Resistance and tolerance in juvenile Interior Douglas-fir trees *Pseudotsuga menziesii* var. *glauca* artificially inoculated with *Armillaria ostoyae*.

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Summary

Plants utilize two general strategies to cope with pathogen attack. They either limit or resist the pathogen (termed '*resistance*') or they cope with the disease by surviving and growing (termed '*tolerance*'). Both strategies tend to increase plant fitness; however, there are possible costs, trade-offs, and interactions associated with each strategy. This study focused on five half-sib Interior Douglas-fir families [*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco] that were putatively classified as either resistant or susceptible to *Armillaria ostoyae* in a previous greenhouse study of seedling families challenged with *A. ostoyae*. We compared their survival rates in the greenhouse study with results of juvenile trees from the same five families that were artificially inoculated in field conditions. Both resistance and tolerance appeared to be operating in the field test trees and a possible trade-off between resistance and tolerance was detected. Significant differences were detected among the five families for stem radial growth following infection at the tree root collar. Compared to the putatively susceptible families, resistant families had smaller lesions and lower proportional root collar girdling. Tolerant families generally had larger lesions but demonstrated better growth when diseased than resistant families. One tolerant family that was a good survivor in the greenhouse survival study presented vertically shaped lesions that were large in area but had greatly reduced proportional root collar girdling. In contrast, a second family had poor survival in the greenhouse study but showed tolerant traits in the field study, presented horizontally spreading lesions that produced high root collar girdling. Lesion shape and lesion size both may be associated with host tolerance in this system. Survival rankings of the five families in the greenhouse study mostly agreed with results from the field study based on the proportion of collar girdling among families. These host responses are discussed with respect to stability, quantity, and quality of stands and products.

1) Introduction

Plants generally possess two main strategies to survive the attack of pathogens: resistance and tolerance. A separate strategy is to prevent attack at the outset called immunity where the pest fails to recognize the host plant (Agrios 2005) and never attacks. The resistant strategy excludes the pathogen completely or reduces the extent of the damage and ultimately reduces pathogen fitness. Resistant plants typically overcome, exclude, or limit the enemy, or they reduce the probability of attack. In coniferous trees, defenses in resistant trees have been classified into preexisting (constitutive) or induced defenses. The constitutive defense system includes resin accumulation, storage of toxins, mechanical barriers, and enzymes. The induced defense system involves activation of a wide range of chemical and structural changes that limit pathogen colonization and contain the affected area (Blanchette and Biggs 1992, Agrios 2005). The tolerance strategy involves coping with the damage and usually lowering the negative effects on pest fitness. Tolerant plants may not limit the pathogen, but have the capacity for greater yield compared to other plants with the same disease severity (Schafer 1971). Although the terminology is different among disciplines, many concepts in plant disease are likely shared across taxonomic kingdoms (Borer et al. 2011) and were most recently applied to the animal kingdom (Baucom and de Roode 2011). Tolerance can also be extended to cover abiotic agents such as herbicides (Baucom and Mauricio 2008), the related effects of drought and salt (Bartels and Sunkar 2005), temperature (Wahid et al. 2007), and potentially any trait that confers a fitness advantage among individuals in a population adapting to environmental gradients.

Although plant tolerance to diseases has long been recognized in agricultural systems, detailed mechanisms are still not clearly understood. Tolerance mechanisms are thought to be related to changes in either resource reallocation or plant architecture (summarized by Stowe et al. 2000). Briefly, tolerant resource reallocation traits could include timing of development, quality of tissue (e.g. thicker cuticle or greater photosynthesis), or differing allocation to storage, growth and reproduction. Tolerance traits related to plant architecture include better resource capture or reduced costs of capture, number of meristems, signaling distance, and ability to reform vascular tissue.

Plant defense mechanisms typically exist along a continuum that can vary within and among populations (Blanchette and Biggs 1992). Moreover, if both resistance and tolerance traits function to allow the plant to survive, it is important to determine their effect at the population level and their role in pest management strategies. van der Meijden et al. (1988) suggest that plant resistance and tolerance traits are redundant strategies with associated costs and benefits. Therefore, expression of both resistance and tolerance attributes in one individual likely would not be maximized together, but instead could be expressed in combination at reduced levels. The importance of each strategy is best understood by their benefits and costs and if they are complimentary. Tolerant traits may lower selection pressure on the pathogen and provide better plant growth or quality in the diseased condition, but may come at a cost in healthy growth. The major disadvantages of tolerance are that it might allow the pathogen to become established and spread, which might also predispose the host to other enemies. Resistance traits tend to

limit pathogen inoculum build-up and viability, host damage, and ultimately pathogen fitness; however, this may also come at a cost in healthy or diseased plant growth. Both strategies may also provide signals for exploitation by other pests (van Dam and Heil 2011). Resistance or tolerance are thought to be prevented absolutely in the population by the costs of these strategies when an enemy is either present or absent. Both tolerance and resistance may be induced mechanisms that allow the plant phenotype to change when an enemy is present, which can reduce costs of a trait by not expressing the trait continuously (Agrawal 1999).

