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 interaction at root contacts between Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] crop trees and stumps colonized by *Armillaria ostoyae* (Romagn.) Herink were investigated at sites in four biogeoclimatic zones 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 among 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 fungi was low on moist hygrotopes. On 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 periodic soil saturation, by drying of the soil, and by host response limiting spread of pathogenic species.

At root contacts between colonized stump roots and crop tree roots, transfer and infection by *A. ostoyae* occurred more frequently in moist biogeoclimatic zones than dry ones. 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 associated with tree bole volume. The results indicate that there will be crop tree mortality following precommercial thinning, especially where inoculum levels are high in the Interior Cedar Hemlock and Interior Douglas-fir biogeoclimatic zones.

INTRODUCTION

Armillaria species cause root disease in natural forests world-wide (Kile et al. 1991), and can cause serious economic loss resulting from mortality and growth reduction. Historically, the disease was attributed to one species, *Armillaria mellea* (sensu lato), which was later divided into several species (Watling et al. 1991). The division helped to explain much of the variation in characteristics seen over large geographic areas. In British Columbia (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 site and have similar appearance in stumps; however, site factors influencing the occurrence of both species are poorly understood.

In western North America, *A*. *ostoyae* is the causal organism associated with disease in conifers (Wargo and Shaw 1985). In the southern one-third of B.C., Armillaria root disease occurs in most biogeoclimatic zones (Morrison et al. 1992). Mortality is common on trees in all age classes in the interior biogeoclimatic zones, but is reported to be rare in trees after age 25 in coastal biogeoclimatic zones (Morrison 1981; Wargo and Shaw 1985). Ecosystem type or micro habitat have been mentioned as factors affecting epidemiology of Armillaria root disease (Byler et al. 1986; Hagle and Goheen 1988; McDonald et al. 1987; Morrison 1981; Ono 1970; Shearer and Tippett 1988).

Precommercial thinning, a type of partial cut, is done in 15-25-year-old plantations and in natural stands to favor certain tree species, reduce stocking to optimum density, and remove trees with defects thus improving form and volume of the remaining 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 (Filip 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. Root contacts established before thinning provide a pathway 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 resulting from *A*. *ostoyae* in the interior but not on the coast. However, studies elsewhere associating disease and precommercial thinning 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 redcedar (*Thuja plicata* Donn ex D. Don). Initially, crop trees showed increased growth, but this declined 5-10 years after thinning, although 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 (*Pinus ponderosa* Douglas ex P. Laws and C. Laws.) reduced

mortality and increased growth of the remaining crop trees, but not statistically significant 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 significantly affect mortality 10 years later compared to controls, but radial growth of crop trees in more than half of the sites did not significantly increase.

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 pathogenic *Armillaria* species in thinning stumps. Thus, the different conclusions reached in these studies may be attributed to variation in ecosystem type, host species, age class, *Armillaria* species, total inoculum levels, or a combination of factors.

The reported differences in behavior of Armillaria root disease between coastal and interior forests (Morrison 1981) were studied with particular reference to: (i) determine the proportion of precommercial thinning stumps colonized by *Armillaria* species, (ii) observe the interaction between *A*. *ostoyae* and Douglas-fir crop trees at root contacts between colonized stumps and crop trees.

MATERIALS AND METHODS

Biogeoclimatic zones and site locations

The biogeoclimatic ecosystem classification system in use in B.C. is based on soils, climate, and indicator plants as described by Braumandl and Curran (1992), Green and Klinka (1994), and Lloyd et al. (1990). Zones, named after the dominant climax plant species, are divided into subzones, the subzones into site series, and the series are located on an edatopic grid which is a two-dimensional display of soil hygrotope (soil moisture regime) and trophotope (soil nutrient regime). 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 (Fig. 1). Study sites were located within subzones of each zone (Table 1 and 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 which are intermediate between the CWH and IDF (Meidinger and Pojar 1991). The snow pack in the ICH reduces summer moisture deficits.

