

Constitutive defenses and damage in Sitka spruce progeny obtained from crosses between white pine weevil resistant and susceptible parents

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Summary

Attack by the white pine weevil has notably reduced Sitka spruce productivity in British Columbia (BC) (Canada) and western US. By the 1970s, the BC Ministry of Forests established provenance trials of Sitka spruce with the objective of detecting usable genetic resistance to weevil. These early trials reported significant weevil resistance and allowed the production of the first (F1) controlled-cross progeny generation with demonstrable weevil resistance (R) or susceptibility (S). This study reports results of the screening for weevil resistance and the levels of constitutive defenses of this F1 Sitka spruce progeny. Progeny from resistant parents (R × R progeny) sustained significantly fewer weevil attacks than progeny from susceptible parents (S × S progeny) or progeny with one resistant and one susceptible parent (R × S progeny). Individual and family heritability estimates of the weevil resistance were 0.5 and 0.9, respectively. Constitutive defenses, measured by resin canal and sclereid cell density in the cortex, were significantly higher in R × R progeny than in R × S or S × S progeny. We observed a negative correlation between the percentage of trees attacked in each cross and the average density of the resin canals or sclereid cells for each cross.

Introduction

The white pine weevil, *Pissodes strobi* Peck (Coleoptera: Curculionidae), is a serious forest pest in young pine and spruce plantations in much of Canada and the northern US (Mitchell *et al.*, 1990; Alfaro *et al.*, 1995). Adults emerge in early spring to begin feeding and egg laying in the secondary phloem of the 1-year-old terminal leader of young trees (Silver, 1968). Upon hatching, larvae mine downward consuming the secondary phloem, girdling and killing the leader (Silver, 1968). This pest causes serious economic impact since leader damage cause large reductions in height growth and severe stem deformations, such as crooks and forks (Alfaro, 1989).

In Western North America, this insect causes damage to Sitka spruce (*Picea sitchensis* (Bong.) Carr) (Alfaro *et al.*, 2000), white spruce (*Picea glauca* (Moench) Voss) (He and Alfaro, 2000), Engelmann spruce (*Picea engelmannii* Parry) (Mitchell *et al.*, 1990) and interior spruce (*Picea glauca* × *Picea engelmannii*) (Taylor *et al.*, 1996). Among these species, Sitka spruce is particularly vulnerable to weevil damage. During the past decades, thousands of hectares of young Sitka spruce were planted annually in Canada and the US, but damage by the white pine weevil has forced managers to severely reduce the planting of this species. For example, in British Columbia (BC), Sitka spruce plantings have been reduced from a historic level of 10 million to fewer than 1 million trees annually (King and Alfaro, 2009).

Several chemical, silvicultural and biological control measures have been tested against *Pissodes strobi*, but none of them has been entirely effective (Alfaro *et al.*, 1995). Genetic resistance offers a promising alternative for weevil management and successful spruce reforestation (Alfaro *et al.*, 1996; King *et al.*, 1997; Alfaro *et al.*, 2004). Strong genetic variation in resistance, both among and within provenances, has been reported for Sitka spruce (Alfaro *et al.*, 2008) and interior spruce (King *et al.*, 1997). In the case of Sitka spruce, there has been already a substantial investment in genetic improvement in BC, with the first provenance trials of Sitka spruce established at the beginning of the 1970s by the BC Ministry of Forests. Results of these early trials showed a notable variation in resistance to this weevil among spruce provenances (Alfaro and Ying, 1990). Resistant provenances that were identified in these studies include the Haney provenance (Lower Fraser Valley), the Big Qualicum provenance (East Vancouver Island) and the Sitka-white spruce hybridization zone in the Skeena River area (Ying, 1991; King *et al.*, 2004; King and Alfaro, 2009), all of them in BC. This early work allowed the selection of a number of individual parent trees with high resistance (King and Alfaro, 2009) and the production of a first (F1) progeny generation resulting from controlled crosses using identified resistant (R) and susceptible (S) parents. This F1 progeny is the subject of this study. The establishment of this F1 progeny trial provided the opportunity to investigate the inheritance of resistance in Sitka spruce, the resistance rankings of the controlled cross F1 progeny generation and the anatomical traits that may act as defensive mechanisms against the weevil.

