# INCIDENCE OF *ARMILLARIA* SPECIES IN PRECOMMERCIAL THINNING STUMPS AND SPREAD OF *A. OSTOYAE* TO ADJACENT DOUGLAS-FIR TREES

by

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**Title of Thesis/Project/Extended Essay**

Incidence of Armillaria species in precommercial thinning stumps and spread of A. ostoyae to adjacent Douglas-fir trees.

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**INCIDENCE OF *ARMILLARIA* SPECIES IN PRECOMMERCIAL THINNING STUMPS AND SPREAD OF *A. OSTOYAE* TO ADJACENT DOUGLAS-FIR TREES.**

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## Abstract

The frequency of *Armillaria* species in precommercial thinning stumps and the reaction of *A. ostoyae* at root contacts between Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) crop trees and thinning stumps colonized by *A.ostoyae* (Romagnesi) Herink were investigated at sites along a transect from the coast through the southern interior of British Columbia. The frequency of stumps colonized by *A. ostoyae* and *A. sinapina* (Bérubé and Dessureault ) varied along lower, mid and upper slope transects. On coastal sites, *A. sinapina* dominated fresh hygrotopes and *A. ostoyae* dominated slightly dry hygrotopes, and the frequency of both was reduced at lower slopes. In interior sites *A. ostoyae* was found over all hygrotopes, but with lower frequency on the driest sites. The distribution of the two *Armillaria* species on sites is apparently determined by anoxia associated with periodically saturated soil and periodically drying soil. At root contacts between colonized stump roots and crop tree roots, transfer and infection by *A. ostoyae* occurred more frequently at moist sites than dry sites. Lesion size on crop tree roots was related to inoculum volume at some sites and to stump root diameter at others. The percentage of lesions on roots at which crop trees formed callus was related to tree bole volume. The results indicate that there will be crop tree mortality following precommercial thinning, especially in the Interior Cedar Hemlock and Interior Douglas-fir biogeoclimatic zones where inoculum levels are high enough.

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# CHAPTER 1 - INTRODUCTION

## 1.1 History and Economic Importance

The first recorded observation of Armillaria root disease, also known as shoestring rot, mushroom rot, crown rot, and oak fungus disease (Agrios 1988) was probably by Micheli (1729). Hartig (1874) first described *Armillaria mellea* (*sensu lato*) as a pathogen of forest trees and described its life cycle, making the connection between its two chief forms, rhizomorphs and mycelial fans. The fungus was assumed to be an opportunistic pathogen because of the widespread occurrence of rhizomorphs in soil without disease symptoms in the surrounding trees. These observations led forest pathologists at the time to believe that disease was linked to the presence of predisposed trees (Day 1929) or to differences in virulence within the pathogen (Childs and Zeller 1929). Developments in genetics allowed *A. mellea* (*sensu lato*) to be separated into several distinct species with different distributions and pathogenicities (Anderson and Ullrich 1979, Hintikka 1973, Korhonen 1978). This helped to explain much but not all of the variation in behavior as described by early workers.

In western North America, Armillaria root disease is a primary causal disease agent on conifers, and occurs most extensively in Oregon, Washington, northern Idaho, western Montana, southern British Columbia (B.C.) (Kile et al. 1991). Smith (1984) estimated average annual loss to five major root diseases to be 6.7 million $m^3$. Shaw et al. (1976) found volume loss in ponderosa pine to have increased from 9 $m^3$/ha to 24 $m^3$/ha in 14 years on managed land in south-central Washington. Filip (1977) in Oregon found 7% of trees in a mixed confer stand to be infected, comprising 32% of stand volume and Filip and Goheen (1982, 1984) in Oregon found annual mortality to be greater than 3 $m^3$/ha on some sites.

In B.C., annual loss to Armillaria root disease is estimated to be 105,000 $m^3$ (Taylor 1986). Armillaria root disease has been described to be of little consequence after age 25 in coastal stands, but can cause serious damage in interior stands, with infection centers covering several hectares and affecting more than 25% of a stand (Morrison 1981, Wargo and Shaw 1985). Reduced incrument increased with increasing basal resinosis and with early infection (Bloomberg and Morrison 1989). Armillaria root disease may predispose conifers to bark beetle attack (Cobb 1988).

More recently, the forest industry in B.C. has recognized Armillaria root disease in planted stands and rated it top priority for forest health research (Nevill and Winston 1994). This is in part due to new forest regulations and a shrinking land base combined with a strong demand for wood products and increasing silvicultural inputs by the forest industry. In addition, partial cuts of the canopy are being proposed for widespread use in B.C. by the environmental movement (Kimmins 1993), and by wildlife managers as a solution to manage habitat (Chambers 1990). This has led to concern among some foresters because of the possibility that partial cuts could exacerbate the effects of Armillaria root disease.

## 1.2 Taxonomy and Nomenclature

The genus *Armillaria* probably contains about 40 species (Watling et al. 1991), most of which cause root diseases of plants. *Armillaria* species belong to the subdivision Basidiomycetes, class Hymenomycetes, order Agaricales, which are fungi with an important and unique niche in their ability to degrade lignin and cellulose (Agrios 1988). Until recently, confusion surrounding the taxonomy and nomenclature of the genus hindered the understanding of the biology and epidemiology. It was generally assumed that *Armillaria mellea* (*sensu lato*) was a single highly variable species with world-wide

distribution. Originally, basidomes were used to describe most of the species, but more recent methods using biological species based on pairing in culture (Anderson and Ullich 1979), DNA (Harrington and Wingfield 1995), and rhizomorph morphology (Mallett 1989) have been developed.

A description of the morphological characteristics of the basidiomes of two common species in B.C., *A. ostoyae* and *A. sinapina*, were given by Bérubé and Dessureault (1988). In *A. ostoyae,* the pileus is 5-10 cm wide, obtusely parabolic then convex and finally plane, generally lightish brown to darker shades of brown; stipe central, 5-20 cm long, 1.5 cm at apex, light brown to mahogany with a thick annulas. In *A. sinapina,* the pileus is 2-6 cm wide, campanulate then convex and finally plano-convex, cinnamon sometimes with dark shades of red and brown; stipe central, 4-8 cm long, 0.5-1.0 cm at apex cream to alabaster with a very thin annulus. Basidiome production is not necessarily associated with actual distribution in nature (Banik et al. 1995) and therefore is less useful for identification purposes in the field.

Biological species tests became possible when Hintikka (1973) observed that single-spore isolates (haploid) produced a culture with fluffy white aerial mycelium, while the colonies from the basidiome (diploid) produced a flat crustose appearance. He also showed that *Armillaria* species were heterothallic, operating on a bifactorial incompatibility system with several alleles at loci A and B. Biological species tests are conducted using a known monosporous (haploid) tester paired with either an unknown monosporous isolate or an unknown diploid isolate from the vegetative mycelium or basidiome. When monosporous cultures of the same compatible species were confronted in culture, the colony morphology changed from white fluffy to flat crustose. Four possible outcomes can occur from this paring: 1) incompatible ($A_1B_1$ x $A_1B_1$) where both colonies do not intermingle and remain with fluffy aerial mycelium; 2) compatible ($A_1B_1$ x

$A_2B_2$) where both colonies intermingle and the fluffy colony becomes crustose in morphology; 3) hemicompatible ($A_1B_1$ x $A_1B_2$) and ($A_1B_1$ x $A_2B_1$) where one of these combinations appears as the incompatible; the other type forms a "barrage zone" between the colonies where sometimes crustose mycelium can be found and aerial mycelium is sparse.

Korhonen (1978) in Finland and Anderson and Ullrich (1979) in North America separated *Armillaria mellea* (*sensu lato*) into several distinct species using haploid-diploid parings. North American biological species were proposed and designated as NABS followed by a roman numeral. In B.C., six North American biological species (NABS) occur, five of which have been described: *A. ostoyae* (Romagnesi) Herink (NABS I), *A. sinapina* Bérubé and Dessureault (NABS V), *A. gallica* Marxmüller and Romagnesi (NABS VII), *A. nabsnona* Volk and Burdsall sp. *nov.* (NABS IX), *A. cepistipes* Velenovsky (NABS XI), and NABS X (Morrison et al. 1985, Watling et al. 1991). *Armillaria ostoyae* and *A. sinapina* occur most frequently in southern B.C. (Morrison et al. 1992). Both species can occupy a single site and have similar appearance in stumps; however, site factors influencing the occurrence of both species are poorly understood.

