RESISTANCE OF SITKA SPRUCE TO THE WHITE PINE WEEVIL

by

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Resistance of Sitka spruce to the white pine weevil.

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ABSTRACT

Because of potential damage by the white pine weevil, *Pissodes strobi* (Peck), planting of Sitka spruce, *Picea sitchensis* (Bong) Carr., is not recommended in coastal areas of British Columbia. There are no direct control techniques used for the weevil. The desirability of developing trees that are genetically resistant to the weevil has long been recognized, and several resistant Sitka spruce genotypes have been identified. My objective was to identify multiple resistance traits in Sitka spruce for the development of a multicomponent resistance index for use in selecting parent trees and screening progeny for breeding programs. Multiple resistance traits are desirable for stable, polygenetic resistance. Characteristics of resistant and susceptible trees in four provenance trials and one clonal outplanting were compared. These included constitutive defenses such as morphological attributes of the leader, and amount and composition of volatile foliar terpenes, cortical diterpene resin acids and condensed tannins. Adult feeding and oviposition behaviour were tested in three types of bioassay, and orientation to host odour was evaluated in a y-tube olfactometer. Capacity for induced resinosis was assessed by histological examination of the bark and wood, and the effects of resin constituents on egg hatch and larval development were evaluated. Resistance was associated with a high density of outer resin ducts, extremely high or low levels of foliar terpenes, and high levels of cortical resin acids. Feeding and oviposition deterrency (or lack of stimulation) were expressed by a few resistant genotypes, and clones differed in their degree of repellency. The capacity for

induced resinosis varied by clone, and this may be less important for Sitka than other spruces. Resin acids stimulated egg hatch, but may increase larval mortality. Different genotypes clearly possessed different combinations of traits. A resistance index compiled from the data predicted the status of all clones except one.

DEDICATION

To Scott and Sarah

"But the Ichneumon cannot destroy the species, nor can man himself, the most effective remedy then in our power is, to cut off the leading shoot in August, or as soon as it is perceived to be dead, an inch or two below the dead part and commit it to the fire."

W.D. Peck, ESQ. 1817

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I. GENERAL INTRODUCTION

The white pine weevil, *Pissodes strobi* (Peck), is the most serious pest of regenerating Sitka spruce, *Picea sitchensis* (Bong) Carr., on the Pacific coast of North America (Mitchell *et al.* 1990; Ying 1991), and of eastern white pine, *Pinus strobus* L., in the northeastern United States and Canada (Wallace & Sullivan 1985). Direct control techniques for the weevil such as overstory shading, leader clipping and disposal, augmentation of natural enemies and insecticide sprays are either ineffective, impractical or too costly to be used on a large scale (Stiell & Berry 1985; McMullen *et al.* 1987; Alfaro & Omule 1990; deGroot & Helson 1993; Hall 1994; Rankin & Lewis 1994). Planting of Sitka spruce is currently not recommended in most coastal areas of British Columbia (Alfaro *et al.* 1995).

In coastal British Columbia, adult *P. strobi* overwinter in the duff near the tree from which they emerged the previous fall (Alfaro 1994), or in the tree crown (McMullen 1976). They feed on lateral branches in early spring and occasionally in the winter on warm days. In March-April they fly or crawl to leaders where they feed, mate and oviposit. Eggs are laid just below the apical bud in feeding punctures which are then capped by a faecal plug. Larvae hatch, and feed downwards, consuming phloem and girdling the leader. They pupate in the xylem and emerge as adults in late summer and early fall. The adults feed and then disperse before overwintering. Repeated leader destruction reduces height growth and causes stem deformities which significantly reduce tree value (Alfaro *et al.* 1995).

Host resistance is generally regarded as the ideal method for pest control (Hanover 1975a; Wiseman 1994), and the desirability of developing trees that are genetically resistant to terminal weevils has long been recognized (Gerhold 1966). Resistant cultivars characteristically provide substantial dollar returns on investment (Wiseman 1994). Resistance can be defined as the collective heritable characteristics by which a plant species, race, clone or individual may reduce the probability of successful utilization of that plant as a host by an insect species, race, biotype or individual (Hanover 1975b), and by which a plant species, race, clone or individual recovers from attack without serious defect.

In British Columbia a number of resistant Sitka spruce genotypes have been identified through replicated provenance trials and one clonal outplanting (Alfaro & Ying 1990; Ying 1991). Given the lack of success of direct control methods, genetic resistance may be the most promising tool for managing *P. strobi* (Alfaro *et al.* 1995). If the mechanisms by which these trees resisted or tolerated attack were known, it would provide a basis for selecting parent trees, and evaluating their progeny in weevil-resistance breeding programs (Brooks & Borden 1992).

Characteristics of plants which can affect their selection as hosts by insects include plant morphological traits, secondary chemical constituents, nutritional factors, phenology, and age (Feeny 1975; Hanover 1975b; Rhoades 1975; Levin 1976; Fernandes 1990). Painter (1968) divided resistance mechanisms of plants into three categories: non-preference or antixenosis (Kogan & Ortman 1978), antibiosis and tolerance. Antixenosis involves mechanisms such as the presence or absence of

volatile attractants, feeding and oviposition stimulants and deterrents, and morphological characteristics of plants affecting preference. Antibiosis involves mechanisms that have an adverse effect on insect life history (Painter 1968), such as reduced growth rate, survival or fecundity. Tolerance is a resistance mechanism whereby the plant is able to withstand herbivore damage without significant negative effects. In addition, plants may possess 'token' traits that could be assessed, such as the presence of certain chemical constituents that may not be involved in resistance *per se*, but could be indicators of resistance (Gerhold 1966; Brooks *et al.* 1987a). The type of defense employed by a plant may depend to a large degree on its life history. Feeny (1975) distinguished between two main types of plants which he called 'apparent' and 'unapparent' plants. Apparent plants are long-lived, persistent and certain to be found by herbivores. Their main defenses are thought to be debilitating ones affecting basic functions which are difficult to overcome, even by specialized herbivores, because of their general nature. These quantitative defenses involving chemicals such as lignins, resins, tannins, silica and other phenolics (Rhoades 1983) tend to act in a dose-dependant manner. Unapparent plants are not likely to be found by insects, and their main defense is unpredictability in space and time. They tend to use qualitative defenses such as the production of steroidal glycoalkaloids, glucosinolates, cardenolides, cyanogenic substances and non-protein amino acids (Rhoades 1983), that are toxic in minute amounts. These substances, while metabolically less expensive to produce and store than quantitative defense compounds, are generally effective only against highly coevolved herbivores (Rhoades

1983). Some persistent plants that are part of low-density populations may have characteristics of both apparent and unapparent plants, and the distinction between qualitative and quantitative defenses is not absolute (Fox 1981)

Resistance may be further classified as constitutive or induced (Klement & Goodman 1967; Levin 1976). Constitutive defenses are present in the plant prior to contact with an insect or microorganism. Induced resistance is based on the accumulation and modification of normal host metabolites as a consequence of physical and chemical interactions between plant and attacker (Levin 1976). During the process of induction, an insect or microorganism attack sets in motion, or accelerates a sequential and complicated series of metabolic disturbances which may affect both proximate and distant tissues (Kozlowski 1969).

Conifers are long-lived plants, have a high likelihood of being attacked by a variety of parasitic and pathogenic organisms (Christiansen & Bakke 1988), and thus would be considered apparent, and most likely to use non-specific, quantitative defenses such as phenolics and terpenes. Conifers have been observed to employ both constitutive and induced defences (Lieutier & Berryman 1988). The former is a passive flow of preformed resin from severed resin ducts which functions to cleanse and seal wounds, and may act as a deterrent to invading insects (Berryman & Ashraf 1970). The exudation pressure probably depends to a large extent on the storage capacity, which would be related to the number and size of the resin ducts (Christiansen & Bakke 1988). The latter is the hypersensitive reaction; a dynamic secretion of secondary resins synthesized by parenchyma cells in response to fungal

invasion. Rapid cellular desiccation, necrosis around the wound site, and the synthesis and release of terpenes, phenols and possibly other compounds from dying parenchyma cells are all observed (Berryman 1972). This results in resinosis of host tissues in advance of the infection boundary. The importance of constitutive and induced resin varies with tree species (Lewinsohn *et al.* 1991a; Raffa 1991) and the type of attacking herbivore.

Resistance in plants can be controlled by a single gene or by multiple genes (Tomiyama 1963). Plants possessing single gene resistance, or vertical resistance (Levin 1976), are usually highly resistant to specific races of pathogens, but susceptible to other races of the same species (Tomiyama 1963). Plants possessing polygenic, or horizontal resistance (Levin 1976), tend to be not so highly resistant to specific pathogens, but resistant to a greater variety of pathogenic races or species (Tomiyama 1963). Polygenic resistance should be quite stable over the lifetime of a long-lived plant such as a conifer, because the tree would have the genetic capacity to respond to changing environmental conditions, and relatively faster adaptation of insects and pathogens (Tomiyama 1963; Wiseman 1994).

My overall objective was to investigate the mechanisms of resistance and susceptibility of Sitka spruce to the white pine weevil in order to develop a multicomponent resistance index for two uses: 1) selecting parent trees for breeding programs, with the aim of achieving polygenic resistance; and 2) screening progeny for resistance characteristics. A number of different characteristics were compared between resistant and susceptible trees, related to weevil feeding and oviposition

behaviour, and for those characteristics that reflected observed patterns of weevil attack, compiled into a multicomponent resistance index. Characteristics that involve antixenosis and antibiosis, that are both constitutive and induced were investigated as itemized below:

1) Constitutive defenses,

    a) morphological attributes of the leader,

    b) resin chemistry (volatile foliar terpenes and cortical diterpene resin acids),

    c) amount of condensed tannin in bark,

    d) effect of host tissues on adult feeding and oviposition behaviour, and orientation to host odour;

2) Induced defenses,

    a) histological changes occurring in bark and xylem following wounding, and

    b) effect of resin constituents on egg hatch and larval development.

II. STUDY SITES AND SELECTION OF TREES

I examined resistant and susceptible Sitka spruce at five sites in British Columbia: provenance trials at Sayward, Head Bay, Kitimat, Nass River and a clonal outplanting at Fair Harbour (Ying 1991). Provenance refers to the geographic location to which the parent trees are native, and within which their genetic constitution has been developed through natural selection (Society of American Foresters 1964). The provenance trials at Head Bay, Kitimat and Nass River were established in 1975 involving 10 provenances, with six overlapping between sites. These trials were established as part of an international cooperative experiment organized by the International Union of Forest Research Organizations (IUFRO) (Ying 1991). The provenance trial at Sayward was established in 1974, and involved 38 Alaskan and British Columbian provenances. In this trial, the identity of individual wind-pollinated families was maintained (Ying 1991). These four sites were heavily attacked by *P. strobi* beginning in 1981, and assessments of weevil damage have been made since 1988 (Ying 1991).

The clonal test at Fair Harbour was established in 1984 with grafted scions, from 36 trees in eight provenances selected from Sayward with varying degrees of weevil resistance, and two trees originating from a plantation at the Green Timbers Nursery in Surrey, B.C. (Ying 1991). The Green Timbers plantation was established with several thousand seedlings from an unknown provenance in the Queen Charlotte

Islands. The two resistant trees cloned at Fair Harbour comprise material from two of five individuals that were the only Green Timbers trees not attacked by *P. strobi* in more than 30 years (Alfaro 1982).

Comparison of trees by provenance utilized material collected at all five sites. At Fair Harbour and Sayward, trees were sampled that had been previously analyzed for foliar monoterpenes by Brooks & Borden (1992). At these locations, trees that were not attacked by weevils, and had good growth form and height at the time of selection, were classified as putatively resistant. Susceptible trees were usually within 2 m of resistant trees, and had been attacked by weevils at least once, and usually two or more times. At Kitimat, Nass R., and Head Bay, trees in each of 10 different provenances were planted in nine blocks of nine trees per block (810 trees total). Trees were systematically selected from each block, with care taken to obtain a representative range of tree heights, growth forms and incidence of weevil attacks from each previously designated resistant or susceptible provenance (Ying 1991). Thus samples consisted of both damaged and undamaged resistant and susceptible trees. Tables 1 and 2 respectively indicate the trees selected for analysis by provenance, and the mean numbers of weevil attacks per provenance, averaged across sites. It was not possible to sample equal numbers of resistant and susceptible provenances, as only a small number of resistant provenances have been identified. To compare specific genotypes, 10 replicates of 10 resistant clones from Fair Harbour were examined and compared with 10 wild susceptible trees. These wild trees were attacked by weevils at least twice, and were within 10 m of the clonal outplanting.

Two unattacked trees of the Big Qualicum provenance were selected from Sayward to complete the comparison of resistant trees by clone. Clones are ranked by numbers of weevil attacks in Table 3.

Table 1. Number of trees of each provenance measured at each study site. Blank spaces occur when a provenance was not represented at a site.

Provenance and susceptibility (S) or resistance (R) status	Fair Harbour	Head Bay	Kitimat	Nass River	Sayward
Necanicum, OR (S)		9	9	9	
Hoquium, WA (S)		9	9		
Forks, WA (S)		9	9	9	
Big Qualicum, B.C. (R)		9	9	10	2
Tahsis, B.C. (S)				9	3
Holberg, B.C. (S)		9	9	9	
Link Rd., B.C. (S)		9	9	9	
Inverness, B.C. (S)		9	9	9	
Usk Ferry, B.C. (S)		9	9		4
Kitwanga, B.C. (R)				9	
Ward L., AK (S)		9	10		
Duck Cr., AK (S)		9	9		
Yakutat, AK (S)				9	
Cedarvale, B.C. (R)	8				
Cranberry R., B.C. (S)					1
Zolap Cr., B.C. (S)					1
Fulmar Cr., B.C. (S)					1
Kitsumkalum L., B.C. (S)					2

Table 1 continued

Aberdeen, B.C. (S)	2	1
Massett Sd., B.C. (S)		1
Moresby Camp, B.C. (S)	1	
Tasu Cr., B.C. (S)	3	
Fair Harbour, B.C. (S)	5	2
Port Renfrew, B.C. (S)		2
Muir Cr., Sooke, B.C. (S)	1	
Green Timbers (R)	7	
Haney, B.C. (R)	10	

Table 2. Resistant and susceptible provenances of trees sampled at up to five sites in British Columbia and the severity of weevil attacks.

Provenance	Resistant (R) or susceptible (S) status	Number of trees sampled	Number of weevil attacks per tree ($\bar{x}$ ± S.E.)
Haney, B.C.	R	10	0.64±0.04
Cedarvale, B.C.	R	8	0.75±0.48
Muir Creek , Sooke, B.C.	S	1	1.00±1.35
Green Timbers, B.C.	R	7	1.17±0.55
Kitwanga, B.C.	R	9	1.22±0.45
Big Qualicum, B.C.	R	30	1.22±0.45
Usk Ferry, B.C.	S	18	2.00±0.45
Aberdeen, B.C.	S	3	2.70±0.78
Inverness, B.C.	S	27	3.00±0.45
Hoquium, WA	S	18	3.11±0.45
Ward Lake, AK	S	19	3.20±0.43
Necanicum, OR	S	27	3.33±0.45
Forks, WA	S	27	3.33±0.45
Tasu Creek, B.C.	S	3	3.33±0.78
Yakutat, AK	S	9	3.44±0.45
Link Road, B.C.	S	27	3.56±0.45
Holberg, B.C.	S	27	3.56±0.45
Duck Creek, AK	S	18	3.80±0.43
Fair Harbour, B.C.	S	5	4.00±0.68
Tahsis Inlet, B.C.	S	12	4.38±0.48

Table 3. Source of material sampled at Fair Harbour and Sayward (Big Qualicum provenance only) for analysis of specific genotypes. N=10 in each case except for Big Qualicum where N=1 for each tree.

Provenance	Family	Clone	Ranked number of weevil attacks per tree ($\bar{x}$ ± S.E.)
Haney	1	1	0.00±0.00
Big Qualicum	2, 15	6, 2	0.00±0.00
Haney	UK	5	0.10±0.10
Haney	UK	7	0.20±0.13
Cedarvale	12	4	0.30±0.15
Green Timbers	-	2	0.30±0.21
Haney	1	2	0.50±0.22
Kitwanga	13	8	0.50±0.17
Kitwanga	13	2	1.50±0.17
Cedarvale	12	5	1.60±0.16
Green Timbers	-	3	2.30±0.33

III. CONSTITUTIVE DEFENCES: MORPHOLOGY

INTRODUCTION

A number of researchers have examined the relationship between leader morphology and the susceptibility of Sitka spruce or eastern white pine, *Pinus strobus* L., to the white pine weevil. Wilkinson (1983) observed that the bark thickness, depth of resin ducts and leader diameter were positively correlated with susceptibility of eastern white pine to the white pine weevil. Similarly, the number of weevil attacks was partially dependant on the bark thickness at breast height (Kriebel 1954), and bark thickness and leader diameter were positively correlated with weevil attack (Stroh & Gerhold 1965). Weevils preferred thick leaders, regardless of their length, and leader thickness and bark thickness were highly correlated (Sullivan 1961). VanderSar & Borden (1977a,b) observed that weevils oriented to vertical or near vertical silhouettes about 3 cm in width, and fed preferentially on large diameter branches of Sitka spruce. Harris *et al.* (1990) hypothesized that spruce needles have a positive thigmotactic effect on weevils. It has also been suggested that weevils are attracted to the most vigorous trees (Plank & Gerhold 1965; Silver 1968).