Resistance to insect herbivory is closely linked to the presence of evolved defensive mechanisms which are commonly subjected to strong genetic control (Núñez-Farfán *et al.*, 2007). Defensive traits associated with phloem feeding insects, such as chemical composition of tree resin, the amount of resin flow, the number and density of resin canals and sclereid cell density have been shown to be under genetic control in several conifer species (Wainhouse and Ashburner, 1996; Roberds *et al.*, 2003; Rosner and Hannrup, 2004; Gerson *et al.*, 2009). The constitutive resin canal system present in the bark of many conifer species is regarded as the first stage and one of the most important defense mechanisms against stem-invading insects (Tomlin and Borden, 1994; Alfaro, 1995; Alfaro *et al.*, 2004; Franceschi *et al.*, 2005) and it has been reported to be a potential trait for selection and breeding (Alfaro *et al.*, 1997, 2004; King and Alfaro, 2009; King *et al.*, 2011). The resin canal system fundamentally acts as physical barrier against herbivore insects, with the resin contained in the canals acting as a chemical defense, causing repellency and toxicity, which alters the feeding behavior of herbivores and increases larval mortality (Phillips and Croteau, 1999; Trapp and Croteau, 2001). Sclereid cells can be found in the secondary phloem, forming small and hard bundles that act as a physical barrier to penetration by phloem invading organisms (Casab, 1998). Alfaro *et al.* (2002) and King *et al.* (2011) presented evidence of higher number of sclereid cells in trees

from open-pollinated spruce provenances with resistance to weevil than in susceptible provenances.

In this study, we present results of the screening for weevil resistance of an F1 Sitka spruce controlled-cross progeny, aiming at determining the inheritance of resistance in this important forest species. We also studied the role of the constitutive defenses, measured by resin canal and sclereid cell density, in weevil resistance, in order to investigate the possibility of using these defensive traits in further screening for weevil resistance in Sitka spruce.

Materials and methods

Study area

The study area was located in southwestern Vancouver Island (British Columbia, Canada). The trial consisted of a controlled-cross F1 progeny trial of Sitka spruce near Jordan River (48° 25' N, 124° 01' W; 31 m a.s.l.; 3 km from sea). The climate in this area is Maritime variant 2 (biogeoclimatic zone CWHxm2) (Klinka *et al.*, 1991), with annual precipitation of ~2800 mm and mean annual temperature of 12.0°C. Soil in this area is Humo-Ferric Podzol with brownish coloured B horizons at least 10 cm thick that are enriched with amorphous material composed of humified organic matter and iron (Soil Classification Working Group, 1998).

Plant material and experimental design

The control-pollinated progeny trial was established using 1-year-old container seedlings of Sitka spruce in the winter of 2004 planted at 2.5 m spacing and was located in a moderate to high weevil hazard area, in order to secure a favorable environment for weevil population development, which would result in effective resistance screening. Forty-two female and 28 male parents of three different provenances were crossed to generate 110 full-sib controlled-crossed F1 progenies (not every male parent was crossed with every female parent) (Table 1). Individual parents for the F1 progenies were selected based on their weevil resistance rankings as determined in earlier screening studies of open-pollinated and clonal trials (Alfaro *et al.*, 2008; King and Alfaro, 2009). The selected parents belong to three provenances that differed in their resistance to weevil: parents from Big Qualicum River (BQ) and Haney (H) provenances, which are classified as resistant to weevil (R) based on earlier well-replicated experiments (King and Alfaro, 2009), and parents from Queen Charlotte Islands (QCI, these islands are now named Haida Gwaii), which are classified as weevil susceptible (S) based on known susceptibility and thus are used as 'susceptible' controls (King and Alfaro, 2009). Thus, in the F1 progeny trial, we had six types of controlled-cross progenies based on parent provenance (H × H, H × BQ, BQ × BQ, as R × R crosses—the major part of the trial, H × QCI, BQ × QCI, as R × S crosses—to create segregating crosses and QCI × QCI, as S × S

Table 1: Resistance ranking of white pine weevil for progeny obtained by controlled crosses of putatively resistant and susceptible Sitka spruce