The identification of field isolates using biological species pairings is time consuming and can lead to ambiguous results. As a result the search continued for a more reliable identification method. Anderson and Stasovski (1992) reported partial DNA sequences for the ribosomal RNA operon for most of the *Armillaria* species in the northern hemisphere. Sequence variation among a limited number of isolates suggested that restriction enzyme digests may be able to discriminate among species. Harrington and Wingfield (1995) used a polymerase chain reaction (PCR)-based method to differentiate *Armillaria* species from North America and Europe. This was based on the

intergenic spacer of the ribosomal RNA operon digested with *Alu I* and electrophoresed on an agarose gel. The DNA was amplified directly in the PCR reaction tube from mycelium grown on malt extract. Unambiguous results may be gained in one day and widespread ecological studies are now feasible.

The growth of rhizomorphs in the soil varies among species; branching is either monopodial or dichotomous (Morrison 1982, 1989). Dichotomously branched species tend to be more pathogenic than monopodially branched species (Morrison 1989). Mallett (1989) could distinguish between *A. ostoyae* (dichotomous) and *A. sinapina* (monopodial) using *in vitro* techniques. Rhizomorph branching pattern can provide additional information complementary to the other identification methods, especially when they produce ambiguous results, and may be useful in the field.

### 1.3 Sexual Life Cycle

In a typical basidiomycete, the result of a compatible mating between two homokaryons is a heterokaryotic mycelium with two or more haploid nuclei in each cell. For *Armillaria* species, the result is a diploid mycelium with uninucleate cells, although older portions of the mycelium, rhizomorphs, and basidiomes are multinucleate (Guillaumin et al. 1991). The current understanding of the sexual cycle is reviewed by Guillaumin et al. (1991). Two haploid monokaryotic mycelium cells originating from spores mate and form a dikaryotic stage with binucleate cells and clamp connections. This stage is transitory in most *Armillaria* species, and within a few days, the dikaryotic cells become monokaryotic by somatic nuclear fusion, diploidization, and then mitosis. The diploid tip cell continues to grow and produces monokaryotic diploid cells. *Armillaria* species are the only known hymenomycete in nature with a diploid vegetative stage.

When conditions are ideal, the diploid thallus produces basidiomes for the purpose of spore production. In *Armillaria* species, the basidiome is diploid monokaryotic; however, in most species the subhymenial cells appear to return to a dikaryotic haploid condition before meiosis occurs. One subhymenial cell donates two haploid nuclei, which undergoes meiosis, to the basidium. The resulting four haploid nuclei migrate into four spores formed on the basidium.

### 1.4 Inoculum and Infection

The primary source of inoculum from which *Armillaria* species spread and cause infection are stumps and dead trees that become colonized from preexisting lesions or by entry from an epiphytic position on the roots (Kile 1980). Stumps can also become colonized by basidiospores (Rishbeth 1970, 1988; Worrall 1994) although artificial inoculations with basidospores have failed. This is apparently an uncommon event, but provides genetic diversity and long range spread. Evidence that colonization by spores does occur is obtained by spatially isolated individuals that are present on some sites and also the colonization newly planted stands not previously used for forestry (Worrall 1994). Moisture may be required for establishment of basidiospores (Rishbeth 1970).

Inoculum longevity is assessed by incubation of woody material to determine if mycelium and rhizomorphs are present, and then by quantifying the rhizomorphs produced. Longevity of the fungus is likely to vary due to host species, stump size, *Armillaria* species, and the ecosystem and microclimate in which the stumps occur. Shaw (1975) found that 30-year-old stumps contained viable inoculum that could produce rhizomorphs. Rishbeth (1985) isolated viable inoculum of *A. bulbosa* from stumps that were 53 years old and still producing rhizomorphs. It seems probable that inoculum could remain on site and be the source for new infections for quite some time.

Individual clones of *Armillaria* species may survive for centuries (Shaw and Roth 1976) and it follows that a series of hosts must be colonized in order for the fungus to survive. Spread of a genotype from one host to the next then becomes important and clonal spread can occur by rhizomorphs and by root contacts. Observations in western North America suggest that rhizomorphs are more common in coastal forests than in interior forests, where root contacts become most important (Morrison 1981, Wargo and Shaw 1985). However, rhizomorph distribution in soil or epiphytically on roots does not assure that this species occupies the stump, although in most instances this is the case (Worrall 1994).

Inoculum potential was defined as the energy of growth of a parasite available for infection of a host, at the surface of the host organ affected (Garrett 1970). Inoculum size, distance between inoculum and host, and the influence of the environment, were the most important determinants of inoculum potential (Garrett 1970). Hypothetically, under field conditions, inoculum is maximal where healthy roots and inoculum are in close contact. When this distance is bridged by rhizomorphs, inoculum potential diminishes with increasing distance. Inoculum quality may also affect inoculum potential because hardwood stumps produce more and longer rhizomorphs than conifer stumps under field conditions (Morrison 1972) and may be a superior food base.

In addition to inoculum potential, the site of infection on the host can be an important factor in disease development. Non-lethal rhizomorph attacks on ponderosa pine (*Pinus ponderosa* Douglas ex. P. Laws & C. Laws.) were associated with lateral roots, while lethal attacks were associated with the root collar (Shaw 1980). A similar pattern was noticed for Douglas-fir (Buckland 1953). Infections on lateral roots can eventually spread to the root collar and tap root and girdle the root collar given suitable conditions for the fungus.

Infection by rhizomorphs is similar in deciduous and conifer hosts and was reviewed by Morrison et al. (1991). Infection begins when the rhizomorph encounters a host and it firmly attaches itself by secreting a mucilaginous substance. A single hypha then penetrates the outer layer of cork cells to anchor the rhizomorph to the root. On smooth bark, rhizomorph branches are formed, colonize the root, and anchor themselves at various points along the root, and the fungus penetrates the cork tissues below by chemical and mechanical means. Beneath the cork, rhizomorphs branch laterally and radially. In scaly bark, rhizomorphs run tangentially under the bark scales and may emerge and colonize new scales successively. Mycelial fans may develop infection wedges beneath the scales and host cell walls turn brown in advance of the fans. At root contacts, mycelial fans initially colonize a patch of outer bark and the patch increases in size and then the fans penetrate the host. Healthy bark is acted upon by toxic substances and enzymes and shallow brown spots appear in the outer parenchyma. Flakes of dead cork cells are sloughed off and new cork layers are formed or the pathogen penetrates the periderm. Bark tissues become necrotic in advance of the mycelial fans.

### 1.5 Physiology of *Armillaria* Pathogenicity and Host Response

Most work on this subject was done before *Armillaria mellea* (*sensu lato*) was separated into several biological species; therefore, the results of some of these experiments may be misleading. Host exudates have been associated with lesions in conifer species (Rykowski 1975, Buckland 1953, Redfern 1978). Physical wounding can also stimulate resin production and studies directly linking this phenomenon are unclear. Alpha-pinene, a constituent of tree resin, was shown to inhibit the growth of *A. ostoyae* and *A. gallica* (Entry and Cromack 1989) and volatile extracts in Scots pine resin reduced growth of *Armillaria* species by one-half (Rishbeth 1972). However, powdered

wound resin from ponderosa pine significantly increased growth of *Armillaria* species (Shaw and Roth 1976).

Compounds produced and stored in the bark tissues may play a role in host defense. An increase in phenol concentration occurs in the inner bark tissue in response to fungal invasion in deciduous species (Wargo 1988). *In vitro* studies have shown that some phenols found in conifers and in deciduous species, such as gallic acid and tannins, can inhibit fungal growth (Wargo 1980). The growth inhibition of *Armillaria* species in the presence of phenols was removed when glucose and ethanol were added to the media, and seemed to be linked to the pathogen's ability to oxidize the phenolic compounds. Factors such as defoilation and drought can change the composition of host root chemicals such as sugars, nitrogens, and alcohols and may affect the host's ability to keep tissues in a reduced rather than oxidized state (Wargo and Shaw 1985). Host-produced enzymes, such as chitinase, have also been found in the bark of oaks and sugar maples and could potentially act on chitin contained in fungal walls (Wargo 1975, 1976).

