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Resistance of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) to white pine weevil (*Pissodes strobi* Peck): characterizing the bark defence mechanisms of resistant populations

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Summary

It has long been known that strong expressions of resistance to the white pine weevil (*Pissodes strobi* Peck) exist in certain Sitka spruce (*Picea sitchensis* (Bong.) Carr.) populations, particularly among trees originating from the Fraser Valley and the Qualicum area of British Columbia (BC). In this paper, we characterize how resistance is manifested in these known resistant populations. Specifically, using cloned individuals, we investigated resistant traits associated with repellency, constitutive resin canals (CRC) and sclereid or stone cells. Results indicate significant population differences in the level of these traits between these two populations and susceptible populations. Fraser Valley populations had four times the sclereid density of susceptible populations. Although the Big Qualicum (East Vancouver Island) population had the same high resistance as the Haney (Fraser Valley) population, it was expressed primarily through increased CRC. Sclereid cell density had the strongest correlation to weevil attack followed by CRC. We discuss pathways by which two distinct resistant populations may have developed in this high weevil hazard region of south-west BC.

Introduction

White pine weevil (*Pissodes strobi* Peck) is one of the most devastating pests of young spruce (*Picea* spp.) and pines (*Pinus* spp.) in North America (Alfaro *et al.*, 2002). This weevil is a native insect that occurs across Canada and the northern United States. In eastern North America, it is a major pest of eastern white pine (*Pinus strobus* L.) and introduced Norway spruce (*Picea abies* (L.) Karst.), but in the west it mainly attacks spruce species (Humble *et al.*, 1994). In Sitka spruce (*Picea sitchensis* (Bong.) Carr.), a particularly susceptible species, damage is so severe that young plantation trees often become stunted and bushy as terminal leaders are repeatedly killed and young trees fail to achieve apical dominance. This damage and lost productivity result in significant economic losses (Heppner and Turner, 2006).

In spring, adult weevils emerge from overwintering in the forest floor, climb to the top of young saplings, feed, mate and oviposit in the bark of the upper section of the previous year's terminal shoot. The larvae then mine down under the bark, consuming the phloem, severing the cambial layer and girdling and eventually killing the leader. Larvae pupate in chambers excavated in the xylem and emerge as adults to feed in the summer and autumn, returning to the forest floor to overwinter (Silver, 1968).

In Sitka spruce, strong phenotypic expressions of resistance to the white pine weevil in some populations were first

observed in the International Union of Forest Research Organizations (IUFRO) provenance experiments on Vancouver Island, British Columbia (BC) (Alfaro and Ying, 1990; Ying, 1991). These studies described three populations in which resistance was shown: (1) the area of natural hybridization between Sitka and white spruce in northwest BC; (2) Haney in the Fraser Valley and (3) Big Qualicum on East Vancouver Island (EVI). These latter two are pure Sitka spruce populations from south-west BC from what is considered a high-weevil hazard area defined: EVI, South-west Mainland BC and Puget Sound, Washington (Figure 1; Ying, 1991; King *et al.*, 2004; Krakowski, 2010). This resistance was subsequently confirmed in a series of clonal and open-pollinated trials in coastal BC that formed the basis of a selective breeding programme primarily for this resistance (King and Alfaro, 2009). Results of all the screening done in Sitka spruce to date indicate that the rates of weevil attack on the Big Qualicum and Haney sources were similar to that of the hybrid white-Sitka spruce population of northwest BC, and consistently are about half of those on susceptible sources (typically Haida Gwaii (Queen Charlotte Islands), Western Vancouver Island or Coastal Oregon or Washington – King and Alfaro, 2009). Another observation from these trials is that the resistance found in individuals from south-west BC is localized close to the two source populations identified in the IUFRO trials and does not appear to be widely dispersed throughout this high hazard area (Figure 1) (King *et al.*, 2004, King and Alfaro, 2009) as had been previously hypothesized (King, 1994).