Resistance ranking	Cross	Male parent	Female parent	Provenance (male × female)*	Resistance (male × female)†	Cumulative attack of F1 progeny (%)
1	31	1179	898	H × H	R × R	0.0
2	56	889	1172	H × H	R × R	0.0
3	57	1172	1172	H × H	R × R	0.0
4	61	1253	898	H × H	R × R	3.3
5	72	898	1020	H × BQ	R × R	3.3
6	30	1152	898	H × H	R × R	3.5
7	38	898	1179	H × H	R × R	3.6
8	67	855	868	BQ × BQ	R × R	3.6
9	29	1139	898	H × H	R × R	6.7
10	41	898	1209	H × H	R × R	6.7
11	44	1210	1209	H × H	R × R	6.7
12	58	898	848	H × BQ	R × R	6.7
13	63	889	1018	H × BQ	R × R	6.7
14	51	1172	1156	H × H	R × R	6.9
15	71	1152	889	H × H	R × R	6.9
16	81	1253	1114	H × BQ	R × R	6.9
17	2	855	848	BQ × BQ	R × R	7.1
18	75	868	1020	BQ × BQ	R × R	7.1
19	16	868	860	BQ × BQ	R × R	7.4
20	40	1210	1179	H × H	R × R	10.0
21	52	1253	1156	H × H	R × R	10.0
22	60	889	898	H × H	R × R	10.0
23	62	898	866	H × BQ	R × R	10.0
24	77	868	1187	H × BQ	R × R	10.0
25	45	1156	889	H × H	R × R	10.3
26	46	889	1075	H × H	R × R	10.3
27	73	868	892	H × BQ	R × R	10.3
28	37	1210	1152	H × H	R × R	10.7
29	22	862	868	BQ × BQ	R × R	13.3
30	32	898	1139	H × H	R × R	13.3
31	49	1253	1075	H × H	R × R	13.3
32	83	1156	1178	H × H	R × R	13.3
33	85	1139	1158	H × H	R × R	13.3
34	48	1159	1075	H × H	R × R	13.8
35	68	849	1234	BQ × BQ	R × R	13.8
36	70	866	1210	H × BQ	R × R	13.8
37	53	889	1159	H × H	R × R	14.3
38	50	1159	1156	H × H	R × R	16.0
39	93	1139	209	H × QCI	R × S	16.1
40	19	1226	862	BQ × BQ	R × R	16.7
41	59	1253	848	H × BQ	R × R	16.7
42	64	1253	866	H × BQ	R × R	16.7
43	21	860	868	BQ × BQ	R × R	17.2
44	43	1152	1209	H × H	R × R	17.2
45	54	1172	1159	H × H	R × R	17.2
46	82	868	1178	H × BQ	R × R	17.2
47	24	860	872	BQ × BQ	R × R	17.9
48	6	849	855	BQ × BQ	R × R	18.5
49	8	866	855	BQ × BQ	R × R	20.0
50	11	866	856	BQ × BQ	R × R	20.0
51	35	1210	1139	H × H	R × R	20.0
52	10	855	856	BQ × BQ	R × R	20.7
53	47	1156	1075	H × H	R × R	20.7
54	84	1253	1178	H × H	R × R	20.7
55	65	1253	849	H × BQ	R × R	21.4
56	99	898	259	H × QCI	R × S	21.4
57	4	856	849	BQ × BQ	R × R	23.3
58	66	889	1139	H × H	R × R	23.3
59	7	856	855	BQ × BQ	R × R	24.1

Table 1: Continued

Resistance ranking	Cross	Male parent	Female parent	Provenance (male × female)*	Resistance (male × female)†	Cumulative attack of F1 progeny (%)
60	20	1234	862	BQ × BQ	R × R	24.1
61	78	868	1241	BQ × BQ	R × R	24.1
62	79	866	1167	H × BQ	R × R	24.1
63	87	866	1020	BQ × BQ	R × R	24.1
64	97	1210	253	H × QCI	R × S	25.9
65	3	866	848	BQ × BQ	R × R	26.7
66	14	866	1018	BQ × BQ	R × R	26.7
67	23	1234	868	BQ × BQ	R × R	26.7
68	26	868	872	BQ × BQ	R × R	26.7
69	80	866	1153	H × BQ	R × R	26.7
70	12	848	1018	BQ × BQ	R × R	27.6
71	55	1253	1159	H × H	R × R	27.6
72	15	862	860	BQ × BQ	R × R	28.6
73	17	860	862	BQ × BQ	R × R	29.6
74	86	849	1007	BQ × BQ	R × R	29.6
75	9	848	856	BQ × BQ	R × R	30.0
76	27	1234	872	BQ × BQ	R × R	30.0
77	28	860	1234	BQ × BQ	R × R	30.0
78	18	868	862	BQ × BQ	R × R	31.0
79	25	862	872	BQ × BQ	R × R	31.0
80	13	849	1018	BQ × BQ	R × R	32.1
81	36	1139	1152	H × H	R × R	33.3
82	95	898	249	H × QCI	R × S	33.3
83	42	1139	1209	H × H	R × R	34.5
84	69	848	1139	H × BQ	R × R	34.5
85	34	1179	1139	H × H	R × R	37.9
86	96	898	256	H × QCI	R × S	37.9
87	1	849	848	BQ × BQ	R × R	41.4
88	39	1139	1179	H × H	R × R	43.3
89	88	866	1151	H × BQ	R × R	43.3
90	98	1253	253	H × QCI	R × S	46.7
91	74	862	1007	BQ × BQ	R × R	48.2
92	5	866	849	BQ × BQ	R × R	48.3
93	33	1152	1139	H × H	R × R	50.0
94	91	1018	259	BQ × QCI	R × S	50.0
95	76	1152	915	H × H	R × R	51.9
96	106	259	259	QCI × QCI	S × S	51.9
97	101	1253	259	H × QCI	R × S	53.6
98	94	848	209	BQ × QCI	R × S	55.2
99	100	1210	259	H × QCI	R × S	70.0
100	90	866	259	BQ × QCI	R × S	71.4
101	89	866	253	BQ × QCI	R × S	72.4
102	107	259	259	QCI × QCI	S × S	79.3
103	108	259	316	QCI × QCI	S × S	79.3
104	102	253	249	QCI × QCI	S × S	80.0
105	105	249	253	QCI × QCI	S × S	82.1
106	103	259	249	QCI × QCI	S × S	82.8
107	92	849	237	BQ × QCI	R × S	83.3
108	109	259	316	QCI × QCI	S × S	85.2
109	104	249	253	QCI × QCI	S × S	90.0
110	110	316	316	QCI × QCI	S × S	96.2

Crosses developed using parent 898, a well-known resistant parent, are presented in bold.

