



# Effect of Swiss needle cast on Douglas-fir stem ethanol and monoterpene concentrations, oleoresin flow, and host selection by the Douglas-fir beetle

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## Abstract

Douglas-fir growing on the western slopes of the Oregon Coast Range are experiencing an unprecedented outbreak of Swiss needle cast (SNC) caused by the fungus *Phaeocryptopus gaeumannii*. SNC can produce substantial physiological stress in host trees by reducing needle gas exchange and enhancing premature needle abscission, resulting in slower growth. Based on the frequent link between stressed trees and insect activity, we explored the potential influence of SNC on Douglas-fir beetle, *Dendroctonus pseudotsugae*, activity and some tree physiological parameters that may influence beetle attraction (i.e., constitutive ethanol and monoterpene contents of woody tissues) and host susceptibility (i.e., wound-induced resin flow). Woody tissue ethanol concentrations, wound-induced resin flow, and beetle attraction were all reduced as SNC severity increased. Although trees affected by SNC attracted fewer beetles, the number of attacks did not decline, the attacks were more likely to penetrate to sapwood depth, and the galleries were longer than in healthier trees, most likely due to a weakened oleoresin defense. However, there have been no current reports of increased Douglas-fir beetle activity on SNC stressed trees, and no rapid increases in beetle population numbers, or outbreaks associated with these diseased forests. SNC stressed trees may remain free from attacks because pioneering beetles have difficulty recognizing them as being stressed with low ethanol concentrations. Furthermore, beetle populations may not be increasing since stressed trees appear unsuitable for reproduction, as no eggs, larvae, or adult beetles were observed in excavated galleries on any attacked trees. However, if large volumes of host materials became available as a result of some catastrophic event (e.g. wildfire or wind-throw), and the beetles can reproduce successfully enough to increase population densities then the potential for a devastating outbreak of Douglas-fir beetle in SNC stressed trees might be exacerbated because they have compromised oleoresin defense systems, and may be killed with fewer beetle attacks.

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**Keywords:** Bark beetles; Stress; Kairomones; Disturbance

## 1. Introduction

Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, growing on the western slopes of the Oregon Coast

Range is experiencing an unprecedented outbreak of Swiss needle cast (SNC) caused by the fungus *Phaeocryptopus gaeumannii* (Rhode) Petrak (Hansen et al., 2000). Visual symptoms include chlorosis and premature loss of foliage, leaving just the current-year needles attached to the ends of branches. Needle loss, and the reduction in photosynthetic capacity of the remaining needles (Manter et al., 2000), is accompanied by

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a reduction in height and diameter growth in trees of all ages and sizes (Maguire et al., 2002). It is readily apparent that these trees are severely stressed.

The Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins, is the most important bark beetle species throughout the range of Douglas-fir (Furniss and Carolin, 1977). During an outbreak they can attack and kill healthy trees, but when beetle population densities are low, they typically act more like secondary beetles and select host trees stressed (Shore et al., 1999) by root disease (Goheen and Hansen, 1993), water deficits (Stoszek, 1973), insect defoliation (Wright et al., 1984; Lessard and Schmid, 1990; Negron, 1998), or with severed stems, such as logs, stumps, wind-throw, and slash (Kingham, 1957; Wright and Lauterbach, 1958; Lejeune et al., 1961; Johnson and Belluschi, 1969; Powers et al., 1999). Trees severely stressed by SNC would seem a suitable host. Yet, there is no evidence to suggest SNC diseased trees are more susceptible to attack and colonization from Douglas-fir beetles than they were prior to the disease outbreak.

Ethanol functions as a primary attractant for various secondary bark beetles when released from severely stressed, dying, or recently killed trees, or insect traps (Moeck, 1970; Klimetzek et al., 1986; Schroeder and Lindelöw, 1989; Byers, 1992; Lindelöw et al., 1993; Kelsey, 1994; Kelsey and Joseph, 1999b, 2001, 2003). Furthermore, the presence of ethanol will often enhance or synergize their response to host monoterpenes, pheromones, or various combinations of these two (Pitman et al., 1975; Tilles et al., 1986; Schroeder and Lindelöw, 1989; Byers, 1992; Lindelöw et al., 1993; Ross and Daterman, 1995). Douglas-fir beetles respond more strongly to traps releasing ethanol with their pheromones, compared to traps releasing just the pheromones alone (Ross and Daterman, 1995). In addition, ethanol has been found in various types of stressed trees and woody residues (Kelsey, 1994; Kelsey et al., 1998b; Kelsey and Joseph, 1998, 1999a,b, 2001) that Douglas-fir beetles are likely to select as hosts when beetle numbers are low. Therefore, ethanol might play some role in their recognition of a suitable host.

Trees infected with SNC could remain free from attack if the beetles are unable to detect the presence of stress. Alternatively, beetles may detect and initiate attacks on diseased trees, but attacks are not successful

because the oleoresin system, which plays a primary role in host defense (Berryman, 1972; Cates and Alexander, 1982; Matson and Hain, 1985; Hain et al., 1985), has not been adversely impacted by the disease. The objectives of this study were to: (i) examine the influence of SNC on ethanol, monoterpenes, and wound-induced resin flow from four sets of Douglas-fir trees with a range of disease, and (ii) compare beetle attraction and attack behavior in trees with and without SNC.