Meristematic activity leading to cork and callus formation has generally been considered a secondary mechanism of host resistance as a result of some previous biochemical defense process. Successful resistance by a host to rhizomorph infection was associated with the formation of a secondary periderm that halted the infection (Thomas 1934). In Scots pine roots, resistant host trees formed secondary cork which was sloughed off (Rykowski 1975). Necrophylactic periderm (NP), a suberinized layer laid down subsequent to reformation of the periderm at the edges of lesions, was always associated with wounds or pathogen invasion (Mullick and Jensen 1973). NP develops internal to a non-suberinized but ligninified water-impervious tissue to restore the periderm continuity (Mullick 1975). Host susceptibility could result from successful pathogen interference with this regeneration process. Mullick (1977) considered that

phytoalexins were components of the periderm restoration process occurring in cells at the interface between dead and live cells, and their presence was a result of this process. Woodward (1992) proposed a model for healing of Sitka spruce following wounding or attack. Bark phenolic compounds such as stilbenes and terpenes provide the first line of defense following a break in the bark surface by forming a temporary barrier to pathogen spread. Ligninified and suberinized tissues are then formed, followed by necrophylactic periderm behind these layers to establish an intact periderm. In uncontained lesions, the growth of the pathogen is faster than the formation of periderm and the fungus outgrows the host or penetrates directly through periderms (Rykowski 1975).

*Armillaria* species can degrade suberin (Swift 1965), but the importance of suberin degradation has not been determined. *Armillaria* species also produce phenol oxidases during the infection process. In susceptible deciduous trees, oxidation of tissues ahead of the infection front probably occurs by peroxidase and laccase (Wargo 1983, 1984). Oxidized phenols in colonized bark were at least three times higher than in uncolonized bark (Wargo 1984); however, no association between quantity of phenol-oxidizing enzymes and pathogenicity was noticed in five *Armillaria* species, including *A. ostoyae* and *A. sinapina* (Marsh and Wargo 1989).

### 1.6 Factors Affecting Disease Development

In western North America, Armillaria root disease causes more extensive damage in interior regions than on the coast, even though the pathogenic species *A. ostoyae* occurs in both areas (Morrison et al. 1992). Morrison et al. (1985) hypothesized that variation in virulence within *A. ostoyae* could account for this difference in behavior between coast and interior. McDonald et al. (1987a) suggested that differences in disease between the two regions could be accounted for by differing levels of stress in natural

undisturbed forests and that disease was worse in transition zones from wet to dry because the trees were maladapted to the area. They also suggested that in highly productive interior regions, the fungus was in balance with the climax vegetation and that disease occurred only as a result of forest management activities. Elsewhere, disease has been reported to be severe in Mediterranean climates or continental forests (Kile et al. 1991). It was hypothesized that the large food base available to the fungus in wet coastal forests was balanced by the weak pathogenicity of resident *Armillaria* species. In harsh environments that occur in continental type forests, stress has selected for more pathogenic species which can survive on a more limited food base.

*Armillaria* species distribution has not been studied adequately but may help to explain the distribution of disease further since pathogenic and non-pathogenic species occur together but are difficult to separate under field conditions. High levels of sand and low clay content seemed to be linked to the presence of *A. ostoyae* and *A. sinapina* in New York (Blodgett and Worrall 1992). Both species were found together under similar conditions, except that *A. ostoyae* was associated with conifers and *A. sinapina* had no host preference. Sandy soils were associated with *A. ostoyae* in the Netherlands (Termorshuizen and Arnolds 1994) and with mortality due to *A. ostoyae* in pot experiments containing lodgepole pine (Blenis and Mugala 1989). Clay soils overlying a restricting layer were associated with Armillaria root disease in Larch in Japan (Ono 1970) and disease was worst at the edges of hollows where moisture collected. Whitney (1978) in Ontario noticed that mortality due to *A. ostoyae* was most frequent in soils with high sand and low clay content but he also suggested a link to soil moisture. Williams and Marsden (1982) also noticed the probability of *A. ostoyae* center occurrence was linked to soil moisture holding capacity and aspect in Idaho, and suggested that disease might be limited to a particular soil moisture regime.

### 1.7 Precommercial Thinning and Disease

Precommercial thinning, a type of partial cut, is done in 15-25-year-old plantations and in natural stands to favor certain tree species, to reduce stocking to optimum densities, and to remove trees with defects thus improving form and volume of the crop trees. However, precommercial thinning can increase inoculum levels following colonization of the stumps by a pathogenic *Armillaria* species, and colonized stumps are associated with disease in crop trees (Fillip 1979, Kellas et al. 1987). Following stump creation, mycelium can spread proximally and distally from existing lesions on roots (Shaw 1980) to colonize the root system. Mycelium transfers across root contacts established before thinning. These existing root contacts provide a mechanism for the fungus to move from stump to crop tree while inoculum potential is at a maximum.

In B.C., anecdotal evidence suggests that precommercial thinning increases mortality due to *A. ostoyae* in the interior but not on the coast. However, studies elsewhere associating precommercial thinning with disease have provided conflicting results. Koenigs (1969) found that incidence of disease symptoms was greater and that lesions on roots were larger and more numerous in thinned compared to unthinned western red cedar (*Thuja plicata* Donn ex D. Don). Crop trees initially showed increased growth, but this declined 5-10 years after thinning, even though there was apparently ample growing space. In contrast, Johnson and Thompson (1975) and Filip et al. (1989) found that thinning a stand of low density ponderosa pine reduced mortality and increased growth of the remaining crop trees, but these values were statistically insignificant compared to those for controls. They concluded that precommercial thinning did not increase root disease. Filip and Goheen (1995) found that precommercial thinning in four different stand types did not statistically affect mortality

10 years later compared to controls. Nearly one-half of thinned plots showed a trend towards increased mortality compared to controls, radial growth after thinning was less than expected, and stand type was a significant factor in explaining mortality. However, they concluded that thinning did not affect mortality due to root disease and that tree growth increased significantly in the study area.

To date, studies on the effects of thinning have not included excavations to expose root contacts between thinning stumps and crop trees, observations of below-ground infections, and confirmation of the presence of *Armillaria* species in thinning stumps. Thus, the different conclusions reached in these studies may be due to variation in ecosystem type, host species, age class, *Armillaria* species, and initial inoculum levels.

# CHAPTER 2 - THE STUDY

## 2.1 Study Objectives

The reported differences (Morrison 1981) in behaviour of Armillaria root disease between coastal and interior forests were studied with particular reference to: i) determining the proportion of precommercial thinning stumps colonized by *Armillaria* species, and ii) observing the interaction between *A. ostoyae* and Douglas-fir crop trees at root contacts between colonized stumps and crop trees. Experimental sites were selected in biogeoclimatic zones along a transect from the southern coast to the southern interior of B.C ( Figure 1).

## 2.2 MATERIALS AND METHODS

### 2.21 Biogeoclimatic Zones and Site Locations

The biogeoclimatic ecosystem classification system in use in B.C. is based on soils, climate, and indicator plants (Braumandl and Curran 1992, Green and Klinka 1994, Lloyd et al. 1990). Zones are divided into subzones, the subzones into sites series, and the series are located on an edatopic grid comprised of soil hygrotope and trophotope. Study sites were located in four zones along a transect from the coast through the southern interior, the Coastal Western Hemlock (CWH), Coastal Douglas-fir (CDF), Interior Cedar Hemlock (ICH), and Interior Douglas-fir (IDF) zones. Study sites were located within subzones of each zone during 1993 and 1994 (Tables 1, 2). In general, the CWH is the wettest zone, characterized by cool summers with frequent warm dry spells, and mild wet winters; the CDF has warm dry summers and mild wet winters; the IDF has hot dry summers with frequent moisture deficits and cold winters; and the ICH has warm dry summers and cold wet winters, with annual precipitation levels intermediate between

