal.
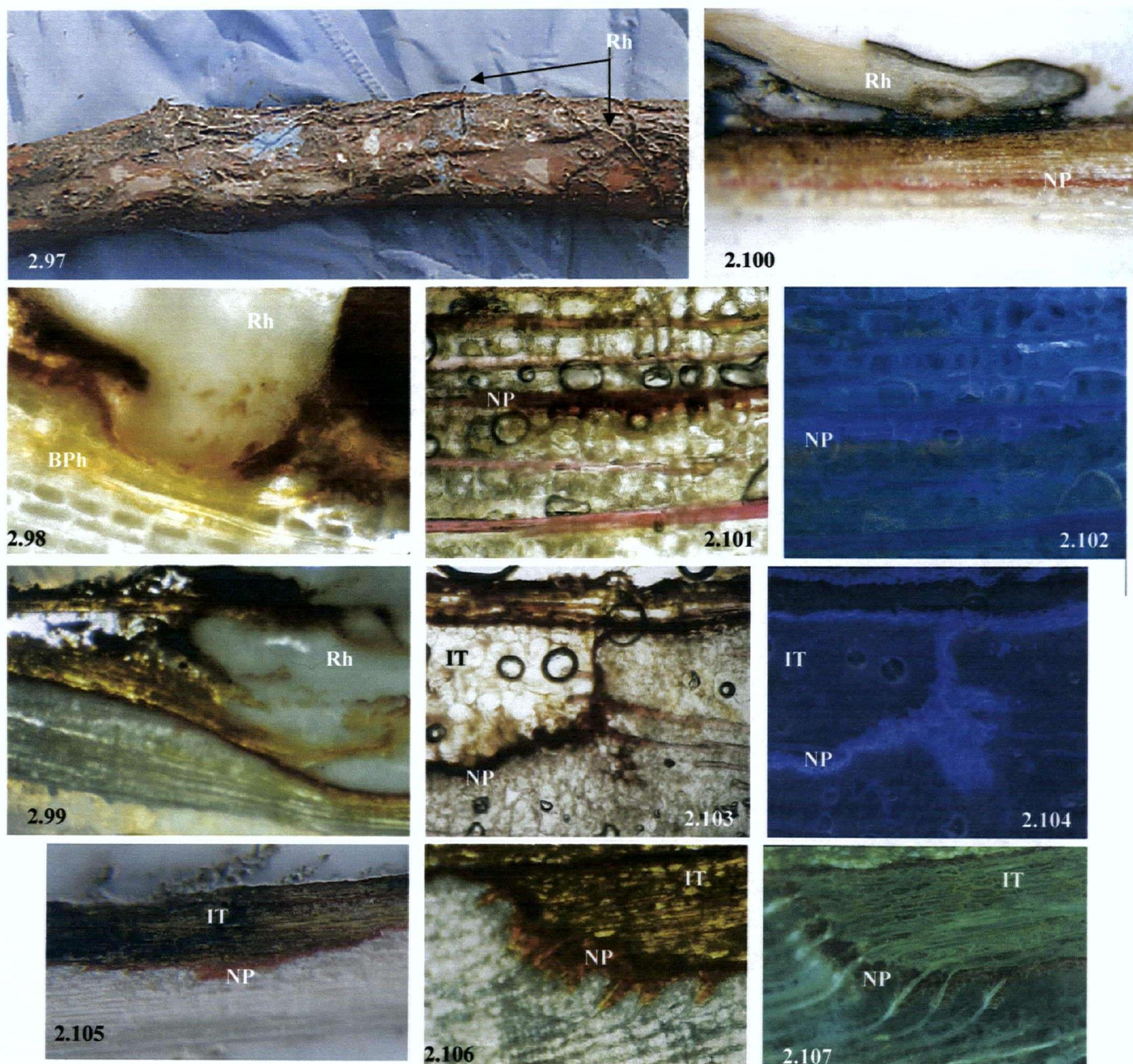
Fig. 2.97. A western redcedar root sampled 1-year following inoculation with *A. ostoyae*. Symptoms of diseased roots are quite inconspicuous other than irregularities in the bark surface. **Fig. 2.98**. A cryofixed section of a cedar root showing rhizomorph penetration of the inner bark. **Fig. 2.99**. A cryofixed section of a western redcedar root showing rhizomorph penetration via enzymatic degradation and mechanical ingress of the fungus resulting in the collapse of the underlying phloem. **Fig. 2.100**. A photomacrograph of a cedar root showing expansion of necrosis in the bark following infection by *A. ostoyae*. **Fig. 2.101**. A phloroglucinol-HCl treated section of a western redcedar root showing lignification of the fibers and thin-walled phellem. The fiber internally abutting the newly formed phellem stained more strongly for lignin, BF. **Fig. 2.102**. The same section shown in Fig. 2.101 but viewed under UV. The same lignified phellem here appear suberized. Fig. 2.103. A phloroglucinol-HCl treated section of a cedar root showing NP formation in the bark in response to infection by *A. ostoyae*. The NP is comprised of 1-2 layers of thin-walled lignified phellem, BF. **Fig. 2.104**. The same section shown in Fig. 2.103 but viewed under UV showing the same lignified phellem fluorescing bright blue-violet indicating suberization. Note prolific formation of phellem around fibers in the vicinity of the newly differentiated periderm. **Fig. 2.105**. A photomacrograph showing a typical resistance response involving NP formation in the bark following invasion by *A. ostoyae*. **Fig. 2.106**. A cryofixed section of cedar root bark showing necrotic tissue bound by a NP. The phellem accumulate red pigments, BF. **Fig. 2.107**. The same section shown in Fig. 2.106 but viewed under BL.

A unique localized host reaction was induced in cedar following inoculation with *A. ostoyae*. Adjacent to an area of infected tissue where the host initially formed a NP, large zones of phloem parenchyma became markedly hypertrophied, increasing bark thickness by 2-3 times normal (Figure 2.108). Large zones appeared non-fluorescent suggesting hyperplasia and meristematic activity. The walls of hypertrophied cells appeared thin and some cells developed cross walls. Cells within the zone of excessive hypertrophy underwent changes in fluorescence characteristics which were indicative of the early stages of tissue dedifferentiation (Figure 2.109). A new periderm was formed on either side of the lesion, and extended for some distance beyond the primary wounded tissue bound by a NP (Figure 2.110). This particular host reaction resembled rhytidome formation and was observed in 27 of 57 (47%) of cedar roots that showed penetration by the fungus. However, this particular host reaction differed from normal rhytidome formation with respect to the intensity of the hypertrophy and hyperplasia occurring in tissue and the extent to which the successive periderm extended along the length of the root to the point at which it became continuous with the original periderm. The new periderm that establishes underneath the zone of hypertrophy and hyperplasia (Figure 2.111) either joined with the initially formed NP at or near its deepest point, or else it laid wholly below (and to either side) of the initially formed NP. In both cases it joined the pre-existing periderm some distance distal and proximal to the infection point. The intensity of the localized response that was expressed depended on the extent of colonization of the bark tissue by the fungus. For example, the lesion size or the length at which induced rhytidome formation occurred beyond the obvious infection front depended on the extent of necrosis in the bark. Thus for shallow bark injuries to one-third or one-half the bark thickness, the host usually responded by forming a NP around the necrotic tissue. Slightly deeper injuries also induced NP formation; however, an expanding zone of necrosis stimulated the induced rhytidome response. In roots showing extensive colonization of the living bark upon initial penetration by the fungus, induced rhytidome formation extended for up to 20 cm, either proximal or distal from the lesion. Roots showing this type of response were sometimes recognized prior to sampling by massive swelling of the outer bark around inoculum contact (Figure 2.112). This particular host reaction is coined 'induced rhytidome' in this thesis.

Apoptosis, otherwise known as a type of cell death program induced in plants, is sometimes described in association with a hypersensitive response that may be triggered by toxic microbial products or elicitors of host origin (Kacprzak *et al.* 2001). Several studies have shown that mycotoxins secreted by fungal species are capable of inducing apoptosis in plant and animal tissues (Gilchrist 1998, Heath 1988, Khurana *et al.* 2005). In this study, cedar responded to invasion of the living tissues by initiating a cell death program in the specific form of induced rhytidome formation to restrict further penetration of the pathogen. In no case did *A. ostoyae* penetrate beyond the induced rhytidome layer.

Hence, when cedar bark was injured, the host formed a necrophylactic periderm to confine the infection and then induced rhytidome formation on either side of the lesion so that one continuous rhytidome layer would be formed and eventually be sloughed from the surface of the root. Roots sampled at 5 months and 1 year following inoculation already displayed *en masse* sloughing of necrotic tissue, particularly in the zone of excessive hypertrophy (= induced rhytidome). Thus it appears that induced rhytidome formation not only served to confine the fungus within a new periderm barrier, but it also facilitated sloughing of infected tissue from the surface of the root thereby reducing the risk of subsequent infections from inactive lesions.

Induced rhytidome formation was only observed in two roots from the first inoculation trial. At the time of examination, these roots did not display the pronounced effect of bark hypertrophy seen in many of the other cedar root samples in subsequent years. However, it did show early development of a new NP differentiating underneath necrotic phloem proximal to the area of bark necrosis (Figure 2.113 and 2.114). Re-examination of the micrographs of these samples revealed anatomical changes in the bark and changes in fluorescence characteristics indicative of induced rhytidome formation, similar to what was observed in subsequent years.
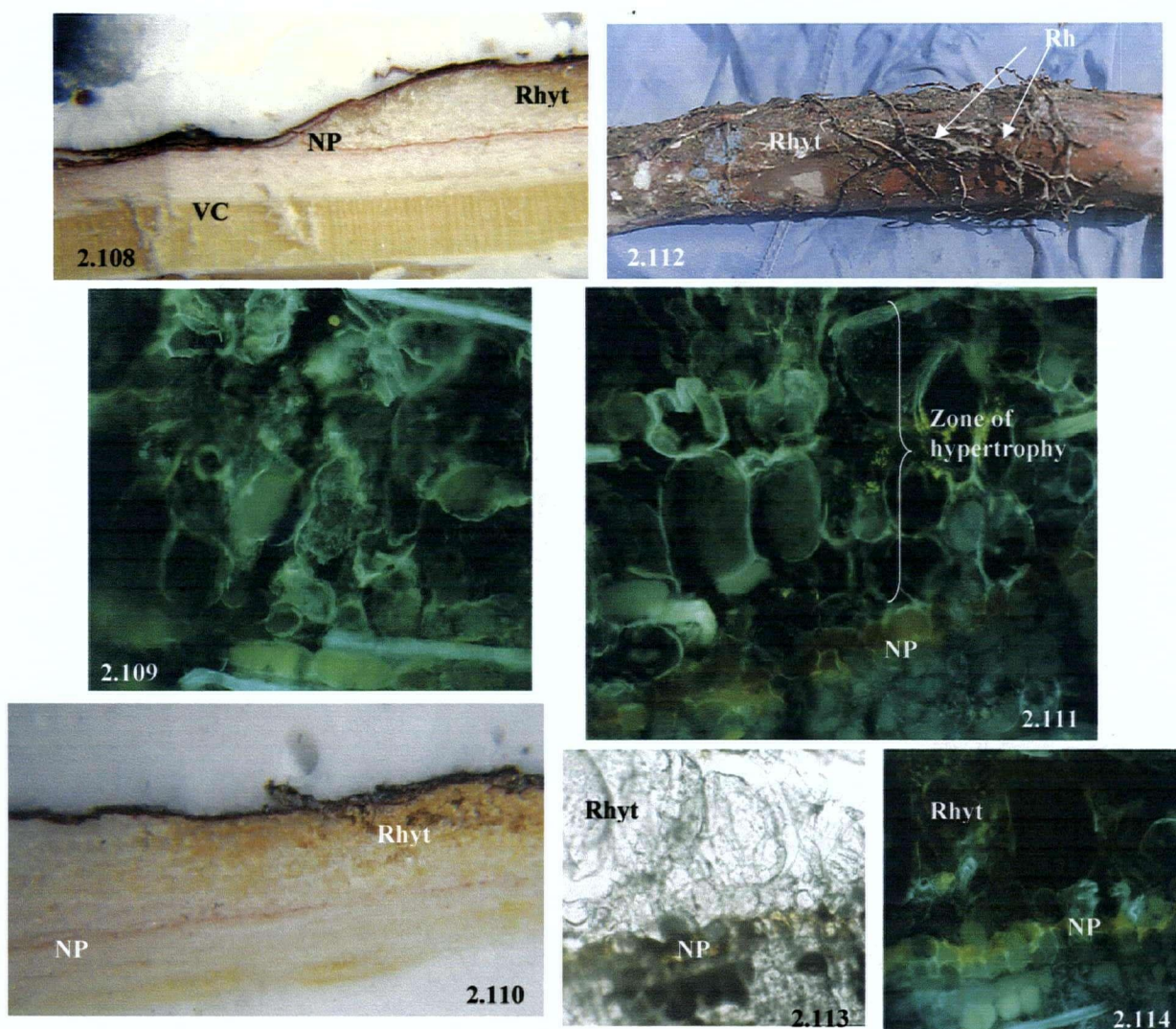
Fig. 2.108. A photomacrograph of a western redcedar root showing induced rhytidome formation in the bark. The excessive bark hypertrophy results in an increase in bark thickness up to 3X the original thickness of the bark. **Fig. 2.109.** A cryofixed section of a western redcedar root showing changes in florescence characteristics in the zone of excessive hypertrophy indicative of the early stages of dedifferentiation. **Fig. 2.110.** A photomacrograph showing induced rhytidome formation in cedar extending for some distance beyond the primary wounded tissue (the original NP). **Fig. 2.111.** A cryofixed section of a western redcedar root showing excessive hypertrophy and dedifferentiation in the phloem proximal and distal to the primary wounded tissue. A new meristematic phellogen differentiates immediately abutting the zone of hypertrophy and produces radial files of thin-walled lignified phellem. **Fig. 2.112.** A western redcedar root showing massive swelling and irregularities in the otherwise smooth bark surface following inoculation with *A. ostoyae*. Note rhizomorphs adhering to the outer surface of the bark. **Fig. 2.113.** A cryofixed section of a western redcedar root showing successive periderm formation deeper in the bark under a zone of bark hypertrophy, BF. **Fig. 2.114.** The same section shown in Fig. 2.113 viewed under BL. The newly formed periderm is comprised of 1-2 layers of thin-walled phellem.

Another unique phenomenon induced in cedar following inoculation with *A. ostoyae* was the formation of traumatic resin ducts in the phloem (Figure 2.115 and 2.116). Western redcedar trees form neither normal nor traumatic resin canals in the xylem and resin canals are rarely observed in healthy phloem root tissue. The formation of traumatic resin ducts in western redcedar has not been described in the literature. However, similar defense responses have been reported in another conifer belonging to Cupressaceae, namely in Japanese cypress trees (Yamada *et al.* 2003, Yamanaka 1984, 1989).

Various stages of development in the formation of traumatic phloem resin ducts (TPRD) were observed in numerous root samples infected by *A. ostoyae*. Because resin ducts do not form synchronously, early stages of development in the formation of TPRD's, as well as fully differentiated resin ducts were observed. This permitted a complete characterization of the various stages of development in TPRD's in western redcedar roots.

Detailed examination of cryofixed bark samples and serial sections of paraffin embedded cedar bark revealed that TPRD's arise by reorganization of preexisting phloem parenchyma in close proximity to the vascular cambium. Assuming that the youngest mature parenchyma cells are physiologically more sensitive to stimulation, expansion and division of cells leading to the formation of TPRD's may occur preferentially in this zone.

Unlike induced rhytidome, this zone of cells did not appear to be excessively hypertrophied. However, it appeared to undergo hyperplasia as evidenced by the numerous small cell derivatives within a relatively narrow zone of tissue. Excessive proliferation of cells, followed by schizogenous and lysigenous separation of cells, resulted in a series of longitudinal resin ducts arranged in tangential bands in the inner and mid-phloem region. Stages of development of traumatic phloem resin ducts and their corresponding photomicrographs are as follows:

1. A zone of pre-existing axial and ray phloem parenchyma cells, closest to the vascular cambium, undergoes hyperplasia and begins to divide and expand radially, forming small regularly spaced clusters of redifferentiating tissue (RZ) (Figure 2.117).

2. The zones of meristematic activity are seen as large areas of non-fluorescence when under BL (Figure 2.118). Recent derivatives repeatedly divide periclinally such that tissue in this meristematic zone is comprised of numerous small parenchyma cells which are capable of further division.

3. Cells situated in the centre of a mass of hyperplasic tissue will begin to separate schizogenously (separation of cells occur along their middle lamellae forming intercellular spaces). Schizogenous separation may also occur concomitantly with lysigeny (dissolution of the common middle lamellae), expanding to create a cavity in the central region (Figure 2.119 and 2.120).

4. The space in the canals becomes enlarged over time via additional periclinal and also anticlinal divisions of parenchyma cells surrounding the canals. Schizogeny and lysigeny subsequently follow.

5. Cells lining the newly formed canals become epithelial cells (Figure 2.121). These cells typically have thin walls and participate in the synthesis of resin.

6. Eventually, circular or elliptic resin canals are formed in tangential rows, separated by phloem ray parenchyma (Figure 2.122). Phloem fibers may appear in the zone of redifferentiating tissue (Figure 2.123). The size of a resin canal varies depending on its developmental stage, although, a fully differentiated resin canal is approximately 100-300 μm in diameter and between 2-5 mm in length (Figure 2.124).

7. Where TPRD's are formed in close proximity to a newly differentiated NP, epithelial cells surrounding the lumen of a resin duct may accumulate phlobaphenes and cell wall constituents, namely lignin and suberin that are normally found in phellem (Figure 2.124-2.127). This is particularly evident where displaced phloem fibers in the zone of phellogen restoration merge with parenchyma cells involved in the formation of TPRD's. Parenchyma cells surrounding the resin duct may be influenced by metabolic and restorative processes occurring within neighbouring cells. However, with increasing distance from a NP, resin ducts appear normal.

8. In transverse section, resin canals appear as neat tangential rows (Figure 2.128) and in tangential longitudinal section, sections may appear as an anastomosing network of resin ducts (Figure 2.129).
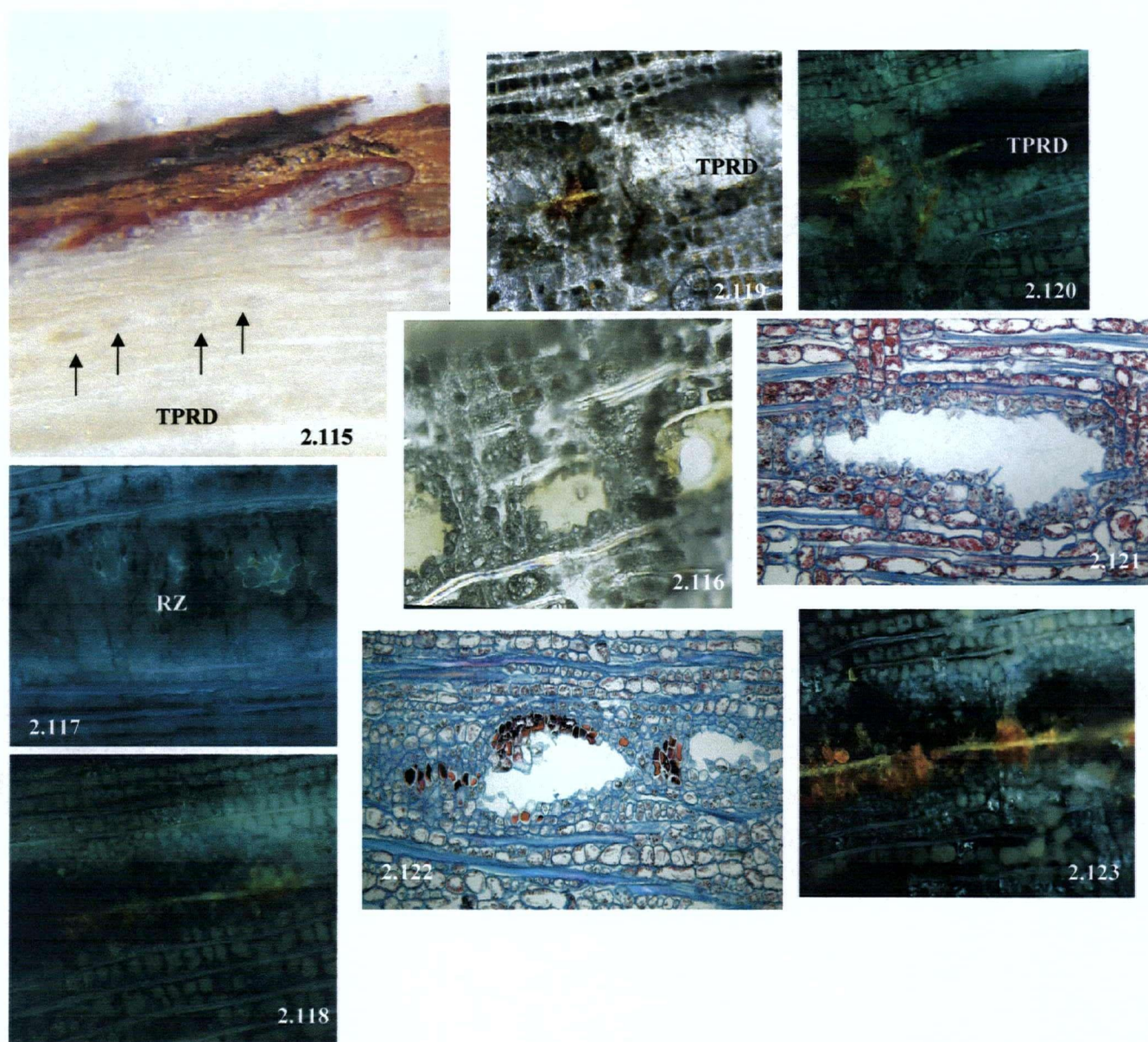
Fig. 2.115. A photomacrograph of a western redcedar root showing a NP formation in the bark and a series of small circular or elliptical zones of tissue (arrows) which differentiated into traumatic resin canals in the phloem. Fig. 2.116. A cryofixed section of cedar root bark shown in Fig. 2.115 showing fully differentiated resin ducts in the phloem surrounding the margin of the lesion. Fig. 2.117. A cryofixed section of cedar root bark showing the initiation of traumatic resin duct formation in the phloem via excessive hyperplasia of phloem parenchyma either in mid-phloem or closer to the vascular cambium, BL. Fig. 2.118. Zones of actively dividing cells are non-fluorescent when viewed under BL. Fig. 2.119. A cryofixed section of cedar showing the cavity of a fully differentiated resin duct resulting from schizogeny and lysigeny of the expanding hyperplasic tissue, BF. Fig. 2.120. The same section shown in Fig. 2.119 but viewed under BL. x45. Fig. 2.121. A paraffin embedded section of a cedar root showing epithelial cells lining the lumen of the resin duct, BF. Fig. 2.122. A paraffin embedded section showing traumatic resin ducts separated by axial and ray phloem parenchyma, BF. Fig. 2.123. A cryofixed section showing phloem fibers fixed in the resin ducts. Note phellem-like cells wrapped around fibers, BL.
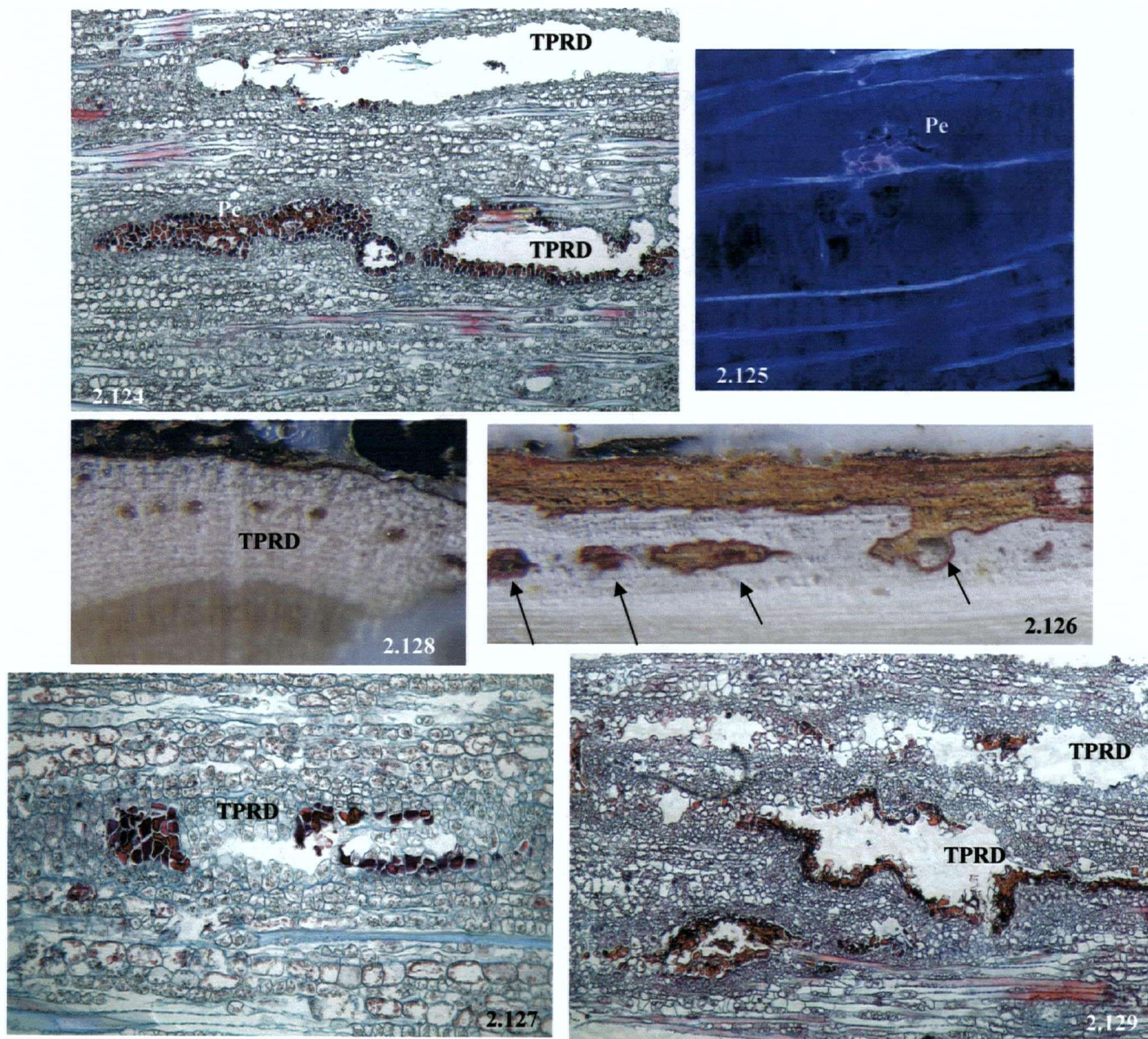
Fig. 2.124. A tangential section of a cedar root showing traumatic resin duct formation in the phloem following inoculation with *A. ostoyae*. The length of resin ducts varies but is generally between 2-5 mm, while the width ranges between 100-300 µm, BF. **Fig. 2.125**. A cryofixed section of a cedar root showing phloem fibers fixed in the resin duct with pigmented phellem-like cells wrapped around the fiber, UV. **Fig. 2.126**. A photomacrograph of a cedar root showing NP formation and traumatic resin duct formation deep in the phloem tissue. The tissue surrounding the differentiating resin ducts appear as small circular or elliptical zones of necrotic tissue bound by phellem-like (arrows) cells that accumulate the normal cell wall constituents, namely lignin and suberin, as well as the pigmented phlobaphene cell contents. With increasing distance from the area of primary wounding where a NP had differentiated, traumatic resin ducts appear normal. **Fig. 2.127**. A paraffin-embedded section of cedar root bark showing traumatic resin canal formation in the phloem adjacent to a newly differentiated NP. The parenchyma cells surrounding the lumen of the duct acculumated phellem-like pigments, BF. **Fig. 2.128**. A transverse section of cedar root bark collected from the root collar area of a healthy tree showing tangential series of traumatic resin canals in the phloem, BF. **Fig. 2.129**. A tangential section of cedar root bark adjacent to a newly differentiating NP showing an anastomosing network of resin ducts of varying lengths and irregular accumulation of phellem-like pigments in the epithelial and/or parenchyma cells lining the resin ducts, BF.

Yamada *et al.* (2002) found that traumatic resin canals were induced in the functional phloem of *Chamaecyparis obtusa* Endlicher (Hinoki cypress) trees following artificial wounding. The authors also reported the presence of traumatic resin canals in the phloem in diseased stems of *C. obtusa* infected with the Rooshi pitch canker (*Cistella japonica* Suto et Kobayashi). Early stages of resin canal formation were evident after just one month (Yamanaka 1984).

In this study, the formation of traumatic resin ducts in the phloem was not an immediate host response to wounding. Its initiation usually coincided with the completion of NP around the primary wounded tissue. Hence, TPRD's would not be evident until at least several weeks following initial penetration by the fungus. No cedar roots inoculated with *A. ostoyae* sampled at 5, 8, 9 or 11 weeks showed evidence of TPRD's in the bark. However, TPRD were observed in 43% (n=23) and 75% (n=12) of the infected cedar roots sampled at 5 months and 1 year following inoculation, respectively. Only 3 of 30 (10%) abiotically wounded roots showed evidence of TPRD's in the bark after 1 year. The lack of both induced rhytidome formation and TPRD's observed in the majority of the freezing injuries may be due to the shallow depth of the bark injuries which are more often walled out by a single NP.

Yamanaka (1989) reported that within 11 days of wounding in the phloem of *C. obtusa*, the conducting system for resin develops within the proliferating parenchyma cells. Nagy *et al.* (2000) also suggested that traumatic resin duct formation in the bark requires up to 2 weeks for formation of structures with secretory capacity. In this study, the margin of the lesions was clearly defined at 5 months and 1 year following inoculation with *A. ostoyae*, and TPRD's extended up to 6-cm proximal and distal from the point where the periderm associated with induced rhytidome became continuous with the original periderm. Presumably the function of resin synthesis in infected roots had already transpired and the fungus would be interacting not only with chemicals involved in the process of phellogen renewal associated with NP and induced rhytidome formation, but also with resin synthesis in response to the wounding. There has been some work suggesting that resin canals induced under pathological stimulation contain a resin with

quantitatively and qualitatively different composition compared to regular resin canals (e.g. the resin may have a higher concentration of diterpene resin acids and monoterpene olefins) (Gref and Ericsson 1985, Fink 1999). Axial resin duct activation was induced by methyl jasmonate in "incipient" phloem resin ducts of *Cupressus macrocarpa* Gord. (Hudgins *et al.* 2004). Dormant axial resin ducts in the secondary phloem of other conifers responded to similar chemical stimuli that mimics part of the wound response by activating epithelial cells lining the resin ducts to produce resin (Hudgins *et al.* 2004).

It is interesting that TPRD's in cedar occurred proximally, distally and tangentially around the periphery of the *Armillaria*-caused lesion. Lesions on cedar typically do not girdle the root. Since the development of TPRD's occurs in conjunction with or immediately following the development of a NP to confine the fungus to a lesion in the bark, the presence of these TPRD's in the phloem may play some secondary role in terms of limiting the spread of the fungus in root tissue. Yamanaka (1984) reported that the metabolic response of resin synthesis and flow may be promoted secondarily by the same stimuli on a continuous basis and epithelial cells can remain active for years. Hence, if *A. ostoyae* infection induced traumatic phloem resin canals, further stimulation by an expanding zone of necrosis or an advancing mycelium would interact with mechanisms already in place for resin synthesis which may cause lysis of fungal material. It is well known that resin production in Douglas-fir and western hemlock does little to deter the advance and colonization of host tissue by *A. ostoyae*. Precisely if or how oleoresin in cedar differs from that found in Douglas-fir or western hemlock and the nature of the biochemical induction of terpene synthesis in cedar with respect to its resistance against *A. ostoyae* warrants further investigation.

In this study, field observations on a small sample of cedar trees revealed that resin canals in the phloem were usually associated with older tissue located at or near the root collar (Figure 2.128). This was not surprising since the minute amount of resinosus observed on cedar trees usually occurs at the base of the stem. These observations suggest that on 22-28-year old cedar trees, resin canals are normally found at the root collar but at approximately 25-30 cm from the root collar no resin canals are usually found.

However, when roots are injured, TPRD's may be induced in large numbers in phloem tissue that normally lacks resin canals. Yamanaka (1984) reported similar results in the phloem of *C. obtusa*, in association with the 'shoe-string rot', *Armillaria mellea* where resin canals appeared to be concentrated in the lower stem. In this study, resin canals in the phloem seem to occur naturally in older tissue and they are frequently formed in roots infected with *A. ostoyae*, and to a lesser extent following abiotic injury. Thus, the response appears to be non-specific.

Like the induced rhytidome, the intensity of the response with respect to the induction and the distal and proximal expansion of TPRD seemed to depend on the extent of injury to the bark. Observations on infected roots of cedar suggested that the expansion of resin ducts occurs far beyond the periphery of the induced rhytidome (i.e. 20 + cm beyond the already expanded lesion). The mechanisms by which such responses are transmitted to distant tissues are unknown. However, Franceschi *et al.* (2000) suggests signals through polyphenolic parenchyma (PP) cells and interconnected radial rays may be one means by which this occurs. Conceivably, this could also be attributed to fungal elicitors or stimuli as *A. ostoyae* colonizes and advances in host tissue. Yamanaka (1984) reported that the formation of phloem resin canals in the branches of *C. obtusa* was accelerated by the insertion of pins coated with paraquat or with cycloheximide. Cheniclet (1987) also showed higher levels of terpene production in tissues following fungal invasion compared to abiotic wounding. Hence, the distance over which these resistance responses are induced in roots inoculated with *A. ostoyae,* over and above that which was observed in abiotically wounded roots, suggests that some chemical stimuli specific to the host-pathogen interaction in cedar has a fundamental and key role in the resistance of that host species to *A. ostoyae.*

Ninety-two percent (n=24) of cedar roots that showed killing to the cambium exhibited compartmentalization of the infection and displayed active callusing around the margin of the lesion (Figure 2.130). Callus was formed at the margin of the lesion by progressive hyperplasia of both cambial initials and phloem parenchyma located in close proximity to the vascular cambium. In the outer periphery of the callus tissue a NP developed, while

in the inner- or mid-region of the callus a new vascular cambium regenerated, probably differentiating from the point where the original vascular cambium was still intact and amalgamated with the original vascular cambium (Figure 2.131). Eventually, the tissue derived from callus developed secondary cell walls and became lignified.

Examination of the barrier zone induced in cedar following cambial invasion revealed some unique characteristics. The anatomy of cedar wood parallels that of Douglas-fir and western hemlock with respect to normal tracheid and ray parenchyma tissue. However, unlike the other conifers, cedar does not form traumatic resin ducts in the xylem following injury of the cambium. Instead, a high density of axial parenchyma was formed adjacent to the killed cambium which appeared occluded with various deposits. The chemistry of the secondary metabolites that accumulate in this tissue and their biological significance in relation to wound defense, specifically against *A. ostoyae*, remains to be determined. However, several reports suggest that a complex range of polyphenolic compounds are involved that are implicated as having an antimicrobial role against pests and diseases (Eyles *et al.* 2003, Woodward and Pearce 1988).

Secondary metabolites appeared to accumulate in the parenchyma cells derived from callus as well as the parenchymatous zone formed by the uninjured cambium restricting the spread of infection to adjacent healthy cambial tissue (Figure 2.132). The intensity of the response diminished with increasing tangential distance from the area of killed cambium. Like in other conifers, tracheids derived from a newly differentiating vascular cambium in cedar may be oriented in a variety of planes (radial, tangential, and transverse), however tissues became progressively more organized with increasing cell division and distance from the callus edge (Figure 2.133).

Cedar appeared to have a superior ability to compartmentalize and develop protective boundaries than the other conifers. This notion was supported by the fact that cedar roots were rarely girdled by the fungus and that longitudinal necrosis in the cambium was considerably less in cedar than in hemlock or Douglas-fir. The wood underlying the area of killed cambium showed slight discoloration, however deposits in the ray parenchyma

in the wood were less frequent than in those formed after injury. The high frequency of compartmentalization observed in cedar roots may be attributed to the barrier zone comprised of a higher density of axial parenchyma cells that accumulate deposits (presumably phenolic in nature) which act as a biological deterrent to the fungus (Figure 2.134).

Comparison of Douglas-fir, western hemlock and western redcedar

Susceptibility has been defined as the 'inability to defend itself against, or to overcome the effects of invasion by a pathogenic organism' (as cited in Bos and Parlevliet 1995). Thus susceptible hosts do not possess the ability to impede attack (growth or development) of a parasite (Bos and Parlevliet 1995). In this study, resistance or susceptibility was determined by a trees' ability to complete NP formation or compartmentalization, or both, while under the influence of the fungus within the frame in which the tree was sampled. Susceptible reactions included situations where there was no visible host response in the bark or cambial region, breaching of either NIT or NP and lack of subsequent barrier formation, or evidence of compartmentalization and callusing but while the fungus was still considered progressive in the bark.

Resistance refers to the ability of a host to hinder growth and activity of a parasite and encompasses a range of mechanisms including resistance to the ingress, establishment and spread of the parasite itself (Bos and Parlevliet 1995). Resistant reactions were classified as such when the infection was contained within a NP or when sections of woody tissue showed barrier zone formation in the wood and callusing at the margin of the wound. Table 2.5 shows the frequency of successful resistance reactions following invasion by *A. ostoyae* for Douglas-fir, western hemlock and western redcedar at the different harvest dates in each field trial.

Chi-square analyses of the frequency of the different stages/events involved in host reactions and the frequency of successful resistance reactions by all three species was only attempted where a sufficient number of instances (i.e. predicted frequency of the phenomenon in question > 1) was available for analysis. As stated earlier, cases where
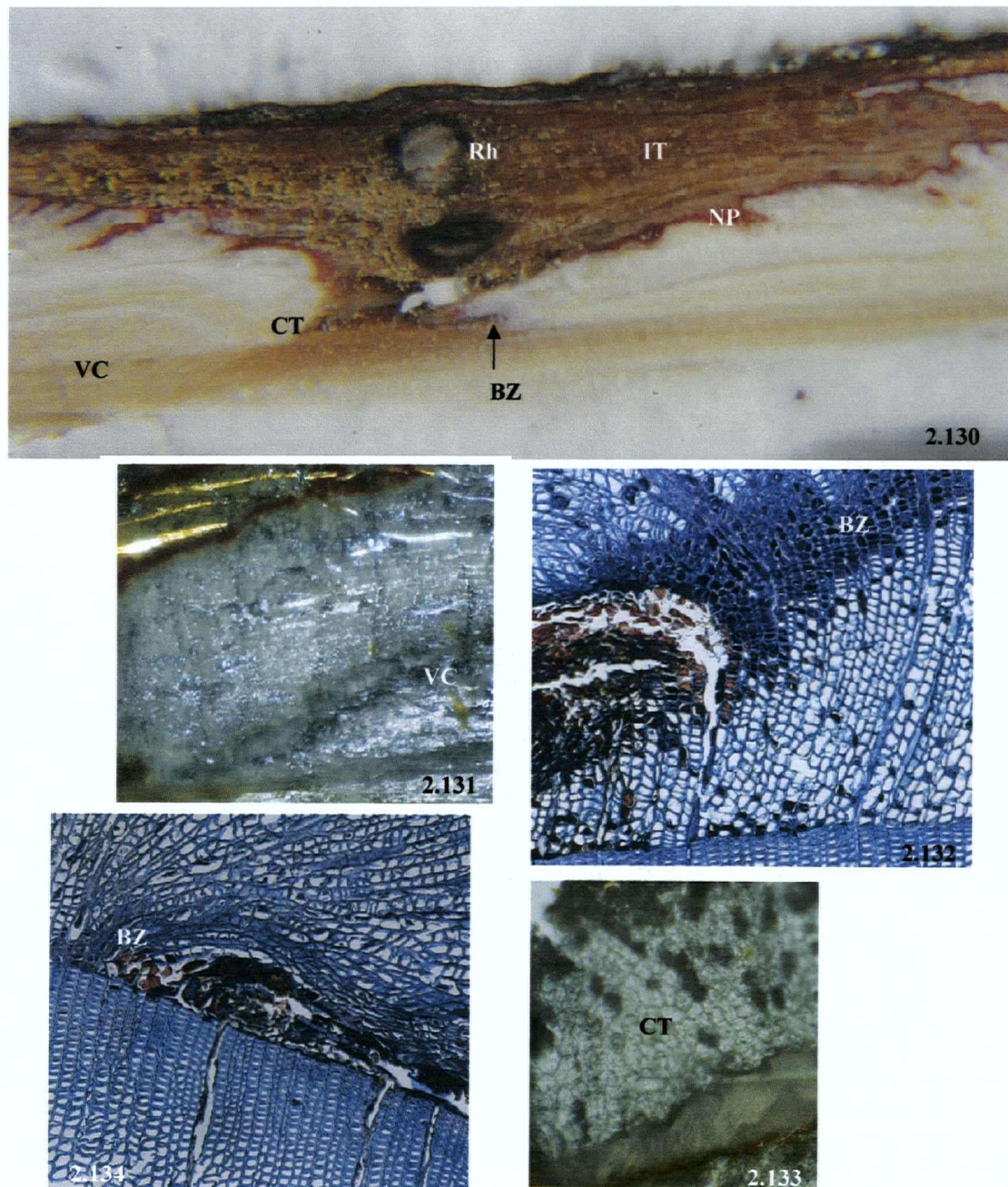
Fig. 2.130. A photomacrograph of a cedar root showing effective compartmentalization and callusing following cambial invasion by *A. ostoyae*. Note rhizomorph embedded within phloem tissue. A NP formed around necrotic tissue in the bark and lateral ingrowth of callus is evident at the margin of the killed cambium. **Fig. 2.131.** A cryofixed section of leading callus edge of the lesion shown in Fig. 2.130 viewed under polarized light. A NP developed in the outer periphery of the callus tissue and a new vascular cambium regenerated within the callus (shown as a darker zone of tissue) and amalgamated with the original vascular cambium. **Fig. 2.132.** A typical barrier zone in cedar is comprised of a higher than average number of axial parenchyma that accumulate polyphenolic deposits. The zone of polyphenolic parenchyma tissue is generally confined to a small zone of tissue immediately adjacent to an area of killed cambium and does not extend tangentially around the circumference of the root. **Fig. 2.133.** A cryofixed showing misalignment of cells at the leading edge of the callus. Cells become oriented in the appropriate plane with increasing distance from the callus edge and/or following fusion of opposing vascular cambia. **Fig. 2.134.** A cedar root showing a barrier zone formed by the uninjured cambium comprised on axial parenchyma with pigmented phenolics. Lateral ingrowth of callus and woundwood extend over the area of killed cambium.

Table 2.5. Frequency of successful resistance reactions following infection by *A. ostoyae* in the roots of Douglas-fir, western hemlock, and western redcedar observed from four separate field trials 2002-2004.

Field Trial	Year	Harvest Time	Frequency of Successful Resistance Reactions as a proportion of successful penetrations by *A. ostoyae*		
			Douglas-fir	Western hemlock	Western redcedar
Hidden Lake	2002	11 weeks	0.33 (n=9)	0.07 (n=14)	0.67 (n=6)
		1 year	0.30 (n=10)	0.00 (n=5)	1.00 (n=4)
Kingfisher	2003	5 weeks	- (n=0)	0.00 (n=1)	- (n=0)
		9 weeks	- (n=0)	0.33 (n=3)	0.50 (n=2)
		1 year	0.20 (n=10)	0.35 (n=23)	0.75 (n=8)
Kingfisher	2004	9 weeks	- (n=0)	0.00 (n=1)	1.00 (n=1)
		5 months	0.11 (n=9)	0.36 (n=11)	0.94 (n=16)
		1 year	0.08 (n=12)	0.42 (n=12)	0.80 (n=10)
Nakusp	2004	8 weeks	- (n=0)	0.50 (n=2)	1.00 (n=1)
		5 months	0.00 (n=2)	0.00 (n=6)	0.71 (n=7)
		1 year	0.00 (n=2)	0.50 (n=2)	1.00 (n=2)

n = the number of cases of successful penetration of roots by *A. ostoyae*.

the pathogen failed to penetrate the root (initial infection) were excluded from analysis of frequency of NIT and NP formation and breaching. Failure to penetrate was not treated as a form of resistance, but rather attributed, in most instances to failure of proper inoculum development or to soil and environmental factors which prevented normal rhizomorph development and adhesion to root surfaces. Thus, 5 week and 9 week harvest dates from the second, third and fourth inoculation trials were excluded from the analysis due to the small sample size and lack of infection in some species on those dates, predominantly in Douglas-fir.

Table 2.6 shows which differences in the frequency of successful resistance reactions between species and at different times and places were statistically significant. Chi-square tests revealed there were significant differences in the frequency of successful resistance reactions between western redcedar and either or both of Douglas-fir and western hemlock on the different harvest dates and field trials (Appendix IV).

Table 2.6. Individual species comparisons of the frequency of successful resistance reactions following inoculation with *A. ostoyae* in Douglas-fir (Fd), western hemlock (Hw) and western redcedar (Cw) for different harvest dates and inoculation trials.

Species Comparisons	Significance[1] of Species Comparisons for each Field Trial and Harvest Date						
	HL (2002)		KF (2003)	KF (2004)		Nakusp (2004)	
	11 wks	1 yr	1 yr	5 mths	1 yr	5 mths	1 yr
Fd/Hw	ns	ns	ns	ns	$p < 0.1$	nt	ns
	(n=9/14)	(n=10/5)	(n=10/23)	(n=9/11)	(n=12/12)	(n=2/6)	(n=2/2)
Fd/Cw	ns	$p < 0.025$	$p < 0.025$	$p < 0.001$	$p < 0.001$	$p < 0.1$	$p < 0.05$
	(n=9/6)	(n=10/4)	(n=10/8)	(n=9/16)	(n=12/10)	(n=2/7)	(n=2/2)
Hw/Cw	$p < 0.01$	$p < 0.01$	$p < 0.05$	$p < 0.01$	$p < 0.1$	$p < 0.01$	ns
	(n=14/6)	(n=5/4)	(n=23/8)	(n=11/16)	(n=12/10)	(n=6/7)	(n=2/2)

[1] The p-value denotes the significance level of which the null hypothesis (i.e. no difference between species in the frequency of successful resistance reactions) would be rejected.
ns = not significant at $\alpha = 0.05$; nt = not tested
n = the number of cases of successful penetration of roots by *A. ostoyae*

Successful resistance reactions following infection by *A. ostoyae* ranged between 0-33% and 0-50% for Douglas-fir and western hemlock trees, respectively. Low frequencies of resistant responses were consistent in Douglas-fir and hemlock roots on each sampling date in each of the four inoculation trials. Hemlock and Douglas-fir did not differ with respect to their ability to contain infections within a lesion in the bark or wood.

Although western red cedar showed a significantly higher proportion of successful resistance reactions than Douglas-fir on all harvest dates in every field trial (Table 2.5), the difference was not significant in the 2002 Hidden Lake trial at 11 weeks (χ^2, p < 1) or at 5 months in the 2004 Nakusp trial (χ^2, < 0.1). Similarly, cedar consistently showed a significantly higher percentage of successful resistance reactions compared to western hemlock, but the difference was less significant at 1 year in the 2004 Kingfisher trial (χ^2, p < 0.1) and at 1 year in the 2004 Nakusp trial (χ^2, p < 0.1).

There was no significant difference in the frequency of successful resistance reactions between the different harvest dates for each species. Furthermore, no difference in the frequency of successful resistance reactions was detected between the different field trials/sites for each species at 1 year.

The above results are interpreted to indicate that resistance did not depend on the length of time that the root remained in the ground in contact with the inoculum block nor did any site factors influence the frequency of the resistance reactions formed in a given host species. For this reason, frequency data for all field trials showing the different stages/events involved host responses leading to NP formation in the bark and compartmentalization in the wood on different harvest dates and in different field trials were pooled for each species and analyzed as such. Table 2.7 shows the frequency of NIT and NP formed, NIT and NP breached, the number of roots that showed cambial invasion and compartmentalization, and the number of roots with successful resistance reactions as a percentage of successful penetrations by *A. ostoyae* in Douglas-fir, western hemlock and western redcedar for all field trials combined. Frequencies of the different stages of host response are not strictly sequential. For example, not all instances of NIT

Table 2.7. The number of root inoculations that resulted in successful penetration by *A. ostoyae*, the frequency of roots showing no visible or ineffective host response, initiation and breaching[a] of non-suberized impervious tissue (NIT) and necrophylactic periderm (NP), killed cambium and compartmentalization, and the number and percentage of roots showing successful resistance reactions in Douglas-fir, western hemlock, and western redcedar trees for all field trials combined.

Species	No. of Successful penetrations	NHR	NIT initiated	NIT breached	NP formed	NP breached	No. roots with killed cambium[b]	CODIT & callusing	No. of successful resistance reactions	Successful resistance reactions as a percentage of successful penetrations
Douglas-fir	54	29	25	10	20	11	20	2	10	**18.52%**
Western hemlock	80	29	51	18	43	22	37	3	21	**26.25%**
Western redcedar	57	1	56[c]	3	53	11	24	22	47	**82.46%**

NHR, no visible or ineffective host response; NIT, non-suberized impervious tissue; NP, necrophylactic periderm; CODIT, compartmentalization of decay in trees

[a] breaching refers to direct penetration through barriers as well as circumvention in places where the host did not complete the formation of the barrier.

[b] killing of the vascular cambium was primarily a reflection of the time and the location of the colonizing fungus in the tissue at the time of sampling. The killing of cambium resulted from a combined number of some, but not all roots showing NHR and breaching of barriers in the bark.

[c] although NIT was generally indistinguishable in cedar, it is the provisional view of this author that in all places where a NP formed, NIT had developed internally abutting that zone.

breaching will result in NP not being formed. NP formation occurs within a few days of NIT initiation and both NIT and NP will be complete in some areas (e.g. central region of the wounded tissue) but incomplete in others (e.g. contiguous with the original periderm). Table 2.8 shows which differences in frequency of host reactions shown in Table 2.7 were significant. The corresponding Chi-square tests can be found in Appendix V.

Table 2.8. Individual species comparisons of the frequency of roots showing NIT initiated, NIT breached, NP formed, NP breached and compartmentalization following inoculation with *A. ostoyae* in Douglas-fir (Fd), western hemlock (Hw), and western redcedar (Cw) trees for all harvest dates in four inoculation trials.

Type of host response	Significance[a] level of differences between pairs of species for different stages/events of host reactions in the bark and wood		
	Fd/Hw	Fd/Cw	Hw/Cw
NIT initiated	(p < 0.05) (n[b]=54/80)	(p < 0.001) (n=54/57)	(p < 0.001) (n=80/57)
NIT breached	ns (n=25/51)	(p < 0.001) (n=25/56)	(p < 0.001) (n=51/56)
NP formed	ns (n=54/80)	(p < 0.001) (n=54/57)	(p < 0.001) (n=80/57)
NP breached	ns (n=20/43)	(p < 0.01) (n=20/53)	(p < 0.01) (n=43/53)
CODIT & callusing	ns (n=20/37)	(p < 0.001) (n=20/24)	(p < 0.001) (n=37/24)

[a] The p-value denotes the significance level of which the null hypothesis (i.e. no difference between species in the frequency of a particular host reaction) would be rejected.
ns = not significant at $\alpha = 0.05$

[b] the sample size (n) changes for different stages of host reactions whereby for NIT initiated and NP formed, n = the number of cases of successful penetration of roots by *A. ostoyae*; for NIT and NP breached, n = the number of cases that resulted in NIT initiation and NP formation, respectively; for CODIT & callusing, n = the number of cases in which the vascular cambium was killed as a combined result of some, but not all roots showing no visible or ineffective host response and breaching of barriers in the bark

Chi-square tests revealed significant differences among species in the frequency of NIT initiation (χ^2, p< 0.001). Western redcedar differed significantly from both Douglas-fir and western hemlock. Although NIT formation in Douglas-fir was slightly lower than western hemlock, the difference was still significant. A higher percentage of western hemlock and Douglas-fir roots showed no visible host response following invasion by *A. ostoyae* than cedar.