Hartig (1874) described the resistance defense reactions to *Armillaria mellea* (*sensu lato*) in trees and was the first to link these reactions with mortality and growth reduction in conifers. Tolerance to disease was probably first described not long after this in agricultural crops (Cobb 1894) and then much later in deciduous trees (Sinclair et al. 1997). *Armillaria* root disease is caused by a globally distributed multi-species fungus that covers the host range of many woody plants (Kile et al. 1991). Root systems act as a food base from which the fungus can attack and spread to new hosts. Single attacks rarely cause tree mortality but instead interfere with potential tree growth because growth reduction accumulates annually following the first infection. Reduction in potential growth of infected trees therefore can have significant impact even in stands with low mortality (Cruickshank et al. 2011). Mortality usually takes place after multiple attacks that eventually reach and girdle the root collar; hence, trees remain alive for some time. The disease epidemiology causes many more infected trees to remain alive but infected below ground than are dead (Morrison et al. 2000, 2001). Therefore, the costs of defence reactions against this fungus are of importance. Previous work has suggested that population variation in survival and growth to this disease agent might be related to resistance and tolerance (Cruickshank et al. 2010, 2011). However, resistance and tolerance can only be separated under specific conditions (Råberg et al. 2009), and survival alone cannot distinguish between the two mechanisms (Kause and Ødegård 2012).

The present study investigated host resistance and tolerance in a small group of 17- and 18-year-old field-grown half-sib families of Interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco). The families were identified through survival analysis of a larger seedling population that had been challenged by *Armillaria ostoyae* (Romagn.) Herink in a greenhouse (Cruickshank et al. 2010). The objectives of the present study were to: 1) compare the results of field inoculation of five juvenile half-sib families to the results of the same families in a separate greenhouse seedling survival study; 2) evaluate how Armillaria root disease affects growth of juvenile trees within and among families consisting of both infected and healthy trees; and, 3) gain a better understanding of the resistance-tolerance mechanisms in conifers. We measured tolerance using a reaction norm relating host growth to disease intensity (i.e. root collar girdling) among genetically related individuals (Simms and Triplett 1994).

2) Materials and Methods

2.1 Inoculum production

Inoculum units were prepared at the Canadian Forest Service, Pacific Forestry Centre (PFC), Victoria, B.C. from freshly cut 1.5 kg blocks of 15 to 25-year-old paper birch (*Betula papyrifera* Marsh.) harvested from the BC interior. The blocks were between 10 and 15 cm in diameter and length. Sequentially, all blocks were covered in warm water, autoclaved for 45 min (121^{o} C), transferred to autoclavable bags, and autoclaved at the same temperature for another 180 min. The blocks in the bags were cooled overnight in a sterile laminar flow hood and then inoculated with an isolate of *Armillaria ostoyae* (Romagn.) Herink obtained from Morrison and Pellow (2002). Inoculation occurred by transferring a piece of the fungus from a 3 % malt extract broth agar plate (1.7 % agar) onto the bark at both ends of each block. The bagged inoculated blocks were stored in plastic storage boxes with a loose fitting lid for 2-3 years at 17^{o} C. After colonization, a 15-19 mm diameter by 100-mm-long living Garry oak branch segment (*Quercus garryana* Douglas) was inserted tightly into a hole drilled perpendicular in one end of each block. Oak branches were prepared by removal of lichens with a pressure washer and a scrubbing brush when needed, cut to length, surface sterilized in 10 % bleach for 10 minutes, and rinsed with tap water. The inoculum units were then stored in moist sand-filled plastic bins until the oak branch cambium became either colonized with mycelium or rhizomorphs were formed at the end of the stick (approximately 4 mon). The units were then placed in moist vermiculite and transported to the BC Ministry of Forests, Lands and Natural Resource Operations Interior Douglas-fir progeny test near Duncan Lake, BC (http://www.for.gov.bc.ca/rsi/research/nsites/ea976024.htm).

2.2 The test site and inoculation

The progeny trial near Duncan Lake (lat. 50^{o} 21’ 57.3” N, long. 116^{o}54’45.4” W, elevation 640 m) is a gently sloping site with a southwest aspect situated in the Interior Cedar-Hemlock biogeoclimatic ecosystem (Meidinger and Pojar 1991). The test was planted in 1987 with wind-pollinated seedling families from the West Kootenay low elevation seed planning zone. Biologically, these seed zones are surrogates for the physical environment in which seedlings grow to their genetic potential, and moving beyond these areas usually results in maladaptation (Ying and Yanchuk 2006). The test was established as a randomized complete block experiment with 8 replicate blocks and 256 4-tree row plots per replicate block. Families were randomly assigned to plots and trees were planted at 2 x 2 m spacing. Five families from this progeny trial, which represented a range from poor to good survival in a previously reported greenhouse survival study of the same West Kootenay Low families, were chosen for study (Cruickshank et al. 2010). Summary data for the number of trees inoculated and un-inoculated, tree height, and diameter at breast height of the trees used in the study are presented in Table 1.

Field trees were initially inspected for infection by removing the soil around the lateral roots and root collar up to 1 m from the bole and checking for lesions caused by root disease including un-inoculated trees. Larger roots were excavated up to 2 m away. The organic layers were kept separate from the mineral soil. On 15 trees per family, inoculum units were placed on both sides of the tree bole with the transfer stick touching

the root collar, and inoculum units kept about 2 cm below the original mineral soil level. The mineral soil was back filled and the organic layer reestablished over the mineral soil. Inoculum units were placed in October 2004 for families 421 and 423 and October 2005 for families 422, 521, and 620. Trees with natural *A. ostoyae* infections and stem defects were avoided. The inoculated trees were evaluated five years after inoculum placement (Table 1).