Stump surveys-occurrence of *Armillaria* species in thinning stumps

The 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; Fig.1). The amount of disease on a site was not a selection criterion. Sites had Douglas-fir as the leading species, and had been thinned at least two years previously to allow time for stump colonization to occur. Average site area was 2.4

ha. Sites were stratified into upper, mid, and lower slope positions to capture variation over the slope. The mean distance between transect lines was about 100 meters. 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. The number of stumps sampled on a transect varied with availability. Soil was removed from around the primary roots for a distance of 40 cm from the stump base, and the occurrence of rhizomorphs on root surfaces and subcortical mycelial fans typical of *Armillaria* species was recorded. For each stump, a wood sample was taken from one root with subcortical mycelial fans 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 (Klinka et al. 1989) was then read from tables in Green and Klinka (1994), and Lloyd et al. (1990) to estimate the growing season soil moisture balance and enable comparisons to be made between sites with respect to soil moisture in either coastal or interior zones.

Stump excavations-transfer of *A. ostoyae* from thinning stumps to crop trees

Stumps were excavated on three sites in each of the CWH and ICH, and two sites in the IDF (Table 1 Fig. 1). 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 from which actual soil hygrotope was read, as described for stump surveys. Stumps for excavation were selected using the following criteria: representing a similar range of stump diameters at each site, occurring in areas with disease symptoms indicative of *A. ostoyae*, and having probable root contacts with living Douglas-fir crop trees. Stumps were excavated by hand to minimize disturbance to root contacts, and roots were exposed down to 5 mm diameter. The diameter of the stump root at the contact with the crop tree root and the distance from the root contact to the stump root collar along the root were recorded.

The total root volume and colonized root volume were determined for each stump. Roots were separated into segments and divided into two root diameter classes, 5-10 mm and >10 mm. The volume of each root segment was calculated from length and diameter measurements using the formula for volume of a frustum of a cone. Segments were classified as colonized or not based on the presence of subcortical mycelial fans. Stump bole volume was calculated by length and diameter measurements noting fungal presence from the soil line down.

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

Crop tree measurements

Tree height and diameter at 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 mycelial fans and necrotic tissue (lesion area) on crop tree roots resulting from contacts with stump roots was calculated. Lesion areas, proximal and distal to the infection point, 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 girdled were called proximal lesions because they were still surrounded by healthy tissue.

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

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 on malt extract broth (MEB), 2 mg/l (a.i.) benomyl and botran (Worrall 1991) amended with 200 mg/l streptomycin, pH 5. All isolates were identified to species using dimon mating tests as described by Korhonen (1978) except that pairings were done on 1.5% MEB. *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). Isolates from stump surveys and stump excavations were also identified using analysis of restriction fragment length polymorphisms of the intergenic region between the 26s and 5s regions of the ribosomal RNA genes (after Harrington and Wingfield 1995).

Statistical analysis

Statistical analyses were done using the SAS statistical package (SAS Institute Inc., Cary, NC). Frequency data for callus formation, transfer of mycelium, and infection of crop tree roots at root contacts were analyzed by a Chi–square test. Frequency data for species found in stump surveys was analyzed by Multivariate analysis (MANOVA) and then by ANOVA (Proc GLM). Transect lines with the proportion of stumps of either *Armillaria* species were used as replicates. A square root transformation was applied to data for *A. ostoyae* in interior hygrotopes and IDF slope positions, and *A. sinapina* for CWH slope positions because of heteroscedasticity. Mean frequency of both species within hygrotopes and slope positions was tested with paired difference t test.

Differences in the proportion of root segments colonized for each stump was analyzed by ANOVA between biogeoclimatic zones. A paired difference t test was used to detect differences between root diameter classes within zones. All multiple comparisons used the Bonferroni t test at $\propto$=0.05.