The above results are interpreted to indicate that the capacity for trees to trigger the process of phellogen renewal is less frequent in Douglas-fir and western hemlock than in western redcedar. Of the total number of western redcedar roots sampled at various intervals over the course of 3 years, only 1 of 57 (2%) roots did not develop NIT compared to 36% (n=80) in western hemlock and 54% (n=54) in Douglas-fir.

The breaching of NIT depends on its speed of formation relative to the speed at which the fungus or the toxins produced by the fungus move through host tissue. NIT was breached in all species although breaching was consistently more frequent in Douglas-fir and western hemlock than western redcedar.

NP formation varied at each sampling date and was primarily dependent on whether the host developed NIT. In most cases, both NIT and NP were observed in roots examined on each harvesting date. A NP can differentiate within several days following the establishment of NIT, even when a fully differentiated zone of NIT is not continuous with the original periderm. Hence, one may find both NIT and NP differentiated in the mid-phloem underlying necrotic tissue while NIT, and therefore NP's are absent or still in the developing stages near the junction of the original periderm.

Although NIT was difficult to discern histologically, it's generally assumed that all species that formed NIT also formed a NP. Western redcedar always formed NP's with higher frequency than either Douglas-fir and western hemlock (Table 2.8). Similar to NIT formation, the frequency of NP formation in cedar was significantly greater than in Douglas-fir and western hemlock. Again, there was no difference between Douglas-fir and western hemlock.

The fact that NIT and NP were initiated in nearly all cedar roots penetrated by the fungus and those barriers showed significantly less breaching by the fungus suggests that Douglas-fir and western hemlock may be slower at completing host defense structures in response to infection by *A. ostoyae* than cedar. A slower rate at which host defenses are induced in Douglas-fir and western hemlock are also suggested by the wider zone of NIT in those species and incomplete differentiation of tissue leading to NP formation.

Breaching of NPs was common among all species, particularly in Douglas-fir and western hemlock. Chi-square tests revealed significant differences between cedar and hemlock and between cedar and Douglas-fir. The frequencies at which NPs were breached in either Douglas-fir or western hemlock were similar.

There were no differences among species in the frequency of killed cambium following successful penetration by *A. ostoyae*. Overall, *A. ostoyae* advanced to and killed the cambium in 37% (n=54), 46% (n=80), and 42% (n=57) of the Douglas-fir, western hemlock and western redcedar roots, respectively. In most situations, cambial invasion was more frequent on roots harvested at 5 months and 1 year compared to the first 11 weeks following inoculation. However, strong differences were evident among species in the frequency of compartmentalization and callusing (χ^2, p< 0.001).

Compartmentalization took place in only 2 of 20 (10%) Douglas-fir roots and in only 3 of 37 (8%) western hemlock roots. These low percentages are in large contrast to the 92% (n=24) of western redcedar roots that showed compartmentalization following cambial invasion with *A. ostoyae*. Species comparison revealed significant differences between cedar and hemlock (χ^2, p< 0.001) and between cedar and Douglas-fir (χ^2, p< 0.001). There was no difference in the frequency of compartmentalization between Douglas-fir and western hemlock.

Due to the fact that nearly all cedar roots initiated host defense responses leading to NIT and NP in the bark and compartmentalization of infected tissue following cambial invasion by the fungus, and few roots showed breaching of those barriers, it was not surprising that the species comparisons for the frequency of host responses showed fairly

significant differences between western redcedar and the other conifers (Table 2.8). Resistance, defined in this thesis as the ability of trees to trigger and complete defense responses to contain the fungus to a lesion on the root, was 3 to 4 times more effective in western redcedar than in western hemlock (χ^2, p< 0.001) and Douglas-fir (χ^2, p< 0.001) trees, respectively (Figure 2.135).
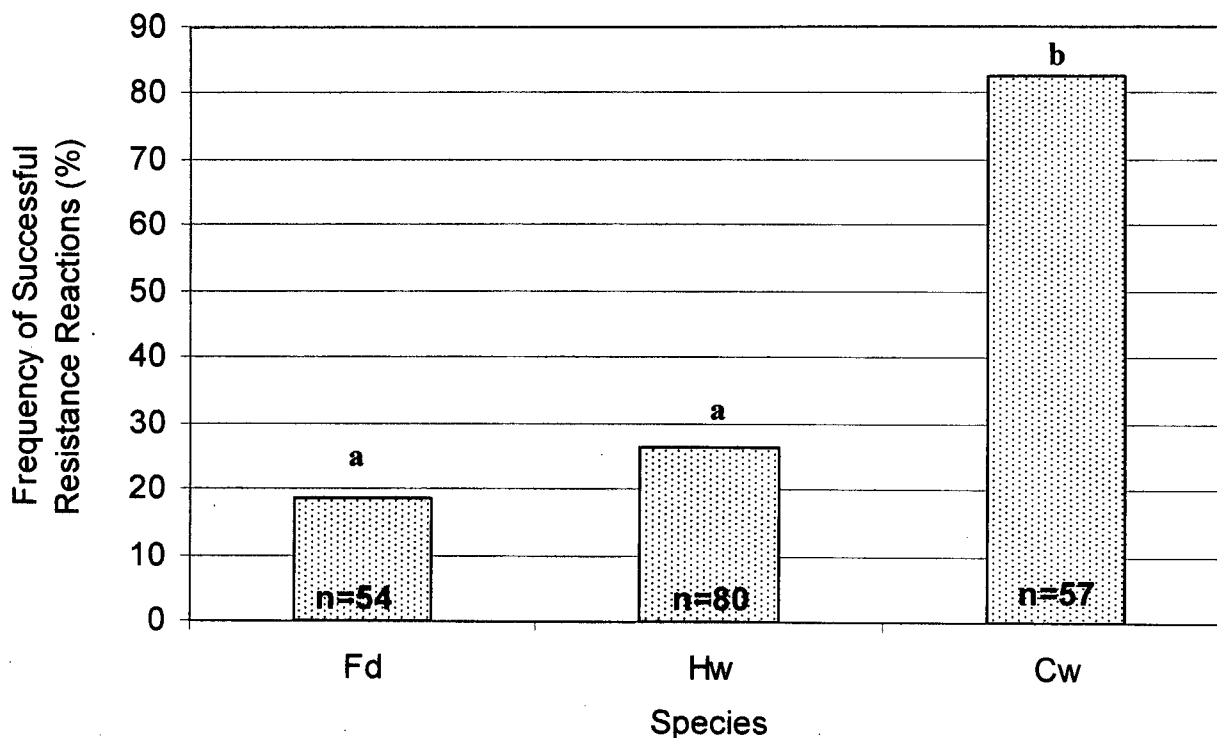
Fig. 2.135. Successful resistance reactions as a proportion of the total number of roots showing successful penetration by the *A. ostoyae* in Douglas-fir (Fd), western hemlock (Hw) and western redcedar (Cw) trees. Note: successful penetration includes cases where the original periderm is not breached but there was evidence of browning of the living phloem. Different letters denote significant differences at $\alpha = 0.001$.

2.3.6. HOST VARIABLES

There is some evidence in the literature, supported by field observations in this study suggesting that the location of an infection on the root system may influence the type of host response and the ability of trees to resist progressive advance of the fungus in host tissue. Although a number of different root variables were measured at the time of sampling, only two root variables that were part of the original design, namely root diameter and distance from the root collar will be discussed here. Figures 2.136 and 2.137 give the proportion of successful resistance reactions by species in relation to the root diameter and distance from the root collar, respectively.

In many cases, host variables such as root size, distance from the root collar, and also root age were confounded. This was certainly the case for Douglas-fir which typically forms a well-developed lateral root system. Larger diameter roots located at or near the root collar are most likely older, while smaller diameter roots located at some distance from the root collar are younger. However, in western hemlock this is less likely the case, and perhaps even less so in western redcedar where dense, profuse networks of smaller diameter roots are common. Thus, the number of cases within each size class between species differed in that hemlock and cedar generally had a larger number of cases occupying the smaller-intermediate root diameter size classes whereas Douglas-fir had a larger number of cases occupying the intermediate-larger root diameter size classes. Furthermore, the lack of infection in the 2003 Kingfisher trial (the year in which a larger and evenly divided number of cases were selected for inoculation) also contributed to the lower number of cases in the largest root diameter size class.

Root diameter apparently had no effect on the ability of trees to resist the advancing fungus (Figure 2.136). A slight declining trend in the frequency of successful resistance reactions was seen with increasing root diameter particularly for Douglas-fir and western hemlock roots. However, the difference in proportion of roots that formed successful resistance reactions between root diameter size classes was relatively small.
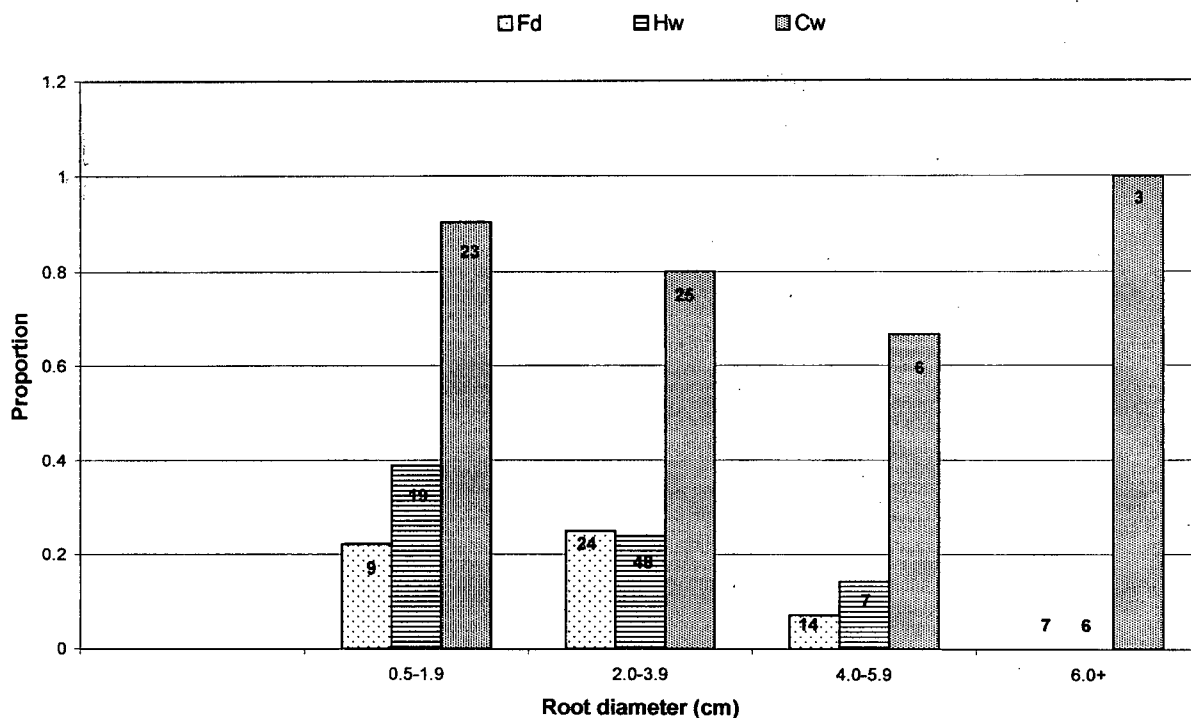
Fig. 2.136. Successful resistance reactions as a proportion of roots penetrated by *A. ostoyae* in relation to root diameter. Number of samples is noted in each bar graph. Fd, Douglas-fir; Hw, western hemlock; Cw, western redcedar
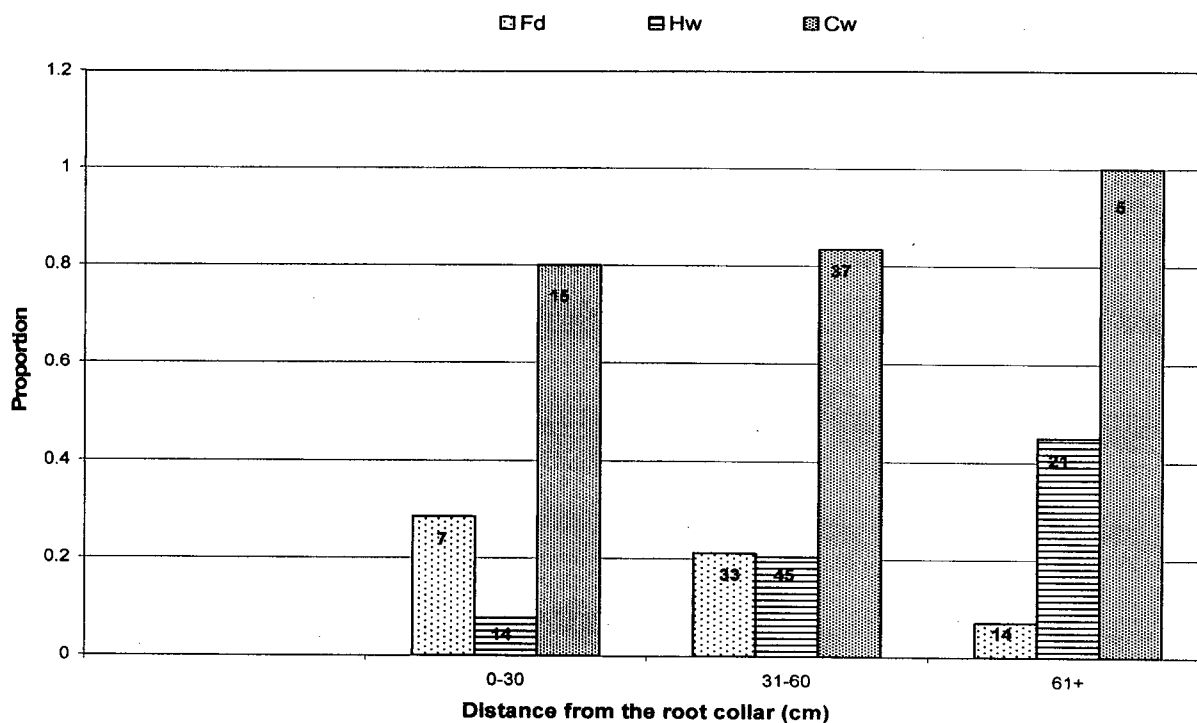
Fig. 2.137. Successful resistance reactions as a proportion of roots penetrated by *A. ostoyae* in relation to the distance from the root collar. Number of samples is noted in each bar graph. Fd, Douglas-fir; Hw, western hemlock; Cw, western redcedar

The largest diameter class had the lowest proportion of successful resistance reaction (0%) for both Douglas-fir and western hemlock. These roots failed to produce a NP in the bark or compartmentalize the infection in the wood following inoculation with *A. ostoyae*.

However, examinations on western hemlock roots naturally infected with *A. ostoyae* as described in the following Section 2.3.8, revealed that the species frequently checked infections by forming a NP in the bark or via compartmentalization when the fungus advanced to the root collar area. NP's formed in this area more often consisted of bands of thick- and thin-walled phellem. Similarly, this type of NP is more frequently found on older and larger Douglas-fir roots.

Robinson (1997) suggested that NP's comprised of bands of thick- and thin-walled phellem was a structural characteristic that helped impart increased resistance to the spread of the *Armillaria* in western larch roots. Although this type of NP was frequently associated with older and larger roots of western hemlock and Douglas-fir, the ability of either host to contain the infection within a new NP barrier was lacking.

Lethal attack by *A. ostoyae* necessarily involves killing of the root collar. The root collar area is also the area where the more resistant types of periderms (i.e. NPs with multiple bands of thick- and thin-walled phellem) are produced in some species like western larch and Douglas-fir (Robinson 1997), and in this study, western hemlock. However, the majority of Douglas-fir and western hemlock roots in the largest root diameter size class (6.0 cm +) had progressive lesions on their roots. Other determining factors may be the inoculum potential of the fungus, thicker bark tissue which may offer a substantial food base for the fungus, and changes in the inoculum potential as the fungus advances in the host. The fact that the phloem of hemlock roots contains large clusters of sclereids likely serves as an obstacle to periderm development enabling the fungus to either breach or circumvent a zone of redifferentiating tissue. In this study, western redcedar showed much higher proportions of successful resistance reactions compared to the other conifers

in all root diameter size classes. Consequently, determining the relationship between host variables and the frequency of successful resistance reaction for species which tend to represent the two extremes is difficult.

The same problem arises in determining a relationship between the frequency of successful resistance reactions and the location of the infection on the root (distance from the root collar) (Figure 2.137). With the low proportion of successful resistance reactions in both western hemlock and Douglas-fir no clear trends could be discerned. As distance from the root collar increases, the root diameter decreases, particularly for western hemlock and Douglas-fir, and NPs with thin-walled phellem were more common. However, the graph for western hemlock showed increasing resistance with increased distance from the root collar which suggests that hemlock roots tend to be less resistant with increasing root size. These observations do not fit well with observations from naturally infected hemlock roots which tend to show infections more often contained to a lesion in the bark when the fungus is located at or near the root collar.

In Douglas-fir, there was a slight decreasing trend in the frequency of successful resistance reactions with increasing distance from the root collar which is what might be expected for this species. However, the 0-30 cm distance class also contained some of the larger diameter roots which generally showed fewer resistance reactions.

There was no relationship between root age, inner bark thickness, tree size and tree age with respect to the frequency of successful resistance reactions in either species (Appendices VI-IX). In Douglas-fir, a slight trend in increasing susceptibility was shown with tree size, but no distinct trend could be detected in western hemlock. Average tree size (DBH) ranged between 112-224 mm, 60-150 mm, and 90-147 mm, for Douglas-fir, western hemlock and western redcedar, respectively. Similarly, no distinct relationship could be detected with tree age. Tree age ranged between 22-31, 20-27, and 19-29 years for Douglas-fir, western hemlock and western redcedar, respectively. Had the study encompassed a wider range of ages, relationships might have been more clearly defined. Root age and inner bark thickness are confounded with root diameter, probably more so for western hemlock and Douglas-fir than western redcedar.

In this study, the lesion size on roots infected with *A. ostoyae* varied among species and reasons for this are numerous. Although the intent was to have uniform inoculum potential, there was likely some variation in potential of inoculum blocks between individuals which can be influenced by the environment in which the fungus was growing (i.e. interactions with the microbial root surface communities or drought conditions reducing rhizomorph growth and development). Furthermore, the type of host response and resulting lesion may also be influenced by inoculum potential during the initial stages of infection as well as any changes in inoculum potential as the fungus advances through the host tissue. Smaller lesion sizes may be a direct result of infection of the host via fungal exudates at surface contact with the root in the absence of direct penetration by mycelium. Variation in lesion size could also result from multiple rhizomorph infections at inoculum contact versus penetration of the host root by a single rhizomorph. Thicker phloem tissue, common in some species like Douglas-fir, may be more favourable to the fungus as it offers a suitable and preferential food base in which the fungus may thrive. In contrast, induced rhytidome formation in western redcedar caused a 2-3 fold increase in bark thickness, although the fungus was rarely seen to be colonizing this tissue and never penetrated beyond the newly established periderm.

Nagy *et al.* (2005) considered Norway spruce [*Picea abies* (L.) Karst.] clones infected by the blue-stain fungus *Ceratocystis polonica* (Siem.) C. Moreau that produced the shortest lesion lengths to be the most resistant. In this study the situation is markedly different for western redcedar. Cedar initiates a localized response involving induced rhytidome formation whereby periderm formation extends up to 20 cm proximally and distally from the primary infected tissue to form one continuous lesion on the surface of the root. Generally, lesions bounded by a NP in western hemlock and Douglas-fir were smaller than those roots showing no host response. However, western redcedar induced a long-range resistance response involving rhytidome and successive periderm formation which ultimately leads to longer lesion lengths. Due to the nature of host reactions in the different conifer species, lesion length is not a good measure of resistance for inter-species comparisons.

One factor influencing the success of host reactions following attack by plant pathogens is the physiological condition of the host plant. Host vigour describes the overall robustness or physiological condition of the tree as indicated by its relative growth rate within a particular environment. Tree variables such as crown position, crown volume, and the mean bole diameter increment are commonly used to classify tree vigor, however they provide very little information about the current state of health of a tree (Wargo and Harrington 1991). Host vigor may influence the rate at which defense mechanisms are initiated and the overall susceptibility of a host tree to killing by the pathogen. On the other hand, host vigour may act primarily by increasing the rate of healing in trees (Neely 1988). Pearce *et al.* (1986) reported that more vigorous, dominant trees were killed less frequently by *Armillaria* than the less vigorous, suppressed trees. Redfern (1978) also showed that tree vigour did not confer resistance in small trees.

Rosso and Hansen (1998) measured tree vigor in Douglas-fir as the above-ground biomass increment per unit of photosynthetic tissue or growth efficiency. Growth efficiency (GE) is a function of photosynthetic efficiency and is thought to influence carbon allocation and the ability of a tree to produce defense compounds (Rosso and Hansen 1998). They postulated that trees with higher GE are less susceptible to disease than those with lower GE. Of the methods used to assess vigor, the visual rating of the crown appearance is most often used in practice (Kostka and Sherald 1982). More specific methods may include a measurement of cambial electrical resistance (CER) or direct measurement of the radial growth increment (RG). Rosso and Hansen (1998) calculated both GE and RG to test whether there were any significant effects of thinning, fertilization and pruning treatments on the vigour of Douglas-fir trees after 10 years. The authors found that RG and GE responses were statistically similar in all cases. Therefore, the current radial growth increment should give an adequate measurement of tree vigour. In this study, tree vigour was measured as the average current annual increment divided by the 5-year average from cores taken at four cardinal directions at the root collar.

The proportion of successful resistance reactions in relation to tree vigor is shown in Figure 2.138. Results showed an increasing trend in the proportion of successful

resistance reactions with increasing tree vigor for all species. Again, cedar showed consistently higher proportions than the other two conifers. Interestingly, the largest increase occurred in hemlock where the proportion of successful resistance reactions in the high vigor class was nearly double that of the intermediate class (Figure 2.138).
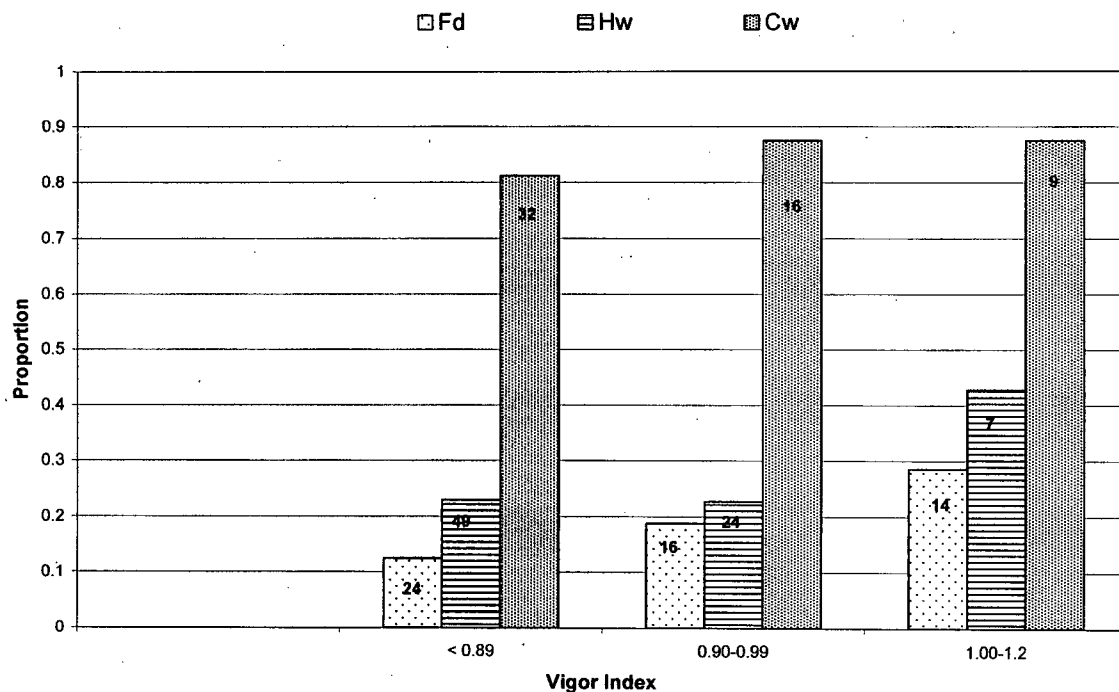
Fig. 2.138. Successful resistance reactions as a proportion of roots penetrated by *A. ostoyae* in relation to tree vigour. Number of samples is noted is each bar graph. Fd, Douglas-fir; Hw, western hemlock; Cw, western redcedar

Lower tree vigour, or stress, can alter the ability of trees to resist infection by modifying the relationship between host and fungus. For example, chemical changes induced in the host caused by certain stress factors may promote susceptibility by producing compounds that stimulate growth and pathogenesis or limit the production of fungal inhibitors by the host (Wargo 1984, Garraway *et al.* 1991). Variation in resistance to *A. ostoyae* within a host species may also be attributed to the characteristics of the planting site and host genetics. It is interesting that the proportion of successful resistance reactions increased with increasing tree vigour for all species, especially given the consistently low frequencies of successful resistance reactions in both Douglas-fir and western hemlock. Hence, tree vigor may be one of the major factors involved in determining the degree of

host resistance to disease development on roots. Tree vigor is determined by a tree's physiological performance within a particular environment, and this performance depends upon the tree's genetic capacity (Wargo and Harrington 1991), as well as microsite conditions and available growing space. Genetic variation gives a range of physiological performance and therefore a range of vigor indices under a given set of environmental conditions.

The data showed a range of vigor indices in all three species and reasons for such are complex. Western redcedar and western hemlock were always in the understory. Thus, the 5-year increment is also reduced and the ratio need not be lower than in the overstory. More than half the number of western hemlock and western redcedar trees had vigor indices less than or equal to 0.89. Nevertheless, in the lowest vigor index class, cedar showed a significantly higher proportion of trees with successful resistance reactions compared to western hemlock and Douglas-fir. The relative susceptibility of the host species, and the influence of tree vigour play an integral role in the ability of host trees to form resistant reactions in adjacent tissues following attack by *A. ostoyae*.

2.3.7 WINTER INOCULATION TRIAL

Field observations and examinations of naturally infected Douglas-fir trees suggest that older trees that succumb to mortality within 1 or 2 years of infection may result from the fungus advancing and killing host tissue during the growing season, as well as in the winter months when the tree is dormant and unable to initiate defense responses (B. van der Kamp, pers. comm.). Unlike dormancy in stem tissues whereby no cell division takes place, root tissues continue to grow, albeit slowly due to low soil temperatures. Although growth of roots tips may continue, the vascular cambium and phellogen activity virtually cease in the winter.

In British Columbia, abiotic injury to the bark of Douglas-fir and western larch roots in the winter showed no response with respect to phellogen renewal when sampled 2-months later (Robinson *et al.* 2004a). Similarly, Douglas-fir roots mechanically

wounded to the xylem in October and March (during tree dormancy) did not produce traumatic resin ducts until cambial activity resumed in the late spring of the next growing season (Cruickshank *et al*. 2006). The authors also noted traumatic resin duct formation soon after cambial awakening in Douglas-fir roots following inoculation with *A. ostoyae* (Cruickshank *et al*. 2006). Invasion of the vascular cambium in the root collar area of 13-year-old *Pinus radiata* D. Don by *A. novae-zelandiae* or *A. limonea* (Stevenson) Boesewinkel during the dormant season was evidenced by the dead xylem surface being continuous with the end of an annual ring (van der Kamp and Hood 2002).

In this study, examinations of the root collar on Douglas-fir naturally infected with *A. ostoyae* also suggest cambium invasion during a period of dormancy as evidenced by the presence of latewood at the xylem surface killed by the pathogen and the formation of traumatic resin ducts in the early part of the next annual ring (Figure 2.139). Thus, invasion of the vascular cambium at the root collar probably occurs most frequently between the cessation of xylem growth at the end of the growing season and its initiation the following spring. Thus, the time of year at which trees become infected by *A. ostoyae* may also have an influence the extent of damage observed on host species.
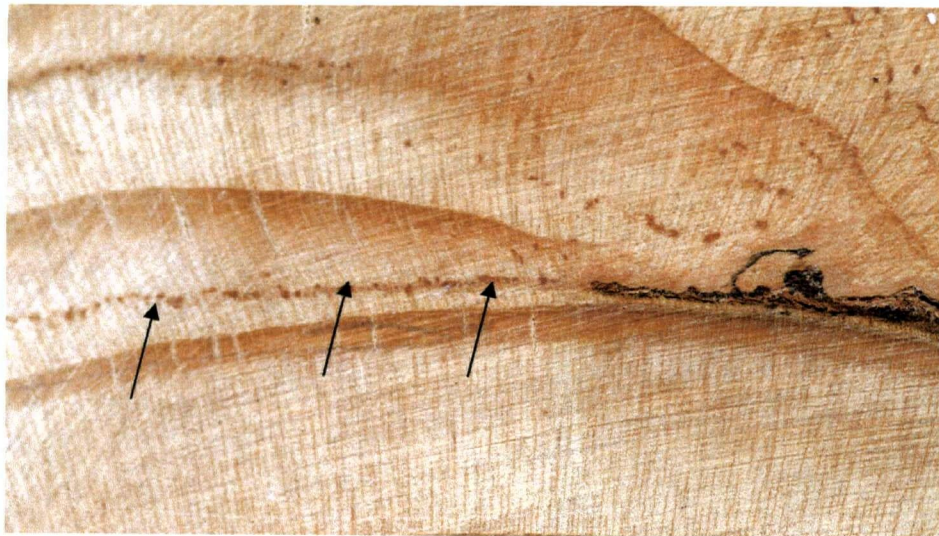
Fig. 2.139. A Douglas-fir root infected with *A. ostoyae* and showing traumatic resin canals (arrows) formed in the next annual growth ring soon after the onset of growth in the next annual ring.

Bannan (1936) showed that following injuries to stems of *Tsuga canadensis* (L.) Carr. during dormancy traumatic resin canals were differentiated as the first newly formed cells at the beginning of the next growing season. Shigo and Tippett (1981) also suggested that the position of the barrier zone in the earliest portion of the growth ring indicated spread of *A. mellea* during a period of dormancy or soon after the onset of growth.

Results from three inoculation trials (methods described in Section 2.2.3.1) investigating whether *A. ostoyae* could successfully penetrate and cause infection in hosts during the winter months are given in Table 2.9 which shows the number roots inoculated with *A. ostoyae* over a period of dormancy for each species and the frequency of infection in hosts on each of the harvest dates.

No roots showed any evidence of infection during the 2002 and 2004 field trials. In the 2003 trial, 33% (n=6) and 40% (n=5) of the roots sampled in the spring showed slight necrosis in the bark at inoculum contact in western hemlock and western redcedar, respectively. No rhizomorphs were associated with those infections. Necrosis likely resulted from fungal toxins exuding from the cambial end of the inoculum block at root contact. In the 2003 Kingfisher trial, the majority of roots were harvested in the early spring of 2004, but some roots were left in place to assess the potential for infection throughout the growing season. Harvesting of roots 2-3 months following the spring sampling revealed an increase in the frequency of infection with time. The frequency of infection on these later dates did not differ significantly among species and did not appear to occur sooner on some species than others. These results are in agreement with those reported in the field inoculation trials (Section 2.3.5).

There was no significant difference in the frequency of infection among all field trials in Douglas-fir for roots sampled in the spring, however the difference was significant for western hemlock (χ^2, p <0.001) and western redcedar (χ^2, p < 0.001), as a result of the few roots that showed slight browning in the inner bark at inoculum contact at spring sampling in the 2003 field trial. The lack of infection by *A. ostoyae* over a period of dormancy (approximately 7 months) contrasts with that observed in the four separate

Table 2.9 The number of Douglas-fir, western hemlock and western redcedar inoculated with *A. ostoyae* in the fall and the frequency of the resulting infection the next spring.

Species	Field Trial	Date Inoculated	Date Harvested	No. of root inoculations	No. of roots infected	Frequency of infection
Douglas-fir						
	Hidden Lake	11/04/2002	06/16/2003	16	0	0.00
	Kingfisher	10/18/2003	05/20/2004	8	0	0.00
			6/30-7/24/2004	11	1	0.09
			9/24/2004	16	5	0.31
	Kingfisher	09/12/2004	4/25/2005	45	0	0.00
Western hemlock						
	Hidden Lake	11/04/2002	06/16/2003	14	0	0.00
	Kingfisher	10/18/2003	05/20/2004	6	2	0.33
			6/30-7/24/2004	9	4	0.44
			9/24/2004	15	8	0.53
	Kingfisher	09/12/2004	4/25/2005	37	0	0.00
Western redcedar						
	Hidden Lake	11/04/2002	06/16/2003	14	0	0.00
	Kingfisher	10/18/2003	05/20/2004	5	2	0.40
			6/30-7/24/2004	11	5	0.45
			9/24/2004	14	9	0.64
	Kingfisher	09/12/2004	4/25/2005	28	0	0.00

inoculation trials initiated at the onset of the growing season (Section 2.3.5). Limiting factors such as low temperature may have had an adverse effect on rhizomorph growth and development because few if any additional rhizomorphs were produced during the winter months, thus reducing the likelihood of infections on roots.

Rishbeth (1978) reported that low temperatures may be a limiting factor on rhizomorph initiation and growth in forest soils in the north temperate zone during the winter months. Pearce and Malajczuk (1990) also found minimal growth of rhizomorphs during the winter/spring season compared to the summer months.

The position of the traumatic resin canals in an annual ring on the roots of Douglas-fir and western hemlock provides an indication of the time of year the cambium was invaded by *Armillaria*. However, the fungus may exist as a lesion in the bark for a given period of time before cambial invasion occurs. Damage caused by *A. ostoyae* over the course of the dormant season may be similar to that observed on the basal stem disk from the naturally infected Douglas-fir as shown in Figure 2.138. Separate cambial invasion events probably resulted from extension of pre-existing lesions in which the fungus had been held in check.

Similarly, Wilbur *et al.* (1972) reported the killing of peach trees during the winter months following inoculation with *A. mellea*, although the incidence of mortality was significantly less than what was reported for spring and summer. Hence, *Armillaria* behaves very much like a perennial canker pathogen, invading and killing bark and cambial tissue during both the growing and dormant seasons. At times, Armillaria may behave like a diffuse canker whereby no effective host resistance occurs at any time of the year, particularly on younger trees and trees that have been severely stressed by some other means. However, further research is needed to substantiate this hypothesis.

2.3.8 LESION ANALYSIS OF NATURALLY INFECTED INDIVIDUALS

In 2002, examination of naturally infected roots of Douglas-fir, western hemlock and western redcedar showed distinct differences among species in host reactions and the ability of the host to contain the fungus within a lesion on the root (Methods described in Section 2.2.3.2).

Table 2.10 shows the number of trees sampled and the total number of lesions examined on host roots of each species. Observations on excavated roots systems revealed that the frequency of mycelial transfer at root contacts between Douglas-fir and western hemlock roots was generally higher than that observed between root crossings of cedar and another species. In this study, cedar roots inoculated with *A. ostoyae* and sampled at 5 months and 1 year later already showed evidence of sloughing of infected tissue from the surface of the root. Therefore, it is highly probable that this, too, occurred in several of the trees naturally infected with *A. ostoyae* such that any evidence of previous infections on roots has disappeared.

Table 2.10. The average age, average tree size, total number of trees sampled and number of lesions examined from the roots of Douglas-fir , western hemlock, and western redcedar trees naturally infected with *A. ostoyae* for all species at both sites near Hidden Lake

Tree Species	Average age (yrs)	Average DBH (mm)	No. of Trees Sampled	No. of Lesions Examined
Douglas-fir	28	133	5	25
Western hemlock	24	24	13	51
Western redcedar	27	158	11	26

Both *A. ostoyae* and *A. sinapina* were found inhabiting the sites. Sometimes, rhizomorphs of both species were found on the same host tree. *A. sinapina* is considered weakly pathogenic. Monopodial rhizomorphs appeared to be abundant, most often seen growing epiphytically over root systems anchoring themselves to the outer rhytidome or running underneath a bark scale. However, *A. sinapina* rhizomorphs were never observed to penetrate the living bark directly.

All isolations made from *Armillaria* infected roots were believed to be caused by *A. ostoyae*. Confirmation of species identification was done by pairing the wild-type isolate with *A. ostoyae* Isolate 87-01, the isolate used in the inoculation trials. The sub-sample of roots from which *Armillaria* was isolated in pure culture showed incomplete intermingling of mycelia at the opposing edges of the mycelial colonies (Figure 2.140). No dark demarcation zone developed in the media with the Isolate #87-01, however when paired against the known isolate of *A. sinapina* (Merritt Isolate), pigmentation between the two opposing mycelia was distinct. Thus, mating tests revealed that the isolates obtained from infected and recently killed trees were *A. ostoyae* (Figure 2.141).
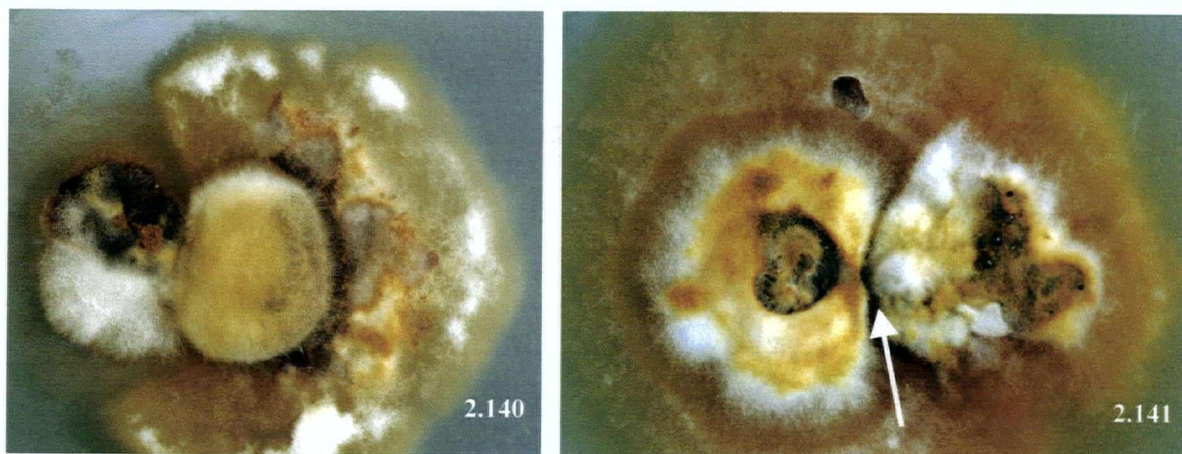
Fig. 2.140. An isolate obtained from a naturally infected western redcedar tree (tree #7905) paired with *A. ostoyae* isolate 87-01. After 8 weeks, incomplete intermingling of mycelia between the two opposing colonies was evident but lacked pigmentation in the media. **Fig. 2.141.** Isolate obtained from the same tree identified in Fig. 2.140 but paired against *A. sinapina* isolate (Merritt). A dark demarcation line (arrow) developed in the pigment between the two opposing colonies indicating interspecific crosses (isolates of two different species of *Armillaria*).

In most cases, distinct *Armillaria*-lesions were evident at root contacts, especially transfers between Douglas-fir and western hemlock trees and were confirmed by the presence of mycelial fans underneath the bark. The frequency of mycelial transfer at root contact between an infected Douglas-fir and/or hemlock tree and a western redcedar tree was noticeably less. Tissue samples at root contact on cedar trees frequently showed slight browning in the inner phloem, but lacked mycelial transfer. The necrosis in the bark may be a case of indirect penetration by *A. ostoyae* via fungal exudates.

Douglas-fir

Douglas-fir in contact with a recently killed tree typically showed resinous lesions where the roots crossed. Infections were quite often initiated on smaller diameter roots where the inner bark is extremely thin and incapable of confining an infection within an NP barrier. Progressive infections on smaller diameter roots may be checked at the junction with a larger primary or secondary root. Further examinations of lesions on Douglas-fir roots revealed that healing over wounds by the formation of callus and woundwood can take up to 10 years. A couple of the infected trees sampled had more than two-thirds of their stem girdled at the base of the tree but lacked above ground symptoms of disease (i.e. chlorosis and reduction in height growth). However, frequently trees show markedly reduced height increment (and possibly chlorosis) the year following invasion but normal height growth 2 or 3 years later following callusing of basal lesions (van der Kamp, pers. comm.). Basal resinosus was common.

Progressive lesions had large wedges of mycelium advancing in the inner bark and cambial zone (Figure 2.142). Necrotic tissue occurred ahead of mycelial wedges (Figure 2.143) and the boundary between necrotic and apparently healthy tissue was either sharp, showing a distinct demarcation in the phloem (Figure 2.144), or diffuse, showing sporadic browning of tissue in the phloem ahead of the primary lesion (Figure 2.145). At the infection front on roots showing no or ineffective host response, adjacent host tissue showed little or no cell hypertrophy.

Root collar examinations on a couple trees revealed that trees sustained repeated mycelial advances over a long period of time (Figure 2.146). Infections advancing to the root collar area usually killed the cambium but trees were not completely girdled. Observations of lesions at the root collar suggested that the fungus advanced and killed the vascular cambium while the tree was dormant. Latewood tracheids at the end of the annual ring and a resin barrier produced by the uninjured cambium in the early portion of the next annual ring suggested that invasion of the cambium took place between the end of the previous growing season and the initiation of growth the following year. (Figure 2.147). Single or double resin barriers were also observed within a growth ring. Callus tissue was formed around the killed tissue and still appeared to be growing over the wound after several years.

The effect of resin on mycelial growth of *Armillaria* is varied. Rykowski (1975) observed that resin-soaked lesions on Scots pine limited spread of *Armillaria*. Similarly, young, vigorously growing Douglas-fir trees checked infections by laying down a callus and resin barrier, and forming a latent canker (Buckland 1953). Observations in this study suggest that resinosus in the phloem does little to deter the advance of *A. ostoyae* in those tissues. Barrier zone formation comprised of traumatic resin ducts in the uninjured wood may slow the spread of *A. ostoyae*, however this type of barrier zone is not always effective at containing the infection and the fungus may infect new tissue formed after this barrier.

NP's were sometimes formed in advance of a penetrating mycelium (Figure 2.148). However, in most cases browning occurred in host tissue beyond the NP which indicated breaching or incomplete differentiation of the newly formed barrier. NP's with multiple bands of thick- and thin-walled phellem were commonly formed on older roots closer to the root collar, however as most trees with lesions at or near the root collar had several major lateral roots infected, the inoculum potential was sufficiently high to overwhelm any type of host response. If the host is able to form such barriers, the fungus commonly breaches it and continues to advance to the root collar where it may or may not be compartmentalized.
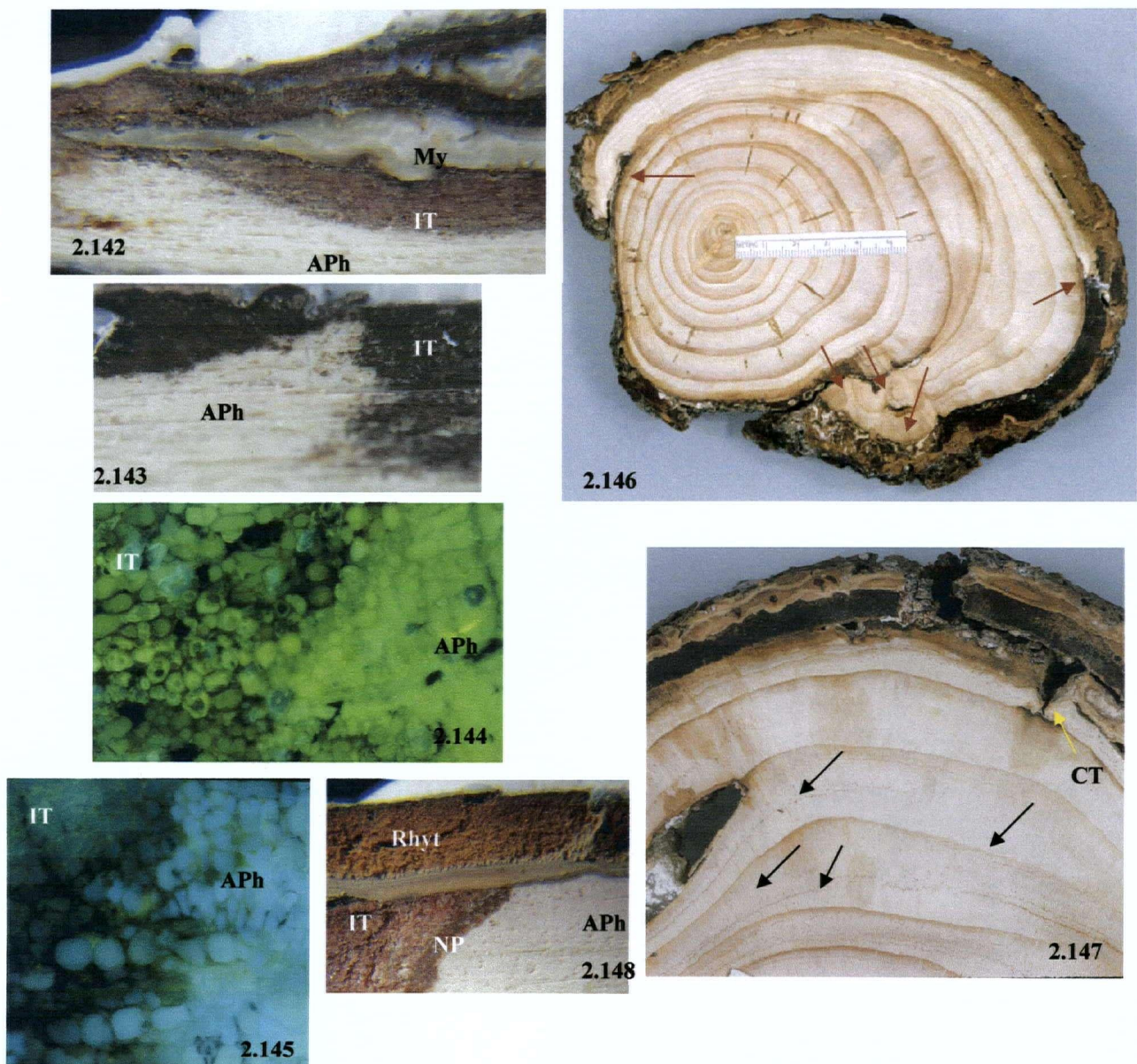
Fig. 2.142. A photomacrograph of a progressive lesion on a Douglas-fir root naturally infected with *A. ostoyae*. Large wedges of mycelium are seen advancing in the inner bark and cambium. Browning tissue in advance of mycelial colonization is seen. **Fig. 2.143.** Another photomacrograph showing typical necrosis in the bark in advance of a penetrating mycelium. **Fig. 2.144.** A cryofixed section of a Douglas-fir root showing sharp demarcation between infected and adjacent phloem tissue, BL.. **Fig. 2.145.** A cryofixed section of a Douglas-fir root showing a diffuse boundary between necrotic and apparently healthy tissue. Adjacent phloem appears hypertrophied but lacked any further differentiation of tissue leading to NIT development, BL. **Fig. 2.146.** A basal disk collected from the base of an infected Douglas-fir tree revealed repeated infections over several years (red arrows). The fungus killed the cambium, most likely during a period of dormancy and then the host formed a barrier zone in the spring to contain the infection. Subsequent formation and breaching of barriers in the bark and wood continued for several years. Note mycelial fans in the bark and cambial zone. **Fig. 2.147.** Another disk collected from a Douglas-fir naturally infected with *A. ostoyae* revealed a resin barrier and callus formation at the onset of a new annual growth ring (yellow arrow) as well as single and double resin barriers (black arrows) within growth rings from previous years. **Fig. 2.148.** A photomacrograph of a Douglas-fir root naturally infected with *A. ostoyae* where the host formed a NP in advance of the penetrating mycelia. Note thick rhytidome tissue external to the last formed periderm.

Western hemlock

Infections on western hemlock were frequently initiated at or near root contacts via rhizomorph penetration. Rhizomorphs typically spread around the entire root resulting in multiple lesions. Mycelial fans were common throughout the inner bark and cambial zone (Figure 2.149). Variations in host response with respect to roots showing no or ineffective host response and NP formation were observed. Progressive infections were seen as mycelial fans spreading laterally in the outer bark tissues. Discoloration of tissue occurred in advance of mycelial spread in the bark. The adjacent tissue showing no or ineffective host response was typical of that described on the other species in this study.

Roots infected with *A. ostoyae* commonly showed bark hypertrophy, even when the fungus appeared to be advancing rapidly. NIT comprised several layers of phloem parenchyma, similar to what was observed in inoculated roots and significantly more than that which occurred in abiotically wounded roots (Figure 2.150). NPs with thin-walled phellem were common on roots located further than 10 cm from the root collar (Figure 2.151). Although NPs were formed initially, the fungus was rarely contained within a new NP barrier (Figures 2.152). Multiple NPs were observed in several infected root samples following breaching of the first NP barrier. Large clusters of sclereids in the phloem often resulted in discontinuities in NP formation and expansion of necrosis internal to a cluster of sclereids was common. However, in older roots, the ability of trees to form a NP with multiple bands of thick- and thin-walled phellem enabled the host to halt the advance of the fungus to adjacent healthy tissue (Figure 2.153 and 2.154).