A second aspect of leader morphology is the anatomy of the resin canal system. Resinosis may be the primary cause of mortality for white pine weevils (Overhulser & Gara 1981a,b). Both longitudinal and transverse resin canals are a constant feature in the bark of *Pinus, Picea, Larix* and *Pseudotsuga spp.* (Core *et al.*

1976); the distribution, size and density of these resin canals determine the amount of resin produced (Hanover 1975b). Blanche *et al.* (1992) found that resin flow in loblolly pine, *Pinus taeda* L., was moderately correlated with vertical resin duct density. Fewer outside resin ducts were found in eastern than in western white pine, *Pinus monticola* Dougl., the less susceptible of the two hosts (Soles *et al.* 1970). Stroh & Gerhold (1965) observed that weevils preferred thick bark, and that shallow cortical resin ducts were associated with shallow and narrow feeding cavities on eastern white pine. They found significant positive correlations between the depth of inside and outside resin ducts and occurrence of successful weevil attacks. There was a very low correlation with the number of resin ducts present, although the most ducts were found in the leaders of heavily weevilled trees (Wilkinson 1983), suggesting that traumatic resin canals (Berryman 1972), may have developed in response to weevilling.

The objective of this study was to determine if various aspects of leader morphology are correlated with resistance of Sitka spruce to the white pine weevil.

METHODS

Measurements were made on trees from both the four provenance trials and the clonal outplanting at Fair Harbour (Tables 1,3). The number of attacks per tree, diameter at breast height (dbh=1.3 m) and height of trees were considered to be indications of the overall vigour of the trees, and a measurement of how severely they had been attacked. Leader length and diameter were measured because previous

studies suggest this may be a visual cue involved in host selection (VanderSar & Borden 1977b). Leader length and diameter were not measured for the clones at Fair Harbour. Needle density was measured to test the hypothesis that resistant trees may have a high density of needles on the leader that might interfere with feeding, or a low density that might deprive weevils of a thigmotactic stimulus. The angle of oppression of needles to the stem was measured to test the hypothesis that resistant trees might have needles pressed closely to the stem that could interfere with feeding. Heights of trees and lengths of leaders were measured using a Senskin© height pole (Neville Crosby Industries, Vancouver, B.C.), and dbh was measured using a diameter tape. The previous year's leader and height were measured because measurements were made from June to October, 1992, when current year's leaders were growing. Vernier callipers were used to measure the diameter of a first-whorl branch cut from the base of the leader, as the actual leaders were frequently too high to reach. The number of needles on the distal 5 cm portion of a first-whorl branch were counted. The branch circumference was calculated from its measured diameter to obtain the surface area from which needle density could be calculated. The angle of oppression to the stem of 10 of these needles was measured using a protractor.

To protect leaders from damage, measurements were made on branches. A comparison of measurements between leaders and laterals indicated no significant differences in needle density or length between first-whorl branches and leaders (Table 4). Although there was a significant reduction in diameter and an increase in needle angle in branches when compared to leaders, and branches had smaller inner resin

Table 4. Comparison of morphological characters (mean ± S.E.) between resistant and susceptible branches and leaders. Data were collected from Sayward and Fair Harbour, using 20 resistant trees and 30 susceptible trees.

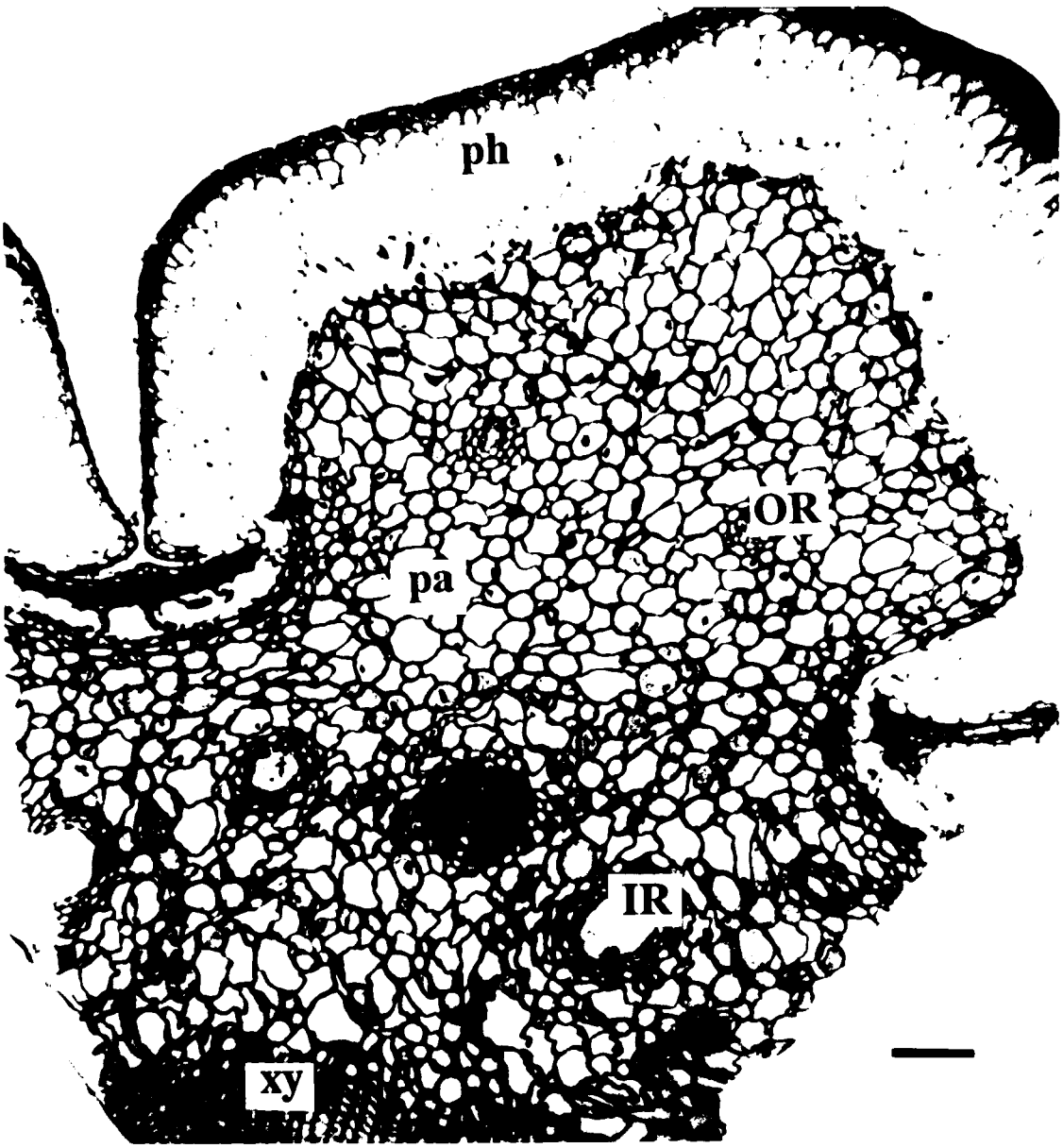
Variable	Status	Branches	Leaders[a]
Needle density (number/cm^2)	Resistant	6.17±1.96	3.68±3.86
	Susceptible	6.61±1.96	7.39±1.96
Diameter (cm)	Resistant	0.74±0.04	1.01±0.08*
	Susceptible	0.68±0.04	0.93±0.04*
Length (cm)	Resistant	59.98±4.51	62.06±8.28
	Susceptible	66.58±4.43	68.7±4.68
Needle angles (degrees)	Resistant	61.26±3.11	49.2±6.09*
	Susceptible	66.12±3.11	55.67±3.11*
Number outer resin ducts	Resistant	19.05±1.52	19.26±1.36
	Susceptible	7.43±1.48	7.44±1.29
Size of inner resin ducts (mm)	Resistant	0.1±0.004	0.24±0.01*
	Susceptible	0.1±0.004	0.21±0.0*

[a] Asterisk indicates a significant difference (ANOVA, $P<0.05$) between leaders and branches.

ducts than leaders, significant differences of comparable magnitude occurred in both resistant and susceptible trees (Table 4). There were no significant differences between leaders and branches with respect to other resin duct measurements such as depth and spacing. For simplicity, only the number of outer resin ducts and the size of inner resin ducts are compared in Table 4. Analysis of variance (PROC GLM) (SAS Institute 1988), further justified measurements made on branches by showing that there was no significant interaction between resistance or susceptibility, and measurements made on leaders or branches.

The number, depth and spacing of resin ducts were measured to test the hypothesis that resistant trees might have larger, shallower or more numerous resin ducts than susceptible trees, and thus a greater capacity for resinosis. To examine the resin duct morphology, the distal portion of an upper lateral branch was collected from every tree. About 50 mm^2 of bark tissue from the distal 5 cm of the branch was excised and placed in formalin-acetic acid-alcohol (FAA) solution (Johansen 1940), and fixed for at least two days. Sections were cut with a scalpel blade, stained in methyl violet, and mounted in glycerine. Measurements were made with an ocular micrometer. The ring of large, uniformly sized resin ducts that were observed closest to the xylem tissue were called the inner resin ducts. All other resin ducts, peripheral to the inner resin ducts, were called outer resin ducts (Fig. 1). The numbers of inner and outer resin ducts, depth and diameter of inner and outer resin ducts, distance between inner ducts and distance between outer ducts, and the radial thickness of the cortical layer (bark thickness) were measured. The depth of the ducts was measured

Figure 1. Cross section (7 μm thick) of Sitka spruce cortical tissue indicating the position of inner and outer resin ducts. Section prepared by fixation in FAA, embedding in Paraplast, sectioning on a rotary microtome and counterstaining with safranin and fast green. IR=inner resin duct, OR=outer resin duct, xy=xylem tissue, ph=phelloderm, pa=parenchyma cell. Scale bar =100 μm.


ph
OR
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xy

using a line parallel to the xylem rays extending to the surface of the bark through the middle of the duct. All measurements of depth and spacing were made to the outer ring of parenchyma cells surrounding the resin ducts. For each tree the value recorded was the mean of five separate measurements. The densities of inner and outer ducts were expressed as the number per cm of circumference. The analysis of the cortical resin canal system was repeated on specific genotypes collected at Fair Harbour (Table 3) the following year. The diameters of the snouts of 59 weevils were also measured using an ocular micrometer so that weevil morphology could be compared with the resin duct placement on the host.

The means of each variable were compared between provenances and between resistant and susceptible provenances for each site using analysis of variance (PROC GLM) or a *t*-test (SAS Institute 1988). Following analysis of variance, Bonferonni *t* tests were used to compare means in order to control the experiment-wise error rate (Schlotzhauer & Littell 1988). In the analysis of clones at Fair Harbour, traits of resistant clones were compared with those of susceptible trees using Dunnett's means test (SAS Institute 1988). Depth of inner and outer resin ducts was regressed against bark thickness for the Fair Harbour clones (PROC GLM, SAS Institute 1988). The numbers of weevil attacks per tree were compared between provenances using a Kruskall Wallis test for non-parametric variables (PROC NPAR1WAY) (SAS Institute 1988). In all cases α=0.05. Provenances were not compared at the Sayward site because there were too few replicates of each, but trees were compared based on their classification as resistant or susceptible. Where n=1, standard errors were estimated

by PROC GLM (SAS Institute 1988).

RESULTS AND DISCUSSION

From the provenance trials, trees labelled as resistant had fewer attacks by weevils than susceptible trees (Table 5). These results indicate that trees were correctly classified as resistant or susceptible. There were no differences in leader length or diameter between provenances.

Trees from the Usk Ferry provenance at Head Bay and the Kitwanga provenance at Nass River had needles that were pressed significantly more closely against the stems than for two and five other provenances, respectively (Table 6). Kitwanga is one of the five acknowledged resistant provenances (Table 1), and trees from the Usk Ferry provenance had the second lowest percentage of trees attacked by *P. strobi* at Head Bay (46%) (Ying 1991). The needles on these trees could physically interfere with feeding or oviposition behavior or could chemically deter weevils (Harris *et al.* 1990). In this study there was no difference in the density of needles between provenances, although Silver (1968) observed that some unattacked, healthy leaders in the Nitinat Valley had an above-average number of needles.

Analysis of the anatomy of the resin duct system revealed three significant trends involving the size of the inner and outer resin ducts, the number of outer resin ducts, and bark thickness.

Table 5. Mean values (± S.E.) for three characteristics of resistant and susceptible provenances of Sitka spruce at five study sites. R=resistant, S=susceptible, df varies from 14 to 91.

Variable[a]		Fair Harbour	Head Bay	Kitimat	Nass R.	Sayward
No. Times attacked[b]	R	0.22±0.43a	0.74±0.76a	1.22±1.09a	1.74±1.63a	0.55±0.53a
	S	2.84±1.30b	1.52±0.80b	3.22±1.16b	4.05±1.31b	4.67±2.12b
Height (m)	R	5.15±0.18a	6.65±0.37a	5.72±0.92a	6.32±0.46a	6.45±0.38a
	S	2.99±0.17b	5.38±0.19b	3.83±0.09b	3.98±0.24b	3.59±0.38b
dbh (cm)	R	8.56±0.41a	11.65±0.82a	11.49±0.89a	15.66±1.83a	12.06±1.07a
	S	5.38±0.39b	10.71±0.41a	9.77±0.29a	10.55±0.95b	7.48±1.07b

[a] Comparisons between resistant and susceptible trees followed by the same letter, within each site, are not significantly different, $P>0.05$, t test.

[b]Means followed by the same letter are not significantly different ($P>0.05$, Kruskal-Wallace test)

Table 6. Mean angle of oppression (degrees ± S.E.) of needles in trees from provenances at Head Bay and Nass River. Means followed by the same letter within each site are not significantly different (P>0.05, Bonferonni t test), N=9.

Provenance and susceptibility (S) or resistance (R) status	Head Bay[a]	Nass River[a]
Necanicum, OR (S)	68.9±4.5a	76.33±7.7a
Hoquium, WA (S)	62.2±4.5ab	
Forks, WA (S)	62.8±4.5ab	71.0±7.7ab
Big Qualicum, B.C. (R)	52.9±4.7ab	74.65±7.7a
Yakutat, AK (S)		69.12±7.7ab
Holberg, B.C. (S)	60.8±4.7ab	68.7±7.7ab
Link Rd., B.C. (S)	60.1±4.5ab	73.7±7.7a
Inverness, B.C. (S)	61.8±4.5ab	73.73±7.7a
Usk Ferry, B.C. (S)	48.2±4.5b	
Kitwanga, B.C. (R)		55.3±7.7b
Ward L., AK (S)	72.5±4.5a	
Duck Cr., AK (S)	59.2±4.5ab	
Tahsis Inlet, B.C. (S)		72.9±6.7a

[a] For Head Bay, F=1.03, df=9,78, P=0.01, and for Nass River, F=2.90, df=9,73, P=0.01.

First, there were significant differences in the size of the inner resin ducts between provenances at Fair Harbour. The smallest ducts were found in the two most susceptible provenances (Moresby Camp and Muir Creek, Sooke) (Ying 1991) (Table 7), suggesting that smaller than average resin ducts could be characteristic of susceptible trees, although the data do not suggest that large ducts are characteristic of resistant trees. Moresby camp is in the Queen Charlotte Islands where *P. strobi* does not occur, and is by far the most susceptible of the provenances examined by Ying (1991). The clones from Fair Harbour (Table 3) differed significantly with respect to the size of inner resin ducts (F=4.07; df=11,92; P<0.0001). Two resistant clones, 18-12-5 (Cedarvale) and 6-13-2 (Kitwanga), had significantly larger inner ducts than the susceptible trees. Resin is considered to be the primary mechanism of defense for conifers (Berryman 1972), and the size of resin ducts is related to the capacity for carrying resin (Christiansen & Bakke 1988).

There was also a strong relationship between the density of outer resin ducts and resistance. When provenances were compared within sites, there were significant differences in the density of outer resin ducts between provenances at Fair Harbour (F=7.53, df=7,36, P<0.0001), and Nass R. (F=2.09, df=9,73, P=0.05), and a nearly significant difference between resistant and susceptible trees at Sayward (t=2.003, df=16, P=0.06). At Fair Harbour, trees from the Haney and Cedarvale provenances had more outer resin ducts than the others, and at Nass R., trees from the Kitwanga, B.C. and Necanicum, OR provenances had the most resin ducts while Link Rd., B.C. had the least. Trees from Necanicum, OR represent an anomaly because they possess

Table 7. Ranked mean diameters of inner resin ducts in upper branches from provenances at Fair Harbour. Means followed by the same letter are not significantly different ($P>0.05$, Bonferonni t test). N varies from 1 to 10.