2.3 Lesion sampling and measurement

After five years, the inoculum units were excavated and examined for the presence of rhizomorphs leading from the stick to the tree. For families 421 and 423, the root systems were completely excavated and removed after mapping the inoculum block location and the lesions arising from it. Wood disks (5 cm thick) were collected from inoculated and un-inoculated trees at stem breast height (1.3 m) for these families and brought back to the lab at PFC. Trees in families 422, 512 and 620 were excavated and mapped in a similar manner; however, the trees were left *in-situ*. For inoculated and un-inoculated trees, two 12 mm cores were taken at breast height (1.3 m) across slope by boring through the pith. Lesions on all trees were traced onto paper and digitized for area using a digitizing tablet (GTCO Corp., Columbia, MD.). Horizontal lesion spread and root collar circumferences were measured with a tape measure. The sum of the horizontal lesion spread divided by the total circumference was used to calculate proportional collar girdling. Lesion spread times for families 421 and 423 were calculated by sectioning each lesion horizontally and counting the number of rings to the first callus tissue. A piece of bark, wood, or rhizomorph from of each lesion was tested for compatibility with the original isolate (Guillaumin et al. 1991) by pairing on 2% malt extract broth (1.7% agar). We found 10 trees with non-compatible lesions (Table 2), meaning that they originated from a different *A. ostoyae* genotype and the trees were removed from further analysis. Ring widths on two radii of the stem disks (families 421 and 423) and on the two cores per tree (families 422, 512 and 620) were measured with a dendrochronometer (digital positiometer manufactured by L. Kutschenreiter, Austria). Diameters for trees prior to the sample date were determined by summing the ring widths for the tree.

2.4 Statistical methods

The SAS system (Ver. 9.2; SAS Institute Cary, NC, USA) was used for all statistical analyses. Models were fit using maximum likelihood via the MIXED procedure, and model selection was based on likelihood ratio tests and Akaike's information criteria. All variables were scaled between one and twenty before analysis to help model convergence. Residuals were checked for normality and homoscedasticity against predicted and explanatory variables, and observed versus predicted values were plotted which indicated good fit (Fig. 1).

Ring widths of the test trees beginning in 1993 were compared to the corresponding level of damage at the root collar according to:

$$Y_{ijkl} = \beta_0 + \beta_1 \text{gird}_{jl} + \beta_2 \text{year}_l + \sum_{i=1}^{5} \beta_3 d_k \text{family} + \sum_{i=1}^{5} \beta_4 d_k \text{family} * \text{gird}_{jl} + \sum_{i=1}^{5} \beta_5 d_k \text{family} * \text{year}_l + a_j + b_j + c_{ij} + \varepsilon_{ijkl} \quad [1]$$

where: Y_{ijkl} is the ring width for radii *i* within tree *j* for family *k* at year *l;* β_0 is the overall mean; β_1 is the continuous fixed effect of the arcsine of the square root of the proportion of root collar girdling in tree *j* which is a time related covariate taking the value of zero until inoculum placement at year *l*; β_2 is the continuous fixed effect of the calendar year *l*; $\beta_{31,}\beta_{32,.....}\beta_{35}$ are the fixed effect parameters associated to differentiate the five half-sibling families via dummy variables d_1, d_2,……$d_{5;}$ $\beta_{41,}\beta_{42,.....}\beta_{45}$ are parameters associated with the interaction between family k and girdling in tree *j* in year *l*; $\beta_{51,}\beta_{52,.....}\beta_{55}$ are the parameters associated with the interaction of family *k* and year *l*; a_j is the random intercept for tree *j*; b_j is the random effect for the slope between the ring widths and collar girdling for tree *j*; c_{ij} is the intercept for random effect of radii *i* within tree *j*; ε_{ijkl} is the residual error, assumed normally distributed. A first order autoregressive covariance structure was applied for ring widths within tree which implies that correlation between any two adjacent elements is ρ^t, where t is the lag time, and ρ is constrained so that $-1 < \rho < 1$. This allows for greater potential correlation between observations that also occur closer together. Covariance was also accommodated between random parameters a_j and b_j.

Ring widths were not averaged within trees because root disease can cause significant variation on ring growth based on the radii position within stems (Cruickshank 2002). This random variation was better explained by other random variables, but the term was left in the model to show that it was accounted for. We also considered an alternate model to Eq. 1 by including the fixed effect of the year of inoculation, but found this term not significant. Half-sibling family normally would be considered a random effect; however, since the families were selected from a previous survival study relating to the best and worst survivors they are considered fixed effects. In addition to the fixed family effects, random family intercepts, slopes, and covariance were also tested but were not significant.

Correlations among variables were assessed using SAS proc CORR Spearman's rank procedure, chi-square tests were done with proc FREQ Fisher's exact test, and t-tests were done with proc TTEST. Statistical comparisons between family means in Table 1 were done using one-way ANOVA and differences of least squares means within proc Mixed.