The fungus commonly advanced to and killed the vascular cambium. Traumatic resin canals (Figure 2.155) and callus tissue (Figure 2.156) were observed as a result of indirect injury to the cambium as the fungus advanced deeper in the bark. Sometimes, phloem parenchyma accumulated pigmented phenolic deposits in advance of a penetrating mycelium however this was ineffective at halting further spread of the fungus in the bark (Figure 2.157). Smaller diameter secondary or tertiary roots often showed very little resistance to the fungus and were usually girdled. Adventitious rooting sometimes resulted from girdling of smaller roots (Figure 2.158). The fungus was

commonly checked at the junction of a larger diameter root (Figure 2.159) suggesting that root diameter or the precise location of the infection on the root may play a role in the expression of resistance in western hemlock. Where the fungus was compartmentalized, a barrier zone comprised of a tangential series of traumatic resin ducts was formed by the uninjured cambium and lateral ingrowth of callus expanded over the injured tissue (Figure 2.160)

The naturally infected western hemlock trees were considerably smaller than the hemlock trees used in the inoculation trials (average DBH = 24 mm for naturally infected trees vs. average DBH = 120 mm for inoculated trees). Thus, it could be speculated that the host responses in smaller trees are indicative of the higher susceptibility exhibited by most trees of that size. Naturally infected hemlock exhibited the same degree of susceptibility as the inoculation trials except that a higher number of naturally infected trees showed resistant reactions at the root collar involving NP formation with multiple bands of thick- and thin-walled phellem.
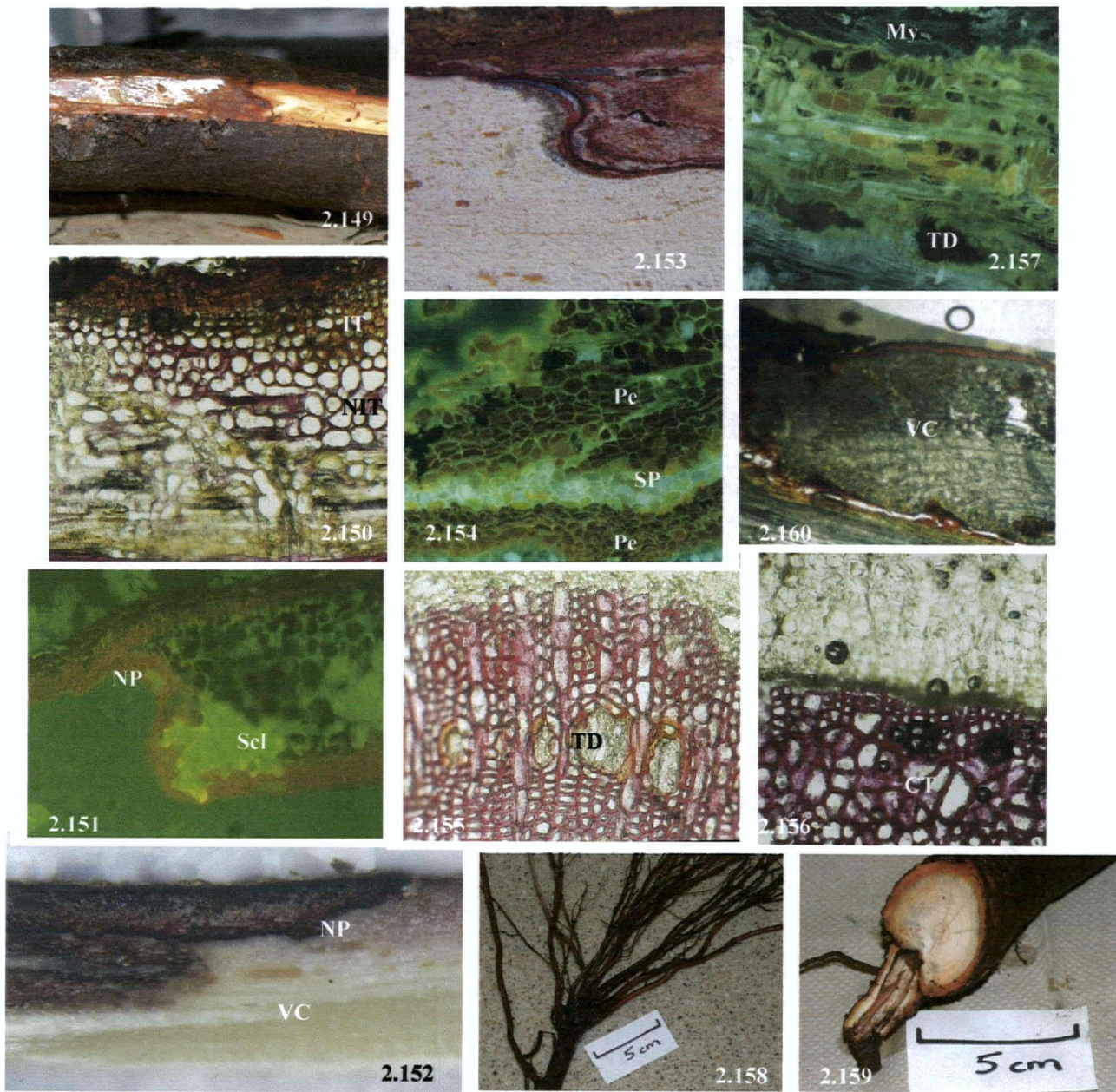
Fig. 2.149. A hemlock root naturally infected with *A. ostoyae* showing mycelial fans and necrosis in the inner bark and cambial zone. **Fig. 2.150.** A phloroglucinol-HCl treated section of a western hemlock root naturally infected with *A. ostoyae* showing a large zone of hypertrophied and heavily lignified NIT internal to a zone of necrotic tissue, BF. **Fig. 2.151.** A Sudan III treated section of western hemlock root showing NP formation in the bark. Note phellem wrapped around large cluster of sclereids, BL. **Fig. 2.152.** A photomacrograph of a western hemlock root naturally infected with *A. ostoyae*. The fungus was initially walled off within a NP but then the fungus breached the barrier and continued to progressively advance in the inner bark. **Fig. 2.153.** A photomacrograph of a larger diameter western hemlock root showing a resistant reaction involving NP formation with multiple bands of thick and thin-walled phellem. **Fig. 2.154.** A cryofixed section of a western hemlock root shown in Fig. 2.153 showing up to 4-5 layers of thick-walled phellem between bands of radially compressed thin-walled phellem. No breaching of these types of barriers was observed, BL. **Fig. 2.155.** A phloroglucinol-HCl treated section of an infected hemlock root showing a short series of traumatic resin ducts (TD) formed in the wood. Normal tracheid production occurred immediately following, suggesting a temporary disruption of cambial activity as the fungus advanced in the bark. **Fig. 2.156.** A phloroglucinol-HCl treated section of an infected hemlock root showing callus tissue (irregularly hypertrophied cells) embedded between normal tracheids in an annual growth ring, also suggesting a temporary disruption of normal cambial activity. The tissue derived from callus eventually developed secondary walls and become lignified. **Fig. 2.157.** A cryofixed section of an infected western hemlock root showing pigmented phenolic deposits in the phloem parenchyma and traumatic resin duct (TD) formation as the mycelium advances in close proximity to the vascular cambium. Such defense reactions were incapable of halting further spread of the fungus. **Fig. 2.158.** Adventitious rooting in western hemlock in response to infection by *A. ostoyae*. **Fig. 2.159.** Infection of a smaller diameter root is confined at the junction of a larger diameter root. Note callus surrounding the central column of decay. **Fig. 2.160.** Compartmentalization in western hemlock and subsequent expansion of callus over the face of the lesion.

Western redcedar

No progressive lesions were noticed on western redcedar roots. Of the 11 trees sampled, only one tree showed evidence of mycelial fans in the bark (Figure 2.161). All other trees had older lesions denoted by a zone of necrotic tissue that was either contained within a NP in the bark or compartmentalized in the wood (Figure 2.162). At the infection front of the progressive lesion observed in Figure 2.161, the necrotic tissue was delimited by traumatic resin ducts in the phloem and no further expansion of necrosis extended beyond that (Figure 2.163). This further suggests a possible role of the traumatic phloem resin ducts in resisting further spread of *A. ostoyae* in host tissue. Traumatic resin ducts were commonly observed in older, larger diameter roots located close to the root collar (Figure 2.164), however, they also appear to be induced on younger, smaller diameter roots in response to infection by *A. ostoyae*.

The barrier zone induced in cedar in response to natural infection was similar to that described in response to inoculation with *A. ostoyae*. There was an immediate chemical response which resulted in the accumulation of phenolic compounds in a zone of parenchymatous tissue adjacent to an area of killed cambium (Figure 2.165 and 2.166). On small roots, lateral ingrowth of callus and woundwood expanded over the face of the wound and healed over completely within 1-3 years, much more rapidly than was observed on western hemlock and Douglas-fir. Following compartmentalization of *A. ostoyae* in cedar, no further breaching of barriers was observed, unlike that observed in Douglas-fir and western hemlock. The barrier zone formed in cedar is effective at containing the fungus and it is rare that the fungus will escape or breach this barrier once formed (Figure 2.167).
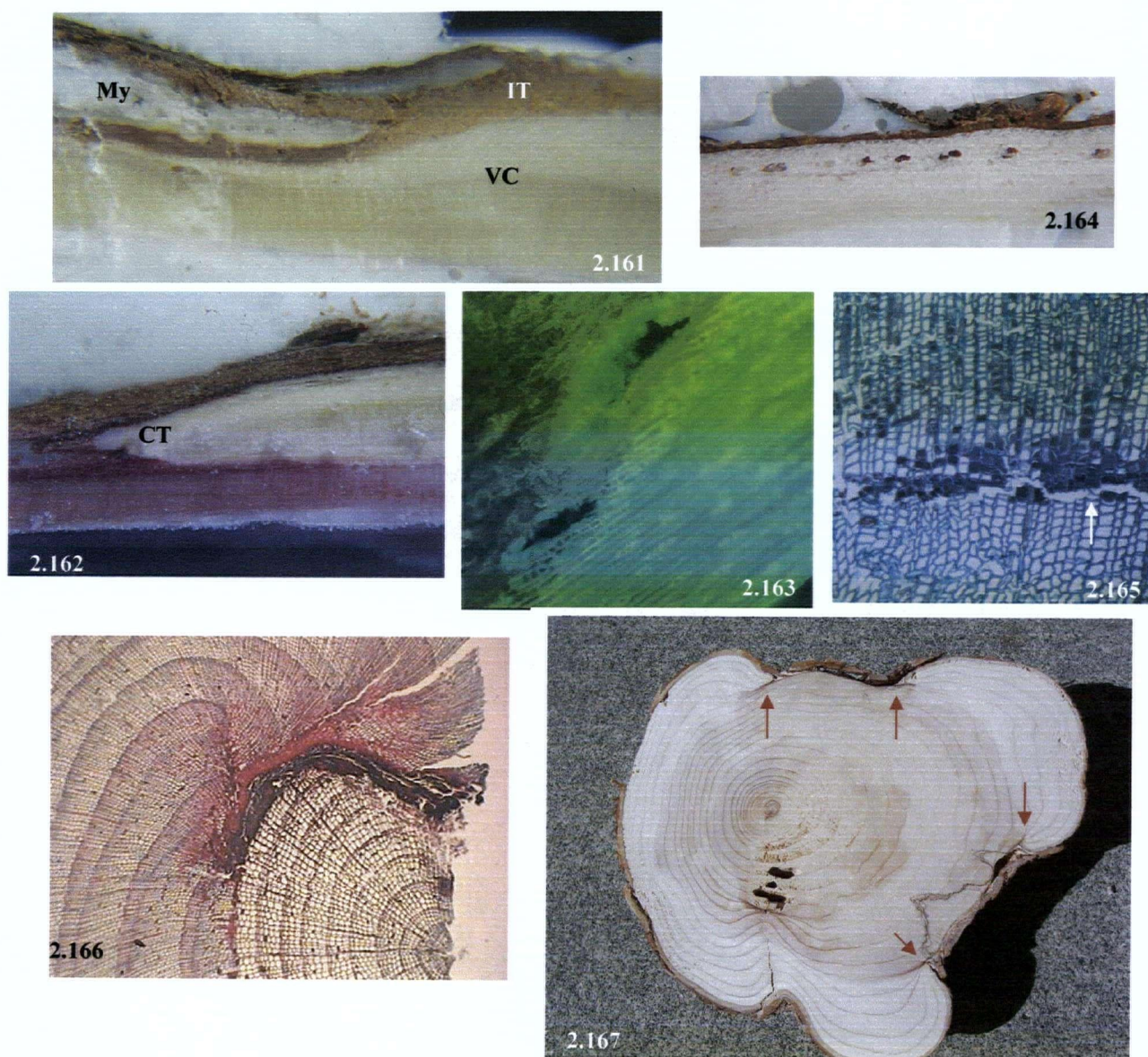
Fig. 2.161. A photomacrograph of a western redcedar root naturally infected with *A. ostoyae*. Note large mycelial fans in the inner bark and browning of phloem in advance of mycelial colonization. **Fig. 2.162.** A photomacrograph showing a typical resistant reaction in western redcedar as NP formation in the bark and effective compartmentalization with the callus edge growing over the face of the wound. **Fig. 2.163.** A cryofixed section of the western redcedar root in Fig. 2.161 showing traumatic resin duct formation in the phloem bordering a zone of necrosis. **Fig. 2.164.** A photomacrograph of a cedar root collected from the root collar area of a healthy tree showing normal resin duct formation in the phloem associated with older tissue at or near the root collar. **Fig. 2.165.** Barrier zone formation in western redcedar comprising a distinct zone of polyphenolic-rich parenchyma adjacent to an area of killed cambium. Callus is formed by the uninjured cambial initials and a new meristematic vascular cambium regenerates within the callus tissue to produce normal xylem and phloem derivatives. **Fig. 2.166.** An older lesion on a western redcedar root shows evidence of the barrier zone formed in response to infection as pigmented deposits within axial parenchyma. **Fig. 2.167.** A basal disk obtained from a western redcedar tree naturally infected with *A. ostoyae*. Compartmentalization is shown as a barrier zone immediately adjacent to the area of killed cambium (barely visible at this magnification as a distinct pink/red hues in the wood (arrows). Note black zone lines formed by the fungus in the underlying wood. Annual growth does not appear to be impeded by the fungus. The fungus was never shown to breach the barrier zone in subsequent years unlike the other conifer species in this study.

Few root lesions were found in western redcedar at root contact with an infected individual, except where compartmentalization and callusing were evident. It is highly probable that any infection to the bark of cedar would be quickly contained within a NP and if the resulting lesion caused induced rhytidome formation, it might not have been evident even one year later because the outer dead tissue containing the fungal mycelia would be sloughed from the surface of the root.

Variations in host response exhibited by all species is likely influenced by the inoculum potential of the fungus, host vigour, the timing and season of infection, none of which could be determined by looking at natural infections. However, trends with respect to host reactions parallel those which were observed in the field inoculation trials for all species.

2.4. CONCLUSIONS

In this study, there was a strong relationship between the ability of *A. ostoyae* inoculum blocks to produce rhizomorphs and the extent of damage as evidenced by the frequency of infection on host roots originating from rhizomorph penetration. A single rhizomorph was capable of establishing a progressive infection in the root. However, penetration may also occur in the absence a rhizomorph through diffusion of fungal toxins/enzymes from the mycelium in the inoculum segment into the root causing cell degradation.

Colder temperatures inhibited rhizomorph initiation and growth in fall and winter and greatly reduced the ability of *A. ostoyae* to infect host trees during winter months when the trees are unable to initiate host defense mechanisms. Thus, damage which is commonly seen occurring over a period of dormancy, as indicated by the position of the series of traumatic resin canals in the next annual growth ring, probably arises from pre-existing root lesions as opposed to direct penetration and infection by the fungus during winter months.

Furthermore, arid soil conditions in the summer of 2003 prevented rhizomorph initiation on inoculum blocks. Thus, drought conditions may not only have an adverse effect on trees (i.e. increasing host susceptibility) but may also affect the inoculum potential and thus the infective capability of the fungus.

Stimulation of defense responses including phellogen renewal associated with NP formation and compartmentalization of infected tissue are ubiquitous responses among all woody plants when injured or infected. These responses were induced in all three species studied herein. Although the frequency of infection was generally similar in all host species, host reactions following invasion of the fungus differed significantly among species.

Anatomical responses that were induced following invasion by *A. ostoyae* were similar to responses initiated by abiotic wounding in that both treatments typically induced NIT and NP formation around a zone of necrotic tissue. However, the rate of NP formation in abiotically wounded tissue was more rapid than in roots inoculated with *A. ostoyae*, especially in Douglas-fir and western hemlock roots. Host tissues under the influence of an advancing mycelium are exposed to pathogen toxins which may slow or inhibit, or sometimes stimulate the development of dedifferentiating tissue leading to phellogen renewal. NIT development in infected hemlock and Douglas-fir roots consisted of a larger number of cells than in abiotically wounded roots. The larger zones of NIT in roots infected with *A. ostoyae* suggest that the host may be continuously stimulated as the fungus progresses in the inner bark. In western hemlock, clusters of sclereids cause delayed development of complete NIT and NP allowing the fungus to breach these areas before the boundaries are complete.

On western hemlock, NPs with thick- and thin-walled phellem were usually formed on larger diameter roots closer to the root collar. Traumatic resin duct formation in the xylem was stimulated when the fungus advanced to but had not yet killed the cambium. This response was observed in both western hemlock and Douglas-fir roots where the cambium became somewhat deranged, similar to what may be seen in frost damaged

tissue, and produced either traumatic resin ducts or callus tissue. The vascular cambium was eventually restored and resumed normal cambial activity.

Shallow bark injuries on cedar trees were immediately walled off by a NP barrier in the bark and the rate of healing did not appear to differ considerably between abiotic and *A. ostoyae* wounds. NIT was inconspicuous in cedar roots and may be masked by the lignification observed in thin-walled phellem immediately abutting it. A typical host response involving rhytidome formation was induced in cedar either simultaneously with or after completion of a NP barrier. The lack of NIT formation and the induced rhytidome response in cedar roots contrasts with the generally accepted model of non-specific host responses following injury to the bark (Mullick 1977). These observations suggest a potentially important caveat for Mullick's model for cedar and possibly other species in the Cupressaceae family.

Induced rhytidome formation extends for some distance proximally and distally from the primary wounded tissue and eventually becomes continuous with the original periderm. In addition, traumatic phloem resin ducts are formed in tangential bands surrounding the margin of the expanded lesion. The resulting *Armillaria* lesion will appear elongated, but the fungus rarely girdles the root.

The formation of this tissue facilitated in the *en masse* sloughing of infected tissue from the surface of the root thereby limiting development of the pathogen in the host tree. Subsequently, traumatic resin ducts were initiated in the phloem surrounding the margin of this new periderm barrier. TPRD's continued to differentiate and expand proximally and distally to the perimeter of where the NP, associated with rhytidome formation, joined the original periderm. Cedar trees showed similar host responses around the margin of the *A. ostoyae*-caused lesions in all field inoculation trials across a wide range of conditions (different sites, genetics, host vigor), and in response to abiotic injury suggesting the responses are non-specific and an inherent characteristic of the species.

Effective compartmentalization in cedar was achieved by producing a barrier zone comprised of a higher than average number of axial parenchyma that accumulate

polyphenolic deposits. The combination of these host-mediated defense mechanisms resulted in a significantly higher frequency of effective resistance reactions in western redcedar trees than in western hemlock or Douglas-fir trees.

Lesion size is not a good measure of resistance in roots infected with *A. ostoyae* because some of the most resistant reactions involving NP and the long-range resistance response were always associated with longer lesion lengths. Compartmentalization and callusing in cedar was particularly successful following invasion of the cambial tissue by *A. ostoyae*. Hence, the host defense responses in western redcedar were effective against *A. ostoyae* and consequently led to the significantly higher frequency of successful resistance reactions than in Douglas-fir and western hemlock.

CHAPTER THREE:

HOST RESPONSE TO INFECTION BY *ARMILLARIA SINAPINA* IN THE ROOTS OF DOUGLAS FIR, WESTERN HEMLOCK AND WESTERN REDCEDAR

3.1 INTRODUCTION

Of the six species of *Armillaria* occurring in British Columbia, only *A. ostoyae* and *A. sinapina* are common throughout the southern interior region. Both species are facultative parasites, usually surviving on woody substrates (i.e. roots and stumps), but under certain circumstances, they may become parasitic and attack living plants. *A. sinapina* is preferentially saprotrophic and is generally considered to be weakly pathogenic (Morrison *et al.* 1985). *A. sinapina* may assume a role as a secondary parasite, colonizing tissue only after it has been weakened or killed by other micro-organisms, including *A. ostoyae* whereas *A. ostoyae* acts as a primary parasite on a variety of tree species, especially conifers (Allen *et al.* 1996, Morrison *et al.* 1985, 1992).

A. sinapina may be easily mistaken for *A. ostoyae* in the field because both species produce mycelial fans in the phloem and fruiting bodies appear similar. However, rhizomorph branching patterns of the two species differ (Morrison 2004). *A. ostoyae* branches dichotomously while *A. sinapina* has monopodial branching. Monopodial branched species of *Armillaria*, including *A. sinapina*, produce an extensive network of rhizomorphs and have a distinct advantage of position during the exploitation of woody substrates in the soil. However, penetration and infection by *A. sinapina* may be inferior to that of *A. ostoyae* which may be related to its inherent pathogenicity, inoculum potential of the fungus, or both.

Recent studies suggest that *A. sinapina* may be slightly more pathogenic than previously thought. In the central interior of B.C., Dettman and van der Kamp (2001a) reported some instances of *A. sinapina* killing coniferous hosts, particularly when *A. ostoyae* was absent. Morrison (2004) found that among the monopodially branched species of *Armillaria*, *A. sinapina* was one of the most virulent, killing about 25% of potted

Douglas-fir seedlings over a two year period. Unlike *A. ostoyae, A. sinapina* required up to 2 years to kill the majority of trees. Morrison's (2004) results suggest that *A. sinapina* may be more of an opportunistic pathogen that infects only the most susceptible (young regenerating trees) or trees that have been weakened by other stress factors.

Little is known about the variation in host susceptibility and host response to infection by *A. sinapina* in older trees. The frequency of infection and the proportion of roots showing successful resistance reactions on roots of western redcedar, western hemlock and Douglas-fir to infection by *A. ostoyae* were reported in the previous chapter. Pathogenicity is the capability of a pathogen to cause disease in the host (Agrios 1997, Holliday 2001). In this context, the pathogenicity involves not only the ability of the fungus to successfully penetrate the living host plant, but also to continue to develop and spread within the host. In this study, the pathogenicity of *A. sinapina* was assessed by comparing the frequency of infection and the frequency of successful resistance reactions with that of *A. ostoyae* on Douglas-fir, western hemlock and western redcedar trees.

One of the study objectives was to compare the frequency of infection of *A. sinapina* occurring on inoculated roots of Douglas-fir, western hemlock and western redcedar with that of *A. ostoyae*. A second objective was to examine host response to infection on roots infected with *A. sinapina* and to compare the frequency of successful resistance reactions induced following invasion by the fungus among host species. Finally, species differences in relation to the different stages of host reactions following invasion by *A. sinapina* were compared to the same differences shown for *A. ostoyae*.

3.2 MATERIALS AND METHODS

3.2.1 STUDY SITES

Three inoculation trials were conducted at two sites: Hidden Lake and Kingfisher. Both sites were also used for the *A. ostoyae* inoculation trials. However, trees selected for inoculation with *A. sinapina* were located in a different section of the stand to ensure the

trials were independent of each other. Information on site locations and stand characteristics is given in Table 2.1.

3.2.2 INOCULUM BLOCK PREPARATION, INOCULATION AND SAMPLING TECHNIQUE

The method of inoculum block preparation was similar to that of *A. ostoyae* previously outlined in Section 2.2.2. The *A. sinapina* isolate (provided by D. Morrison, CFS) used to make starter discs in the lab was collected near Merritt, B.C (CCCM No. 64). Segments of Garry oak branchwood inoculated with *A. sinapina* and buried in coarse soil for several months produced an extensive rhizomorph system.

Inoculation and sampling techniques were similar to those outlined in Section 2.2.3. Root diameter and distance from the root collar were in the range of 2-3 cm and 30-60 cm, respectively. No abiotic wounding (freezing injuries) or controls (uninfected fresh segments of Garry oak) were used in the *A. sinapina* field trials because adequate data from samples in the *A. ostoyae* could be used for comparison. In the Hidden Lake trial, inoculum segments were placed in the soil at the end of September 2002 and sampled in June 2003 and June 2004. In the 2003 and 2004 field trials at Kingfisher, trees were inoculated in May and sampled 3-4-months and 1-year later.

Harvested roots were carefully examined for infection at the point where the inoculum block was placed against the root and all areas showing rhizomorph attachment in the rhytidome. Macroscopic observations of host response (i.e. NP formation in the bark, resin exudation, compartmentalization and callusing) were described. Lesion length and the distance at which the resulting lesion occurred either proximally, distally or both to the inoculum block were noted.

In the lab, frozen tissue samples from infected roots were sectioned on a cryostat and paraffin-embedded woody samples were sectioned on a rotary microtome according to the methodology outlined in Section 2.2.4. Host response to infection was described at the macroscopic and microscopic level for all tissue samples.

3.2.3 RE-ISOLATION OF *A. SINAPINA* FROM LESIONS

A. sinapina was isolated from a sample of roots showing distinct lesions at inoculum contact according to the methodology outlined in 2.2.5. Somatic compatibility tests were performed between isolates from infected roots and the isolate used to inoculate the segments of Garry oak. Isolates from infected roots were also paired with a different isolate of *A. sinapina* (Isolate #29-2-8C provided by M. Cruickshank, CFS) and *A. ostoyae* (Isolate #87-01, CCCM No. 63) to demonstrate incompatibility reactions resulting from intra- and inter-specific crosses between isolates.

3.2.4. STATISTICAL ANALYSIS

Chi-square tests were used to examine various differences in frequency of infection, successful resistance reactions, and the different stages of host reactions (Appendix X-XII). Successful resistance reactions in this study are defined as the ability of a host to complete necrophylactic periderm (NP) formation in the bark, compartmentalization in the wood, or both, in order to halt further spread of the fungus in host tissue. Host reactions are given as (a) non-suberized impervious tissue initiated in root bark, NIT initiated; (b) breaching of the newly formed NIT barrier, NIT breached; (c) NP formation, NP formed; (d) breaching of the newly formed NP barrier, NP breached; and (e) compartmentalization and callusing evident following invasion of cambial tissue, CODIT and callusing. Significant differences between paired species using Chi-square analysis are summarized in separate tables.

3.3. RESULTS

3.3.1 INOCULATION TRIAL: FREQUENCY OF INFECTION

Segments of Garry oak branchwood inoculated with *A. sinapina* produced an extensive network of rhizomorphs which was especially noticeable after 1-year. Figure 3.1 shows a profuse network of *A. sinapina* rhizomorphs on an inoculum block removed from the soil. Rhizomorphs extended along the length of a root up to 30-cm from the inoculum block.

Fig 3.1. Extensive rhizomorph production of *A. sinapina* from inoculum segments 1-year following inoculation of roots.

The number of roots inoculated and sampled on each harvesting date and the frequency of infection caused by *A. sinapina* on the roots of Douglas-fir, western hemlock and western redcedar are shown in Table 3.1. Any roots that were not considered to be 'challenged' by the fungus because of lack of rhizomorphs or mycelium at root contact, or both, were eliminated from the dataset.

In the 2002 Hidden Lake trial, trees were inoculated at the end of the growing season (late September) and then sampled the following June. No infection occurred over the winter. Results from three separate field trials showed that *A. ostoyae* was also unable to penetrate and cause infection on host trees over the winter (Section 2.3.7.). Based on these results, it may be conceivable that in the 2002 Hidden Lake trial, *A. sinapina* infection on hosts was limited by low soil temperature in the winter season and likely resumed its infective capacity in the spring, approximately 7-8 months after blocks were initially placed in the soil which generally coincided with the time at which subsequent inoculation trials commenced (May). Thus, the starting date for the 2002 trial (i.e. the date at which trees would initially be 'challenged' by the fungus) was in reality, the spring of 2003. This is supported by the fact that in June 2003 (9-months post inoculation) roots lacked infection at inoculum contact. The blocks were still viable and actively producing rhizomorphs. The few rhizomorphs that adhered to the outer surface of the bark were superficial. Observations of inoculum blocks *in situ* suggested that

Table 3.1 Number of root inoculations on Douglas-fir, western hemlock and western redcedar trees and the number and proportion of roots that showed infection by *A. sinapina* at various harvest times in three field trials.

Species	Field trial	Harvest time	No. of root inoculations	No. of successful penetrations	Frequency of Infection
Douglas-fir	Hidden Lake (2002)	9 months	7	0	0.00
		21 months	7	6	0.86
		Total:	14	6	0.43
	Kingfisher (2003)	3 months	2	0	0.00
		1 year	12	5	0.42
		Total:	14	5	0.36
	Kingfisher (2004)	4 months	4	3	0.75
		1 year	7	4	0.57
		Total:	11	7	0.64
Western hemlock	Hidden Lake (2002)	9 months	2	0	0.00
		21 months	2	2	1.00
		Total:	4	2	0.50
	Kingfisher (2003)	3 months	2	0	0.00
		1 year	13	10	0.77
		Total:	15	10	0.67
	Kingfisher (2004)	4 months	5	5	1.00
		1 year	10	8	0.80
		Total:	15	13	0.87
Western redcedar	Hidden Lake (2002)	9 months	0	0	-
		21 months	13	2	0.15
		Total:	13	2	0.15
	Kingfisher (2003)	3 months	3	0	0.00
		1 year	8	4	0.50
		Total:	11	4	0.36
	Kingfisher (2004)	4 months	5	2	0.40
		1 year	9	6	0.67
		Total:	14	8	0.57

lateral branches formed points of attachment on the root but not enough time had lapsed for infection to occur. Moreover, fewer and shorter rhizomorphs were produced from inoculum blocks from those sampled the following year. Therefore, the data collected in June 2004 (21-months post inoculation) was used as the 1-year old data in the 2002 Hidden Lake trial and analyzed as such.

There was no difference in the frequency of infection among tree species with *A. sinapina* in the 2003 and 2004 Kingfisher trials. However differences in infection rates among species were observed in the Hidden Lake trial (χ^2, $p < 0.01$). Infection in western redcedar differed significantly from both Douglas-fir (χ^2, $p < 0.01$) and western hemlock (χ^2, $p < 0.025$). Similar results were obtained for cedar in the *A. ostoyae* trial. At the Hidden Lake site, initial infection of cedar was much lower than of Douglas-fir and hemlock, while at the other two sites, in both 2003 and 2004 such differences were not observed. Possible differences in microsite conditions or microbial root surface communities between cedar and the other conifers may help explain the difference in infection rates at the Hidden Lake site.

Infection frequency on all conifers generally increased with time in all field trials although this relationship was only significant between the 3-months and 1-year harvest date for western hemlock in 2003 (χ^2, $p < 0.05$). Similar results were observed on hemlock inoculated with *A. ostoyae* during the same year. Increases in infection rates were not observed between 4-months and 1-year. The earlier harvest date coincides with the end of the growing season and limiting factors such as temperature apparently reduced the infective capability of *A. sinapina* until growth resumed the following year. Similarly, little differences in the frequency of infection were observed on trees within the same time frame following inoculation with *A. ostoyae* in the Kingfisher and Nakusp trials in 2004. Thus, when trees are inoculated in the spring, no significant difference in infection frequencies were detected at 1-year than at 4-5 months.

Similar to the *A. ostoyae* field trial, much lower infection rates were observed on Douglas-fir and western redcedar than on western hemlock after 1 year in the 2003 trial.

The lack of infection may be associated with periodic drying of the soil as a result of drought conditions that summer. The spread and survival strategy of *A. sinapina* depends to a large extent on the proliferation of rhizomorphs in the soil. Thus, low soil moisture conditions associated with drought may adversely impact rhizomorph growth and development reducing the ability of the fungus to seek out and capture nutrient resources in the soil. The summer of 2004 was wetter than the previous year especially during the beginning of the field season and the rate of infection was higher in all species after 4-months compared to that at 3 months in the previous year. However, this difference was only significant, once again, in western hemlock (χ^2, $p < 0.01$) and to a lesser extent in Douglas-fir (χ^2, $p < 0.1$).

There was no difference in the frequency of infection among the different field trials after 1 year for either Douglas-fir or western hemlock. However, the frequency of infection occurring in western redcedar differed significantly among field trials (χ^2, $p < 0.05$). Infection on cedar at the Hidden Lake site was significantly lower than at the Kingfisher site in 2003 (χ^2, $p < 0.1$) and 2004 (χ^2, $p < 0.025$).

There was no significant difference in the frequency of infection within a species infected with either *A. sinapina* (Table 3.1) or *A. ostoyae* (Table 2.2, page 56) after 1 year in any of the field trials. Therefore, *A. sinapina* causes infection on host roots as frequently as *A. ostoyae* does.

3.3.2 HOST RESPONSE TO INOCULATION WITH *A. SINAPINA* IN ROOTS

The macro- and micrograph samples featured in Figures 3.5–3.41 are radially sectioned and oriented so that the rhytidome is at or above the top of the figure and the vascular cambium is at or below the bottom of the figure. The magnification of all macrographs and cryofixed sections is x15 and x35 unless otherwise stated. Acronyms are defined on page xxviii of this thesis.

Douglas-fir and western hemlock roots infected by *A. sinapina* showed resin exudation at the surface of the root (Figure 3.2 and 3.3). Similar to roots infected with *A. ostoyae*,

western redcedar showed virtually no symptoms of disease other than irregularities on the surface of the root, a result of excessive bark hypertrophy associated with rhytidome formation (Figure 3.4).

The mode of penetration by *A. sinapina* was essentially the same as that for *A. ostoyae*. The fungus formed a lateral branch which penetrated the outer phellem as a larger unit by means of mechanical force and enzymatic degradation of the host tissue. Rhizomorphs of *A. ostoyae* that penetrated the outer rhytidome tissue were usually successful at penetrating deeper into the living phloem than *A. sinapina*. Lateral branches of *A. sinapina* rhizomorphs typically formed a point of attachment, anchored the rhizomorph to the outer phellem layers and continued to grow epiphytically on the root (Figure 3.5). The inability of *A. sinapina* to penetrate the living bark at most points of attachment may be associated with low inoculum potential.

Douglas-fir roots showed suppression or stimulation of phellogen activity in the zone of tissue immediately underlying or adjacent to a penetrating rhizomorph, respectively (Figure 3.6). Lesions on roots infected with *A. sinapina* were always initiated via rhizomorph penetration. Indirect penetration via extracellular secretions of cell-wall degrading enzymes or toxic metabolites from the inoculum block was reported in roots inoculated with *A. ostoyae*. However, this type of damage was not observed on roots inoculated with *A. sinapina*.

Once *A. sinapina* penetrated the phellogen and underlying phloem tissues, lateral and tangential proliferation of mycelial fans colonize the inner bark and may advance to the vascular cambium killing host tissue in advance of mycelial colonization (Figure 3.7 and 3.8). Host reactions following invasion by *A. sinapina* appeared to be more successful at halting further spread of the fungus in host tissue than roots infected with *A. ostoyae* for all species. It appeared as though *A. sinapina* advanced through bark tissue at a much slower rate compared to *A. ostoyae*. *A. sinapina* mycelial fans were rarely seen at the edge of an advancing infection front in contrast to what was commonly observed in roots infected with *A. ostoyae* (Figure 3.9).
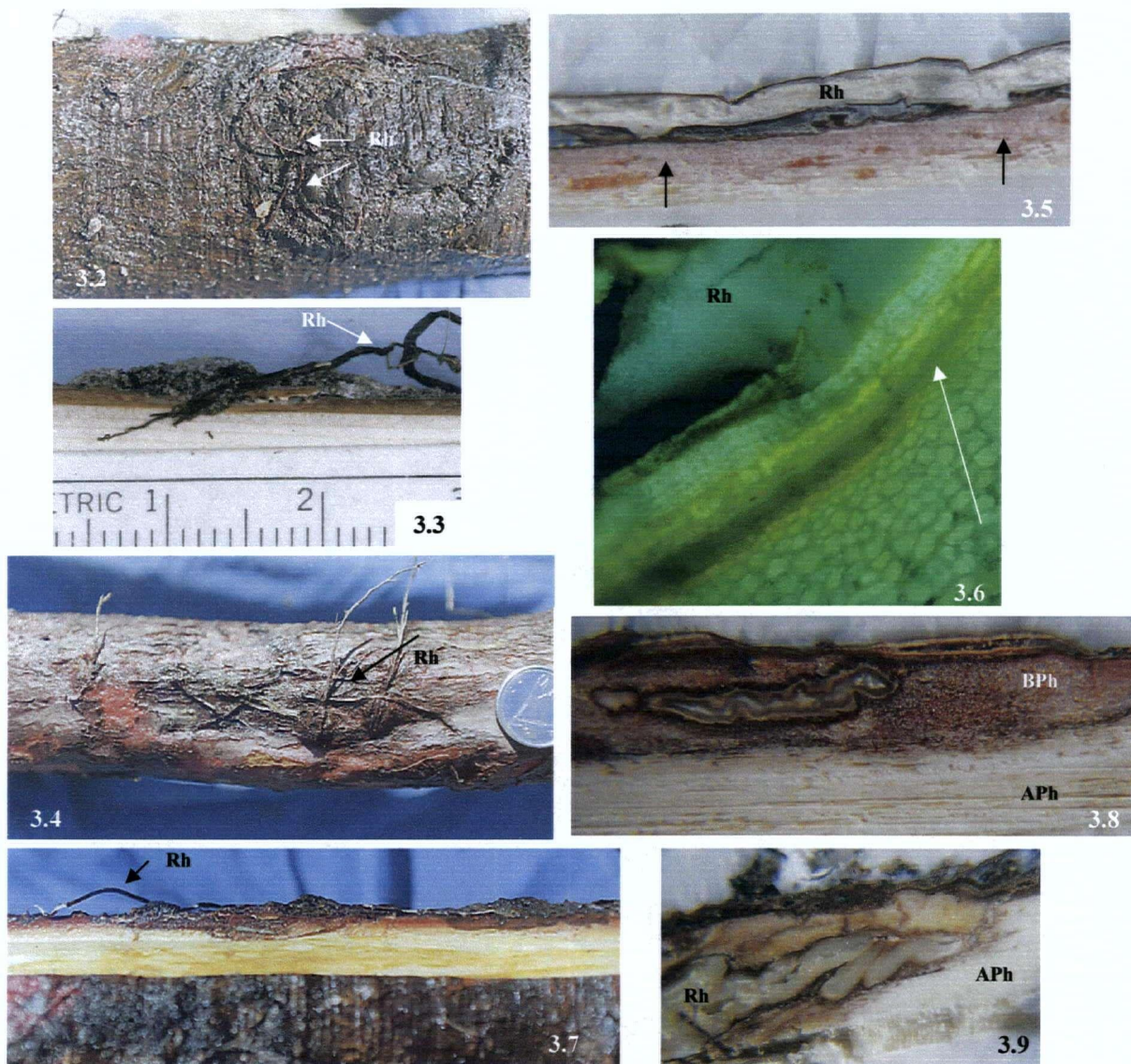
Fig. 3.2. A Douglas-fir root showing *A. sinapina* rhizomorphs adhered to the outer bark (arrows) and exudation of pitch on the surface of the root. **Fig. 3.3.** A western hemlock root showing pitch and rhizomorphs on the root surface four months following inoculation with *A. sinapina*. **Fig. 3.4.** A western redcedar root showing bark swelling (at bottom) associated with the hypersensitive response involving rhytidome formation. Several *A. sinapina* rhizomorphs are showing on the surface of the root. **Fig. 3.5.** A western hemlock root showing epiphytic growth of an *A. sinapina* rhizomorph on the root. Lateral branches of the rhizomorph formed two points of attachment to the outer periderm (arrows). Penetration of the inner bark was lacking. **Fig. 3.6.** A cryofixed section of a Douglas-fir root showing an *A. sinapina* rhizomorph penetrating the outer phellem as it formed a point of attachment. The underlying phellogen zone, shown as the narrow zone of non-fluorescence, appeared narrow suggesting the suppression of phellogen activity by the fungus. Note the contiguous phellogen zone is at least 2-3 times wider than that occurring immediately underneath the penetrating rhizomorph, BL. **Fig. 3.7.** A Douglas-fir root showing two separate rhizomorph-initiated lesions following inoculation with *A. sinapina*. Note rhizomorphs on the surface of the root. Wedges of mycelium can be seen within the necrotic tissue. The fungus killed host tissue down to the vascular cambium. **Fig. 3.8.** A photomacrograph of a Douglas-fir root showing mycelium of *A. sinapina* immobilized within a larger zone of necrotic tissue. Hypertrophy of cells within the necrotic zone is evident. **Fig. 3.9.** A photomacrograph of a Douglas-fir root inoculated with *A. ostoyae* showing the mycelial fan located at the leading edge of an advancing infection front.

Lesion size on host roots inoculated with *A. sinapina* varied according to the proximity of the rhizomorph-initiated lesion to the inoculum block. Multiple lesions on the host roots resulting from separate individual rhizomorph penetrations were common. The largest lesions always occurred at inoculum contact where several rhizomorphs emerged from the cambial end of the inoculum block and penetrated the bark directly. Lesions sizes in tissue underlying the inoculum block ranged between 1.0–6.5 cm in length. On any given root, a profuse network of *A. sinapina* rhizomorphs resulted in several points of attachment and attempted penetrations by the fungus. In addition to the large lesion at inoculum contact, roots had up to 6 additional lesions either proximal or distal to the inoculum block. Lesions located between 5-10 cm from the inoculum source ranged between 0.5-2.2 cm in length and penetrated to one-third or one-half the bark thickness while those located 10+ cm were 2-6 mm in length and typically shallow. These results suggest that the smaller rhizomorph-initiated lesions or lack of penetration by rhizomorphs at increasing distances from the point of inoculation may be due to lower inoculum potential at distal tips of the rhizomorph.

Table 3.2 shows the frequency of successful resistance reactions following invasion by *A. sinapina* for Douglas-fir, western hemlock and western redcedar at the different harvest dates in each field trial. Successful resistance reactions are defined in this study as the complete formation of a NP in the bark or compartmentalization following vascular cambium killing within the time frame from which the root was sampled and those barriers being sufficient to halt further spread of the fungus to adjacent healthy tissue.

Although western hemlock had a lower proportion of roots that showed successful resistance reactions compared to Douglas-fir and western redcedar, the difference among species harvested 1 year following inoculation with *A. sinapina* in all field trials was not significant (χ^2, p < 0.1). Following invasion by *A. sinapina*, Douglas-fir and western hemlock triggered defense reactions to resist further spread of the fungus, but to a lesser degree than western redcedar. There was no difference in the frequency of successful resistance reactions among field trials for a given species. Resistance responses within each species were rather consistent from year to year.

Table 3.2. Frequency of successful resistance reactions following infection by *A. sinapina* in the roots of Douglas-fir, western hemlock, and western redcedar observed from three separate field trials 2002-2004.

Field Trial	Year	Harvest Time	Frequency of successful resistance reactions as a proportion of penetrations by *A. sinapina*		
			Douglas-fir	Western hemlock	Western redcedar
Hidden Lake	2002	1 year[1]	1.00 (n=6)	0.50 (n=2)	1.00 (n=2)
Kingfisher	2003	3 months	-	-	-
		1 year	0.80 (n=5)	0.40 (n=10)	0.75 (n=4)
Kingfisher	2004	4 months	0.66 (n=3)	0.40 (n=5)	1.00 (n=2)
		1 year	0.75 (n=4)	0.87 (n=8)	1.00 (n=6)

[1] samples obtained from roots harvested in June 2004

n = the number of cases of successful penetration of roots by the pathogen

There was no difference in the frequency of successful resistance reactions induced following invasion by *A. sinapina* on roots harvested at 4-months and 1-year post inoculation for either western redcedar, western hemlock or Douglas-fir in the 2004 Kingfisher trial. Results indicated that no more infection occurred at 1-year than what was detected at 4-5 months post-inoculation in both *A. sinapina* and *A. ostoyae* field trials. For this reason, total numbers were used to compare the differences in the frequency of successful resistance reactions induced in host species following invasion by *A. sinapina* and *A. ostoyae*. Data were pooled from the three *A. sinapina* field trials and from the four *A. ostoyae* field trials using data collected at 4-5 months and 1 year for each tree species to formulate a new data set by which to compare the two species of *Armillaria* (Table 3.3.)

Table 3.4 compares the association between individual species for the different types of host reactions induced following invasion by *A. sinapina* and *A. ostoyae*.

Table 3.3. Frequency data from Douglas-fir, western hemlock and western redcedar roots inoculated with *A. sinapina* and *A. ostoyae* harvested at 4-5 months and 1-year from all field inoculation trials.

Species	Armillaria species	No. of infected roots	NHR	NIT initiated	NIT breached[a]	NP formed	NP breached[a]	No. roots with killed cambium[b]	CODIT and callusing	No. roots showing successful resistance reactions
Douglas-fir	A. sinapina	18	4	14	1	14	1	5	5	15
	A. ostoyae	45	27	18	9	14	8	20	2	7
Western hemlock	A. sinapina	25	5	20	1	18	4	11	5	14
	A. ostoyae	59	24	35	12	31	13	27	3	18
Western red cedar	A. sinapina	13	1	12[c]	0	12	1	5	4	12
	A. ostoyae	47	1	46[c]	2	44	8	23	21	40

NHR, no visible or ineffective host response; NIT, non-suberized impervious tissue; NP, necrophylactic periderm; CODIT, compartmentalization of decay in trees

[a] breaching refers to direct penetration through barriers as well as circumvention in places where the host did not complete the formation of the barrier.

[b] killing of the vascular cambium was primarily a reflection of the time and the location of the colonizing fungus in the tissue at the time of sampling. The killing of cambium resulted from a combined number of some, but not all roots showing NHR and breaching of barriers in the bark.

[c] although NIT was generally indistinguishable in cedar, it is the provisional view of this author that in all places where a NP formed, NIT had developed internally abutting that zone.

Table 3.4. Individual species comparisons of the frequency of the type of host response induced following infection by *A. sinapina* and *A. ostoyae* in Douglas-fir, western hemlock and western redcedar trees.

Type of host response	Douglas-fir/Hemlock		Douglas-fir/Cedar		Hemlock/Cedar	
	A. sinapina	*A. ostoyae*	*A. sinapina*	*A. ostoyae*	*A. sinapina*	*A. ostoyae*
NIT Initiated	(n=18/25)	(n=45/59)	(n=18/13)	**$p < 0.001$** (n=45/47)	(n=25/13)	**$p < 0.001$** (n=59/47)
NIT breached	(n=14/20)	(n=18/35)	(n=14/12)	**$p < 0.001$** (n=18/46)	(n=14/12)	**$p < 0.001$** (n=35/46)
NP formed	(n=14/20)	(n=18/35)	(n=14/12)	**$p < 0.05$** (n=18/46)	(n=14/12)	(n=35/46)
NP breached	(n=14/18)	(n=14/31)	(n=14/12)	**$p < 0.01$** (n=14/44)	(n=14/12)	**$p < 0.025$** (n=31/44)
CODIT & callusing	**$p < 0.05$** (n=5/11)	(n=20/27)	(n=5/5)	**$p < 0.001$** (n=20/23)	(n=11/5)	**$p < 0.001$** (n=27/23)

Significance of species comparisons of the different stages/events of host reactions in the bark and wood for roots infected with *A. sinapina* and *A. ostoyae*

$n = \underline{\#/\#}$ are the number of cases of successful penetrations by either *A. sinapina* or *A. ostoyae* in the two species that were tested.
blank cells indicate interactions that were not significantly different at $\alpha = 0.05$

Strong differences in the frequency of different host responses induced following invasion by the fungus were particularly evident between western redcedar and the other two conifers infected with *A. ostoyae*. Cedar was always more efficient than Douglas-fir and western hemlock at containing the fungus to a lesion in the bark. On the contrary, species comparisons in relation to the type of host response induced following invasion

by *A. sinapina* in the bark did not differ significantly from each other. Following cambial invasion, the frequency at which western hemlock roots compartmentalized infections was significantly lower than Douglas-fir (χ^2, p < 0.05) which resulted in an overall lower frequency of successful resistance reactions for western hemlock compared to the other species (Appendix XII).

Results for the frequency of successful resistance reactions induced in Douglas-fir and western hemlock roots inoculated with *A. sinapina* strongly contrasts with that shown following invasion by *A. ostoyae* (Figure 3.10).
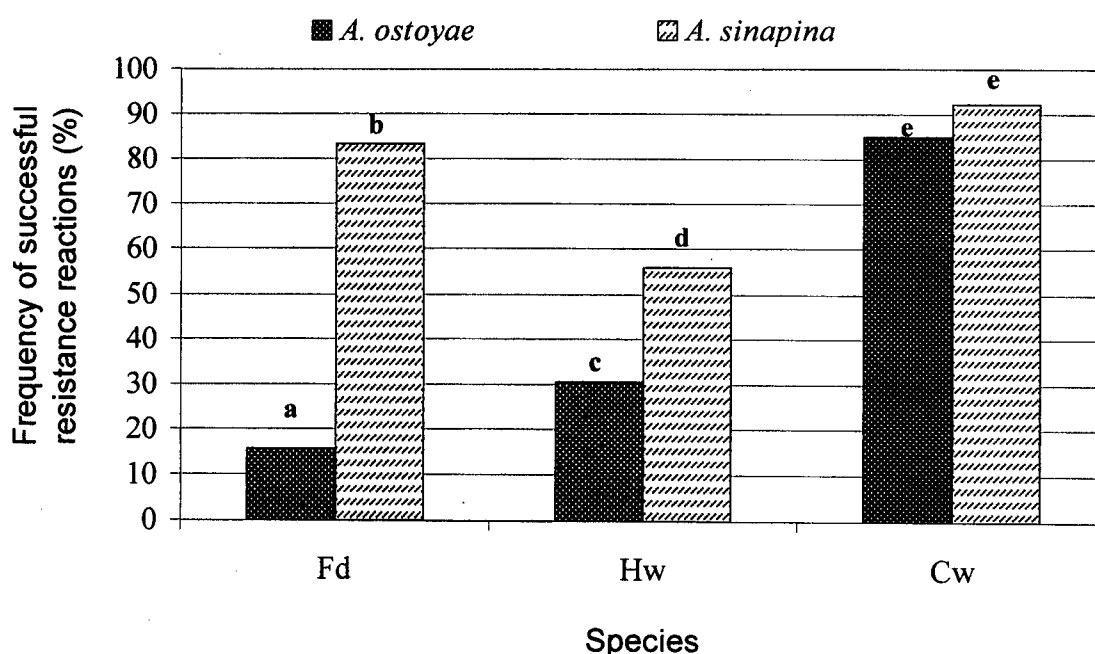
Figure 3.10. The frequency of successful resistance reactions formed in the roots of Douglas-fir (Fd), western hemlock (Hw) and western redcedar (Cw) trees following invasion by *A. sinapina* and *A. ostoyae* as a proportion of the total number of roots that showed penetration at 4-5 months and 1 year for all field trials combined. Different letters denote significant differences within a species.

Similar to *A. ostoyae*, the frequency of successful resistance reactions in western hemlock inoculated with *A. sinapina* was not significantly different from Douglas-fir, but it was significantly different from western redcedar (χ^2, p < 0.025). Within a species, the frequency at which successful resistance reactions were induced in roots following invasion by *A. sinapina* differed significantly from that induced in response to *A. ostoyae* in Douglas-fir (χ^2, p < 0.001) and western hemlock (χ^2, p < 0.05) trees. Douglas-fir roots infected with *A. sinapina* were on average five times more successful at containing the fungus within a NP in the bark or via compartmentalization than roots infected with *A. ostoyae*. Similarly, western hemlock roots infected with *A. sinapina* were nearly twice as efficient as those infected by *A. ostoyae* at halting the spread of the fungus. No differences in the frequency of successful resistance reactions were found between cedar roots infected with either *A. sinapina* or *A. ostoyae*. Cedar consistently formed resistant reactions involving NP and induced rhytidome formation in the bark and showed compartmentalization in the wood following cambial invasion by either fungus.

Douglas-fir

Roots of Douglas-fir trees typically showed resinosus in the bark which exuded at the outer surface of the root. The mode of infection and colonization strategies were similar for both species of *Armillaria* (Figure 3.11 and 3.12), although the frequency at which *A. sinapina* advanced in host tissue lagged behind that of *A. ostoyae*. For example, only 4 of 18 (22%) of Douglas-fir roots infected with *A. sinapina* showed no host response compared to 27 of 45 (60%) in roots infected with *A. ostoyae* (refer to definition of no host response p.80). At least half the roots showing no host response also had significant hypertrophy at the infection front and in some cases, the zone of hypertrophy was quite pronounced, encompassing several cell layers (Figure 3.13). Despite the hypertrophy in the adjacent phloem, sections did not stain positively for lignin following treatment with phloroglucinol-HCl. The boundary between the necrotic zone and the apparently healthy tissue at the infection front on Douglas-fir roots infected with *A. sinapina* was always quite abrupt (Figure 3.14). Under fluorescence, the zone of necrosis appeared as an amorphous mass of tissue with bright yellow-green cell walls.