Alfaro *et al.* (2002) used the resistance of Sitka spruce to white pine weevil as a model of how resistance mechanisms have evolved by trees against shoot infesting insects. They described three general types of resistance categories: (1) repellence or hindrance, (2) phenology and synchronicity and (3) toxicity. Attack hindrance through resin canals is an important resistance mechanism that includes both increases in the density of resin canals naturally found in the bark (constitutive resin canals (CRC); Tomlin and Borden, 1994; Alfaro *et al.*, 1997), and the induced formation of rings of traumatic resin canals (TRC) caused by the feeding and oviposition punctures of the weevils (Alfaro, 1995). These resin canal mechanisms generally hinder feeding, oviposition and can drown eggs and young larvae (Tomlin and Borden, 1994; Alfaro, 1995; Alfaro *et al.*, 2002). Sclereid or lignified stone cells (Fahn, 1969; Esau, 1977) also form a major hindrance resistance mechanism in this general category (Chakravarthy *et al.*, 1985; Alfaro *et al.*, 2002). Indeed, it is suggested that these lignified stone cells, or sclereids, may be as significant a defence against insects and pathogens as resin flow (Wainhouse *et al.*, 1990, 2009; Wainhouse and Ashburner, 1996). Besides offering a physical hindrance, these cells can damage the digestive passages of insect larvae. Other categories of resistance include the synchronization of growth and defensive cycles of the tree relative to the life cycles of the weevil and its predator and parasite populations (Hulme, 1994; Alfaro *et al.*, 2000; Poulin *et al.*, 2006). Moreover, some element (antibiotic or antixenotic) associated with ovary regression is also cited in the observed resistance on some genotypes (Sahota *et al.*, 1994; Leal *et al.*, 1997).

In spite of our general understanding of the types of resistance mechanisms associated with shoot infesting insects, we know very little about how specific defence mechanisms act in the resistant populations and individuals which we have identified in the BC Sitka spruce tree breeding programme. Resistance in this programme is based on the phenotypic expression of leader kill, which is usually measured as mean annual attack (MAA) rate (King and Alfaro, 2009). In this paper, we examine the two major bark hindrance traits: the constitutive resin cells (CRC) and sclereids or lignified stone cells, from the known resistant provenances of Sitka spruce, and describe their respective roles as resistance mechanisms to weevil attack. We concentrate here on the two pure Sitka spruce provenances known to be resistant to the weevil (Big Qualicum on EVI and Haney in the Fraser Valley) and compare them to susceptible sources (Figure 1). Both populations have shown marked resistance to the weevil, but the specific mechanisms conferring this resistance in these particular populations have not yet been identified. We characterized and compared bark traits of trees from these two resistant populations with more susceptible sources to determine which of these traits, or combination of traits, have the strongest influence on this phenotypic expression of resistance. In our sampling, we studied these bark traits in both sections of leaders as well as corresponding laterals. Another objective was to see if sampling laterals could be used as an effective substitute for the destructive sampling of the tree leader.

Materials and methods

Plant material

Branch samples for the measurement of bark traits were taken from ramets established at clonal trials on Vancouver Island. The ortet parents of these trials consisted of 60- to 100-year-old Sitka spruce trees from natural stands located close to the known resistant provenances of Big Qualicum and Haney; and 17- to 24-year-old trees from resistant and susceptible provenances at the IUFRO trials described in the Introduction. Clones from older ortets were established as grafts, but clones from younger ortets were often established by rooting juvenile cuttings. No noticeable differences between these types were observed and the grafts grew as vigorously as the rooted cuttings. These trials were established with four replications of four tree plots making a total of 16 ramets per clone. Further details of these trials are published in King and Alfaro (2009). For this study, stem samples were taken from both leaders and top-most laterals, from resistant and susceptible clones at two of the trial sites where a range of clonal material was available and had come under reasonable attack rates (Armishaw Road and Espinosa Creek, King and Alfaro 2009). The constitutive traits of resin canal density and sclereid cell density (SCLERD) were examined in a total of 394 samples from 47 different clones representing susceptible and resistant clones from the Biq Qualicum and Haney provenance as well as from known susceptible populations of Haida Gwaii,