## 2. Methods

### 2.1. Study sites

Three 16–20 year-old Douglas-fir plantations with varying levels of SNC infection (evaluated as described below), located in the northern Oregon Coast Range, were selected for quantification of tree physiology measurements. Sample trees were randomly selected at two plantations; one located at Juno Hill (45°29'11"N; 123°48'29"W, 116 m elevation) near Tillamook ( $n = 13$ ) with severe disease (J-S), and the other located at North Fork (45°46'18"N; 123°52'25"W, 49 m elevation) near Nehalem ( $n = 18$ ) with heavy disease (N-H). At a third plantation near Beaver (45°17'14"N; 123°48'05"W, 158 m elevation), trees with low (B-L, created by previous applications of the fungicide Bravo Weatherstik 720 at 1.25 l ha<sup>-1</sup>) and moderate (B-M, no fungicide application) disease were selected from three sets of paired plots. The fungicide was applied aerially for five consecutive years (1996–2000) to three 2 ha plots, shortly after bud break, or when shoots averaged 5 cm, and again 2 weeks later (A. Kanaskie, Oregon Department of Forestry, personal communication). Six trees were selected at each of the Beaver plots, giving a total of 18 B-M and 18 B-L trees. At the Beaver plots, trees were selected with similar diameters at breast height (DBH) to minimize the potential influence of bole size on Douglas-fir beetle host selection (Baker and Trostle, 1973).

### 2.2. Evaluating infection levels

Fungal colonization was determined for the two youngest needle age classes (2001 and 2000) sampled

April 2002 from two sun-exposed secondary-lateral branches (ca. 5th whorl) by visual assessments of the percent of stomata with *P. gaemannii* pseudothecia (i.e., infection index). Fifty needles from each sample were randomly drawn, affixed to index cards with double sided adhesive tape, and examined under the dissecting microscope to determine the proportion of needles bearing pseudothecia (incidence of infection). The first 10 needles on each card with pseudothecia present were then used to determine the proportion of stomata occluded by pseudothecia (severity) from three 2.6 mm × 0.26 mm sections on each needle. Infection index was calculated as the product of incidence × severity and expressed as a percent.

### 2.3. Ethanol and monoterpene analysis

For ethanol and monoterpene analysis, wood cores (0.5 cm dia.) were removed from each tree at two positions (north and south aspect at breast height) on 16 April 2002. Each core was separated into sapwood (outer 2.0 cm) and phloem tissues and sealed in 4 ml glass vials with Teflon<sup>®</sup> (PTFE) lined screw caps. Vials were immediately frozen with dry ice for transport to the laboratory where they were stored frozen (−36 °C). In preparation for analysis, samples were thawed on ice, weighed into headspace autosampler vials (22 mm × 75 mm, 22 ml volume), sealed with PTFE lined butyl rubber septa, and heated for 30 min at 102 °C to deactivate any remaining enzyme activity. Constitutive ethanol and monoterpenes concentrations were analyzed by the same general method outlined in Kelsey and Joseph (1998), but with newer instruments (Perkin Elmer, Wellesley, MA) and modified settings.

The headspace autosampler was a Turbomatrix 110 with vial oven, needle, and transfer line temperatures set at 70, 75 and 100 °C, respectively. The vials were thermostated for 30.0 min, and then pressurized for 4.0 min with helium at 124 kPa (18 psi), injected for 0.04 min, vented for 0.08 min, and the needle withdrawn in 0.1 min. Helium flow in the transfer line was 1.0 ml min<sup>−1</sup>. The gas chromatograph was a PE Auto-system XL equipped with a J&W scientific BD-Wax column (30 m × 0.32 mm, 0.25 μm film), and flame ionization detector. The injector and detector temperatures were 200 and 250 °C, respectively. The helium carrier gas was set at a constant flow of 2.0 ml min<sup>−1</sup>. The oven temperature was initially held at 50 °C for

2.4 min, and then increased to 120 °C at 45 °C min<sup>−1</sup>, with a final hold for 0.5 min.

Each vial was analyzed twice with venting between runs in order to calculate ethanol and monoterpene concentrations in the tissues using the multiple headspace extraction procedure (Kolb, 1982; Kolb et al., 1984). External standard curves with three calibration levels were prepared for ethanol and α-pinene by diluting ethanol (100%, Apper Alcohol & Chemical Co., Shelbyville, KY) with deionized water, or diluting α-pinene (99+%, Aldrich # 27,439-9) with ethyl acetate (Fisher Scientific, HPLC grade). A syringe was used to deliver 5.0 μl of each solution into separate autosampler vials that were analyzed interspersed with the tissue samples. Replicates of these solutions were averaged for each calibration level in the curve.

### 2.4. Resin flow

Resin flow was measured in duplicate at about 1.3 m on each side of the barrier trap (described below) for trees on the Beaver plots, and on opposite sides of the trees at Juno Hill and North Fork. All phloem and cambial tissues were removed using a cork borer (15 mm diameter). In the center, an 8 mm × 25 mm hole was drilled in the sapwood at a 10° upward angle. A snug fitting, pre-weighed, flexible plastic straw (7.0 mm × 207 mm) was inserted 5–6 mm into the hole and the distal end turned up in an L-shape. To avoid pressurizing the straw as it filled with resin, a pin-sized hole was made on the upper-side near the bole. After 5 days, 12–17 July, the straws were removed and reweighed to determine the mass of resin.

### 2.5. Growth rates

Growth rates were analyzed with a subset of 10 trees from each group (i.e., J-S, N-H, B-M and B-L), but they were not necessarily the same trees sampled for physiological measurements. Two cores (north and south aspect at breast height) and DBH measurements were used to construct the 5-year average annual area increment (cm<sup>2</sup> per year) for 1997–2001.