* H = Haney provenance parents (resistant), BQ = Big Qualicum River provenance parents (resistant) and QCI = Queen Charlotte Islands provenance parents (susceptible).

† R = resistant parent, S = susceptible parent.

crosses—used as susceptible controls) (Table 1). The trial was established in a randomized complete-block design with 30 blocks, each containing one tree from each

controlled-cross. The total number of trees in the trial was 3300, corresponding to 110 controlled-cross progenies × 30 blocks.

Weevil resistance

A natural population of *Pissodes strobi* caused sporadic low-level attacks in the controlled-cross progeny trial during the first 3 years after plantation (from 2004 to 2007). Each tree was scored as attacked (value = 1) or not attacked (value = 0) in September 2007. We considered an attack as successful when weevil oviposition and larval feeding resulted in the death of the leader. Dead leaders due to weevil were easily identified since they contained oviposition punctures and emergence holes of the new adults and the phloem was consumed. This 2004–2007 weevil damage was identified as ‘Old Attack’. The 2007 survey indicated that only 0.5 per cent of the trees had sustained Old Attack. For this reason, in order to ensure sufficient population pressure for effective screening, the weevil population in the trial was augmented using the techniques described by Alfaro *et al.* (2008). Briefly, for weevil augmentation, weevils were reared from infested leaders collected in late summer 2006 from infested regeneration near trial site. Adult weevils were maintained in 5 gallon bucket in wire mesh cages with spruce branches for food until the next spring. Water was provided by misting. In September 2007, three to four weevils were released onto each tree in the trial.

Total height of each tree was assessed in September 2008, 1 year after weevil release. For height measurement, we excluded trees with dead or damaged leaders in any previous year. Weevil damage of each tree was again assessed in September 2008 and 2009, 1 and 2 years after the release of weevils into the plantation. Weevil damage in these years was evaluated following the same procedure as for the measurement of the Old Attack. To calculate a final measure of resistance level, we noted the presence or absence of attack from 2004 to 2009, scoring each tree as attacked in any year (value = 1) or never attacked (value = 0).

Constitutive defenses

In order to explore the relevance of constitutive defenses in determining Sitka spruce resistance to pine weevil, we studied a subsample of spruce seedlings within the trial. To avoid possible modification of the shoot anatomy or induction of defenses by weevil attack, all leader samples within a cross were chosen from trees that had escaped weevil attack (i.e. never attacked from 2004 to 2009). We sampled apical leader tissue in five replicates of eight controlled-cross progenies from resistant (R × R) parents (four from H × H parent provenance and four from BQ × BQ parent provenance), eight controlled-cross progenies derived from resistant and susceptible parents (R × S) (four from H × QCI parent provenance and four from BQ × QCI parent provenance) and four controlled-cross progenies from susceptible parents (QCI × QCI). Total sample size was 100, corresponding to 20 controlled-cross progenies × 5 replicates.

In May 2010, terminal leaders (2009 growth) from these 100 trees were destructively sampled. Total length and diameter of each terminal leader was measured in the lab.

Samples were immediately placed in glass vials and fixed in formalin acetic acid for 48 h and then transferred to 70 per cent EtOH for storage until sectioning and staining. Cross sections, 90 µm thick, were made using a sliding microtome. Sections were stained with 0.1 per cent aqueous Safranin according standard procedures (Ruzin, 1999; Moreira *et al.*, 2008). Photographs were taken with a Leica Digital Sight DFC320, mounted on a Leica MS5 light binocular microscope at ×10 magnification. Digital image analysis was used to measure constitutive defenses on a randomly selected quarter of cross section of the leader shoot, with the Phloemalyzer v.2.12 image analysis software developed at the Pacific Forestry Centre (Victoria, British Columbia, Canada).

The following variables were calculated for each cross section sample: (1) inner, outer and total resin canal density, measured by the number of resin canals per square millimeter of bark and by the percentage of bark area occupied by them and (2) sclereid cell density, determined by the number of groups of sclereid cells per square millimeter of bark. Inner resin canals are the ring of large uniformly distributed ducts closest to the cambium and outer ducts refer to all other ducts, which are generally smaller and located close to the edge of the cross section (Figure 1). Additional descriptions of inner and outer resin canals can be found in Tomlin and Borden (1994). Total resin canal density was the sum of inner and outer resin canals. Sclereids occur throughout the cortex but are often associated with vascular bundles. Cortical vascular bundles occur in the cortex of primary shoots of conifers and may or may not be associated with lateral buds or needles. Inner and outer resin canals and groups of sclereid cells are shown in the Figure 1.