Nearly twice the frequency of Douglas-fir roots infected with *A. sinapina* formed NIT compared to those infected with *A. ostoyae*. Distinct zones of NIT were recognized following staining with phloroglucinol-HCl. One sample from the Hidden Lake trial showed two distinct zones of NIT whereby the fungus had breached the initial barrier and a second zone of NIT was formed deeper in the bark tissue. Given that the lesion length was less than 2-cm, it suggests that upon initial penetration by *A. sinapina*, the fungus was advancing slowly enough to allow a second zone of NIT to be formed. In 22% of roots, NIT development was either not triggered or incomplete and the fungus advanced to the vascular cambium (Table 3.3). Following inoculation with *A. ostoyae*, this happened in 62% of all roots with initial infection.

The frequency of NIT formation in roots of Douglas-fir following infection by *A. sinapina* was not significantly different from western hemlock (78%, n=18 vs. 80%, n=25). Thus, both species triggered the process of phellogen renewal at relatively the same rate. Although, western hemlock had a slightly lower frequency of successful resistance reactions than did Douglas-fir, these two species generally did not differ (Figure 3.10). In contrast to the *A. ostoyae* inoculation trial, the frequency of NIT formation in Douglas-fir roots infected with *A. sinapina* did not differ from that in western hemlock.

Breaching of NIT was observed in only 1 of 14 (7%) of Douglas-fir roots infected with *A. sinapina* whereas half the roots that initially formed NIT were breached by *A. ostoyae* at the junction with the vascular cambium. Subsequently, the uninjured cambium formed a barrier zone in the wood comprised of a tangential series of traumatic resin ducts and callus formed at the margin of the wound. The frequency of NIT breaching in Douglas-fir roots infected with *A. sinapina* did not differ significantly from that in western hemlock or western redcedar.

All roots with NIT (78%) also formed a NP (Figure 3.15). A distinct zone of clear/white tissue underlying the necrotic tissue indicated the new phellogen zone (Figure 3.16 and

3.17). Interestingly, the newly restored phellogen produced significantly more layers of thin-walled phellem after 4-months or 1-year (which also coincided with the end of the growing season the year prior), than that reported for the same harvest times on roots infected with *A. ostoyae*. Excessive phellem production was commonly observed on either side of a penetrating rhizomorph or wedge of mycelium. In at least 3 roots, up to 22-layers of thin-walled phellem were observed (Figure 3.18), almost three times that observed from NPs formed around tissue killed by *A. ostoyae*.

Stone phellem were rarely observed in newly formed NP's. Thus, infection by *A. sinapina* appeared to, at times, stimulate phellogen activity resulting in a higher than average number of phellem cells being produced. No significant difference in the frequency of NP formation was detected among species infected with *A. sinapina*. In contrast to the *A. ostoyae* trials, Douglas-fir infected with *A. sinapina* formed NP's just as often as western redcedar.

Only one instance of breaching of a fully formed NP was observed. This is in large contrast to the 57% (n=14) of breaching which occurred in roots infected with *A. ostoyae*. This single root was breached in the same area as the developing NIT zone: at the junction of the vascular cambium. All NP's formed were capable of resisting further penetration by the fungus. The frequency of breached NP's did not differ significantly among species infected with *A. sinapina*.

A. sinapina advanced to and killed the vascular cambium in 28% (n=18) of the total number of Douglas-fir roots sampled. All roots showing cambial invasion by *A. sinapina* showed evidence of compartmentalization and callusing compared to only 10% (n=20) in the *A. ostoyae* field trials. The rate at which compartmentalization and callusing occurred in roots infected with *A. sinapina* did not differ from that in western redcedar. However, Chi-square tests revealed a significant difference between Douglas-fir and western hemlock (χ^2, p < 0.05) where less than half the number of hemlock roots that were killed to the cambium were compartmentalized.
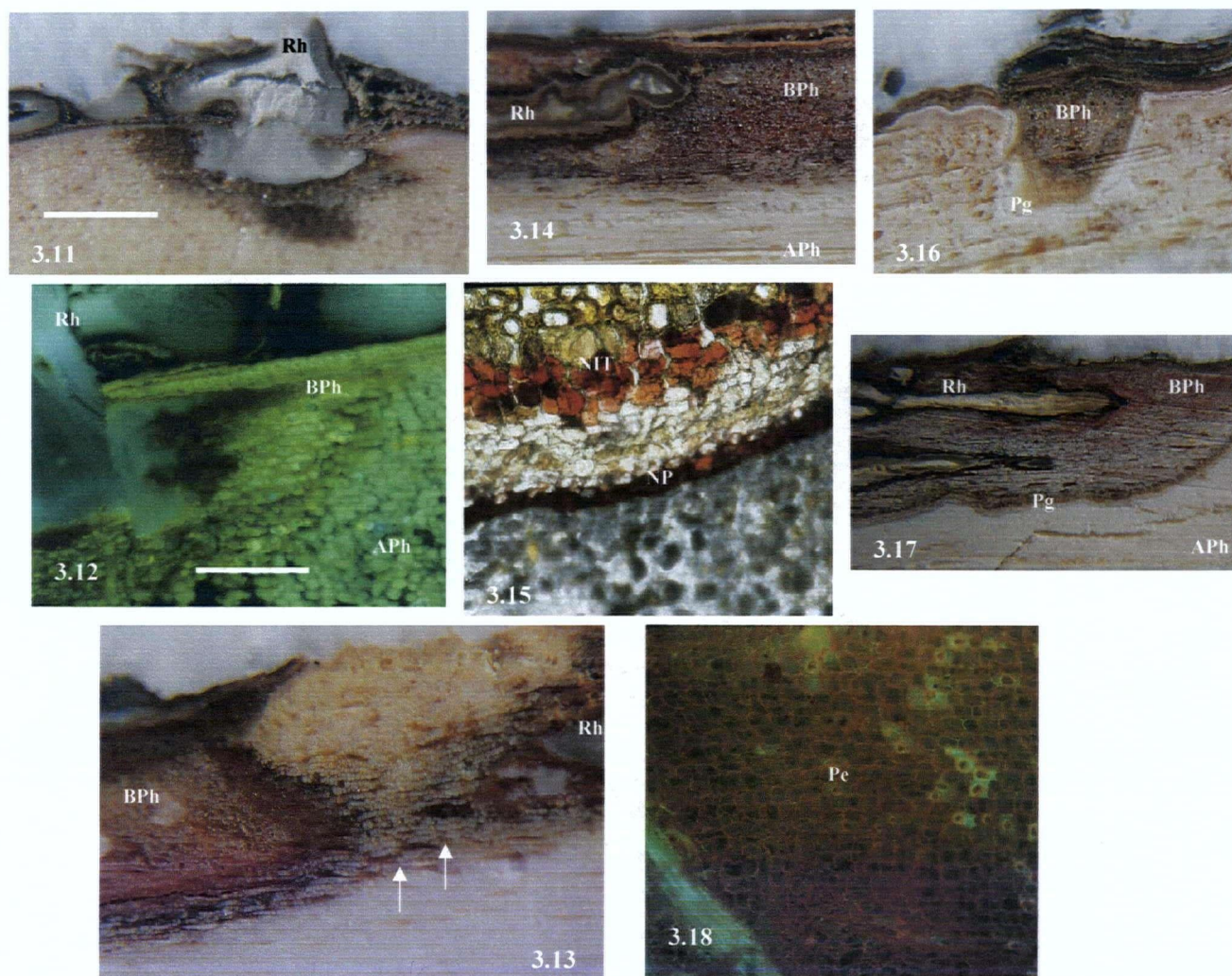
Fig. 3.11. A photomacrograph of a Douglas-fir root showing invasion of the inner bark tissue by *A. sinapina*. Fig. 3.12. A cryofixed section of the same root shown in Fig. 3.11 viewed in BL. Necrosis can be seen in advance of the penetrating mycelium and no visible host response was observed in the adjacent phloem at the infection front. Fig. 3.13. A photomacrograph of a Douglas-fir root that showed no host response following invasion by *A. sinapina*. Large zone of phloem appeared hypertrophied, clearly visible at the macroscopic level (arrows), but a distinct zone of NIT was not identified. Fig. 3.14. A photomacrograph of a Douglas-fir root infected with *A. sinapina* showing a sharp demarcation between the boundary of infected and adjacent, healthy tissue. Fig. 3.15. A cryofixed section of a Douglas-fir root showing NP formation in the bark following invasion by *A. sinapina*, BF. Fig. 3.16. A photomacrograph of a Douglas-fir root which identified the zone of clear-white tissue immediately underlying necrotic phloem as the newly restored NP. Fig. 3.17. Another photomacrograph of a Douglas-fir root showing two wedges of mycelium in the zone of necrotic tissue. A new phellogen zone was restored around killed tissue. Fig. 3.18. A Sudan III stained section of a Douglas-fir root with up to 22 layers of thin-walled, suberized phellem comprised the newly formed NP, BL. Bar on photomacrographs and photomicrographs are 1 mm and 25 µm, respectively, and are applicable to successive photomacrographs and photomicrographs in this Chapter unless stated otherwise.

Barrier zones formed by the uninjured cambium in roots infected with *A. sinapina* did not differ from those described for *A. ostoyae.* The barrier zone was comprised of traumatic resin canals which typically occurred in tangential series and ray parenchyma and tracheids surrounding resin ducts appeared occluded with deposits.

A. sinapina did not interfere with the initiation of active defense mechanisms involving NP formation and compartmentalization to the extent that *A. ostoyae* did. The ability of the host to form these barriers and the low frequency of breaching of these barriers by the fungus enabled 83% (n=18) of roots infected by *A. sinapina* to contain the fungus within a lesion on the root.

Western hemlock

Resinosus was a common symptom on western hemlock roots infected with *A. sinapina*. However it did little to deter the advance of the fungus in host tissue. Phloem necrosis always preceded colonization by the fungus (Figure 3.19).

The proportion of hemlock roots showing no or ineffective host response following invasion by *A. sinapina* was similar to Douglas-fir, but lower than that which occurred on hemlock following infection by *A. ostoyae*. Overall, 20% (n=25) of western hemlock roots showed no or ineffective host response following inoculation with *A. sinapina* compared to 40% (n=59) of hemlock roots inoculated with *A. ostoyae*. Similarly, roots lacking any visible host reaction typically showed no or only patchy cell hypertrophy in adjacent phloem cells at the infection front and did not stain positively for lignin following treatment with phloroglucinol-HCl (Figure 3.20 and 3.21). The boundary between the necrotic zone and the adjacent phloem was either quite diffuse or abrupt (Figure 3.20). Under BL fluorescence, the necrotic zone fluoresced bright yellow-green. The lack of any visible host response leading to NIT and NP formation enabled the fungus to advance to and kill the vascular cambium (Figure 3.19).

Eighty percent (n=25) of western hemlock roots developed NIT in response to invasion by *A. sinapina*. The number of cell layers involved in the development of NIT was

noticeably fewer in roots infected with *A. sinapina* than *A. ostoyae*, but higher than that observed on abiotically wounded roots. These observations suggest that once *A. sinapina* penetrates living phellogen, the rate at which the fungus advances in the living bark is less rapid than that observed for *A. ostoyae*. The delay in NP formation on roots infected with *A. ostoyae* resulted in larger zones of NIT. This suggests that a general stimulation of the lignification process involved in NIT formation occurs when the bark is injured. However, *A. sinapina* did not 'challenge' the host root to the extent that *A. ostoyae* did and the process of phellogen restoration was not delayed. The proportion of western hemlock roots that developed NIT in response to invasion by *A. sinapina* was not significantly different from Douglas-fir or western redcedar.

Breaching of NIT occurred in only one root where both NIT and NP were incomplete around a cluster of sclereids (Figure 3.22). The proportion of western hemlock roots showing breaching of NIT did not differ from Douglas-fir or western redcedar. However, the frequency of NIT breaching in western hemlock roots infected with *A. sinapina* was significantly less than with *A. ostoyae* (χ^2, $p < 0.025$).

Of the total number of hemlock roots sampled, 72% (n=25) formed a NP. Although this proportion was higher than that observed in roots infected with *A. ostoyae*, it was not highly significant (χ^2, $p < 0.1$). At the end of the growing season, lesions bound by a NP in the bark had produced 2-6 layers of thin-walled phellem (Figures 3.23 and 3.24), slightly fewer than that reported in roots infected with *A. ostoyae*. No stone phellem cells were associated with any of the roots sampled. However diameters of inoculated roots were smaller than those in which one would expect to find thick-walled phellem. In general, all cases where NIT formed, a NP followed (Table 3.3).

Only 4 of the 18 (22%) hemlock roots that initially formed a NP were breached by the fungus (Figure 3.25). Similar to *A. ostoyae*-infected roots, breached periderms occurred mostly around clusters of sclereids but also at the junction of the NP and vascular cambium (Figure 3.26). Although NIT and NP formation in roots infected with *A. sinapina* were clearly more efficient than in those infected with *A. ostoyae*, the length of

time required for phellogen restoration to occur around clusters of sclereids was longer than the time required for the fungus to penetrate beyond this developing zone. However, this applied to only 4 of 18 cases (Table 3.3). The proportion of hemlock roots with breached NP's was not significantly different from Douglas-fir or cedar. In those cases in which the NP barrier was breached, further necrosis developed deeper in the bark tissue.

A. sinapina advanced to and killed the vascular cambium in 44% (n=25) of western hemlock roots penetrated by the fungus. Of those roots with killed cambium, only 45% (n=11) showed compartmentalization and callusing from the margin of the lesion. The lower frequency of compartmentalization occurring in western hemlock roots differed significantly from that of Douglas-fir (χ^2, $p < 0.05$) where all of the Douglas-fir roots showing cambial invasion were compartmentalized. Although the frequency of compartmentalization and callusing were nearly twice as high in western redcedar as western hemlock, the relationship was not significant (χ^2, $p < 0.2$). However the proportion of western hemlock roots infected with *A. sinapina* that compartmentalized the fungus was significantly higher than those infected with *A. ostoyae* (χ^2, $p < 0.025$). The barrier zone in hemlock was similar to that reported following invasion by *A. ostoyae* (Figure 3.27). The lower frequency of compartmentalized lesions in hemlock led to an overall lower frequency of successful resistance reactions compared to the other conifers. However, this proportion was still sufficiently low to show significant difference between western hemlock and western redcedar (Figure 3.10).

A. sinapina interfered with the initiation of host defense mechanisms to a lesser extent than in roots infected with *A. ostoyae*. There was no difference in the frequency of NIT and NP formation among species. Similar to Douglas-fir, western hemlock roots were more efficient at containing the fungus within a NP in the bark than when they were infected with *A. ostoyae*. Discontinuities in NIT and NP occurred around clusters of sclereids, occasionally allowing the fungus to breach or circumvent these barriers and advance to the vascular cambium. Hemlock was less efficient at compartmentalizing infections by *A. sinapina* than Douglas-fir or western redcedar, yet more efficient than hemlock roots infected with *A. ostoyae*.
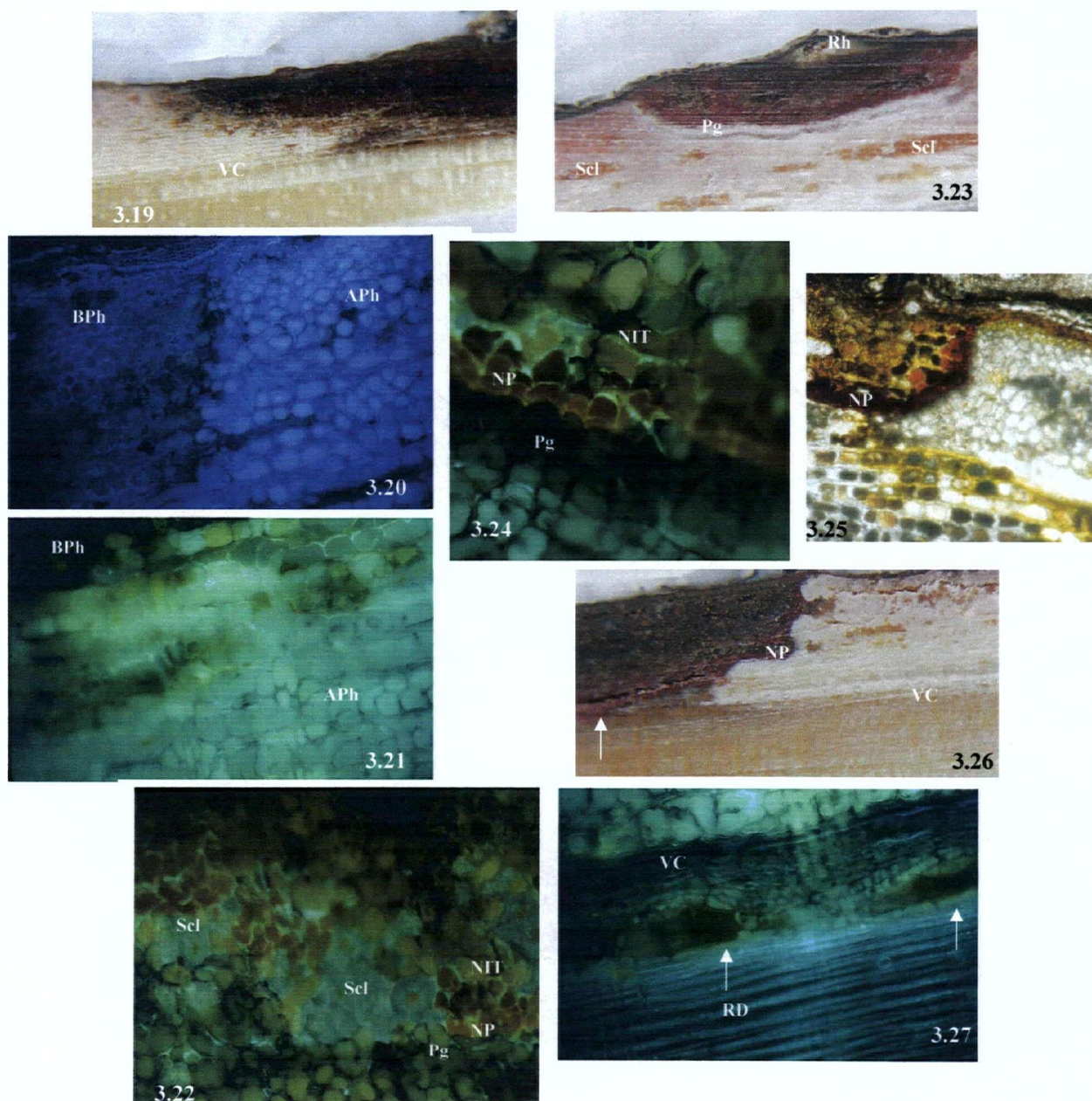
Fig. 3.19. A photomacrograph of a western hemlock root showing necrosis in the bark and cambial zone following invasion by *A. sinapina*. **Fig. 3.20**. A cryofixed section of a hemlock root showing no visible host response and lack of cell hypertrophy in the adjacent phloem at the infection front. The boundary between the necrotic zone and the adjacent living phloem was abrupt, UV. **Fig. 3.21**. A cryofixed section of a western hemlock root showing no host response. Irregular hypertrophy occurred in the adjacent phloem at the infection front and the boundary between necrotic and living tissue was rather diffuse, BL. **Fig. 3.22**. A cryofixed section of a western hemlock root showing incomplete differentiation of NIT and NP around large clusters of sclereids. These areas were commonly breached by the fungus, BL. **Fig. 3.23**. A photomacrograph of a hemlock root sampled 1-year following inoculation with *A. sinapina*. The rhizomorph did not penetrate to great depth in the living bark before the host contained the fungus within a new NP. **Fig. 3.24**. A cryofixed section of a hemlock root showing a recently formed NP around killed tissue. The new phellogen appears as the zone of non-fluorescence internally abutting the two layers of thin-walled phellem. The NIT zone was relatively inconspicuous as it only comprised 1-2 layers of hypertrophied and lignified phloem externally abutting the phellem. **Fig. 3.25**. A cryofixed section of a hemlock root showing browning of tissue in the adjacent phloem internal to the newly formed NP. Breaching of NP's were common through clusters of sclereids, BF. **Fig. 3.26**. A photomacrograph of a hemlock root inoculated with *A. sinapina* showing breaching of the NP barrier at the junction with the vascular cambium (arrow). **Fig. 3.27**. A cryofixed section of a hemlock root showing barrier zone formation in the wood comprised of traumatic resin ducts (arrows), BL.

Western redcedar

A. sinapina rhizomorphs were typically abundant on the surface of western redcedar roots (Figure 3.28). No external symptoms were evident other than some irregularity in the otherwise smooth bark surface. Infection on cedar by *A. sinapina* was similar to that described for *A. ostoyae*. Mycelial fans were seen in the inner bark and cambial tissue. Unlike Douglas-fir and western hemlock, suppression and/or stimulation of phellogen activity was not observed underlying or adjacent to a penetrating rhizomorph in cedar.

Of the 13 cedar roots showing infection by *A. sinapina*, only one (8%) root showed no visible host response, compared to 20% (n=25) and 22% (n=18) in western hemlock and Douglas-fir roots, respectively (Table 3.3).

NP formation was observed in 92% (n=13) of cedar roots infected with *A. sinapina* (Figure 3.29). Similar to that reported in cedar roots infected with *A. ostoyae*, the NIT zone was difficult to discern following staining with phloroglucinol-HCl (Figure 3.30). The NIT zone is typically one cell layer wide and lignification that occurs in that zone may be masked by that which forms in the thin-walled phellem immediately abutting the NIT (Figure 3.31). The newly restored phellogen produced 1-2 layers of phellem which eventually became continuous with the original periderm (Figure 3.32). Only one root showed breaching of the NP barrier and necrosis extended down to the vascular cambium.

Species comparisons of the frequency of host responses showed no significant differences between western redcedar and Douglas-fir or western hemlock (Table 3.4). Thus, the frequency at which defense responses were initiated in all species infected with *A. sinapina* was relatively similar. This is in strong contrast to the response to infection with *A. ostoyae* wherein cedar was markedly different from the other species in all host reaction categories.

A localized response involving rhytidome formation was formed in cedar following infection by *A. sinapina*. Large zones of excessively hypertrophied phloem formed

adjacent to a newly formed NP (Figure 3.33 and 3.34). Initially, the zone of hypertrophy appeared non-fluorescent, suggesting that cells within this zone were actively dividing (Figure 3.35). Enlarged cells had thin cell walls and some developed cross walls. Soon thereafter, cells underwent changes in fluorescence characteristics and were moribund (Figure 3.36). A new periderm established under the zone of hypertrophy and amalgamated with the recently formed NP near the centre of the lesion or continued to form immediately underneath the NP and merged with the original periderm (Figure 3.34).

Induced rhytidome was formed in 62% (n=13) and 55% (n=26) of the western redcedar roots infected with *A. sinapina* and *A. ostoyae*, respectively. Both shallow and deep lesions to the bark induced rhytidome formation. For example, initial penetration of the inner bark by *A. sinapina* was quickly walled off by a NP, resulting in a 2-mm length lesion less than one-third the thickness of the bark. Subsequently, induced rhytidome formation was triggered which resulted in a total lesion length of 14-mm. Thus, the induced rhytidome following invasion by *A. sinapina* can cause even the smallest lesions to be expanded up to five times in length. Where *A. sinapina* penetrated and killed the cambium, the fungus was compartmentalized. Callus tissue was formed by the uninjured vascular cambium as well as by phloem parenchyma located close the vascular cambium. A NP was formed along the outer periphery of the callus and a new vascular cambium differentiated within the callus and merged with the original vascular cambium. The NP enveloped necrotic phloem and eventually merged with the original periderm.
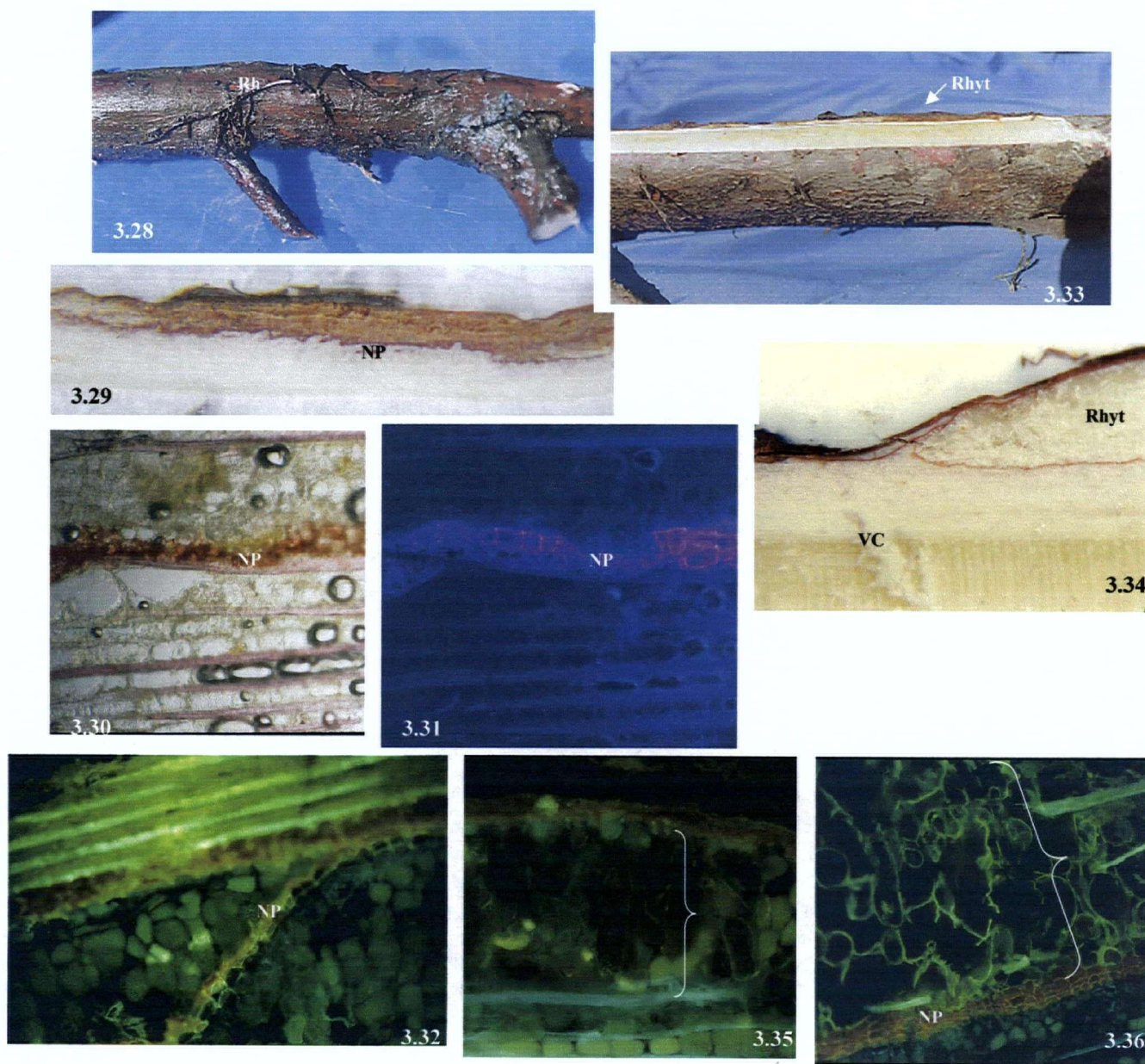
Fig. 3.28. A western redcedar root showing abundant rhizomorphs growing on the surface of the root following inoculation with *A. sinapina*. **Fig. 3.29.** A photomacrograph of a western redcedar root showing NP formation around killed tissue following invasion by *A. sinapina* in the bark. **Fig. 3.30.** A phloroglucinol-HCl treated section of a cedar root showing NP formation in the bark. The NIT zone is difficult to discern as thin-walled phellem also stain positive for lignin, BF. **Fig. 3.31.** The same section shown in Fig. 3.30 viewed under UV fluorescence showing the same lignified thin-walled phellem also to be suberized. **Fig. 3.32.** A cryofixed section of a western redcedar root showing NP formation in the bark. Killed tissue fluoresces bright yellow-green and the phellem comprising the newly formed barrier are typically 2 cells wide, BL. **Fig. 3.33.** A western redcedar root showing large zones of hypertrophied phloem following inoculation with *A. sinapina* (arrow). **Fig. 3.34.** A photomacrograph of a western redcedar root showing the degree of hypertrophy in the inner bark induced by the HR associated with rhytidome formation. This image shows the rhytidome at the proximal end of the lesion where it becomes continuous with the original periderm. **Fig. 3.35.** A cryofixed section of a cedar root showing the large zone of excessive hypertrophy involved in rhytidome formation in the bark. This section shows the hypertrophied cells as non-fluorescent indicating the onset of meristematic activity, BL. **Fig. 3.36.** A cryofixed section of a cedar root showing a later stage of rhytidome formation with a newly restored periderm internally abutting the zone of hypertrophy. Cells are now moribund and fluoresce bright yellow. The structure of cells within this zone deteriorates which facilitates in the *en masse* sloughing of necrotic tissue from the surface of the root, BL.

The formation of phellem by a newly restored phellogen often appear convoluted, wrapping around fibers located in the vicinity of a newly formed NP or in a zone of redifferentiation. Fibers often appeared displaced because of hyperplasia immediately adjacent and internal in the zone of phellogen restoration (Figure 3.37 and 3.38). Adjacent to a NP, rhytidome formed deeper in the bark and extended up to 6-cm from the primary wound and facilitated the sloughing of infected tissue from the surface of the root. The increase in bark thickness caused by rhytidome formation in cedar roots infected with *A. sinapina* was similar to that reported for *A. ostoyae* (Figure 3.39). However, the length to which rhytidome extended beyond the primary wounded tissue was greater in roots challenged by *A. ostoyae* than by *A. sinapina*. For example, on approximately equal diameter roots and extent of killed cambium, the length to which rhytidome formation extended away from the primary lesion (either proximally or distally) was up to three times longer in roots infected by *A. ostoyae* compared to *A. sinapina*. The fact that both shallow and deep bark injuries caused by *A. sinapina* induced rhytidome formation demonstrates that this host reaction can be a non-specific defense mechanism in cedar trees induced following any degree of injury in the bark, be it mechanical or abiotic injury, or invasion by weak or strong pathogenic fungi. However, this non-specific mechanism was only invoked about half the time.

Similar to the *A. ostoyae* trial, cedar roots infected with *A. sinapina* formed traumatic resin ducts in the phloem (TPRD). The development of TPRD in *A. sinapina*-infected roots was similar to that described for *A. ostoyae*: hyperplasia of phloem parenchyma in close proximity to the vascular cambium, followed by schizogenous and lysigenous separation of cells, forming a tangential series of vertical resin ducts in the inner- and mid-phloem (Figure 3.40). The frequency at which TPRD formed following invasion by *A. sinapina* was comparable to that of *A. ostoyae*; 38% (n=13) and 40% (n=47), respectively. However, like the induced rhytidome formation in *A. sinapina*-infected roots, the distance at which TPRD occurred beyond the primary and secondary NP was greater for *A. ostoyae* than for *A. sinapina*. Thus, the stronger host response as evidenced by more extensive resistance reactions (induced rhytidome and TPRD) following invasion by *A. ostoyae* may be a direct result of the host reacting to fungal elicitors or

some other chemical stimuli specific to the interaction of cedar with a more pathogenic fungus as opposed to one that challenges the host to a lesser degree.

Eighty percent (n=5) of the cedar roots that showed killing to the cambium by *A. sinapina* were compartmentalized. The frequency of compartmentalization in cedar was similar in the *A. sinapina* and *A. ostoyae* trials. Furthermore, the proportion of cedar roots showing compartmentalization following cambial invasion by *A. sinapina* did not differ from those for Douglas-fir or hemlock trees (Table 3.4). This is distinctly different from the frequency of these reactions in the three hosts following invasion by *A. ostoyae*. Barrier zone formation in roots infected with *A. sinapina* was similar to that reported for cedar infected with *A. ostoyae*, however the number of axial parenchyma cells in the barrier zone induced by *A. sinapina* was fewer than for *A. ostoyae* (Figure 3.41). These observations suggest a direct relationship between the degree of pathogenicity of the invading *Armillaria* species and the intensity of the host response to infection.
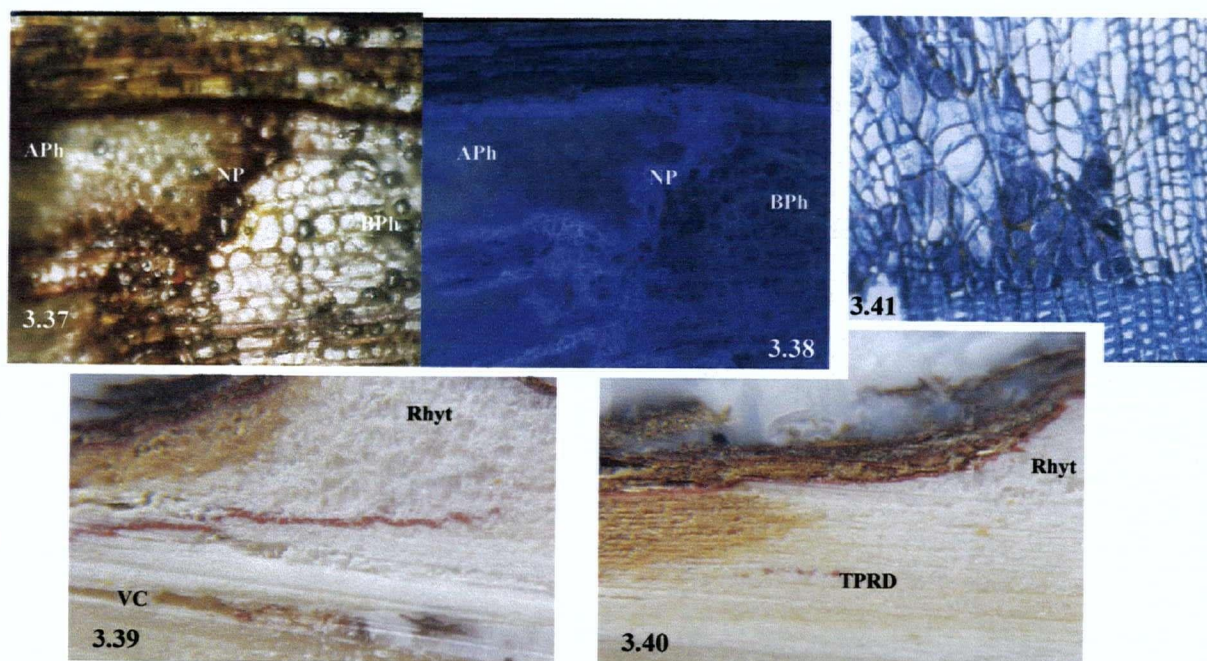
Fig. 3.37. A phloroglucinol-HCl treated section of a western redcedar root showing lignification of the thin-walled phellem comprising the newly formed NP barrier in the bark, BF. **Fig. 3.38.** The same section in Fig. 3.37 viewed under UV. This image shows the degree of difficulty a newly restored phellogen has forming around killed tissue when phloem fibers occur within the zone of redifferentiation. Newly formed phellem wrap around the phloem fibers, sometimes enveloping the entire length of the cell protruding from the necrotic zone until the phellogen becomes continuous. **Fig. 3.39.** Another photomacrograph of a cedar root showing induced rhytidome formation in the bark and extent of bark hypertrophy. **Fig. 3.40.** A western redcedar root showing browned tissue in the inner bark and traumatic resin ducts in the phloem at the margin of the necrotic tissue. Slight pigmentation (shown in red) can be seen in this image. Parenchyma cells differentiating into epithelial cells surrounding the lumen of the resin duct may accumulate phellem-like pigments, particularly where resin ducts are differentiating in close proximity and in conjunction with a newly formed periderm barrier. **Fig. 3.41.** Barrier zone formation in cedar following invasion by *A. sinapina* comprised fewer polyphenolic-rich parenchyma than that induced following invasion by *A. ostoyae*.

3.3.3 RE-ISOLATION OF *A. SINAPINA* FROM LESIONS

All isolates obtained from *Armillaria*-caused lesions were compatible with the *A. sinapina* isolate used in the inoculation trial. Figure 3.42 shows the pairing of the isolate from the inoculated root with the known *A. sinapina* isolate used in the inoculation trial. Complete intermingling of mycelia at the opposing edges of the colonies confirmed compatibility. Similar tests of the same isolate with a different *A. sinapina* (Isolate 29-2-8C) and *A. ostoyae* (Isolate 87-01) isolate, showed incomplete intermingling of mycelia and in the latter case, a dark demarcation zone developed in the media between the two opposing colonies (Figure 3.43).

Fig. 3.42. An isolate obtained from an infected western redcedar tree (tree #12993) paired with the known *A. sinapina* isolate used in the inoculation trial (on the right). After several weeks, complete intermingling of mycelia and rhizomorphs within the culture was evident. **Fig. 3.43**. An example of an incompatible reaction between two different isolates of *A. sinapina*. The isolate on the left is the known isolate used in the inoculation trial paired against *A. sinapina* isolate #29-2-8C on the right. Incomplete intermingling of mycelia between the two opposing colonies was evident after several weeks. Growth at the leading edge of the mycelial colony was inhibited.

3.4 DISCUSSION

The level of infection achieved in inoculation trials with *A. sinapina* in this study may be artificially high compared to that under natural conditions. Under such conditions, an extensive rhizomorph network of a monopodially-branched species of *Armillaria* may have lower inoculum potential at the rhizomorph tips and hence a reduced capacity to penetrate the living bark on host roots. More often than not, this is usually what is seen *in situ*. *A. sinapina* rhizomorphs grow epiphytically on the surface of the root, running underneath a bark scale, but merely forming an attachment in the outer rhytidome and cause little or no damage on the host root. Examination of roots naturally infected with *A. ostoyae* (Section 2.3.8) revealed several *A. sinapina* rhizomorphs on the root surface but never were the rhizomorphs seen to penetrate the living bark.

The above results are interpreted to say that the pathogenicity of *A. sinapina* is determined in part by inoculum potential. In this study, the energy available to the fungus to infect the host at inoculum contact was considerably higher than what might occur under natural conditions. A large cluster of rhizomorphs emerging from the cambial end of the inoculum block would have a superior ability to infect a host root than a single rhizomorph. Lesions on roots underneath the inoculum block were a result of multiple rhizomorph infections which coalesced into a single, large lesion. High inoculum potential is very much localized to the point of inoculation on the root. Inoculum potential declines with increasing distance from the inoculum source (Garrett 1970). In this study, it is possible that *A. sinapina* rhizomorphs had a reduced ability to penetrate and cause infection on roots at greater distances from the inoculum block.

The frequency of infection on host species is not the only determinant of pathogenicity. The classical definition of pathogenicity lacks recognition of the host-pathogen interaction as a function of the ability of a pathogen to cause disease (Casadevall and Pirofski 1999). Contributions of both host (i.e. resistance reactions) and pathogen (i.e. increases in inoculum potential following colonization of host tissue and virulence) play an important role in disease development and the ability of the fungus to spread from tree

to tree. In this study, pathogenicity arises from host reactions, not the inability of *A. sinapina* to penetrate.

Host responses exhibit a broad range of temporal variability in the frequency with which resistant barriers are formed depending on the pathogenicity of the fungus (influenced by inoculum potential and virulence), the susceptibility of the host species (influenced by genetics, tree size, age and the growing conditions), and the influence of environment on all the above factors. In this study, the frequency of infection among host species infected with *A. sinapina* was not significantly different from that of *A. ostoyae*. However, the frequency at which host reactions were induced following invasion by *A. sinapina* was markedly different.

Similar host responses were seen in roots following invasion by *A. sinapina* to those reported for abiotic wounding and inoculation with *A. ostoyae*. However, the frequency at which NP formation and compartmentalization occurred in roots infected with *A. sinapina* was higher than those infected with *A. ostoyae*, especially in Douglas-fir and western hemlock roots. *A. ostoyae* challenges host roots to a higher degree than *A. sinapina* resulting in slower phellogen restoration and NP formation. This was evidenced by the larger proportion of roots that lacked NIT formation or had larger zones of NIT formed under continuous stimulation by *A. ostoyae* advancing in the inner bark and cambial tissue. Of the three conifers studied, western hemlock was the least efficient at containing lesions within a NP. Breached barriers were common in roots inoculated with both species of *Armillaria*. Clusters of sclereids resulted in discontinuities in NIT development and NP formation allowing the fungus to breach these areas before the boundaries were complete. Compartmentalization was more common in western hemlock and Douglas-fir trees infected with *A. sinapina* than with *A. ostoyae*. However, hemlock was slightly less efficient at compartmentalizing infections than Douglas-fir.

Invasion of western redcedar trees by *A. sinapina* triggered a series of non-specific defense responses which resulted in the fungus being contained within a barrier in the bark or in cases involving vascular cambium killing, bark and wood. Some roots that

were abiotically wounded or infected with either *A. ostoyae* or *A. sinapina* induced a localized response involving rhytidome formation. In each case, large zones of phloem parenchyma adjacent to a zone of necrotic tissue underwent excessive hypertrophy and hyperplasia and a phellogen was restored underneath. The new periderm either joined with the initially formed NP at or near its deepest point or else it lay wholly below the primary lesion. In both cases, the new periderm joined the pre-existing periderm some distance distal and proximal to the primary lesion. The distance to which the induced rhytidome response extended from the primary lesion in roots infected with *A. sinapina* was less than that induced under the influence of *A. ostoyae* invasion. Rhytidome formation caused by abiotic wounding was minor compared to that induced by either *Armillaria* species. A similar situation can be claimed for TPRD in cedar. The intensity of the response involving TPRD in roots infected with *A. ostoyae* is stronger than in roots infected with *A. sinapina*. Similarly, compartmentalization in cedar roots infected with *A. ostoyae* exhibited a higher than average number of pigmented parenchyma cells (the contents of which are presumably phenolic in nature) than following invasion by *A. sinapina*. Thus, in cedar, strongly pathogenic fungi, like *A. ostoyae*, induce stronger host responses that characteristically involve further expansion of the induced rhytidome response and TPRD in the phloem and a larger proportion of polyphenolic-rich parenchyma in the barrier zone than weaker pathogens like *A. sinapina*, while the responses to abiotic wounding are weakest of all.

A. sinapina is generally less pathogenic than *A. ostoyae* and under the same inoculum potential, penetration by *A. sinapina* did not result in the same amount of damage in host tissue as *A. ostoyae* did. Additionally, penetration and lesions cause by *A. sinapina* were reduced at greater distances from the inoculum block which implies lower inoculum potential at more distal rhizomorph tips in *A. sinapina* than in *A. ostoyae* thereby reducing its infective capability. The rate at which *A. sinapina* spreads in host tissue following penetration of the living phellogen appears to be much slower than *A. ostoyae*, and the production of toxic metabolites may also vary between the two pathogens enabling the host to initiate and complete the formation of new barriers in the bark and wood while under the influence of the fungus.

Infection that progresses slowly may be increasingly checked by host resistance (Morrison 1972). In this study, host species did not appear to be 'challenged' by *A. sinapina* to the extent they were with the more pathogenic fungus, *A. ostoyae*. Field observations under this study suggest that a single rhizomorph penetration by *A. sinapina* was unable to initiate a progressive infection in a root, probably due in large part to the lower inoculum potential of the fungus. Changes in the inoculum potential may occur following initial penetration by the fungus. For example, as the fungus advances in a host species colonizing more bark and cambial tissue, inoculum potential may increase to the point that it may overwhelm any type of host response to halt further spread of the fungus. This is frequently the case for *A. ostoyae*, but occurs to a lesser extent with *A. sinapina* where mycelial wedges appeared immobilized within necrotic tissue and were seldom observed at the leading edge of an infection front.

Lesion size on host roots inoculated with *A. sinapina* varied according to their proximity to the inoculum source. There was a noticeable trend in decreasing lesion size and depth of penetration with increasing distance between the inoculum block and the rhizomorph tips. For example, at inoculum contact *A. sinapina* caused necrosis of the living bark down to the vascular cambium whereas more distal points of attempted penetration resulted in very shallow lesions in the bark or lacked any penetration of the outer bark at all.

With increasing distance between the apical tip of the rhizomorph and the inoculum block the rate at which nutrients can be mobilized at the growing apex is reduced (Garrett 1956). The largest cross-sectional area of the fungus in contact with the root surface was that underlying the inoculum block which always showed the largest lesions. Thus, the density of the fungal hyphae (i.e. rhizomorphs) per unit area of host surface and the vitality of those rhizomorphs, as influenced by the size and proximity to the inoculum source, determines the inoculum potential of the fungus (Garrett 1970). In this study, woody inoculum of a sufficient volume was adequate for infection to take place at the point of contact with the root. *A. sinapina* had a reduced capacity to infect host roots at further distances from the inoculum source and when *A. sinapina* was able to penetrate

the host, it had a reduced ability to grow and spread within host tissue and was usually contained within a NP barrier in the bark or compartmentalized. There were considerably more rhizomorph attachments that lacked penetration of the outer periderm than attachments which were able to penetrate and initiate the process of phellogen renewal.

Several individual *A. sinapina* rhizomorph attachments, depending on their relative position in the outer rhytidome, appeared to suppress phellogen activity in the zone immediately underlying a penetrating rhizomorph. For example, where a rhizomorph had formed a lateral branch (attachment) and was actively degrading some of the outer phellem, while the underlying phellogen was unharmed, the phellogen zone appeared narrower directly below the penetrating rhizomorphs than contiguous phellogen on either side. It was not clear whether the fungus suppressing phellogen activity subsequently induced a stimulatory effect in the adjacent phellogen resulting in a higher than average number of phellem produced. There was clearly a lot of variation within any given sample since some rhizomorphs which were nestled between bark scales deeper in the rhytidome induced no such response in the underlying phellogen zone. However, it is interesting that up to 22 layers of phellem cells were produced around lesions infected with *A. sinapina*, at least double that shown in roots infected with *A. ostoyae*.

Rhizomorphs located on the surface of the rhytidome were strictly superficial and had no effect on the phellogen. It is possible that fungal elicitors at surface contact with the host may trigger the initiation of defense responses although Mullick (1977) suggests that the process of phellogen renewal occurs only after the phellogen becomes non-functional. If this were true, then the host would have a distinct advantage over the fungus at containing potential infections quickly and resisting lateral and tangential spread in the root. This idea is not confirmed but warrants further investigation; however, the chief difficulty is sampling the roots at precisely the right time as the rhizomorph is invading the phellem, but has not yet injured the underlying phloem.

Previous reports indicate that *A. sinapina* is a weak pathogen, generally not capable of attacking living conifers. *A. sinapina* has the ability to search out moribund but still living substrates and may at times assume the role as a secondary parasite by invading trees that have been weakened by other biotic or abiotic factors. However, recent studies have reported varying degrees of pathogenicity exhibited by *A. sinapina* depending on the host substrate, inoculum potential of the fungus, the mode of infection and interspecific competition for host resources (Dettman and van der Kamp 2001a, Morrison 2004). In the central interior of B.C., 72% of *A. sinapina* isolates obtained from trees were collected from roots of standing conifers that were still alive (Dettman and van der Kamp 2001a). Given the fact that these trees were found to be in the 'early stages of infection' it is highly probable that the length of time required for *A. sinapina* to kill a tree is longer than *A. ostoyae*, or that the lower virulence of *A. sinapina* enabled trees to produce host reactions that contained the fungus. In this study, excavations of root systems of trees naturally infected with *Armillaria* revealed an abundance of *A. sinapina* rhizomorphs in the soil. Isolations from lesions infected with *Armillaria* followed by somatic incompatibility tests using known isolates of *A. ostoyae* and *A. sinapina* revealed that the species causing infection on roots was indeed *A. ostoyae*. Similarly, Dettman and van der Kamp (2001b) did not isolate *A. sinapina* from any trees in the same general area at Hidden Lake as this study, suggesting that *A. ostoyae* is the dominant, primary parasite. Nevertheless, the authors suggest that in the central interior of B.C., *A. sinapina* may be more pathogenic to coniferous hosts than previously thought, particularly when *A. ostoyae* is absent. Prospero *et al.* (2006) supports this premise by reporting that stump colonization by *A. cepistipes*, generally considered a saprotrophic species, was inhibited by the presence of *A. ostoyae* on Norway spruce following dual inoculation of seedlings in pots compared to trees that had single inoculation with either species of *Armillaria*.

Pankuch *et al.* (2003) reported that 93% of *A. sinapina* infections found on aspen roots were associated with wounding following mechanical site preparation in a boreal mixedwood site, more than three times the number of wounded roots that became colonized by *A. ostoyae*. Thus, *A. ostoyae* was more frequently isolated from unwounded root tissue than *A. sinapina* (Pankuch *et al.* 2003). The authors also reported a low

frequency of compartmentalization to contain the fungus in roots infected with *A. sinapina*. The results obtained in this study contrast with those of Pankuch *et al.* (2003) such that nearly all *A. sinapina* infections were successfully contained within a new periderm barrier, or compartmentalized, or both.

Following harvesting, *A. sinapina* will colonize moribund tissue in stumps more easily than in living trees offering this 'weak pathogen' a large amount of energy to potentially infect new hosts. Isolations from precommercially thinned stumps in the ICH Zone in the southern interior of B.C. showed that 13% were colonized by *A. sinapina* (Cruickshank *et al.* 1997). Although colonization of stumps by *A. ostoyae* was nearly four times that of *A. sinapina*, the results suggest that colonization by *A. sinapina* is not uncommon. Newly planted crop trees are more susceptible to killing by *Armillaria* residing in large woody substrates particularly when the crop trees are situated in close proximity to the inoculum source where rhizomorphs have high inoculum potential. Under high inoculum potential, young, newly regenerating trees may offer little resistance to even a weak pathogen such as *A. sinapina*. Thus, it is possible that some of the mortality observed in young plantations may in fact be caused by *A. sinapina*.

The fact that the two species overlap suggests that their dissemination strategy is somewhat different and that *A. ostoyae* does not generally compete with *A. sinapina* for host substrates. The survival strategy of *A. sinapina* relies on the propagation of rhizomorphs in the soil giving it advantage of position in the capture of potential resources. However, *A. ostoyae* is more efficient as a primary resource capturer because it is better able to infect and kill live hosts and utilize the woody tissue as a food base, allowing for increased inoculum levels for the fungus. Dettman and van der Kamp (2001a) showed that *A. sinapina* genets were much smaller and more numerous than *A. ostoyae* clones, suggesting more frequent spore infection for *A. sinapina*. Prospero *et al.* (2003) found that when *A. cepistipes* and *A. ostoyae* occurred in the same pathosystem, *A. ostoyae* was more frequently found occupying stumps and *A. cepistipes* was more frequently found existing as rhizomorphs in the soil. Cruickshank *et al.* (1997) suggested that the distribution of *A. sinapina* and *A. ostoyae* on the same site may be partially

determined by anoxia associated with periodically saturated soil that limits survival of inoculum in woody substrates and periodic drying of the soil that limits rhizomorph growth and transfer of mycelium at root contacts. Thus, periodic drying of the soil would more strongly affect spread of *A. sinapina* than *A. ostoyae* (Cruickshank *et al.* 1997). In this study, the frequency of infection caused by *A. ostoyae* and *A. sinapina* did not differ in any of the species 1-year after the summer drought in 2003. Thus, adverse environmental conditions associated with periodic drying of the soil negatively affected rhizomorph growth and development by both species.