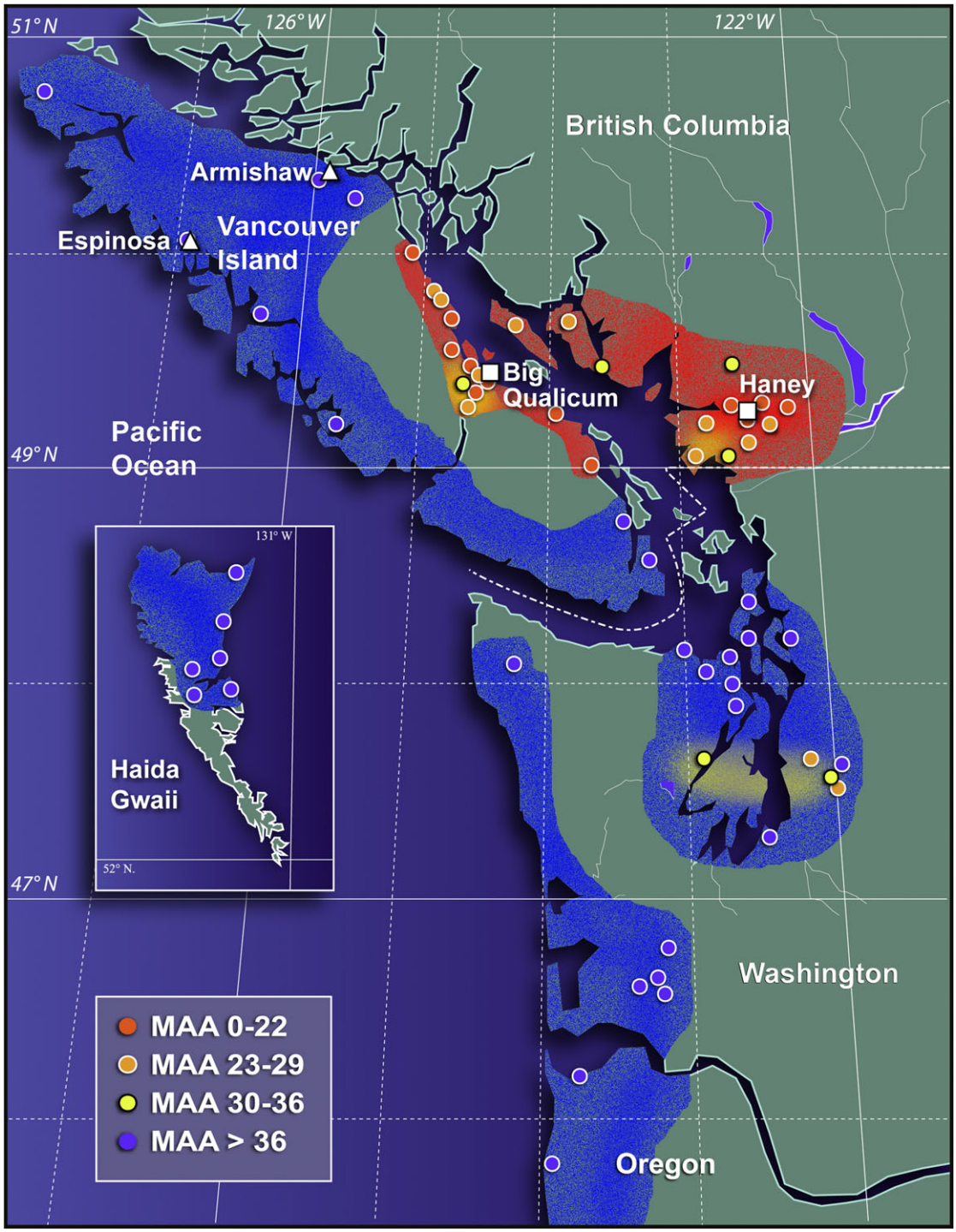

Figure 1. Distribution of widely sampled resistant provenance source (circles) sampled in the large O.P. Trials (King and Alfaro 2009). Showing the original IUFRO provenances of Big Qualicum and Haney (squares) and the clonal trial sites reported here at Armishaw and Espinosa (triangles) (revised from Figure 9 – King and Alfaro, 2009).

Western Vancouver Island, coastal Oregon and Washington States. Lateral branches were taken from six to seven ramets of each clone (287 samples) and leaders from two to three ramets (107) to make up the total of 394 samples.