### 2.6. Beetle assessments

Only the paired B-M and B-L plots from the Beaver plantation were used to assess the relationship

between SNC and Douglas-fir beetle activity. At the center of each 2 ha plot, we attached a 16-funnel Lindgren trap (Lindgren, 1983) to an aluminum L-shaped rod, inverted and stuck into the ground. The overhead arm of the rod was about 2 m above ground. On 23 April 2002 pheromone baits consisting of 200 mg frontalin and 50 mg seudenol suspended separately in 5% plastisol (PVC) rods (Daterman, 1974; Ross and Daterman, 1995) were added to the central Lindgren funnel trap.

Six Douglas-fir trees nearest the Lindgren trap, 5 m or less on all plots, were then outfitted with a barrier trap attached with its top at a height of 1.5 m on the main stem. They had been pruned of all dead branches up to approximately 2 m to insure the beetles had a clear bole silhouette. Traps were oriented to directly face the central Lindgren trap. A plastic catch cup was attached to the funnel and a 2 cm × 5 cm piece of insect pest strip added to the cup (Revenge Bug Strip, Bonide Products, Inc., Oriskany, NY; active ingredient: 18.6% 2,2-dichlorovinyl dimethyl phosphate).

Each barrier trap was constructed from two pieces of fluted plastic sheeting, one cut and folded into a funnel, and the other a rectangular barrier (25.4 cm × 52 cm). The funnel was stapled to a small piece of wood (25 cm × 4 cm) on the outside. The rectangular barrier was inserted into the funnel and also stapled to the wooden piece, with 40 cm of barrier protruding above the funnel (about 1000 cm<sup>2</sup> surface area). A plastic catch cup (14 cm tall × 10.8 cm wide) containing a 2 cm × 5 cm insect pest strip was attached beneath the funnel.

Bi-weekly surveys of trapped beetles were made from 23 April to 12 July 2002. On 13 August 2002, the number of attacks reaching the sapwood, and gallery length were recorded for each clearly discernable attack (i.e., entrance holes with visible red frass) over the entire bole between the ground and 2 m for each sample tree.

### 2.7. Statistical analysis

Infection index DBH, annual area increment, ethanol,  $\alpha$ -pinene, and resin flow for trees at each infection level (J-S, N-H, B-M and B-L) were analyzed as a completely randomized design (CRD) with a one-way analysis of variance (ANOVA) using Proc GLM in

SAS (Vers. 8.1, SAS Institute, Inc., Cary, NC). Infection index were transformed to natural logarithms for analysis to meet the assumptions of homogeneous variances and normal distribution. Their least-squares means and limits defined by  $\pm 1$  pooled S.E. were back-transformed for presentation. Ethanol,  $\alpha$ -pinene, and resin flow were rank transformed for analysis and their medians are presented with the 25th and 75th percentiles. In all analyses, group means were compared for significant differences using Fisher's protected least-significant difference (LSD, model  $\alpha = 0.05$ ). Differences in beetle activity (total number of beetles capture in the Lindgren traps or the tree barrier traps, total number of attacks per plot, attacks as a percentage of the beetle number captured in tree traps, number of attacks reaching the sapwood, and gallery length) between the B-M and B-L trees were transformed to natural logarithms and analyzed as a randomized complete block design (Proc GLM, SAS), where the experimental unit is each 2 ha plot with the six trees as sub-samples, and each block was comprised of adjacent pairs of B-M and B-L plots.

## 3. Results

### 3.1. Disease

The presence of the SNC pathogen, *P. gaemannii*, was different among the four sets of trees. For the 2001 needle age class, all groups were significantly different and ranked as follows: J-S > N-H > B-M > B-L (Fig. 1A). For the 2000 needle age class, the difference between J-S and N-H was no longer present (Fig. 1B) and is consistent with greater levels of needle abscission in the older, more heavily diseased needles at the J-S site (Hansen et al., 2000) leaving only the less infected needles available for assessment. Differences in DBH and the 5-year average annual area increments at breast height were detected (Fig. 1C and D). In general, as disease increased, tree growth declined. Our study was initiated 2 years after the last spray treatment on the B-L trees, and they were still the least diseased among the four groups, but their level of infection was increasing. This was indicated by a marginal difference in the 2002 annual area increment between B-M and B-L trees (data not shown).