In this study, inoculum potential and host-pathogen interactions were key determinants of pathogenicity of *A. sinapina* on Douglas-fir, western hemlock and western redcedar trees. Damage was considerably less on all species infected with *A. sinapina* than *A. ostoyae*, particularly for Douglas-fir and western hemlock. Host-mediated defense mechanisms resulted in a significantly higher frequency of effective resistance reactions induced in Douglas-fir and western hemlock trees infected with *A. sinapina* than *A. ostoyae*. Western redcedar consistently formed resistance reactions to contain the fungus. Where damage ensued, it was always associated with high inoculum potential. Under natural conditions however, *A. ostoyae* and *A. sinapina* have a neutralistic co-existence with each other. Unlike *A. ostoyae*, *A. sinapina* poses little risk to conifers in the forests of the southern interior region of British Columbia. Pathogenicity of *A. sinapina* is generally low and it is not likely to cause significant damage to trees unless they have been weakened by some other biotic or abiotic stress factor.

CHAPTER FOUR:

ABOVE GROUND SYMPTOM DEVELOPMENT AND MORTALITY RATES BY SPECIES IN JUVENILE MIXED CONIFER STANDS

4.1 INTRODUCTION

Armillaria root disease occurs in most biogeoclimatic zones in the southern interior of BC. In undisturbed mature stands, the incidence of diseased trees can range from about 10% in the IDF to about 80% in the ICH (Morrison *et al.* 2001). However, only about one-quarter of the trees with belowground infection can be detected aboveground (Morrison *et al.* 2001). *A. ostoyae* inoculum is universally present in all but the driest subzone of the ICH. A large number of plantations in the ICH consist of pure Douglas-fir or Douglas-fir mixed with lodgepole pine (*Pinus contorta* Dougl. *ex* Loud.) and other conifers. Infection of plantation trees usually begins around ages 5-7 from carry-over inoculum in stumps and the incidence of diseased trees increases gradually to 30-35% by age 20 (Morrison *et al.* 2000). Cumulative mortality can be as much as 20% by age 25, resulting in undesirable stocking in juvenile stands (Morrison and Pellow 1994). The probability of infection increases with increasing tree size (Morrison 2000). Hence, additional mortality and growth loss on trees that sustain non-lethal infections will occur throughout a rotation especially on susceptible conifers like Douglas-fir. Ultimately, these losses can lower the level of sustainable harvest on infested sites. Damage caused by Armillaria root disease has meaning only in the context of our expectations of yield and the goal of maximizing stand productivity for the next rotation of timber. Armillaria root disease has always been a natural component of forest ecosystems and the yield from mature stands where the fungus is known to be present is probably lower than future expectations of yield from such stands. Silvicultural practices that focus on rapid regeneration with species that are highly susceptible to killing by *A. ostoyae* and various stand tending operations including brushing, precommercial and commercial thinning upset natural balances that develop over time and result in conditions that favour survival and spread of Armillaria in plantations.

Few options are available to mitigate potential losses due to Armillaria root disease. Removal of stumps from the ground is a very effective means of reducing the amount of woody inoculum that would otherwise be available to the fungus. Thirty years after removal of stumps infected with *A. ostoyae* and *P. sulphurascens* Pilat (= *P. weirii*, Douglas-fir form), cumulative mortality from the two root diseases was less than 4% in stumped plots compared to more than 20% in untreated plots for most tree species (Morrison 1998). Another less intrusive option for managing stands where root disease is of concern is to plant conifer species that have a low susceptibility to killing by *A. ostoyae*, either alone, or in mixtures with susceptible conifers to impede disease transfer between roots of susceptible species.

Generally, all conifers less than 15-years-old are highly susceptible to killing by *A. ostoyae* and after this age some conifers become more tolerant of the fungus (Morrison and Mallet 1996). For example, resistance in western larch against *A. ostoyae* becomes more pronounced when trees reach the age of 20-25 years. Robinson (1997) attributed this increased resistance to more frequent formation of necrophylactic periderms with multiple bands of thick- and thin-walled phellem, a structural characteristic of periderms which helps impart increased resistance to the spread of *A. ostoyae* in host tissue. However, before this age, western larch shows considerable mortality, approaching and sometimes exceeding that of Douglas-fir. In a species trial in the southern interior of B.C., *Armillaria*-caused mortality in western larch after 15-years was higher than all other conifers (H. Merler, unpublished data). The trial also showed western redcedar to have the lowest proportion of trees killed by *A. ostoyae*, comparable to that observed in hardwoods. Birch and poplars are generally resistant to the fungus, but may become increasingly susceptible to killing by *A. ostoyae* after the age of 40 particularly where they are mixed with conifers and become overtopped. The ideal scenario to mitigate damage caused by Armillaria root disease with mixed conifer plantations is to select species that are more resistant to the fungus, for example a higher frequency of callusing, and plant those species in intimate mixtures (i.e. not clumped) with other susceptible conifers. The species chosen should show effective resistance to *A. ostoyae*, express this

resistance at an earlier age than perhaps western larch, and be able to tolerate infections without significant growth losses.

In the southern interior of British Columbia, DeLong (1997) observed a low incidence of decay caused by *A. ostoyae* on advanced regeneration of western redcedar trees. Morrison *et al.* (2000) reported the actual belowground disease incidence in western redcedar was 20-27% while infection in western hemlock and Douglas-fir was 45-53%. After 20 years, mortality caused by *A. ostoyae* in western redcedar was about one-tenth that observed in other conifers (B. van der Kamp, unpublished data). Together these findings clearly establish lower disease incidence in western redcedar relative to other conifers.

The results from field inoculation trials and examination of western redcedar trees naturally infected with *A. ostoyae* (Section 2.3.5. in Chapter 2) show that cedar is more resistant to the fungus than Douglas-fir and western hemlock. However, a question remains: does the observed resistance in western redcedar at the individual root level translate into reduced infection and mortality in mixed species stands? The objective of this study was to assess the potential for using western redcedar as a primary species choice for regenerating infested sites by examining symptom development and mortality rates caused by *A. ostoyae* in cedar and comparing these to a known susceptible species, Douglas-fir.

4.2   MATERIALS AND METHODS

4.2.1 SITE SELECTION

The selection of sites for surveying disease incidence in stands of mixed conifers was based on several criteria. Stands were to be located in the moist-warm (mw) subzone of the ICH in the southern interior of British Columbia. However, candidate sites located in the moist-cool (mk) or wet cool (vk) subzones were also considered. All stands were to have Douglas-fir as the leading species with a significant component and intimate

mixture of western redcedar in the understory so that nearly equal proportions of the two species across all sites could be compared. Stands were to fall within an age range of 15 to 30 years and have moderate to high levels (approximately 10%) of above ground signs and/or symptoms attributed to Armillaria root disease.

Forest cover maps were provided by the B.C. Ministry of Forests, Tolko Industries Ltd. (formally Riverside Forest Products), and Pope and Talbot, Inc. Prospective sites that met the selection criteria with respect to species and age were selected from maps and then each site was visited. Sites were assessed by walking through the stand to determine whether the levels of aboveground signs and symptoms and species proportions met the selection criteria. In many cases, silviculture labels on forest cover maps depicting occurrence and proportions of cedar were not accurate and local knowledge of foresters was used to supplement map information. Limited access to sites because of decommissioned roads or road washouts also reduced the number of prospective sites.

4.2.2   PLOT SELECTION AND MEASUREMENT OF TREES

For each site, transects were run across the slope in order to capture any variation in position across the block. Transects started at least 30-m from the stand edge, landing, or road on a chosen compass bearing. The minimum distance between transect lines was 50-m so as to not overlap with adjacent plots. At each 50-m stop position, the first dead conifer tree greater than 3-cm diameter at breast height (DBH) encountered within a 20-m search radius was considered for potential plot center tree. A 10-m radius plot was centred around a potential plot centre tree provided a second set of criteria were satisfied: these were based on visual assessment to determine whether (1) there was at least 10% infection by *A. ostoyae* (lethal and non-lethal) in conifers in the plot, (2) a minimum number of trees (Douglas-fir and western redcedar) per plot each comprised approximately 30% of the species composition, and (3) the total number of trees tallied would yield between 100-150 trees per plot.

Infected trees along the transect line were considered for 'plot centre tree' until a suitable plot location was found. Within a plot, each tree (living and dead) greater than 3-cm DBH was identified and recorded by species, DBH, and disease status. Down trees were also counted and recorded as killed by *A. ostoyae* only when evidence of the fungus was present. *Armillaria* infection was confirmed by sub-cortical mycelial fans or impressions of such fans in resin-soaked or necrotic bark, or both, in all trees suspected of being infected or killed by *A. ostoyae*. The plot center tree was not part of the sample. Soil was removed from the root collar area on all trees and the root collar was examined for evidence of old or current basal lesions caused by *A. ostoyae*. Infections at the root collar of living trees were classified as either progressive (i.e. where the fungus was advancing in the inner bark and cambial tissue) or callused (i.e. the fungus was compartmentalized and spread of the fungus had been stopped). The percentage of root collar circumference showing resinosus was used as an estimate of percent girdling of the root collar by *A. ostoyae* for all species except western redcedar and hardwoods. Trees showing 100% girdling around the base of the tree lacking crown symptoms were classified as killed by *A. ostoyae*. Standing dead or down trees killed by other biotic or abiotic agents were also recorded.

4.2.3. STATISTICAL ANALYSIS

The proportion of trees killed or infected by *A. ostoyae* was determined by species for each plot and site. Statistical analysis was done using SPSS statistical package (SPSS Inc., Chicago, IL). Frequency data for species mortality were analyzed by Chi square tests. A logistic regression model was fitted to the data [i.e. $log\ p/(1-p) = a + b\ (dbh)$] where 'p' is the probability of mortality and 'a' and 'b' are regression co-efficients for the two species tested. The Hosmer-Lemeshow goodness of fit test was used to test whether the model fit the data adequately.

4.3 RESULTS

A total of 20 juvenile mixed conifer stands were surveyed for mortality incidence in the Okanagan Shuswap and Arrow Boundary Forest Districts in the southern interior forest region (Figure 4.1). Table 4.1 lists the characteristics of all sites numbered on the map in Figure 4.1.

Map source: www.maps.gov.bc.ca
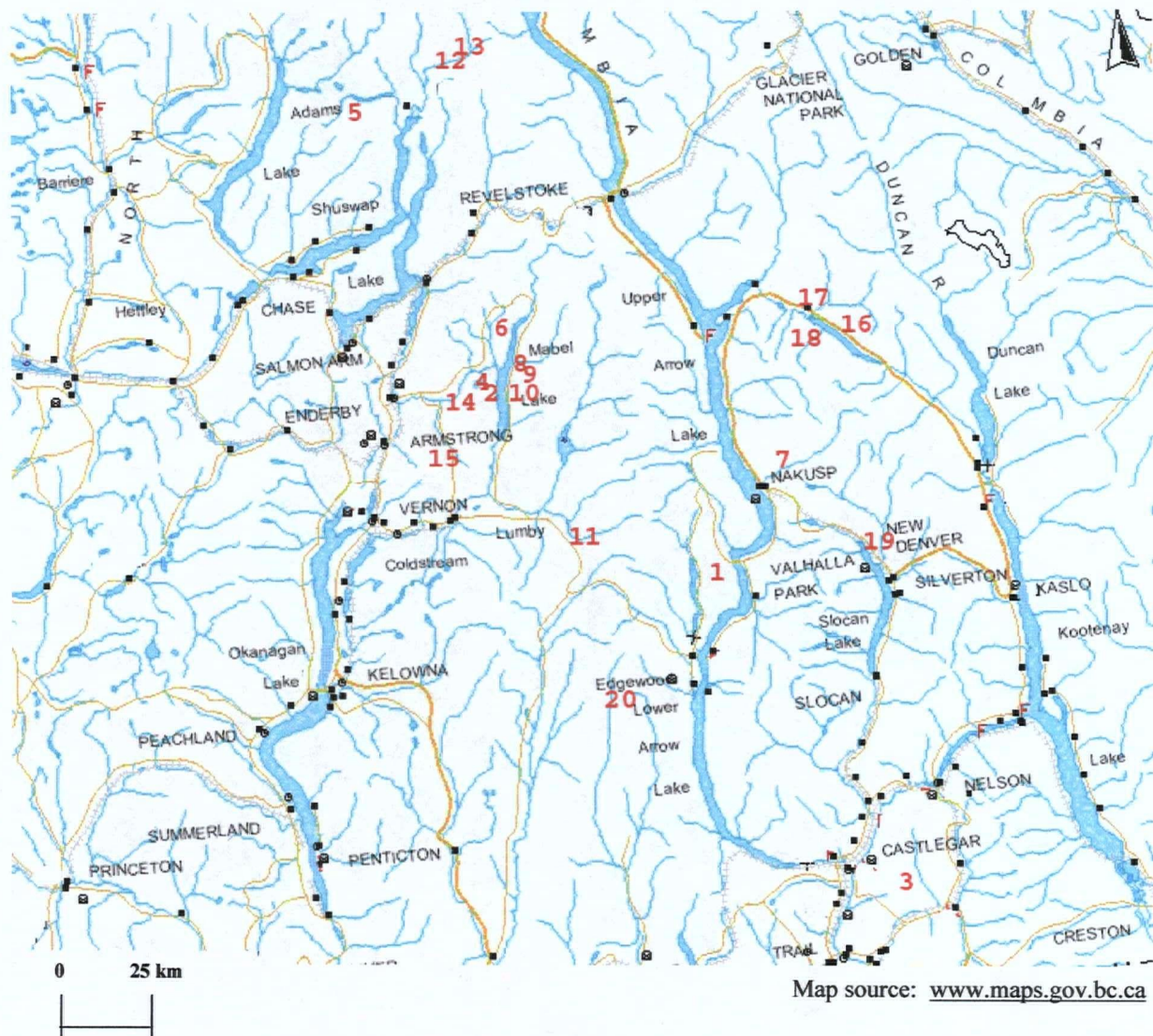
Fig. 4.1. Location of study sites numbered 1 to 20 used for surveying species mortality caused by Armillaria root disease in mixed conifer stands in the southern interior of British Columbia.

Table 4.1. Characteristics of the twenty sites surveyed for incidence of infection and mortality by *A. ostoyae* in juvenile mixed conifer stands in the Okanagan-Shuswap and Arrow-Boundary Forest Districts in the southern interior of B.C.

Site No.	Site Name	Location	Elevation (m)	BEC Zone[1]/ subzone	Stand Age	Species[2] Composition	No. of Plots	Total No. of trees	% F	% C	% H	% Above-Ground Infection[3]	Cumulative Mortality (%)
1	Arrow Park	50°12'N 118°0' W	1000	ICHmw2	15	PwFC(H)	1	154	17.5	40.3	6.5	16.2	11.0
2	Baird Lk.	50°31'N 118°48'W	1000	ICHmw2	18	FE(LC)	3	452	29.4	29.0	3.7	17.9	13.1
3	Erie Cr.	49°17'N 117°22W	1200	ICHmw2	16	FCAc(Pw)	4	421	44.9	26.6	3.3	10.2	8.6
4	Hidden Lk	50°33'N 118°49'W	700	ICHmw2	28	EFC(HAc)	3	341	34.0	26.4	26.4	12.9	8.8
5	Humamilt Lk.	51°16'N 119°7' W	800	ICHmw3	21	AcCF(H)	2	268	27.2	29.1	5.9	12.7	9.7
6	Kidney Lk	50°45'N 118°38'W	800	ICHmw2	22	FCH(E)	2	534	15.0	19.5	59.5	16.9	7.7
7	Kuskanax	50°17'N 117°44'W	900	ICHmw2	24	SFPwAc(C)	3	353	50.7	30.9	5.4	23.2	14.2
8	Mabel Lk.Icebox	50°36'N 118°38'W	800	ICHmw2	20	FCH(L)	3	438	39.0	33.6	3.0	15.8	11.4
9	Mabel Lk.Simard	50°32'N 118°42'W	800	ICHmw2	21	EF(CH)	3	466	22.1	32.4	25.5	17.0	9.4
10	Mabel Lk_2	50°38'N 118°39'W	800	ICHmw2	16	EFC	2	357	57.1	24.1	2.0	28.0	25.2
11*	Monashee Cr.	50° 9' N 118°28'W	1000	ICH mk1	21	CF(H)	2	340	35.3	38.5	5.3	15.6	10.9
12	Northfork_55	51°22'N 118°46'W	600	ICHmw3	17	CHF	3	313	26.5	37.1	10.2	13.1	4.5
13	Northfork_60	51°22'N 118°43W	600	ICHmw3	21	CFH	4	472	32.0	37.9	11.0	16.1	11.2
14	Toledo	50°32'N 118°52'W	600	ICHmw2	23	CH(FPwAcE)	2	393	10.4	15.5	53.4	6.1	5.9
15	Trinity Valley	50°27'N 118°59W	700	ICHmw2	15	FEAcC	2	450	71.3	20.9	0.2	35.8	32.2
16	Trout Lk._1	50°36'N 117°26'W	1000	ICHmw2	21	CFPw(H)	2	152	38.8	28.3	28.3	18.4	13.2
17	Trout_Lk._2	50°39'N 117°33'W	1000	ICHmw2	25	FC	2	246	26.4	50.0	20.3	22.0	6.9
18	Trout Lk._3	50°37'N 118°59'W	1000	ICH wk1	25	FC(H)	3	302	26.2	48.7	9.9	13.2	5.0
19	Wilson Cr.	50°5' N 117°23'W	1100	ICHmw2	17	EF(C)	2	350	26.9	25.7	22.2	10.3	4.3
20	Worthington	49°44'N 118°16'W	1000	ICHmw2	33	HSFC	3	373	29.0	30.8	22.2	19.3	15.6
						TOTAL:	51	7175	MEAN: 33.0	31.3	16.2	17.0	11.4

* = naturally regenerated stand

[1] See Meidinger and Pojar (1991), Braumandl and Curran (1992).

[2] Species codes follow silviculture labels on forest cover maps. Species are listed in order of their frequency and 'minor' components are placed in brackets.
Pw, western white pine; F, Douglas-fir; C, western redcedar; H, western hemlock; E, white birch; L, western larch; Ac, cottonwood; S, Englemann spruce

[3] Percent aboveground infection includes both mortality and infection on living trees

The sample population was comprised of 51 plots across 20 sites and a total of 7175 trees (Table 4.1). The number of plots per site depended on the number of acceptable plots that fit the criteria listed above, the number of trees per plot and the length of time required to survey each plot. Across all sites, the mean proportion for Douglas-fir and western redcedar was similar at 33% and 31.3%, respectively. However, tree size distribution differed between these species. Figure 4.2 shows that the number of stems in the 30-50 mm DBH size classes for western redcedar was approximately double the number of stems for Douglas-fir, primarily due to differences in growth rates, shade tolerance, and the timing of establishment between the two species.

There were significant differences ($p < 0.0001$) in the incidence of mortality between Douglas-fir and western redcedar over all sites. Cumulative mortality ranged between 4.3% and 32.2 % with an average of 11.4% (Table 4.1). Within a given plot, the aboveground incidence of *A. ostoyae* was generally patchy, occurring as small infection foci comprised of 2-4 trees and probably associated with patchy distribution of inoculum. Aboveground disease incidence in trees varied by site and was up to 2-3 times more than percent cumulative mortality. However, actual incidence (belowground) was undoubtedly higher than what was detected in this study since entire root systems were not examined. Interestingly, the two sites with the highest proportion of Douglas-fir (Mabel Lake_2 and Trinity Valley) also showed the highest cumulative mortality (mainly Douglas-fir) and the smallest difference between mortality and aboveground incidence (Table 4.1).

Table 4.2 shows the total number of conifer trees tallied for all sites in different disease status categories. Disease status and tree measurements were also recorded for hardwood species at each site (Appendix XIII).
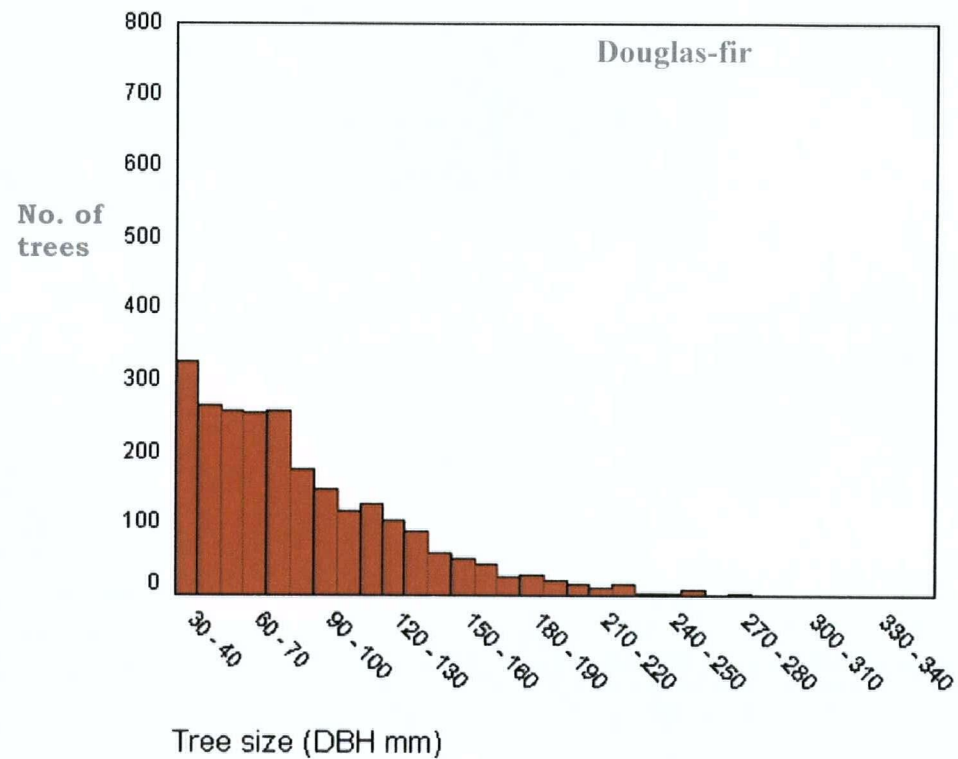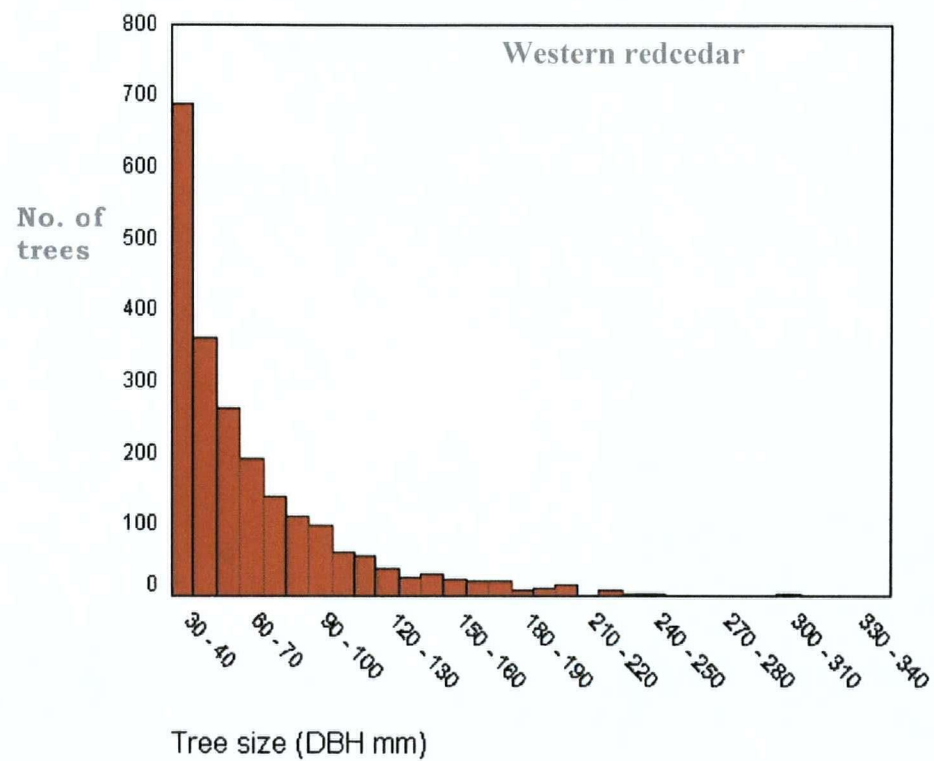
Fig. 4.2. Tree size distribution as number of trees per 10 mm DBH class for western red cedar and Douglas-fir trees for all 20 sites combined.

Table 4.2. Total number of conifer trees tallied by species in different disease status categories for all twenty sites combined.

	CONIFER SPECIES[a]								
DISEASE STATUS	Fd	Hw	Cw	Bg	Lw	Pl	Se	Pw	TOTAL
Healthy	1642	952	2050	24	65	8	120	155	5016
Infected by *A. ostoyae* (PROGRESSIVE)[b]	136	88	4	0	2	0	8	6	244
Infected by *A. ostoyae* (CALLUSED)[c]	25	50	71	0	0	0	1	0	147
Dead (KILLED by *A. ostoyae*)	591	130	42	0	21	2	10	35	831
Dead (unknown/other factors)	2	0	2	0	2	1	0	46[d]	53
Total	2396	1220	2169	24	90	11	139	242	6291

[a] Fd = Douglas-fir; Hw = western hemlock; Cw = western redcedar; Bg = grand fir; Lw = western larch; Pl = lodgepole pine; Se = Engelmann spruce; Pw = western white pine.

[b] Progressive lesions are defined as infections at the root collar that lack evidence of NP formation in the bark or compartmentalization. Lesions appeared as browned tissue in advance of mycelial colonization.

[c] Callused lesions are defined as infections at the root collar that were compartmentalized and spread of the fungus had been stopped.

[d] 19% of the mortality in western white pine was caused by *Cronartium ribicola* (J.C. Fisch).

The number of trees tallied by species and disease status for each of the twenty sites is given in Appendix XIV. Other agents of mortality on conifers included drought dieback, Warren's root collar weevil (*Hylobius warreni* Wood), and *Cronartium ribicola* (J.C. Fisch). The proportion of conifers other than Douglas-fir and western redcedar varied among sites. For example, more than half the number of western larch trees was found at a single site (Baird Lake). In this particular case, mortality in 18-year-old western larch was 34% (n=49) compared to 25.5% (n=133) in Douglas-fir of similar age (Appendix XIV).

However, the number of trees for conifers other than Douglas-fir, western hemlock and western redcedar was too low to make statistical comparisons among species. The main comparison of interest was between Douglas-fir and western redcedar. It was not possible to find sites with nearly equal proportions of Douglas-fir, cedar and hemlock. Thus, hemlock was considered separately and comparisons between hemlock, Douglas-fir and western redcedar were based on sites which comprised a minimum of 10% hemlock in the stand (10 sites in total).

Comparison between Douglas fir and western redcedar

Table 4.3 shows the proportion of Douglas-fir and western redcedar trees by disease status category. Significant differences were found in the percentage of trees killed by *A. ostoyae* between species across all sites surveyed. Western redcedar mortality was always lower than Douglas-fir.

Table 4.3. The proportion of the total number of trees by disease status category for Douglas-fir and western redcedar.

	Species	
Disease Status	Douglas-fir	Western redcedar
Healthy	0.69	0.95
Infected by *A. ostoyae* (PROGRESSIVE)[a]	0.06	tr^b
Infected by *A. ostoyae* (CALLUSED)[c]	0.01	0.03
Dead (KILLED by *A. ostoyae*)	0.25	0.02
Dead (unknown/other factors)	tr^d	tr^d
Total	1.00 (n=2396)	1.00 (n=2169)

[a] Progressive lesions are defined as infections at the root collar that lack evidence of NP formation in the bark or compartmentalization. Lesions appeared as browned tissue in advance of mycelial colonization.

[b] *tr* = proportion of trees with progressive lesions at the root collar was less than 0.005

[c] Callused lesions are defined as infections at the root collar that were compartmentalized and spread of the fungus had been stopped

[d] *tr* = proportion of trees killed by unknown/other factors was less than 0.005

Overall, 25% (n=2396) of the Douglas-fir trees were killed by *A. ostoyae* compared to only 2% (n=2169) of the western red cedar trees (p<0.0001).

The logistic model which compared the probability of mortality between Douglas-fir and western redcedar showed species to be significant. This implies that the probability of western redcedar trees being killed by Armillaria is significantly lower than for Douglas-fir trees ($\beta = -2.683$, $p < 0.0001$). The Hosmer-Lemeshow test was not significant suggesting that the logistic model adequately fits the data ($\chi^2 = 3.733$, $p = 0.880$). The logistic model calculated the corresponding odds ratio which indicated that in young juvenile stands the odds of a Douglas-fir being killed (Exp^β) is 14.6 times greater (95% C.I. = 10.57, 20.71) than a western red cedar tree (Appendix XV).

The percentage of infected trees with progressive lesions at the root collar differed between species (Table 4.4). Only 4 of 117 (3%) of western redcedar trees had progressive lesions at the root collar compared to 136 of 752 (18%) of the Douglas-fir trees. Progressive lesions were analogous to the classification of 'susceptible' host reactions discussed previously in Chapter 2 (Section 2.3.5) in that trees show no evidence of necrophylactic periderm (NP) formation in the bark or compartmentalization following invasion of the vascular cambium. The infection front of progressive lesions appeared as browning of tissue in advance of the fungus. Mycelial fans were also frequent. Basal resinosus was common in Douglas-fir but not in western redcedar. In many cases, basal resinosus occurred around the entire circumference of the root collar yet trees lacked any distinctive crown symptoms indicative of root disease (i.e. chlorosis of foliage, crowns with shorter and fewer number of needles, distress cones, and reduction in growth of current year's leader). Thus, crown symptoms were not always good indicators of root disease presence. These observations are consistent with that reported by Filip (1986) and Morrison *et al.* (2000) who reported that the majority of trees infected belowground lacked evidence of aboveground symptoms of disease.

Table 4.4. Incidence of progressive infections, callused infections and mortality in Douglas-fir and western redcedar as a proportion of the total number of trees with above-ground signs or symptoms of *A. ostoyae*.

	SPECIES	
DISEASE STATUS	Douglas-fir	Western redcedar
Infected by *A. ostoyae* (PROGRESSIVE)[a]	0.18	0.03
Infected by *A. ostoyae* (CALLUSED)[b]	0.03	0.61
Dead (KILLED by *A. ostoyae*)	0.79	0.36
Total	1.00 (n=752)	1.00 (n=117)

[a] Progressive lesions are defined as infections at the root collar that lack evidence of NP formation in the bark or compartmentalization. Lesions appeared as browned tissue in advance of mycelial colonization.
[b] Callused lesions are defined as infections at the root collar that were compartmentalized and spread of the fungus had been stopped

The proportion of trees showing compartmentalization and callusing at the root collar differed among species (Table 4.4). The incidence of compartmentalization and callusing at the root collar was significantly higher in cedar than Douglas-fir (Table 4.4). Callused lesions at the root collar on cedar typically appeared as a flattened area at the base of the stem and the tree exhibited fluting of its trunk as a result of lateral ingrowth of callus from both sides (Figure 4.3). In that figure, following formation of the barrier zone by the uninjured vascular cambium, further lateral spread of the fungus did not occur and no breaching of this barrier was observed in subsequent years. In contrast, Douglas-fir exhibited continuous formation and breaching of its barrier zone in subsequent years and at times even within the same growing season (Figure 4.4). Sometimes, single or double bands of traumatic resin ducts were formed in annual growth rings in succeeding years indicating a continuous stimulation of this non-specific host defense response by the fungus (Figure 4.5). These observations suggest that cedar shows more effective compartmentalization of *A. ostoyae* at the root collar than Douglas-fir.
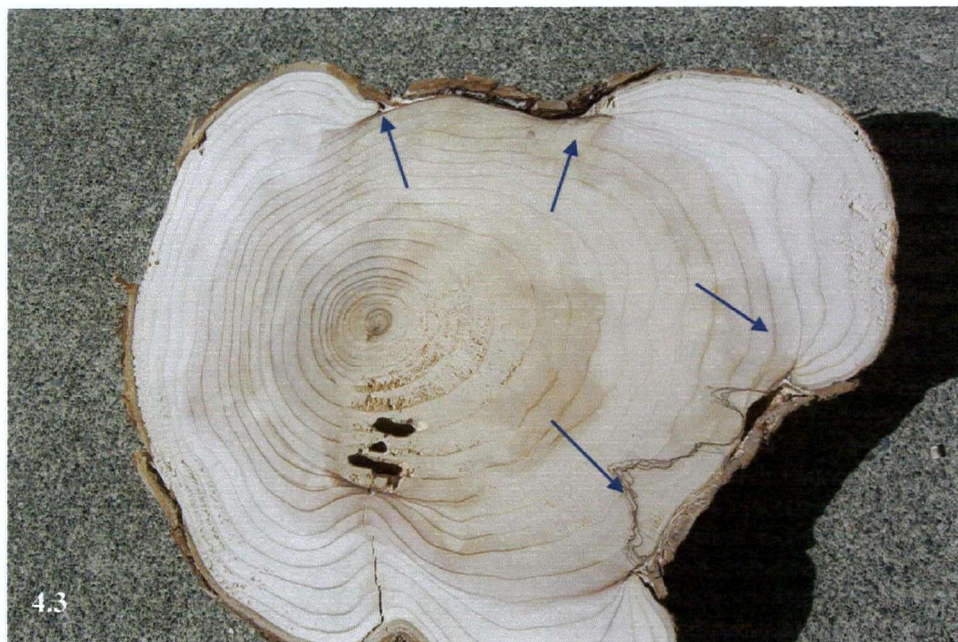
Fig. 4.3. Compartmentalization of *Armillaria*-caused lesions in the root collar area on a western redcedar tree. Two basal lesions resulted from an infection advancing on a single lateral root. The infection was checked once it reached the root collar. Barrier zone formation can be seen as a pinkish hue in the injured sapwood (arrows). Trunk fluting appears as a result of lateral ingrowth of callus over the face of the basal lesion. Note zone line formation in the infected sapwood.
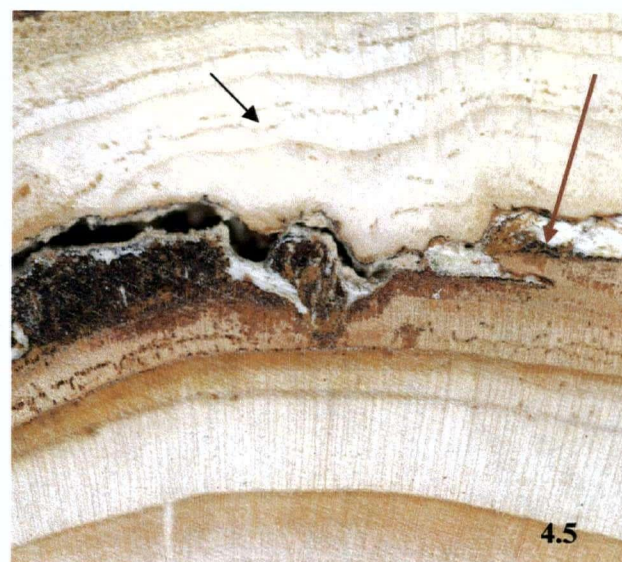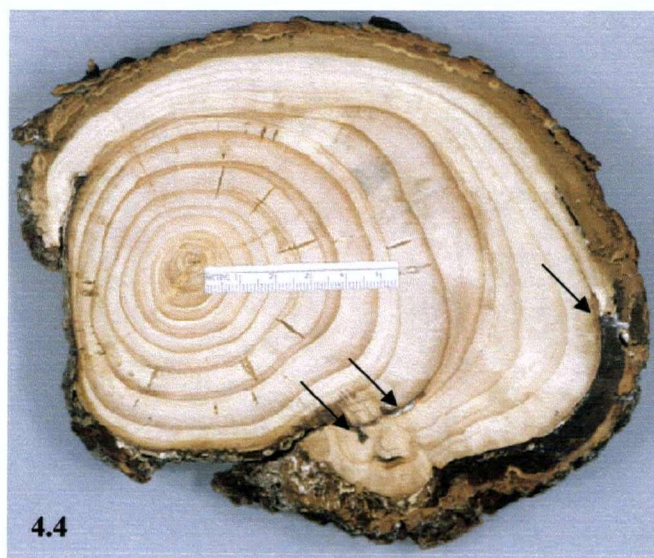
Fig. 4.4. Compartmentalization of *A. ostoyae* infection in the root collar area on Douglas-fir (also shown as Fig. 2.146 in Section 2.3.8 of Chapter 2). Continuous formation and breaching of barriers in subsequent years is evident (arrows). Traumatic resin canals extended tangentially around the circumference of the stem the year that the lesion was compartmentalized. Subsequent years also show shorter tangential series of traumatic resin canals. **Fig. 4.5.** A basal stem disk of Douglas-fir shows resin canal formation in the year prior to cambial invasion at the root collar. Note mycelial fans and resin embedded within infected bark (red arrow). Callus formation and vascular regeneration enabled the tree to grow over the infected tissue, yet the formation of traumatic resin canals was continuously stimulated in subsequent years (arrows). The fungus breached the temporary barriers in subsequent years to colonized additional cambial tissue.

The probability of mortality among trees infected by *A. ostoyae* depended on both species and tree size ($p < 0.001$). The incidence of mortality was significantly greater in the smaller diameter size classes than in the larger size classes for both species and cedar mortality was consistently lower than Douglas-fir. Although the risk of mortality decreased with increasing tree size in both species, the rate of decrease was noticeably greater among cedar compared to Douglas-fir trees (Figure 4.6).

There was an increasing trend in the proportion of infected trees showing compartmentalization and callusing with tree size for both western redcedar and Douglas-fir, but the increase was markedly greater for cedar than Douglas-fir trees (Figure 4.7).

The proportion of the total number of lesions at the root collar that were progressive differed between species; for Douglas-fir it increased with increasing diameter class whereas for western redcedar it was nearly constant across diameter classes (Figure 4.8). Of the four western redcedar trees that showed progressive lesions in the root collar, one tree occurred in each diameter class.
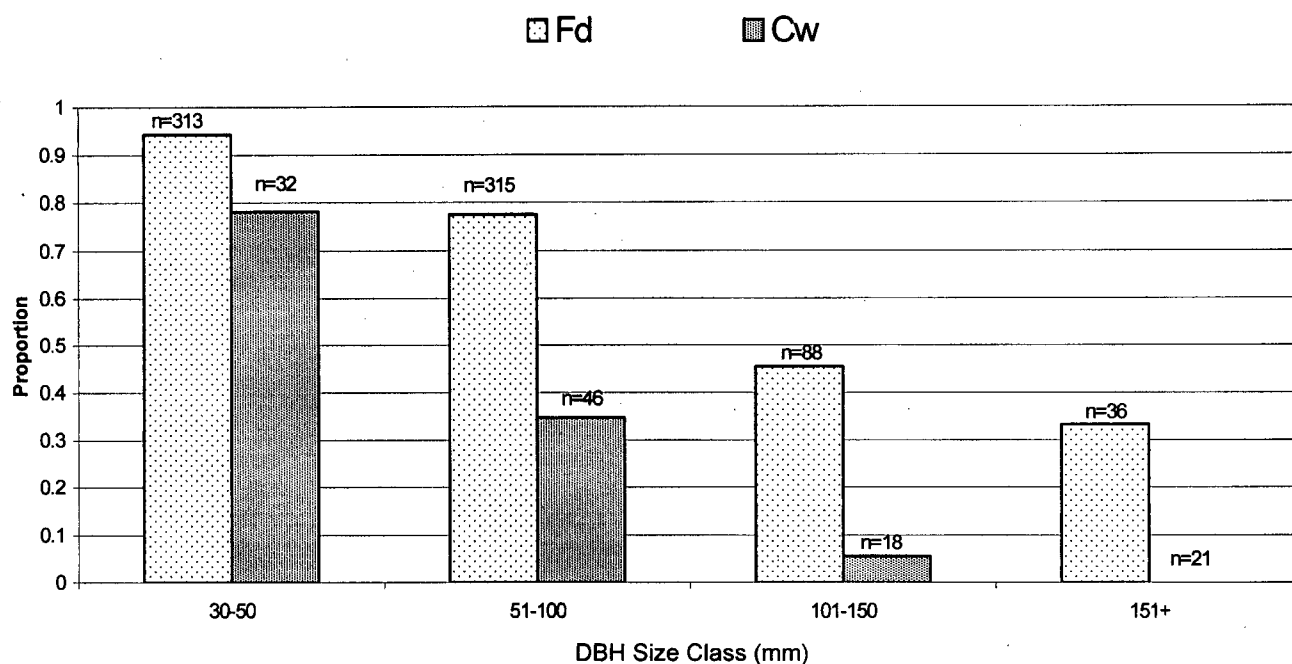
Figure 4.6. Mortality in Douglas-fir (Fd) and western redcedar (Cw) as a proportion of the total number of trees with above-ground signs or symptoms of *A. ostoyae* by size class
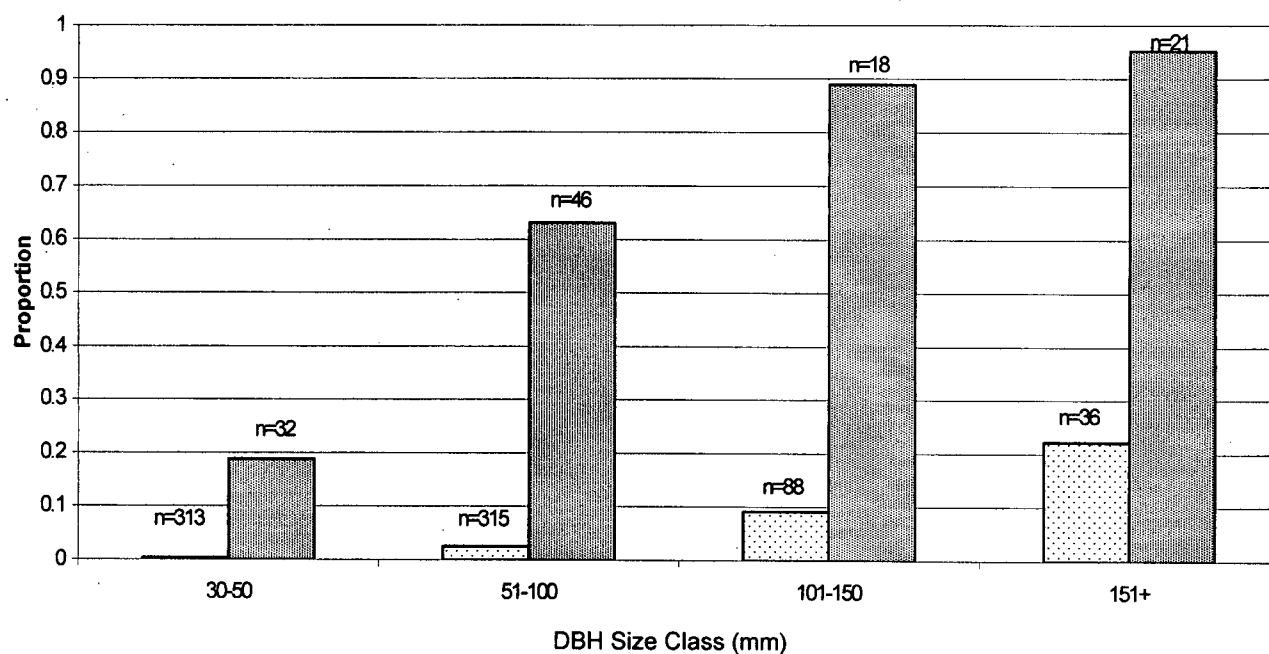
Figure 4.7. Callused lesions at the root collar in Douglas-fir (Fd) and western redcedar (Cw) as a proportion of the total number of trees with above-ground signs or symptoms of *A. ostoyae* by size class
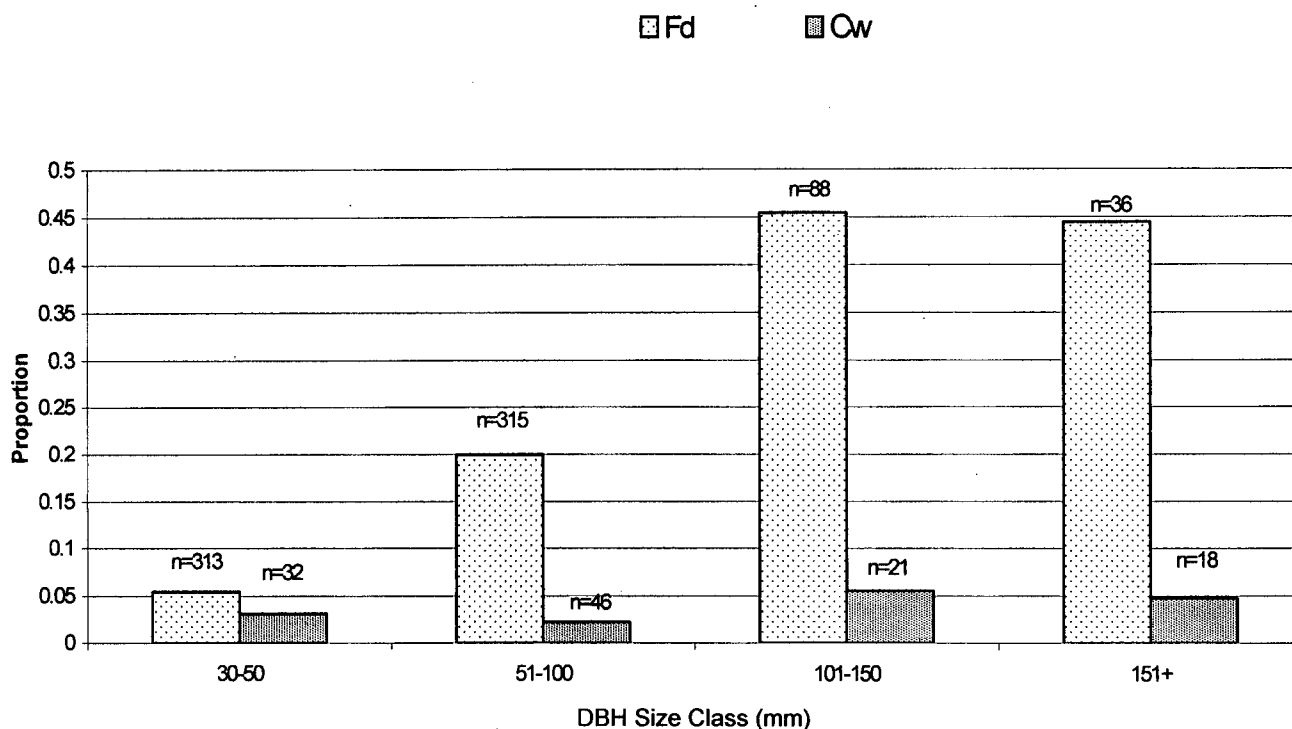
Figure 4.8. Progressive lesions at the root collar on Douglas-fir (Fd) and western redcedar (Cw) trees caused by *A. ostoyae* expressed as a proportion of the total number of infected trees by size class

Comparison among Douglas fir, western hemlock and western redcedar

Comparisons among Douglas-fir, western hemlock, and western redcedar were done using a dataset comprised of sites 4 (Hidden Lake), 6 (Kidney Lake), 9 (Mabel Lake_Simard), 12-14 (Northfork_55 and 60, and Toledo), 16-17 (Trout Lake_1 and 2), and 19-20 (Wilson Crk. and Worthington) in which hemlock comprised at least 10% of the stand composition (Table 4.1). Tree size distribution for western hemlock was similar to that of western redcedar with the majority of stems occurring in the smaller diameter size classes (Appendix XVI). Table 4.5 shows the proportion of Douglas-fir, western hemlock and western redcedar trees by disease status category. All species differed significantly from each other (χ^2, $p < 0.001$) and ranked in decreasing incidence of mortality as Douglas-fir (19%), hemlock (10%) and cedar (1%) (Appendix XVII). Progressive lesions at the root collar represent the risk of future mortality in host trees. Figure 4.9 shows the anticipated impact of Armillaria on Douglas-fir, hemlock and cedar as the cumulative effect of mortality and progressive lesions at the root collar. Significantly more Douglas-fir trees were impacted by Armillaria than hemlock and cedar (χ^2, $p < 0.001$) (Appendix XVIII).

Table 4.5. The proportion of the total number of trees by disease status category for Douglas-fir, western hemlock and western redcedar from ten sites.

DISEASE STATUS	SPECIES		
	Douglas-fir	Western hemlock	Western redcedar
Healthy	0.73	0.79	0.95
Infected by *A. ostoyae* (PROGRESSIVE)	0.07	0.07	tr^a
Infected by *A. ostoyae* (CALLUSED)	0.01	0.04	0.04
Dead (KILLED by *A. ostoyae*)	0.19	0.10	0.01
Dead (unknown/other factors)	tr^b	tr^b	tr^b
Total	1.00 (n=900)	1.00 (n=1075)	1.00 (n=1072)

[a] tr = proportion of trees showing progressive lesions at the root collar was less than 0.005
[b] tr = proportion of trees killed by unknown/other factors was less than 0.005
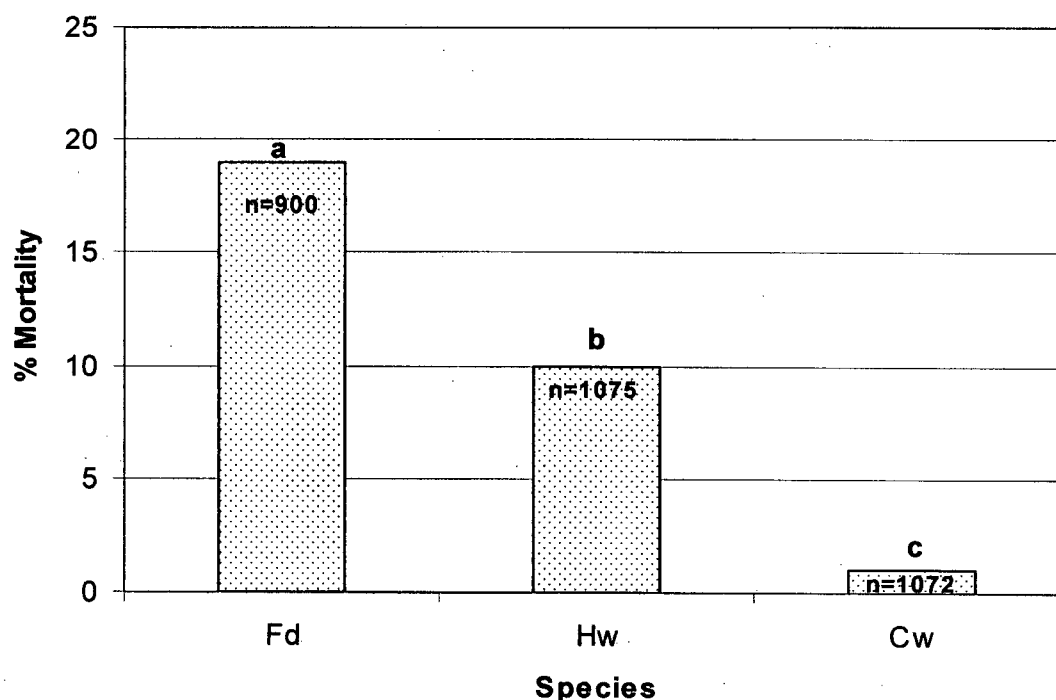
Fig. 4.9. Percent dead and dying Douglas-fir (Fd), western hemlock (Hw) and western redcedar (Cw) as a proportion of the total number of trees tallied. Different letters denote significant differences at $\alpha = 0.001$

Table 4.6 shows the proportion of infected trees with progressive lesions, callused lesions, and mortality caused by *A. ostoyae* by species for the 10 sites. Only 1 of 56 (2%) of western redcedar trees showed a progressive lesion at the root collar. This was significantly different from the 26% (n=244) and 33% (n=222) of progressive lesions on Douglas-fir and western hemlock, respectively (χ^2, p < 0.001). The difference was not significant between hemlock and Douglas-fir (Appendix XIX). Basal resinosus was a common symptom in hemlock and Douglas-fir, but not cedar. The higher percentage of progressive lesions in hemlock and the fact that nearly half the infected trees were killed by *A. ostoyae* are consistent with results from field inoculation trials (Section 2.3.5) showing a high frequency of unsuccessful resistance reactions in hemlock compared to the other species. Compartmentalization and callusing of lesions in the root collar area was significantly higher in western hemlock (17%, n=222) than in Douglas-fir (3%, n=243) (χ^2, p < 0.001). However, 40 of 56 (71%) of callused lesions on western redcedar was significantly higher than both Douglas-fir and western hemlock (χ^2, p<0.001).