3) Results

3.1) Inoculum transfer and survival rankings

The number of inoculum blocks where the fungus transferred to the target tree and caused lesions is summarized in Table 2. In total, 150 inoculum units were applied to test trees

(5 families x 15 trees/family x 2 inoculum units /tree). Unfortunately, not all units resulted in fungus-caused lesions, and no family had every tree with fungus-caused lesions from both inoculum units. Nine trees from three families had both units with no compatible fungal-caused lesions at the root collar, while 66 trees presented fungal-caused lesions from at least one unit (Table 2). Therefore, field infection success was 88%. Of the 66 trees with lesions associated with at least one unit, eleven trees (17%) from three families had units associated only with bark infections (i.e. no penetration to cambium); ten trees (15%) had at least one non-compatible lesion indicating a different fungal genotype than inoculated; and data for one tree went missing. Therefore, the total number of trees removed from the analysis was 22 (Table 2). After elimination of these 22 trees, only 5 trees remained in the analysis with one unit causing lesions, one in each of the families. The total number of trees used in the analysis was 74 (Table 1 and 2; 30 healthy, 44 infected).

3.2) Comparison between greenhouse seedling survival and lesion measures on field inoculated trees

Spearman rank correlations between family survival in the greenhouse seedling study and the family mean proportion of root collar girdled or root collar lesion area in this study were -0.7 and -0.5, respectively. This indicated that the root collar lesions on the juvenile trees were best related to the seedling survival results as a lesion measure relative to the circumference of the juvenile tree (proportion of root collar girdled) compared to lesion area on the tree. The proportion of root collar girdling was used as a covariate in Eq. 1, since it is also the main prerequisite to tree death for root disease. The relationship between lesion area and proportion of collar girdling pooled among families was positively correlated (Spearman's $r = 0.75$, $p<0.0001$, $n = 44$, infected only). This correlation also varied by family with families 421 and 423 having the lowest correlations ($r=0.55$ and 0.49, respectively) because of the variation in lesion shape versus area described in section 3.3.

Only family 514 ranked slightly higher in the field study having lower than expected proportional collar girdling compared to the survival rank in the previous seedling survival study (Table 1). Family 514 had the largest number of field trees associated with bark lesions (7- Table 2) with no fungal penetration to the cambium ($p<0.0001$, Fisher's exact). The average 5-year ring width post inoculum placement for the trees in this family with bark infections only (removed from analysis) was 2.7 mm, which compares with average healthy tree growth for this family over the same time period at 2.3 mm. This family had some of the largest trees pre and post inoculum placement in the study (Table 1).

3.3) Lesion spread time and shape

Lesions in all trees were contained (callused) by the sampling date 5 years after inoculum placement. All trees from families 421 and 423 were excavated and the time that the fungus spread horizontally in the lesions before callusing was determined. The two

families were marginally different (p=0.07, t-test) in their horizontal root collar lesion time to callus (421=2.2 years vs. 423=1.7 years). This was consistent with their rankings in the greenhouse survival study in that trees that callus sooner have better survival. In general, families with low survival rank in the greenhouse study (e.g. family 421) had lesions that spread horizontally in the field study (Fig. 2). Families with higher survival rank in the greenhouse study (e.g. family 423) had lesions that tended to spread mainly vertically (Fig. 2) or that were restricted horizontally and vertically. Family 423 had the largest mean lesion area (175.2 cm^2) but relatively low mean horizontal lesion spread (33^o), while family 421 had high lesion area (147.5 cm^2) and large horizontal lesion spread (50^o) (Table 1). On the other hand, families 620 and 422, presented small lesion areas and low horizontal lesion spread in this study, and high survival in the greenhouse study (Table 1).

3.4) Annual ring width in healthy and infected trees

The calendar year was added to account for increasing competitive interference and consistent ring width decline in all trees with time ($p<0.0001$, Table 3). Averaged over all trees, annual ring width as a function of the proportion of root collar girdling (Eq. 1) was negatively related (p=0.008, Table 3) suggesting that root collar damage reduced growth. Likewise, there were significant family differences in mean healthy annual ring given by the family intercepts (p=<0.0001, Table 3). The interaction between half-sibling family and the proportion of collar girdling on annual ring width indicates variation in host tolerance. The more negative the slope (smaller coefficient) the lower the tolerance. This interaction was significant (p=0.03, Table 3) with decreasing tolerance in order for families 421, 423, 620, 514, and 422 (Table 4, family x girdling). Although having moderate growth in the healthy condition, family 422 had the second worst diseased growth for most levels of girdling (Fig. 3); however, this family also limited lesion area, consistent with resistance (Table 1). Family 514 was the best grower in the healthy or diseased condition but had the next lowest tolerance to root collar girdling (Fig. 3); however, it had low survival in the previous greenhouse seedling study (Table 1) and was considered susceptible. Family 620 was also considered resistant (low collar girdling), and was intermediate in tolerance. Family 421, was the slowest growing in the healthy and diseased condition, but had no impact of collar girdling on radial growth (i.e. completely tolerant, Fig. 3). Family 421 had the largest collar girdling which also corresponded to the low survival ranking from the greenhouse study (Table 1) making it susceptible. Family 423 had the second highest growth in the healthy and diseased condition indicating its tolerance to root collar damage (Fig. 3); moreover, its proportional collar girdling was moderate (Table 1) which corresponded to the moderate ranking in the seedling survival study.