Proximal lesion size was correlated to inoculum volume (Proc GLM) for the CWH and ICH using the model $y = b_0 + b_1x_1 + b_2x_2$ where y= proximal lesion size, b_0 = intercept, x_1 = root inoculum volume, and x_2 = bole inoculum volume. However, this model was not satisfactory for the IDF and a second model was used of the form $y = b_0 + b_1x_1$ where x_1= 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 proportion of the bole was associated with each lesion. For the second model each root was treated separately.

RESULTS

Stump surveys

Incidence of *Armillaria* species in precommercial thinning stumps

Two species, *Armillaria sinapina* and *A. ostoyae,* were isolated from stumps at 11 sites (Table 2). 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 most often in stumps of the CWH (33%), followed by the ICH (13%), the CDF (8%), and IDF (6%). *Armillaria ostoyae* was found most often in stumps of the ICH (51%), followed by the IDF (30%), CWH(22%), and CDF (12%).

The incidence of *A. sinapina* and *A. ostoyae* in stumps differed among lower, mid, and upper slope transects and among coastal and interior locations (Fig. 2) but differences were significant only for *A. sinapina* in the CWH and *A. ostoyae* in the IDF. In the CWH, both *Armillaria* species were found less often in stumps at lower slopes than in those at higher slope positions. At mid slope in the CWH, *A. sinapina* was significantly more common than at upper slope ($F=5.64$ $P=0.05$), while *A. ostoyae* was more common at upper slopes. In the CDF, *A. sinapina* was only found at lower slopes and *A. ostoyae* at lower and mid slopes. In the ICH, the frequency of *A. ostoyae* was greatest at lower slopes and least at upper slopes, but remained high throughout sites, while *A. sinapina* was only found at mid and upper slopes. In the IDF, *A. ostoyae* was significantly higher at lower slopes ($F=5.52$ $P=0.05$) 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 (Fig. 3). However, on the coast the frequency of both species was also lower on moist hygrotopes but differences were not significant. On the coast, *A. sinapina* was absent from moderately dry hygrotopes, and was significantly more common on fresh hygrotopes ($F=7.84$ $P=0.009$), while *A. ostoyae* was most common on slightly dry hygrotopes but differences were not significant. In the interior, *A. ostoyae* was more prevalent over all hygrotopes compared to *A. sinapina* but differences were not significant. On the very dry hygrotopes *A. sinapina* was not present and *A. ostoyae* was significantly lower ($F=4.29$ $P=0.02$) than most of the other hygrotopes.

Colonization of excavated stumps

Over all zones, 83-89% of the volume of stumps was colonized by *A. ostoyae*. *Armillaria ostoyae* was found less frequently (F=5.62 P=0.007) in roots 5-10 mm diameter in the IDF and CWH than in the ICH (Table 3). The same trend was noticed for roots >10 mm diameter but differences were not significant (F=2.61 P=0.085). There were no significant differences between root diameter classes or study sites within a biogeoclimatic zone.

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 necrosis of cambial tissues. Mycelium from colonized stumps transferred across root contacts and 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 between colonized stump roots and crop tree roots found in humus layers of the CWH. Root contacts were absent from 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 roots 5-10 mm diameter and >10 mm diameter were similar, while in the CWH and IDF, there were twice as many roots in the >10 mm class (Table 4). At root contacts, both the percentage of stump roots transferring mycelium (χ^2=2.26 P=0.32 for roots 5-10 in diameter and χ^2=4.89 P=0.08 for roots >10 mm in diameter) and the percentage of stump roots causing infection on crop tree roots (χ^2=0.99 P=0.61 for roots 5-10 in diameter and χ^2=6.1 P=0.04 for roots >10 mm in diameter) were greatest in the ICH and least in the IDF (Table 4); however, differences were only significant for infection at roots >10 mm between zones. Except for a greater transfer of mycelium at stump roots >10 mm in diameter (χ^2=4.82 P=0.028) compared to roots 5-10 in the CWH, there were no significant differences for transfer or infection between root diameter classes within a zone.

