

Attack by *Hylobius warreni* on grafted lodgepole pine and its relationships with monoterpene composition and scion : rootstock diameter ratio

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- Abstract**
- 1 A lodgepole pine seed orchard at the Prince George Tree Improvement Station (PGTIS), with up to 60% of grafted trees attacked by the Warren root collar weevil *Hylobius warreni* was investigated to determine whether relative monoterpene composition or scion : rootstock interactions of grafts affected susceptibility to attack.
 - 2 There was a significant relationship between relative levels of α -pinene, β -thujene, β -pinene, δ -3-carene and limonene in scion and rootstock in unattacked trees, indicating a potential effect of the scion monoterpene composition on their composition in the rootstock.
 - 3 Relative content of δ -3-carene and β -phellandrene differed significantly in root stocks of attacked and unattacked trees but, for individual clones, a significant difference was only detected for β -phellandrene in one clone. δ -3-Carene levels may have been too low in the examined trees to exert a strong effect.
 - 4 Interestingly, attack status was significantly associated with two scion monoterpenes: α -thujene and α -terpinolene, both of which had higher levels in unattacked than in attacked trees.
 - 5 Warren root collar weevils appear largely unaffected by monoterpene content, but further study is required to determine whether high levels of δ -3-carene imparts some level of resistance to attack, and to verify whether the observed effects of scion monoterpenes are real or artefacts of the analysis.
 - 6 *Hylobius warreni*-attacked trees had smaller scion : rootstock diameter ratio (i.e. a large rootstock diameter relative to the scion diameter) than unattacked trees. This effect was consistent among clones, and was not due to the absolute diameter of the rootstock or the scion. Trees with increased diameter-growth at the root collar (e.g. some grafted trees) may have increased susceptibility to attack by *H. warreni*, or diameter-growth at the root collar is affected by the attack.

Keywords Coleoptera, Curculionidae, grafted trees, monoterpenes, *Pinus contorta*, root stock, scion, Warren root collar weevil.

Introduction

The Warren root collar weevil *Hylobius warreni* Wood is a common pest of lodgepole pine *Pinus contorta* var. *latifolia* in British Columbia (Grant, 1966; Cerezke, 1994). Adult

females oviposit on or near the root collar of trees, and larvae mine the inner bark and the surface of the sapwood, resulting in scarring and copious resinosis (Cerezke, 1994). Girdling may cause mortality in trees less than 5 cm diameter at the root collar but, in larger-diameter trees, larvae feed mainly on major roots rather than on the root collar. Despite the low overall levels of mortality due to *H. warreni* attack, considerable losses in growth and stability may result. Girdling weakens the root, making attacked trees unstable, and growth reductions occur above the girdled area of the root collar and

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below the girdled area on lateral roots. Reduction in height growth appears to be the main long-term effect of weevil attack. However, such losses have yet to be quantified on a stand and temporal basis (Cerezke, 1994).

Lodgepole pine is one of the most important commercial tree species in the interior of British Columbia. Several lodgepole pine seed orchards have been established to cover the demand for seeds. The Prince George Tree Improvement Station (PGTIS), located just south of Prince George, contains seven lodgepole pine seed orchards. Seed produced from these orchards is quite valuable, and there is a low tolerance threshold for damage in the seed orchards. The lodgepole pine seed orchards at the PGTIS have experienced high levels of *H. warreni* infestation. In a survey recently conducted by B. S. Lindgren (unpublished data), the overall *H. warreni* attack rates in four lodgepole pine seed orchards at the PGTIS were 62.3%, 24.4%, 12.7% and 5%, with the older orchards showing the greatest proportion of attacks. Based on the high monetary value of each tree, these infestation rates are unacceptable.

The lodgepole pine seed orchards at the PGTIS consist of clones grafted onto lodgepole pine rootstock of unknown origin (i.e. within any one orchard, rootstock trees are from one or several unknown seedlots). Grafting leads to more or less pronounced physiological stress, depending on the level of compatibility between rootstock and scion. Incompatibility is sometimes evident as differential growth rates between the scion and rootstock, resulting in a swelling either above or below the graft union.