A negative correlation between coefficients for family intercept and the coefficients for the interaction term of family by collar girdling (Table 4) indicates that high tolerance is associated with low growth in the undamaged condition (i.e. a cost when healthy). Family intercepts represent fitness or vigor in the undamaged state, and the slopes (collar damage) represent how growth varied with damage. At the family level, this relationship between fixed effect coefficients was the correct sign but not significant (Spearman's r =

-0.50, p = 0.39, n=5). At the tree level, random intercepts ring width variation for individual tree vigor in a pathogen free environment was 0.42 (Table 4 and Eq. 1). The random slope (proportion collar girdling) represented genetic variation for tolerance for all trees was 0.001 (Table 4). The covariance between the slope (infected) and intercept (healthy) was -0.01 (Table 4) and represented that tree fitness (ring width) was a trade-off with tolerance. The negative covariance indicated a trade-off because the largest trees in the healthy condition had greater ring width reduction post infection but lower collar girdling. Adding these three random effects had a significant effect to the model (likelihood ratio test $p<0.0001$, d.f.=3). The covariance for individuals was calculated within family, and then pooled. With more data, this covariance could be calculated separately for each family.

The least square ring width estimates for families from Eq. 1 include the years before and after infection and account for all the model fixed and random effects (Table 1). The smallest standard errors occurred for the two families that had the lowest change in ring width post inoculation.

4) Discussion

After damage, plants must re-form the phellogen (bark cambium), cambium, and sapwood tissues (Mullick 1977). In this study, all of the field-inoculated trees with necrotic lesions would require repair of these tissues. As in other studies of plant disease, we found both tolerance and resistance to be operating simultaneously (Koskela et al. 2002, Stowe 1998). We found that resistant trees generally limited pathogen colonization (damage) through smaller lesion size and lower proportional root collar girdling, which also limits pathogen inoculum and ultimately pathogen fitness. However, limiting pathogen damage was also associated with reduced stem radial growth. Reduced radial growth may not necessarily be associated with lower fitness, assuming the mechanism provides an advantage when it is induced at least some of the time (i.e. greater survival and lower damage). We also found susceptible trees with less effective resistant attributes. Tolerant trees on the other hand, contained a set of attributes distinct from resistant trees. They allowed greater disease damage, good survival, and little or no radial growth impact compared to healthy trees.

Diseased trees of family 423 presented large fungal lesion area but sustained low growth impact from fungal damage. Interestingly, these trees also limited the proportion of root collar girdling by restricting lesion spread horizontally and forming vertical shaped lesions. These vertically shaped lesions may have allowed family 423 to be a moderately good survivor in the previous greenhouse study. Compared to lesions that spread more horizontally, it would take many more attacks at the root collar to girdle a stem with vertically shaped lesions. These vertically shaped lesions also likely cause less damage to vascular tissue compared to horizontal spreading lesions. Limiting horizontal but not vertical lesion spread also appeared to have low growth impact compared to healthy trees in this family. These vertically inverted ‘V’ shaped lesions formed after attack from

Armillaria root disease have been described in *Eucalyptus* (Shearer and Tippett 1988) and Western redcedar (Koenigs 1969).

All families except family 421 displayed reduced growth post inoculation, even at low collar girdling. Similarly in a previous study of volume growth reduction in Douglas-fir, the greatest rate of reduction was immediately following infection, even in lightly damaged trees (Cruickshank et al. 2011). This growth reduction continued many years after infection and occurred most strongly in larger trees. Cruickshank and Filipescu (2012) found that the number of years a Douglas-fir tree had been infected was also related to allometric architecture shifts suggesting resource reallocation in response to disease. Therefore, damaged roots alone are probably not acting as a physical restriction on tree growth.

A number of trees in family 514 were able to confine the fungus to bark lesions only. Trees from family 514 probably had increased capacity for phellogen formation of wound periderms (phellem), which if produced quickly enough, may slough off the pathogen before it can penetrate the cambium. Previous studies have shown that young interior Douglas-fir roots up to age six years allowed frequent penetration of *A. ostoyae* to the cambium (Robinson and Morrison 2001). Trees as old as 19 years showed increasing frequency of multiple bands of phellem (cork) that contained the fungus to the bark, while larger roots with thicker bark showed an increasing ability to form multiple bands of phellem (Robinson and Morrison 2001). Bark periderm formation could be considered an induced resistance reaction. Growth was impacted in family 514 trees when the fungus penetrated to the cambium, and although untested statistically, growth in trees only with bark infections was similar to healthy trees. The production of multiple phellem bands may not be produced effectively in seedlings, and this may explain the slightly lower survival ranking in the greenhouse study compared to their ranking based on lesion size in this field study.