Table 4.6. Incidence of progressive infections, callused infections and mortality in Douglas-fir, western hemlock and western redcedar as a proportion of the total number of trees with above-ground signs or symptoms of *A. ostoyae* in 10 sites.

	SPECIES		
DISEASE STATUS	Douglas-fir	Western hemlock	Western redcedar
Infected by *A. ostoyae* (PROGRESSIVE)	0.27	0.33	0.02
Infected by *A. ostoyae* (CALLUSED)	0.03	0.17	0.71
Dead (KILLED by *A. ostoyae*)	0.70	0.49	0.27
Total	1.00 (n=244)	1.00 (n=222)	1.00 (n=56)

The probability of mortality and compartmentalization among Douglas-fir, western hemlock and western redcedar infected by *A. ostoyae* depended on both species and tree size (Figure 4.10 and 4.11). Similar to Douglas-fir and cedar, the incidence of mortality in hemlock was significantly greater in the smallest diameter size class than in the larger size classes (χ^2, p < 0.001) (Figure 4.10). In general, mortality decreased with increasing tree size for all species. Although the proportion of hemlock trees killed by *A. ostoyae* was lower than Douglas-fir, the rate of decrease between the two species was similar across the first three size classes. No mortality occurred in larger hemlock trees whereas mortality in Douglas-fir trees greater than 100 mm DBH was constant. An increasing trend in the proportion of infected trees showing compartmentalization and callusing with increasing tree size was evident for both western hemlock and western redcedar and compartmentalization was consistently higher in cedar than in hemlock. Although both species showed a marked increase in the frequency of callused lesions with tree size, this increase occurred much sooner on cedar than on hemlock (Figure 4.11). Douglas-fir showed little or no difference in the frequency of callused lesions among the different size classes.

Progressive lesions at the root collar also differed among species (Figure 4.12). More progressive lesions were observed on smaller hemlock trees than on larger hemlock trees. In general, the proportion of infected trees with progressive lesions at the root collar declined as tree size increased. These results indicate that as tree size increases, the ability of hemlock to halt advance of the fungus also increases. These results are consistent with the increased proportion of callused lesions with increasing trees size in Figure 4.11. In contrast, progressive lesions at the root collar on Douglas-fir generally increased with size. In this dataset, only 1 of 56 (2%) of western redcedar trees showed a progressive lesion at the root collar.
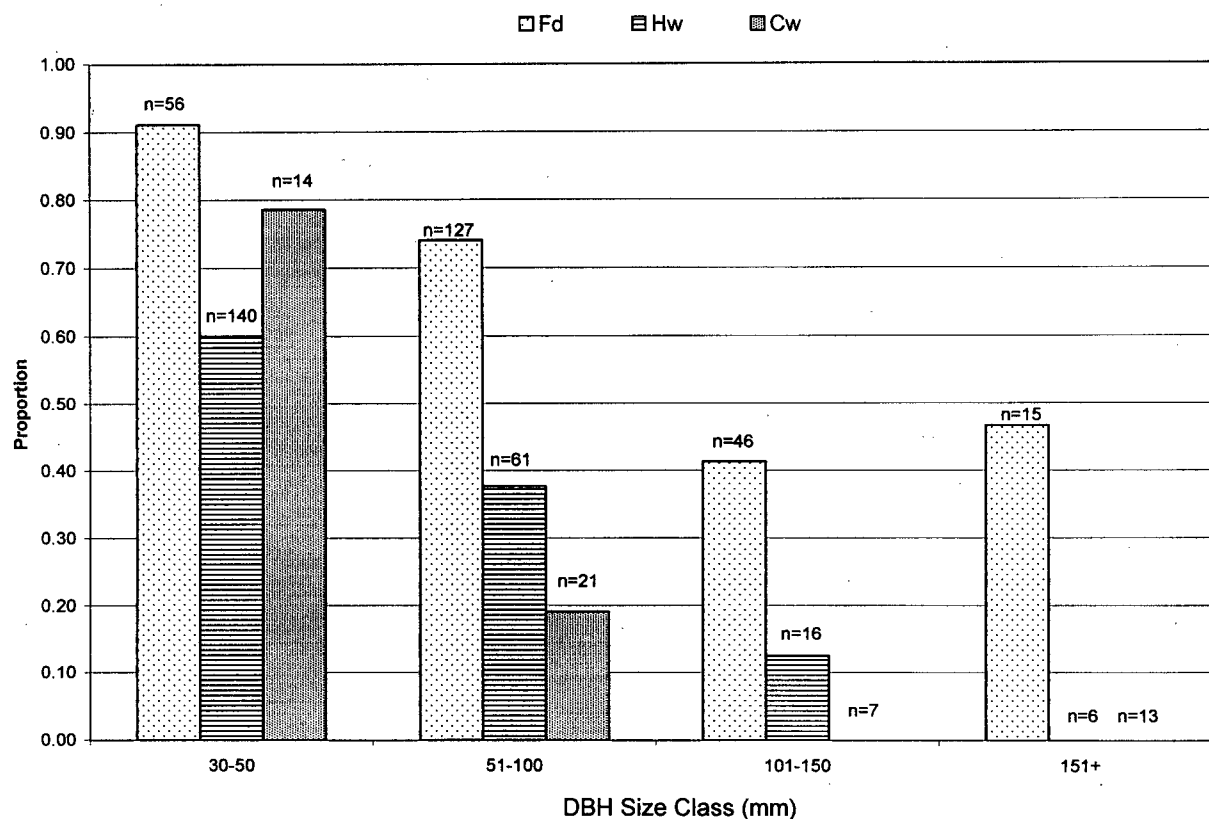
Fig. 4.10. Mortality in Douglas-fir (Fd), western hemlock (Hw) and western redcedar (Cw) as a proportion of the total number of trees with above-ground signs or symptoms of *A. ostoyae* by size class
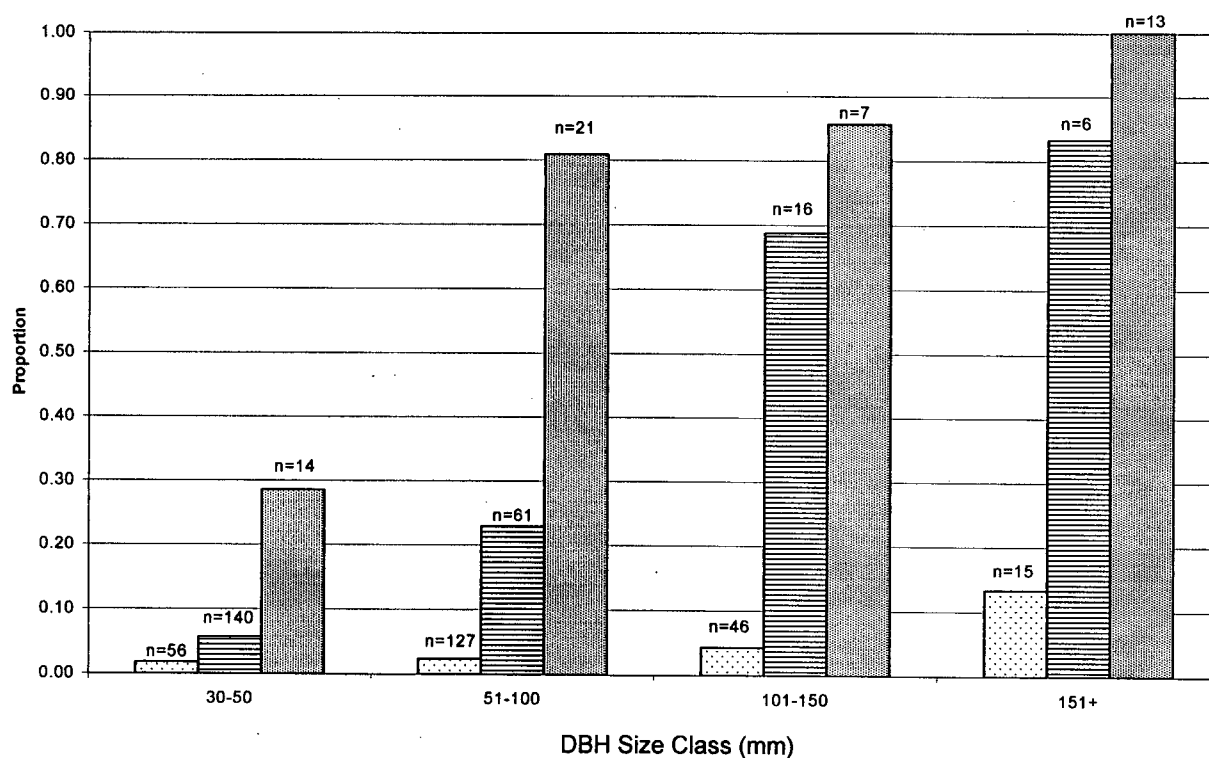
Fig. 4.11. Callused lesions at the root collar in Douglas-fir (Fd), western hemlock (Hw) and western redcedar (Cw) as a proportion of the total number of trees with above-ground signs or symptoms of *A. ostoyae* by size class
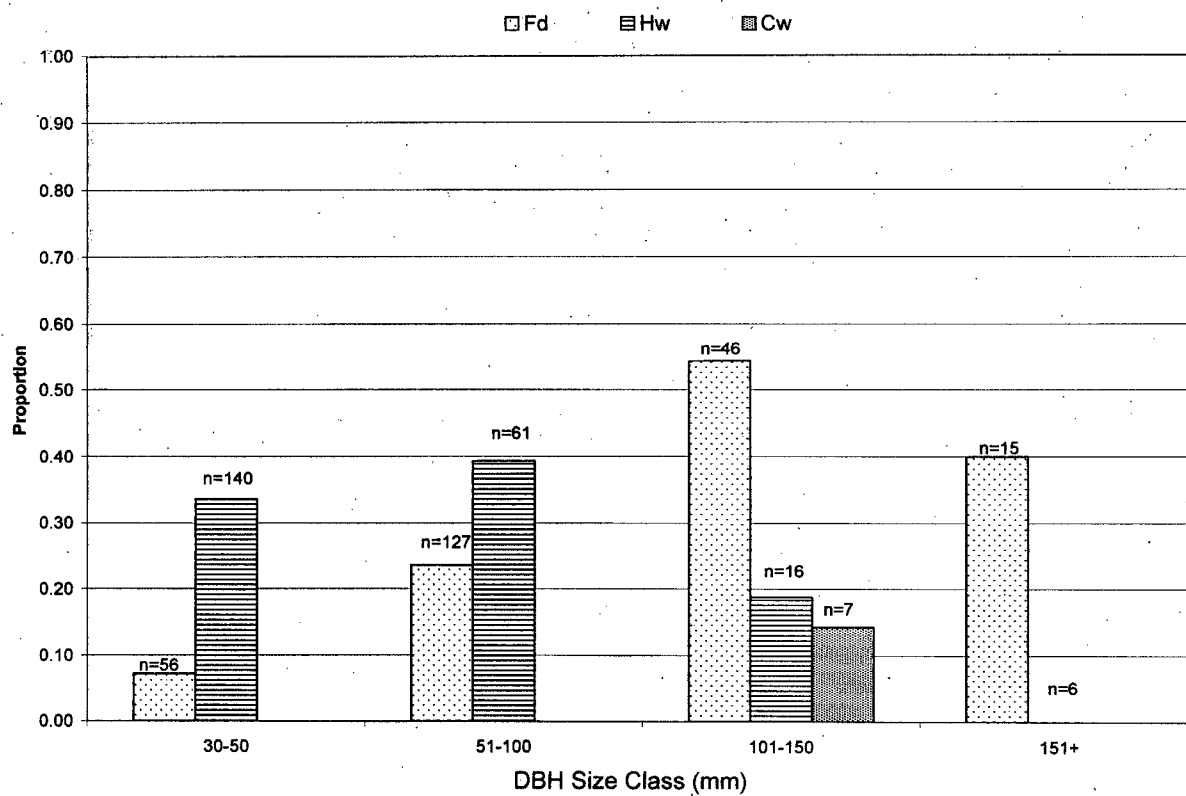
Fig. 4.12. Progressive lesions at the root collar in Douglas-fir (Fd), western hemlock (Hw) and western redcedar (Cw) as a proportion of the total number of trees with above-ground signs or symptoms of *A. ostoyae* by size class

4.4 DISCUSSION

The large variation in aboveground disease symptoms among plots and sites with similar species composition and age class reflects the natural variation in disease incidence observed on a larger landscape level throughout much of the southern interior forest region. This variation may be due to differences in the stage of development of disease within the stand, the availability and spatial distribution of inoculum and susceptible host species, management history and site factors (e.g. slope or soil moisture) that may directly affect the inoculum. Belowground disease incidence was not determined in this survey, although previous studies suggest that the actual incidence in similar-type juvenile stands can be 4-5 times higher than what can be detected aboveground (Morrison *et al.* 2000). However, this survey was not designed to yield information about overall amount of *A. ostoyae* infection, nor was it designed to explain the variation in incidence of infection by plots or site. In the search for candidate sites, moderately to heavily infested areas with the appropriate mixture of Douglas-fir and western redcedar were sampled in order to make direct comparisons of symptom development and mortality rates between the two species. The disease incidence levels reported in this study do not represent the average levels of aboveground infection since sites with low levels of infection were eliminated.

The cumulative mortality in mixed conifer stands in this study was variable although mortality rates parallel those reported by Morrison and Pellow (1994) for juvenile Douglas-fir plantations. The two sites in this study that showed the highest cumulative mortality (mainly in Douglas-fir) also showed the smallest difference between mortality and aboveground incidence. These results are interpreted to indicate that high inoculum levels maintain active disease centres and nearly all Douglas-fir trees showing evidence of aboveground symptoms of disease are killed soon after these appear. On sites having larger differences between cumulative mortality and aboveground disease incidence, trees are likely to live and tolerate the fungus for a longer period of time before being killed. However, the negative effects of non-lethal infections on growth of trees during this period of time can be substantial.

Given similar incidence of infection, the frequency of mortality for a particular host species is a direct measure of its susceptibility to killing by *A. ostoyae* and the ability of a particular species to survive in the presence of inoculum. This survey revealed new information regarding the relative susceptibility of western redcedar and western hemlock to killing by *A. ostoyae*. Comparisons between Douglas-fir and western redcedar in twenty sites throughout the southern interior showed that mortality in Douglas-fir was 12-14 times that in western redcedar. Comparisons among species in 10 sites showed significantly less mortality in cedar than hemlock and Douglas-fir although mortality in hemlock was midway between Douglas-fir and cedar. These results are interpreted to indicate that the population of trees in the ICH that was sampled, western redcedar is killed much less frequently than Douglas-fir and western hemlock. Similar results are reported by Merler (unpublished), van der Kamp (unpublished), and Woods (1994).

Much of the incidence of *A. ostoyae* on cedar was recorded as callused lesions at the root collar rather than direct mortality. Koenigs (1969) reported a high frequency of *A. mellea* Vahl. (Quél.) (= *A. ostoyae*, D. Morrison, pers. comm.) infections on 80+ year old western redcedar trees following thinning treatment of the stand. Of the infected cedar, only 22% of the circumference of the stem was infected with *A. mellea* and basal cankers (i.e. compartmentalized lesions at the root collar). Furthermore, no mortality occurred in the western red cedar trees 20 years following thinning, even though extensive root systems were likely established (Koenigs 1969).

In the majority of conifer plantations in the ICH, western redcedar regenerates naturally, so that trees are smaller than the planted species. In this study, tree size distribution for western redcedar was similar to that of 19-year-old planted cedar in the Skimikin trial (D. Morrison, unpublished) whereby a higher proportion of stems occurred in the small diameter size classes and the distribution appeared skewed. However, differences in tree size between Douglas-fir and cedar may be due more to differences in growth rates than differences in age. Cedar would have probably been established shortly after the sites had been harvested or burned and it survives being overtopped because it is shade

tolerant and during this stage cedar has slower growth rates relative to the other planted conifers. However, during this period of time trees develop a profuse network of fine roots (Minore 1983).

The above results might suggest that mortality in the planted Douglas-fir has been happening for at least a few years longer than in cedar. Nonetheless, reduced mortality rates exhibited in even the intermediate size classes and the higher frequency of callusing observed in the smallest diameter size class indicates that western redcedar is more resistant to infection by *A. ostoyae* and this resistance occurs much earlier than most other conifers, even when tree size is relatively small.

Morrison (2000) reported that the probability of infection increases with increasing tree size. However, rooting characteristics of different conifer species also play an important role in contacting potential sources of *A. ostoyae* inoculum in the soil. Reynolds and Bloomberg (1982) suggested that the number of roots is probably the most important overall attribute determining the probability of root contact between individual trees. In the ICH, Douglas-fir generally develops large, lateral root systems (D. Morrison, pers. comm..) and intra-tree root grafts are common. Cedar on the other hand grows slowly for the first 10-12 years in the understory as it is quickly overtopped by faster growing species like Douglas-fir, lodgepole pine, western larch, and birch. Thus, cedar may at times escape contact with *Armillaria* inoculum. However, cedar also produces a large number of smaller diameter roots which are usually shallower than Douglas-fir. Root size and rooting depth of western hemlock is intermediate between that of Douglas-fir and western redcedar. These rooting characteristics mean that cedar and hemlock also have a high probability of contacting potential inoculum sources in the soil. Because western redcedar develops a profuse network of roots and root characteristics of western hemlock are similar to both cedar and Douglas-fir, it is believed that the probability of root contact between cedar or hemlock and other conifers should be similar to that of Douglas-fir and other conifers.

The largest proportion of mortality caused by *A. ostoyae* among all sites occurred in the smallest diameter class for both Douglas-fir and western redcedar. These results are in agreement with those reported by Woods (1994) who showed Douglas-fir to be significantly more heavily infected than western redcedar and western hemlock across similar diameter size classes. The incidence of mortality declined with increasing tree diameter for all species. However, the rate of decrease was markedly greater for cedar than Douglas-fir or western hemlock. The decline in cedar mortality is likely attributable to host reactions induced in response to invasion by the fungus that become increasingly effective at halting the spread of the fungus on a root as tree size increases.

The smaller percentage of progressive lesions at the root collar on western redcedar relative to Douglas-fir and western hemlock suggests that fewer cedar trees are at risk of being killed by *A. ostoyae*. It is probable that the majority of infections occurring on cedar roots did not advance to the root collar because they were confined to a lesion further out on the root system.

Western redcedar trees that showed progressive infections at the root collar lacked any crown symptoms or basal resinosus indicative of root disease presence. Resin exudation on cedar in not nearly as copious as that normally observed on other conifers like Douglas-fir, western hemlock, or lodgepole pine. Small droplets of resin may be observed on the surface of the bark at the base of a western redcedar tree. This symptom appears to be more frequent in older, mature cedar trees. However, previous literature documenting resin exudation as a symptom of Armillaria root disease may have been misinterpreted as normal resin exudation that occurs in tissue at the base of the stem or in the root collar area, whereas in cedar resin does not arise from resin ducts formed in the wood (as in the case of Douglas-fir), but from vertical resin ducts in the phloem. The formation of resin ducts in the phloem of cedar is a non-specific response to injury of the bark tissue and occurs naturally in older stem tissue at the base of trees (as described in Chapter 2).

When Armillaria invades the bark tissue on roots of cedar, trees frequently form a necrophylactic periderm to contain the infection. Cedar appears to form these "resistant" reactions more frequently than Douglas-fir and western hemlock. Results from inoculation trials (Section 2.3.5) and examinations of naturally infected roots (Section 2.3.8) suggests that when cedar becomes infected, it forms a NP barrier in the bark and may induce a response involving rhytidome formation proximally and distally to the primary lesion. Successive periderm formation at either end of an *Armillaria*-caused lesion extends for some distance along the root so that over time one continuous rhytidome layer is formed which may eventually be sloughed from the surface of the root.

Basal lesions at the root collar on western redcedar usually resulted from a progressive infection advancing on a single lateral root. Once the fungus approaches the root collar the host forms a barrier zone to compartmentalize the infection. The appearance of this barrier zone was described previously in Section 2.3.5 in this thesis. The pigmented contents of the axial parenchyma cells comprising the barrier zone appear as a pinkish hue in the sapwood. In addition, the polyphenolic-rich parenchyma cells comprising the barrier zone are limited to a relatively small zone of tissue immediately adjacent to the area of killed cambium. When cedar compartmentalizes an *Armillaria* infection at the root collar, it typically appears as a flattened area at the base of the stem and the tree exhibits fluting of its trunk as a result of lateral ingrowth of callus from both sides. Moreover, once the host forms this barrier zone, the fungus rarely breaches it. This is in marked contrast to Douglas-fir where continual formation and breaching of such barriers may occur within the same growing season or in successive years. The barrier zone in Douglas-fir is comprised of a series of traumatic resin canals that extends tangentially for some distance away from the area of killed cambium (Section 2.3.5). In many cases, single or double bands of traumatic resin ducts are continuously formed in the annual growth rings in subsequent years indicating a continuous stimulation of this non-specific host defense response by the fungus.

Johnson *et al.* (1972) suggested that the ability of conifers to compartmentalize infections as lesions on roots or at the root collar increases between the ages of 5-20 years. Results

from this study suggest that cedar has a superior ability to compartmentalize infections, and that it does so much earlier than most other conifers.

Among the twenty sites that were surveyed, Douglas-fir had a higher proportion of infected trees than western redcedar. However, it is possible that a large number of the cedar trees surveyed were in fact infected belowground, but resistance mechanisms triggered soon after initial invasion by the fungus could have resulted in infections being confined to tissue immediately surrounding an initial point of penetration (Section 2.3.5) limiting the proportion cedar showing aboveground symptoms of disease.

Crown symptoms in Douglas-fir trees were usually prominent only after a significant portion of the root system was infected or decayed and this effect appeared to be more pronounced in trees that sustained repeated infection over a number of years. However, some Douglas-fir trees did not exhibit any crown symptoms despite abundant resinosus and girdling around the circumference of the stem. Many of these trees showed no height reduction but some appeared to have less radial growth than surrounding trees in the plot. Chronic (i.e. non-lethal) infections may not result in easily detected above ground symptoms however significant increment losses can occur which will differ by species.

Western hemlock has high susceptibility to killing by *A. ostoyae*, particularly when young or when trees are relative small but susceptibility tends to decrease as trees get older and larger as evidenced by the lower frequency of progressive lesions and the higher frequency of compartmentalized lesions at the root collar. These observations are consistent with those reported for naturally infected hemlock in Section 2.3.8 in this thesis. There is some evidence from this study that western hemlock may be less susceptible to killing by Armillaria root disease than Douglas-fir, but still considerably more susceptible than western redcedar.

Management of second-growth stands in the southern interior region of B.C. without any regard for Armillaria root disease can result in serious losses in timber production. Individual-tree volume growth losses in diseased juvenile Douglas-fir in as much as 61%

compared to healthy trees and overall growth losses ranging between 7-13% for juvenile stands (Cruickshank 2006) are alarming. Further work is needed to account for such yield reductions in growth and yield models for mature timber types throughout the ICH in the southern interior of B.C.

Data collected in this study documenting symptom development and mortality rates for cedar is in agreement with that reported from field inoculation trials (Section 2.3.5). In this study, mortality occurred in small patches throughout most of the individual plots and sites, and in areas with high mortality incidence understocking of trees was evident. Given the number of infected Douglas-fir and western hemlock in the smaller diameter size classes, mortality will likely continue for several years. As these stands age, root systems will continue to overlap thereby increasing the risk of disease transfer to individual healthy trees. Whether the incidence of mortality declines or remains constant in subsequent years will primarily depend on host resistance to the fungus and the general increase in resistance with age, the proportion of trees with progressive infections, and any increases in inoculum potential that would enable the fungus to remain active throughout the rotation of the stand. Results from this study showed that the proportions of infected Douglas-fir trees that have progressive lesions at the root collar and cumulative mortality in the larger diameter size classes are sufficiently high to pose considerable risk to residual trees by serving as a continuous supply of inoculum. This risk may be reduced by regenerating stands with species that show a higher frequency of callusing at Armillaria-caused lesions such as western redcedar. Planting cedar in higher proportions (e.g. 30-50%) along side other susceptible species like Douglas-fir will form a fully stocked stand and likely reduce spread and transfer of the pathogen (i.e. reduced root contact or wider spacing) between susceptible hosts like Douglas-fir.

In the ICH, Douglas-fir is replaced by cedar and hemlock, the climax species of this zone. Armillaria root disease in the ICH probably has some influence on accelerating species succession by selectively removing more susceptible conifers like Douglas-fir and creating gaps in the stand that may be filled in by cedar, hemlock or deciduous species

such as birch or aspen. Morrison and Pellow (1994) reported that expanding root disease centres in juvenile Douglas-fir stands in the ICH had filled in with hemlock and redcedar. In this study, western redcedar shows effective resistance against *A. ostoyae*. This resistance appears to increase over time to the point where the host reaches a relatively stable equilibrium with the fungus. The time at which this equilibrium is attained probably occurs much sooner compared to other conifers within the same stand. The lower mortality rates in cedar in response to infection by *A. ostoyae* and the lack of other damaging biotic agents occurring on western redcedar relative to seral conifers in the ICH allows cedar to maintain its position through to maturity.

The practical implications of using western redcedar for mitigating damage caused by *A. ostoyae* in the ICH are significant. In stands with moderate to high levels of infection, cedar can be used to restrict vegetative spread of *A. ostoyae* by reducing the number of root contacts between susceptible conifer species. It is more likely that infection by *A. ostoyae* on cedar will induce formation of resistance mechanisms to confine the fungus to a lesion in the bark or wood. Resistance in one particular species has direct implications on adjacent crop trees including lowering overall mortality rates in newly regenerating trees, and reducing the amount of secondary inoculum which would otherwise be available to the fungus to infect new hosts.

CHAPTER FIVE:
SUMMARY AND CONCLUSIONS

In this study, host response to infection by *A. ostoyae* and *A. sinapina* was examined at the tissue and cellular level on the roots of Douglas-fir, western hemlock and western redcedar trees, particularly with respect to the types of barriers that are formed in the bark and in the wood in response to infection by either species. Several important conclusions may be made based on the results of this research with regards to the interaction of either *A. ostoyae* or *A. sinapina* on juvenile (15-30 year old) trees.

First, western redcedar shows a higher degree of resistance to *A. ostoyae* than Douglas-fir and western hemlock. The results from examinations of trees naturally infected and inoculated with *A. ostoyae* (Chapter 2) showed cedar to be more effective at containing infections within a NP barrier than Douglas-fir and western hemlock. Breaching of barriers by the fungus was more common in the other conifers in this study. Following cambial invasion, barrier zone formation in cedar provided a permanent barrier to spread by the fungus, unlike the barrier zones initiated in Douglas-fir and hemlock.

Second, unique resistance mechanisms involving programmed cell death in the bark associated with the induced rhytidome response and the formation of traumatic resin ducts induced in the phloem of western redcedar may impart increased resistance to the spread of *A. ostoyae* in host tissue. This is the first study to report resistance mechanisms of this kind operating in western redcedar. The induced rhytidome response, in particular, may facilitate sloughing of infected tissue from the surface of the root which could eliminate any evidence of previous infections within a relatively short period of time (e.g. 1 year). Further study on the precise nature of resistance offered by traumatic phloem resin ducts against *A. ostoyae* is warranted.

Third, differences in anatomy of the bark and wood, the chemical nature of cell wall constituents comprising the thin-walled phellem (lignified and suberized), the lack of a

distinguishable zone of NIT, and differences in the types of host responses to infection (e.g. induced rhytidome, TPRDs) between cedar and the other conifers suggests that cedar does not follow the conventional and generally accepted model of non-specific host response to wound healing in trees: an important caveat for Mullick's (1977) model.

This is also the first study to examine the infection biology of *A. sinapina* and host response to infection by *A. sinapina*, generally known as a weak parasite or saprophyte, on 20-30 year-old trees and compare host reactions with those induced in response to invasion by a more aggressive pathogen, *A. ostoyae*. The frequency of infection caused by *A. sinapina* was not significantly different from that of *A. ostoyae*. However, damage was considerably less on all species infected by *A. sinapina* than those species infected by *A. ostoyae*, particularly western hemlock and Douglas-fir. The fourth conclusion based on the field inoculation studies is that inoculum potential and host-pathogen interactions were key determinants of pathogenicity of *A. sinapina*. Under natural conditions, the pathogenicity of *A. sinapina* is much lower than *A. ostoyae* and *A. sinapina* accounts for very little damage in trees unless they have been stressed by other biotic or abiotic factors.

Fifth, inoculum potential has an influence on the type of host response induced in trees following invasion by the fungus. This was particularly evident in cedar roots where the intensity of the host response (i.e. induced rhytidome and TPRD formation) was more pronounced in roots infected with *A. ostoyae* than abiotically wounded roots, or roots infected with *A. sinapina*. Similarly, larger lesions caused by *A. sinapina* were always associated with higher inoculum potential. Lesion size was positively correlated with numerous rhizomorph penetrations and the distance from the inoculum source. The significance of inoculum potential as a major determining factor of disease development and the variation in host response of a given species under varying amounts of inoculum potential deserves more research.

Sixth, environmental factors such as moisture and temperature have an influence on the ability of the fungus to become established in the roots of trees. Low soil moisture

associated with drought in the summer of 2003 inhibited rhizomorph development and growth from the inoculum blocks in the soil which resulted in low infection rates on hosts in the months following inoculation. Thus, drought may have an effect not only on the vigour of trees, but also on the vigour of the fungus not already established in the host. These results may help explain the variation in root disease mortality following periodic episodes of drought. The inability of *A. ostoyae* to cause infection during the winter months was probably due to low soil temperatures that limited rhizomorph initiation and growth. Thus, killing of the vascular cambium by *A. ostoyae* on trees during a period of tree dormancy when the host is unable to initiate host defenses is more likely caused by the fungus which is already established in the host prior to tree dormancy as opposed to direct penetration of the fungus.

The seventh conclusion based on the results of surveying symptom development and mortality incidence caused by Armillaria root disease in juvenile stands relates to the superior ability of western redcedar to survive in the presence of inoculum relative to other common conifers. Aboveground disease incidence surveys revealed significantly lower mortality rates and a higher frequency of compartmentalization and callusing in cedar trees compared to Douglas-fir and western hemlock trees. The general conception that all conifers are highly susceptible to killing by *A. ostoyae* when less than about 15-years old still holds true, but the results of this research suggests that resistance against *A. ostoyae* in cedar probably occurs much sooner than this critical age, even when the trees are relatively small. Due to the similarity in host response to infection by *A. ostoyae* in western redcedar across a wide geographic range, results of this work are applicable throughout the range of western redcedar in the ICH zone in the southern interior region.

Armillaria root disease poses a serious threat to sustainable forest management throughout much of the southern interior region of B.C. In diseased plantations, losses in timber production as a direct result of mortality and growth repression in susceptible conifers like Douglas-fir, will persist throughout a rotation so long as a continuous supply of inoculum is maintained and transmission of inoculum takes place between infected and healthy crop trees. Sustainable forest management of such diseased stands requires

recognition of the nature of root disease and prescription of appropriate practices to minimize long-term impacts in plantations.

Options to reduce the impact of Armillaria root disease in plantations include stump removal at harvest and planting species with low susceptibility to killing by the fungus. Resistant species may serve as a barrier to disease spread between susceptible species, minimize the risk of individual tree mortality and potentially reduce secondary inoculum in the stand that would otherwise increase the risk of infection to residual or regenerating trees. Results from this study have supported the development of extension material for Armillaria root disease and forest management for the southern interior region of B.C., including a revised table of susceptibility ratings for conifers and recommendations for the use of western redcedar when regenerating sites infested with root disease (Appendix XX).

Major amendments to the host susceptibility table from that described in Morrison *et al.* (1992) include the reclassification of western redcedar and western hemlock from moderate susceptibility to low and high, respectively. Despite observations of increased frequency of compartmentalization and callusing on hemlock trees with increasing tree size, the low proportion of infected hemlock roots that showed successful resistance reactions following inoculation by *A. ostoyae* and the high rates of mortality in juvenile mixed conifer stands suggests that susceptibility of hemlock to killing by the fungus should be classified as high. In contrast, this study has demonstrated that the relative susceptibility of western redcedar to killing by *A. ostoyae* is quite opposite from that observed in other common conifers.

Rapid regeneration following harvesting of mature stands in the ICH ensures that young conifers are exposed to inoculum when it is at or near its peak potential, resulting in considerable mortality. Results from this study suggest that early survival of susceptible host species like Douglas-fir could be enhanced if planted in uniform mixtures containing higher proportions (30-50%) of resistant hosts such as western redcedar. Management recommendations that include resistant host species such as western redcedar in mixed

conifer plantations have major implications for maintaining free growing stands, minimizing losses due to root disease in immature stands, alleviating the long-term impacts of Armillaria root disease on growth loss and restoring site productivity. Further investigation into disease epidemiology in mixed conifer stands that include higher proportions of western redcedar is warranted.

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APPENDIX I

Map highlighting the Okanagan-Shuswap and Arrow-Boundary Forest Districts in the southern interior of British Columbia where inoculation trials were conducted.

Source: http://www.for.gov.bc.ca/mof/maps/regdis/regdis.htm

APPENDIX II

CHI-SQUARE TESTS FOR THE FREQUENCY OF INFECTION AMONG AND BETWEEN SPECIES AT DIFFERENT HARVEST TIMES IN EACH FIELD TRIAL

F(infection) **among** species at *11 weeks* in HL-2002 trial

	Failure	Success	Total
Fd	2	9	11
Hw	5	14	19
Cw	13	6	19
Total	20	29	49

Degrees of freedom: 2;   Chi-square = 9.980;   p ≤ 0.01;   The difference is significant.

F(infection) **between** species at *11 weeks* in HL-2002 trial

	Failure	Success	Total
Fd	2	9	11
Hw	5	14	19
Total	7	23	30

Degrees of freedom: 1;   Chi-square = 0.257;   For significance at the .05 level, chi-square should be ≥ to 3.84;   p ≤ 1; The difference is not significant.

F(infection) **between** species at *11 weeks* in HL-2002 trial

	Failure	Success	Total
Fd	2	9	11
Cw	13	6	19
Total	15	15	30

Degrees of freedom: 1;   Chi-square = 7.033;   p ≤ 0.01;   The difference is significant.

F(infection) **between** species at *11 weeks* in HL-2002 trial

	Failure	Success	Total
Hw	5	14	19
Cw	13	6	19
Total	18	20	38

Degrees of freedom: 1;   Chi-square = 6.755;   p ≤ 0.01;   The difference is significant.

F(infection) **among** species at *1 year* in HL-2002 trial

	Failure	Success	Total
Fd	0	10	10
Hw	1	5	6
Cw	6	4	10
Total	7	19	26

Degrees of freedom: 2;   Chi-square = 9.565;   p ≤ 0.01;   The difference is significant.

F(infection) **between** species at *1 year* in HL-2002 trial

	Failure	Success	Total
Hw	1	5	6
Fd	0	10	10
Total	1	15	16

Degrees of freedom: 1   Chi-square = 1.778;   For significance at the .05 level, chi-square should be ≥ to 3.84;   The difference is not significant;   p ≤ 0.20.

F(infection) **between** species at *1 year* in HL-2002 trial

	Failure	Success	Total
Cw	6	4	10
Fd	0	10	10
Total	6	14	20

Degrees of freedom: 1;   Chi-square = 8.571;   p ≤ 0.01;   The difference is significant.

F(infection) **between** species at *1 year* in HL-2002 trial

	Failure	Success	Total
Hw	1	5	6
Cw	6	4	10
Total	7	9	16

Degrees of freedom: 1   Chi-square = 2.861;   For significance at the .05 level, chi-square should be ≥ 3.84;
The difference is not significant.   p ≤ 0.10.

F(infection) **among** species at *5 weeks* in KF-2003 trial

	Failure	Success	Total
Fd	2	0	2
Hw	6	1	7
Cw	10	0	10
Total	18	1	19

Degrees of freedom: 2;   Chi-square = 1.809;   For significance at the .05 level, chi-square should be ≥ 5.99;   The difference is
not significant.   p ≤ 1.

F(infection) **among** species at *9 weeks* in KF-2003 trial

	Failure	Success	Total
Fd	4	0	4
Hw	5	3	8
Cw	3	2	5
Total	12	5	17

Degrees of freedom: 2;   Chi-square = 2.189;   For significance at the .05 level, chi-square should be ≥ 5.99;   The difference is
not significant;   p ≤ 1.

F(infection) **among** species at *1 year* in KF-2003 trial

	Failure	Success	Total
Fd	20	10	30
Hw	7	23	30
Cw	16	8	24
Total	43	41	84

Degrees of freedom: 2;   Chi-square = 14.494;   p ≤ 0.001.   The difference is significant.

F(infection) **between** species at *1 year* in KF-2003 trial

	Failure	Success	Total
Cw	16	8	24
Fd	20	10	30
Total	36	18	54

Degrees of freedom: 1   Chi-square = 0;   For significance at the .05 level, chi-square should be ≥ 3.84;   The difference is not
significant.   p ≤ 1.

F(infection) **between** species at *1 year* in KF-2003 trial

	Failure	Success	Total
Cw	16	8	24
Hw	7	23	30
Total	23	31	54

Degrees of freedom: 1;   Chi-square = 10.239   p ≤ 0.01.   The difference is significant.

F(infection) **between** species at *1 year* in KF-2003 trial

	Failure	Success	Total
Fd	20	10	30
Hw	7	23	30
Total	27	33	60

Degrees of freedom: 1;   Chi-square = 11.380;   p ≤ 0.001.   The difference is significant.

F(infection) **among** species at *9 weeks* in KF-2004 trial

	Failure	Success	Total
Fd	5	0	5
Hw	6	1	7
Cw	3	1	4
Total	14	2	16

Degrees of freedom: 2;   Chi-square = 1.306;   For significance at the .05 level, chi-square should be ≥ 5.99;
The difference is not significant.   p ≤ 1.

F(infection) **among** species at *5 months* in KF-2004 trial

	Failure	Success	Total
Fd	5	9	14
Hw	3	11	14
Cw	4	16	20
Total	12	36	48

Degrees of freedom: 2;   Chi-square = 1.219;   For significance at the .05 level, chi-square should be ≥5.99.
The difference is not significant.   p ≤ 1.

F(infection) **among** species at *1 year* in KF-2004 trial

	Failure	Success	Total
Fd	1	12	13
Hw	5	12	17
Cw	2	10	12
Total	8	34	42

Degrees of freedom: 2   Chi-square = 2.315;   For significance at the .05 level, chi-square should be ≥ 5.99.
The difference is not significant.   p ≤ 1.

F(infection) **among** species at *8 weeks* in Nakusp-2004 trial

	Failure	Success	Total
Fd	2	0	2
Hw	1	2	3
Cw	1	1	2
Total	4	3	7

Degrees of freedom: 2   Chi-square = 2.236;   For significance at the .05 level, chi-square should be ≥ 5.99.
The difference is not significant.   p ≤ 1.

F(infection) **among** species at *5 months* in Nakusp-2004 trial

	Failure	Success	Total
Fd	0	2	2
Hw	1	6	7
Cw	0	7	7
Total	1	15	16

Degrees of freedom: 2   Chi-square = 1.371;   For significance at the .05 level, chi-square should be ≥ 5.99.
The difference is not significant. $p \leq 1$.

F(infection) **among** species at *1 year* in Nakusp-2004 trial

	Failure	Success	Total
Fd	0	2	2
Hw	1	2	3
Cw	0	2	2
Total	1	6	7

Degrees of freedom: 2   Chi-square = 1.556;   For significance at the .05 level, chi-square should be ≥ 5.99.
The difference is not significant. $p \leq 1$.

CHI-SQUARE TESTS FOR THE FREQUENCY OF INFECTION AMONG AND BETWEEN HARVEST DATES FOR EACH SPECIES

F(infection) **between** diff. harvest times in *Fd* for HL-2002 trial

	Failure	Success	Total
11 weeks	2	9	11
1 year	0	10	10
Total	2	19	21

Degrees of freedom: 1   Chi-square = 2.009;   For significance at the .05 level, chi-square should be ≥ 3.84.
The difference is not significant. p is ≤ 0.20.

F(infection) **among** diff. harvest times in *Fd* for KF-2003 trial

	Failure	Success	Total
5 weeks	2	0	2
9 weeks	4	0	4
1 year	20	10	30
Total	26	10	36

Degrees of freedom: 2   Chi-square = 2.769;   For significance at the .05 level, chi-square should be ≥ 5.99.
The difference is not significant. $p \leq 1$.

F(infection) **among** diff. harvest times in *Fd* for KF-2004 trial

	Failure	Success	Total
9 weeks	5	0	5
5 months	5	9	14
1 year	1	12	13
Total	11	21	32

Degrees of freedom: 2   Chi-square = 13.659; $p \leq 0.01$.   The difference is significant.

F(infection) **between** diff. harvest times in *Fd* for KF-2004 trial

	Failure	Success	Total
9 weeks	5	0	5
5 months	5	9	14
Total	10	9	19

Degrees of freedom: 1   Chi-square = 6.107; $p \leq 0.025$.   The difference is significant.

F(infection) **between** diff. harvest times in *Fd* for KF-2004 trial

	Failure	Success	Total
9 weeks	5	0	5
1 year	1	12	13
Total	6	12	18

Degrees of freedom: 1   Chi-square = 13.846; $p \leq 0.001$.   The difference is significant.

F(infection) **between** diff. harvest times in *Fd* for KF-2004 trial

	Failure	Success	Total
5 month	5	9	14
1 year	1	12	13
Total	6	21	27

Degrees of freedom: 1   Chi-square = 3.062;   For significance at the .05 level, chi-square should be ≥ 3.84.
The difference is not significant. $p \leq 0.10$.

F(infection) **among** diff. harvest times in *Fd* for Nakusp-2004 trial

	Failure	Success	Total
8 weeks	2	0	2
5 months	0	2	2
1 year	0	2	2
Total	2	4	6

Degrees of freedom: 2   Chi-square = 6; $p \leq 0.05$.   The difference is significant

F(infection) **between** diff. harvest times in *Hw* for HL-2002 trial

	Failure	Success	Total
11 weeks	5	14	19
1 year	1	5	6
Total	6	19	25

Degrees of freedom: 1   Chi-square = 0.233;   For significance at the .05 level, chi-square should be ≥ 3.84.
The difference is not significant. $p \leq 1$.

F(infection) **among** diff. harvest times in *Hw* for KF-2003 trial

	Failure	Success	Total
5 weeks	6	1	7
9 weeks	5	3	8
1 year	7	23	30
Total	18	27	45

Degrees of freedom: 2;   Chi-square = 11.255; $p \leq 0.01$.   The difference is significant

F(infection) **between** diff. harvest times in *Hw* for HL-2003 trial

	Failure	Success	Total
5 weeks	6	1	7
9 weeks	5	3	8
Total	11	4	15

Degrees of freedom: 1;   Chi-square = 1.029;   For significance at the .05 level, chi-square should be ≥ 3.84.
The difference is not significant. $p \leq 1$.

F(infection) **between** diff. harvest times in *Hw* for KF-2003 trial

	Failure	Success	Total
5 weeks	6	1	7
1 year	7	23	30
Total	13	24	37

Degrees of freedom: 1   Chi-square = 9.691; $p \leq 0.01$.   The difference is significant.

F(infection) **between** diff. harvest times in *Hw* for KF-2003 trial

	Failure	Success	Total
9 weeks	5	3	8
1 year	7	23	30
Total	12	26	38

Degrees of freedom: 1   Chi-square = 4.484; $p \leq 0.05$.   The difference is significant.

F(infection) in Hw **among** diff. harvest times for KF-2004 trial

	Failure	Success	Total
9 weeks	6	1	7
5 months	3	11	14
1 year	5	12	17
Total	14	24	38

Degrees of freedom: 2   Chi-square = 9.018; $p \leq 0.025$.   The difference is significant

F(infection) in Hw **between** diff. harvest times for KF-2004 trial

	Failure	Success	Total
9 weeks	6	1	7
5 months	3	11	14
Total	9	12	21

Degrees of freedom: 1   Chi-square = 7.875; $p \leq 0.01$.   The difference is significant.

F(infection) in Hw **between** diff. harvest times for KF-2004 trial

	Failure	Success	Total
9 weeks	6	1	7
1 year	5	12	17
Total	11	13	24

Degrees of freedom: 1   Chi-square = 6.331; $p \leq 0.025$.   The difference is significant.

F(infection) in Hw **between** diff. harvest times for KF-2004 trial

	Failure	Success	Total
5 month	3	11	14
1 year	5	12	17
Total	8	23	31

Degrees of freedom: 1   Chi-square = 0.255   For significance at the .05 level, chi-square should be ≥ 3.84.
The difference is not significant. $p \leq 1$.

F(infection) in Hw **among** diff. harvest times for Nakusp-2004 trial

	Failure	Success	Total
8 weeks	1	2	3
5 months	1	6	7
1 year	1	2	3
Total	3	10	13

Degrees of freedom: 2   Chi-square = 0.660   For significance at the .05 level, chi-square should be ≥ 5.99.
The difference is not significant.   p ≤ 1.

F(infection) in Cw **between** diff. harvest times for HL-2002 trial

	Failure	Success	Total
11 weeks	13	6	19
1 year	6	4	10
Total	19	10	29

Degrees of freedom: 1   Chi-square = 0.2056   For significance at the .05 level, chi-square should be ≥ 3.84.
The difference is not significant.   p ≤ 1.

F(infection) in Cw **among** diff. harvest times for KF-2003 trial

	Failure	Success	Total
5 weeks	10	0	10
9 weeks	3	2	5
1 year	16	8	24
Total	29	10	39

Degrees of freedom: 2   Chi-square = 4.734   For significance at the .05 level, chi-square should be ≥ 5.99.
The difference is not significant.   p ≤ 0.10.

F(infection) in Cw **among** diff. harvest times for KF-2004 trial

	Failure	Success	Total
9 weeks	3	1	4
5 months	4	16	20
1 year	2	10	12
Total	9	27	36

Degrees of freedom: 2   Chi-square = 6.044   p ≤ 0.05.   The difference is significant.

F(infection) in Cw **between** diff. harvest times for KF-2004 trial

	Failure	Success	Total
9 weeks	3	1	4
5 months	4	16	20
Total	7	17	24

Degrees of freedom: 1   Chi-square = 4.881   p ≤ 0.05.   The difference is significant.

F(infection) in Cw **between** diff. harvest times for KF-2004 trial

	Failure	Success	Total
9 weeks	3	1	4
1 year	2	10	12
Total	5	11	16

Degrees of freedom: 1   Chi-square = 4.752   p ≤ 0.05.   The difference is significant.

F(infection) in Cw **between** diff. harvest times for KF-2004 trial

	Failure	Success	Total
5 month	4	16	20
1 year	2	10	12
Total	6	26	32

Degrees of freedom: 1   Chi-square = 0.0547   For significance at the .05 level, chi-square should be ≥ 3.84.
The difference is not significant. p ≤ 1.

F(infection) in Cw **among** diff. harvest times for KF-2004 trial

	Failure	Success	Total
8 weeks	1	1	2
5 months	0	7	7
1 year	0	2	2
Total	1	10	11

Degrees of freedom: 2   Chi-square = 4.95.   For significance at the .05 level, chi-square should be ≥ 5.99.
The difference is not significant.   p ≤ 0.10

F(infection) for *Fd at 1 year* **between** field trials

	Failure	Success	Total
HL 2002	0	10	10
KF 2003	20	10	30
KF 2004	1	12	13
Nakusp 2004	0	2	2
Total	21	34	55

Degrees of freedom: 3   Chi-square = 22.845   p ≤ 0.001.   The difference is significant.

F(infection) for *Fd at 1 year* **among** field trials

	Failure	Success	Total
HL 2002	0	10	10
KF 2004	1	12	13
Nakusp	0	2	2
Total	1	24	25

Degrees of freedom: 2   Chi-square = 0.962   For significance at the .05 level, chi-square should be ≥ 5.99.
The difference is not significant.   p ≤ 1.

F(infection) for *Hw at 1 year* **among** field trials

	Failure	Success	Total
HL 2002	1	5	6
KF 2003	7	23	30
KF 2004	5	12	17
Nakusp 2004	1	2	3
Total	14	42	56

Degrees of freedom: 3   Chi-square = 0.554   For significance at the .05 level, chi-square should be ≥ 7.82.
The difference is not significant.   p ≤ 1.

F(infection) for *Cw at 1 year* **among field trials**

	Failure	Success	Total
HL 2002	6	4	10
KF 2003	16	8	24
KF 2004	2	10	12
Nakusp 2004	0	2	2
Total	24	24	48

Degrees of freedom: 3   Chi-square = 10.4 $p \leq 0.025$.   The difference is significant.

F(infection) for *Cw at 1 year* **among field trials**

	Failure	Success	Total
HL 2002	6	4	10
KF 2004	2	10	12
Nakusp 2004	0	2	2
Total	8	16	24

Degrees of freedom: 2   Chi-square = 5.7   For significance at the .05 level, chi-square should be ≥ 5.99.
The difference is not significant. $p \leq 0.10$.

F(infection) for *Cw at 1 year* **among field trials**

	Failure	Success	Total
KF 2003	16	8	24
KF 2004	2	10	12
Nakusp 2004	0	2	2
Total	18	20	38

Degrees of freedom: 2   Chi-square = 9.922 $p \leq 0.01$.   The difference is significant.

CHI-SQUARE TESTS FOR THE FREQUENCY OF INFECTION AMONG SPECIES FOR WINTER INOCULATION TRIAL

F(infection) winter inoculations – HL. Nov. 4/02 – June 16/03

	Failure	Success	Total
Fd	16	0	16
Hw	14	0	0
Cw	14	0	0
Total	44	0	44

Total Chi-square = 0… not testable.

F(infection) winter inoculations – KF 2003. Oct. 18/03 – May 20/04

	Failure	Success	Total
Fd	8	0	8
Hw	4	2	6
Cw	3	2	5
Total	15	4	19

Degrees of freedom: 2;   Chi-square = 3.758. For significance at the 0.05 level, chi-square should be $\geq$ 5.99. The difference is not significant. $P \leq 0.20$.

F(infection) winter inoculations – KF 2004. Oct. 18/03 – July 4/04

	Failure	Success	Total
Fd	10	1	11
Hw	5	4	9
Cw	6	5	11
Total	21	19	31

Degrees of freedom: 2;   Chi-square = 4,189. For significance at the 0.05 level, chi-square should be ≥ 5.99. The difference is not significant. P ≤ 0.20.