Screening and scoring for resistance

An outline of the resistance screening programme, which often involved augmentation of the local weevil population at the screening trial, to achieve uniform weevil pressure

throughout each site, is reported in King and Alfaro (2009). Genotypes: families, or clones as in this instance, are assigned a nominal resistance value based on the MAA rate (King and Alfaro, 2009). MAA is assessed based on the average of leader kills measured on a yearly basis during the peak years, usually after artificial infestation (Alfaro *et al.*, 2008), and is reported as a percentage. A zero score indicates no attack, but so far very few clones have in fact expressed this 'total' resistance over a wide variety of sites and conditions; the maximum rate observed (or most susceptible cases) is ~ 50 per cent attack rate. To compare screenings done in different years, with different populations on different sites, MAA rate is adjusted for the site hazard using a mixed linear effects model (King and Alfaro, 2009). Site hazard is a measure of the suitability of the site for sustaining high weevil populations and is based on degree-day models developed by McMullen (1976) and updated recently (Krakowski, 2010).

In this study, the Armishaw Road trial was augmented in the fall of 1996 with weevils and weevil attack on each tree was assessed in the fall of 1997 and 1998, as either 1 = kill or 0 = no kill and averaged during the peak years of attack after augmentation (2 of the 3 years assessed). Espinosa Creek, however, was not augmented with weevils, but we relied on natural weevil build-up during a 5-year period of assessment (years 1994–1998), four of which (1994–1997) were considered peak years, and were used to calculate MAA.

Methods for studying the constitutive bark traits were based on Alfaro *et al.* (2004). A single 5-cm section of each leader and lateral sampled (one lateral was chosen from the top most whorl) was collected during April when the weevils emerged and was immediately preserved in 70 per cent ethanol. Cross-sections 80- to 100-μm thick were collected from each of these samples using a sliding microtome stained with 0.1 per cent aqueous safranin, and mounted on glass slides with glycerine. A representative quarter of each cross-section was digitized using a video camera connected to a compound microscope and monitor with digital image analysis software (SigmaScanPro, Systat Software Inc., San Jose, CA). Detailed measures in the scan included total area of cross-section occupied by the bark and number and area of inner and outer resin canals. From this, we calculated the density of resin canals, expressed by the number and area of resin canals per square millimetre of bark (CRCD – constitutive resin canal density). Another quarter of each cross-section was assessed in a similar manner for the density of sclereid cells (SCLERD). Also measured were the radius to the outer perimeter of the bark, the radius to the inner perimeter of the bark and the radius to first and second growth rings. From these, we calculated second-year growth radius increment (GROW2) and bark thickness (BKTHIK). Figure 2 shows an image comparing two ramets for sclereid density. Further details of the microscopy and digital image technique are outlined by Alfaro *et al.* (2004).

Statistical analysis

Correlations were made between bark traits (CRCD, SCLERD, and BKTHIK), growth (GROW2) and the resistance measure (MAA) with the lateral dataset, the smaller leader dataset and the clonal mean datasets. We also investigated partial correlations between some of the bark and growth characteristics to further investigate the interrelationships between these variables (Steele and Torrie, 1980). Linear regression was used to evaluate which of the independent bark traits could best predict the resistance score – MAA – and to compare the reliability of lateral samples *vs* leaders. This was achieved by examining the fit (R^2) of all possible linear regression models. We report adjusted R^2s (SAS Institute Inc., 2004) as a way of reducing the impact of superfluous variables and multicollinearity.

Analyses of the bark traits were also made using a linear mixed-effects model that included the effects of provenance (P), trial (T) site and clone (C). The P (provenance) effect has three levels: the two resistant sources of Big Qualicum and Haney (for these clonal trials, only trees from these original IUFRO sources were used – the resistant areas were subsequently enlarged – Figure 1; King and Alfaro, 2009), and 'susceptible' known highly susceptible sources (using the original IUFRO information) from coastal Washington , Western Vancouver Island and Haida Gwaii (Table 1; Ying, 1991; Ying and Ebata, 1994; King *et al.*, 2004). The T effect defines the collection site of individual samples, while the C effect includes the cloned replicates. In the mixed model, clones are unique to each combination of site and provenance, therefore, C was nested in both T and P.

The MIXED procedure of SAS (SAS Institute Inc., 2004; Littell *et al.*, 2006) was used to analyze REML estimates of variance components associated with random effects T and C, and the significance of the fixed effect P. The bark characteristics, percentage of the bark area occupied by resin canals (CRCD) and sclereid cell percentage (SCLERD), were arcsine square-root transformed to produce more normally distributed standardized residuals. Back-transformed least squares means (to the original percentage scale) are used in discussions in the text. The significance of each random effect was tested by individually removing it from the model and assessing the change in the model fit via the observed log likelihood. Since the reduced model lies on the boundary of the parameter space for the variance component, the P-value associated with these likelihood ratio tests was determined using the technique described in Verbeke and Molenberghs (2000: Section 6.3.4).