In a study conducted in one of the seven orchards at the PGTIS, Rocchini *et al.* (2000) found that the relative amount of δ -3-carene explained over 40% of the variation in attacks on stems (scions) of grafted lodgepole pines by the Douglas-fir pitch moth, *Synanthedon novoarvensis* (Lepidoptera: Sesiidae), when regressed against relative amounts of monoterpenes. Because the composition of resin produced is under genetic control (Smith, 1964; Bernard-Dagan, 1988), analysis of monoterpenes may reveal why some clones appear to be more resistant to weevil attack, and therefore would provide a potential means of chemotyping resistant trees (Tomlin *et al.*, 1997).

In the present study, we investigated the relative monoterpene content of the rootstock and scion, and whether these levels correlated across the graft union. We also measured the diameters of the root stock and scion associated with grafting, and calculated their ratio as a potential index of tree-response to grafting. The study aimed to determine whether either of these parameters could explain susceptibility to attack on the rootstock of grafted lodgepole pine by the Warren root collar weevil.

Materials and methods

Phloem samples were collected using a 10-mm leather punch (Arch Punch, C.S. Osborne and Co., Harrison, New Jersey) from trees in the Willow-Bowron #220 seed orchard at the PGTIS (53°46'N, 122°43'W), 10–15 October, 2000. Eight to 10 ramets (four to six attacked and four to six unattacked) from each of six different clones (980, 986, 1542, 1583, 1584, 2000) were sampled. All trees in the selected clones

were surveyed for attack to confirm their status. Due to the cryptic habits of this insect and the propensity for repeated attacks over many years, and hence the difficulty in assessing the number of larvae present, we only considered presence or absence of attacks. Four phloem samples were taken from each tree, two per aspect (north and south), with one from above the graft union and the other from the rootstock, as close to the ground as possible. Because the graft union is sometimes indistinct, the scion samples were taken on the trunk approximately 10–15 cm above the graft union to ensure that only clonal scion-tissue was sampled. Depending on the height of the graft union on the tree, this was equivalent to 20–40 cm above ground level. Root stock samples were taken so that they were not directly adjacent to attack to avoid sampling of secondary monoterpenes. The punch was cleaned between each scion and rootstock sample and between each tree. The outer bark was discarded and the phloem placed into a labelled 2 × 3 inch paper envelope. The envelope was sealed, labelled and placed in liquid nitrogen until samples could be transferred to a –80 °C freezer, where they were stored until monoterpenes were extracted for analysis by coupled gas chromatography–mass spectrometry (GC–MS). Only the south samples were extracted for the purpose of this study. Diameters above and below the graft union of each tree were measured approximately 10 cm above the highest and below the lowest point of the graft union, respectively, using a standard diameter tape (Lufkin 6 m C106TPM diameter tape, Lufkin, Texas).

For monoterpene extractions, each phloem sample was chopped into coarse pieces and placed in labelled 10-mL glass scintillation vials (Wheaton Scientific Products, Millville, New Jersey). Five mL of hexane with 0.02% undecane as an internal standard was added, and the samples left at approximately 22 °C for 23 h. The extract was then filtered using a 10-mL syringe (Beckon-Dickinson, Singapore) fitted with a Gelman nylon Acrodisc 13, 0.2- μ m filter (Gelman Sciences, Ann Arbor, Michigan). The filtered extract from each sample was split between two 2-mL GC vials (National Scientific Company, Lawrenceville, Georgia) to allow separate runs on two different columns. The analyses were performed using a Hewlett-Packard (Palo Alto, California) HP 5890 Series 11 GC fitted with a 30 m × 0.25 mm × 0.25 μ m DB 5 or DB 23 capillary column and connected to a Hewlett Packard HP 5989B mass spectrometer. The two columns were utilized because some monoterpenes coelute on each column (e.g. β -phellandrene and limonene on DB 5). One μ L of each sample was injected using a HP6890 Series injector autosampler. The carrier gas used was helium at a flow rate of 1.3 mL/min for both columns. For the DB5 column, the injection temperature of 40 °C was maintained for 2 min, then ramped at 2 °C/min to 70 °C, followed by 1 °C/min to 80 °C and finally 30 °C/min to 250 °C, where it was held for 7 min. For the DB23 column, the injection temperature of 30 °C was maintained for 2 min, then ramped at 2 °C/min to 55 °C, at 30 °C/min to 250 °C, where it was held for 4 min. All peaks exceeding 0.1% of total monoterpene content were identified using a Wiley MS library and comparisons with known standards. A hexane blank containing 0.02% of the internal standard was run