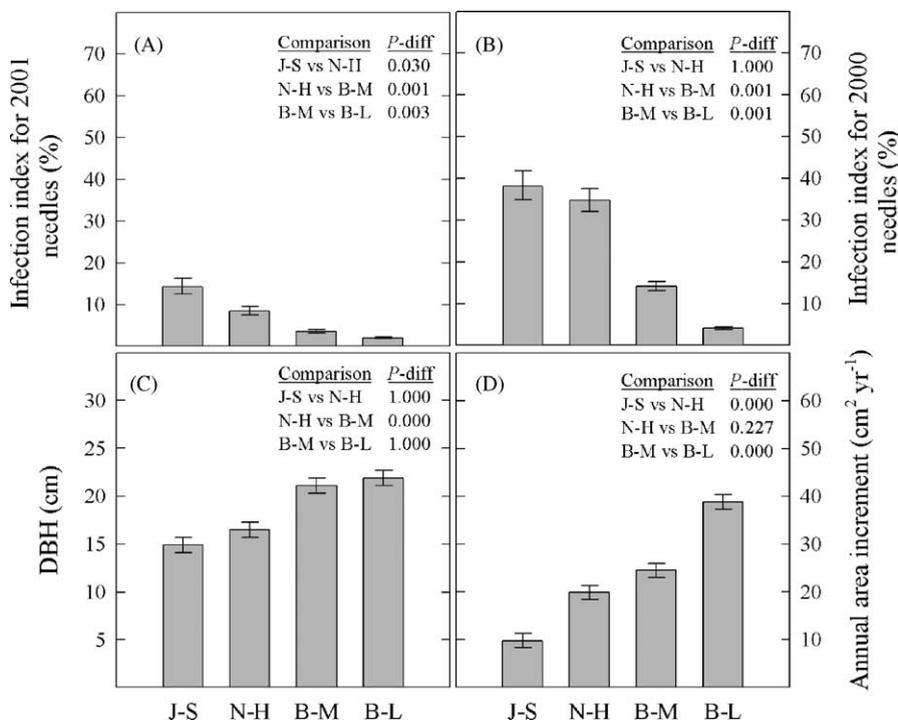


Fig. 1. Disease and growth characteristics of Douglas-fir trees from four plantations with varying levels of Swiss needle cast infection (J-S, N-H, B-M and B-L). Disease was quantified by the percent of stomata with visible pseudothecia or infection index for the 2001 (panel A) and 2000 (panel B) needle age classes; and growth was quantified as DBH (panel C; B-M and B-L trees were selected with similar diameters) and the average annual area increment for the 1997–2001 period (panel D). Details of the statistical analysis are described in the text. Bars in panel A and B are the back-transformed least-square means and their limits defined by  $\pm$ pooled standard errors. Bars in panels C and D are least-square mean with  $\pm$ pooled standard errors.

### 3.2. Ethanol

In general, sapwood tissues contained small amounts of ethanol (Fig. 2A), but differences were detectable between infection groups with the median ranging from 0.00  $\mu\text{mol g}^{-1}$  fresh weight (FW) in the high disease (J-S) trees to 0.28  $\mu\text{mol g}^{-1}$  in the low disease (B-L) trees. A similar trend was observed for the phloem with the median ranging from 0.03  $\mu\text{mol g}^{-1}$  FW in (J-S) trees with the highest disease to 2.78  $\mu\text{mol g}^{-1}$  FW in the low disease (B-L) trees. For the latter trees, the phloem (Fig. 2B) contained 10 times more ethanol than the sapwood (Fig. 2A). On a tree basis, sapwood and phloem ethanol contents were significantly correlated (Table 1), because ethanol is non-ionic and readily diffuses between tissues when a gradient exists. Ethanol per gram FW was highly correlated with concentrations per gram dry weight (DW) in both the sapwood and phloem (Table 1). Diffusion of ethanol in tissue water

makes calculations on an FW basis more appropriate for expressing concentrations than the DW basis.

### 3.3. Monoterpenes and resin flow

Sapwood  $\alpha$ -pinene contents (Fig. 2C) in the two groups with the highest infection (J-S and N-H, ca. 1.2  $\mu\text{mol g}^{-1}$  FW) were significantly lower than in the two with the lowest infection (B-M and B-L, ca. 3.0  $\mu\text{mol g}^{-1}$  FW). In contrast, there were no differences in phloem  $\alpha$ -pinene concentrations among the four infection levels (Fig. 2D). Unlike ethanol,  $\alpha$ -pinene and total monoterpene concentrations in the sapwood were not strongly correlated with concentrations in the phloem (Table 1). Total monoterpene contents were significantly correlated with  $\alpha$ -pinene contents (Table 1) for both the sapwood and phloem tissues, and  $\alpha$ -pinene comprised  $65 \pm 2$  and  $74 \pm 2\%$  of the total monoterpenes in the phloem and sapwood tissues,

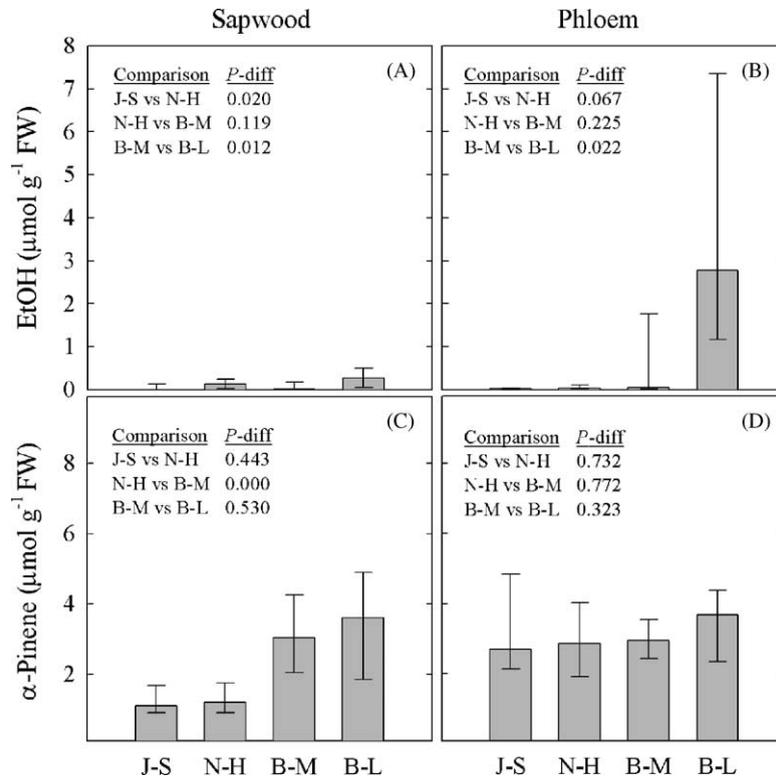


Fig. 2. Ethanol (panels A and B) and  $\alpha$ -pinene (panels C and D) contents of sapwood (panels A and C) and phloem (panels B and D) tissues from the four Douglas-fir plantations with varying levels of Swiss needle cast infection (J-S, N-H, B-M and B-L). Details of the statistical analysis are described in the text. Bars for all panels are the median with the 25th and 75th percentiles.