Our study also suggests that tolerance at the individual tree level may come at a cost. Averaged over all families, trees with lower than average healthy growth sustained the least growth impact when diseased, which suggests a cost for maintaining tolerance traits when healthy. It also suggests there is little cost to maintaining resistance traits as long as the tree remains healthy, after which growth impacts occur following infection. These trends were indicated by a negative covariance between intercepts (healthy) and slopes (damage vs. growth). A positive covariance would have indicated a cost of resistance and no cost for tolerance, while zero covariance would indicate no cost for tolerance or resistance. With larger sample size, we would be able to calculate this relationship for trees within each family separately. Further, we found the same trend at the family level, but this was not significant probably due to the few numbers of families in the study. Although probably logistically prohibitive in field studies, future studies would benefit from including more families and more trees per family as well as including family as random effect. A potential trade-off between resistance and tolerance has long been recognized as a type of cost (Mauricio et al. 1997, Rausher 1996). Since resistance and tolerance both increase fitness, they can be considered redundant strategies and the cost of maintaining two similar strategies could outweigh the value of expressing both traits at

high levels (van der Meijden et al. 1988). In our small population sample, no trees were found to express both traits at high levels; however, some trees within and between each family had varying amounts of each trait. Other mechanisms that might cause a trade-off between resistance and tolerance traits are: 1) pleiotrophy where one gene can affect multiple phenotypic traits, or 2) linkage disequilibrium where one or more alleles for a phenotypic trait are not randomly present in the population (Stowe et al. 2000). A larger study is needed to estimate the frequency of tolerance or resistance genes in the population and to confirm a possible trade-off between the two.

Since coniferous trees are long-lived plants that typically grow in heterogeneous environments, they must evolve adaptive strategies to cope with these complex environmental pressures (Lazzaro et al. 2009). Genes that influence both tolerance and resistance in these complex environments would be beneficial at the individual and population level. For example, tradeoffs between costs and benefits for pest resistance could occur in response to abiotic conditions such as drought, which is a common growth limiting factor throughout the range of Interior Douglas-fir. Plants resistant to root disease benefit by having lower root damage, and probably greater survival and growth in drought conditions. On the other hand, in years when growth is not limited by other factors, disease tolerant plants may benefit from better growth under disease limiting conditions. Moreover, disease tolerant neighbours may be able to compensate for the reduced growth of resistant neighbours while at the same time benefitting from lower fungal inoculum caused by resistant neighbours. Therefore, these two traits could be complementary over longer time periods at the stand level especially under changing stand conditions. Tolerance as a host response has been suggested as a platform on which other mechanisms that may be less stable but have superior and more specific control, can be added to the population (Schafer 1971). Survival to multiple selection pressures might also help explain the apparent need for tolerance and resistance in a population. Future studies might also test how leaf area and longevity, crown length, and drought tolerance relate to disease resistance and tolerance.

One of the primary strengths of *Armillaria* species is their ability to persist on a site for centuries in part through mobility of rhizomorph movement within and between roots and trees. This results in long-term, persistent and sustained disease in host plants. Since most Interior Douglas-fir trees eventually exist in a diseased state (Morrison et al. 2000, 2001) disease tolerance would be attractive from several practical standpoints. Lack of timber product uniformity is one of the greatest problems faced by wood-use industries (Bowyer et al. 2007), and wood quality, product recovery, and piece size, all related to growth, are important determinants of Douglas-fir product value. Uniformity of ring width within stems and rings is strongly disrupted by root disease in the bottom several meters of the tree (Cruickshank 2002), lowering timber product value through piece size and ring heterogeneity (Cruickshank 2010) and possibly through increased stem taper (Cruickshank and Filipescu 2012). Disease tolerance might reduce this heterogeneity. We noted that the slowest growing family (421), and one of the fastest growing families (423) with the least growth impacts from damage, both shared the lowest growth ring heterogeneity over the study period. On the negative side, larger lesions produced on tolerant trees might act as infection courts that predispose the tree to other problems such

as butt rot decay, but this is usually not a problem in Interior Douglas-fir. Vertically elongated lesions may help to minimize this problem. Ideally, selection for both resistance and tolerance traits in the population might be preferable because of the potentially positive effects of both traits. Breeding programs that focus solely on high levels of resistance, especially in populations where resistance appears as a trade-off with tolerance, might not be the best solution (Clark 1966).

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Table 1 – Summary of Interior Douglas-fir families field-inoculated with *A. ostoyae* and infection results. Letters following numbers indicate significant difference at $p<0.05$.

Family	421	422	423	514	620
Sample year (inoculation yr.)	2008 (2004)	2009 (2005)	2008 (2004)	2009 (2005)	2009 (2005)
Number trees (healthy, infected)	5, 14	4,7	6, 13	9, 4	6,6
Mean proportionate collar girdling – diseased (min, max)	0.35a (0.01,0.6)	0.06b (0.01,0.14)	0.2b (0.01,0.7)	0.15b (0.01,0.4)	0.12b (0.03,0.3)
Mean horizontal degrees collar lesion spread (min, max)	50 (7,93)	18 (1,42)	33 (10,83)	34 (3,76)	23 (5,36)
Mean collar lesion area (cm^2) (min,max)	147.5a (2.0,363.7)	18.2b (0.7,42.1)	175.2a (7.9,552.6)	43.03b (3.6,138.5)	21.59b (1.8,52.4)
Proportion of seedlings surviving [1]	0.12	0.47	0.35	0.07	0.44
Mean dia. (cm) 1.3 m under bark at 2003 (min,max)	9.6 (6.7,11.9)	10.2 (7.2,13.2)	11.0 (7.9,14.2)	10.4 (7.4,14.5)	9.5 (5.5,13.9)
Mean dia. 1.3 m over bark (cm) at sample year (min,max)	12.7 (8.7,15.5)	13.9 (9.7,17.6)	15.1 (10.2,19.8)	14.8 (9.5,21.3)	13.1 (8.2,19.8)
Mean final height (cm) (min,max)	1284 (1015,1530)	1316 (908,1685)	1330 (854,1675)	1486 (1149,1860)	1377 (954,1615)
Mean ring width ± SE (LS means from eq. 1)	2.90±0.15	3.18±0.20	3.51±0.15	3.38±0.19	3.02±0.19_

[1] families were selected based on early results in a greenhouse *A. ostoyae* resistance screening study (Cruickshank et al. 2010)

Table 2. Infection summary of Interior Douglas-fir families field-inoculated with *A. ostoyae*. Non-compatible lesions are those with a fungal genotype other than the one in the inoculum block.