through the GC–MS every ten samples to ensure that retention times did not drift throughout the analysis and that the column was not contaminated.

The relative concentration of each monoterpene was calculated by dividing the area of each monoterpene peak in the chromatogram by the total area of all monoterpene peaks and multiplying by 100. Thus, monoterpene concentration was expressed as a percentage of total monoterpene content (Squillace, 1976).

Regression analyses were conducted to determine whether there were any relationships between monoterpene contents in scion and rootstock. The relative monoterpene content in the rootstock was used as the dependent variable based on the findings by Mirov (1945). All remaining data were analysed by multivariate analysis of variance (MANOVA) to determine whether there were significant differences in monoterpene composition between clones and to determine whether the monoterpene composition of the scion and rootstock differed significantly. Clone was included as a categorical variable to test for potential effects of scion genotype on rootstock susceptibility. The first hypothesis of no effect by attack status on rootstock monoterpene composition was analysed by MANOVA for each monoterpene separately using only rootstock data because the weevil attacks trees at or below ground level. These analyses used attack as the dependent variable, and clone, monoterpene percent and their interaction as the independent variables. The second null hypothesis of no effect of scion or rootstock diameters, or their ratio, on *H. warreni* attack was analysed by analysis of variance (ANOVA) with clone, diameter or diameter ratio, and their interaction as the independent variables. All statistical tests were performed using SYSTAT 9.0 (SPSS Inc. Chicago, Illinois) or JMP for Windows (SAS Institute Inc. Cary, North Carolina).

Results

We initially intended to select the clones to have three each with relatively lower and higher attack percentage, respectively, based on data from the seed orchard. However, these data did not correspond well with actual attack rates, which were relatively high for all six clones (range 35.7–60% of ramets in each clone). The attack rates and total number of trees for each of the clones are shown in Table 1.

Mean percentages of each monoterpene in the scions and rootstocks of sampled lodgepole pines at the PGTIS are shown in Table 2. Five monoterpenes, β -phellandrene, β -pinene, α -pinene, δ -3-carene and limonene, accounted for 90.8% of total monoterpene content in the scion tissue sampled. Similarly, in rootstocks, the four monoterpenes β -phellandrene, β -pinene, δ -3-carene and α -pinene accounted for 89.1% of total monoterpene content. All other monoterpenes were present in mean relative amounts of 3.4% or less (Table 2). There was a significant relationship between relative levels of α -pinene ($P < 0.001$, $r^2 = 0.641$, $n = 26$), β -thujene ($P = 0.05$, $r^2 = 0.151$, $n = 26$), β -pinene ($P = 0.035$, $r^2 = 0.173$, $n = 26$), δ -3-carene ($P = 0.011$, $r^2 = 0.239$, $n = 26$) and limonene ($P = 0.01$, $r^2 = 0.247$, $n = 26$) in scion and rootstock.