Table 1

Pearson correlation coefficients ( $r$ ) between the chemical constituents in the woody tissues of Douglas-fir trees from the four sites with varying levels of Swiss needle cast infection (J-S, N-H, B-M and B-L)

	$r$	P-value
Fresh weight (FW) vs. dry weight ( $\mu\text{mol g}^{-1}$ )		
Sapwood ethanol	0.994	<0.001
Phloem ethanol	0.998	<0.001
Sapwood $\alpha$ -pinene	0.981	<0.001
Phloem $\alpha$ -pinene	0.980	<0.001
$\alpha$ -Pinene vs. total terpenes ( $\mu\text{mol g}^{-1}$ FW)		
Sapwood	0.969	<0.001
Phloem	0.833	<0.001
Sapwood vs. phloem ( $\mu\text{mol g}^{-1}$ FW)		
Ethanol	0.834	<0.001
$\alpha$ -Pinene	0.215	0.087
Total terpenes	0.105	0.414

respectively. FW and DW  $\alpha$ -pinene concentrations were strongly correlated (Table 1) so only FW  $\alpha$ -pinene concentrations are presented like those of ethanol.

Wound-induced resin flow declined with increasing SNC presence and was significantly different among all tree groups except B-M and B-L (Fig. 3). Since  $\alpha$ -pinene is a major resin component it was significantly related with resin flow (Fig. 4). Their curvilinear relationship was probably caused largely by volume limitations of the straws used to collect the resin rather than an actual physiological limit. Furthermore, regression analysis showed that resin flow was not significantly related to any other tree characteristic (i.e., ethanol, DBH).

### 3.4. Beetles

Douglas-fir beetle adults were captured on all collection dates over the entire 3-month study period, and

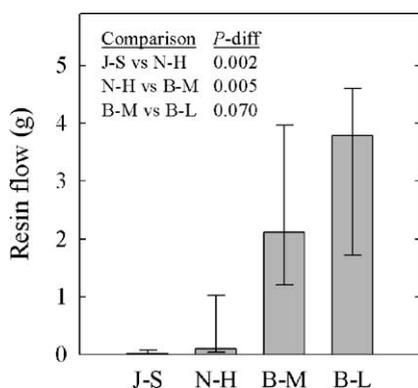


Fig. 3. Sapwood wound-induced resin flow in Douglas-fir trees from the four plantations with varying levels of Swiss needle cast infection (J-S, N-H, B-M and B-L). Details of the statistical analysis are described in the text. Bars are the median with the 25th and 75th percentiles.

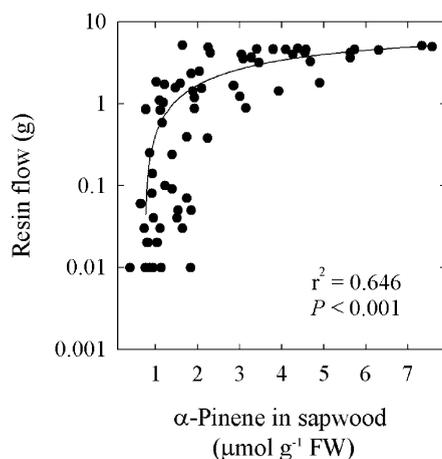


Fig. 4. The relationship between sapwood wound-induced resin flow and  $\alpha$ -pinene content in Douglas-fir trees from the four plantations with varying levels of Swiss needle cast infection (J-S, N-H, B-M and B-L).

for both trap-types approximately twice as many adults were captured in the B-L than in B-M plots (Fig. 5A and B). Most attacks occurred between ground level and 1.8 m up the bole where the stem diameter and bark were thickest, in the same zone where mass attacks were initiated on boles of larger trees (Prenzel et al., 1999). Attacks from Douglas-fir beetles were identified by the characteristic red frass piled outside gallery entrances and bark fissures (Furniss and Carolin, 1977), and probably represent a

minimal number, as there were holes in the bark without frass or that were dripping resin, but they could not be reliably attributed to these beetles and were not counted. Although fewer beetles were captured on B-M than on B-L trees they had the same number of attacks (Fig. 5C) suggesting the more diseased B-M trees might be at greater risk of attack. The B-M trees had a higher percentage of attacks than B-L trees relative to the number of beetles captured in their barrier traps, but the difference was not significant (Fig. 5D).

Douglas-fir beetles normally bore their galleries down to the sapwood and then remain at this depth, lightly etching the sapwood as the gallery excavation proceeds (Furniss and Carolin, 1977). The number of galleries penetrating to sapwood depth (Fig. 5E) and the gallery lengths (Fig. 5F) were reduced in the B-L trees compared to the B-M trees, with the latter difference being the strongest statistically. However, no live adult beetles, eggs, larvae, or larval galleries were observed in any of the galleries excavated on 13 August, regardless of the SNC infection level, indicating the adult beetles may have abandoned them before eggs were laid.

Of all the variables measured (i.e., DBH,  $\alpha$ -pinene, and total monoterpenes), only the phloem ethanol content was significantly correlated with the number of beetles captured on an individual tree, or plot basis (Fig. 6). In addition, the best predictor of attacks penetrating to the sapwood was sapwood  $\alpha$ -pinene content (Fig. 7).