Statistical analyses

Total tree height was analyzed for statistical differences by Block, Resistance class, Provenance and Cross using the PROC-MIXED procedure of the SAS System (Littell *et al.*, 2006) using the following mixed model:

$$Y_{ijkl} = \mu + B_i + R_j + P(R)_{kj} + C(P \times R)_{lkj} + \varepsilon_{ijkl}, \quad (1)$$

where Y_{ijkl} is the continuous response variable tree height; μ is the general mean, B_i , R_j , P_k and C_l are the main effects of block i ($i = 1-30$), resistance class j ($j = 1-3$, corresponding to R × R, R × S and S × S crosses), provenance k ($k = 1-3$) and controlled-cross progeny l ($l = 1-88$) and ε_{ijkl} is the residual error. Provenance was nested within resistance, $P(R)_{kj}$ and controlled-cross was nested within provenance and resistance, $C(P \times R)_{lkj}$. All these factors were considered fixed effects.

Weevil damage was modeled with PROC-GLIMMIX (binary distribution) according to:

$$Y_{ijkl} = 1/(1 + \exp(\mu + B_i + R_j + P(R)_{kj} + C(P \times R)_{lkj})), \quad (2)$$

where Y_{ijkl} is a binary response (attacked or not) and all other model terms are the same as those used for total tree height above.

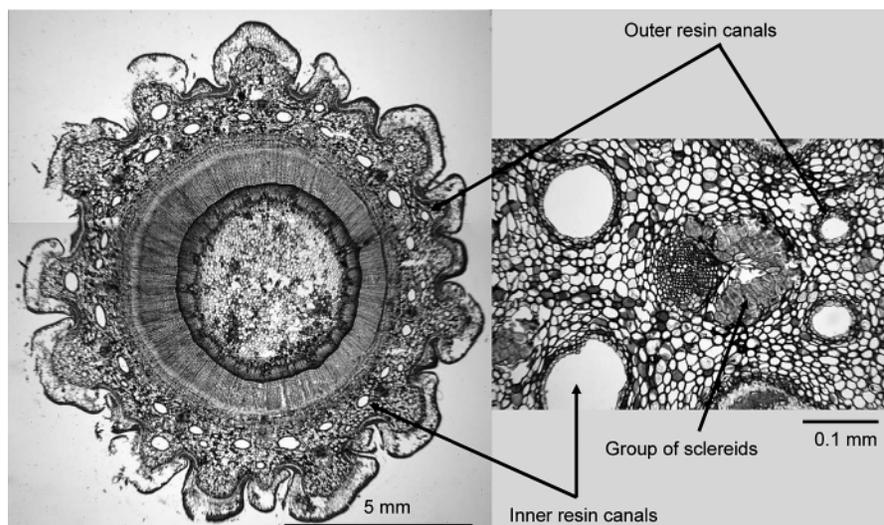


Figure 1. Cross-section of a Sitka spruce terminal leader, indicating inner and outer resin canals and showing a group of sclereid cells adjacent to a vascular bundle.

Length and diameter of the terminal leaders and density of resin canals and sclereid cells were analysed using the PROC MIXED procedure of the SAS System (Littell *et al.*, 2006) and the following mixed model:

$$Y_{ijk} = \mu + R_i + P(R)_{ji} + C(P * R)_{kji} + \varepsilon_{ijk}, \quad (3)$$

where Y_{ijk} is a continuous response variable for resin canal and sclereid density or for the percentage of resin canal area relative to bark area; μ is the general mean, R_i , P_j and C_k are the main effects of resistance level i ($i = 1-3$), parent provenance j ($j = 1-3$) and controlled-cross progeny k ($k = 1-8$) and ε_{ijk} is the residual error. Parent provenance was nested within resistance, and controlled-cross progeny was nested within provenance and resistance. All these factors were considered fixed effects. Diameter of terminal leader was used as covariate in the analysis of resin canal and sclereid cell density.

When needed, normality was achieved by log-transformation (sclereid cell density) and by arcsin-transformation (resin canal density measured by the percentage of bark area occupied by them) of the original variables. When main effects were significant, differences among means were tested for significance using the LSMEAN statement of the SAS System (Littell *et al.*, 2006).

Individual (b_i^2) and family (b_f^2) heritabilities for weevil resistance and constitutive defenses (density of resin canals and sclereids) were estimated according to Wright (1976):

$$b_i^2 = 2\sigma_f^2 / (\sigma_f^2 + \sigma_e^2) \quad (4)$$

$$b_f^2 = \sigma_f^2 / (\sigma_f^2 + \sigma_e^2 / nb), \quad (5)$$

where σ_f^2 is family variance, σ_e^2 is the residual variance, n is the number of trees per family and block ($n = 1$) and b is the number of blocks ($b = 30$ for weevil resistance and $b = 5$ for constitutive defenses).