Table 1 Number of lodgepole pine trees attacked by *Hylobius warreni* for each of six clones

Clone no.	<i>N</i>	Number attacked trees	% Attacked
980	30	18	60.0
986	19	10	52.6
1542	28	10	35.7
1583	33	18	54.5
1584	32	15	46.9
2000	24	10	41.7

Willow-Bowron #220 seed orchard, Prince George Tree Improvement Station, Prince George, British Columbia, September to October 2000.

MANOVA analysis of the scion monoterpene data revealed a significant effect at the whole model level ($F_{121,250.92} = 9.792$, $P < 0.0001$, Wilks' $\lambda = 1.1 \times 10^{-6}$). In addition, the difference in the mean relative monoterpene content was significant at the model level for all clones ($F_{55,142.45} = 38.133$, $P < 0.0001$, Wilks' $\lambda = 2.9 \times 10^{-6}$). Monoterpene content among the scions also differed significantly ($F_{5,40} = 2.9-239.2$, $P < 0.05$ for all clones).

Attack status was significantly associated with two scion monoterpenes: α -thujene ($F_{1,1} = 4.236$, $P = 0.0461$) and α -terpinolene ($F_{1,1} = 16.344$, $P = 0.0002$) (Table 3). For α -terpinolene, there was also a significant attack by clone interaction ($F_{5,5} = 2.960$, $P = 0.0231$). In all clones except clone 1542, mean (least squares) relative α -thujene content was greater in unattacked than attacked trees. For α -terpinolene, mean relative content was greater in scions of unattacked than attacked trees for all clones except clone 980. ANOVA and Tukey's HSD test for individual clones showed no significant difference in α -thujene for any single clone. α -Terpinolene content was significantly lower in unattacked than in attacked trees in clone 1542, but did not differ for any other clone.

Table 2 Mean and standard deviation of relative cortical monoterpene levels (%) in scions and rootstock from six lodgepole pine clones ($n = 52$)

Scion	Rootstock, % (mean \pm SD)	Monoterpene, % (mean \pm SD)
α -thujene	0.3 \pm 0.2	0.2 \pm 0.2
α -pinene	9.5 \pm 5.6	8.6 \pm 3.8
camphene	0.5 \pm 0.2	0.4 \pm 0.5
β -thujene	1.3 \pm 0.5	1.6 \pm 1.4
β -pinene	16.3 \pm 6.9	16.8 \pm 6.3
β -myrcene	2.4 \pm 0.5	1.9 \pm 0.7
α -phellandrene	1.6 \pm 0.4	1.3 \pm 0.6
δ -3-carene	8.3 \pm 4.4	13.8 \pm 8.2
β -phellandrene	50.7 \pm 9.3	49.9 \pm 8.8
α -terpinolene	3.1 \pm 1.5	2.1 \pm 1.5
limonene	6.0 \pm 5.3	3.4 \pm 2.5

Willow-Bowron #220 seed orchard, Prince George Tree Improvement Station, Prince George, British Columbia, October 2000.

Table 3 Percent (mean \pm SD) relative monoterpene content of scion phloem tissue, and the probability of these monoterpenes influencing attack by *Hylobius warreni* ($n = 26$)

Monoterpene	Attacked scion	Unattacked scion	Effect on attack	
			<i>F</i>	<i>P</i>
α -thujene	0.3 \pm 0.2	0.4 \pm 0.2	4.24	0.05
α -pinene	9.2 \pm 5.6	9.7 \pm 5.7	0.03	0.87
β -thujene	1.2 \pm 0.4	1.4 \pm 0.6	2.15	0.15
β -pinene	16.7 \pm 6.4	15.9 \pm 7.4	0.05	0.82
camphene	0.4 \pm 0.2	0.5 \pm 0.2	0.65	0.43
β -myrcene	2.3 \pm 0.5	2.5 \pm 0.5	1.42	0.24
α -phellandrene	1.5 \pm 0.4	1.6 \pm 0.4	1.07	0.31
δ -3-carene	8.0 \pm 4.5	8.5 \pm 4.3	0.07	0.79
β -phellandrene	51.0 \pm 8.7	50.4 \pm 10.1	1.11	0.30
α -terpinolene	2.7 \pm 1.6	3.6 \pm 1.2	16.34	<0.001
limonene	6.7 \pm 5.7	5.3 \pm 4.9	0.04	0.84

Willow-Bowron #220 seed orchard, Prince George Tree Improvement Station, British Columbia, October 2000 ($\alpha = 0.05$).