#### 4. Discussion

Within most coastal Oregon Douglas-fir plantations the level of infection by SNC is rather homogeneous. To get the desired range of infection levels, we sampled plantations at different sites that have been under observation and study since the mid-1990s (Hansen et al., 2000), including one where a portion had been treated with fungicide to provide a low infection level. As a consequence, the analyses and interpretations of the physiological parameters with level of infection (Figs. 1–3) are confounded by a potential site effect. However, there is no confounding site effect on the Beaver plantation paired-plots for the comparison of physiological parameters, or

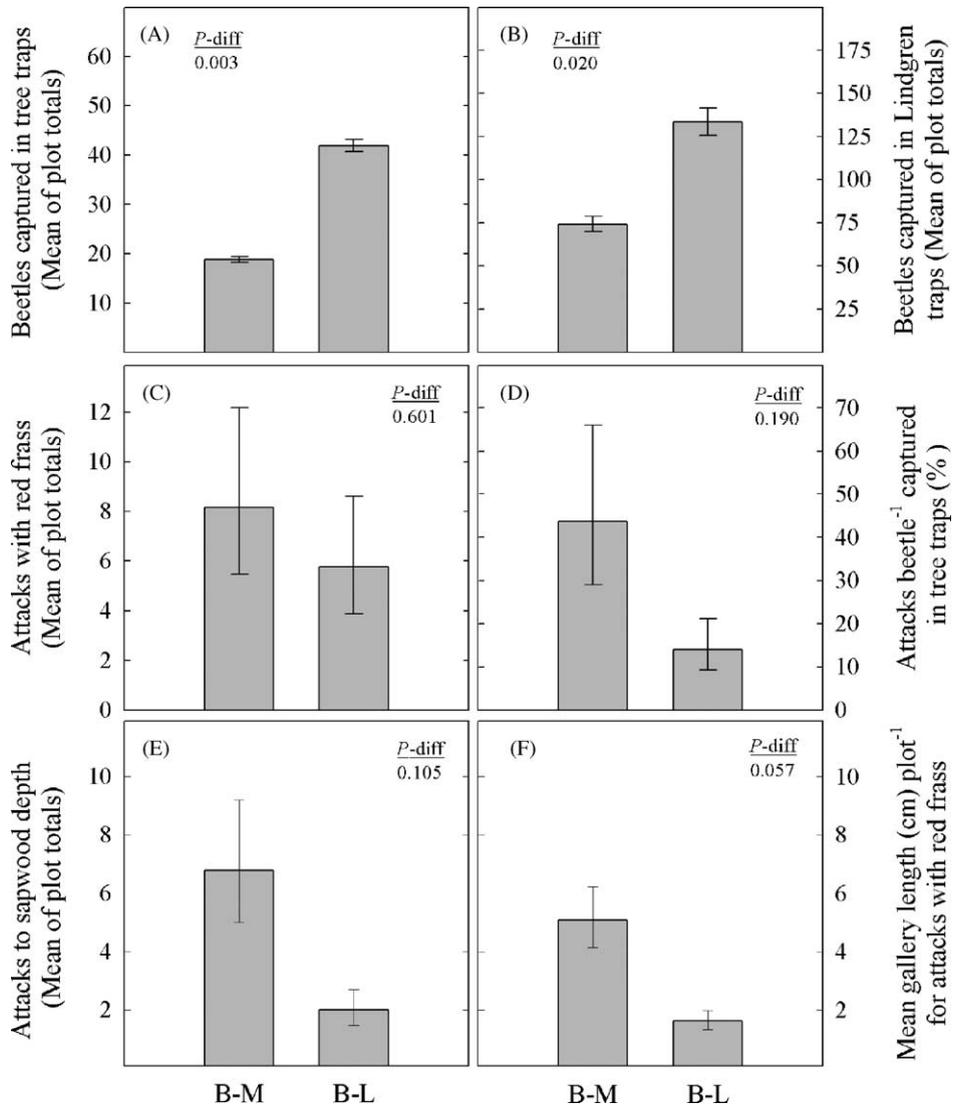


Fig. 5. The mean total number of beetles captured per plot in passive barrier traps on six trees surrounding the Lindgren trap (panel A), and captured in the Lindgren trap at the plot center (panel B), the mean total number of attacks per plot with red frass (panel C), the mean total number of attacks on trees with passive barrier traps as a percentage of the mean number of total beetles captured in the tree traps per plot (panel D), the mean total number of galleries reaching the sapwood per plot (panel E), and the mean gallery length per plot from attacks with red frass (panel F) for the B-M and B-L trees at the Beaver site. Details of the statistical analysis are described in the text. Bars in all panels are the back-transformed least-square means and their limits defined by  $\pm$ pooled standard errors.

beetle studies. We suggest that the level of SNC infection is the major factor influencing the physiological parameters at all sites for the following reasons: (i) the responses are consistent with the known physiological impacts of SNC (as discussed below) and (ii) the patterns within the Beaver plantation

paired-plots (where site is not a confounding factor) are consistent with the results seen at the other two plantations (J-S and B-H). For example, wound-induced resin flow was greatest in the healthier B-L trees, and declined in the order of disease severity B-M > N-H > J-S trees.

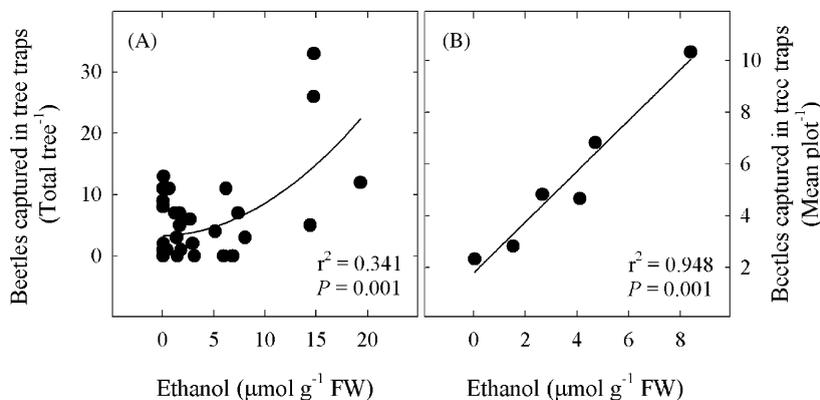


Fig. 6. Relationship between phloem ethanol content and beetles captured in the barrier traps using individual tree totals (panel A) and plot averages (panel B) for the B-M and B-L plots at the Beaver site.