Results

The MAA on the two resistant provenances (Big Qualicum and Haney) and the susceptible mixed provenances is shown in Table 1; for comparison, we also included the equivalent numbers from the combined analysis over all sites and series of the parallel open-pollinated screenings reported by King and Alfaro (2009). These latter results represent close to 500 open-pollinated (OP) families and seedlots screened and scored for weevil resistance from eight test sites covering a range of environments and weevil attack intensities (King and Alfaro, 2009). In Table 1, the

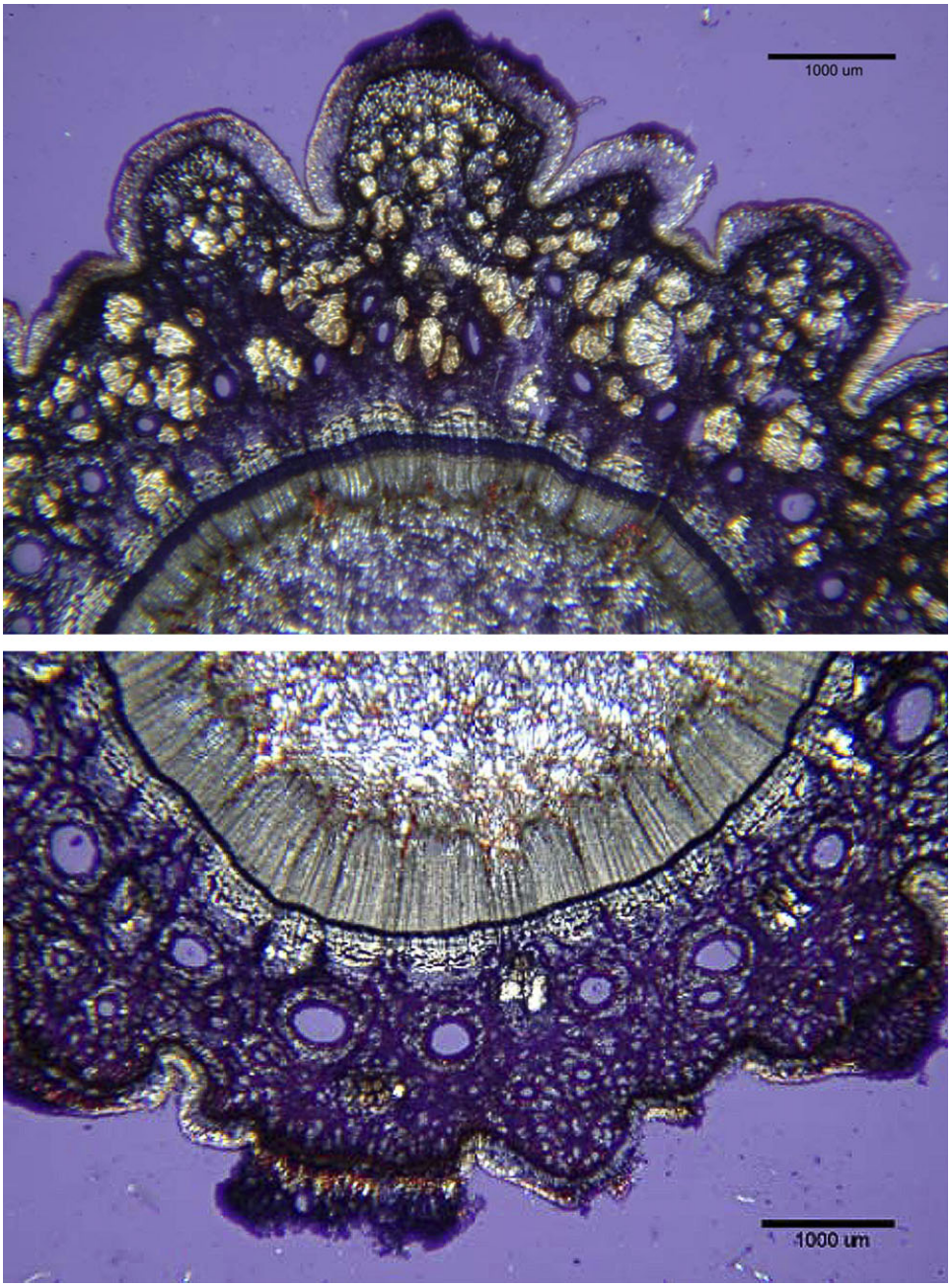

Figure 2. Above high-density sclereids sample 1229-4; below low-density sclereids sample 1217-2.