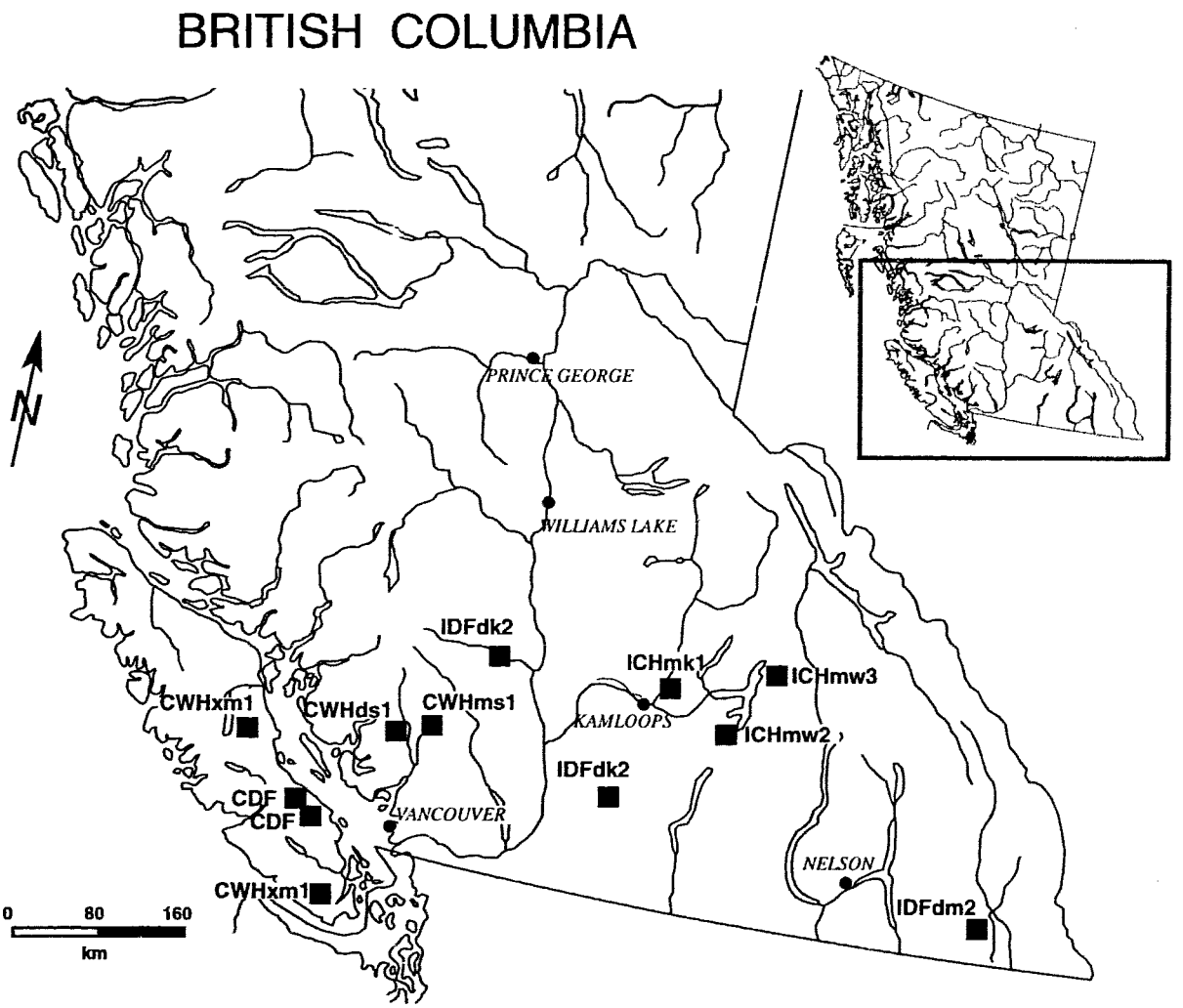


Figure 1 - Location of study sites within British Columbia for stump excavation and stump surveys.

Table 1 - Characteristics of sites chosen for thinning stump excavation to examine *A. ostoyae* stump colonization, transfer of mycelium and lesions formed on crop tree roots.

| Site | Zone/ subzone | Tree age at study | Years since thinning | No. stumps excavated | Actual hygrotope |
|---|---|---|---|---|---|
| 1 | CWHms1 | 26 | 4 | 6 | slightly dry |
| 2 | CWHxm1 | 23 | 5 | 5 | slightly dry |
| 3 | CWHds1 | 26 | 6 | 6 | fresh-moist |
| 4 | ICHmw3 | 22 | 5 | 4 | slightly dry-fresh |
| 5 | ICHmw2 | 24 | 6 | 6 | slightly dry-fresh |
| 6 | ICHmk1 | 23 | 5 | 6 | fresh |
| 7 | IDFdk2 | 25 | 3 | 5 | moderately dry |
| 8 | IDFdk2 | 28 | 5 | 7 | very dry-moderately dry |

Table 2- Characteristics of sites chosen to establish transects for *Armillaria* species frequency in thinning stumps.

| Site | Zone/ subzone | Tree age at study | Years since thinning |
|---|---|---|---|
| 1 | CWHms1 | 22 | 2 |
| 2 | CWHxm1 | 27 | 5 |
| 3 | CWHxm1 | 26 | 4 |
| 4 | CDFmm | 38 | 5 |
| 5 | CDFmm | 28 | 5 |
| 6 | ICHmw3 | 22 | 5 |
| 7 | ICHmw2 | 24 | 4 |
| 8 | ICHmk1 | 23 | 5 |
| 9 | IDFdk2 | 25 | 3 |
| 10 | IDFdk2 | 28 | 5 |
| 11 | IDFdm2 | 70 | 6 |

the CWH and IDF (Meidinger and Pojar 1991). The snow pack in the ICH reduces summer moisture deficits.

### 2.22 Stump Surveys

Surveys for stumps colonized by *Armillaria* species were conducted on three sites in each of the CWH, ICH, IDF and two sites in the CDF (Table 2). Sites had been thinned at least two years previously allowing time for stump colonization to occur. They were stratified into upper, mid, and lower slope positions.

A transect 4 m wide was established across slope at each slope position, and the first 10 conifer stumps encountered that were > 7 cm in diameter at stump height were examined. Soil was removed from the primary roots for up to 40 cm from the stump base, and the occurrence of rhizomorphs on root surfaces and sub-cortical mycelial fans typical of *Armillaria* species was recorded. For each stump, a section of root with sub-cortical mycelial fans was taken for culturing and *Armillaria* species identification. Indicator plants and soils were used to locate all transect lines on the edatopic grid for that subzone. Actual soil hygrotope was then determined (Klinka et al. 1989), to estimate the growing season soil moisture balance (Table 3), enabling comparisons to be made between sites with respect to soil moisture.

### 2.23 Stump Excavations

Stumps were excavated on three sites in each of the CWH and ICH, and two sites in the IDF. Suitable sites could not be located in the CDF. Sites were carefully chosen to minimize variation in crop tree age and years since thinning (Table 1). Each stump was located on the edatopic grid as described for stump surveys, and actual soil hygrotope was determined for the area around each stump as described by Green and

Table 3- Actual soil moisture regime from Klinka et al. (1989).

| Hygrotope | Moisture balance |
|---|---|
| very dry | >105-150 days deficit |
| moderately dry | >45-105 days deficit |
| slightly dry | >0-45 days deficit |
| fresh | no surplus or deficit |
| moist | slight surplus with possible temporary high water table |

Klinka (1994), and Lloyd et al. (1990). Stumps for excavation were selected using the following criteria: similarity of stump diameters within each site, occurrence in areas with disease symptoms indicative of *A. ostoyae*, and presence of possible root contacts to Douglas-fir trees. Crop trees associated with the stumps were alive at the time of excavation. Stumps were excavated by hand to minimize disturbance to root contacts, and roots were exposed down to 5 mm in diameter. The diameter of the stump root at each contact with a crop tree root and the distance from the root contact to the stump root collar along the root were recorded.

The volume of each stump root was calculated from distance-diameter measurements using the formula for a frustum of a cone. The presence of sub-cortical mycelial fans was recorded for each measurement. The total root volume and colonized root volume were determined for each stump. Bole volume was calculated in the same manner, measured from the soil line downward, also noting fungal presence.

A sample was taken from one lateral root of each stump for *Armillaria* species identification. A root sample was taken from lesions on trees for culturing and pairing with the isolate from the contacting stump.

### 2.24 Crop Tree Measurements

Tree height and diameter at breast height, 1.3 m (dbh), were measured for crop trees associated with excavated stumps. These values were used to calculate tree bole volume above ground using the formula for volume of a cone above 1.3 m, and for a cylinder below 1.3 m.

The surface area occupied by fans and necrotic tissue (lesion) on crop tree roots resulting from contacts with stump roots was calculated. Proximal and distal lesion areas were measured separately for each girdled tree root from the point of root contact. Tree roots with lesions on one side of the root only and not girdling the root were called

proximal lesions because they were still surrounded by healthy tissue. The distance along the tree root from the root contact to the crop tree root collar and the tree root diameter at the contact were measured.

Host reaction at the contact point was recorded with respect to callus formation, resin flow, bark hypertrophy and adventitious roots.