Lesion size

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) even though average colonized stump volume was largest in the CWH (10,127 cm^3) and least in the ICH (549 cm^3). The model used to investigate lesion size on crop trees using stump root and bole inoculum volume was correlated for the CWH and ICH (Table 5). In the IDF, this model was not significant so a second model was fitted to the data using stump root diameter at the contact point with the crop tree root as a predictor of lesion size. It showed that the size of lesions on crop tree roots was proportional (Table 5) to the diameter of the contacting stump roots in the IDF. Stump root diameter was not a significant factor in the CWH or ICH.

The regression coefficients for root and bole inoculum volume indicate the magnitude of the relationship between these parameters and lesion size (Table 5). As root or bole inoculum volume increased, lesion size increased more rapidly in the ICH compared to the CWH ($F=25.26$ for root and $F=66.66$ for bole $P=0.0001$).

Host response to infection

Crop trees in the CWH grew more rapidly ($F=20.74$ $P=0.0001$) than trees of a similar age in the other zones as indicated by bole volumes three to four times that of the ICH and IDF, respectively (Fig. 4). The percentage of crop tree roots which formed callus at lesions was associated with tree bole volume and was significantly greater ($\chi^2=27.87$ $P=0.0001$) in the CWH than in the other zones (Fig. 4). Proportion of callused lesions were not different among replicate study sites within a zone ($P>0.05$). However, a trend toward less frequent callus formation was noticed at sites in the CWH transition to the interior, compared to the site located at the coast.

Other crop tree responses to infection were bark thickening for several cm 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 found on roots 3 cm or more in diameter. Bark thickening was generally less frequent on larger diameter roots near 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 least in the IDF (33%), and was most noticeable on larger diameter roots and near 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 most of the smaller roots, the symptoms of host response observed in this study 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 compatible in all cases.

DISCUSSION

Stump survey

The frequency of thinning stump colonization by an *Armillaria* species is a measure of the suitability of the site stratum for survival and spread of the fungus. Limiting factors will determine the frequency of thinning stump colonization.

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. The occurrence of actual hygrotopes on the transects at lower, mid and upper slope positions varies from zone to zone because of precipitation patterns.

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. Presumably 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). Similarly, Ono (1970) reported that 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. Poor rhizomorph growth and loss of viability are probably caused by anoxia. *Phellinus noxius* (Corner) Cunningham, another root disease fungus, survived less than one month in wood blocks under anoxia imposed by flooding (Chang 1996). The critical duration of that condition for survival of *Armillaria* species in woody inoculum is not known, but is presumably similar to that for *P. noxius*.

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* Watling and Kile rhizomorphs in Australian soils (Pearce and Malajczuk 1990) and of *A. gallica* Marxmüller and Romagn. in the upper soil layers of dry sites in Britain (Morrison 1976, 1991). Because *A. sinapina* is weakly pathogenic (Morrison et al. 1985), it 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.

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 (Lloyd et al. 1990). Summer precipitation is higher in the CWH and ICH than in the other zones. Hence, favorable conditions for *Armillaria* species likely 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 distribution of the two *Armillaria* species on sites is at least partly 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. The difference in fungal abundance in stumps between coastal and interior sites may also be related to differences in host response to infection. At the CWH sites, conditions appear to be optimal for rhizomorph growth in soil and on root systems, favoring *A. sinapina* over *A. ostoyae* because the latter produces fewer rhizomorphs. In addition, spread of *A. ostoyae* through crop tree root systems is limited by strong host response. Thus, *A. sinapina* is in a better position to colonize root systems than *A. ostoyae* and is more abundant in thinning stumps in the CWH. In the ICH, the weaker host response is less likely to limit the spread of *A. ostoyae* through

host root systems. Consequently, *A*. *ostoyae* is encountered more frequently in thinning stumps in the ICH.