Family	Trees with 0-2 blocks causing lesions			Trees with bark lesions only	Trees with non-compatible lesions	Total trees used in analysis (healthy + infected)
	Number of blocks					
	0	1	2			
421	0	1	14	0	1	19
422	3	4	8	2	3	11
423	0	1	14	0	2	19
514	3	1	11	7	1	13
620	3	3	9	2	3	12
Sum	9	10	56	11	10	74

Table 3 – Overall tests from regression analyses of Interior Douglas-fir tree size before field-inoculation with *A. ostoyae* and proportion of root collar girdling on family annual stem ring width after inoculum placement (Eq. 1).

Effect	Numerator degrees freedom	Denominator degrees freedom	F value	P type III
Proportionate collar girdling	1	68.9	12.36	0.0008
Family	4	1254	21.18	<0.0001
Calendar year	1	1266	3058.62	<0.0001
Family x proportion girdling	4	46.5	2.82	0.0356
Year*family	4	1254	21.23	<0.0001

Table 4 – Parameter estimates from regression analysis predicting Interior Douglas-fir family annual ring width after inoculum placement of *A. ostoyae* (Eq. 1).

Fixed effects	Coefficient	Std. Error	Probability
Intercept	480.19	20.5582	<0.0001
Proportion collar girdling	-0.0404	0.0445	0.3668
Calendar year	-23.8476	1.0279	<0.0001
Family 421	137.63	28.3302	<0.0001
Family 422	-0.5452	30.3268	0.9857
Family 423	53.5477	27.9375	0.0555
Family 514	-101.77	27.6316	0.0002
Family 620	0	-	-
Family 421*girdling	0.0440	0.0484	0.3673
Family 422*girdling	-0.1454	0.0726	0.0470
Family 423*girdling	0.0123	0.0515	0.8119
Family 514*girdling	-0.0322	0.0642	0.6193
Family 620*girdling	0.0000	-	-
Family 421*year	-6.8870	1.4168	<0.0001
Family 422* year	0.04100	1.5163	0.9784
Family 423*year	-2.6523	1.3971	0.0579
Family 514* year	5.1057	1.3814	0.0002
Family 620* year	0	-	-

Random effects	Variance	Std. Error	Probability
Tree intercept (var. a1)	0.4218	0.0790	<0.0001
Tree proportion collar girdling (var. b1)	0.0010	0.0012	0.1996
Covariance (a1, b1)	-0.0102	0.0091	0.2661
Radius within tree	0	-	-
Correlation among repeated measures (ρ) within tree	0.4311	0.0211	<0.0001
Error	0.5037	0.0192	<0.0001