F(infection) winter inoculations – KF 2004. Oct. 18/03 – Sept./04

	Failure	Success	Total
Fd	11	5	16
Hw	7	8	15
Cw	5	9	14
Total	23	22	45

Degrees of freedom: 2;   Chi-square = 3.439. For significance at the 0.05 level, chi-square should be ≥ 5.99. The difference is not significant. P ≤ 0.20.

F(infection) winter inoculations – KF 2004. Sept. 12/04 – April 25/05

	Failure	Success	Total
Fd	45	0	45
Hw	37	0	37
Cw	28	0	28
Total	110	0	110

Total chi-square = 0. nt.

F(infection) winter inoculations – between field trials for Fd

	Failure	Success	Total
Hl 2002	16	0	16
KF 2003	8	0	8
KF 2004	45	0	45
Total	69	0	69

Total chi-square = 0. nt.

F(infection) winter inoculations – between field trials for Hw

	Failure	Success	Total
Hl 2002	14	0	14
KF 2003	4	2	6
KF 2004	37	0	37
Total	55	2	57

Degrees of freedom: 2.   chi-square = 17.618.   p ≤ 0.001. The difference is significant.

F(infection) winter inoculations – between field trials for Cw

	Failure	Success	Total
HI 2002	14	0	14
KF 2003	3	2	5
KF 2004	28	0	28
Total	45	2	47

Degrees of freedom: 2. chi-square = 17.546. $p \leq 0.001$. The difference is significant.

APPENDIX III

Summary of field inoculation trials investigating host response to infection by *A. ostoyae* in the roots of Douglas-fir, western hemlock and western red cedar

Species	Field Trial	Harvest Time	NHR	NIT initiated	NIT breached	NP formed	NP breached	cambium killed	compart & callusing	resistance reactions	Total # of successful resistance reactions	Percent resistance reactions
Douglas-fir	Hidden Lake (2002)	11 weeks	2	7	1	6	3	0	0	3	6	33.33%
		1 year	3	7	2	5	2	4	0	3	7	30.00%
		Total:	5	14	3	11	5	4	0	6	13	**31.58%**
	Kingfisher (2003)	5 weeks	-	-	-	-	-	-	-	-	-	-
		9 weeks	-	-	-	-	-	-	-	-	-	-
		1 year	7	3	1	3	1	4	0	2	8	20.00%
		Total:	7	3	1	3	1	4	0	2	8	**20.00%**
	Kingfisher (2004)	9 weeks	-	-	-	-	-	-	-	-	-	-
		5 months	7	2	1	1	1	6	2	1	8	11.11%
		1 year	7	5	4	4	3	5	0	1	11	8.33%
		Total:	14	7	5	5	4	11	2	2	19	**9.52%**
	Nakusp (2004)	8 weeks	-	-	-	-	-	-	-	-	-	-
		5 months	1	1	1	1	1	1	0	0	2	0.00%
		1 year	2	0	0	0	0	0	0	0	2	0.00%
		Total:	3	1	1	1	1	1	0	0	4	**0.00%**
Western hemlock	Hidden Lake (2002)	11 weeks	2	12	4	8	7	9	0	1	13	7.14%
		1 year	2	3	1	2	2	2	0	0	5	0.00%
		Total:	4	15	5	10	9	11	0	1	18	**5.26%**
	Kingfisher (2003)	5 weeks	1	0	-	-	-	-	-	0	1	0.00%
		9 weeks	0	3	2	3	2	0	0	1	2	33.33%
		1 year	8	15	6	15	7	14	2	8	15	34.78%
		Total:	9	18	8	18	9	14	2	9	18	**33.33%**

	Kingfisher (2004)	9 weeks	1	0	-	-	-	1	0	0	1	0.00%
		5 months	3	8	3	6	2	5	0	4	7	36.36%
		1 year	5	7	1	6	1	4	1	5	7	41.67%
		Total:	9	15	4	12	3	10	1	9	15	**37.50%**
	Nakusp (2004)	8 weeks	1	1	0	1	0	0	0	1	1	50.00%
		5 months	5	1	1	1	1	2	0	0	6	0.00%
		1 year	1	1	0	1	0	0	0	1	1	50.00%
		Total:	7	3	1	3	1	2	0	2	8	**20.00%**
Western red cedar	Hidden Lake (2002)	11 weeks	0	6	1	5	2	1	1	4	2	66.67%
		1 year	0	4	1	3	0	3	3	4	0	100.00%
		Total:	0	10	2	8	2	4	4	8	2	**80.00%**
	Kingfisher (2003)	5 weeks	-	-	-	-	-	-	-	-	-	-
		9 weeks	0	2	0	2	1	0	0	1	1	50.00%
		1 year	0	8	1	7	3	2	2	6	2	75.00%
		Total:	0	10	1	9	4	2	2	7	3	**70.00%**
	Kingfisher (2004)	9 weeks	0	1	0	1	0	0	0	1	0	100.00%
		5 months	1	15	0	15	1	5	5	15	1	93.75%
		1 year	0	10	0	10	3	6	6	8	2	80.00%
		Total:	1	26	0	26	4	11	11	24	3	**88.89%**
	Nakusp (2004)	8 weeks	0	1	0	1	0	0	0	1	0	100.00%
		5 months	0	7	0	7	1	5	3	5	2	71.43%
		1 year	0	2	0	2	0	2	2	2	0	100.00%
		Total:	0	10	0	10	1	7	5	8	2	**80.00%**

APPENDIX IV

Chi-square tests of the frequency of successful resistance reactions as a percentage of successful penetrations by *A. ostoyae* among species on the different harvest dates and field trials

F successful resist rxns HL'02, 11 wks

	Failure	Success	Total
Fd	6	3	9
Hw	13	1	14
Total	19	4	23

Degrees of freedom: 1; Chi-square = 2.615; For significance at the .05 level, chi-square should be ≥ 3.84.
The difference is not significant. p is ≤ 0.20.

F successful resist rxns HL'02, 1 yr

	Failure	Success	Total
Fd	7	3	10
Hw	5	0	5
Total	12	3	15

Degrees of freedom: 1   Chi-square = 1.875   For significance at the .05 level, chi-square should be ≥ 3.84.   The difference
is not significant. p ≤ 0.20.

F successful resist rxns KF'03, 1 yr

	Failure	Success	Total
Fd	8	2	10
Hw	15	8	23
Total	23	10	33

Degrees of freedom: 1; Chi-square = 0.721; For significance at the .05 level, chi-square should be ≥ 3.84.
The difference is not significant. p is ≤ 1.

F successful resist rxns KF'04, 5 months

	Failure	Success	Total
Fd	8	1	9
Hw	7	4	11
Total	15	5	20

Degrees of freedom: 1; Chi-square = 1.683; For significance at the .05 level, chi-square should be ≥ 3.84.
The difference is not significant. p is ≤ 0.20.

F successful resist rxns KF'04, 1 yr

	Failure	Success	Total
Fd	11	1	12
Hw	7	5	12
Total	18	6	24

Degrees of freedom: 1; Chi-square = 3.555; For significance at the .05 level, chi-square should be ≥ 3.84.
The difference is not significant. p is ≤ 0.10.

F successful resist rxns Nakusp'04, 5 months

	Failure	Success	Total
Fd	2	0	2
Hw	6	0	6
Total	8	0	8

Not testable

F successful resist rxns Nakusp'04, 1 yr

	Failure	Success	Total
Fd	2	0	2
Hw	1	1	2
Total	3	1	4

Degrees of freedom: 1; Chi-square = 1.333; For significance at the .05 level, chi-square should be $\geq$ 3.84. The difference is not significant. p is less $\leq$ 1.

F successful resist rxns HL'02, 11 wks

	Failure	Success	Total
Fd	6	3	9
Cw	2	4	6
Total	8	7	15

Degrees of freedom: 1; Chi-square = 1.607; For significance at the .05 level, chi-square should be $\geq$ 3.84. The difference is not significant. p is $\leq$ 1.

F successful resist rxns HL'02, 1 yr

	Failure	Success	Total
Fd	7	3	10
Cw	0	4	4
Total	7	7	14

Degrees of freedom: 1; Chi-square = 5.6; p is $\leq$0.025. The difference is significant.

F successful resist rxns KF'03, 1 yr

	Failure	Success	Total
Fd	8	2	10
Cw	2	6	8
Total	10	8	18

Degrees of freedom: 1; Chi-square = 5.445; p is $\leq$ 0.025. The difference is significant.

F successful resist rxns KF'04, 5 months

	Failure	Success	Total
Fd	8	1	9
Cw	1	15	16
Total	9	16	25

Degrees of freedom: 1; Chi-square = 17.073; p is $\leq$ 0.001. The difference is significant.

F successful resist rxns KF'04, 1 yr

	Failure	Success	Total
Fd	11	1	12
Cw	2	8	10
Total	13	9	22

Degrees of freedom: 1; Chi-square = 11.589; p is ≤ 0.001. The difference is significant.

F successful resist rxns Nakusp'04, 5 months

	Failure	Success	Total
Fd	2	0	2
Cw	2	5	7
Total	4	5	9

Degrees of freedom: 1; Chi-square = 3.214; For significance at the .05 level, chi-square should be ≥ 3.84.
The difference is not significant. p is ≤ 0.10.

F successful resist rxns Nakusp'04, 1 yr

	Failure	Success	Total
Fd	2	0	2
Cw	0	2	2
Total	2	2	4

Degrees of freedom: 1; Chi-square = 4 ; p is ≤ 0.05. The difference is significant.

F successful resist rxns HL'02, 11 wks

	Failure	Success	Total
Hw	13	1	14
Cw	2	4	6
Total	15	5	20

Degrees of freedom: 1 ; Chi-square = 7.936; p is ≤ 0.01. The difference is significant.

F successful resist rxns HL'02, 1 yr

	Failure	Success	Total
Hw	5	0	5
Cw	0	4	4
Total	5	4	9

Degrees of freedom: 1 ; Chi-square = 9 p is ≤ 0.01. The difference is significant.

F successful resist rxns KF'03, 1 yr

	Failure	Success	Total
Hw	15	8	23
Cw	2	6	8
Total	17	14	31

Degrees of freedom: 1 Chi-square = 3.876; p is ≤ 0.05. The difference is significant.

F successful resist rxns KF'04, 5 months

	Failure	Success	Total
Hw	7	4	11
Cw	1	15	16
Total	8	19	27

Degrees of freedom: 1 ;   Chi-square = 10.295; p is ≤ 0.01.   The difference is significant.

F successful resist rxns KF'04, 1 yr

	Failure	Success	Total
Hw	7	5	12
Cw	2	8	10
Total	9	13	22

Degrees of freedom: 1 ;   Chi-square = 3.315;   For significance at the .05 level, chi-square should be ≥ 3.84.
The difference is not significant. p is ≤ 0.10.

F successful resist rxns Nakusp'04, 5 months

	Failure	Success	Total
Hw	6	0	6
Cw	2	5	7
Total	8	5	13

Degrees of freedom: 1 ;   Chi-square = 6.964; p is ≤ 0.01.   The difference is significant.

F successful resist rxns Nakusp'04, 1 yr

	Failure	Success	Total
Hw	1	1	2
Cw	0	2	2
Total	1	3	4

Degrees of freedom: 1 ;   Chi-square = 1.333;   For significance at the .05 level, chi-square should be ≥ to 3.84.
The difference is not significant. p is ≤ 1.

Successful resistance reactions: Fd - A.o. inoc. at 1 year

	Failure	Success	Total
HL 2002	7	3	10
KF 2003	8	2	10
KF 2004	11	1	12
Nakusp 2004	2	0	2
Total	28	6	34

Degrees of freedom: 3 ;   Chi-square = 2.233;   For significance at the .05 level, chi-square should be ≥ 7.82.
The difference is not significant. p is ≤ 1.

Successful resistance reactions: Hw - A.o. inoc. at 1 year

	Failure	Success	Total
HL 2002	5	0	5
KF 2003	15	8	23
KF 2004	7	5	12
Nakusp 2004	1	1	2
Total	28	14	42

Degrees of freedom: 3 ;   Chi-square = 3.147;   For significance at the .05 level, chi-square should be ≥ 7.82.
The difference is not significant. p is ≤ 1.

Successful resistance reactions: Cw - A.o. inoc. at 1 year

	Failure	Success	Total
HL 2002	0	4	4
KF 2003	2	6	8
KF 2004	2	8	10
Nakusp 2004	1	1	2
Total	5	19	24

Degrees of freedom: 3 ;   Chi-square = 2.173;   For significance at the .05 level, chi-square should be ≥ 7.82.
The difference is not significant. p is ≤ 1.

APPENDIX V

Chi-square tests of the frequency of NIT and NP formed, NIT and NP breached, the number of roots that showed cambial invasion and compartmentalization among species.

NHR – *A.ostoyae* inoculation

	Failure	Success	Total
Fd	25	29	54
Hw	51	29	80
Cw	56	1	57
Total	132	59	191

Degrees of freedom: 2 ;   Chi-square = 36.907;   *p* is ≤ 0.001.   The difference is significant.

NHR - *A. ostoyae* inoculation

	Failure	Success	Total
Fd	25	29	54
Hw	51	29	80
Total	76	58	134

Degrees of freedom: 1 ;   Chi-square = 4.000;   *p* is ≤ 0.05.   The difference is significant.

NHR - *A. ostoyae* inoculation

	Failure	Success	Total
Fd	25	29	54
Cw	56	1	57
Total	81	30	111

Degrees of freedom: 1 ;   Chi-square = 37.944;   *p* is ≤ 0.001.   The difference is significant.

NHR - *A. ostoyae* inoculation

	Failure	Success	Total
Hw	51	29	80
Cw	56	1	57
Total	107	30	137

Degrees of freedom: 1 ;   Chi-square = 23.158;   *p* is ≤ 0.001.   The difference is significant.

NIT initiated - *A. ostoyae* inoculation

	Failure	Success	Total
Fd	29	25	54
Hw	29	51	80
Cw	1	56	57
Total	59	132	191

Degrees of freedom: 2 ;   Chi-square = 36.907;   *p* is ≤ 0.001.   The difference is significant.

NIT initiated - *A. ostoyae* inoculation

	Failure	Success	Total
Fd	25	29	54
Hw	51	29	80
Total	76	58	134

Degrees of freedom: 1;   Chi-square = 4.000; p is ≤ 0.05.   The difference is significant.

NIT initiated - *A. ostoyae* inoculation

	Failure	Success	Total
Fd	25	29	54
Cw	56	1	57
Total	81	30	111

Degrees of freedom: 1;   Chi-square = 37.944; p is ≤ 0.001.   The difference is significant.

NIT initiated - *A. ostoyae* inoculation

	Failure	Success	Total
Hw	51	29	80
Cw	56	1	57
Total	107	30	137

Degrees of freedom: 1;   Chi-square = 23.158; p is ≤ 0.001.   The difference is significant.

NIT breached - *A. ostoyae* inoculation

	Failure	Success	Total
Fd	15	10	25
Hw	33	18	51
Cw	53	3	56
Total	101	31	132

Degrees of freedom: 2   Chi-square = 17.993; p is ≤ 0.001.   The difference is significant.

NIT breached - *A. ostoyae* inoculation

	Failure	Success	Total
Fd	15	10	25
Hw	33	18	51
Total	48	28	76

Degrees of freedom: 1   Chi-square = 0.1596;   For significance at the .05 level, chi-square should be ≥ 3.84. The difference is not significant. p is ≤ 1.

NIT breached - *A. ostoyae* inoculation

	Failure	Success	Total
Fd	15	10	25
Cw	53	3	56
Total	68	13	81

Degrees of freedom: 1   Chi-square = 15.395; p is ≤ 0.001.   The difference is significant.

NIT breached - *A. ostoyae* inoculation

	Failure	Success	Total
Hw	33	18	51
Cw	53	3	56
Total	86	21	107

Degrees of freedom: 1 ;   Chi-square = 15.165; p is $\leq o\ 0.001$.   The difference is significant.

NP formed - *A. ostoyae* inoculation

	Failure	Success	Total
Fd	5	20	25
Hw	8	43	51
Cw	0	53	53
Total	13	116	129

Degrees of freedom: 2 ;   Chi-square = 10.426; p is ≤ 0.01.   The difference is significant.

NP formed - *A. ostoyae* inoculation

	Failure	Success	Total
Fd	5	20	25
Hw	8	43	51
Total	13	63	76

Degrees of freedom: 1   Chi-square = 0.220   For significance at the .05 level, chi-square should be ≥ 3.84.
The difference is not significant. p is ≤ 1.

NP formed - *A. ostoyae* inoculation

	Failure	Success	Total
Fd	5	20	25
Cw	0	53	53
Total	5	73	78

Degrees of freedom: 1 ;   Chi-square = 11.326; p is ≤ 0.001.   The difference is significant.

NP formed - *A. ostoyae* inoculation

	Failure	Success	Total
Hw	33	18	51
Cw	0	53	53
Total	33	71	104

Degrees of freedom: 1;   Chi-square = 50.234; p is ≤ 0.001.   The difference is significant.

NP breached - *A. ostoyae* inoculation

	Failure	Success	Total
Fd	9	11	20
Hw	21	22	43
Cw	42	11	53
Total	72	44	116

Degrees of freedom: 2 ; Chi-square = 12.314; p is ≤ 0.01. The difference is significant.

NP breached - *A. ostoyae* inoculation

	Failure	Success	Total
Fd	9	11	20
Hw	21	22	43
Total	30	33	63

Degrees of freedom: 1 Chi-square = 0.081 For significance at the .05 level, chi-square should be ≥ 3.84.
The difference is not significant. p is ≤ 1.

NP breached - *A. ostoyae* inoculation

	Failure	Success	Total
Fd	9	11	20
Cw	42	11	53
Total	51	22	73

Degrees of freedom: 1 ; Chi-square = 8.0879; p is ≤ 0.01. The difference is significant.

NP breached - *A. ostoyae* inoculation

	Failure	Success	Total
Hw	21	22	43
Cw	42	11	53
Total	63	33	96

Degrees of freedom: 1 ; Chi-square = 9.730; p is ≤ 0.01. The difference is significant.

Killed cambium - *A. ostoyae* inoculation

	Failure	Success	Total
Fd	34	20	54
Hw	43	37	80
Cw	33	24	57
Total	110	81	191

Degrees of freedom: 2 ; Chi-square = 1.123; For significance at the .05 level, chi-square should be ≥ 5.99.
The difference is not significant. p is ≤ 1.

CODIT & callusing - *A. ostoyae* inoculation

	Failure	Success	Total
Fd	18	2	20
Hw	34	3	37
Cw	2	22	24
Total	54	27	81

Degrees of freedom: 2 ;   Chi-square = 52.245; p is ≤ 0.001.   The difference is significant.

CODIT & callusing - *A. ostoyae* inoculation

	Failure	Success	Total
Fd	18	2	20
Hw	34	3	37
Total	52	5	57

Degrees of freedom: 1 ;   Chi-square = 0.058;   For significance at the .05 level, chi-square should be ≥ 3.84.
The difference is not significant. p is ≤ 1.

CODIT & callusing - A. ostoyae inoculation

	Failure	Success	Total
Fd	18	2	20
Cw	2	22	24
Total	20	24	44

Degrees of freedom: 1 ;   Chi-square = 29.346; p is ≤ 0.001.   The difference is significant.

CODIT & callusing - *A. ostoyae* inoculation

	Failure	Success	Total
Hw	34	3	37
Cw	2	22	24
Total	36	25	61

Degrees of freedom: 1 ;   Chi-square = 42.023; p is ≤ 0.001.   The difference is significant.

Successful resistance reactions - *A. ostoyae* inoculation

	Failure	Success	Total
Fd	44	10	54
Hw	59	21	80
Cw	10	47	57
Total	113	78	191

Degrees of freedom: 2   Chi-square = 59.044; p is ≤ 0.001.   The difference is significant.

Successful resistance reactions - *A. ostoyae* inoculation

	Failure	Success	Total
Fd	44	10	54
Hw	59	21	80
Total	103	31	134

Degrees of freedom: 1   Chi-square = 1.084   For significance at the .05 level, chi-square should be ≥ 3.84.
The difference is not significant. p is ≤ 1.

Successful resistance reactions - *A. ostoyae* inoculation

	Failure	Success	Total
Fd	44	10	54
Cw	10	47	57
Total	54	57	111

Degrees of freedom: 1   Chi-square = 45.377; p is ≤ 0.001.   The difference is significant.

Successful resistance reactions - *A. ostoyae* inoculation

	Failure	Success	Total
Hw	59	21	80
Cw	10	47	57
Total	69	68	137

Degrees of freedom: 1   Chi-square = 42.062; p is ≤ 0.001.   The difference is significant.

APPENDIX VI

Relationship between root age and the frequency of successful resistance reactions in Douglas-fir, western hemlock and western redcedar following penetration by *A. ostoyae*.
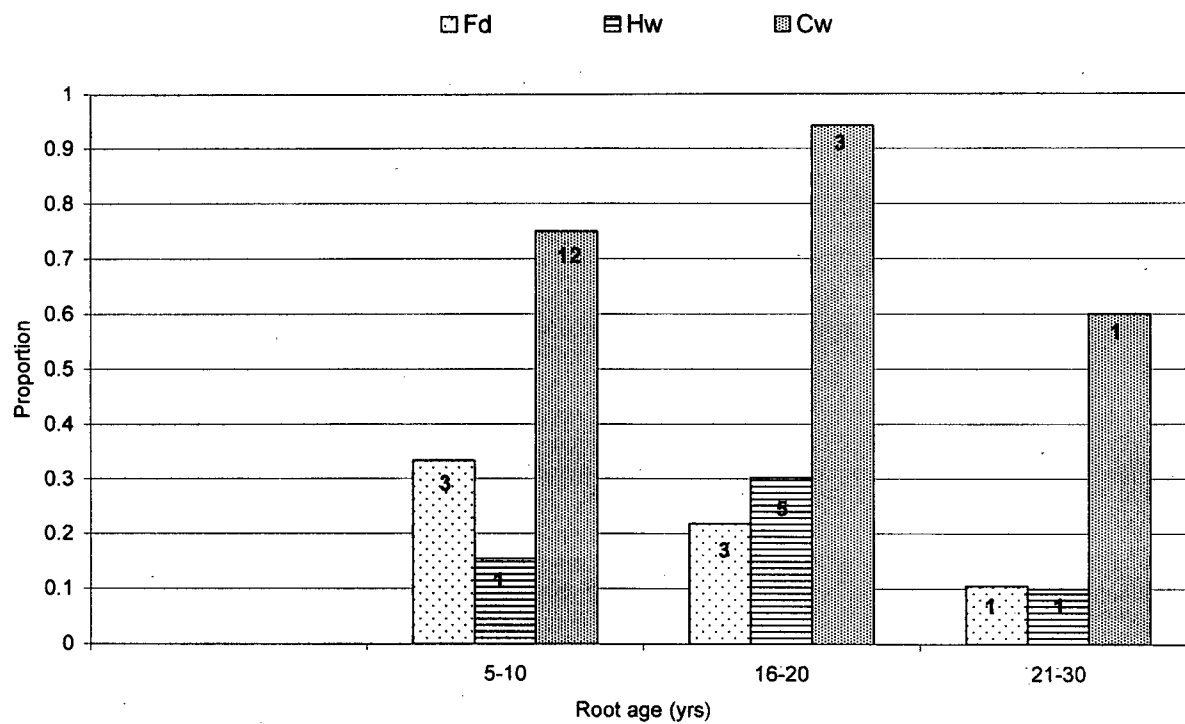
n = no. of observations in bars
Fd, Douglas-fir; Hw, western hemlock; Cw, western redcedar

APPENDIX VII

Relationship between inner bark thickness and the frequency of successful resistance reactions in Douglas-fir, western hemlock and western redcedar following penetration by *A. ostoyae*.

□ Fd   ⊟ Hw   ▦ Cw

Inner bark thickness (mm)
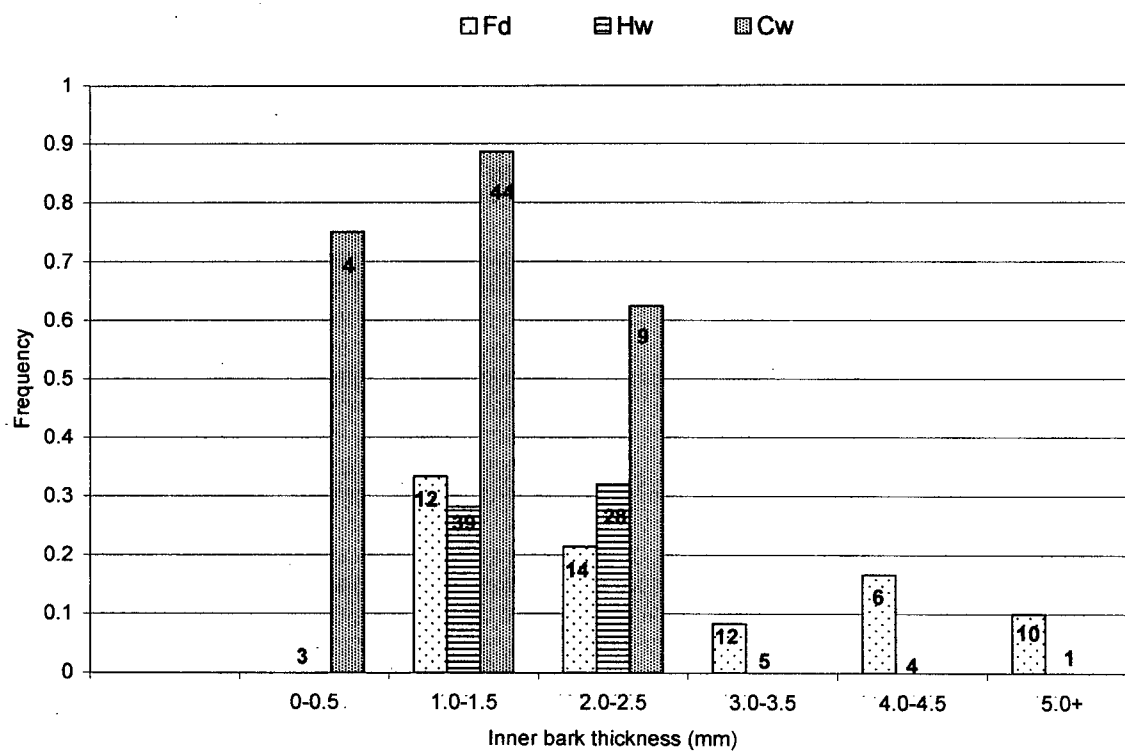
n = no. of observations in bars
Fd, Douglas-fir; Hw, western hemlock; Cw, western redcedar

APPENDIX VIII

Relationship between tree age and the frequency of successful resistance reactions in (i) western hemlock, western redcedar, and (ii) Douglas-fir, following penetration by *A. ostoyae*.

i)
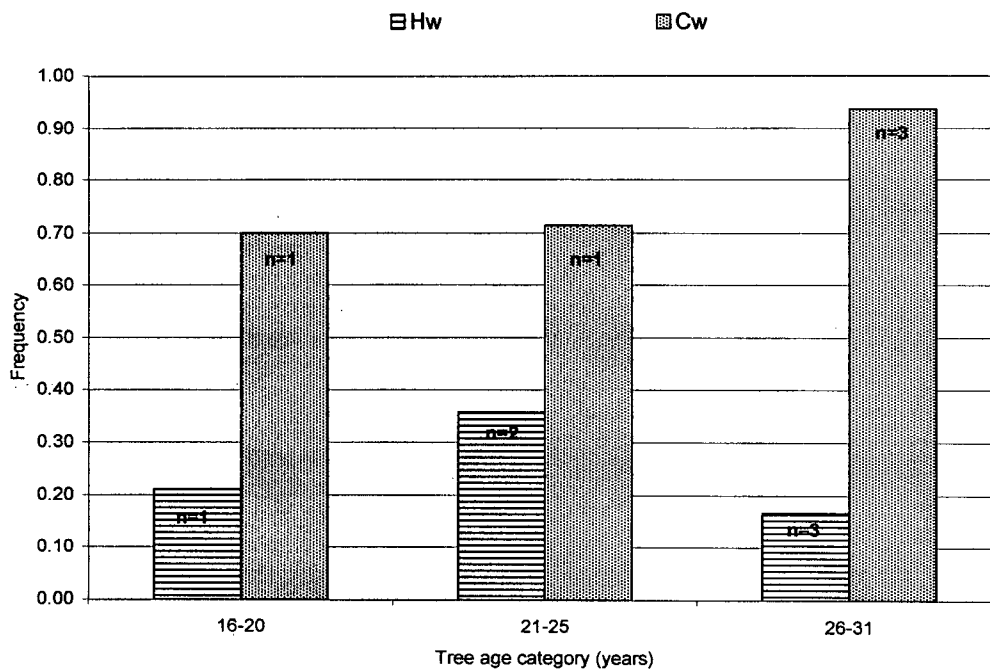
ii)
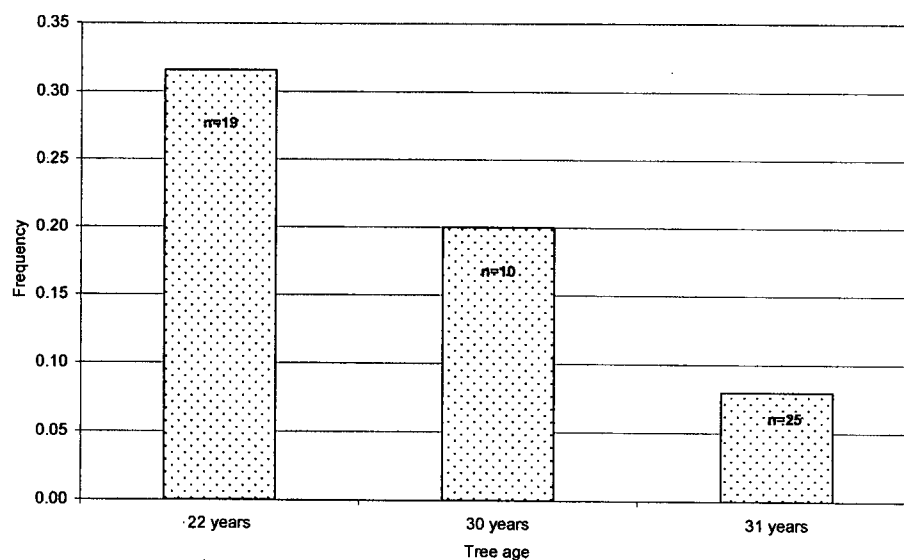
n = no. of observations in bars
Fd, Douglas-fir; Hw, western hemlock; Cw, western redcedar

APPENDIX IX

Relationship between tree size and the frequency of successful resistance reactions in Douglas-fir, western hemlock, and western redcedar following penetration by *A. ostoyae*.
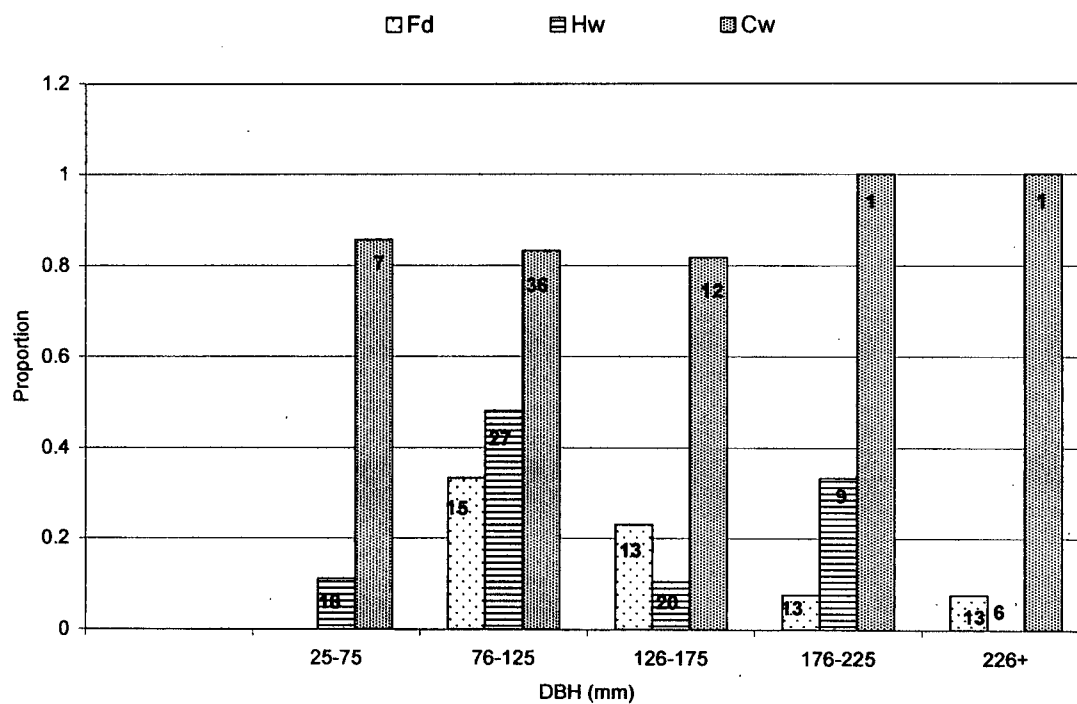
n = no. of observations in bars
Fd, Douglas-fir; Hw, western hemlock; Cw, western redcedar

APPENDIX X

Chi-square tests of the frequency of infection by *A. sinapina* among species for all field trials.

A. sinapina....F (infection) among species – HL 2002

	Failure	Success	Total
Fd	1	6	7
Hw	0	2	2
Cw	11	2	13
Total	12	10	22

Degrees of freedom: 2. Chi-square = 11.717. p ≤ 0.01. The difference is significant.

A. sinapina....F (infection) among species – HL 2002

	Failure	Success	Total
Fd	1	6	7
Hw	0	2	2
Total	1	8	9

Degrees of freedom: 1. Chi-square = 0.321. For significance at the 0.05 level, chi-square should be ≥ 3.84. the difference is not significant. p ≤ 1

A. sinapina....F (infection) among species – HL 2002

	Failure	Success	Total
Fd	1	6	7
Cw	11	2	13
Total	12	8	20

Degrees of freedom: 1. Chi-square = 9.377. p ≤ 0.01. The difference is significant.

A. sinapina....F (infection) among species – HL 2002

	Failure	Success	Total
Hw	0	2	2
Cw	11	2	13
Total	12	10	22

Degrees of freedom: 1. Chi-square = 6.346. p ≤ 0.025. The difference is significant.

A. sinapina....F (infection) among species at 1 year - KF 2003

	Failure	Success	Total
Fd	7	5	12
Hw	3	10	13
Cw	4	4	8
Total	14	19	33

Degrees of freedom: 2. Chi-square = 3.423. For significance at the 0.05 level, chi-square should be ≥ 5.99. the difference is not significant. p ≤ 0.020.

A. sinapina....F (infection) among species at 4 months - KF 2003

	Failure	Success	Total
Fd	1	3	4
Hw	0	5	5
Cw	3	2	5
Total	4	10	14

Degrees of freedom: 2. Chi-square = 4.445 For significance at the 0.05 level, chi-square should be ≥ 5.99. the difference is not significant. p ≤ 0.020.

A. sinapina....F (infection) among species at 1 year - KF 2004

	Failure	Success	Total
Fd	3	4	7
Hw	2	8	10
Cw	3	6	9
Total	8	18	26

Degrees of freedom: 2. Chi-square = 1.0523. For significance at the 0.05 level, chi-square should be ≥ 5.99. the difference is not significant. p ≤ 1.

A. sinapina....F (infection) between different harvest times in Fd – Hl 2002

	Failure	Success	Total
1 year	7	0	7
2 year	1	6	7
Total	8	6	14

Degrees of freedom: 1. Chi-square = 10.5. p ≤ 0.01. The difference is significant.

A. sinapina....F (infection) between different harvest times in Hw – HL 2002

	Failure	Success	Total
1 year	2	0	2
2 year	0	2	2
Total	2	2	4

Degrees of freedom: 1. Chi-square = 4. p ≤ 0.05. The difference is significant.

A. sinapina....F (infection) between different harvest times in Cw – HL/2002

	Failure	Success	Total
1 year	0	0	0
2 year	11	2	13
Total	11	2	13

Not testable

A. sinapina....F (infection) between different harvest times in Fd – KF 2003

	Failure	Success	Total
3 months	2	0	2
1 year	7	5	12
Total	9	5	14

Degrees of freedom: 1. Chi-square = 1.296. For significance at the 0.05 level, chi-square should be ≥ 3.84. the difference is not significant. p ≤ 1.

A. sinapina....F (infection) between different harvest times in Cw – KF 2003

	Failure	Success	Total
3 months	3	0	3
1 year	4	4	8
Total	7	4	11

Degrees of freedom: 1. Chi-square = 2.357. For significance at the 0.05 level, chi-square should be $\geq$ 3.84. the difference is not significant. p $\leq$ 0.20.

A. sinapina....F (infection) between different harvest times in Hw – KF 2003

	Failure	Success	Total
3 months	2	0	2
1 year	3	10	13
Total	5	10	15

Degrees of freedom: 1. Chi-square = 4.615. p $\leq$ 0.05. The difference is significant.

A. sinapina....F (infection) between different harvest times in Fd – KF 2004

	Failure	Success	Total
4 months	1	3	4
1 year	3	4	7
Total	4	7	11

Degrees of freedom: 1. Chi-square = 0.351. For significance at the 0.05 level, chi-square should be $\geq$ 3.84. the difference is not significant. p $\leq$ 1.

A. sinapina....F (infection) between different harvest times in Hw – KF 2004

	Failure	Success	Total
4 months	0	5	5
1 year	2	8	10
Total	2	13	15

Degrees of freedom: 1. Chi-square = 1.154 For significance at the 0.05 level, chi-square should be $\geq$ 3.84. the difference is not significant. p $\leq$ 1.

A. sinapina....F (infection) between different harvest times in Cw – KF 2004

	Failure	Success	Total
4 months	3	2	5
1 year	3	6	9
Total	6	8	14

Degrees of freedom: 1. Chi-square = 0.9333 For significance at the 0.05 level, chi-square should be $\geq$ 3.84. the difference is not significant. p $\leq$ 1.

A. sinapina....F (infection) between different trials in Fd –

	Failure	Success	Total
3 months – KF 2003	2	0	2
4 months – KF 2004	1	3	4
Total	3	3	6

Degrees of freedom: 1. Chi-square = 3 For significance at the 0.05 level, chi-square should be $\geq$ 3.84. the difference is not significant. p $\leq$ 0.1.

A. sinapina....F (infection) between different trials in Hw –

	Failure	Success	Total
3 months – KF 2003	2	0	2
4 months – KF 2004	0	5	5
Total	2	5	7

Degrees of freedom: 1. Chi-square = 7. $p \leq 0.01$. The difference is significant.

A. sinapina....F (infection) between different trials in Cw –

	Failure	Success	Total
3 months – KF 2003	3	0	3
4 months – KF 2004	3	2	5
Total	6	2	8

Degrees of freedom: 1. Chi-square = 1.6 For significance at the 0.05 level, chi-square should be ≥ 3.84. the difference is not significant. $p \leq 1$.

A. sinapina....F (infection) between field trials in Cw –

	Failure	Success	Total
HL 2002	11	2	13
KF 2003	4	4	8
KF 2004	3	6	9
Total	18	12	30

Degrees of freedom: 2. Chi-square = 6.282. $p \leq 0.05$. The difference is significant.

A. sinapina....F (infection) between field trials in Cw –

	Failure	Success	Total
HL 2002	11	2	13
KF 2003	4	4	8
Total	15	6	21

Degrees of freedom: 1. Chi-square = 2.907 For significance at the 0.05 level, chi-square should be $\geq$ 3.84. the difference is not significant. $p \leq 0.1$.

A. sinapina....F (infection) between field trials in Cw –

	Failure	Success	Total
HL 2003	4	4	8
KF 2004	3	6	9
Total	7	10	17

Degrees of freedom: 1. Chi-square = 0.485 For significance at the 0.05 level, chi-square should be $\geq$ 3.84. the difference is not significant. $p \leq 1$.

A. sinapina....F (infection) between field trials in Cw –

	Failure	Success	Total
HL 2002	11	2	13
KF 2004	3	6	9
Total	14	8	22

Degrees of freedom: 1. Chi-square = 6.043. $p \leq 0.025$. The difference is significant.

APPENDIX XI

Chi-square tests of the frequency of successful resistance reactions following inoculation with *A. sinapina* among species.

A. sinapina....F (successful resistance reactions) among species at 1 year - HL

	Failure	Success	Total
Fd	0	6	6
Hw	2	3	5
Cw	2	2	4
Total	4	11	15

Degrees of freedom: 2. Chi-square = 3.75   For significance at the 0.05 level, chi-square should be ≥ 5.99. the difference is not significant. p ≤ 0.20.

A. sinapina....F (successful resistance reactions) among species at 1 year – KF 2003

	Failure	Success	Total
Fd	1	1	2
Hw	3	7	10
Cw	2	6	8
Total	6	14	20

Degrees of freedom: 2. Chi-square = 0.476   For significance at the 0.05 level, chi-square should be ≥ 5.99. the difference is not significant. p ≤ 1

A. sinapina....F (successful resistance reactions) among species at 1 year – KF 2004

	Failure	Success	Total
Fd	0	2	2
Hw	1	3	4
Cw	0	4	4
Total	1	9	10

Degrees of freedom: 2. Chi-square = 1.667   For significance at the 0.05 level, chi-square should be ≥ 5.99. the difference is not significant. p ≤ 1

A. sinapina....F (successful resistance reactions) between field trials for Fd

	Failure	Success	Total
HL 2002	0	6	6
KF 2003	2	3	5
KF 2004	2	2	4
Total	4	11	15

Degrees of freedom: 2. Chi-square = 3.75   For significance at the 0.05 level, chi-square should be ≥ 5.99. the difference is not significant. p ≤ 0.20.

A. sinapina....F (successful resistance reactions) between field trials for Hw

	Failure	Success	Total
HL 2002	1	1	2
KF 2003	7	3	10
KF 2004	6	2	8
Total	14	6	20

Degrees of freedom: 2. Chi-square = 0.476 For significance at the 0.05 level, chi-square should be ≥ 5.99. the difference is not significant. $p \leq 1$.

A. sinapina....F (successful resistance reactions) between field trials for Cw

	Failure	Success	Total
HL 2002	2	0	2
KF 2003	3	1	4
KF 2004	4	0	4
Total	9	1	10

Degrees of freedom: 2. Chi-square = 1.667 For significance at the 0.05 level, chi-square should be ≥ 5.99. the difference is not significant. $p \leq 1$.

APPENDIX XII

Chi-square tests of the frequency of NIT and NP initiated, NIT and NP breached, the number of roots that showed cambial invasion and compartmentalization between species infected with *A. sinapina* and *A. ostoyae*.

A. sinapina....F (NHR) between Fd/Hw

	Failure	Success	Total
Fd	14	4	18
Hw	20	5	25
Total	34	9	23

Degrees of freedom: 1. Chi-square = 0.031   For significance at the 0.05 level, chi-square should be $\geq$ 3.84. the difference is not significant. $p \leq 1$.

A. sinapina....F (NIT initiated) between Fd/Hw

	Failure	Success	Total
Fd	4	14	18
Hw	5	20	25
Total	9	34	43

Degrees of freedom: 1. Chi-square = 0.031   For significance at the 0.05 level, chi-square should be $\geq$ 3.84. the difference is not significant. $p \leq 1$.

A. sinapina....F (NIT breached) between Fd/Hw

	Failure	Success	Total
Fd	13	1	14
Hw	19	1	20
Total	32	2	34

Degrees of freedom: 1. Chi-square = 0.068   For significance at the 0.05 level, chi-square should be $\geq$ 3.84. the difference is not significant. $p \leq 1$.

A. sinapina....F (NP formed) between Fd/Hw

	Failure	Success	Total
Fd	0	14	14
Hw	2	18	20
Total	2	32	34

Degrees of freedom: 1. Chi-square = 1.487   For significance at the 0.05 level, chi-square should be $\geq$ 3.84. the difference is not significant. $p \leq 1$.

A. sinapina....F (codit) between Fd/Hw

	Failure	Success	Total
Fd	0	5	5
Hw	6	5	11
Total	6	10	16

Degrees of freedom: 1. Chi-square = 4.363. $p \leq 0.05$. The difference is significant.

A. ostoyae....F (NHR) between Fd/Hw

	Failure	Success	Total
Fd	18	27	45
Hw	35	24	59
Total	53	51	104

Degrees of freedom: 1. Chi-square = 3.814 For significance at the 0.05 level, chi-square should be ≥ 3.84. the difference is not significant. p ≤ 0.1.

A. ostoyae....F (NIT initiated) between Fd/Hw

	Failure	Success	Total
Fd	27	18	45
Hw	24	35	59
Total	51	53	104

Degrees of freedom: 1. Chi-square = 3.814 For significance at the 0.05 level, chi-square should be ≥ 3.84. the difference is not significant. p ≤ 0.1.

A. ostoyae....F (NIT breached) between Fd/Hw

	Failure	Success	Total
Fd	9	9	18
Hw	23	12	35
Total	32	21	53

Degrees of freedom: 1. Chi-square = 1.227 For significance at the 0.05 level, chi-square should be ≥ 3.84. the difference is not significant. p ≤ 1.

A. ostoyae....F (NP formed) between Fd/Hw

	Failure	Success	Total
Fd	4	14	18
Hw	4	31	35
Total	8	45	53

Degrees of freedom: 1. Chi-square = 1.080 For significance at the 0.05 level, chi-square should be ≥ 3.84. the difference is not significant. p ≤ 1.

A. ostoyae....F (codit) between Fd/Hw

	Failure	Success	Total
Fd	18	2	20
Hw	24	3	27
Total	42	5	47

Degrees of freedom: 1. Chi-square = 0.015 For significance at the 0.05 level, chi-square should be ≥ 3.84. the difference is not significant. p ≤ 1.

A. sinapina....F (NHR/NIT initiated) between Fd/Cw

	Failure	Success	Total
Fd	4	14	22
Cw	12	1	13
Total	30	5	35

Degrees of freedom: 1. Chi-square = 0.734 For significance at the 0.05 level, chi-square should be ≥ 3.84. the difference is not significant. p ≤ 1.

A. sinapina....F (NIT breached) between Fd/Cw

	Failure	Success	Total
Fd	13	1	15
Cw	12	0	12
Total	25	1	26

Degrees of freedom: 1. Chi-square = 0.891 For significance at the 0.05 level, chi-square should be ≥ 3.84. the difference is not significant. p ≤ 1.

A. sinapina....F (NP formed) between Fd/Cw

	Failure	Success	Total
Fd	0	14	14
Cw	0	12	12
Total	0	26	26

Not testable

A. sinapina....F (NP breached) between Fd/Cw

	Failure	Success	Total
Fd	13	1	14
Cw	11	1	12
Total	24	2	26

Degrees of freedom: 1. Chi-square = 0.013 For significance at the 0.05 level, chi-square should be ≥ 3.84. the difference is not significant. p ≤ 1.

A. sinapina....F (codit) between Fd/Cw

	Failure	Success	Total
Fd	0	5	5
Cw	1	4	5
Total	1	9	10

Degrees of freedom: 1. Chi-square = 1.111 For significance at the 0.05 level, chi-square should be ≥ 3.84. the difference is not significant. p ≤ 1.

A. ostoyae....F (NHR/NIT initiated) between Fd/Cw

	Failure	Success	Total
Fd	18	27	45
Cw	46	1	47
Total	64	28	92

Degrees of freedom: 1. Chi-square = 36.367. p ≤ 0.001. The difference is significant.

A. ostoyae....F (NIT breached) between Fd/Cw

	Failure	Success	Total
Fd	9	9	18
Cw	44	2	46
Total	53	11	64

Degrees of freedom: 1. Chi-square = 18.943. p ≤ 0.001. The difference is significant.

A. ostoyae....F (NP formed) between Fd/Cw

	Failure	Success	Total
Fd	4	14	18
Cw	2	44	46
Total	6	58	64

Degrees of freedom: 1. Chi-square = 4.865. $p \leq 0.05$. The difference is significant.

A. ostoyae....F (NP breached) between Fd/Cw

	Failure	Success	Total
Fd	6	8	14
Cw	36	8	44
Total	42	16	58

Degrees of freedom: 1. Chi-square = 8.071 $p \leq 0.01$. The difference is significant.

A. ostoyae....F (codit) between Fd/Cw

	Failure	Success	Total
Fd	18	2	20
Cw	2	21	23
Total	20	23	43

Degrees of freedom: 1. Chi-square = 28.425 $p \leq 0.001$. The difference is significant.

A. sinapina....F (NHR/NIT initiated) between Hw/Cw

	Failure	Success	Total
Hw	20	5	25
Cw	12	1	13
Total	32	6	38

Degrees of freedom: 1. Chi-square = 0.974 For significance at the 0.05 level, chi-square should be $\geq$ 3.84. the difference is not significant. $p \leq 1$.

A. sinapina....F (NIT breached) between Hw/Cw

	Failure	Success	Total
Hw	19	1	20
Cw	12	0	12
Total	31	1	32

Degrees of freedom: 1. Chi-square = 0.619 For significance at the 0.05 level, chi-square should be $\geq$ 3.84. the difference is not significant. $p \leq 1$.

A. sinapina....F (NP formed) between Hw/Cw

	Failure	Success	Total
Hw	2	18	20
Cw	0	12	12
Total	2	30	32

Degrees of freedom: 1. Chi-square = 1.28 For significance at the 0.05 level, chi-square should be $\geq$ 3.84. the difference is not significant. $p \leq 1$.

A. sinapina....F (NP breached) between Hw/Cw

	Failure	Success	Total
Hw	14	4	18
Cw	11	1	12
Total	25	5	30

Degrees of freedom: 1. Chi-square = 1 For significance at the 0.05 level, chi-square should be ≥ 3.84. the difference is not significant. $p \leq 1$.

A. sinapina....F (codit) between Hw/Cw

	Failure	Success	Total
Hw	6	5	11
Cw	1	4	5
Total	7	9	16

Degrees of freedom: 1. Chi-square = 1.667 For significance at the 0.05 level, chi-square should be ≥ 3.84. the difference is not significant. $p \leq 0.20$.

A. ostoyae....F (NHR/NIT initiated) between Hw/Cw

	Failure	Success	Total
Hw	35	24	59
Cw	46	1	47
Total	81	25	106

Degrees of freedom: 1. Chi-square = 21.571 $p \leq 0.001$. The difference is significant.

A. ostoyae....F (NIT breached) between Hw/Cw

	Failure	Success	Total
Hw	23	12	35
Cw	44	2	46
Total	67	14	81

Degrees of freedom: 1. Chi-square = 12.461 $p \leq 0.001$. The difference is significant.

A. ostoyae....F (NP formed) between Hw/Cw

	Failure	Success	Total
Hw	4	31	35
Cw	2	44	46
Total	6	75	81

Degrees of freedom: 1. Chi-square = 1.453 For significance at the 0.05 level, chi-square should be ≥ 3.84. the difference is not significant. $p \leq 1$.

A. ostoyae....F (codit) between Hw/Cw

	Failure	Success	Total
Hw	24	3	27
Cw	2	21	23
Total	26	24	50

Degrees of freedom: 1. Chi-square = 32.000 $p \leq 0.001$. The difference is significant.