At the whole model level, MANOVA analysis revealed a significant effect for rootstock monoterpenes ($F_{121,250,92} = 1.841$, $P < 0.0001$, Wilks' $\lambda = 6.78 \times 10^{-3}$) and mean relative monoterpene content ($F_{55,142,45} = 2.898$, $P < 0.0001$, Wilks' $\lambda = 0.031$), whereas, neither attack ($F_{11,30} = 1.504$, $P = 0.182$) nor clone by attack interaction were significant ($F_{55,142,45} = 1.0202$, $P = 0.451$, Wilks' $\lambda = 0.215$).

Monoterpene variation among clones was significant for all monoterpenes analysed ($F_{5,46} = 2.6$ – 246.4 , $P < 0.05$ for all clones). In the nonclonal rootstocks, variation among clones were significant for α -pinene ($F_{5,40} = 16.219$, $P < 0.0001$), β -pinene ($F_{5,40} = 6.800$, $P < 0.0001$) and δ -3-carene ($F_{5,40} = 3.585$, $P = 0.009$).

Attack was marginally significant for δ -3-carene ($F_{1,40} = 4.063$, $P = 0.051$) and significant for β -phellandrene ($F_{1,40} = 5.020$, $P = 0.031$) (Table 4). Clone by attack interaction was also significant for β -phellandrene ($F_{5,40} = 2.614$, $P = 0.039$) and the significant effect by attack appeared to be confined to two clones, with all other clones being relatively similar.

The diameter of the rootstock was always greater than that of the scion. The ratio of scion to rootstock diameter was significantly different in attacked trees and unattacked trees ($F_{1,40} = 6.4$, $P = 0.02$), with attacks associated with a lower ratio (i.e. a larger difference in diameter) within each clone. There was no attack by clone interaction ($F_{5,40} = 0.3$, $P = 0.9$), indicating that this relationship was consistent in all clones (Fig. 1). Absolute diameter of the rootstock ($F_{1,40} = 1.4$, $P = 0.31$) or the scion ($F_{1,40} = 0.38$, $P = 0.54$) did not affect attack. The size-difference ranged from approximately 14–27% in attacked trees, and from approximately 11–21% in unattacked trees.

Discussion

The Warren root collar weevil attacks the rootstock of grafted trees. Although the rootstock is not clonal, we included clone of the associated scion as a categorical variable in our analysis to account for potential effects by the scion on the rootstock. Mirov (1945) demonstrated that the scion can affect oleoresin composition of the rootstock by grafting

Table 4 Percent (mean \pm SD) relative monoterpene content of rootstock phloem tissue, and the probability of these monoterpenes influencing attack by *Hylobius warreni* ($n = 26$)

Monoterpene	Attacked rootstock	Unattacked rootstock	Effect on attack	
			<i>F</i>	<i>P</i>
α -thujene	0.1 \pm 0.1	0.2 \pm 0.2	1.34	0.25
α -pinene	8.6 \pm 3.5	8.7 \pm 4.2	0.03	0.86
β -thujene	1.3 \pm 0.5	2.0 \pm 1.9	2.48	0.12
β -pinene	17.5 \pm 5.6	16.2 \pm 7.0	0.02	0.90
camphene	0.5 \pm 0.6	0.4 \pm 0.3	0.95	0.34
β -myrcene	2.0 \pm 0.6	2.2 \pm 0.7	0.02	0.88
α -phellandrene	1.3 \pm 0.6	1.9 \pm 0.8	0.20	0.66
δ -3-carene	11.7 \pm 4.5	15.8 \pm 10.4	4.06	0.05
β -phellandrene	51.8 \pm 8.6	48.0 \pm 8.7	5.02	0.03
α -terpinolene	1.7 \pm 1.0	2.5 \pm 1.8	3.10	0.09
limonene	3.6 \pm 2.2	3.1 \pm 2.8	0.12	0.74

Willow-Bowron #220 seed orchard, Prince George Tree Improvement Station, British Columbia, October 2000 ($\alpha = 0.05$).