### 2.25 Isolation and Identification of *Armillaria* Species

Wood chips from root samples taken from survey stumps, excavated stumps and lesions on crop trees were all cultured directly on malt extract broth (MEB) with 1.5% agar, 2 g/l (a.i.) benomyl and botran (Worrall 1991) amended with 2 g/l streptomycin, pH 5. Isolates were subcultured until pure and then transferred to 1.5% MEB with 1.5% agar on slants for storage. All isolates were identified to species using dimon mating tests (Korhonen 1978) except that pairings were done on 1.5% MEB. Monosporous testers of B.C. species were obtained from the Canadian Forest Service. *Armillaria ostoyae* stump isolates from excavations were challenged in a diploid-diploid pairing with corresponding isolates from crop tree lesions to determine genotype similarity (Anderson and Ullrich 1982), based on pigmented zone formation in the agar medium below the mycelial front. In pairings between diploid field isolates and haploid testers, the fluffy white mycelium of the haploid turned crustose when compatible. Isolates from stump surveys and stump excavations were also identified using analysis of restriction fragment length polymorphisms of the intergenic ribosomal region between the 26s and 5.8s genes (Harrington and Wingfield 1995).

### 2.26 Statistical Analysis

Statistical analyses were done using the SAS statistical package (SAS Institute Inc., Cary, NC). In all cases significant results were considered at $P<0.05$. Multiple comparisons were done using the Bonferroni t-test at alpha=0.05.

Frequency data for transfer and infection were analyzed by Chi square tests. Mean frequency data for species found in stump surveys was analyzed by one-way ANOVA, using General Linear Models (GLM) procedure, for effects of hygrotope and slope position on each species separately. A square root transformation on data for *A. ostoyae* in interior hygrotopes and IDF slope positions was used. A paired difference test was used to determine differences between mean frequency of both species within hygrotopes and slope positions.

The colonization of stump roots by *A. ostoyae* was evaluated by calculating the proportion of root segments (usually branch to branch points) containing mycelial fans in each stump root diameter class. A one-way ANOVA using GLM was performed to detect effects of zone on colonization of each root diameter class separately using stumps as replicates. A paired difference test was used to detect differences between root diameter classes within zones.

Proximal lesion size was correlated to inoculum volume using the GLM procedure. The model used for the CWH and ICH was of the form $y = a + bx_1 + cx_2$ where y=proximal lesion size, a=intercept, b= coefficient of root inoculum volume, and c= coefficient for bole inoculum volume; however this was not sufficient for the IDF and a second model was used of the form $y = a + bx$ where b= coefficient of root diameter of the stump at the contact point. For the first model, stump bole inoculum volume and root inoculum volumes were analyzed as separate parameters. Lesion size was calculated by summing areas of the proximal lesions associated with one stump. Each colonized stump root volume associated with a lesion was then summed and used with colonized bole

volume to correlate lesion size with stump inoculum volume. This was necessary because of the difficulty in determining which portion of the bole was responsible for each lesion. For the second model each root was treated separately.

## 2.3 RESULTS

### 2.31 Stump Surveys - Incidence of *Armillaria* species in precommercial thinning stumps

Two species, *A. sinapina* and *A. ostoyae* were isolated from stumps at 11 sites (Table 4 ). The highest incidence of *A. sinapina* occurred in coastal sites (1-5), while *A. ostoyae* occurred most often in interior sites (6-11). *Armillaria sinapina* was found in decreasing frequency in the CWH (36%), ICH, CDF, and IDF zones. *Armillaria ostoyae* was found most often in stumps of the ICH (52%), followed by the CWH and IDF, and finally the CDF.

The incidence of *A. sinapina* and *A. ostoyae* in stumps differed among lower, mid, and upper slope transects and among coastal and interior locations; however, except for *A. ostoyae* in the IDF, significant differences were not found. However, *A. ostoyae* in the CWH and *A. sinapina* in the CDF approached significance (P=0.07 and P=0.06 respectively)(Figure 2). Trends in species distribution were consistent in the IDF, ICH, and CDF towards lower frequency at upper slopes. In the CWH, both species were found less often in stumps at lower slopes than *A. ostoyae* at upper slopes. At midslope, *A. sinapina* was more common than *A. ostoyae*, while the opposite was true at upper slopes. In the CDF, *A. sinapina* was only found at lower slopes and *A. ostoyae* at low

Table 4 - Incidence of *Armillaria sinapina* and *A. ostoyae* in thinning stumps at stump survey sites.

| Site | Zone/ subzone | Number of stumps | % stumps *A. sinapina* | % stumps *A. ostoyae* |
|---|---|---|---|---|
| 1 | CWHms1 | 40 | 33 | 23 |
| 2 | CWHxm1 | 30 | 23 | 20 |
| 3 | CWHxm1 | 29 | 45 | 24 |
| total | CWH | 99 | 33 | 22 |
| 4 | CDFmm | 30 | 13 | 23 |
| 5 | CDFmm | 30 | 3 | 0 |
| total | CDF | 60 | 8 | 12 |
| 6 | ICHmw3 | 31 | 3 | 45 |
| 7 | ICHmw2 | 35 | 3 | 77 |
| 8 | ICHmk1 | 28 | 36 | 28 |
| total | ICH | 94 | 13 | 51 |
| 9 | IDFdk2 | 29 | 3 | 24 |
| 10 | IDFdk2 | 32 | 16 | 47 |
| 11 | IDFdm2 | 30 | 0 | 17 |
| total | IDF | 91 | 6 | 30 |

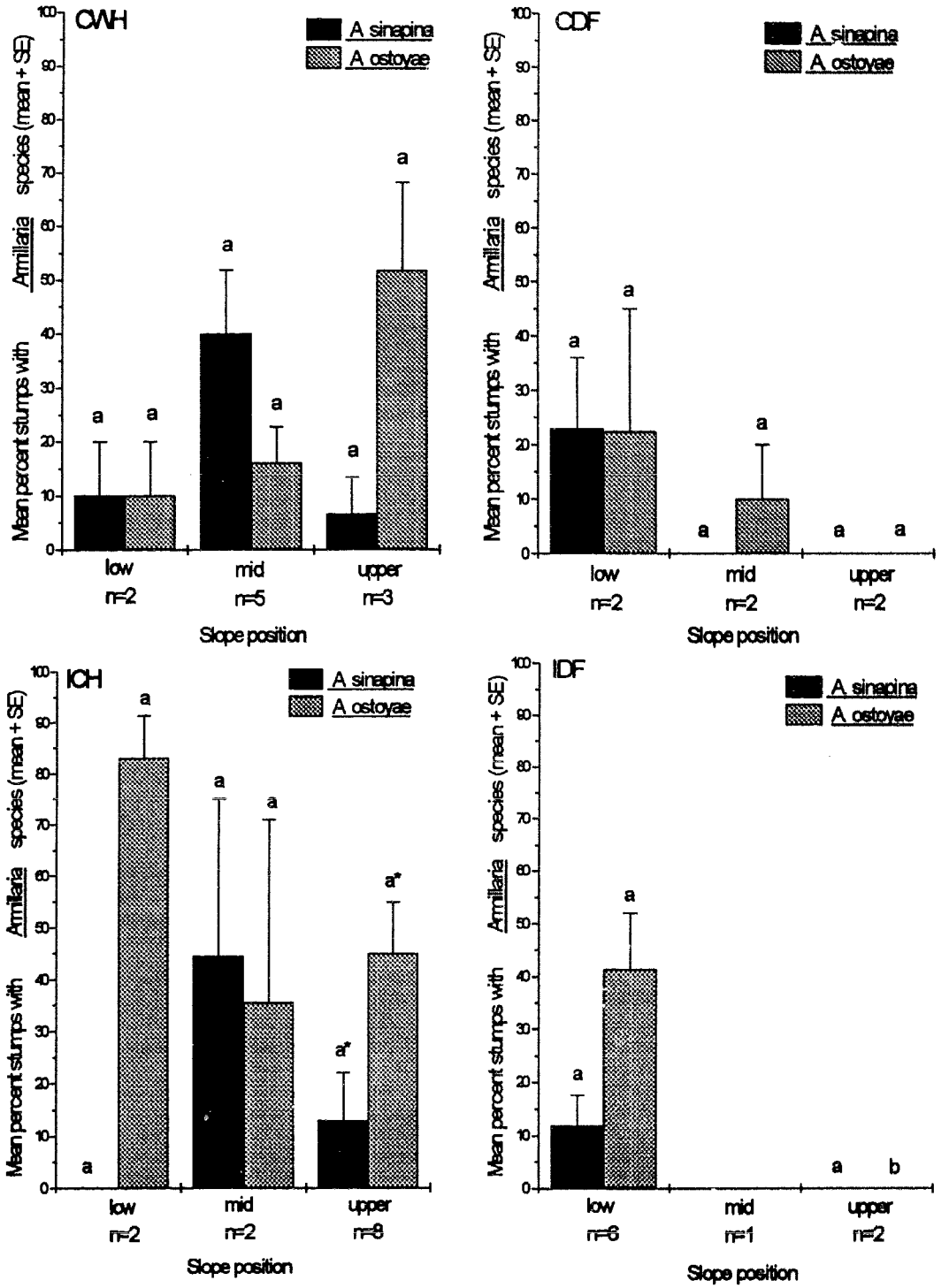


Figure 2 - Percentage of stumps colonized by *Armillaria sinapina* and *A. ostoyae* along lower-, mid-, and upper-slope transects for the CWH , CDF, ICH, and IDF zones. Letters above bars indicate differences between slope positions for one species, * indicates differences between species within slope positions and n=number of transect lines.

and mid slopes. In the ICH, the frequency of *A. ostoyae* was greatest at lower slopes and least on upper slopes, but remained high throughout the sites; however *A. sinapina* was only found at mid and upper slopes and at significantly lower levels than *A. ostoyae* in upper slopes. In the IDF, *A. ostoyae* dominated lower slopes and both species were absent from mid and upper slopes.