Pearson correlations were used to evaluate family relationships between weevil damage (per cent of trees with leader kill) and constitutive resistance of Sitka spruce (density of resin canals and sclereid cells). These analyses were conducted on controlled-cross progeny means ($N = 20$).

Results

Weevil resistance

Following weevil release in the F1 progeny trial, overall mean attack rate increased from 0.5 per cent (± 0.1) (cumulative attack from 2004 to 2007) to 18.7 per cent (± 2.1) in 2008. After that, overall weevil attack rate decreased to 12.8 per cent (± 1.5) in 2009.

Weevil attack level varied at the full-sib family level (controlled-cross progeny). Weevil attack ranged from 0 to 52 per cent among R \times R crosses, from 16 to 83 per cent among R \times S crosses and from 52 to 96 per cent among S \times S crosses (Table 1). The 10 most weevil resistant crosses were R \times R crosses and the 10 least resistant crosses were either R \times S or S \times S crosses (Table 1).

The statistical analysis indicated significant differences in weevil attack among resistance cross level, parent provenances and controlled-cross families (Table 2). Specifically, considering the entire study period, there were significantly fewer leader kills among the R \times R progeny, relative to the R \times S or the S \times S progeny (Figure 2). We also observed significantly fewer leader kills among the R \times S progeny than in the S \times S progeny (Figure 2). No significant differences in total height among resistance levels were observed in 2008, 1 year after weevil release ($F_{2,3031} = 0.21$, $P > 0.05$), indicating that tree growth did not appear to be a factor for weevil preference.

Table 2: Summary of the mixed model used to evaluate white pine weevil resistance (noted as the presence or absence of attack from 2004 to 2009) of the progeny obtained by controlled crosses of putatively resistant and susceptible Sitka spruce

	DF*	F	P
Block	29 3051	2.06	0.001
Resistance	2 3051	25.93	<0.001
Provenance†	4 3051	11.62	<0.001
Cross‡	103 3051	2.14	<0.001

Significance ($P < 0.05$) P values are typed in bold.

* DF = degrees of freedom (numerator, denominator).

† Provenance is nested within resistance.

‡ Controlled-cross is nested within provenance and resistance.

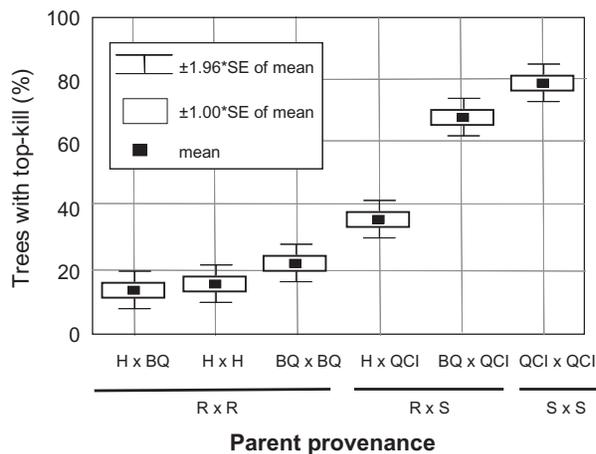


Figure 2. Percent of trees with top-kill resulting from attack by *Pissodes strobi*, in Sitka spruce progeny from controlled crosses of putative weevil resistant and susceptible parents (cumulative attack from 2004 to 2009). Codes for the parent resistant level are: R = resistant parent, S = susceptible parent. Codes for the parent provenance are: H = Haney provenance parents (resistant), BQ = Big Qualicum River provenance parents (resistant) and QCI = Queen Charlotte Islands provenance parents (susceptible).

Considering the provenances of parent trees, H x H, BQ x BQ and H x BQ (progeny obtained by crossing R x R parents) showed a per cent of leader kill lower than the BQ x QCI crosses (progenie from R x S parents) and the QCI x QCI (progeny from S x S parents) (Figure 2). No significant differences were observed for the per cent of leader kill between the provenance of resistant crosses (H x H, BQ x BQ and H x BQ parents) (Figure 2). Among the R x R progeny, the controlled-crosses developed using a Haney-898 parent are of great interest due to their history of high weevil resistance (Ying, 1991; King and Alfaro, 2009). Interestingly, 7 of the 10 most resistant F1 crosses had the genotype 898 among its parents, either male or female (Table 1).

The estimated heritability on a family mean basis was high for weevil damage ($h_f^2 = 0.9$), suggesting that important genetic gain in weevil resistance is possible

through family selection. Individual heritability was also high ($h_i^2 = 0.5$).