Provenance and susceptibility (S) or resistance (R) status	n	Diameter (mm) (mean ± S.E.)
Aberdeen, B.C. (S)	2	0.101±0.01a
Cedarvale, B.C. (R)	8	0.097±0.01ab
Fair Harbour, B.C. (S)	5	0.095±0.01ab
Green Timbers, B.C. (R)	7	0.094±0.01ab
Haney, B.C. (R)	10	0.085±0.01abc
Tasu Creek, B.C. (S)	3	0.061±0.01abc
Muir Creek, Sooke, B.C. (S)	1	0.052±0.01bc
Moresby Camp, B.C. (S)	1	0.044±0.01c

a relatively high density of outer resin ducts (Fig. 2) and yet are from one of the most susceptible provenances (Ying 1991). It is possible that these trees are able to produce large numbers of outer resin ducts in response to weevil infestation. At Sayward, trees from Big Qualicum, B.C. had more outer resin ducts than the average of the susceptible provenances. Trees from Haney and Cedarvale, B.C. have the most outer resin ducts (Fig. 2) of all provenances measured when sites were pooled, and trees from Masset Sound had the least, although only one tree was measured. Masset Sound is also in the Queen Charlotte Islands, and had one of the highest attack rates of all provenances assessed by Ying (1991). With the exception of trees from Usk Ferry and Aberdeen, provenances from the Sitka/white spruce hybridization zone (Ying 1991), tended to have trees with more outer resin ducts than those from other provenances (Fig. 2). Overall, there was a highly significant difference in the density of resin ducts between the pooled resistant and susceptible trees (t=4.48, df=316, P<0.0001) (Fig. 2, inset). It should be noted that this analysis does not take into account any effect site might have on expression of resin canals, as each provenance is not replicated at every site. The number of outer resin ducts also varied among specific genotypes at Fair Harbour. All of the resistant clones, except Big Qualicum, had significantly more outer resin ducts than the susceptible trees (F=5.56; df=11,98; P<0.0001) (Fig. 3). Two Haney clones (29-1-2, 29-1-1) and one Cedarvale clone (18-12-4) had the highest number of outer resin ducts, supporting results obtained in the provenance trials.

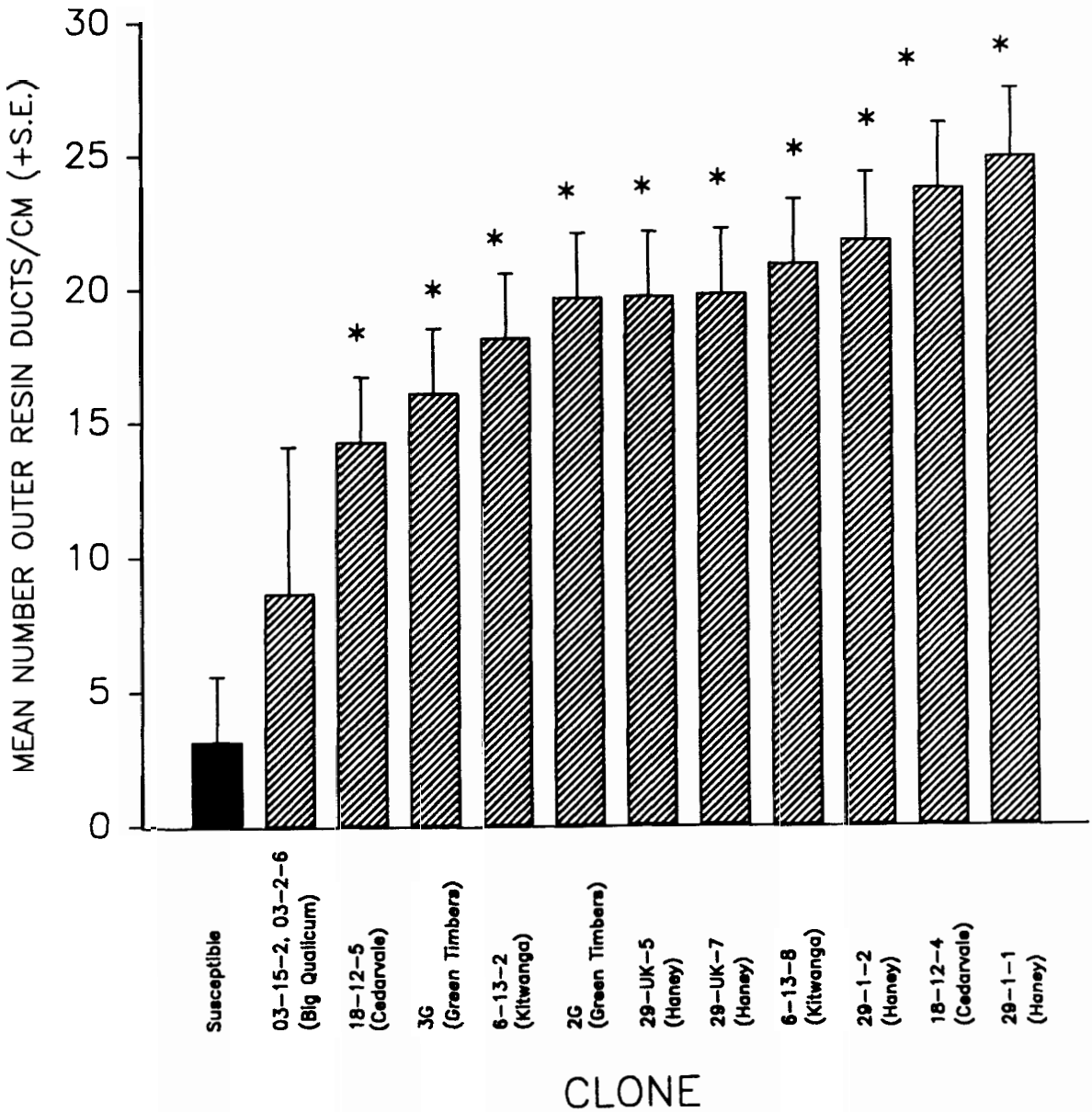
Analysis of clones at Fair Harbour revealed that four resistant clones, 18-12-4,

Figure 2. Graph of number of outer resin ducts per cm averaged over all test sites. Bars lacking a standard error represent a single observation (F=3.14, df=30, 317, P<0.0001). Data from Sayward included to illustrate the ranking of pooled resistant (R) and susceptible (S) trees from this site in comparison with those from individual provenances. Inset indicates the average of all resistant compared with all susceptible provenances at all sites (t=4.48, df=316, P<0.0001).

Mean Number of Outer Resin Ducts Per cm (+ S.E.)
0
5
10
15
20
25
30
35
40
Provenance
Haney, B.C.
* Cedarvale, B.C.
* Fulmar Cr., B.C
* Kitsumkalum L.
Sayward-R
* Cranberry R.,BC
Port Renfrew,BC
* Kitwanga, B.C.
Muir Cr., Sooke
* Zolap Cr., B.C.
Green Timbers
Tahsis, B.C.
Fair Harbour BC
Necanicum, OR
Big Qualicum,BC
Hoquium, AK
Ward L., AK
Forks, WA
* Usk Ferry, BC.
Tasu Cr., B.C.
Sayward-S
* Aberdeen, B.C.
Moresby Camp,BC
Inverness, B.C.
Holberg, B.C.
Duck Cr., AK
Yakutat, AK
Link Rd., B.C.
Masset Sd, B.C.
Resistant
Susceptible
0
2
4
6
8
10
12
14
16
18
Resistant Provenances
Susceptible Provenances
* Provenances from the white/
Sitka spruce hybridization zone

29

Figure 3. Graph of number of outer resin ducts per cm in resistant clones and susceptible trees at Fair Harbour. Asterisks indicate clones that are significantly different from the susceptible trees, Dunnett's test, $P<0.05$.


MEAN NUMBER OUTER RESIN DUCTS/CM (+S.E.)
30
25
20
15
10
5
0
Susceptible
03-15-2, 03-2-6 (Big Qualicum)
18-12-5 (Cedarvale)
3G (Green Timbers)
6-13-2 (Kitwanga)
2G (Green Timbers)
29-UK-5 (Haney)
29-UK-7 (Haney)
6-13-8 (Kitwanga)
29-1-2 (Haney)
18-12-4 (Cedarvale)
29-1-1 (Haney)
CLONE

18-12-5 (Cedarvale), 2G (Green Timbers), and 6-13-2 (Kitwanga) had significantly thicker bark than the susceptible trees (F=4.23; df=11,98; P<0.0001) (Fig. 4). Only one genotype, Big Qualicum (03-15-2 and 03-2-6) had slightly, but not significantly, thinner bark. The depth of inner and outer resin ducts varied slightly, but significantlyamong clones (F=2.97; df=11,98; P=0.0019 and F=3.69; df=11,92; P<0.0001, respectively). Both depth measurements varied positively with bark thickness (Fig. 5). My result are similar to those of Plank & Gerhold (1965), who found that western white pine, an unfavourable host for *P. strobi*, had larger and more numerous outer resin ducts than Engelmann spruce, *Picea engelmannii* Parry ex Engelm. There are several reasons why large numbers of outer resin ducts might confer resistance. If outer ducts are capable of producing traumatic resin, which may contain higher quantities of defensive compounds than constitutive resin (Raffa & Berryman 1982), they would be the first line of defense encountered by weevils feeding or ovipositing on trees in which the production of traumatic resin had been induced. Some resin constituents, such as limonene and piperitone, are deterrent to feeding weevils (Alfaro *et al.* 1980). VanderSar (1978) found more feeding punctures on western white pine than on Engelmann spruce, even though no oviposition occurred, suggesting that weevils would feed extensively on this host, but would not oviposit until they encountered an area free of outer resin ducts. In eastern white pine, Stroh & Gerhold (1965) observed that only 9.3% of 215 feeding cavities contacted the epithelial cells of the outer resin ducts, suggesting an antixenotic effect. In contrast, most feeding cavities came in contact with the inner resin ducts, although they did not

Figure 4. Graph of bark thickness in resistant clones and susceptible trees at Fair Harbour. Asterisks indicate clones that are significantly different from the susceptible trees, Dunnett's test, $P<0.05$.

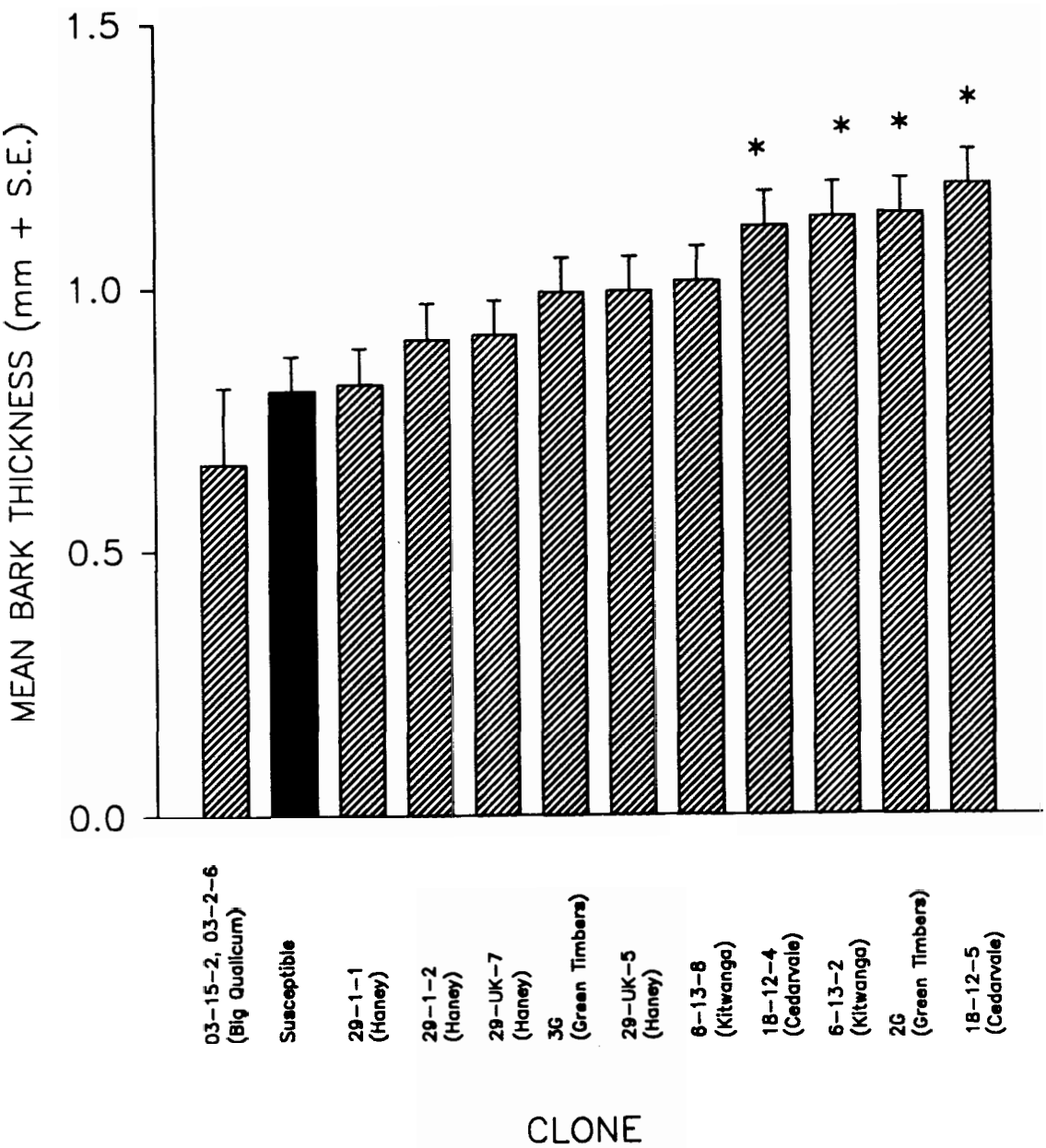
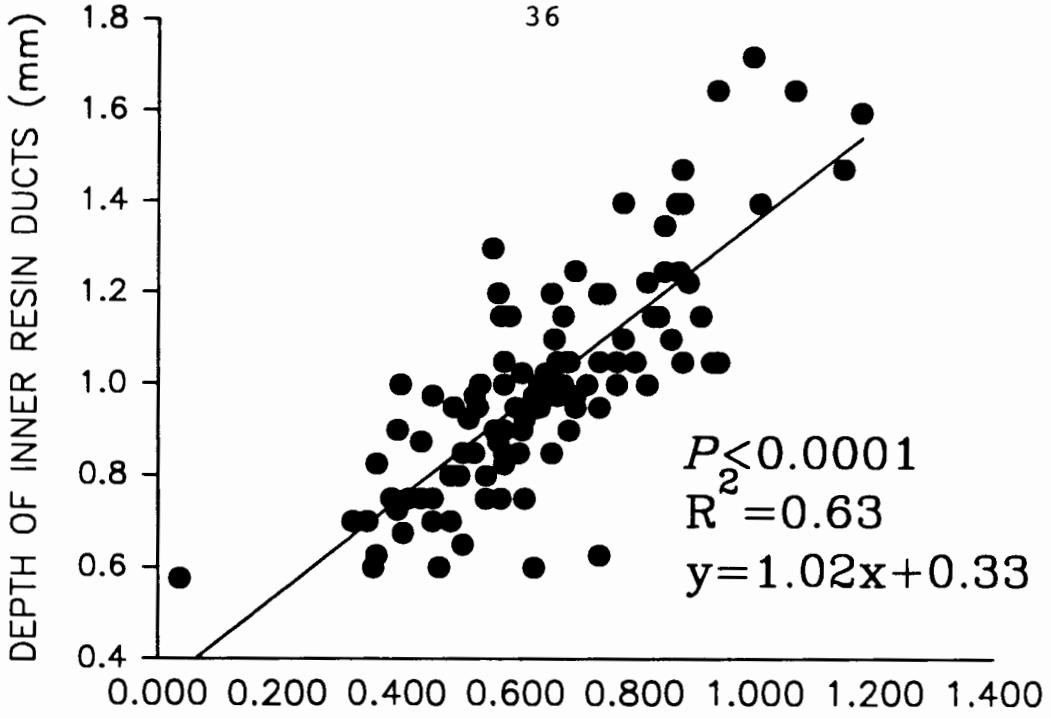
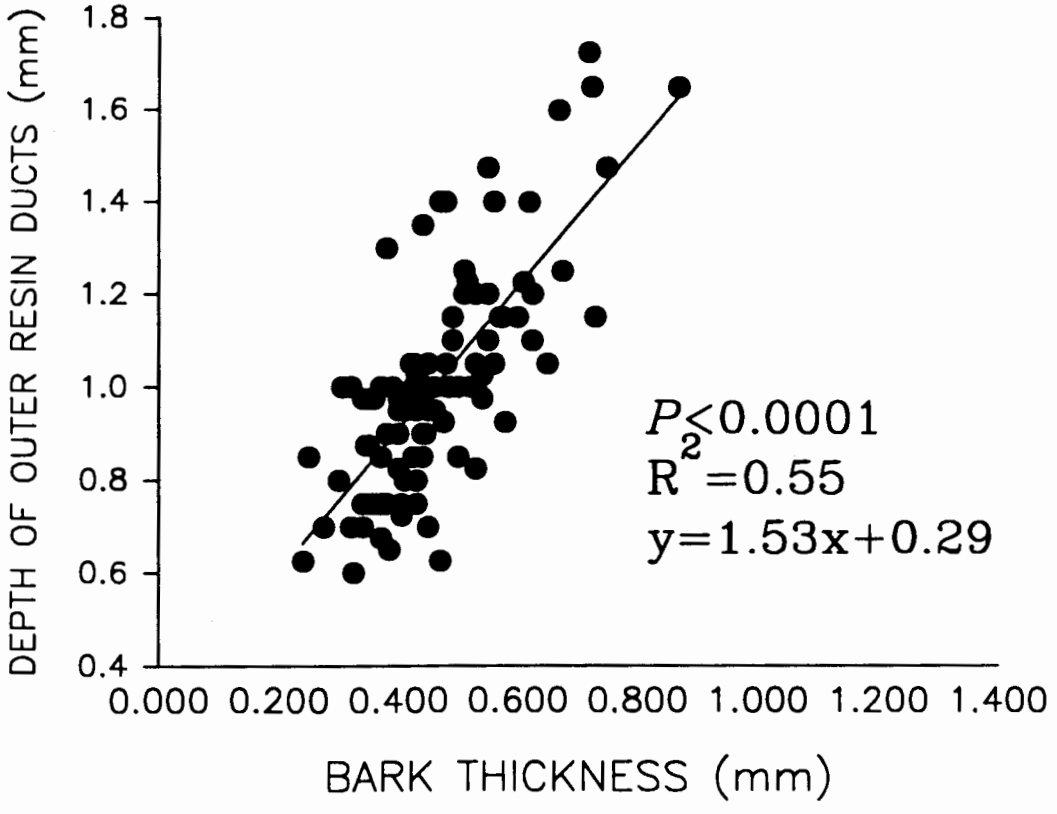

MEAN BARK THICKNESS (mm + S.E.)
1.5
1.0
0.5
0.0
*
*
*
*
03-15-2, 03-2-6 (Big Qualicum)
Susceptible
29-1-1 (Haney)
29-1-2 (Haney)
29-UK-7 (Haney)
3G (Green Timbers)
29-UK-5 (Haney)
6-13-8 (Kitwanga)
18-12-4 (Cedarvale)
6-13-2 (Kitwanga)
2G (Green Timbers)
18-12-5 (Cedarvale)
CLONE

Figure 5. Relationship between bark thickness and depth of inner and outer resin ducts in clones sampled at Fair Harbour.