The information on *Armillaria* species distribution reported here is in general agreement with that of Whitney (1984) and McDonald et al. (1987). The latter reported that on the National Forests of the northern Rocky Mountains in the United States, *Armillaria* species were absent from warm-dry and cold-wet habitat types. Whitney found that conifer species were more heavily attacked on dry than on wet sites. 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 suggesting a link to soil moisture regime. Interestingly, the incidence of mortality caused by *Phellinus weirii* (Murr.) Gilbn., another root disease fungus, increased with position up slope in stands of Douglas-fir on the Oregon coast (Kastner et al. 1994).

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 more affected by the surrounding soil environment than larger roots because of their large surface to volume ratio. A higher proportion of 5-10 mm diameter roots was colonized in the ICH than in the other zones likely due to more uniform soil moisture conditions during the year. In the CWH, roots 5-10 mm in diameter were colonized to a lesser degree compared to larger roots, probably because wet soil conditions in the spring and fall can cause the death of small roots (Day 1963, Eis 1970), which then become colonized by other fungi. In the IDF, the driest zone, both root diameter classes were not colonized as frequently as in other zones because repeated drying associated with low soil moisture likely 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. Rooting pattern may affect spread of root pathogens. 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 deeper in the soil profile. The root systems in the CWH had a greater number of roots covering 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 of contacts 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, an effect not apparent in the ICH where a large proportion of the lesions were associated with small stump roots.

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. 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). 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 root diameters, unlike the other zones.

The vigorous growth of trees in the CWH is likely responsible for the higher rate of callusing at lesions. Advance of the fungus was checked by a callus and resin barrier in trees of good vigor (Buckland 1953). 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.

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 12-52% 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 following thinning because of the increase in inoculum potential at root contacts following *A. ostoyae* stump colonization. However, any flush should be brief because the rapid juvenile growth of trees in this zone resulted in 70% of root lesions being callused. A small 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 enable stocking targets to be met. This should leave the smallest stumps in contact with the largest most resistant trees and minimize mortality.

In the ICH and IDF, the limited ability of most trees to produce callus at infections is likely 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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Fig. 1. Location of study sites within British Columbia for stump surveys and stump excavations.

Fig. 2. Percentage of conifer stumps colonized by *Armillaria sinapina* and *A. ostoyae* along lower, mid, and upper slope transects for the a) Coastal Western Hemlock (CWH), b) Coastal Douglas-fir (CDF), c) Interior Cedar Hemlock (ICH), and d) Interior Douglas-fir (IDF) biogeoclimatic zones. Letters above bars indicate significant difference between slope positions within species, and * indicates significant difference between species within slope positions ($P \leq 0.05$). n= number of transect lines.

Fig. 3. Percentage of conifer stumps colonized by *Armillaria sinapina* and *A. ostoyae* in locations classified according to actual soil hygrotope (Klinka et al. 1989) for a) coastal (CWH, CDF) and b) interior sites (ICH, IDF). No sites in very dry hygrotopes on the coast were sampled. Letters above bars indicate significant difference between hygrotopes within species, and ***** indicates significant difference between species within hygrotopes ($P \leq 0.05$). n= number of transect lines.

Fig. 4. Callus formation (%) at root lesions on Douglas-fir crop trees and mean tree volume for each of the Coastal Western Hemlock (CWH), Interior Cedar Hemlock (ICH), and Interior Douglas-fir (IDF) biogeoclimatic zones. Letters above bars indicate significant difference between zones ($P \leq 0.05$), and n= number of observations.

Table 1. Characteristics of sites chosen for Douglas–fir thinning stump excavation to examine *Armillaria ostoyae* stump colonization, transfer of mycelium, and lesions on roots of Douglas–fir crop trees.

Site	Zone/ subzone[a]	Tree age at study	Years since thinning	No. stumps excavated	Actual hygrotope[b]
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

[a]CWH=Coastal Western Hemlock, ICH=Interior Cedar Hemlock, IDF=Interior Douglas–fir.
See Braumandl and Curran (1992), and Lloyd et al. (1990) for subzones.
[b] See Klinka et al. (1989).