Decreasing constitutive ethanol concentrations in Douglas-fir stem tissues with increasing levels of SNC is consistent with a mechanism where the availability of carbohydrate substrate for both aerobic respiration and fermentation becomes increasingly limited as a consequence of the infection. SNC decreases photosynthate production by reducing needle retention (Hansen et al., 2000; Maguire et al., 2002) and impairing carbon assimilation in the remaining infected needles (Manter et al., 2000); and when pseudothecia densities reach ca. 25%, carbon budgets are predicted to be negative (Manter et al., 2003). Evidence of limited carbohydrates is further supported by the reductions in growth (Fig. 1C and D),  $\alpha$ -pinene contents (Fig. 2C) and wound-induced resin flow (Fig. 3)

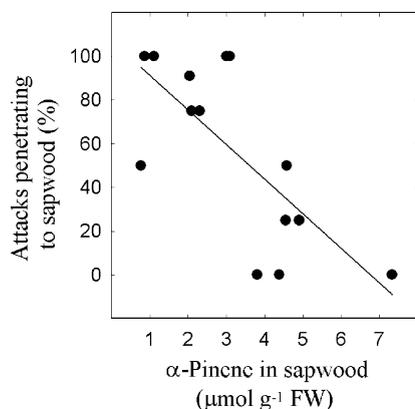


Fig. 7. Relationship between the percent of attacks penetrating to the sapwood and sapwood  $\alpha$ -pinene content for the B-M and B-L trees at the Beaver site.

with increasing levels of SNC, as all of these are dependent on the carbohydrate supply (review, Kozłowski, 1992). Severe defoliation of Douglas-fir by tussock moth, *Orgyia pseudotsugata* (McDunnough) proportionally decreased the amounts of starch stored in the foliage, twigs, and roots (Webb and Karchesy, 1977); and defoliation of grand fir, *Abies grandis* (Douglas) Lindley, by tussock moth caused various changes in the phloem, including a reduction in total sugars the first year, a decrease in starch the second year, and a reduction in the ability to synthesize monoterpenes during the hypersensitive wound response (Wright et al., 1979). When photosynthetic rates of ponderosa pine, *Pinus ponderosa* var. *scopulorum* Engelm., were impaired by stress from intraspecific competition (high basal area), growth and resin flow were both reduced (Kolb et al., 1998).

A limited carbohydrate pool could reduce ethanol production two ways in trees with SNC. First, by limiting aerobic respiration rates. Ethanol biosynthesis may be induced during brief intervals of hypoxia when cambial respiration rates associated with growth are fast enough to deplete tissue  $\text{O}_2$  supplies (Harry and Kimmerer, 1991; Kelsey et al., 1998a; authors' unpublished data). But, if growth is substrate limited then hypoxia from rapid respiration should occur less frequently and produce less ethanol. Secondly, since ethanol biosynthesis also requires carbohydrates (Harry and Kimmerer, 1991; Kelsey et al., 1998a), when hypoxia does occur the amount of ethanol synthesized would be limited by the low carbohydrate supply in trees with SNC.

Douglas-fir beetles respond more strongly to traps releasing ethanol and pheromones simultaneously compared to traps with just the pheromones alone (Pitman et al., 1975; Ross and Daterman, 1995). Since pheromones were present on the Beaver plots, lower ethanol concentrations in the B-M trees might explain why they attracted about half the number of Douglas-fir beetles compared to the less stressed B-L trees. At the tree-level, regression analysis showed that there was a moderate, but significant relationship between phloem ethanol content and beetle attraction. This relationship was greatly improved when analyzed on a plot basis ( $r^2 = 0.948$ ,  $n = 6$ ). Collapsing the data to the plot level improved the relationship because the variability was reduced, and plot means were probably better at accounting for “spill-over,” or those individuals that may have been attracted to the site by the pheromone baits, but landed or wandered to a neighboring tree before being captured in the passive tree traps. When individual trees are baited with pheromones, unbaited trees 20–40 m away may also be attacked as a result of spill-over (Baker and Trostle, 1973; Ringold et al., 1975; Their and Patterson, 1997).

Whether ethanol functions as a primary attractant for pioneering Douglas-fir beetles during their early dispersal phase, before pheromones are released, is not known. However, they typically select and colonize stressed trees, which as described earlier are likely to contain elevated quantities of ethanol. But, due to the different nature of SNC stress, as the level of disease increases ethanol concentrations decline; consequently there is no signal of physiological stress for the beetles to detect resulting in fewer numbers of beetles attracted to SNC-affected trees.