DEPTH OF INNER RESIN DUCTS (mm)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.000 0.200 0.400 0.600 0.800 1.000 1.200 1.400
P<0.0001
R²=0.63
y=1.02x+0.33


DEPTH OF OUTER RESIN DUCTS (mm)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.000 0.200 0.400 0.600 0.800 1.000 1.200 1.400
P<0.0001
R²=0.55
y=1.53x+0.29
BARK THICKNESS (mm)

sever them. Feeding cavity dimensions were correlated positively with the distance between inner and outer ducts.

If thin bark is correlated with resistance (Stroh & Gerhold 1965), then one might question why susceptible trees had bark as thin as that of resistant trees (Fig. 4). In clones such as 29-1-1 (Haney), a combination of thin bark and high density of outer resin ducts (Fig. 3) would increase the chances that weevils would encounter resin ducts, almost surely preventing acceptance of this host by a weevil. In the case of Big Qualicum trees, the bark was thin (Fig. 4), and consequently, the resin ducts were shallow (Fig. 5), but this clone has so few outer ducts (Fig. 3) that they resemble susceptible trees in both traits. The high degree of resistance in these trees (Table 3) must therefore be imparted by other resistance traits. The susceptible trees, however, had almost no outer resin ducts that would have deterred feeding by weevils (Fig. 3). Thus thin bark would be a weak resistance trait unless it was accompanied by a high density of outer resin ducts.

The distance between outer resin ducts ranged from 0.20 mm to 0.80 mm, and the mean diameter (± S.E.) of the snout of *P. strobi* was 0.82±0.1 mm. It is clear that in many cases weevils would need to make several trials before finding a route through the outer resin ducts, and in the most dense distributions of resin ducts, weevils would be unlikely to find sufficient passage to construct an oviposition puncture. Finally, a great number of outer resin ducts may simply aid in the volume of resin production and/or transport. Plank & Gerhold (1965) observed that if insufficient eggs hatch, larvae are killed by resin and the tree recovers from attack.

As suggested for the trees from the Necanicum OR provenance, production of outer resin ducts might actually be stimulated in susceptible provenances. As the outer ducts are encountered first by the weevils, there would be strong selection pressure to produce large numbers of constitutive outer resin ducts or to have the capability of producing them in response to weevilling. It is thus not surprising that there was little variation in the numbers or size (Table 7) of the inner ducts, although Wilkinson (1983) observed that the numbers of both outer and to a lesser extent, inner, resin ducts were highest in the most heavily weevilled eastern white pines. It is obvious that before morphological characteristics of the resin system can be used to assess young trees that have not been exposed to weevilling, it will be necessary to determine which traits are innate and which are induced, wholly or in part, by weevil activity. Although not measured, horizontal resin canals (Core *et al.* 1976), could also be important in resistance by Sitka spruce to *P. strobi.* By the distributions of outer resin ducts of trees in resistant provenances (Fig. 2), and clones (Fig. 3), I hypothesize that density of outer resin ducts is an important resistance mechanism, particularly when this trait is associated with thin bark. A positive relationship between inner duct diameter and outer duct density might also be important.

In developing a multicomponent resistance index (Brooks & Borden 1992), it would be prudent to include a term classifying trees based on their resin systems, using the density of outer resin ducts and bark thickness. High scores would be awarded to trees with the most resin ducts, and the thinnest bark.

IV. CONSTITUTIVE DEFENSES: CHEMISTRY

INTRODUCTION

Resin is generally thought to play an important role in the defense of conifers against stem invading insects and pathogens (Berryman & Ashraf 1970; Berryman 1972; Hanover 1975b). Primary (constitutive) resin is considered to be the first line of defense of conifers, acting as a wound cleansing agent; in some cases it deters further attack and prevents fungal growth (Berryman 1972). Constitutive resin flow may 'buy time' for induced responses to become effective (Christiansen & Bakke 1988). The rate of resin flow is determined largely by the viscosity of the resin, number and size of preformed resin ducts (Hanover 1975b), and the proportion of resin secretory cells which are functional (Matson & Hain 1983), while exudation pressure is regulated more by the water status of the tree (Vité 1961; Hanover 1975a).

Production of resin is metabolically expensive to a tree and results in the loss of carbon that could otherwise be allocated to growth and reproduction (Matson & Hain 1983). Primary resin is present in the bark and foliage of trees regardless of infection, and is constantly broken down and remetabolized. Losses occur when resin ducts are severed (Matson & Hain 1983). Secondary resin is only synthesized in response to injury or infection, but is probably more costly to the tree than primary resin because it contains higher concentrations of monoterpenes (Matson & Hain 1983). Miller & Berryman (1983) observed a 30% decrease in soluble sugars and a

15% decrease in reducing sugars in the wound reaction area of lodgepole pine, *Pinus contorta Dougl.* var. *latifolia*, infected with *Ceratocystis clavigerum* (Robinson & Davidson), a symbiotic fungus of the mountain pine beetle, *Dendrotconous ponderosae* Hopkins.

Primary conifer resin is composed mainly of diterpene resin acids, monoterpenes, sesquiterpenes and terpene alcohols, the predominant fraction being the diterpene resin acids (Hanover 1975a). Chemical constituents of primary oleoresin can act as repellents, feeding and oviposition deterrents, and reduce growth and survival of stem invading insects.

The volatility of monoterpenes causes them to be one of the first host traits that can be assessed by herbivores, and repellency of insects by volatile terpenes might represent an efficient defensive strategy against stem-invading insects by conifers. In particular, foliage occupies a large surface area for dissemination of constitutive terpenes, and losses due to the severing of cortical resin ducts would be avoided or reduced. Some monoterpenes such as myrcene (Bedard *et al.* 1969) and α-pinene (Renwick and Vité 1969), are attractive to bark beetles, but more often they are repellent to bark beetles (Berryman & Ashraf 1970; Smith 1975; Bordasch & Berryman 1977), the white pine weevil (Alfaro *et al.* 1980) and some mammalian herbivores (Zhang & States 1991; Duncan *et al.* 1994).

Resin acids may deter some insects. Björkman & Gref (1993) observed that European pine sawfly, *Neodiprion sertifer* Fourcroy, pre-pupae preferred to spin cocoons in the feces from larvae fed on needles containing high amounts of resin acid

in their needles. These feces contained more resin acids than those of larvae feeding on needles with low amounts of resin acids, perhaps providing the pre-pupae with protection from natural enemies, because of repellency of the resin acids.

If insects are not initially repelled by the odour of either bark or foliage, feeding or oviposition deterrency may occur in response to resin flow or gustatory stimuli. Success of the fir engraver, *Scolytus ventralis* LeConte, on grand fir, *Abies grandis* (Dougl.)Lindl., was determined by the rapidity of the host's resin flow, which in itself was deterrent to the beetles (Berryman & Ashraf 1970). Hodges *et al.* (1979) correlated resistance of southern pines to the southern pine beetle, *Dendroctonus frontalis* Zimmerman, with total resin flow, flow rate, viscosity and time for resin to crystallize. When topically applied to tamarack, *Larix laricina* (Du Roi) K. Kock, several resin acids caused decreased foliage consumption by larch sawflies, *Pristiphora erichsonii* (Hartig) (Wagner *et al.* 1983).

In the absence of feeding or oviposition deterrency, resin constituents may affect the growth and survival of insects. Reid & Gates (1970) found that direct contact with resin greatly reduced the egg hatch of mountain pine beetle, and limonene was found to be toxic to the southern pine beetle (Hodges *et al.* 1979). The oleoresin of ponderosa pine, *Pinus ponderosa* Laws., had broad antimicrobial activity; monoterpenes were fungistatic, and diterpene resin acids were toxic to gram-positive bacteria (Himejima *et al.* 1992). Negative effects on microorganisms associated with stem-invading insects might limit the survival of eggs and growth of larvae if thes microoganisms are required to overcome host defenses. Larvae of the European pine

sawfly exhibited long development times and high mortality rates when fed on clones of Scots pine, *Pinus sylvestris* L., containing high levels of resin acids (Larsson *et al.* 1986).

In addition to direct effects on herbivores, conifer resins or their constituents may be correlated with resistance and may be useful in 'chemotyping' trees. von Rudloff (1964) compared the foliar terpenes of Sitka and Engelmann spruce, *Picea engelmanni* Parry ex Engelm., and concluded that oxygenated monoterpenes were of both taxonomic and phylogenetic value. Similarly, von Rudloff & Holst (1968) demonstrated the hybrid nature of Rosendahl spruce by comparing its foliar terpenes with those of its parents, white spruce, *Picea glauca* (Moench)Voss and black spruce, *Picea mariana* (Mill.)B.S.P., and Hanover & Wilkinson (1969) showed that hybrids of blue spruce, *Picea pungens* Engelm., and white spruce could be identified on the basis of cortical monoterpenes. Some provenances of *Pinus merkusii* de Vriese were separated on the basis of xylem turpentine and resin acid composition (Coppen *et. al.* 1993a). Three varieties of *Pinus caribaea* Morelet were separated on the basis of the xylem monoterpenes α-pinene and β-phellandrene, and isopimaric acid, a diterpene resin acid. Additional separation was achieved on the basis of bornyl acetate, longifolene and several sesquiterpenes (Coppen *et al.* 1993b). Smith (1983) compared two races of lodgepole pine, and observed significant differences on the basis of β-pinene, 3-carene and β-phellandrene. In addition, Mergen *et al.* (1955) observed that the oleoresin yield of slash pine, *Pinus elliotti* Engelm., is inherited, and that viscosity of oleoresin, determined primarily by resin acid content and composition (Santamour

1965), is under strong genetic control. The quantitative composition of monterpenes is also under strong genetic control (Hanover 1975b). von Rudloff (1975) concluded that quantitative leaf oil analysis was extremely useful for discriminating among both species and subspecies, and Zavarin *et. al* (1969) suggested that differences in turpentine composition of pines was controlled by a limited number of genes with major effects. Thus foliar terpenes and cortical resin acids are potential genetic-based markers of resistance (Wilkinson 1980), and good candidates for incorporating into tree breeding programs.

Volatile terpenes have been variously related to resistance of both Sitka spruce and eastern white pine, *Pinus strobus* L., to *P. strobi.* Brigden *et al.* (1979) observed no differences in the cortical terpenes of resistant and susceptible eastern white pine. Hanover (1975b) found quantitative differences in the foliar and cortical terpene composition of eastern and western white pine, *Pinus monticola* Dougl., with similar patterns of variation in both tissues. Eastern white pine, the preferred host of eastern *P. strobi,* tended to have higher levels of α-pinene and camphene and lower amounts of β-pinene than western white pine. In contrast, Hunt *et al.* (1990) found lower amounts of the sesquiterpene β-caryophyllene in western than eastern white pine; bornyl acetate and β-elemene were absent in eastern white pine. Wilkinson (1980) observed higher amounts of α-pinene and lower amounts of limonene in unattacked eastern white pine trees when compared with heavily attacked trees. Hrutfiord & Gara (1989) found the leaders (presumably bark and foliage combined) of fast-growing, susceptible Sitka spruce to have low amounts of myrcene and an unknown peak not

observed in more resistant, slow-growing trees. Amounts of β-phellandrene were lower, and β-pinene and 3-carene higher in the bark of resistant than susceptible Sitka spruce trees (Harris *et al.* 1983). Brooks *et al.* (1987a) observed higher amounts of α-pinene, β-pinene, camphor and camphene in the foliage of resistant than susceptible Sitka spruce; amounts of isoamyl and isopentenyl isovalerate were consistently low in resistant trees.

There is also evidence that the composition or amount of diterpene resin acids could be involved in resistance of spruces or pines against the white pine weevil. Hanover (1975b) observed that the composition of resin acids differed between eastern and western white pine. Western white pine was characterized by having more pimaric, palustric and isopimaric acid, and less sandaracopimaric acid than eastern white pine, and eastern white pine had less dehydroabietic, strobic, neoabietic acid and total amount of resin acid than western white pine. In addition, the resin of eastern white pine was twice as viscous as that of western white pine. Wilkinson (1979) found that strobic acid was positively correlated with the rate of crystallization of eastern white pine resins, and the proportions of abietic and palustric acids were negatively correlated. Highly weevilled trees had the highest frequencies of non-crystallizing resin, although differences between resistant and susceptible trees with respect to rates of crystallization were small. In contrast, van Buijtenen & Santamour (1972) observed that out of 20 eastern white pines with non-crystallizing resin, only three were successfully attacked, but that 95 of 189 (50%) of the remaining trees with crystallizing resin were attacked. Santamour (1965) suggested that crystallization of

resin, which is the result of precipitating resin acids, may deactivate it as a defense mechanism. He suggested that the degree of crystallization was probably dependent on the total resin acid concentration, as well as the proportions of individual resin acids.

In addition to oleoresin constituents, tannins may be important in defense against herbivores and pathogens in conifers. Tannins are traditionally thought to be the main defensive compounds of long-lived, conspicuous (apparent) plants (Feeny 1975), where they supposedly act to reduce the digestibility of the plant. A review of the literature, however, reveals a great deal of variation in insect-tannin relationships, and several hypotheses regarding the defensive role of tannins. Tannins have been hypothesized to act as antibacterial and antifungal agents (Hillis & Inoue 1968; Kosuge 1969; Rhodes & Wooltorton 1978; Swain 1979), feeding deterrents (Bennett 1965; Swain 1979; Marks *et al.* 1988), digestibility reducers (Feeny 1968; Feeny & Bostock 1968; Feeny 1970; Bernays *et al.* 1981), toxins (Bennett 1965) and as structural defenses (Haslam 1988). Only condensed tannins occur in conifers (Swain 1979), specifically stilbenes in the case of Sitka spruce (Forrest 1975; Woodward & Pearce 1988; Underwood & Pearce 1991; Werner & Illman 1994).

Clonal differences in the antifungal stilbenes astringen and astringenen (Woodward & Pearce 1988) were observed by Forrest (1975). These stilbenes increased during the hypersensitive response of Sitka spruce to both mechanical wounding and inoculation with a blue stain fungus (Werner & Illman 1994). If clones resistant to the white pine weevil contain higher amounts of total condensed tannins

than susceptible clones, they might impart feeding deterrency, increase bark toughness, reduce bark digestibility or inhibit or kill any microorganisms that could be associated with *P. strobi.*

Objectives:

My objective was to compare the volatile foliar monoterpenes, cortical diterpene resin acids, and total cortical condensed tannins between both resistant and susceptible Sitka spruce provenances, and between known genotypes of Sitka spruce to determine if there is a genetically-based relationship between these compounds and resistance or susceptibility of Sitka spruce to the white pine weevil.

METHODS

Collection of Material

Branches were collected from provenance trials and the clonal outplanting at Fair Harbour (Tables 2, 3). Samples were removed from the first whorl of branches below the leader, and the previous year's growth was examined in each case. Because foliar terpenes change throughout the summer (Hrutfiord *et al.* 1974; Brooks *et al.* 1987b), samples were collected no earlier than late August and stored in the dark at 0°C until ready for analysis. Although volatile cortical terpenes do not vary much throughout the year (Forrest 1980) it is not known if diterpene resin acids vary.

Sample Preparation

Branches were dipped in liquid nitrogen, causing the needles to fall readily

from the branch. Bark was peeled from the branches and the foliage and bark ground separately in liquid nitrogen with a mortar and pestle. The ground foliage was used in analysis of volatile terpenes, and the bark in analysis of diterpene resin acids and condensed tannins. A portion of each tissue before extraction was retained for dry weight determination.