Classification of stumps according to actual hygrotope showed that *Armillaria* species were absent or infrequent in dry hygrotopes in both coastal and interior regions (Figure 3). However, on the coast the frequency of both species was also low on moist hygrotopes. On the coast, *A. sinapina* was absent from moderately dry hygrotopes, and was most common on fresh hygrotopes, while *A. ostoyae* was most common on slightly dry and moderately dry hygrotopes. In the interior, *A. ostoyae* was more prevalent over all hygrotopes compared to *A. sinapina*, and *A. sinapina* was absent on the very dry hygrotopes.

### 2.32 Colonization of Excavated Stumps

Over all zones, a mean ranging from 83-89% of stump volume was colonized by *A. ostoyae*. This species was found less frequently ($P<0.05$) in roots 5-10 mm diameter in the IDF and CWH than in the ICH. The same trend was evident for roots > 10 mm diameter but differences were not significant ($P=0.08$) (Table 5). No differences were detected between root diameter classes within a zone. Differences in mean frequency of *A. ostoyae* in stump root segments could not be attributed to a single site.

### 2.33 Transfer of *A. ostoyae* Across Root Contacts

In this study, transfer is defined as the movement of mycelium from a colonized stump root into the bark of the crop tree. Infection is defined as visible necrosis of cambial tissues. Mycelium from colonized stumps transferred across root contacts and

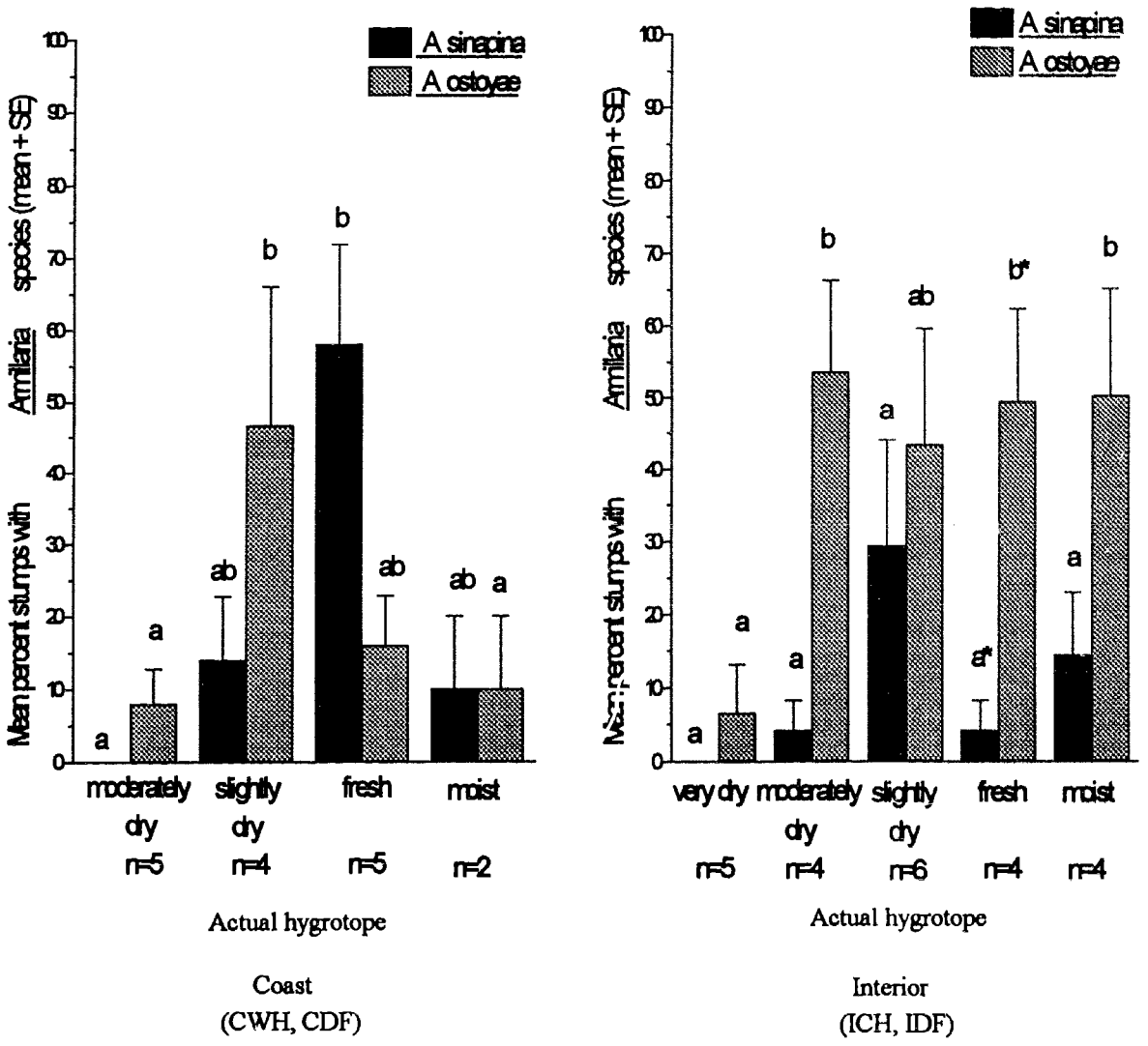


Figure 3 - Percentage of stumps colonized by *Armillaria sinapina* and *A. ostoyae* in locations classified according to actual soil hygrotope (Klinka et al. 1989) for coastal and interior sites. No sites for very dry hygrotopes were sampled on the coast. Letters above bars indicate differences between hygrotopes for one species and * indicate differences between species within hygrotopes, both at $P<0.05$.

Table 5 - Mean percent of stump root segments 5-10 mm and >10 mm diameter colonized by *A. ostoyae*. Letters following percentages indicates differences between zones and * indicates differences between root classes, both at $P<0.05$.

| Zone | Number of stumps | Mean % root segments colonized 5-10mm | Mean % root segments colonized >10 mm |
|---|---|---|---|
| CWH | 17 | 72a | 76a |
| ICH | 16 | 91b | 89a |
| IDF | 12 | 72a | 76a |

initially formed a patch of ectotrophic mycelium in the outer bark of the crop tree root. This was followed by penetration of the periderm. Transfer of mycelium did not occur at root contacts found in humus layers of the CWH. Root contacts were absent in this layer in other zones, since this horizon was poorly developed and roots were found in the underlying mineral soil.

In the ICH, the numbers of stump root contacts for roots 5-10 mm in diameter and >10 mm diameter was similar, while in the CWH and IDF, there were twice as many in the >10 mm diameter class (Table 6). At root contacts, the percentage of stump roots transferring mycelium and the percentage of stump roots causing infection on crop tree roots were greatest in the ICH and least in the IDF (Table 6); however, these differences were not significant. In the CWH only, both transfer of mycelium and infection of crop trees occurred more frequently ($P<0.05$) for large than small stump roots.

### 2.34 Lesion Size

The GLM procedure revealed a significant correlation between lesion size and inoculum volume for the ICH and CWH ($P<0.01$) but not the IDF (Table 7). Average lesion size on crop tree roots was largest in the CWH (562 $cm^2$) and ICH (549 $cm^2$), and least in the IDF (49 $cm^2$); however, average colonized stump volume was largest in the CWH (10,127 $cm^3$) and least in the ICH (4803 $cm^3$). Stump root and bole volume were not good predictors of lesion size in the IDF so a second model was fitted to the data (Table 7). The second model used stump root diameter at the contact point with the crop tree root as a predictor of lesion size. Lesion size on crop tree roots was proportional to the diameter of the contacting stump roots in the IDF. Stump root diameter was not a significant factor in the CWH or ICH.