Constitutive concentrations of  $\alpha$ -pinene and total monoterpenes were influenced by the presence of SNC in Douglas-fir sapwood, but not the phloem. Significant differences were detected between the sapwood contents from the two lowest and the two highest disease levels. Constitutive monoterpenes in the B-L and B-M trees were not different and therefore not a likely contributor to the beetle's greater attraction to B-L trees. Furthermore, traps baited with ethanol and pheromones need not contain monoterpenes to capture large numbers of Douglas-fir beetles (Ross and Daterman, 1995, 1997). Pioneering beetles

rely on monoterpene odors to identify the appropriate host-species (Heikkinen and Hrutford, 1965; Rudinsky, 1966b; Furniss and Schmitz, 1971), since they normally attack only Douglas-fir and western larch, *Larix occidentalis* Nutt. (Furniss and Carolin, 1977). Monoterpenes released from fresh cut logs can induce a rapid response from pioneering beetles (McMullen and Atkins, 1962; Rudinsky, 1966b), and when monoterpene volatilization declines as logs age, beetle attraction is diminished (McMullen and Atkins, 1962). Traps baited with 2.5% Douglas-fir resin, 1%  $\alpha$ -pinene, or 1% camphene, each diluted with 95% ethanol, attracted more beetles than a fresh cut log (Rudinsky, 1966a). Douglas-fir beetles responded synergistically to traps releasing Douglas-fir resin or  $\alpha$ -pinene in combination with the pheromone, frontalin, compared to traps releasing each component separately (Furniss and Schmitz, 1971). However, when undiluted  $\alpha$ -pinene and camphene were added 1:1 to pheromone baits (frontalin + seudenol) they had limited impact on beetle attraction (Pitman et al., 1975).

Although trees stressed by SNC attracted fewer Douglas-fir beetles in the presence of pheromones, the number of attacks were the same for B-M and B-L trees, indicating their risk of being attacked may have actually increased, although the percentage of attacks relative to the number of beetles captured in tree trapped was not significantly different. There was stronger evidence to show attacks were more successful (i.e., number of attacks reaching the sapwood and gallery length) in trees with higher disease levels, most likely because they have impaired oleoresin defense systems compared with the less diseased trees. Galleries reaching the sapwood were best predicted by sapwood  $\alpha$ -pinene contents, but not wound-induced resin flow. The lack of a significant relationship with wound-induced resin flow may be due to either (i) the error associated with resin collection as discussed above or (ii)  $\alpha$ -pinene is a strong contributor to resin toxicity toward the Douglas-fir beetle. For conifers, oleoresins play a primary role in influencing gallery length and beetle colonization success (Berryman, 1972; Cates and Alexander, 1982; Matson and Hain, 1985; Hain et al., 1985). This activity has been attributed to factors such as the quantity of wound-induced monoterpenes produced (Wright et al., 1979), resin exudation pressure (Rudinsky, 1966b), oleoresin composition (Heikkinen and Hrutford, 1965), and

vapor toxicity (Jantz and Rudinsky, 1965). In laboratory olfactometers, Douglas-fir beetles were repelled by vapors from Douglas-fir oleoresin (Jantz and Rudinsky, 1965) and by  $\beta$ -pinene (Heikkinen and Hrutfiord, 1965). Beetle mortality was observed after 3 days of continuous exposure to resin vapors, or immediately upon immersion in resin (Jantz and Rudinsky, 1965).

With the outbreak of SNC in the Oregon Coast Range, it has been questioned why stressed trees of suitable size are not being more heavily attacked and why there has not been a corresponding outbreak of Douglas-fir beetle, since susceptibility of Douglas-fir to beetle attacks is associated with reduced growth from stress (Shore et al., 1999), including defoliation by insects. For example, trees defoliated 95–100% by Douglas-fir tussock moth were selected as hosts by Douglas-fir beetles during the 2 years of active defoliation (Wright et al., 1984). This stimulated a rapid increase in beetle population densities for 2 years following the defoliation. In Colorado, outbreaks of Douglas-fir beetle were preceded by defoliation from western spruce budworm, *Choristoneura occidentalis* Freeman, that caused measurable declines in growth (Lessard and Schmid, 1990; Negron, 1998). However, the beetle outbreaks occurred as the trees were recovering from defoliation and growth was increasing. Douglas-fir beetles may respond differently to trees defoliated heavily and quickly by insects than those defoliated more slowly and repeatedly by SNC, if the physiological responses of the trees are not similar.

The absence of a bark beetle outbreak in Douglas-fir defoliated by SNC in the Oregon Coast Range may be the result of several factors. First, pioneering Douglas-fir beetles may not readily recognize trees stressed by SNC because of their low ethanol concentrations. Additional studies are needed to clearly evaluate the influence of ethanol on host selection by pioneering Douglas-fir beetles. Second, in the vicinity of our study sites it appears that the endemic beetle population density is low, probably because much of the area is covered with relatively young forest plantations and the amount of suitable host material (e.g., large susceptible trees, stumps, and down woody debris from wind storms or harvesting) required for rapid increases in beetle population densities is limited. Past outbreaks of the Douglas-fir beetle in the Oregon Coast Range have

been typically associated with catastrophic events such as wind and fire, or harvesting that generated large volumes of suitable host materials from otherwise vigorous trees (Wright and Lauterbach, 1958; Johnson and Belluschi, 1969). By the end of our study, we had less than 140 beetles per baited trap. In central Idaho where endemic population densities are higher, trap catches were 100 times greater using pheromone/ethanol baits that contained twice the pheromone levels used in this study (Dodds et al., 2000).

Finally, although the attacks in trees infected with SNC appeared more successful based on gallery size, there were no eggs, larvae, or adults observed in the excavated galleries. Douglas-fir defoliated by SNC infection is not likely to be an optimal host for beetle reproduction because of nutritional limitations in their tissues resulting from stress. The number of offspring per female produced in trees defoliated by tussock moth decreased progressively as the cumulative defoliation increased from 40–49 to 70–79% (Wright et al., 1984). If defoliation by SNC has a similar effect on beetle reproduction then the rate of increase in their population densities are likely to be much slower, compared to stands without SNC. However, if future beetle populations do increase as a result of some catastrophic event (e.g. wildfire or wind-throw), and their reproductive success is not completely impaired by SNC, then the potential for a devastating Douglas-fir beetle outbreak might be exacerbated because the SNC stressed trees have compromised oleoresin defense systems, and they could be killed with fewer beetle attacks.

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