OP families results were just those from the original Big Qualicum and Haney locations (similar to the clonal origins) and susceptible were from Haida Gwaii, Western Vancouver Island or Coastal Washington. It can be seen that the attack level on the samples collected from the clonal trials in this study match closely the results of both the large screening data in King and Alfaro (2009) and the original IUFRO provenance results published 18 years earlier by Ying (1991). These random samples from the two resistant populations of Big Qualicum and Haney match each other in attack level but sustain less than half of the attack of susceptible sources.

Table 2 shows the means and ranges of the untransformed bark traits measured in both leaders and laterals samples. Only our estimation of the sclereid densities showed any marked difference between leader and lateral samples. Clonal mean correlations between the larger lateral dataset and the smaller leader dataset are very strong for sclereids ($r = 0.745$, $P < 0.0001$) but although significant are less impressive for resin canals ($r = 0.422$, $P = 0.0031$). The regressions of MAA on the transformed bark traits are shown in Table 3 , where we looked at separate regressions for the leader and the lateral datasets. With the

smaller leader dataset, only sclereids (SCLERD) are a significant model factor (CRCD is not significant); in the larger lateral dataset, CRCD does enter the model but SCLERD is the first model factor. The adjusted R^2 square of laterals is 64 per cent of that of the directly measured leaders but including CRCD is 82 per cent that of the leaders for predicting MAA.

The correlation between sclereids and MAA was the strongest indicator of resistance (Table 4 , $r = -0.45$, $P < 0.0001$). The correlation between MAA and CRCD was also significant ($r = -0.21$, $P = 0.0003$) but as mentioned above this was non-significant in the leader data set. Both BKTHIK and growth rate show significant negative correlations ($P < 0.0001$) with weevil attack rates. In other words, thicker barked trees and faster growth show less attack (this growth rate effect was not shown in the leader dataset ($P = 0.2386$) and no correlation is observed between growth rate and BKTHIK. Partial correlations, controlling for BKTHIK or growth rate, did not affect these relationships much except that controlling for BKTHIK sclereid correlations were reduced from −0.45 to −0.38 for MAA (Table 4). The strong correlation between BKTHIK and sclereids indicates that higher sclereid densities are found in thicker barked trees.

Analysis of variance using the mixed model on the lateral dataset indicated significant differences in CRCD and sclereid density by provenance and by clones within provenance (Table 5). The Big Qualicum provenance had a significantly ($P < 0.05$), though not strongly expressed, higher CRCD (3.148 per cent of the total bark area) than both the susceptible and Haney provenances, whereas the CRCD of the Haney provenance (2.699 per cent) did not differ significantly from that of susceptible sources (2.537 per cent) (Table 5). The mixed-model analysis for sclereids (Table 5) indicated that the fixed provenance effect of Haney was over threefold higher (9.794 vs 2.833; $P < 0.005$) than the susceptible sources, and higher but non-significantly different than Big Qualicum (7.290).

These results indicate that although the populations from EVI (Big Qualicum) and the Fraser Valley (Haney) show equally strong resistance level, this resistance may be expressed by emphasizing different mechanisms. The Fraser Valley resistant population is marked by a strong expression of sclereid cells, whereas the EVI (Big Qualicum) appears to have higher CRCD. Both of these populations also have significant genetic variation for these constitutive bark traits, shown by the significant clone effects (Table 5).

Table 1: Mean annual weevil attack MAA ± standard errors and number of clones *N* (families or seedlots in the case of the overall analysis) assessed for two resistant (Big Qualicum and Haney) and susceptible Sitka spruce provenances

	Armishaw Road		Espinosa Creek		Overall[a]	
Provenance	MAA	*N*	MAA	*N*	MAA	*N*
Big Qualicum (EVI)	20 ± 1.4	10	17 ± 1.8	10	21 ± 4.4	98
Haney (Fraser Valley)	18 ± 1.7	11	16 ± 1.2	10	24 ± 4.8	47
Susceptible sources[b]	45 ± 0.5	3	48 ± n.c.	3	42–48 ± n.c.	60

n.c., not calculated.