Table 6- Number of stump roots containing *A. ostoyae* in root contact with crop trees and the percentage of those roots transferring mycelium to crop trees and causing infection. Letters following numbers indicate differences between zones and * indicates differences between root classes, both at $P<0.05$.

| Zone | No. stump roots with mycelium and contacts to crop trees | | %Stump roots transferring mycelium to tree roots | | %Stump roots causing infections on tree roots | |
|---|---|---|---|---|---|---|
| | 5-10 mm dia | >10 mm dia | 5-10 mm dia | >10 mm dia | 5-10 mm dia | >10 mm dia |
| CWH | 25 | 62 | 44 a* | 71 a* | 44 a* | 63 a* |
| ICH | 28 | 23 | 64 a | 69 a | 57 a | 69 a |
| IDF | 10 | 22 | 50 a | 48 a | 40 a | 39 a |

Table 7- Correlation between *A. ostoyae* proximal lesion size on crop tree roots and inoculum volume of the contacting colonized stump. Model is of the form $y = a + bx_1 + cx_2$ where a=intercept, b=coefficient for stump root inoculum volume, and c=coefficient for stump bole inoculum volume. This model was not significant for the IDF as shown and a second model of the form $y = a + bx$ was used where b=coefficient for stump root diameter.

| Zone | P (model) | $R^2$ | n | Intercept | Source | Coefficient | P type III |
|---|---|---|---|---|---|---|---|
| CWH | 0.0038 | 0.67 | 13 | 139.67 | root | 0.11 | 0.004 |
| | | | 13 | | bole | 0.08 | 0.015 |
| ICH | 0.0001 | 0.94 | 15 | -313.42 | root | 0.43 | 0.0001 |
| | | | 15 | | bole | 0.55 | 0.0001 |
| IDF | 0.4524 | 0.33 | 8 | 8.46 | root | 0.005 | 0.7426 |
| | | | 8 | | bole | 0.005 | 0.5019 |
| IDF | 0.0054 | 0.69 | 11 | -6.91 | root diameter | 24.58 | 0.0054 |

The regression coefficients for root and bole inoculum volume indicate the magnitude of the relationship between these parameters and lesion size (Table 7). As root or bole inoculum volume increased, lesion size increased more rapidly in the ICH than in the CWH ($P<0.01$). Differences in lesion size were not influenced by a single site within a zone.

**2.35 Host Response to Infection**

Crop trees in the CWH grew more rapidly, 3 times the ICH and 4 times the IDF, ($P<0.05$) than trees of a similar age (Figure 4). The percentages of roots at which the tree formed callused lesions were associated with tree bole volume (Figure 4) and were significantly greater ($P<0.05$) in the CWH than in the other zones. Significant differences between study sites within a zone were not detected.

Other crop tree responses to infection were bark thickening several centimeters proximal to the lesion front, adventitious roots, and resin production. Bark thickening was a common response of many infected roots in IDF (80%), CWH (67%) , and the ICH (44%). Lesions with callus but without bark thickening were all found on roots $\geq 3$ cm in diameter. Bark thickening was generally least on large diameter roots and at the root collar. The presence of resin near the margin of infection on roots was most common in the CWH (86%) and ICH (80%), and lowest in the IDF (33%); it was most noticeable near large diameter roots and the bole. Adventitious roots arising from infected roots occurred most frequently in the ICH (25%), followed by the CWH (17%), and the IDF (6%). On almost all of the small roots, the symptoms of host response were not evident until the lesion had reached at least 4-6 year old root tissues.

Pairs of *A. ostoyae* isolates from stump roots and corresponding crop tree lesions were found to be compatible in all cases.

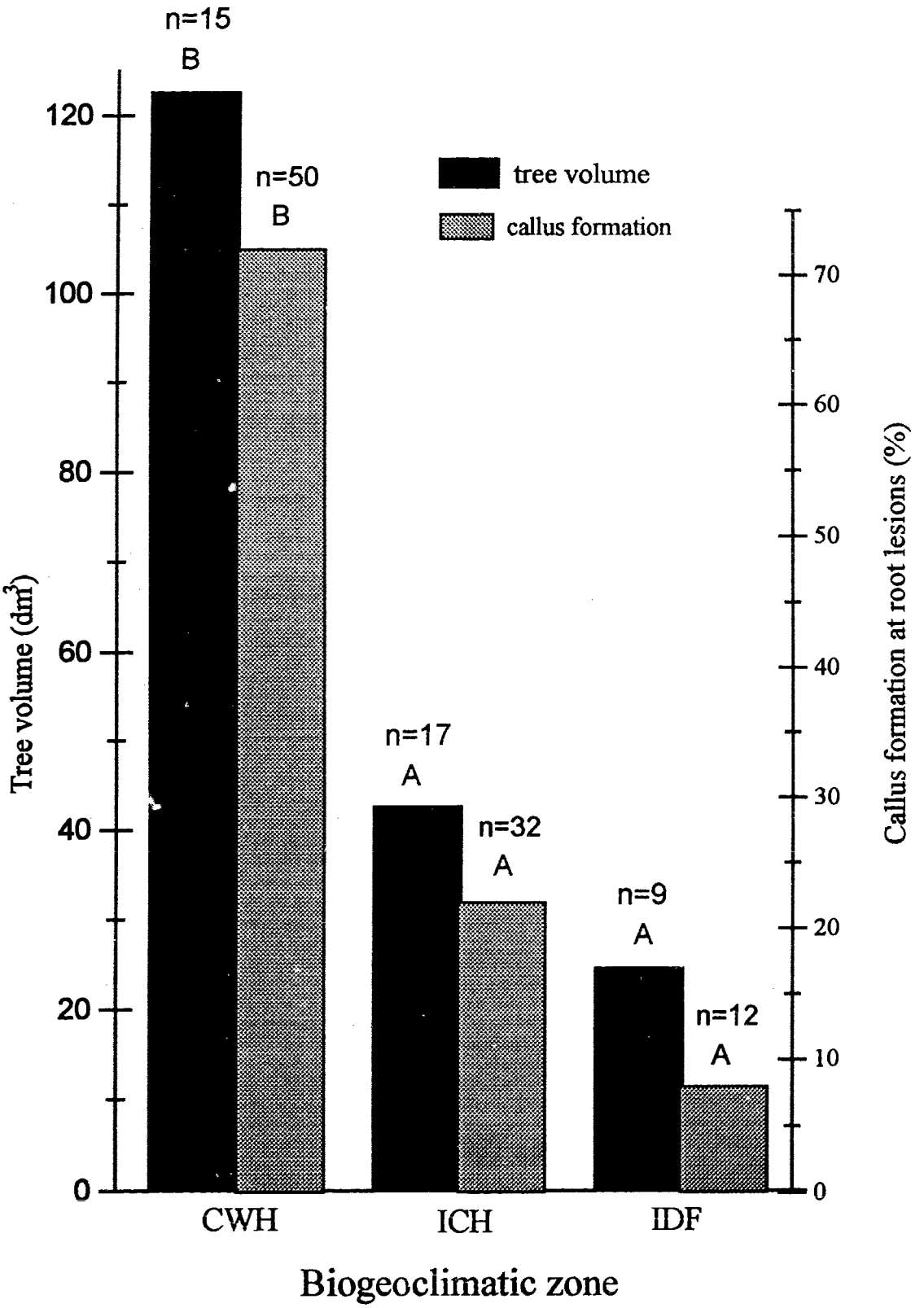


Figure 4 - Callus formation at root lesions on crop trees and tree volume for each zone. Different letters indicate significant differences at $P<0.05$.

## 2.4 DISCUSSION

### 2.41 Stump survey

Actual soil moisture regimes or hygrotopes (Klinka et al. 1989) are based on the annual water balance and the depth of the growing season water table. In this study they facilitated comparison of frequency data for *Armillaria* species in stumps among sites in each of the coastal and interior regions.

The frequency of thinning stump colonization by an *Armillaria* species is a measure of the suitability of the site stratum (hygrotope) for survival and spread of the fungus. Fresh hygrotopes were optimal for *A. sinapina* on CDF and CWH sites and on slightly dry hygrotopes in IDF and ICH sites. The species was absent or infrequent in stumps on very to moderately dry hygrotopes and low on the wettest ones of interior zones. Coastal sites optimal for *A. ostoyae* occurred in slightly dry hygrotopes, while on interior sites a broad range of hygrotopes was optimal.