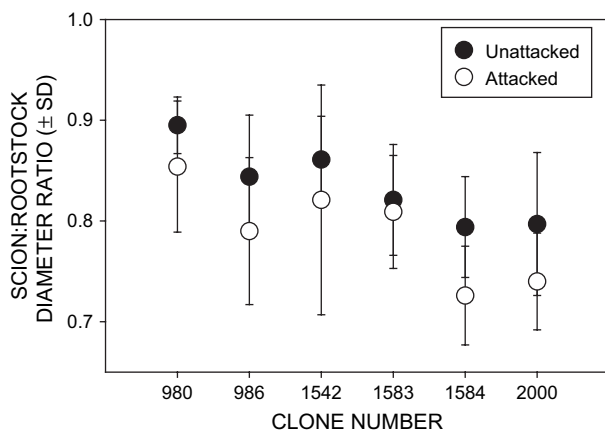


Figure 1 Plot showing the mean \pm SD diameter ratios (scion/rootstock) for attacked and unattacked clones sampled at the Prince George Tree Improvement Station, Prince George, British Columbia, October 2000.

digger pine *Pinus sabiniana* and Jeffrey pine *Pinus jeffreyi* onto rootstock of ponderosa pine *Pinus ponderosa*. The former species are unique among pines in that the volatile portion of their oleoresin is composed primarily of normal heptane with small amounts of aldehydes rather than terpenoids (Mirov, 1945). Aldehydes were present in ponderosa pine rootstock when either of the other species were used as scions, whereas no translocation occurred to ponderosa pine scions grafted onto either digger or Jeffrey pine rootstock. We found a significant relationship of relative monoterpene levels in the scion with that in the rootstock for five of the 11 monoterpenes analysed. Thus, it is conceivable that the scion can affect monoterpene composition in the rootstock, and hence its susceptibility to root insects. However, we found no effect of clone on attack, which may have been because relative monoterpene composition was relatively similar overall.

Our results for relative monoterpene levels were similar to those obtained by Rocchini *et al.* (2000) in a study conducted at the same tree improvement station. The lodgepole pines in the orchards examined in both studies are consistent with Forrest's (1980) Type B based on monoterpene profiles. Type B contains β -phellandrene in greater concentration than β -pinene, which is present in greater concentration than α -pinene.

Hylobius abietis and several other *Hylobius* species rely primarily on olfactory cues to locate their hosts, and monoterpenes are generally important (Tilles *et al.*, 1986a; Nordenhem & Eidmann, 1991; Nordlander, 1991; Rieske & Raffa, 1991; Lindelöw *et al.*, 1992; Nordenhem & Nordlander, 1994). *Hylobius abietis* is attracted to α -pinene on its own, or a terpene blend consisting of α -pinene, β -pinene and δ -3-carene (Lindelöw *et al.*, 1993). The combination of α -pinene and ethanol is strongly attractive to *H. abietis*, and *Hylobius pales* (Tilles *et al.*, 1986a, b; Rieske & Raffa, 1991). Ethanol is found in relatively high amounts in dead or weakened conifers, and is absent or present in very small amounts in healthy trees. Because *H. abietis* breeds in

dead or dying roots, its attraction for an ethanol/terpene combination is reasonable (Tilles *et al.*, 1986b).