[a] Combined sites analysis from King and Alfaro (2009) using open-pollinated families, Table 5.

[b] Based on combination of Western Vancouver Island, Haida Gwaii (Queen Charlotte Islands) and Coastal Washington from original IUFRO trials.

Discussion

The high degree of resistance found originally in the IUFRO tests and the OP screening trials for the Big Qualicum and Haney populations from south-west BC has been confirmed by this study. Our results parallel to those reported by Wainhouse *et al.* (1997, 2009) indicate that sclereid cells are at least as significant a defence factor as resin flow. We also suggest growth rate is not traded off for resistance. This confirms our earlier paper, investigating a large open-pollinated trial, showing that phenotypic correlations can indicate a positive relationship between vigorous growth and weevil attack, but genetic correlations are the opposite – in other words, more resistant genotypes are vigorous and can effectively defend themselves without trade-off to growth rate (King *et al.*, 1997). Our results also confirm Tomlin and Borden (1997) who found BKTHIK was less a direct resistance trait and that resistance had more to do with the density of the outer resin ducts (CRCD) that they measured – and in our case also the density of sclereid cells.

Table 2: Bark trait means and ranges for Sitka spruce leaders and lateral samples, and estimated Pearson correlation coefficients *r* and *P*-values (below) between the lateral and leader samples based on the clonal means

	Sclereids, mean (range)	CRCD, mean (range)	BKTHIK, mean (range)	GROW2, mean (range)
Leaders	21.4 (1–70)	2.64 (0.77–5.3)	1.77 (0.88–3.23)	0.11 (0–0.36)
Laterals	7.95 (0–40)	2.88 (0.96–5.8)	1.11 (0.52–2.09)	0.081 (0–0.36)
Correlation	0.745, $P < 0.0001$	0.422, $P = 0.0031$	n.c.	n.c.

Sclereids, density of sclereid cells of total bark area as %; CRCD, constitutive resin canal density of total bark area as %; BKTHIK, bark thickness (mm); GROW2, second-year growth increment (mm); n.c., not calculated.

The EVI resistance found around Big Qualicum appears to lay emphasis on CRC, whereas in the Haney (Fraser Valley) resistance appears to result from significantly higher SCLERD in the outer bark – over threefold the amount found in susceptible sources. Both populations contain significant genetic variation (shown by the replicated clonal effects) for these bark traits.

It is interesting to postulate why these populations (Big Qualicum (EVI) and Haney (Fraser Valley)), although both expressing high levels of resistance, appear to emphasize different bark characteristics. Interestingly, it also appears that the Haney provenance has unique terpene profiles, different from the Big Qualicum provenance that may also contribute to resistance. Tomlin *et al.* (1997) reported significant distinct associations in Haney clones for specific monoterpenes and Roberts *et al.* (2010) report that two monoterpene compounds, (+)-3-carene and terpinolene, appear to be significantly associated with resistance in genotypes originating in the Haney region – these associations were non-significant in the Big Qualicum samples. It is often assumed that wind pollinated and dispersed conifers with widespread distributions and high outcrossing

Table 3: Multiple regression of MAA by the white pine weevil on the Sitka spruce bark traits (abbreviations as per Table 2)

Model	Adjusted R^2	Model R^2	Significance of model factor
MAA laterals (n = 287)			
Sclereid	0.220	0.223	$P < 0.0001$
Sclereid, CRCD	0.249	0.254	$P = 0.0006$
Sclereid, CRCD, GROW2	0.283	0.291	$P = 0.001$
MAA leaders (n = 107)			
Sclereid	0.345	0.350	$P < 0.0001$

Table 4: Correlations between the bark and resistance scores on the lateral dataset with significance levels (in brackets the clonal mean dataset) using just the lateral samples