....F (successful resistance reactions) between Fd/Hw

	Failure	Success	Total
Fd	38	7	45
Hw	41	18	59
Total	79	25	104

Degrees of freedom: 1. Chi-square = 3.126 For significance at the 0.05 level, chi-square should be $\geq$ 3.84. the difference is not significant. $p \leq 0.1$...

....F (successful resistance reactions) between Hw/Cw

	Failure	Success	Total
Hw	41	18	59
Cw	7	40	47
Total	48	58	106

Degrees of freedom: 1. Chi-square = 31.473 $p \leq 0.001$. The difference is significant.

APPENDIX XIII

Total number of hardwood trees tallied by species in different disease status categories

DISEASE STATUS	HARDWOOD SPECIES[a]						
	W	Ac	At	Alder	Maple	Ep	TOTAL
Healthy	206	93	18	2	7	479	805
Infected by *A. ostoyae* (PROGRESSIVE infection at the root collar)	0	0	0	0	0	0	0
Infected by *A. ostoyae* (CALLUSED infection at the root collar)	0	0	0	0	0	0	0
Dead (KILLED by *A. ostoyae*)	6	0	0	0	0	0	6
Dead (unknown/other factors)	65	1	1	0	0	6	73
Total	277	94	19	2	7	485	884

[a] W = willow; Ac = cottonwood; At = trembling aspen; alder = red alder; maple = Douglas-maple; Ep = paper birch

APPENDIX XIV

The number of trees tallied by species and disease status for all twenty sites.

Arrow Park Plot #1

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	21	8	59	1			14		1	1		24	129
Infected (*A. ostoyae*)	3											1	4
Infected (callused/recovered)													0
Dead (*A.ostoyae*)	3	2	1									11	17
Dead (unknown/other factors)			2				1					1	4
Total	27	10	62	1	0	0	15	0	1	1	0	37	154

Baird Lake Plot #1

Disease Status	Fd	Hw	Cw	W	Ac	Number of Trees At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	34	5	38	1		1	34		7		1	3	124
Infected (*A. ostoyae*)	5												5
Infected (callused/recovered)		1											1
Dead (*A.ostoyae*)	7		1						1				9
Dead (unknown/other factors)												4	4
Total	46	6	39	1	0	1	34	0	8	0	1	7	143

Baird Lake Plot #2

Disease Status	Fd	Hw	Cw	W	Ac	Number of Trees At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	30	4	52	1			25		17	1	2	8	140
Infected (*A. ostoyae*)	1	1							1		1		4
Infected (callused/recovered)	1	1	3										5
Dead (*A.ostoyae*)	16	1	3						11		2		33
Dead (unknown/other factors)												3	3
Total	48	7	58	1	0	0	25	0	29	1	5	11	185

Baird Lake Plot #3

Disease Status	Fd	Hw	Cw	W	Ac	Number of Trees At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	27	2	33				11		6		17	3	99
Infected (*A. ostoyae*)	1	1							1		3		6
Infected (callused/recovered)		1											1
Dead (*A.ostoyae*)	11		1						5				17
Dead (unknown/other factors)												1	1
Total	39	4	34	0	0	0	11	0	12	0	20	4	124

Erie Creek Plot #1

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	35	4	28	1		2	1	4	1		2	2	80
Infected (*A. ostoyae*)													0
Infected (callused/recovered)	1	1											2
Dead (*A.ostoyae*)	3		1										4
Dead (unknown/other factors)													0
Total	39	5	29	1	0	2	1	4	1	0	2	2	86

Erie Creek Plot #2

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	maple	S	Pw	Total
Healthy	45	4	22	1	3	2			1	2	2	11	93
Infected (*A. ostoyae*)	1												1
Infected (callused/recovered)													0
Dead (*A.ostoyae*)	12		1									1	14
Dead (unknown/other factors)									1			1	2
Total	58	4	23	1	3	2	0	0	2	2	2	13	110

Erie Creek Plot #3

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	35		44	2	2	1	1	4	3		7	8	107
Infected (*A. ostoyae*)	1	1											2
Infected (callused/recovered)													0
Dead (*A.ostoyae*)	12	1							1				14
Dead (unknown/other factors)												2	2
Total	48	2	44	2	2	1	1	4	4	0	7	10	125

Erie Creek Plot #4

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	40	3	16	6	7	1	5	7	4		3	2	94
Infected (*A. ostoyae*)	1												1
Infected (callused/recovered)													0
Dead (*A.ostoyae*)	3								1		1		5
Dead (unknown/other factors)													0
Total	44	3	16	6	7	1	5	7	5	0	4	2	100

Hidden Lake Plot #1

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
						Number of Trees							
Healthy	25	39	30		1		8				1		104
Infected (*A. ostoyae*)	3	1											4
Infected (callused/recovered)			1										1
Dead (*A.ostoyae*)	4	7											11
Dead (unknown/other factors)							1						1
Total	32	47	31	0	1	0	9	0	0	0	1	0	121

Hidden Lake Plot #2

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
						Number of Trees							
Healthy	31	18	42		3		14				1	3	112
Infected (*A. ostoyae*)													0
Infected (callused/recovered)													0
Dead (*A.ostoyae*)	2	4	1										7
Dead (unknown/other factors)							1						1
Total	33	22	43	0	3	0	15	0	0	0	1	3	120

Hidden Lake Plot #3

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
						Number of Trees							
Healthy	42	10	15		1		11						79
Infected (*A. ostoyae*)	5	3											8
Infected (callused/recovered)			1										1
Dead (*A.ostoyae*)	4	8											12
Dead (unknown/other factors)													0
Total	51	21	16	0	1	0	11	0	0	0	0	0	100

Hummamilt Lake Plot #1

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	22	6	46	16	22		3			1	2		118
Infected (*A. ostoyae*)	3	2	1										6
Infected (callused/recovered)			1										1
Dead (*A.ostoyae*)	10	4	1	3									18
Dead (unknown/other factors)				6	1								7
Total	35	12	49	25	23	0	3	0	0	1	2	0	150

Hummamilt Lake Plot #2

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	30	4	29	29	7	3	1			1	2		106
Infected (*A. ostoyae*)	1												1
Infected (callused/recovered)													0
Dead (*A.ostoyae*)	7											1	8
Dead (unknown/other factors)				3									3
Total	38	4	29	32	7	3	1	0	0	1	2	1	118

Kidney Lake Plot #1

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	21	134	33	5	4							2	199
Infected (*A. ostoyae*)	5	7										1	13
Infected (callused/recovered)	1	4											5
Dead (*A.ostoyae*)	7	9	1										17
Dead (unknown/other factors)	1												1
Total	35	154	34	5	4	0	0	0	0	0	0	3	235

Kidney Lake Plot #2

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	30	125	70	3	6		4				1	2	241
Infected (*A. ostoyae*)	5	25										1	31
Infected (callused/recovered)													0
Dead (*A.ostoyae*)	10	14											24
Dead (unknown/other factors)				3									3
Total	45	164	70	6	6	0	4	0	0	0	1	3	299

Kuskanax Plot #1

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	50		33				2				4		89
Infected (*A. ostoyae*)	2												2
Infected (callused/recovered)	2												2
Dead (*A.ostoyae*)	11											1	12
Dead (unknown/other factors)							1					5	6
Total	65	0	33	0	0	0	3	0	0	0	4	6	111

Kuskanax Plot #2

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	34	2	41								6	6	89
Infected (*A. ostoyae*)	3	2											5
Infected (callused/recovered)	2	1	2										5
Dead (*A.ostoyae*)	17		1									4	22
Dead (unknown/other factors)												5	5
Total	56	5	44	0	0	0	0	0	0	0	6	15	126

Kuskanax Plot #3

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	31	9	31				1						72
Infected (*A. ostoyae*)	11	2										1	14
Infected (callused/recovered)	2	2											4
Dead (*A.ostoyae*)	14	1	1										16
Dead (unknown/other factors)												10	10
Total	58	14	32	0	0	0	1	0	0	0	0	11	116

Mabel Lake_Icebox   Plot #1

Disease Status	Fd	Hw	Cw	W	Ac	Number of Trees At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	38	4	43		1		32					2	120
Infected (*A. ostoyae*)	4												4
Infected (callused)			3										3
Dead (*A.ostoyae*)	10		2									1	13
Dead (unknown/other factors)												1	1
Total	52	4	48	0	1	0	32	0	0	0	0	4	141

Mabel Lake_Icebox   Plot #2

Disease Status	Fd	Hw	Cw	W	Ac	Number of Trees At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	31	1	35	3			13					2	85
Infected (*A. ostoyae*)	2												2
Infected (callused)	1		2										3
Dead (*A.ostoyae*)	11												11
Dead (unknown/other factors)				1								1	2
Total	45	1	37	4	0	0	13	0	0	0	0	3	103

Mabel Lake_Icebox   Plot #3

Disease Status	Fd	Hw	Cw	W	Ac	Number of Trees At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	50	3	59				35		5			6	158
Infected (*A. ostoyae*)	4											1	5
Infected (callused)			2										2
Dead (*A.ostoyae*)	20	5	1										26
Dead (unknown/other factors)												3	3
Total	74	8	62	0	0	0	35	0	5	0	0	10	194

Mabel Lake_Simard Plot #1

| | | | | | | Number of Trees | | | | | | | |
Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	25	31	39	4			43		2			4	148
Infected (*A. ostoyae*)	4	8											12
Infected (callused)													0
Dead (*A.ostoyae*)	7	6		1					1				15
Dead (unknown/other factors)				2			1						3
Total	36	45	39	7	0	0	44	0	3	0	0	4	178

Mabel Lake_Simard Plot #2

| | | | | | | Number of Trees | | | | | | | |
Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	18	29	33				25		1			1	107
Infected (*A. ostoyae*)	4	4										1	9
Infected (callused)		2	5										7
Dead (*A.ostoyae*)	3	5	2									2	12
Dead (unknown/other factors)													0
Total	25	40	40	0	0	0	25	0	1	0	0	4	135

Mabel Lake_Simard Plot #3

| | | | | | | Number of Trees | | | | | | | |
Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	28	25	71						1		1	3	129
Infected (*A. ostoyae*)	3	2											5
Infected (callused)		1	1										2
Dead (*A.ostoyae*)	11	6											17
Dead (unknown/other factors)													0
Total	42	34	72	0	0	0	0	0	1	0	1	3	153

Mabel Lake_2 Plot #1

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	51		35				18						104
Infected (*A. ostoyae*)	3												3
Infected (callused/recovered)			1										1
Dead (*A.ostoyae*)	43		7										50
Dead (unknown/other factors)													0
Total	97	0	43	0	0	0	18	0	0	0	0	0	158

Mabel Lake_2 Plot #2

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	67	6	41				15		3			19	151
Infected (*A. ostoyae*)	5												5
Infected (callused/recovered)	1												1
Dead (*A.ostoyae*)	34	1	2									3	40
Dead (unknown/other factors)												2	2
Total	107	7	43	0	0	0	15	0	3	0	0	24	199

Monashee Creek Plot #1

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	alder	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	52	3	66	1	1	2	28		4	1	3	4	165
Infected (*A. ostoyae*)	1												1
Infected (callused/recovered)			2										2
Dead (*A.ostoyae*)	11	1	1						1		1		15
Dead (unknown/other factors)													0
Total	64	4	69	1	1	2	28	0	5	1	4	4	183

Monashee Creek Plot #2

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	35	5	60				10		1		3	4	118
Infected (*A. ostoyae*)	5	3											8
Infected (callused/recovered)		3	1								1		5
Dead (*A.ostoyae*)	16	3	1									2	22
Dead (unknown/other factors)												4	4
Total	56	14	62	0	0	0	10	0	1	0	4	10	157

Northfork_55 Plot #1

Disease Status	Fd	Hw	Cw	W	Ac	Number of Trees At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	26	2	27	19							1	7	82
Infected (*A. ostoyae*)	5												5
Infected (callused)													0
Dead (*A.ostoyae*)	3	2											5
Dead (unknown/other factors)				10									10
Total	34	4	27	29	0	0	0	0	0	0	1	7	102

Northfork_55 Plot #2

Disease Status	Fd	Hw	Cw	W	Ac	Number of Trees At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	8	14	60	19								7	108
Infected (*A. ostoyae*)	1	5											6
Infected (callused)		2	5										7
Dead (*A.ostoyae*)	3	1									1	2	7
Dead (unknown/other factors)				7									7
Total	12	22	65	26	0	0	0	0	0	0	1	9	135

Northfork_55 Plot #3

Disease Status	Fd	Hw	Cw	W	Ac	Number of Trees At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	29	4	24	1								6	64
Infected (*A. ostoyae*)	7	2											9
Infected (callused)													0
Dead (*A.ostoyae*)	1											1	2
Dead (unknown/other factors)												1	1
Total	37	6	24	1	0	0	0	0	0	0	0	8	76

Northfork_60 Plot #1

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	26	22	26	1	7		2				4	1	89
Infected (*A. ostoyae*)	3	3											6
Infected (callused/recovered)	1	1											2
Dead (*A.ostoyae*)	12	2	1									1	16
Dead (unknown/other factors)				13									13
Total	42	28	27	14	7	0	2	0	0	0	4	2	126

Northfork_60 Plot #2

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	32	8	55	9	1	1						1	107
Infected (*A. ostoyae*)	1		1										2
Infected (callused/recovered)		1	5										6
Dead (*A.ostoyae*)	10	4	5									1	20
Dead (unknown/other factors)				3									3
Total	43	13	66	12	1	1	0	0	0	0	0	2	138

Northfork_60 Plot #3

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	31	3	53	6							1		94
Infected (*A. ostoyae*)	1												1
Infected (callused/recovered)	1	1											2
Dead (*A.ostoyae*)	7		3										10
Dead (unknown/other factors)				4									4
Total	40	4	56	10	0	0	0	0	0	0	1	0	111

Northfork_60 Plot #4

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	17	5	30	21		2					1	1	77
Infected (*A. ostoyae*)	2	1											3
Infected (callused/recovered)		1											1
Dead (*A.ostoyae*)	7												7
Dead (unknown/other factors)				9									9
Total	26	7	30	30	0	2	0	0	0	0	1	1	97

Toledo Plot #1

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	19	132	35	1	2		20					1	210
Infected (*A. ostoyae*)		1											1
Infected (callused/recovered)													0
Dead (*A.ostoyae*)	3	13	1										17
Dead (unknown/other factors)													0
Total	22	146	36	1	2	0	20	0	0	0	0	1	228

Toledo Plot #2

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	16	61	25	23	20		11					2	158
Infected (*A. ostoyae*)													0
Infected (callused/recovered)													0
Dead (*A.ostoyae*)	3	3											6
Dead (unknown/other factors)				1									1
Total	19	64	25	24	20	0	11	0	0	0	0	2	165

Trinity Valley Plot #1

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	67		38				10		3	1	1		120
Infected (*A. ostoyae*)	4												4
Infected (callused/recovered)	2		4										6
Dead (*A.ostoyae*)	75		1										76
Dead (unknown/other factors)							1						1
Total	148	0	43	0	0	0	11	0	3	1	1	0	207

Trinity Valley Plot #2

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	104	1	48				10		2	1		1	167
Infected (*A. ostoyae*)	4												4
Infected (callused/recovered)			2										2
Dead (*A.ostoyae*)	65		1						1			2	69
Dead (unknown/other factors)												1	1
Total	173	1	51	0	0	0	10	0	3	1	0	4	243

Trout Lake_1 Plot #1

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	25	12	22			1					1	3	64
Infected (*A. ostoyae*)	2										1		3
Infected (callused/recovered)		3	1										4
Dead (*A.ostoyae*)	8	6											14
Dead (unknown/other factors)													0
Total	35	21	23	0	0	1	0	0	0	0	2	3	85

Trout Lake_1 Plot #2

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	22	17	20									1	60
Infected (*A. ostoyae*)													0
Infected (callused/recovered)	1												1
Dead (*A.ostoyae*)	1	5											6
Dead (unknown/other factors)													0
Total	24	22	20	0	0	0	0	0	0	0	0	1	67

Trout Lake_2   Plot #1

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	maple	Lw	Pl	S	Pw	Total
Healthy	8	27	61	2				1			1		100
Infected (*A. ostoyae*)	2	5											7
Infected (callused/recovered)	1	8	9										18
Dead (*A.ostoyae*)	8	5	1										14
Dead (unknown/other factors)													0
Total	19	45	71	2	0	0	0	1	0	0	1	0	139

Trout Lake_2 Plot #2

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	maple	Lw	Pl	S	Pw	Total
Healthy	38	5	45	3				1					92
Infected (*A. ostoyae*)	4												4
Infected (callused/recovered)	1		7										8
Dead (*A.ostoyae*)	3												3
Dead (unknown/other factors)													0
Total	46	5	52	3	0	0	0	1	0	0	0	0	107

Trout Lake_3   Plot #1

Disease Status	Fd	Hw	Cw	W	Ac	Number of Trees At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	19	13	31	3							5	1	72
Infected (*A. ostoyae*)	3										1		4
Infected (callused)	1	3	2										6
Dead (*A.ostoyae*)	1	1		1									3
Dead (unknown/other factors)													0
Total	24	17	33	4	0	0	0	0	0	0	6	1	85

Trout Lake_3   Plot #2

Disease Status	Fd	Hw	Cw	W	Ac	Number of Trees At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	20	3	66	10	1						3		103
Infected (*A. ostoyae*)	3	1	1										5
Infected (callused)			1										1
Dead (*A.ostoyae*)	4		1										5
Dead (unknown/other factors)				4									4
Total	27	4	69	14	1	0	0	0	0	0	3	0	118

Trout Lake_3   Plot #3

Disease Status	Fd	Hw	Cw	W	Ac	Number of Trees At	Ep	maple	Lw	Pl	S	Pw	Total
Healthy	18	9	41	7				2			6		83
Infected (*A. ostoyae*)	1										1		2
Infected (callused)	3		4										7
Dead (*A.ostoyae*)	6											1	7
Dead (unknown/other factors)													0
Total	28	9	45	7	0	0	0	2	0	0	7	1	99

Wilson Creek Plot #1

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	42	41	49				34		1		6	1	174
Infected (*A. ostoyae*)	3	1											4
Infected (callused/recovered)		3											3
Dead (*A.ostoyae*)	9	2											11
Dead (unknown/other factors)													0
Total	54	47	49	0	0	0	34	0	1	0	6	1	192

Wilson Creek Plot #2

Number of Trees

Disease Status	Fd	Hw	Cw	W	Ac	At	Ep	maple	Lw	Pl	S	Pw	Total
Healthy	34	22	39	2			36	1	1		4		139
Infected (*A. ostoyae*)	1	5											6
Infected (callused/recovered)	2	4	2										8
Dead (*A.ostoyae*)	3										1		4
Dead (unknown/other factors)												1	1
Total	40	31	41	2	0	0	36	1	1	0	5	1	158

Worthington Plot #1

Disease Status	Fd	Hw	Cw	W	Ac	Number of Trees At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	26	32	56			1		4	1		10		130
Infected (*A. ostoyae*)	0	1									1		2
Infected (callused)		3	2										5
Dead (*A.ostoyae*)	16	1									2	1	20
Dead (unknown/other factors)													0
Total	42	37	58	0	0	1	0	4	1	0	13	1	157

Worthington Plot #2

Disease Status	Fd	Hw	Cw	W	Ac	Number of Trees At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	19	10	35	3	2	3	2	3		1	10	1	89
Infected (*A. ostoyae*)													0
Infected (callused)		1	1										2
Dead (*A.ostoyae*)	17	3								2	1		23
Dead (unknown/other factors)						1				1			2
Total	36	14	36	3	2	4	2	3	0	4	11	1	116

Worthington Plot #3

Disease Status	Fd	Hw	Cw	W	Ac	Number of Trees At	Ep	Bg	Lw	Pl	S	Pw	Total
Healthy	18	25	21	2	2			2			8	2	80
Infected (*A. ostoyae*)	2												2
Infected (callused)		3											3
Dead (*A.ostoyae*)	10	4									1		15
Dead (unknown/other factors)													0
Total	30	32	21	2	2	0	0	2	0	0	9	2	100

Logistic Regression Model Analysis for Douglas-fir and Western redcedar

Case Processing Summary

Unweighted Cases[a]		N	Percent
Selected Cases	Included in Analysis	4565	100.0
	Missing Cases	0	.0
	Total	4565	100.0
Unselected Cases		0	.0
Total		4565	100.0

a. If weight is in effect, see classification table for the total number of cases.

Dependent Variable Encoding

Original Value	Internal Value
other (healthy+infected_	0
killed	1

Classification Table[a],[b]

			Predicted		
			disease		
Observed			other (healthy+i nfected_	killed	Percentage Correct
Step 0	disease	other (healthy+infected_	3932	0	100.0
		killed	633	0	.0
	Overall Percentage				86.1

a. Constant is included in the model.

b. The cut value is .500

Categorical Variables Codings

		Frequency	Parameter coding																		
			(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)
site	1	89	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	2	264	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	3	301	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	4	206	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	5	151	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	6	184	.000	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	7	288	.000	.000	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	8	318	.000	.000	.000	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	9	254	.000	.000	.000	.000	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	10	290	.000	.000	.000	.000	.000	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	11	251	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	12	199	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000
	13	330	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000
	14	102	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000
	15	415	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	.000	.000	.000	.000	.000
	16	102	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	.000	.000	.000	.000
	17	188	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	.000	.000	.000
	18	226	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	.000	.000
	19	184	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	.000
	20	223	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000
species	Douglas-fir	2396	.000																		
	western red ce	2169	1.000																		

Block 0: Beginning Block

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 0	Constant	-1.826	.043	1818.796	1	.000	.161

Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	site	297.884	19	.000
		site(1)	.193	1	.661
		site(2)	2.824	1	.093
		site(3)	13.132	1	.000
		site(4)	.495	1	.482
		site(5)	3.437	1	.064
		site(6)	.132	1	.716
		site(7)	.000	1	.987
		site(8)	5.213	1	.022
		site(9)	64.635	1	.000
		site(10)	1.189	1	.275
		site(11)	18.658	1	.000
		site(12)	.016	1	.900
		site(13)	4.285	1	.038
		site(14)	158.291	1	.000
		site(15)	3.169	1	.075
		site(16)	9.194	1	.002
		site(17)	14.576	1	.000
		site(18)	8.659	1	.003
		site(19)	5.758	1	.016
		species(1)	492.446	1	.000
Overall Statistics			671.249	20	.000

Block 1: Method = Enter

Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	753.781	20	.000
	Block	753.781	20	.000
	Model	753.781	20	.000

Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	2921.321[a]	.152	.275

a. Estimation terminated at iteration number 7 because parameter estimates changed by less than .001.

Hosmer and Lemeshow Test

Step	Chi-square	df	Sig.
1	3.733	8	.880

Contingency Table for Hosmer and Lemeshow Test

		disease = other (healthy+infected_		disease = killed		
		Observed	Expected	Observed	Expected	Total
Step 1	1	399	397.891	2	3.109	401
	2	438	437.698	5	5.302	443
	3	489	486.236	6	8.764	495
	4	391	394.994	13	9.006	404
	5	410	410.181	16	15.819	426
	6	407	408.629	51	49.371	458
	7	394	394.959	84	83.041	478
	8	287	289.110	85	82.890	372
	9	344	337.697	111	117.303	455
	10	373	374.605	260	258.395	633

Classification Table[a]

			Predicted		
			disease		
	Observed		other (healthy+infected_nfected_	killed	Percentage Correct
Step 1	disease	other (healthy+infected_	3932	0	100.0
		killed	633	0	.0
	Overall Percentage				86.1

a. The cut value is .500

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I.for EXP(B)	
								Lower	Upper
Step 1	site			155.262	19	.000			
	site(1)	.939	.561	2.805	1	.094	2.558	.852	7.680
	site(2)	.292	.564	.269	1	.604	1.340	.444	4.044
	site(3)	-.379	.616	.379	1	.538	.684	.204	2.291
	site(4)	.706	.592	1.422	1	.233	2.026	.635	6.466
	site(5)	.500	.593	.711	1	.399	1.649	.516	5.272
	site(6)	.690	.558	1.532	1	.216	1.994	.668	5.947
	site(7)	.782	.557	1.973	1	.160	2.185	.734	6.506
	site(8)	.545	.577	.892	1	.345	1.725	.556	5.349
	site(9)	1.546	.547	7.974	1	.005	4.691	1.605	13.714
	site(10)	.678	.569	1.421	1	.233	1.971	.646	6.011
	site(11)	-.553	.659	.703	1	.402	.575	.158	2.093
	site(12)	.939	.557	2.844	1	.092	2.557	.859	7.612
	site(13)	.225	.668	.113	1	.737	1.252	.338	4.635
	site(14)	1.649	.541	9.290	1	.002	5.203	1.802	15.025
	site(15)	.023	.650	.001	1	.972	1.023	.286	3.661
	site(16)	.282	.614	.211	1	.646	1.326	.398	4.421
	site(17)	.061	.612	.010	1	.921	1.063	.320	3.528
	site(18)	-.064	.612	.011	1	.917	.938	.283	3.112
	site(19)	1.359	.561	5.861	1	.015	3.891	1.295	11.688
	species(1)	-2.683	.165	264.726	1	.000	.068	.049	.094
	Constant	-1.939	.530	13.370	1	.000	.144		

a. Variable(s) entered on step 1: site, species.

Logistic Regression

Case Processing Summary

Unweighted Cases [a]		N	Percent
Selected Cases	Included in Analysis	4565	100.0
	Missing Cases	0	.0
	Total	4565	100.0
Unselected Cases		0	.0
Total		4565	100.0

a. If weight is in effect, see classification table for the total number of cases.

Dependent Variable Encoding

Original Value	Internal Value
other (healthy+infected_	0
killed	1

Classification Table[a,b]

		Predicted		
		disease		
Observed		other (healthy+infected_	killed	Percentage Correct
Step 0 disease	other (healthy+infected_	3932	0	100.0
	killed	633	0	.0
Overall Percentage				86.1

a. Constant is included in the model.

b. The cut value is .500

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 0	Constant	-1.826	.043	1818.796	1	.000	.161

Categorical Variables Codings

		Frequency	Parameter coding																		
			(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)
site	1	89	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	2	264	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	3	301	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	4	206	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	5	151	.000	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	6	184	.000	.000	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	7	288	.000	.000	.000	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	8	318	.000	.000	.000	.000	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	9	254	.000	.000	.000	.000	.000	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	10	290	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	11	251	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000
	12	199	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000
	13	330	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000
	14	102	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	.000	.000	.000	.000	.000
	15	415	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	.000	.000	.000	.000
	16	102	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	.000	.000	.000
	17	188	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	.000	.000
	18	226	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	.000
	19	184	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000
	20	223	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
species	Douglas-fir	2396	1.000																		
	western red cedar	2169	.000																		

Variables not in the Equation

				Score	df	Sig.
Step 0	Variables	site		297.884	19	.000
		site(1)		6.675	1	.010
		site(2)		.193	1	.661
		site(3)		2.824	1	.093
		site(4)		13.132	1	.000
		site(5)		.495	1	.482
		site(6)		3.437	1	.064
		site(7)		.132	1	.716
		site(8)		.000	1	.987
		site(9)		5.213	1	.022
		site(10)		64.635	1	.000
		site(11)		1.189	1	.275
		site(12)		18.658	1	.000
		site(13)		.016	1	.900
		site(14)		4.285	1	.038
		site(15)		158.291	1	.000
		site(16)		3.169	1	.075
		site(17)		9.194	1	.002
		site(18)		14.576	1	.000
		site(19)		8.659	1	.003
		species(1)		492.446	1	.000
	Overall Statistics			671.249	20	.000

Block 1: Method = Enter

Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	753.781	20	.000
	Block	753.781	20	.000
	Model	753.781	20	.000

Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	2921.321[a]	.152	.275

a. Estimation terminated at iteration number 7 because parameter estimates changed by less than .001.

Hosmer and Lemeshow Test

Step	Chi-square	df	Sig.
1	3.733	8	.880

Contingency Table for Hosmer and Lemeshow Test

		disease = other (healthy+infected		disease = killed		
		Observed	Expected	Observed	Expected	Total
Step 1	1	399	397.891	2	3.109	401
	2	438	437.698	5	5.302	443
	3	489	486.236	6	8.764	495
	4	391	394.994	13	9.006	404
	5	410	410.181	16	15.819	426
	6	407	408.629	51	49.371	458
	7	394	394.959	84	83.041	478
	8	287	289.110	85	82.890	372
	9	344	337.697	111	117.303	455
	10	373	374.605	260	258.395	633

Classification Table[a]

			Predicted		
			disease		
	Observed		other (healthy+infected	killed	Percentage Correct
Step 1	disease	other (healthy+infected_	3932	0	100.0
		killed	633	0	.0
	Overall Percentage				86.1

a. The cut value is .500

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I.for EXP(B) Lower	95.0% C.I.for EXP(B) Upper
Step 1[a]	site			155.262	19	.000			
	site(1)	-1.359	.561	5.861	1	.015	.257	.086	.772
	site(2)	-.419	.262	2.558	1	.110	.658	.393	1.099
	site(3)	-1.066	.268	15.844	1	.000	.344	.204	.582
	site(4)	-1.738	.366	22.521	1	.000	.176	.086	.361
	site(5)	-.653	.324	4.065	1	.044	.521	.276	.982
	site(6)	-.859	.325	6.964	1	.008	.424	.224	.802
	site(7)	-.669	.255	6.879	1	.009	.512	.311	.845
	site(8)	-.577	.253	5.205	1	.023	.562	.342	.922
	site(9)	-.813	.296	7.560	1	.006	.443	.248	.792
	site(10)	.187	.232	.652	1	.420	1.206	.766	1.899
	site(11)	-.680	.279	5.941	1	.015	.506	.293	.875
	site(12)	-1.911	.434	19.408	1	.000	.148	.063	.346
	site(13)	-.420	.253	2.755	1	.097	.657	.400	1.079
	site(14)	-1.134	.447	6.421	1	.011	.322	.134	.773
	site(15)	.291	.216	1.803	1	.179	1.337	.875	2.044
	site(16)	-1.336	.421	10.073	1	.002	.263	.115	.600
	site(17)	-1.076	.363	8.804	1	.003	.341	.167	.694
	site(18)	-1.298	.359	13.057	1	.000	.273	.135	.552
	site(19)	-1.423	.358	15.757	1	.000	.241	.119	.487
	species(1)	2.683	.165	264.726	1	.000	14.633	10.591	20.217
	Constant	-3.263	.234	194.942	1	.000	.038		

a. Variable(s) entered on step 1: site, species.

APPENDIX XVI

Tree size distribution as number of trees per DBH class of western hemlock trees for all 20 sites combined.

Std. Dev = 30.08
Mean = 56
N = 1218.00

No. of trees

Tree Size (DBH mm)
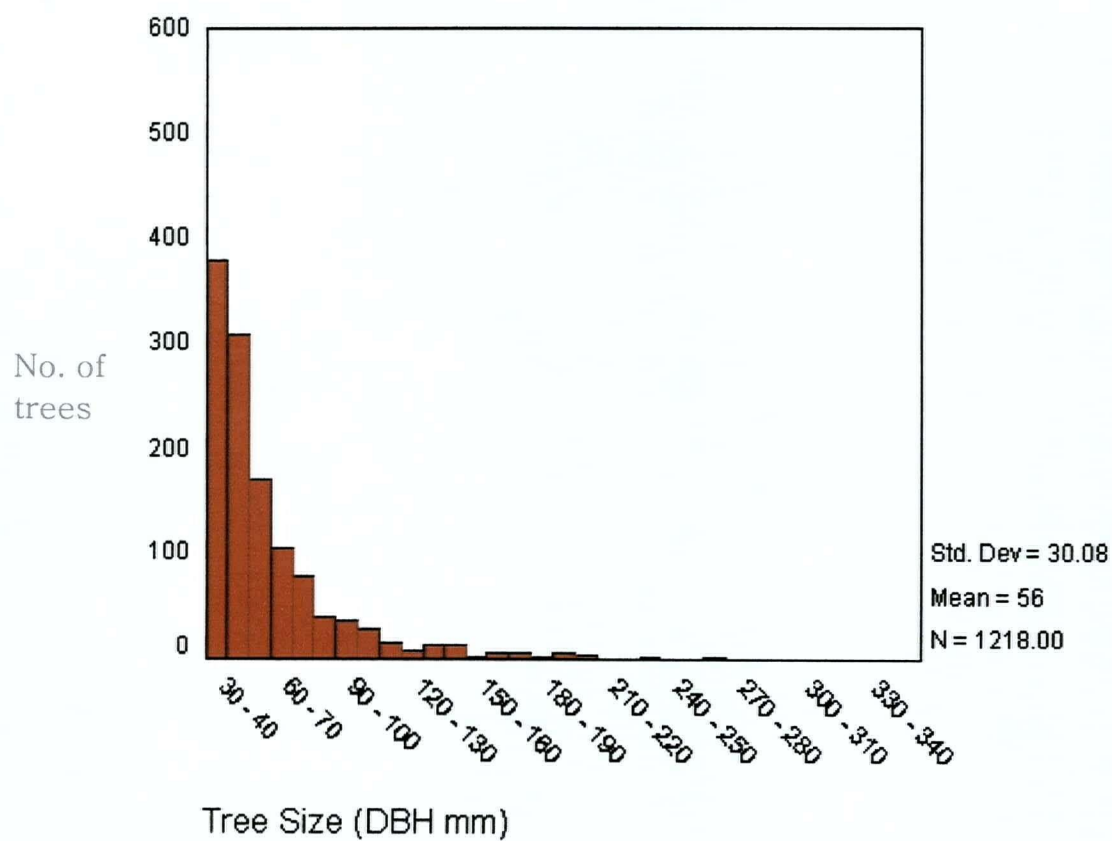
APPENDIX XVII

Chi-square tests of the frequency of mortality caused by *A. ostoyae* among species for 10 sites combined

Frequency of mortality among species for 10 sites combined

	Success	Failure	Total
Fd	171	729	900
Hw	108	967	1075
Cw	11	1061	1072
Total	290	2757	3047

Degrees of freedom: 2 ;   Chi-square = 184.077 ; $p \leq 0.001$.   The difference is significant.

Frequency of mortality between species for 10 sites combined

	Success	Failure	Total
Fd	171	729	900
Hw	108	967	1075
Total	279	1696	1975

Degrees of freedom: 1   Chi-square = 32.372; $p \leq 0.001$.   The difference is significant.

Frequency of mortality between species for 10 sites combined

	Success	Failure	Total
Fd	171	729	900
Cw	11	1061	1072
Total	182	1790	1972

Degrees of freedom: 1 ;   Chi-square = 188.670; $p \leq 0.001$.   The difference is significant.

Frequency of mortality between species for 10 sites combined

	Success	Failure	Total
Hw	108	967	1075
Cw	11	1061	1072
Total	119	2028	2147

Degrees of freedom: 1   Chi-square = 83.420; $p \leq 0.001$.   The difference is significant.

APPENDIX XVIII

Chi-square tests of the frequency of mortality (dead trees) and progressive lesions at the root collar (dying trees) among species

Frequency of dead and dying trees among species

	Success	Failure	Total
Fd	234	666	900
Hw	183	892	1075
Cw	11	1061	1072
Total	428	2619	3047

Degrees of freedom: 2   Chi-square = 264.926; $p \leq 0.001$.   The difference is significant.

Frequency of dead and dying trees between species

	Success	Failure	Total
Fd	234	666	900
Hw	183	892	1075
Total	417	1558	1975

Degrees of freedom: 1   Chi-square = 23.700; $p \leq 0.001$.   The difference is significant.

Frequency of dead and dying trees between species

	Success	Failure	Total
Fd	234	666	900
Cw	11	1061	1072
Total	245	1727	1972

Degrees of freedom: 1   Chi-square = 280.452; $p \leq 0.001$.   The difference is significant.

Frequency of dead and dying trees between species

	Success	Failure	Total
Hw	183	892	1075
Cw	11	1061	1072
Total	194	1953	2147

Degrees of freedom: 1   Chi-square = 167.115; $p \leq 0.001$.   The difference is significant.

APPENDIX XIX

Chi-square tests of the frequency of mortality, progressive lesions at the root collar, and callused lesions at the root collar as a proportion of the total number of trees showing above ground symptoms of disease among species.

Frequency of mortality among infected species

	Success	Failure	Total
Fd	171	73	244
Hw	109	113	222
Cw	15	41	56
Total	295	227	522

Degrees of freedom: 2   Chisquare = 43.382; $p \leq 0.001$.   The difference is significant.

Frequency of mortality between infected species

	Success	Failure	Total
fd	171	73	244
Hw	109	113	222
Total	280	186	466

Degrees of freedom: 1   Chi-square = 21.340 $p \leq 0.001$.   The difference is significant.

Frequency mortality between infected species

	Success	Failure	Total
fd	171	73	244
cw	15	41	56
Total	186	114	300

Degrees of freedom: 1   Chi-square = 36.239; $p \leq 0.001$.   The difference is significant.

Frequency of mortality between infected species

	Success	Failure	Total
Hw	109	113	222
cw	15	41	56
Total	124	154	278

Degrees of freedom: 1   Chi-square = 9.011; $p \leq 0.01$.   The difference is significant.

Frequency of progressive lesions among infected species

	Success	Failure	Total
Fd	66	178	244
Hw	73	149	222
Cw	1	55	56
Total	140	382	522

Degrees of freedom: 2   Chi-square = 22.046 $p \leq 0.001$.   The difference is significant.

Frequency of progressive lesions between infected species

	Success	Failure	Total
fd	66	178	244
Hw	73	149	222
Total	139	327	466

Degrees of freedom: 1   Chi-square = 1.890;   For significance at the .05 level, chi-square should be greater than or equal to 3.84.   The difference is not significant. $p \leq 0.20$.

Frequency of progressive lesions between infected species

	Success	Failure	Total
fd	66	178	244
cw	1	55	56
Total	67	233	300

Degrees of freedom: 1   Chi-square = 16.759; $p \leq 0.001$.   The difference is significant.

Frequency of progressive lesions between infected species

	Success	Failure	Total
hw	73	149	222
cw	1	55	56
Total	74	204	278

Degrees of freedom: 1   Chi-square = 22.139; $p \leq 0.001$.   The difference is significant

Frequency of callused lesions among infected species

	Success	Failure	Total
Fd	7	237	244
Hw	38	184	222
Cw	40	16	56
Total	85	437	522

Degrees of freedom: 2   Chi-square = 157.246 $p \leq 0.001$.   The difference is significant.

Frequency of callused lesions between infected species

	Success	Failure	Total
fd	7	237	244
Hw	38	184	222
Total	45	421	466

Degrees of freedom: 1   Chi-square = 27.049; $p \leq 0.001$.   The difference is significant.

Frequency of callused lesions between infected species

	Success	Failure	Total
fd	7	237	244
cw	40	16	56
Total	47	253	300

Degrees of freedom: 1   Chi-square = 162.039; $p \leq 0.001$.   The difference is significant.

Frequency of callused lesions between infected species

	Success	Failure	Total
hw	38	184	222
cw	40	16	56
Total	78	200	278

Degrees of freedom: 1   Chi-square = 65.350; $p \leq 0.001$.   The difference is significant

The probability of significant damage is a function of both of these factors. Differences in DRA-caused damage among Southern Interior BEC zones are largely attributable to differences in inoculum. In all but the driest and wettest site series in the ICH, DRA is universally present. In the other zones Armillaria is distinctly patchwise distributed, typically within large (at least 1 ha and sometimes >10 ha) patches. Subzones can differ significantly and, in theory, a much more precise ranking could be provided at a subzone level. For instance the IDFmw may be similar to the ICH with respect to DRA distribution and damage. However, in general we lack the necessary information to distinguish among subzones.

Resistance (being the ability to ward off or limit infection after exposure to inoculum) depends in part on tree vigour, which would be generally greater in the ICH compared to the IDF, MS, PP and ESSF zones. A revised table of host susceptibility ratings for species is provided in this extension note (Table 1) and only those species that are well adapted to a particular zone are considered. For instance, Bl is only ranked in the MS, ICH and ESSF.

Forest managers need a tool to help make informed decisions with respect to DRA impacts. We have provided such a tool in the form of a decision key in which the various branches lead to different treatments. The main outcomes (treatments) are: 1) ignore DRA and accept a loss of volume at rotation, 2) select more resistant species or uniform species mixtures containing resistant species, and 3) inoculum removal. Use of the latter two may be limited by site factors or management constraints not related to DRA (e.g. erosion, calcareous soils, ecologically sensitive areas, etc.). The necessity of dealing with DRA, when it is present and threatening, is seldom the only consideration in regeneration decisions.

The decision key (Figure 1) aims to differentiate between the distribution of DRA and the extent of damage within the BEC zones/subzones and then suggests appropriate measures to be taken in order to minimize losses due to DRA in the regenerating stand. It takes the form of a 'decision flowchart'.

The host susceptibility table does not describe the frequency or extent of damage on host species within their corresponding BEC zone. The purpose of this table is to present information about the susceptibility of various species to killing by DRA. It is not however intended to replace the Ministry guidelines of preferred and acceptable species for stand establishment.

[a] See Meidinger and Pojar (1991) for an explanation of Biogeoclimatic Ecosystem Classification (BEC) Zone, subZone, and variant abbreviations

Characteristics of Susceptible Stands:

- Newly established and managed stands of highly susceptible conifers (see Table 1), especially in the ICH, but also the IDF, MS, and ESSF zones.
- Moist climatic regions have higher incidence of infection than wet or dry climatic regions. Furthermore, the wettest and driest site series generally have a lower incidence of *Armillaria* than mesic site series.

Hosts: All conifers, deciduous trees, shrubs and some herbaceous plants are susceptible to infection by the fungus. Relative susceptibility of host trees to killing by *A. ostoyae* is given in Table 1.

Table 1. Host susceptibility[1,2] to killing by DRA in 20-80-year-old trees by BEC zone and Species rated as Low, Medium and High.

Species	BEC ZONES				
	PP	IDF	MS	ICH	ESSF
Fd	M	H	H	H	-
Bl	-	-	H	H	H
Bg	-	H	-	H	-
Hw	-	-	-	H	H
S	-	M-H	M-H	M-H	M-H
Py	M	M	-	M	-
Pw	-	-	-	M	-
Pl	-	M	M	M	M
Lw[3]	-	L	L	L	-
Cw[4]	-	L	-	L	L
Ep[5]	-	L	L	L	-
At5	-	L	L	L	L
Ac	-	L	L	L	L

[1] Susceptibility is not a good single index of damage. For example, in undisturbed stands in the IDF, Fd is as susceptible or more so than in the ICH, but is not exposed to inoculum as often as in the ICH. Hence DRA impact on Fd is much lower in the IDF than in the ICH. Ratings are only provided for species common in and suitable for the respective BEC zones

[2] **All conifer species are quite susceptible to killing when young (with the possible exception of Cw).** The ratings here reflect the degree to which they become resistant with age, usually starting about age 15-20.

[3] Lw becomes increasingly resistant to *A. ostoyae* only after the age of 20 years. On good sites, rapid growth characteristics of Lw at early ages enable trees to contact inoculum sooner than other regenerating conifers which results in high mortality rates for Lw in younger stands, comparable to that of Fd.

[4] Mortality rates for young cedar are significantly lower than other conifers in juvenile stands. Smaller trees exhibit high frequency of compartmentalization and callusing at the root collar and the rate of callusing increases with tree size. Hence, resistance in Cw appears to occur much earlier than other conifers.

[5] Ep and At have low susceptibility to killing until about age 40 or until they are overtopped, then susceptibility increases.

Armillaria Root Disease – Southern Interior Forest Region

Note:

[1] Look for presence of DRA in pre-harvest stand. Evidence most commonly found on younger (all-aged stands) or intermediate and suppressed trees. Also consider local experience in similar cutblocks.

[2] Techniques for delineating strata are described in the Root Disease Management Guidebook.
Note: strata known or assumed to be infected need not be surveyed. Surveys detecting even minimal levels of DRA in stratified blocks should advance to the next level in the decision key bearing in mind that in undisturbed mature stands, only a small proportion of infected trees exhibit above ground symptoms. If Phellinus root disease is present, refer to the corresponding decision aid.

[3] Suitable with respect to performance on site and meets Timber Supply objectives of the management unit.

[4] Resistant species are classified as 'L' in the host susceptibility table (Table 1).

Figure 1. Decision Key for different treatment strategies for Armillaria-infested sites by BEC zone for the southern interior region of BC.

323

General Information:

• *Armillaria ostoyae* is the most widespread and damaging root disease pathogen of conifers in the southern interior of BC, causing mortality and, in trees that sustain non-lethal infections, growth repression.

• *A. ostoyae* is a facultative parasite. During its parasitic phase it invades and kills host tissue (mainly roots and fresh stumps). In undisturbed mature stands in the ICH, especially on moist site series, up to 90% of trees will have Armillaria lesions on their roots. Following harvesting, root systems remain alive for a couple years during which time the fungus can escape from small contained infections and invade the whole stump and root system. Spread within the host is limited to the parasitic phase. During the saprophytic phase it uses the invaded tissues as a food source and produces rhizomorphs that seek out new hosts. In this state, it can survive for many years on larger dead roots and stumps.

• Disease spread occurs belowground by mycelial growth across root contacts between infected tissue and healthy, adjacent trees or via rhizomorphs growing through the soil. Infection by spores is very rare.

• Aboveground symptoms of disease include basal resinosus in most conifers, chlorosis of needles, reduced terminal growth, and stress-induced cone crops. However, aboveground symptoms on individual trees are variable and may only become evident immediately preceding death of the tree, particularly in young stands. Close examination under the bark of roots and root collar area is recommended.

• White mycelial fans can be seen under the bark or along the cambial zone on colonized roots. Another species of Armillaria, *A. sinapina,* co-exists with *A. ostoyae* throughout much of its range. *A. sinapina* is usually considered a weak pathogen that at times assumes the role of a secondary parasite, attacking stressed trees. It is difficult to distinguish between *A. ostoyae* and *A. sinapina* in the field as both species form white mycelial fans. If there is tree mortality, assume you are dealing with *A. ostoyae*.

• Rhizomorphs may be found on infected roots or in the soil. Advanced decay appears as yellow stringy white rot. Clusters of honey mushroom fruiting bodies may be formed in the late summer/early fall at the base of infected trees or stumps or overlying infected roots.

• Armillaria may be associated with other root diseases such as *Phellinus sulphuracens* (= *P. weirii*).

• Distribution of the disease can be uniform or patchy depending on the climatic region and/or BEC zone.

• In young plantations and natural stands the disease is manifest as small groups of two or three symptomatic or killed trees and there may be many such groups per hectare.

Harvesting and Silviculture Considerations:

• Many forestry practices affect the incidence and severity of Armillaria root disease. Any practice that creates stumps, especially large ones, increases the amount of inoculum on a site. Rapid regeneration following harvest ensures that young, highly susceptible conifers are exposed to that inoculum when it is at or near its peak potential, resulting in considerable mortality.

• Conventional logging, which leaves stumps in the ground, will increase the inoculum potential of the fungus on the site. Treatment strategies for managing Armillaria root

disease consist of either inoculum reduction (stump removal), or selection of species for regeneration that have a low susceptibility to killing.
•Treatment strategies for Armillaria root disease management will vary by BEC zone (refer to Decision Key). Sometimes no treatment strategies are suitable/available. In such cases a significant yield reduction is to be expected.
•Following partial cutting the build-up of inoculum in stumps allows the pathogen to invade and kill trees in the residual stand. Mortality usually starts about five years after the partial cut and often continues for many years thereafter since each newly killed tree adds fresh inoculum.
•Surveys are not practical for predicting disease levels, especially during the free-to-grow window because mortality in conifers in the regenerating stand usually begins 5-7 years after stand establishment and does not peak until trees reach the age of 10-12 years.
•In the ICH, surveys of disease incidence in undisturbed (pre-harvest) stands are unreliable. If the disease is detected it is clearly present and probably serious. However, the pathogen can be totally quiescent in such stands, (present only as contained root lesions), but following cutting there can be widespread invasion of stumps and root systems and high inoculum potential.

Regeneration/Establishment:
Site Preparation:
•The impact of Armillaria root disease in new plantations can be reduced by mechanical removal of stumps and major roots during or after harvest. Stumping is not possible everywhere because of various site constraints (i.e. slope, calcareous soils, etc.).

Planting:
•When inoculum removal is not an option, regenerating stands with resistant species is preferred.
•Plant conifers that have a low susceptibility to killing by the fungus (Table 1). Use of species from the High and Moderately susceptible categories in very high proportions is not recommended. Early survival of more susceptible host species (i.e. Douglas-fir) could be enhanced if planted in uniform mixtures containing at least (~ 50%) of a resistant host such as western redcedar. Uniform species mixtures comprising up to 50% or resistant conifers (i.e. Cw, Lw) and no more that 50% of hosts with high susceptibility to killing is recommended. If moderately susceptible hosts are available, the proportion of resistant hosts in mixtures may be reduced to 30% provided the proportion of susceptible hosts is less than 40% of the species composition. Choice of species may be limited by ecological suitability.
•Natural regeneration of resistant hosts (i.e. Cw) may be encouraged to increase stocking and species diversity although relying on the ingress of natural regeneration to meet the minimum proportion of resistant hosts is not recommended because it is unlikely to result in a uniform mixture which is required to achieve reduced mortality and disease spread between trees.
•Faster growing species may die more quickly because they tend to contact inoculum much sooner when the inoculum potential of the fungus is high. Lw is a good example.
•Hardwoods, like cedar, may help mitigate damage caused by Armillaria in new plantations. However, hardwoods may become more susceptible to killing after age 40

particularly in situations where they are mixed with conifers and overtopped. Hardwood stumps can become a source of inoculum for the fungus after they die or are cut down.
•Resistant conifers may be fill-planted in openings caused by the disease to improve stocking levels.

Plantation Maintenance:
•Cleaning or brushing of hardwoods can increase the inoculum and, if unavoidable, is best done as early as possible so that the stumps created are small. Cutting of herbaceous material or woody shrubs apparently has little effect.
•Precommercial or commercial thinning increases the amount of inoculum on site and hence disease incidence and severity. This can be avoided by using techniques like pop-up spacing.
•Multiple stand entries maintain high fungal inoculum potential because the stumps that feed the fungus become available at regular intervals.
•Retain or favour healthy planted or naturally regenerated tree species with high resistance to Armillaria (i.e. western redcedar and paper birch) in order to increase barriers to spread by the fungus between susceptible host species.

Potential Productivity Implications:
•Forest management practices that create stumps and rapid regeneration of sites with susceptible hosts may exacerbate disease levels over and above that which would normally occur in nature by exposing trees to inoculum while its potential is still high.
•Armillaria is universally present in all subzones throughout the southern ICH except perhaps on the driest and wettest sites. The proportion of diseased trees that show above ground symptoms is lower in the ICH than any other zone.
•In the MS, IDF, and ESSF, the distribution of Armillaria can be somewhat patchy, occurring as distinct centres typically within larger (1-10+ hectares) patches. Infected patches are usually characterized by scattered single or small clumps of dead and symptomatic trees; however the actual incidence of infection is always noticeably higher than what can be detected above ground.
•Increased inoculum on sites will lead to mortality or growth loss in trees that sustain non-lethal infections, hence reducing ecosystem productivity. Indirect effects of the disease include increased susceptibility to windthrow and insect damage.
•Cumulative mortality in Douglas-fir stands in the ICH can be as much as 20% by age 20-years resulting in unacceptable stocking in juvenile stands.
•The probability of infection by *A. ostoyae* increases with increasing DBH.
•An operational adjustment factor (OAF) developed for Armillaria root disease and applicable to Douglas-fir managed stands in the ICH showed that for medium severity Armillaria infections, the long-term productivity was reduced by 7.2%.

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