Analysis of Volatile Terpenes

A known weight of ground foliage (1-2 g) was extracted in 8 mL of ether: methanol: water (79:20:1 by volume) with a Tissue Tearer© hand-held homogenizer (Biospec Products Inc., P.O. Box 722, Bartlesville, OK). Heptyl acetate was used as an internal standard. The extracts were centrifuged to remove most particulate matter, and filtered through activated charcoal and cotton to remove chlorophyll, and through DEAE Sephadex in the basic form to remove resin acids (Zinkel & Magee 1991). The extracts were then mixed with hexane, the hexane layer removed, dried over magnesium sulphate, and analyzed by gas chromatography. An HP Ultra 2 capillary column (25 m, 0.20 mm i.d.), with a temperature program of 70°C to 280°C, at 7°C per min was used. Split injector temperature was 180°C with *ca* 100:1 split ratio. The linear velocity of the helium carrier gas was 22.4 cm per sec. Flame ionization detector temperature was 295°C. Compounds were identified and quantified (mg per g dry weight) by the internal standard method using a calibration mixture of 15 terpenes, and the identifications verified by coupled gas chromatography-mass spectrometry. The use of heptyl acetate as an internal standard may have slightly overestimated the

amount of the diterpene, manool, but comparisons between samples for this compound are still valid. The total amount of foliar terpenes was calculated by summing the amounts of identified individual terpenes.

Analysis of Diterpene Resin Acids

A known weight of ground bark (1-2 g) was extracted twice, each time in 8 mL acetone, using an electric hand-held Tissue Tearer® (Biospec Products Inc., P.O. Box 722, Bartlesville, OK). 1,2-Dichlorodehydroabietic acid (Helix Biotech, Vancouver, B.C.) was added as an internal standard. The combined extracts were centrifuged to remove most particulate matter, and then evaporated to dryness under a stream of nitrogen. The residue was redissolved in 1 mL of dichloromethane and the acidic fraction was isolated using NH_2 aminopropyl ion exchange columns (Chen *et al.* 1994). This fraction consists mainly of resin acids and fatty acids (Browning 1967). Filtrates were evaporated to dryness under a stream of nitrogen, redissolved in 2 mL methanol and methylated using excess ethereal diazomethane (Alberta Ministry of the Environment, Method No. AE129.0). Methylated resin acids were evaporated to 1 mL, redissolved in 1 mL methyl *t* butyl ether, filtered through glass microfibre filter paper, dried over $MgSO_4$, and analyzed by gas chromatography. A Hewlett Packard Ultra 2 capillary column (25 m x 0.20 mm i.d.) was used, with a temperature program of 70°C to 200°C, at 10°C per min followed by 200-280°C at 5°C per min. Other analytical conditions were identical to those used in volatile terpene analysis. Resin acids were identified by mass spectrometry and identified peaks were quantified using

the internal standard method with a calibration mixture of pimaric, isopimaric, levopimaric, palustric, dehydroabietic, abietic, and neoabietic acid (Helix Biotech, Vancouver, B.C.). Peaks comprising < 1% of the total peak area, or not identified as resin acids, were ignored. The total amount of resin acid was calculated by summing the amounts of individual identified resin acids. For samples collected from the provenance trials, cortical monoterpenes were also extracted and analyzed by the method described for volatile foliar terpenes. The total amount of extractable resin was calculated by summing the amounts of total terpenes and resin acids, and the amount of resin acid expressed as a proportion of that total.

In addition, the total amount of resin acid was compared with an index of the total volume of resin canals in the samples collected from Fair Harbour. The number and diameter of resin canals (Section III) were used to calculate the total surface area of resin canals in a cross-sectional plane of a different upper lateral branch of the same age that was used for resin acid extractions. This cross-sectional area was used as an index of resin duct volume.

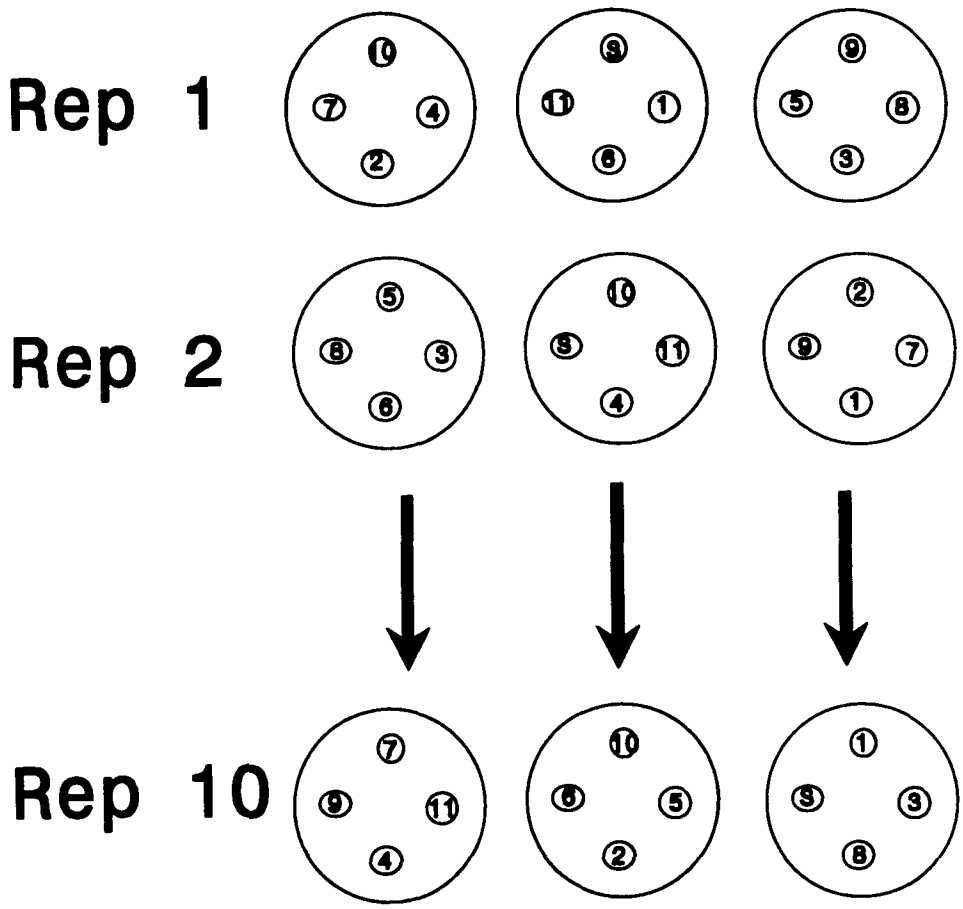
Analysis of Condensed Tannins by Clone

A known weight of ground bark (1-2 g) was extracted in 6 mL acetone:water (60:40, v/v) (Hagerman 1988), using an electric hand-held Tissue Tearer® (Biospec Products Inc., P.O. Box 722, Bartlesville, OK). The bark extracts were centrifuged to remove particulate matter, and stored at 0°C until ready for analysis.

Condensed tannins were quantified using a modified radial diffusion assay

(Hagerman 1987) and modified based on (A.E. Hagerman, Dept. Chemistry, Miami Univ., Oxford, Ohio *pers. comm.*). Agarose gels were prepared by dissolving 4 g of agarose in 400 mL of 0.05 M, acetate buffer, pH 5.0, containing 60 μM ascorbic acid. The boiled agarose solution was cooled to 45°C, and 0.04 g of bovine serum albumin (BSA) was added and dissolved; 9.5 mL of agarose solution was dispensed into each of 30, 100 x 15 mm disposable plastic Petri plates. The plates were maintained on a level surface until the gel hardened. Using a 4.0 mm cork borer, four holes were punched as far apart from each other as possible in each Petri plate. Sixteen μL of the bark extract from a single branch from 10 different replicates of each of 11 Fair Harbour clones and ten susceptible trees was added to each well in a completely randomized block design (Fig. 6). The 11 clones, and one of ten susceptible trees were randomly assigned to wells 1-12, and the 12 wells replicated 10 times. Each replicate represents a separate extraction. The Petri plates were sealed with Parafilm, and the gels were incubated at 30°C for 96 h to allow the condensed tannins in the extract to diffuse through the gel and precipitate the BSA. They were then marked with india ink for identification, removed from the Petri dishes and washed in three changes of 0.3M NaCl to remove any unprecipitated protein from the gel. The gels were stained with Prussian blue reagent for several minutes, causing the tannin-containing rings of precipitate to turn blue-green. The excess stain was poured off, the gels rinsed with 0.10 M HCl, and the diameter of the rings measured immediately on two axes perpendicular to each other. The two measurements were averaged for each well. Tannins were quantified by comparing the diameter of the sample rings to a

Figure 6. Experimental design for the modified radial diffusion assay used in analysis of cortical condensed tannins. Each large circle represents a separate Petri dish, and each small circle represents a well in the agarose gel containing tannin extract from one of the 11 clones and one of ten susceptible trees.


Rep 1
Rep 2
Rep 10

calibration curve created by plotting ring diameter with known concentrations of purified quebracho tannin (Trask Chemical Corporation, Peabody, MA). Quebracho tannin was purified on Sephadex LH20 (Hagerman & Butler 1980), and modified by A.E. Hagerman (*pers. comm.*).

Statistical Analysis

In all cases α=0.05. Differences between resistant and susceptible provenances with respect to individual terpenes or resin acids, total amount of volatile terpenes or resin acids, and proportion of resin acid were compared using t-tests. Principal components analysis (Proc PRINCOMP, SAS Institute 1988), was used to determine if any clustering of trees occurred based on the total complement of terpenes or resin acids. Principal components (PC's) are linear combinations of the original variables with coefficients equal to the eigenvectors of the correlation or covariance matrix (SAS Institute 1988). They account for as much of the variance as is mathematically possible (Bernstein 1988). The magnitude of the principal components scores for each variable indicated their relative importance in separating data points (SAS Institute 1988). Manova and discriminant analysis were not appropriate because there were too many treatment groups and too few replicates.

The differences between clones, and the susceptible trees from Fair Harbour and the two Big Qualicum trees from Sayward, with respect to terpenes and resin acids were compared using Manova and canonical discriminant analysis (Proc GLM and CANDISC, respectively, SAS Institute 1988). This method allows individuals to

be classified into discrete groups on the basis of multiple predictors (Bernstein 1988). The canonical score for each variable (terpene or resin acid) indicates its relative importance in separating treatment groups, and the sign (positive or negative) associated with each variable indicates the nature of its correlation with a given canonical (discriminant) axis. Each canonical variate or axis is a linear combination of variables or predictors (Bernstein 1988) as in the principal components analysis. In addition, a stepwise discriminant analysis was performed (Proc STEPDISC, SAS Institute 1988), as a further method of assessing the relative importance of the different terpenes or resin acids. The total amount of terpenes, and amounts of individual terpenes were compared between clones using analysis of variance (Proc GLM, SAS Institute 1988) followed by Dunnett's means test to determine which resistant clones differed from the susceptible trees (SAS Institute 1988). In all cases, except in the analysis of resin acids by clone, the square root transforms were used for statistical analysis in order to satisfy the assumptions of parametric tests.

Regression analysis was used to determine the relationship between the total amount of resin acid and cross-sectional area of all resin ducts.

The amounts of condensed tannin in bark from Fair Harbour clones were compared using analysis of variance (Proc ANOVA, SAS Institute 1988) followed by Dunnett's means test to determine which resistant clones differed from the susceptible trees (SAS Institute 1988). Data were not transformed.

RESULTS

Analysis of Volatile Terpenes by Provenance

By gas chromatographic analysis of foliage from trees in resistant and susceptible provenances, 15 terpenes were identified and quantified (Fig. 7, Table 8). Manool, a diterpene was identified by GC-MS analysis. Peaks constituting ≤ 1% of the total peak area were ignored. β-Phellandrene and limonene were not separated.

Compared to susceptible provenances, resistant provenances contained significantly lower amounts of most individual terpenes, including α-pinene, β-pinene, myrcene, terpinolene, isoamyl and isopentenyl isovalerate, borneol, and piperitone (Table 8), and on average, resistant provenances had half the amount of total foliar terpenes (11±2.63 mg) than susceptible provenances (24±3.09 mg) (t=3.56, df=128.9, P=0.0005). The amount of myrcene was more variable than other terpenes in resistant provenances.

Principal components analysis revealed some separation of resistant and susceptible provenances based on the whole complement of terpenes (Fig. 8); 49% of the variation is accounted for by scores for PC1 and 12 % by PC2 (Table 9). Susceptible trees are distributed along the whole PC1 axis, while resistant trees are clustered towards the lower end of the axis. No distinct clusters occurred that might indicate distinct provenances (Fig. 8). Along PC1, all of the variables have a positive coefficient (Table 9) indicating a trend for the magnitude of that variable to increase along that axis, reflecting the trends observed in Table 8. Isoamyl and isopentenyl isovalerate, terpinolenes, β-pinene,and β-phellandrene/limonene are the most important

Figure 7. Typical gas chromatographic trace of volatile terpenes extracted from Sitka spruce foliage.

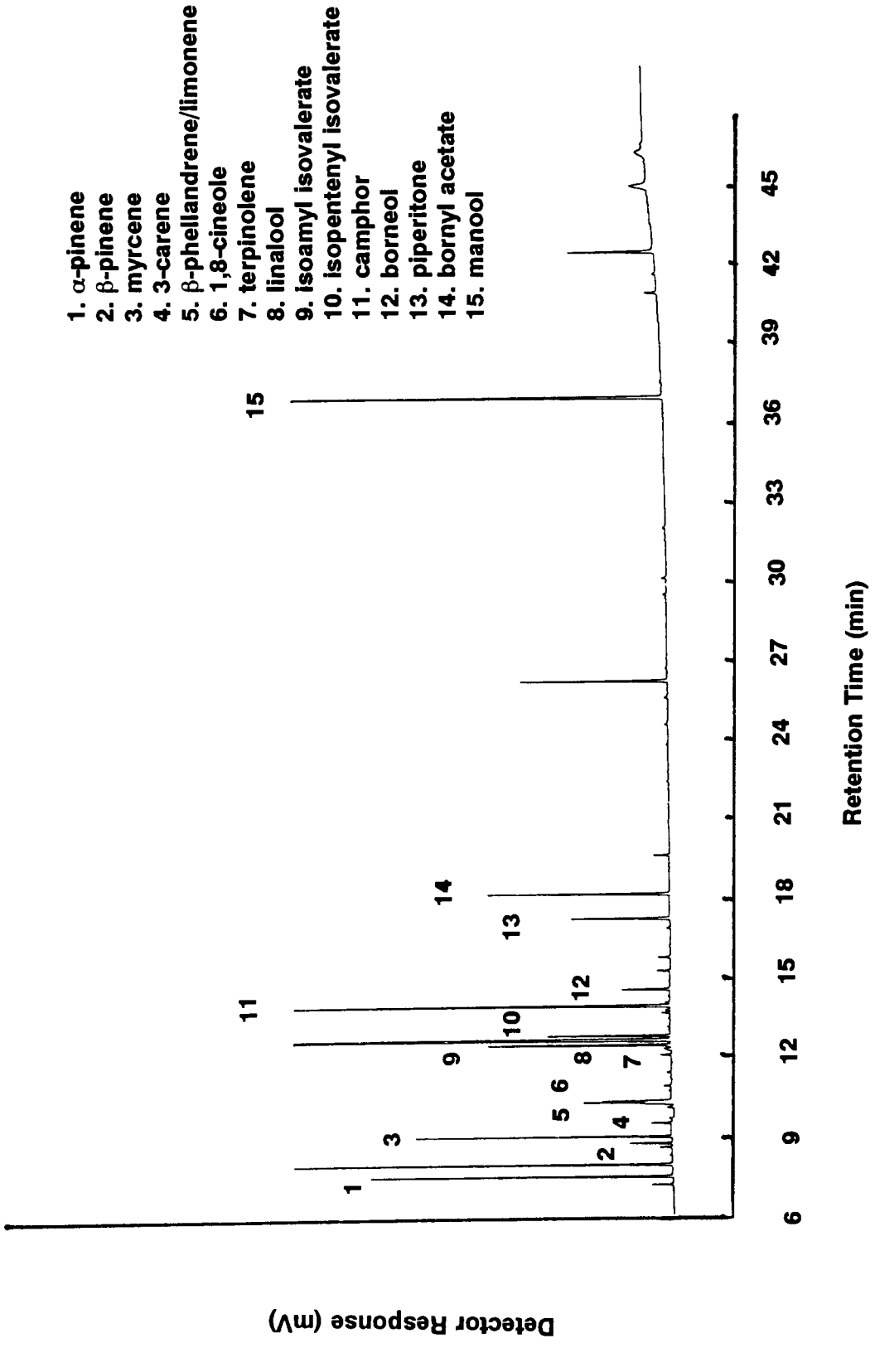

1. α-pinene
2. β-pinene
3. myrcene
4. 3-carene
5. β-phellandrene/limonene
6. 1,8-cineole
7. terpinolene
8. linalool
9. isoamyl isovalerate
10. isopentenyl isovalerate
11. camphor
12. borneol
13. piperitone
14. bornyl acetate
15. manool
Detector Response (mV)
Retention Time (min)
6
9
12
15
18
21
24
27
30
33
36
39
42
45

Table 8. Amounts of individual volatile foliar terpenes, and relative retention times (RRT) for all resistant and susceptible trees by provenance. Analyses were performed on square root transforms. In all cases df=147

Terpene	RRT	Amount ($\bar{x}$ mg/g dry wt ± S.E.)		t-test *P*-value
		Resistant provenances	Susceptible provenances	resistant *vs* susceptible
α-Pinene	0.587	0.23±0.11	0.44±0.04	0.0068
β-Pinene	0.687	0.26±0.14	0.52±0.05	0.0005
Myrcene	0.707	1.39±1.10	3.86±0.39	0.0002
3-Carene	0.762	0.06±0.04	0.02±0.01	0.7742
β-Phellandrene/ limonene	0.809	1.30±0.69	2.53±0.25	0.0001
1,8-Cineole	0.814	0.20±0.14	0.34±0.05	0.1038
Terpinolene	0.954	0.01±0.02	0.07±0.01	0.0033
Linalool	0.968	0.00±0.04	0.07±0.01	0.6443
Isoamyl isovalerate	0.983	0.26±0.24	0.85±0.09	0.0001
Isopentenyl isovalerate	1.012	0.09±0.09	0.32±0.03	0.0001
Camphor	1.11	0.35±0.18	0.37±0.06	0.9114
Borneol	1.15	0.01±0.06	0.16±0.02	0.0016
Piperitone	1.36	0.54±0.31	1.16±0.11	0.0002
Bornyl acetate	1.438	0.12±0.06	0.05±0.02	0.1771
Manool	2.924	5.97±10.78	12.79±3.84	0.0001

Figure 8. Principal components analysis of provenances based on volatile foliar terpenes. Plot of first and second principal components (eigenvectors), accounts for 61% of the variation. Resistant trees are solid circles and susceptible trees are open circles. This analysis was performed on the square root transforms, N=149.