	Sclereids	CRCD	BKTHIK	GROW2
MAA	−0.45 (−0.56)$P < 0.0001$	−0.21 (−0.30) $P = 0.0003$	−0.26 (−0.35) $P < 0.0001$	−0.23 (−0.29) $P < 0.0001$
Control BKTHIK	−0.384 ($P < 0.0001$)	−0.223 ($P < 0.0001$)	n.c	n.c.
Control GROW2	−0.439 ($P < 0.0001$)	−0.241 ($P < 0.0001$)	n.c.	n.c.
Sclereids		0.055 (0.16) $P = 0.3495$	0.62 (0.67) $P < 0.0001$	0.12 (0.17) $P = 0.0348$
CRCD			0.027 $P = 0.3495$	−0.090 (−0.046) $P = 0.1254$
BKTHIK				0.059 (0.039) $P = 0.3232$

The partial correlations between MAA and Sclereids and CRCD controlling for BKTHIK and growth rate with the lateral data (*P*-values in brackets) are also shown (abbreviations as per Table 2).

Table 5: Analysis of variance results of the mixed-effects model for per cent resin canal density (CRCD) and Sclereids in Sitka spruce from two resistant provenances and a susceptible mix

Bark characteristics	CRCD		Sclereid	
Random effects	Estimated variance component (REML)	*P*	Estimated variance component (REML)	*P*
Site	0	n/a	0	n/a
Site × provenance	0	n/a	0	n/a
Clone (site provenance)	2.46×10^{-4}	<0.0001	9.29×10^{-3}	<0.0001
Tree (clone site provenance)	3.52×10^{-4}	<0.0001	5.60×10^{-3}	<0.0001
Fixed effect	*F*-value (df)	*P*	*F*-value (df)	*P*
Provenance	3.98 (2,44)	0.0258	4.51 (2,44)	0.0166
Provenance level	Least-squares mean (original scale)	Confidence limits (original scale)	Least-squares mean (original scale)	Confidence limits (original scale)
Big Qualicum, EVI	3.148	2.888, 3.408	7.290	4.879, 9.701
Haney, Fraser Valley	2.699	2.432, 2.965	9.794	7.324, 12.26
Outer coast (susceptible)	2.537	2.050, 3.024	2.833	−1.680, 7.346
Comparison of provenance levels	Difference in least-squares means (arcsin√ scale)	*P*	Difference in least-squares means (arcsin√ scale)	P
Big Qualicum *vs* Haney	0.0130	0.0212	−0.0479	0.1364
Big Qualicum *vs* susceptible	0.1778	0.0330	0.0911	0.0579
Haney *vs* susceptible	0.0047	0.5602	0.1390	0.0050

As per Table 2, CRCD, constitutive resin canal density as % of total bark area; sclereids, density of sclereid cells as % of total bark area; df, degrees of freedom; n/a, not applicable.

are unlikely to have such small-scale genetic differentiation. Strong genetic divergence however can occur under the influence of strong environmental pressure, as has previously been reported in Sitka spruce (Mimura and Aitken, 2007). The weevil does affect growth and survival and may act an important component of selection. Also it could be conjectured that some of these resistance factors may be controlled by relatively few genes of large and significant effects which may help explain this marked geographic resistance effect (King *et al.*, 2004; J.N. King, unpublished data). The geographic complexity of south-west BC, with island populations and mountain barriers, may further explain how two such different resistant populations could arise across a relatively short distance.

Alfaro *et al.* (2002) discuss the various resistance mechanisms that have been reported for Sitka and white spruce (*Picea glauca* (Moench) Voss). In addition to the two traits studied, it is possible that the Big Qualicum and Haney provenances differ in the level of yet other resistance mechanisms. For example, studies of white spruce populations indicate that another important resistance mechanism is the production of TRC in response to weevil attack. Resin cells are induced by weevil feeding and oviposition punctures, which are also strongly associated with resistance (Alfaro, 1995; Alfaro *et al.*, 1996, 2004). In the natural hybrid zone between Sitka and white spruce in Northwest BC, that also shows high levels of natural resistance, the most significant predictive variable for TRC density was distance from salt water (O'Neill *et al.*, 2002). This indicates that the overall higher resistance found in white spruce and the high susceptibility found in Sitka could be explained by the lower expression of TRC in Sitka compared with white spruce populations.

Understanding the exact nature of the resistance in different provenances and individuals opens the road to the breeding of specialty genotypes; combining different resistance mechanism into single genotypes can provide enhanced protection and reduce the chances of weevil adaptation.

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Conflict of interest statement

None declared.

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