The occurrence of actual hygrotopes on the transects at lower, mid and upper slope positions varies from zone to zone. The frequency of the two *Armillaria* species in hygrotopes gives rise to the patterns at lower, mid and upper slope positions in the zones.

The moisture content of branches lying on the soil showed seasonal fluctuations which were generally correlated with rainfall (Boddy 1983). Their moisture content varied from near fiber saturation in the dry autumn to saturation in the winter. It is reasonable to assume that the moisture content of dead stump roots in soil follows a similar pattern.

Although *Armillaria* species growing in stem segments survived and produced rhizomorphs over a broad range of soil moisture, they lost viability during four months in saturated soil (Pearce and Malajczuk 1990, Redfern 1970). Rhizomorph production from inoculum blocks and the number of infected roots were lower in pots of larch seedlings

with a high water table than in control pots (Ono 1970). Poor rhizomorph growth and loss of viability are probably caused by anoxia. The critical duration of that condition for survival of *Armillaria* species in woody inoculum is not known.

Continued growth of rhizomorph tips through soil depends on the tip being covered by a film of water (Smith and Griffin 1971). Below a critical soil moisture level the tip becomes melanized and growth ceases. Seasonal drying explains the paucity of *A. luteobubalina* rhizomorphs in Australian soils (Pearce and Malajczuk 1990) and of *A. gallica* Marxmüller and Romagnesi in the upper soil layers of dry sites in Britain (Morrison 1976, 1991). Because it is weakly pathogenic (Morrison et al. 1985), *A. sinapina* depends on an extensive rhizomorph network in soil and on root surfaces in order to exploit newly available food bases, like thinning stumps. Consequently, spread of *A. sinapina* would be affected more by seasonal drying than *A. ostoyae* which is strongly pathogenic and thus able to spread through living root systems. The distribution of the two *Armillaria* species on sites is apparently partly determined by anoxia associated with periodically saturated soil and periodic drying of the soil. The ability of trees to form callus and limit the amount of inoculum and spread of pathogens may also affect distribution.

In the wettest month, the CWH receives twice as much precipitation as the other zones (Reynolds 1992), and precipitation and seepage from upslope result in periodic saturation of the soil profile at the lower slope position in autumn, winter and early spring (McMinn 1960). Much of the winter precipitation in the ICH is snow, which melts slowly, thus reducing summer moisture deficits. Summer precipitation is higher in the CWH and ICH than in the other zones. Hence, favorable conditions for *Armillaria* species occur at the mid and upper slope positions in the CWH and at all slope positions

in the ICH. In the drier zones, favorable conditions occur only at lower and mid (CDF only) slope positions.

The information on *Armillaria* species distribution reported here is in general agreement with that of Whitney (1984) and McDonald et al. (1987b). The latter reported that on the National Forests of the northern Rocky Mountains, *Armillaria* species were absent from warm-dry and cold-wet habitat types. Whitney (1984) found that three conifer species were more heavily attacked on dry sites than on wet ones. In addition, Williams and Marsden (1982) found that disease incidence in Idaho was greater on wet sites with low water retention and on dry sites with good water retention. Interestingly, the incidence of mortality in Douglas-fir stands in coastal sites caused by *Phellinus weirii* (Murr.) Gilbn., another root disease fungus, increased with position up slope (Kastner et al. 1994).

### 2.42 Stump colonization by *A. ostoyae* and transfer to crop trees

Although the percentage of stump and root volume colonized by *A. ostoyae* was similar among zones, there were differences in the percentages of roots colonized in the two diameter classes. Roots 5-10 mm in diameter contribute little to inoculum volume, but provide an avenue of inoculum transfer to suscepts. These roots would be affected by the surrounding soil environment to a greater degree than larger roots because of their large surface to volume ratio. Soil moisture conditions in the ICH are more uniform during the year due to late snow melt and summer precipitation (Lloyd et al. 1990); consequently, a higher proportion of 5-10 mm diameter roots were colonized in the ICH than in the other zones. In the CWH, these roots were colonized to a lesser degree than the ICH probably because wet soil conditions in the spring and fall can cause the death of small roots (Day 1963 and Eis 1970), which then become colonized by other fungi. In the IDF, the driest zone, these roots were probably less colonized because repeated

drying associated with low soil moisture probably affects colonization of roots and survival of inoculum.

There is a paucity of information on the rooting habit of juvenile Douglas-fir (McMinn 1963), especially under different growing conditions like those found in the CWH, IDF and ICH. In this study, excavated root systems in the CWH and ICH had well developed lateral roots near the soil surface while in the IDF roots were found deeper in the soil profile. The root systems in the CWH had more roots and covered a larger area than those in the ICH, reflecting the larger size of trees in the former zone. The number of inter-tree root contacts depends on inter-tree distance, rooting depth, and most importantly, number of roots (Reynolds and Bloomberg 1982). These factors affected the frequency of contacts between stump and tree roots so that the number was small for 5-10 mm roots in the IDF and large for >10 mm roots in the CWH.

There was a trend toward lower frequency of *A. ostoyae* transfer and infection at all contacts with crop tree roots in the IDF and at contacts between 5-10 mm stump roots and crop tree roots in the CWH. Periodic low soil moisture in the IDF may affect transfer of mycelium and the initial stages of lesion development on roots in the IDF. In the CWH, many roots 5-10 mm in diameter may lack the inoculum potential to transfer to and infect roots on vigorous crop trees.

The incidence and size of *A. ostoyae* lesions on crop trees is determined by the inoculum potential of the fungus and the resources available to the host for defense. While average lesion size was the same for the CWH and ICH zones, a smaller volume of inoculum was needed to form a lesion in the ICH. Root and bole volume were good predictors of lesion size in the ICH and CWH. Colonized stump volume in the IDF was similar to that in the ICH, but lesions associated with stumps in the IDF were much smaller, suggesting that less of the stump was suitable as a substrate or that the inoculum

potential was lower at root contacts in the IDF. In the IDF, after colonization of stump bole and root tissues, those above and near the ground line dry out, and *A. ostoyae* retreats below ground, as indicated by a series of pseudosclerotial plates on the stump bole. Pseudosclerotial plates are formed in response to fluctuating moisture conditions (Lopez-Real and Swift 1975). While stump root and bole volumes predicted lesion size in the ICH and CWH, they were not suitable predictors in the IDF. Stump root diameter at the contact point was the only significant predictor of lesion size in the IDF, and larger lesions were associated with larger stump roots, unlike the other zones.

Crop tree age in the three zones where stumps were excavated was similar, 22-28 years; yet, bole volume in the CWH was 3-4 times that in the interior zones, reflecting the better growing conditions of the CWH. The vigorous growth of trees in the CWH is likely responsible for the much higher rate of callusing at lesions. Buckland (1953) observed that in trees of good vigor advance of the fungus was checked by a callus and resin barrier. Bark thickening did not show a pattern of frequency across zones that could be related to any characteristic of the tree species or zone. However, the frequency of resinosis at infections and adventitious rooting was higher in the moist zones (CWH and ICH) than in the dry IDF.

### 2.43 Implications for management of young stands

Of the six *Armillaria* species occurring in B.C. only *A. ostoyae* kills conifers in production forests (Morrison et al. 1985). The stump survey showed that, depending on biogeoclimatic zone, from 12 to 52% of precommercial thinning stumps were colonized by *A. ostoyae*. Hence, following precommercial thinning there is an increase in the amount and potential of *A. ostoyae* inoculum on the site.

In the CWH, there may be a flush of infection and mortality because of the increase in inoculum potential. However, any flush should be brief because the rapid

juvenile growth of trees in this zone resulted in 70% of root lesions being callused. An increase in post-spacing stocking density, selection of the most resistant tree species and selection for the largest crop trees regardless of inter-tree distance should minimize mortality.

In the ICH and where adequate moisture occurs in the IDF, about one-half of the conifer stumps were colonized by *A. ostoyae*. About 40% of the stump root-tree root contacts in the IDF and 60% in the ICH result in infection of the crop tree, and at only about 10% and 30% of those infections respectively does the tree produce callus. The limited ability of most trees to produce callus at infections is related to their much slower juvenile growth rate (compared to trees of the same age in the CWH). Crop tree mortality means the quantity and quality of inoculum on site will remain high, and the flush of mortality could continue. Where practicable, pop-up spacing should be practiced in interior zones to prevent inoculum increase.

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