Table 2. Site characteristics and incidence of *Armillaria sinapina* and *A. ostoyae* in conifer thinning stumps at survey sites in the CWH, CDF, ICH, and IDF biogeoclimatic zones.

Site	Zone/ subzone[a]	No. of stumps	% Stumps with *A. sinapina*	% Stumps with *A. ostoyae*	Tree age at study	Years since thinning	
1	CWHms1	40	33	23	22	2	
2	CWHxm1	30	23	20	27	5	
3	CWHxm1	29	45	24	26	4	
total/mean		99	33	22			
4	CDFmm	30	13	23	38	5	
5	CDFmm	30	3	0		8	5
total/mean		60	8	12			
6	ICHmw3	31	3	45	22	5	
7	ICHmw2	35	3	77	24	4	
8	ICHmk1	28	36	28	23	5	
total/mean		94	13	51			
9	IDFdk2	29	3	24	25	3	
10	IDFdk2	32	16	47	28	5	
11	IDFdm2	30	0	17	70	6	
total/mean		91	6	30			

[a]CWH=Coastal Western Hemlock, CDF=Coastal Douglas–fir, ICH=Interior Cedar Hemlock, IDF=Interior Douglas–fir. See Braumandl and Curran (1992), and Lloyd et al. (1990) for subzones.

Table 3. Mean percentage of excavated Douglas–fir stump root segments 5-10 mm and >10 mm diameter colonized by *Armillaria ostoyae*.

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

[a]CWH=Coastal Western Hemlock, ICH=Interior Cedar Hemlock, IDF=Interior Douglas–fir.
[b]Letters following percentages indicate significant difference between zones and ***** indicates significant difference between root classes ($P \leq 0.05$).

Table 4. Number of excavated Douglas–fir stump roots containing *Armillaria ostoyae* in root contact with crop trees and the percentage of those roots transferring mycelium to Douglas–fir crop trees and causing infection.

Zone[a]	No. stump roots with mycelium and contacts to crop trees		%Stump roots transferring mycelium to tree roots[b]		%Stump roots causing infections on tree roots[b]	
	5-10 mm	>10 mm	5-10 mm	>10 mm	5-10 mm	>10 mm
CWH	25	62	44 a*	71 a*	44 a	63 ab
ICH	28	23	64 a	69 a	57 a	69 a
IDF	10	22	50 a	45 a	40 a	36 b

[a]CWH=Coastal Western Hemlock, ICH=Interior Cedar Hemlock, IDF=Interior Douglas–fir.

[b]Letters following percentages indicate significant difference between zones and * indicates significant difference between root classes ($P \leq 0.05$).

Table 5. Correlation between *Armillaria ostoyae* proximal lesion size on Douglas–fir crop tree roots and inoculum volume of the contacting colonized Douglas–fir stump. The first three models are of the form $y = b_0 + b_1x_1 + b_2x_2$ where b_0 = intercept, x_1 = stump root inoculum volume, and x_2 = stump bole inoculum volume. Stump root and bole volumes predicted lesion size in the CWH and ICH but not for the IDF. A second model for the IDF of the form $y = b_0 + b_1x_1$ was used where x_1 = stump root diameter.

Zone[a]	n	P (model)	R^2	Intercept	Source	Coefficient	P type III
CWH	13	0.0038	0.67	139.67	root	0.11	0.004
					bole	0.08	0.015
ICH	15	0.0001	0.94	-313.42	root	0.43	0.0001
					bole	0.55	0.0001
IDF	8	0.4524	0.3274	8.46	root	0.005	0.7426
					bole	0.005	0.5019
IDF	9	0.0054	0.69	-6.91	root diameter	24.58	0.0054

[a]CWH=Coastal Western Hemlock, ICH=Interior Cedar Hemlock, IDF=Interior Douglas–fir.