Constitutive defenses

Length and diameter of the terminal leaders used for histological analysis were not significantly different among resistance levels ($F_{2,76} = 0.80, P > 0.05$ and $F_{2,76} = 2.29, P > 0.05$, respectively).

Results from the mixed model indicated significant differences between resistance levels for inner, outer and total resin canal density measured either by the number of resin canals per square millimeter of bark or by the percentage of bark area occupied by resin canals (Table 3). Specifically, the R x R F1 progeny had significantly greater density of inner, outer and total resin canals than F1 progeny of the other two resistance levels (R x S and S x S) (Table 4).

Results from analysis of variance also indicated significant differences between parent provenances for inner, outer and total resin canal density (Table 3). Especially, controlled-crosses with both parents from Haney provenance showed significantly greater density of resin canals (Table 4). Significant differences among controlled-cross progenies were observed for outer and total resin canal density measured by the percentage of bark area occupied by resin canals (Table 3). Significant differences among resistance levels and controlled-cross progenies were also observed for sclereid cell density (Table 3). Specifically, the R x R progeny had significantly greater density of sclereids (0.26 ± 0.03 sclereids mm^{-2}) than did progeny from the other two resistance levels (0.14 ± 0.04 and 0.12 ± 0.05 sclereids mm^{-2} for R x S and S x S progenies, respectively). However, no significant differences among parent provenances were observed for sclereid cell density (Table 3). The estimated heritability on a family mean basis was high ($h_f^2 = 0.8$) for density of resin canals and for sclereids. Individual heritability was moderate ($h_i^2 = 0.2$) for both traits.

We found a positive and significant correlation at the family level between density of outer and density of inner resin canal (Pearson $r = 0.78, P < 0.001$ for the density measured by the number of resin canals per square millimeter of bark and Pearson $r = 0.62, P = 0.003$ for the density measured by the percentage of bark area occupied by resin canals). We also found a significant and positive family correlation between density of total resin canals and density of sclereid cells (Pearson $r = 0.57, P = 0.009, N = 20$ controlled-cross progenies).

The level of weevil damage was reduced in genotypes with high density of resin canals or sclereid cells. Significant and negative correlations were detected at the family (controlled-cross) level between the percentages of weevil attack and the average density of (1) inner resin canals (Pearson $r = -0.64, P = 0.004$), (2) outer resin canals (Pearson $r = -0.66, P = 0.001$), (3) total resin canals (Pearson $r = -0.61, P = 0.005$) and (4) sclereid cell density (Pearson $r = -0.48, P = 0.032$) expressed by the number of each of them per square millimeter of bark.

Table 3: Significance tests on resin canal and sclereid cell density by resistance class, provenance and control-pollinated families

Effect	DF*	Inner resin canal				Outer resin canals				Total resin canals				Sclereids	
		Number		Percentage		Number		Percentage		Number		Percentage		Number	P
		F	P	F	P	F	P	F	P	F	P	F	P		
Resistance	2, 76	4.18	0.010	2.96	0.046	3.80	0.027	2.16	0.123	3.92	0.024	3.02	0.047	3.68	0.029
Provenance†	2, 76	5.55	0.006	3.34	0.042	5.92	0.004	3.17	0.044	5.97	0.004	3.91	0.025	2.29	0.108
Cross‡	15, 76	1.11	0.359	2.60	0.004	1.37	0.183	0.87	0.597	1.15	0.326	2.62	0.004	1.70	0.044
Leader diameter§	1, 76	15.02	<0.001	0.01	0.942	8.02	0.006	2.61	0.111	15.82	<0.001	0.21	0.645	1.49	0.225

Significant ($P < 0.05$) P values are in bold.

* DF = degrees of freedom (numerator, denominator).

† Provenance is nested within resistance.

‡ Controlled-cross is nested within provenance and resistance.

§ Diameter of the terminal leaders was used as covariate.

Discussion

Weevil resistance

This study showed important genetic variation for white pine weevil resistance among Sitka spruce families. The weevil showed strong preference for some Sitka spruce families—particularly those from the QCI source. Our results showed that progeny from the $R \times R$ controlled-crosses had significantly higher weevil resistance compared with crosses with two susceptible parents ($S \times S$), whereas the $R \times S$ progeny tended to have intermediate levels of weevil resistance, as would be expected under an additive genetic model. These results confirmed previous observations by Kiss and Yanchuk (1991), King *et al.* (1997) and Alfaro *et al.* (2004) for other spruce species.

Our results from the F1's confirm the strong provenance-based resistance that has long been observed (Alfaro and Ying, 1990; King and Alfaro, 2009; King *et al.*, 2011). It has been postulated that extreme selection pressure in high weevil hazard zones increased the proportion of resistant trees in these areas, but it has been noted that resistance is not widely distributed throughout these high weevil hazard zones (King *et al.*, 2004; King and Alfaro, 2009). The high susceptibility of the QCI provenance is probably due to the fact that *Pissodes strobi* does not occur on the Queen Charlotte Islands and therefore natural selection for weevil resistance has not taken place within this provenance.