-2 log likelihood = 4961.7, Akaike information criteria = 4971.7

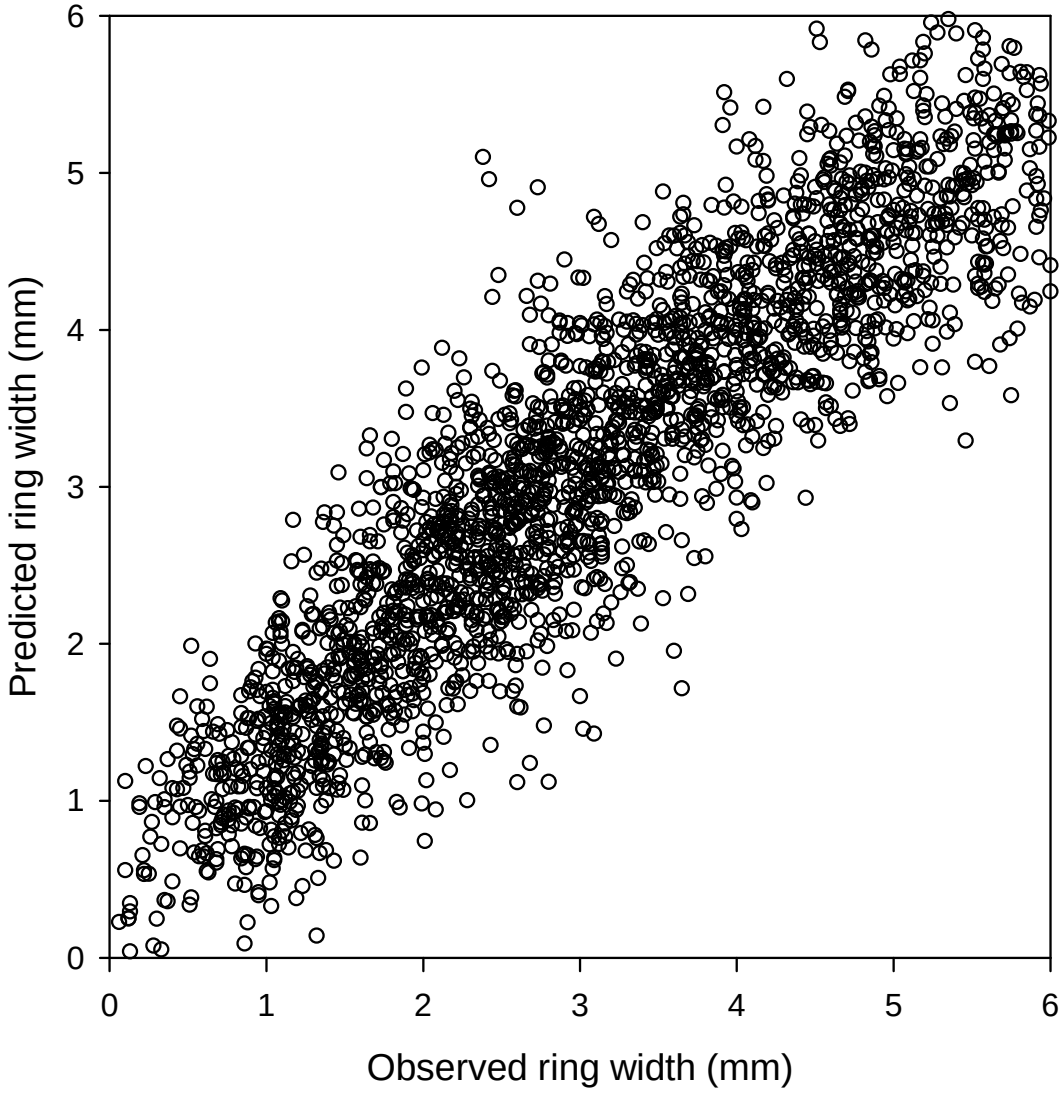

Figure 1. Observed versus predicted values for model Eq. 1.

Figure 2- Tree from family 421(top) showing a horizontally spreading lesion, and tree 423 (bottom) showing a vertically spreading lesion caused by *A. ostoyae* infection at the root collar.

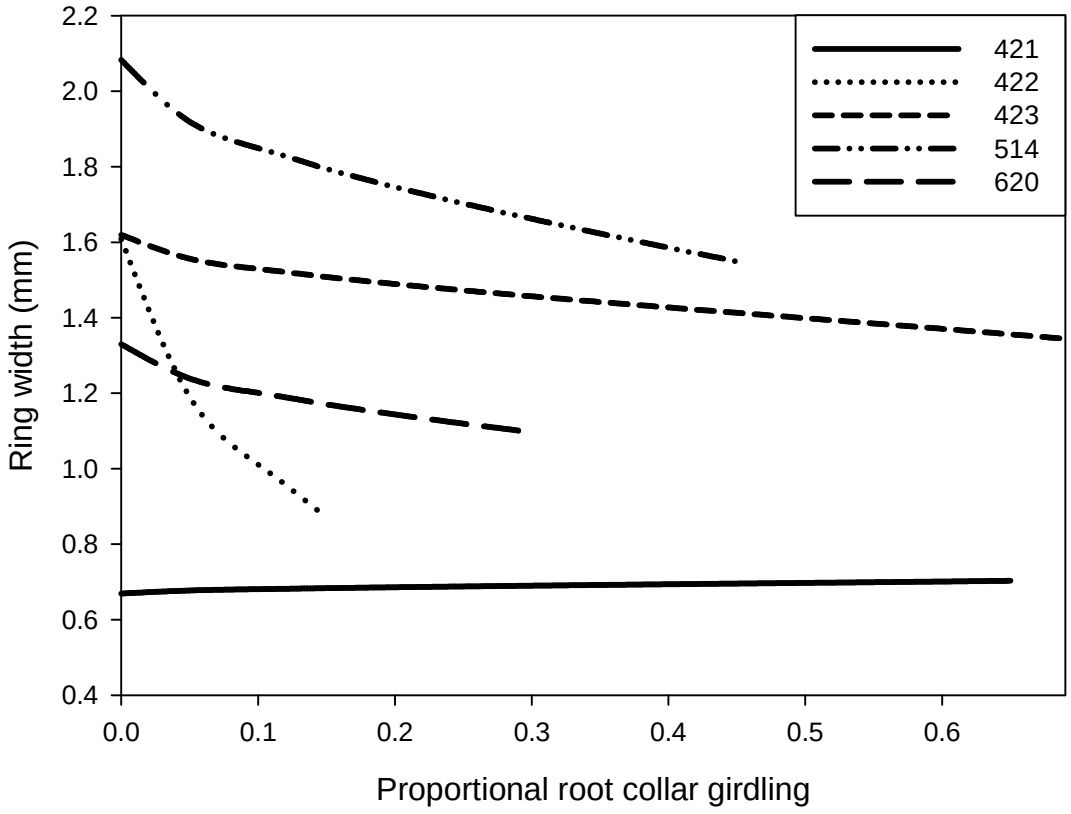

Figure 3 – Relationship between family ring width in 2008 and the proportion of root collar girdling for five Interior Douglas-fir families infected with *A. ostoyae*. Lines represent model fits predicted from Eq. 1.