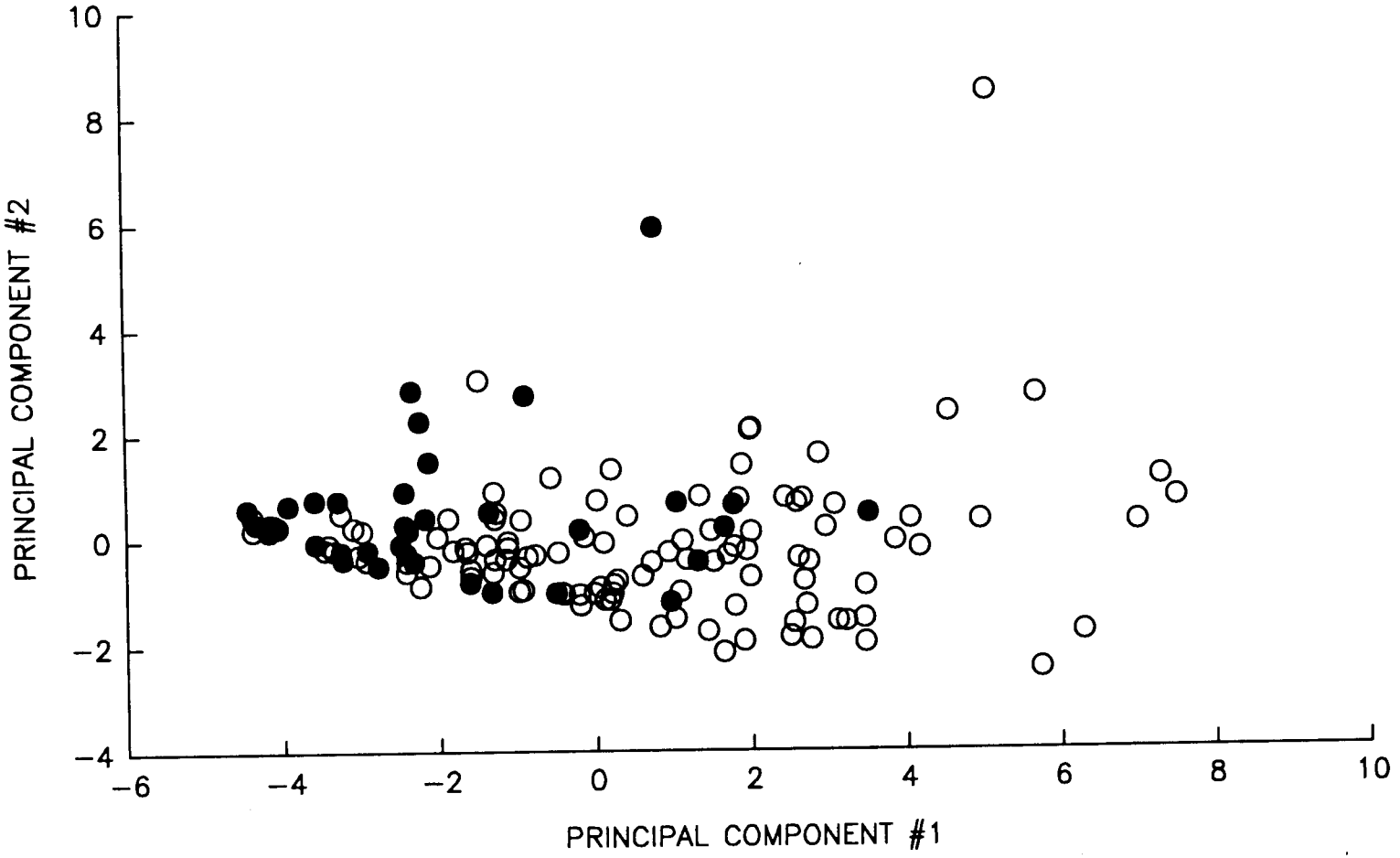

PRINCIPAL COMPONENT #2
10
8
6
4
2
0
−2
−4
−6
−4
−2
0
2
4
6
8
10
PRINCIPAL COMPONENT #1

Table 9. Principal components scores for analysis of volatile foliar terpenes by treatment (resistant or susceptible). Data were log-transformed for statistical analysis.

	Principal components scores	
Terpene	Prin1	Prin2
α-Pinene	0.29	0.32
β-Pinene	0.30	-0.07
Myrcene	0.31	-0.21
3-Carene	0.09	0.04
β-Phellandrene/limonene	0.31	-0.16
1,8-Cineole	0.26	-0.01
Terpinolenes	0.29	0.06
Linalool	0.11	-0.03
Isoamyl isovalerate	0.32	-0.13
Isopentenyl isovalerate	0.31	-0.05
Camphor	0.19	0.50
Borneol	0.19	0.29
Piperitone	0.32	-0.18
Bornyl acetate	0.07	0.62
Manool	0.25	-0.19

variables contributing to the spread along this axis based on the magnitude of their principal components scores (Table 9). There was some spread along the PC2 axis, but to a similar extent by both resistant and susceptible trees (Fig. 8). Increasing magnitude of PC2 scores is correlated with higher amounts of α-pinene, myrcene, bornyl acetate and camphor, and lower amounts of myrcene and manool (Table 9). While some trends are evident, there may be too much variation within provenances to select resistant trees on this basis.

Analysis of Volatile Terpenes by Clone

The total amount of terpenes varied as much as 10-fold between different clones (Fig. 9). Only one Haney clone had trees with significantly greater foliar terpene content than susceptible trees (Dunnett's test, $P<0.05$). Trees in one Cedarvale clone, one Green Timbers clone, and both Kitwanga clones had lower terpene content than susceptible trees from Fair Harbour.

All individual terpenes varied significantly between clones (Table 10). Most varied in a similar pattern to the total amount of terpenes (Table 11) with some exceptions, particularly 3-carene, piperitone, and manool. Manova revealed a significant difference ($F=6.68$; df= 165,781; $P<0.0001$) between clones with respect to the total complement of terpenes. When the canonical discriminant analysis was performed the first eight canonical variates accounted for a significant amount of variation, but for simplicity, only Can 1 to Can 3 will be discussed. These account for 42%, 22% and 10% of the variation, respectively. A plot of the first three canonical

Figure 9. Amount of volatile foliar terpene in resistant and susceptible clones (mg/g dry weight) (F=11.31; df=11,98; P<0.0001). Analysis was performed on square root transforms. Asterisks indicate clones that are significantly different from the susceptible trees, Dunnett's test, P<0.05.

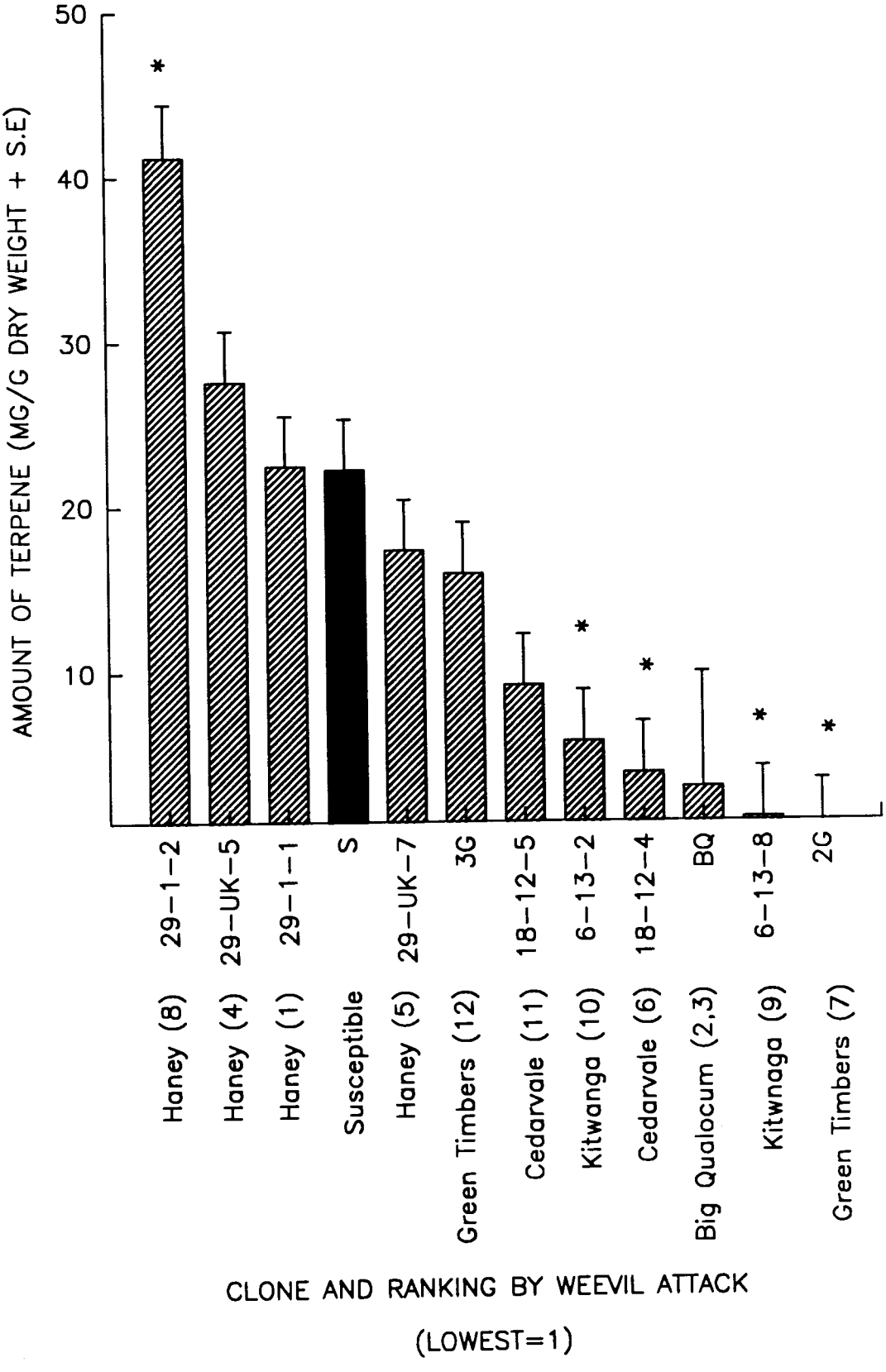

AMOUNT OF TERPENE (MG/G DRY WEIGHT + S.E)
50
40
30
20
10
*
*
*
*
*
Haney (8) 29-1-2
Haney (4) 29-UK-5
Haney (1) 29-1-1
Susceptible S
Haney (5) 29-UK-7
Green Timbers (12) 3G
Cedarvale (11) 18-12-5
Kitwanga (10) 6-13-2
Cedarvale (6) 18-12-4
Big Qualocum (2,3) BQ
Kitwnaga (9) 6-13-8
Green Timbers (7) 2G
CLONE AND RANKING BY WEEVIL ATTACK
(LOWEST=1)

Table 10. Statistics for analysis of volatile foliar terpenes by clone. Data were log-transformed for statistical analysis. df=11, 98.

				Total standardized canonical coefficients		
Terpene	F	P	Stepdisc rank[a]	Can1	Can2	Can3
α-Pinene	18.73	0.0001	5	-1.03	-0.91	0.33
β-Pinene	18.86	0.0001	11	0.69	0.19	0.43
Myrcene	30.48	0.0001	1	3.84	1.36	-0.53
3-Carene	6.68	0.0001	12	0.002	-0.24	-0.76
β-Phellandrene/ limonene	17.54	0.0001	3	-0.88	-0.53	1.03
1,8-Cineole	21.07	0.0001	10	0.66	-0.21	-0.05
Terpinolenes	13.62	0.0001	13	0.23	0.48	0.14
Linalool	4.99	0.0001	-	0.08	0.07	-0.09
Isoamyl isovalerate	16.58	0.0001	8	1.80	1.07	-1.37
Isopentenyl isovalerate	10.46	0.0001	6	-2.18	-0.97	0.17
Camphor	28.37	0.0001	2	0.31	-2.09	-0.13
Borneol	9.49	0.0001	4	0.60	0.84	0.37
Piperitone	14.55	0.0001	14	0.22	0.15	-0.19
Bornyl acetate	14.55	0.0001	9	-1.87	-0.16	1.07
Manool	19.94	0.0001	7	-0.65	0.24	0.31

[a]Refers to the order in which the stepwise discriminant analysis included variables in the model. A dash indicates variables eliminated from the model.

Table 11. Comparison of individual volatile foliar terpenes between clones ranked left to right in the same order as in Fig. 9. Symbols indicate, for each clone, whether the amount of terpene is significantly greater (+) or smaller (-) than in the susceptible trees (S). No significant difference between a clone and susceptible trees is indicated by a space. Comparisons were made within rows by Dunnett's test, $P<0.05$.

	Significant difference (+ or -) from susceptible trees										
Terpene	29-1-2 Haney	29-UK-5 Haney	18-12-5 Cedarvale	29-1-1 Haney	3G Green Timbers	29-UK-7 Haney	6-13-2 Kitwanga	BQ Big Qualicum	18-12-4 Cedarvale	6-13-8 Kitwanga	2G Green Timbers
α-Pinene	+	+							-	-	-
β-Pinene	+			+						-	-
Myrcene	+		-				-		-	-	-
3-Carene	+										
β-Phellandrene/ Limonene								-	-	-	-
1,8-Cineole	+			+			-		-	-	-
Terpinolene	+			+					-	-	-
Linalool			-		-	-			-	-	-

Table 11 continued

Isoamyl isovalerate			–	–		–	–	–
Isopentenyl isovalerate			–			–	–	–
Camphor	+	+		–			–	–
Borneol				–	–	–	–	–
Piperitone								
Manool			–					

variates revealed that the four Haney clones were distinctly separated from all others (Fig. 10), primarily along Can 1. This is the most important trend as Can1 accounts for the greatest amount of variation. Based on Table 10, Haney clones should have higher amounts of myrcene and isoamyl isovalerate, and lower amounts of α-pinene, isopentenyl isovalerate and bornyl acetate than the other clones. There is no evidence, however, that the more susceptible clones separate from the more resistant ones.

The stepwise discriminant analysis ranked myrcene, camphor, β-phellandrene/limonene, borneol, α-pinene, isopentenyl isovalerate, manool, bornyl acetate and cineol as the 10 most important variables to separate clones (Table 10). This analysis eliminated only linalool from the model, indicating that separation between groups is best achieved by a combination of 14 different terpenes.