A similar relationship is unlikely in *H. warreni* because it breeds in live roots rather than dead or dying roots (Byford, 1994; Cerezke, 1994). This insect does not appear to be attracted to α -pinene or ethanol (B. S. Lindgren, unpublished data), which is consistent with our finding that relative amounts of α -pinene did not differ significantly between attacked and unattacked trees (Table 4). The association of *H. warreni* attacks with high levels of β -phellandrene in the rootstocks of some clones could be a host recognition response. However, such a clear-cut relationship is unlikely because *H. warreni* also attacks other conifers (e.g. spruce) (Cerezke, 1994). The preference may be a function of certain monoterpene combinations or a function of absolute monoterpene concentrations, which we did not measure, rather than relative concentrations.

The preference of *H. warreni* for rootstocks with less δ -3-carene over those with greater δ -3-carene levels is consistent with the relatively high toxicity of this compound to many phloeophagous insects (Cook & Hain, 1988). In a study on the sesiid pitch moth *Synanthedon novoarvensis* (Hy. Edwards), which also attacks live trees, Rocchini *et al.* (2000) found that δ -3-carene levels exceeding 10% reduced susceptibility of host trees. Their study was conducted in a different seed orchard but at the same site. In the present study, mean δ -3-carene levels in both attacked and unattacked rootstocks exceeded 10%, and yet we found a marginally significant difference in this monoterpene in attacked and unattacked trees. Selection of rootstocks with very high levels of δ -3-carene may reduce incidence of attack on these trees, but additional research is needed to confirm that the effect we detected is real.

Limonene is toxic to *H. abietis* (Lindgren *et al.*, 1996), and has shown toxicity and/or repellency to several species of bark beetles (Cook & Hain, 1988). Limonene inhibited the attraction of *H. abietis* and *H. pinastri* to α -pinene (Nordlander, 1990, 1991), with concentrations as low as 5% showing activity (Nordlander, 1990). In living tissue, relative limonene levels are often greater than 5%. However, in the present study, no trees had mean relative limonene concentrations above 5% in the rootstock, and limonene levels were not correlated with rootstock attack ($F_{1,40} = 0.12$, $P = 0.74$). Furthermore, relative amounts of limonene did not differ significantly at any level, indicating that this monoterpene was not a factor in host selection by *H. warreni*.

Many factors are involved in host resistance to weevil attack, and monoterpene analysis alone may not provide a clear understanding of the host-insect interaction (Wilkinson, 1980; Nault *et al.*, 1999). This is evident from some conflicting information in the available literature regarding the influence of monoterpenes on tree susceptibility to insect attack. For example, different 'resistant' varieties of Scots pine *Pinus sylvestris* to the pine root collar weevil *Hylobius radicis* had different levels of significant individual monoterpenes (Wilson & Millers, 1983). Nault *et al.* (1999) showed that individual terpenes were of no value for predicting the resistance of Sitka spruce *Picea sitchensis*, Engelmann spruce *Picea engelmannii* and white spruce *Picea glauca* to white

pine weevil *Pissodes strobi* attack. However, absolute and relative monoterpene composition is under genetic control (Smith, 1964; Bernard-Dagan, 1988), and some monoterpenes have shown relatively consistent effects, and the ease with which these compounds can be measured make them potentially useful for screening of resistance in some insect-plant interactions.