We also observed differences in weevil resistance among and within the resistant controlled-cross families, within parent provenances. For example, the high resistance of progenies from parents of the Haney genotype number 898 (Table 1) demonstrated the heritability of weevil resistance since this parent was already known to be highly resistant to weevil from previous studies (King and Alfaro, 2009). Individual and family heritability was high (0.5 and 0.9, respectively) suggesting that important gains can be expected through family selection. These heritabilities are generally higher than previous reports for spruce resistance to white pine weevil or other insects. Individual heritability for interior spruce resistance to *Pissodes strobi* was found to be ~ 0.4 (King *et al.*, 1997). Zas *et al.* (2005) observed that individual heritability for *Pinus pinaster* Ait. resistance to the weevil *Hylobius abietis* L. was ~ 0.2 . Thus, our results confirmed that it is possible to develop a screening program to breed for Sitka spruce for resistance to the white pine weevil using individual putatively resistant parents to create highly resistant pedigree progenies.

Constitutive defenses

Our results showed that controlled-crosses from $R \times R$ parents had greater density of resin canals and sclereid cells than controlled-crosses from $R \times S$ and $S \times S$ parents. Moreover, we found a negative relationship between the level of weevil attack by cross and constitutive defenses (density of resin canals and sclereid cells), that is controlled-cross families which sustained fewer attacks had greater density of these constitutive defenses. These results support previous findings in

Table 4: Least-square means of resin canal density (mm²) by resistance classes and provenances

	Inner resin canals		Outer resin canals		Total resin canals	
	Number	Percentage	Number	Percentage	Number	Percentage
Resistance level*						
R × R	2.10 a	2.98 a	1.60 a	0.82 a	3.73 a	3.81 a
R × S	1.59 b	2.72 ab	1.19 b	0.68 a	2.80 b	3.46 b
S × S	1.51 b	2.36 b	1.10 b	0.61 a	2.60 b	3.14 b
Parent provenance†						
H × H	2.51 a	3.06 a	1.99 a	0.95 a	4.51 a	4.01 a
BQ × BQ	1.68 b	2.91 ab	1.22 b	0.69 b	2.94 b	3.61 ab
H × QCI	1.71 b	3.04 a	1.36 b	0.77 ab	3.07 b	3.91 a
BQ × QCI	1.47 b	2.41 bc	1.03 b	0.60 b	2.52 b	3.01 c
QCI × QCI	1.51 b	2.36 c	1.10 b	0.61 b	3.00 b	3.14 bc

Different letters indicate significant differences at $P < 0.05$.

* R × R = resistant parent crossed with resistant parent, R × S = resistant parent crossed with susceptible parent, S × S = susceptible parent crossed with susceptible parent.

† Haney (H) and Big Qualicum (BQ) provenances are considered as weevil resistant while Queen Charlotte Island (QCI) provenance is considered as weevil susceptible.

an F1 progeny trial of interior spruce studied by Alfaro *et al.* (2004) who found that weevil resistance was strongly correlated to constitutive defenses and highlights the relevance of these traits for weevil resistance within the spruce genus.

We also observed large differences among parent provenances for the density of inner, outer and total resin canals. Controlled-crosses with both parents from Haney provenance (H × H) showed the greatest density of resin canals. Interestingly, the R × R controlled-crosses of Sitka spruce with both parents from Haney provenance showed density of total resin canals nearly 50% greater than R × R controlled-crosses with both parents from Big Qualicum resistant provenance, although they showed similar levels of weevil resistance. These results are in agreement with previous findings by Tomlin and Borden (1994) who found that half-sib families from the Big Qualicum provenance showed high resistance to white pine weevil, but relatively low density of outer resin canals. The high resistance of our controlled-crosses from Big Qualicum, which have lower density of constitutive resin canals and sclereid cells, indicates that these genotypes may rely more on other types of resistance mechanisms to defend against weevil attack, such as the content of phenolic or specific terpenoid compounds in the bark (Franceschi *et al.*, 2000) or the induced production of traumatic resin canals (Alfaro, 1995). Tomlin *et al.* (1997) and Robert *et al.* (2010) reported that resistant trees varied in levels of terpenoid compounds. Further studies of these additional resistance mechanisms are necessary to confirm our hypothesis.

In summary, results of this study indicate that screening of F1 Sitka spruce progeny for white pine weevil resistance can be used for recurrent selection of parents within existing seed orchards of the Sitka spruce tree improvement program or to produce progeny for direct utilization in reforestation. Genetic resistance should be considered as an important strategy for an Integrated Pest Management System against the white pine weevil to help bring back Sitka spruce to the managed forests of western North America.

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Conflict of interest statement

None declared.

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