Diterpene Resin Acids

Figure 11 shows the typical separation of cortical resin acids in Sitka spruce. On average, when compared with susceptible provenances, trees from resistant provenances had significantly higher amounts of pimaric, isopimaric, levopimaric, dehydroabietic, abietic, and neoabietic acid, but not palustric acid (Fig. 12). The total amount of resin acid was also significantly higher in trees from resistant (14.0 ± 2.49 mg per g) than susceptible provenances (6.99 ±0.86 mg per g) (t=3.57; df=29.8; P=0.0012). The proportion of resin acid in the total extractable resin was also higher in resistant (0.74±0.04) than susceptible trees (0.65±0.01) (t=2.28; df=129 P=0.0243). The total amount of resin acid increased significantly as the cross-sectional area of the

Figure 10. Canonical discriminant analysis of clones from Fair Harbour based on volatile foliar terpenes ($P<0.0001$). Plot of the first three canonical variates accounts for 74% of the variation. N=111. Centroids for each clone are plotted. Different clones are represented by the following symbols:

18-12-4  (Cedarvale), 18-12-5  (Cedarvale),

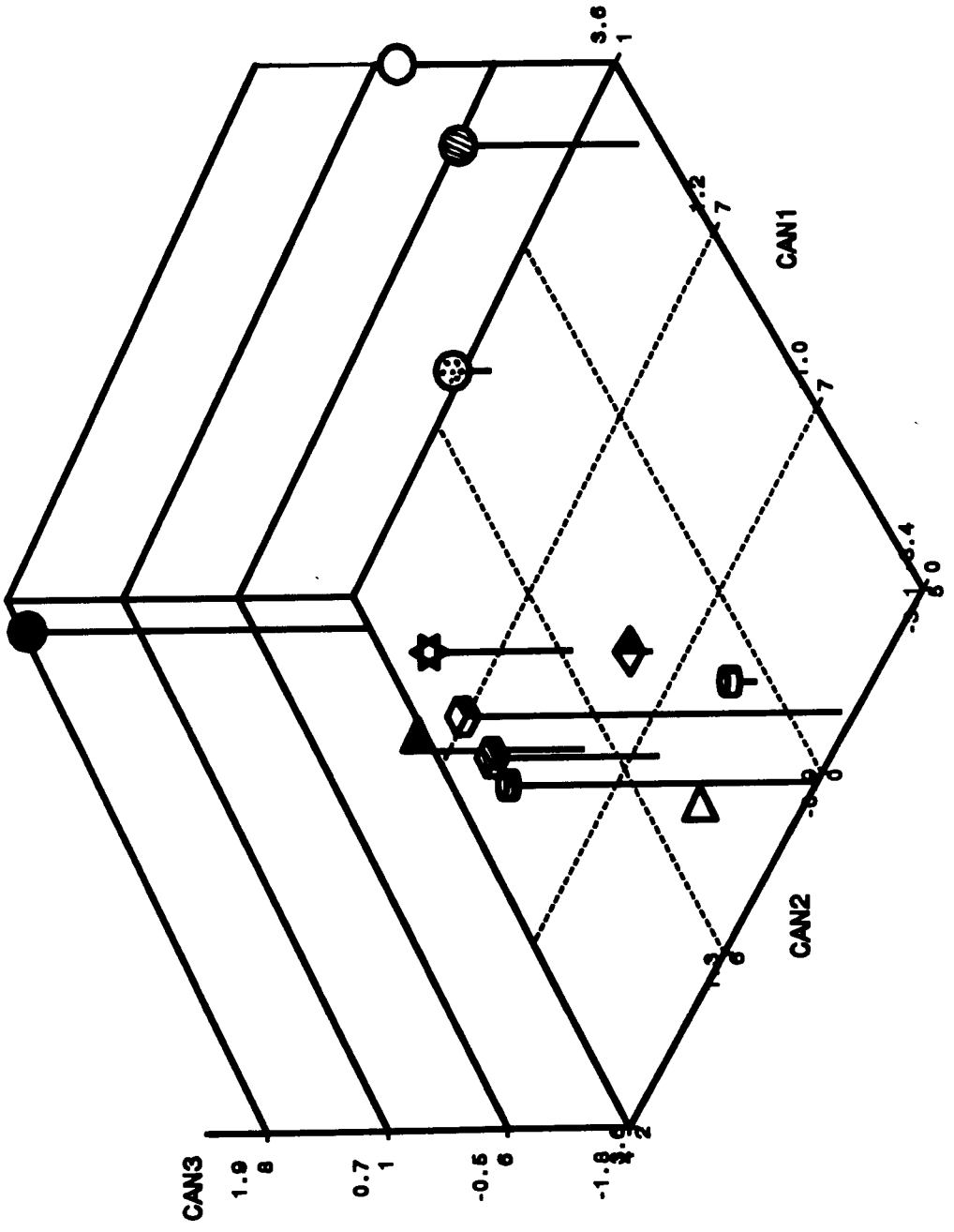
6-13-2  (Kitwanga), 6-13-8  (Kitwanga),

2G  (Green Timbers), 3G  (Green Timbers),

29-1-1  (Haney), 29-1-2  (Haney),

29-UK-5  (Haney), 29-UK-7  (Haney),

3-2-6 and 3-15-2  (Big Qualicum), Susceptible 



70

Figure 11. Typical gas chromatographic trace of cortical diterpene resin acids extracted from Sitka spruce bark.

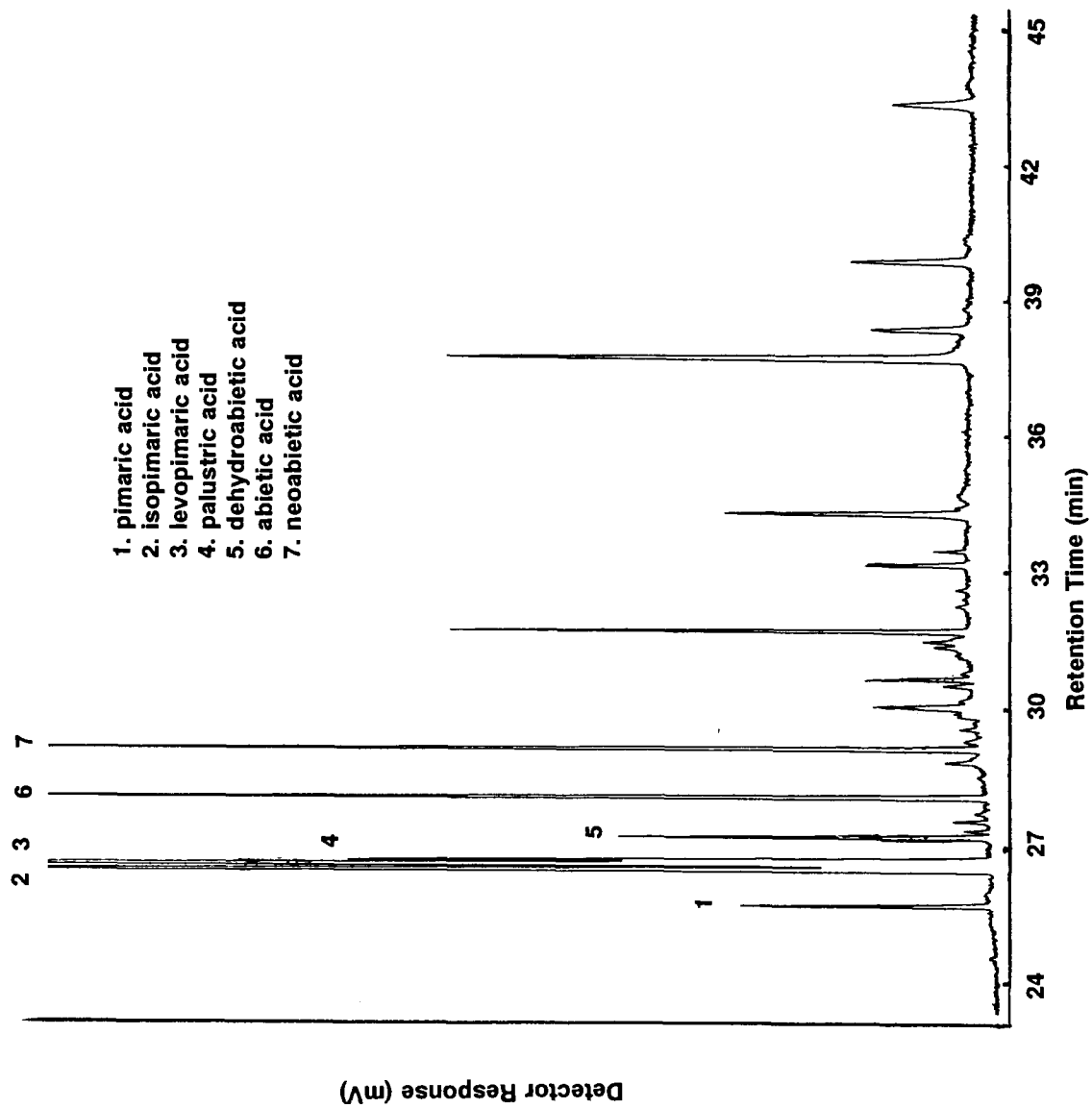
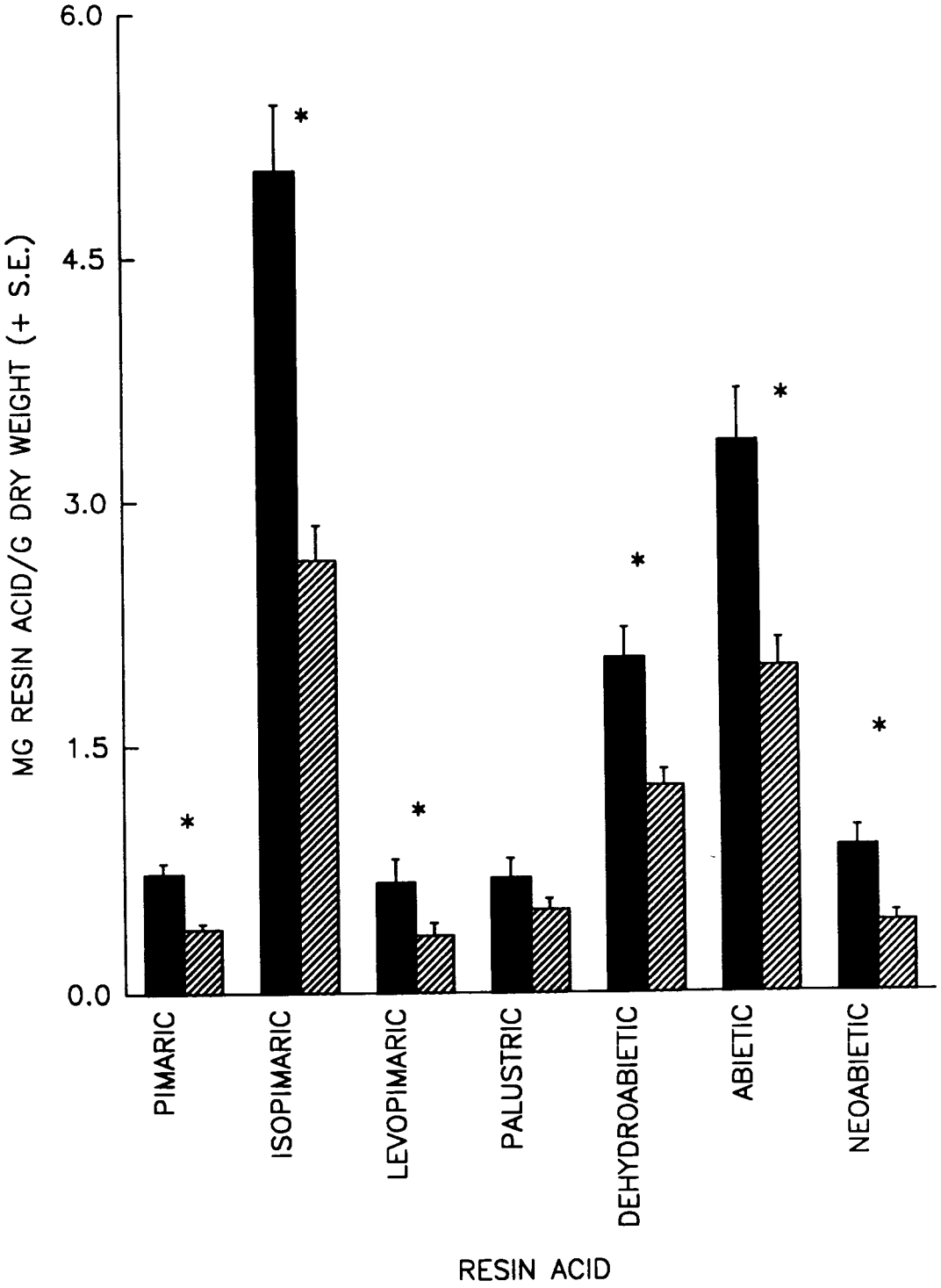

1. pimaric acid
2. isopimaric acid
3. levopimaric acid
4. palustric acid
5. dehydroabietic acid
6. abietic acid
7. neoabietic acid
2 3 6 7
4
5
1
24
27
30
33
36
39
42
45
Retention Time (min)
Detector Response (mV)

Figure 12. Amounts of individual cortical diterpene resin acids for the average of all resistant and susceptible trees by provenance (mg/g dry weight). Resistant clones are indicated by the hatched bars and the susceptible trees by the solid bars. Analyses were performed on square root transforms. In all cases df=135. Asterisks indicate significant differences between paired bars, t-test, $P<0.05$.

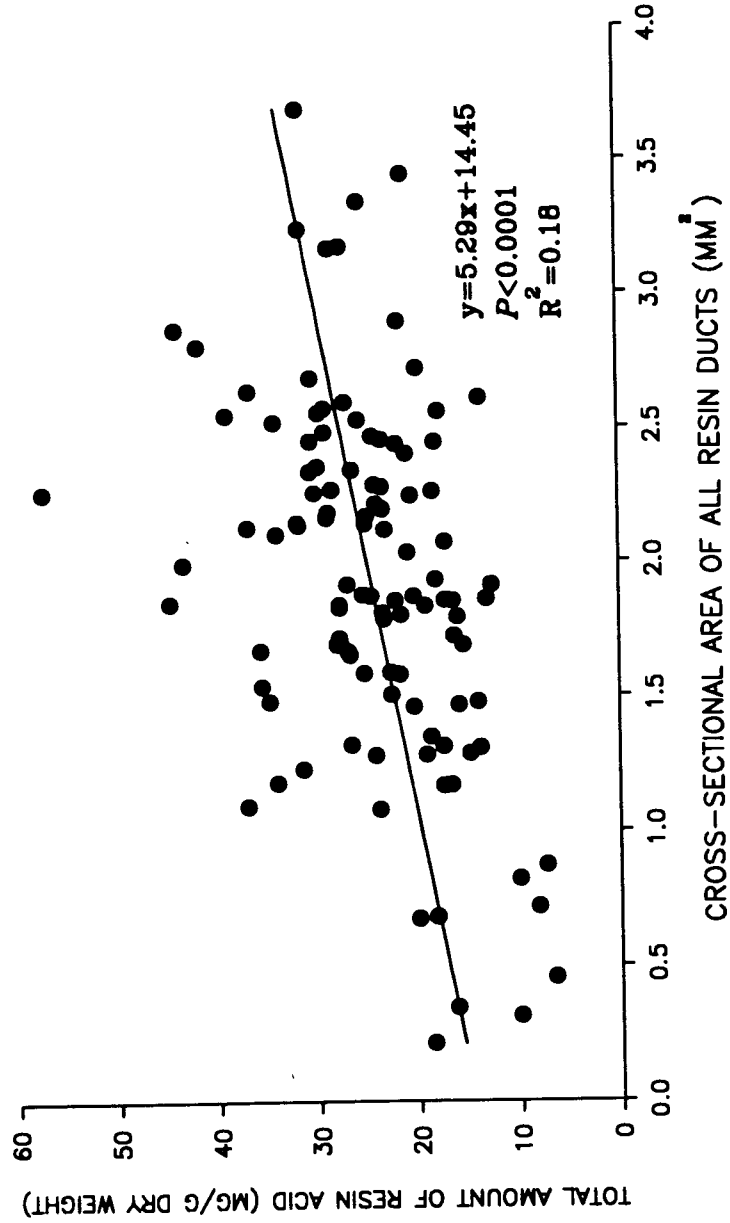

6.0
4.5
3.0
1.5
0.0
MG RESIN ACID/G DRY WEIGHT (+ S.E.)
*
PIMARIC
ISOPIMARIC
LEVOPIMARIC
PALUSTRIC
DEHYDROABIETIC
ABIETIC
NEOABIETIC
RESIN ACID

resin ducts increased (Fig. 13). Only 18% of the variation was explained by the cross-sectional area of resin ducts. Principal components analysis (Table 12) did not reveal any clustering of resistant and susceptible provenances (Fig. 14), suggesting no qualitative differences; 38% of the variation was accounted for by PC1 and 16 % by PC2.

There were significant differences in the total amount of resin acid among clones of resistant genotypes (F=3.82; df=11,109, P=0.001). Five clones, including two from the Kitwanga provenance, and one each from Haney, Green Timbers and Cedarvale, had significantly greater resin acid content than the susceptible trees (Fig. 15). Although none of the other clones had a resin acid content different from that in susceptible trees, the content in the Big Qualicum clone was ≤ 50% of that in the other resistant clones. All of the resin acids except dehydroabietic acid were present in significantly greater amounts in one or more resistant clones than in susceptible trees (Table 13). Isopimaric, abietic and neoabietic were all significantly higher in four different clones. The two highest ranking clones (6-13-2 and 29-UK-7) based on total resin acid content (Fig. 15), were again ranked the highest in terms of number of individual resin acids, each with four resin acids in higher amounts than in susceptible trees. There were differences, however, in the resin acids represented, and only two, levopimaric and neoabietic acid, were in common in the two clones (Table 13).

MANOVA revealed significant differences (F=6.99; df=77,559; P<0.0001) between clones with respect to the full complement of resin acids; 94% of the

Figure 13. Relationship between total amount of cortical diterpene resin acid and cross-sectional area of resin ducts for trees sampled at the provenance trials.