Variation in susceptibility to weevil attack is influenced by the size of the tree (Cerezke, 1994). In a survey of lodgepole pine stands in Alberta, mean tree diameter in each sampled plot was shown to account for a significant amount of variation in the proportion of trees with both old and new *H. warreni* attacks (Cerezke, 1994). Ives & Rentz (1993) determined that, in high productivity sites (i.e. with fast-growing trees), the overall mortality due to girdling caused by *H. warreni* attack was approximately double that in low or medium productivity sites. This suggests that adult females prefer bark with thick phloem on the roots and root collar for oviposition. Bark areas with old weevil attack wounds and resinosis are generally avoided by ovipositing females and feeding larvae (Cerezke, 1994). As a result, larger diameter trees are more likely to be attacked because they can support more larvae (Byford, 1994; Cerezke, 1994). *Hylobius warreni* also appears to prefer faster growing trees (Cerezke, 1994). Fast growth is usually considered to be an indication of vigour. The larger diameter of rootstocks relative to scions at PGTIS may be due to differences in vegetative vigour, or simply to the fact that the rootstock was bigger than the scion prior to grafting. However, it is likely that low-level graft incompatibility or physiological constraints at the graft union resulted in these differences because a noticeable bulge below the graft union was present on many trees. Because this bulge was not associated with the below-ground attacks by the weevil, we do not think that the overgrowth of the rootstock was a result of injury caused by weevil attack. Copes (1989) found that in grafts from five Douglas-fir seed orchards, the annual diameter growth of the scion was greater than the rootstock for all compatibility classes (i.e. incompatible, indeterminate and compatible). In addition, the inequality between scion and rootstock diameter growth was the lowest for compatible grafts (Copes, 1989). Furthermore, Douglas-fir grafts that had died during the study had scion diameters that were an average of 25% greater than that of the rootstock. In the present study, attacked trees of clones 1584 and 2000 had average size difference between rootstock and scion > 25%, but unattacked trees of this clone had average size differences of < 20% (Table 3). The attack rates for clones 1584 and 2000 were 46.9% and 41.7%, respectively. Although these rates are high, they were not the highest we observed (Table 3). Clone 980, with 60.0%, and clone 986, with 52.6% of the ramets attacked had the highest attack rates, with mean size differences between scion and rootstock for attacked trees of 14.58% and 21.03%, respectively (Table 3). Thus, diameter ratio as such does not explain susceptibility to attack. Low-level graft incompatibility may lead to increased growth of the rootstock, which may provide a suitable brood substrate for the weevil if monoterpene levels and other factors are suitable. This would be consistent with the observation that

the Warren root collar weevil preferentially attacks large diameter trees (Cerezke, 1994).

Graft incompatibility in woody plants is usually expressed as an overgrowth in the scion tissue rather than the rootstock (Argles, 1937; Copes, 1967, 1970, 1989). In the Willow-Bowron #220 lodgepole pine seed orchard, all of the rootstocks were larger in diameter than the scion, and graft incompatibility is not considered to be a problem in lodgepole pine (M. R. Carlson, personal communication). The difference between rootstock and scion may be a function of inherent differences in growth rates, as opposed to graft incompatibility in the traditional sense. If the faster growing tissue is the cause of increased susceptibility, then this effect should be a function of absolute diameter of the rootstock. However, we found that it was the rootstock diameter relative to the scion diameter that was significant, indicating some interaction between scion and rootstock. Hence, we contend that if physiological processes are compromised, for example by grafting or root dysfunction (Robert, 2004), susceptibility to insect pests may be affected, even if shoot growth appears to be unaffected. Further study of how tree physiology is affected by grafting is needed to fully understand how trees respond to grafting, and whether trees with no apparent symptoms of graft incompatibility are compromised in any way. Such physiological constraints (e.g. due to grafting or root dysfunction in container-grown or improperly planted seedlings) (Robert, 2004) could potentially affect susceptibility to phloeophagous insects.

Additional research is needed to further elucidate the roles of monoterpenes in host selection by *H. warreni*. We sampled trees late in the autumn, after most oviposition and larval establishment had already taken place. However, we observed many fresh attacks with very small larvae during sampling, and therefore feel that the monoterpene composition measured is biologically meaningful. Nevertheless, future sampling should be conducted at different times during the season of weevil activity. Future studies should also analyse enantiomeric composition and absolute monoterpene content, and clones or seedlots with higher δ -3-carene content than those sampled in the present study should be specifically selected to evaluate the role of this particular monoterpene. To avoid confounding effects caused by induced responses in attacked trees, preselected clones with known monoterpene profiles would be required.

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