TOTAL AMOUNT OF RESIN ACID (MG/G DRY WEIGHT)
CROSS-SECTIONAL AREA OF ALL RESIN DUCTS (MM²)
y=5.29x+14.45
P<0.0001
R²=0.18
0
10
20
30
40
50
60
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0

77

Table 12. Statistics for principal components analysis of cortical diterpene resin acids in provenances by treatment (resistant or susceptible). Data were log-transformed for statistical analysis. df=1, 115.

	Principal components scores	
Resin Acid	Prin1	Prin2
Pimaric	0.38	-0.37
Isopimaric	0.44	-0.13
Levopimaric	0.33	0.48
Palustric	0.39	0.37
Dehydroabietic	0.34	-0.48
Abietic	0.39	-0.24
Neoabietic	0.36	0.42

Figure 14. Principal components analysis of provenances based on cortical diterpene resin acids. Plot of first and second principal components (eigenvectors), accounts for 54% of the variation. Resistant trees are solid circles and susceptible trees are open circles. This analysis was performed on the square root transforms, N=149.

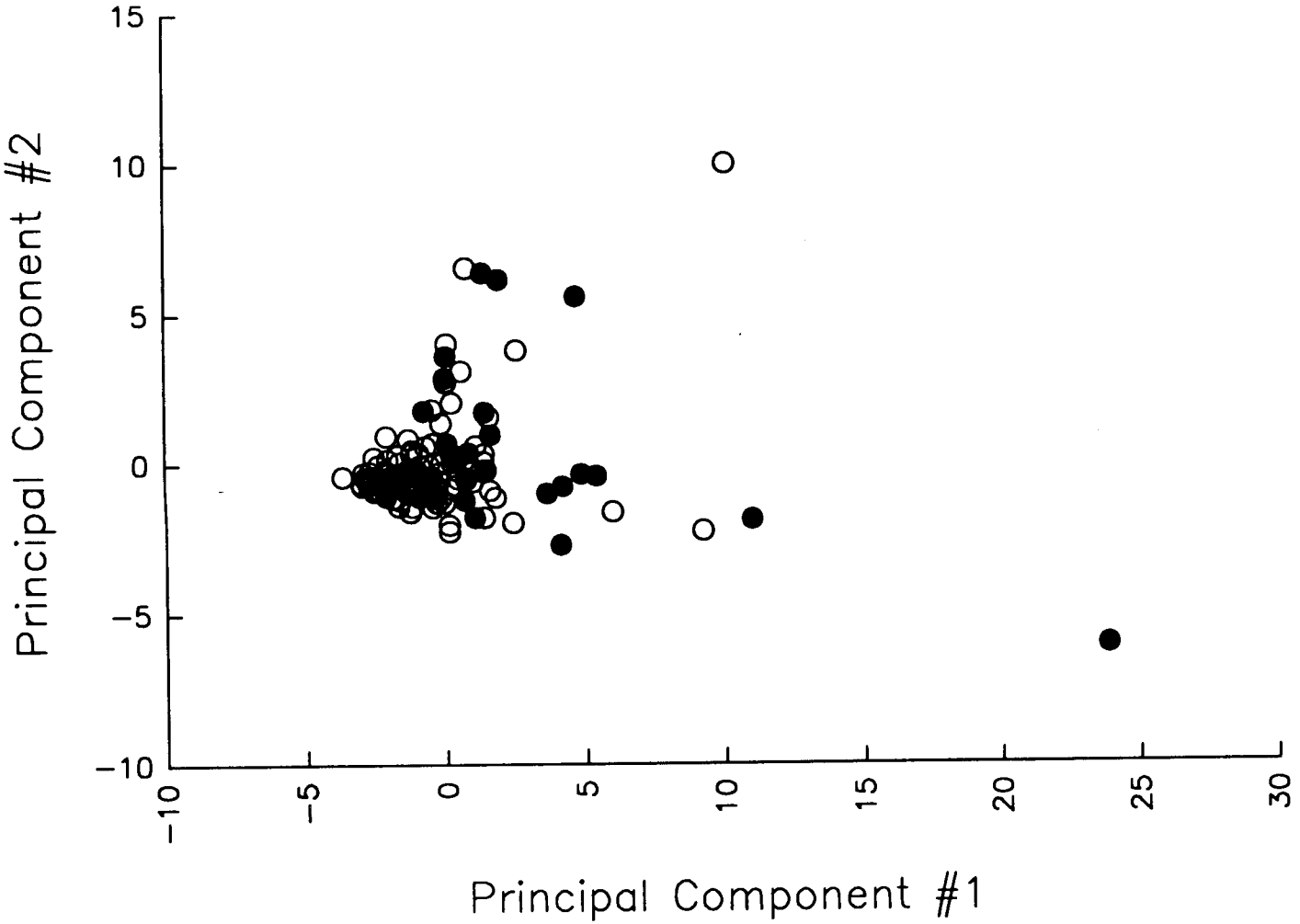

Principal Component #2
15
10
5
0
−5
−10
−10
−5
0
5
10
15
20
25
30
Principal Component #1

Figure 15. Ranked total amounts of cortical diterpene resin acid in resistant clones and susceptible trees (black bar) (F=4.12; df=11,98; P<0.0001). Asterisks indicate clones that are significantly different from the susceptible trees, Dunnett's test, P<0.05.

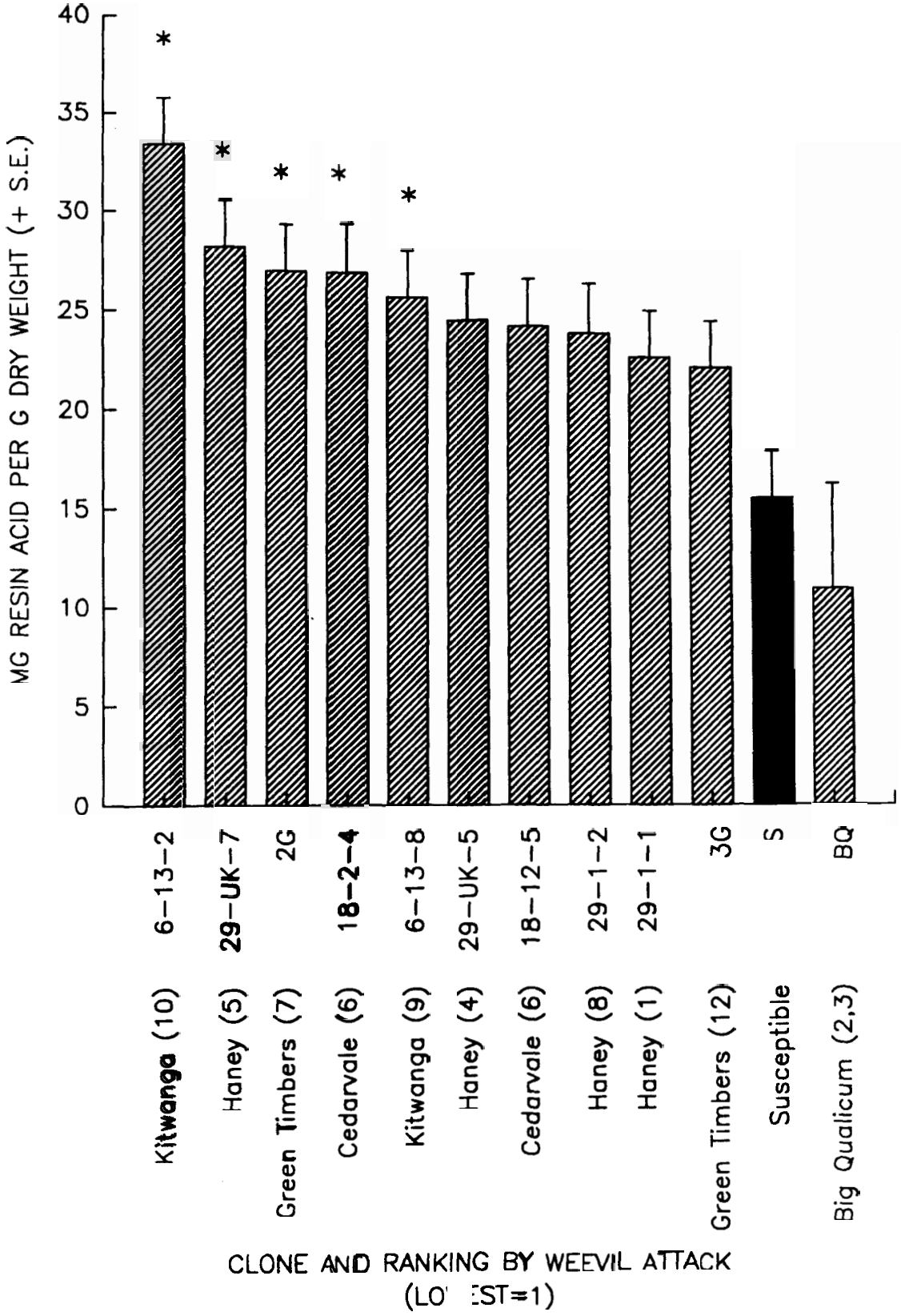

MG RESIN ACID PER G DRY WEIGHT (+ S.E.)
0
5
10
15
20
25
30
35
40
*
*
*
*
*
Kitwanga (10)
Haney (5)
Green Timbers (7)
Cedarvale (6)
Kitwanga (9)
Haney (4)
Cedarvale (6)
Haney (8)
Haney (1)
Green Timbers (12)
Susceptible
Big Qualicum (2,3)
6-13-2
29-UK-7
2G
18-2-4
6-13-8
29-UK-5
18-12-5
29-1-2
29-1-1
3G
S
BQ
CLONE AND RANKING BY WEEVIL ATTACK
(LOWEST=1)

Table 13. Comparison of individual cortical diterpene resin acids among clones. Symbols indicate, for each clone, whether the amount of resin acid is significantly greater (+), Dunnett's test, $P<0.05$, than in the susceptible trees. In no case was there significantly less of any resin acid in resistant clones than in susceptible trees. Blank spaces indicate no significant difference between a clone and susceptible trees.

	Significant difference (+) from susceptible trees										
Resin acid	6-13-2 Kitwanga	29-UK-7 Haney	2G Green Timbers	18-12-4 Cedarvale	6-13-8 Kitwanga	29-UK-5 Haney	18-12-5 Cedarvale	29-1-2 Haney	29-1-1 Haney	3G Green Timbers	BQ Big Qualicum
Pimaric		+	+	+							
Isopimaric	+			+	+		+				
Levopimaric	+	+									
Palustric		+				+					
Dehydroabietic											
Abietic	+		+	+	+						
Neoabietic	+	+	+					+			

variation was accounted for by the first five canonical variates, but only the first three were considered for simplicity. Can 1 to Can 3 represent 51, 26 and 13% of the variation respectively. A plot of the first three canonical variates (Fig. 16) revealed considerable separation between clones. In particular, 6-13-8 (Kitwanga) separates primarily on Can 1, 6-13-2 (Kitwanga) separates on Can 1 and Can 2, and 2G (Green Timbers) and 18-12-4 (Cedarvale) separate primarily on Can 3. The rest of the clones spread out primarily on Can 2. Separation along Can 1 is primarily associated with decreasing amounts of pimaric acid, and increasing amounts of isopimaric acid, palustric acid, and abietic acid (Table 14). Separation along Can 2 is characterized by decreasing amounts of isopimaric acid, palustric acid and abietic acid, and increasing amounts of neoabietic acid. Separation along Can 3 is characterized primarily by decreasing amounts of pimaric and isopimaric acid, and increasing amounts of dehydroabietic acid and levopimaric acid. (Table 14). Thus 6-13-8 (Kitwanga) would tend to have proportionately low amounts of pimaric acid and high amounts of isopimaric, palustric and abietic acids, 6-13-2 (Kitwanga) would share the above characteristics with 6-13-8, but would have lower amounts of isopimaric, palustric and abietic acids and very high amounts of neoabietic acid, and 2G (Green Timbers) and 18-12-4 (Cedarvale) would have proportionately low amounts of dehydroabietic and levopimaric acids and high amounts of pimaric acid. Stepwise discriminant analysis suggests isopimaric, pimaric, neoabietic, palustric and levopimaric acid are the five most important variables (in that order) to separate the clones. No variables were eliminated by the model using stepwise analysis, indicating that the differences

Figure 16. Canonical discriminant analysis of clones from Fair Harbour based on cortical diterpene resin acids ($P<0.0001$). Plot of the first three canonical variates accounts for 90% of the variation. N=109. Centroids for each clone are plotted. Different clones are represented by the following symbols:

18-12-4  (Cedarvale), 18-12-5  (Cedarvale),

6-13-2  (Kitwanga), 6-13-8  (Kitwanga),

2G  (Green Timbers), 3G  (Green Timbers),

29-1-1  (Haney), 29-1-2  (Haney),

29-UK-5  (Haney), 29-UK-7  (Haney),

3-2-6 and 3-15-2  (Big Qualicum), Susceptible 

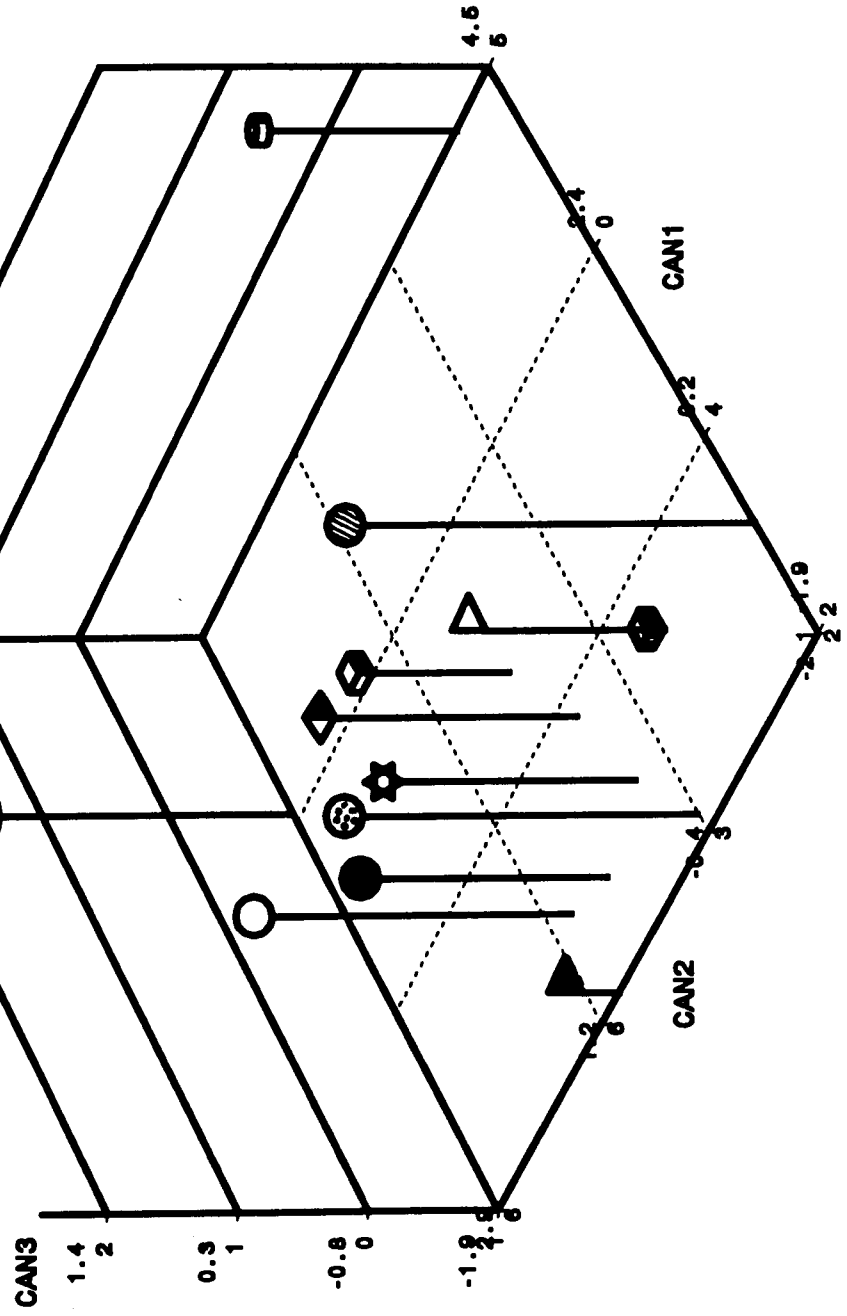


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Table 14. Statistics for canonical discriminant analysis of cortical diterpene resin acids by clone. df=11, 109.

				Total standardized canonical score		
			STEPDISC			
Resin Acid	F	*P*	Rank[a]	Can1	Can2	Can3
Pimaric	4.45	0.0001	2	-2.93	0.42	-1.66
Isopimaric	6.71	0.0001	1	1.94	-0.80	-0.61
Levopimaric	4.55	0.0001	5	0.18	-0.44	1.70
Palustric	2.51	0.0080	4	1.10	-1.76	0.24
Dehydroabietic	1.24	0.2741	6	0.06	0.57	0.93
Abietic	4.25	0.0001	7	0.82	-0.94	0.10
Neoabietic	6.58	0.0001	3	-0.32	2.92	-0.07

[a] Refers to the order in which the stepwise discriminant analysis included variables into the model

between clones with respect to resin acids are not simple. Comparing the pattern of resin acid variation with the weevil attack data (Table 2) there was no clear pattern associated with resistance or susceptibility.

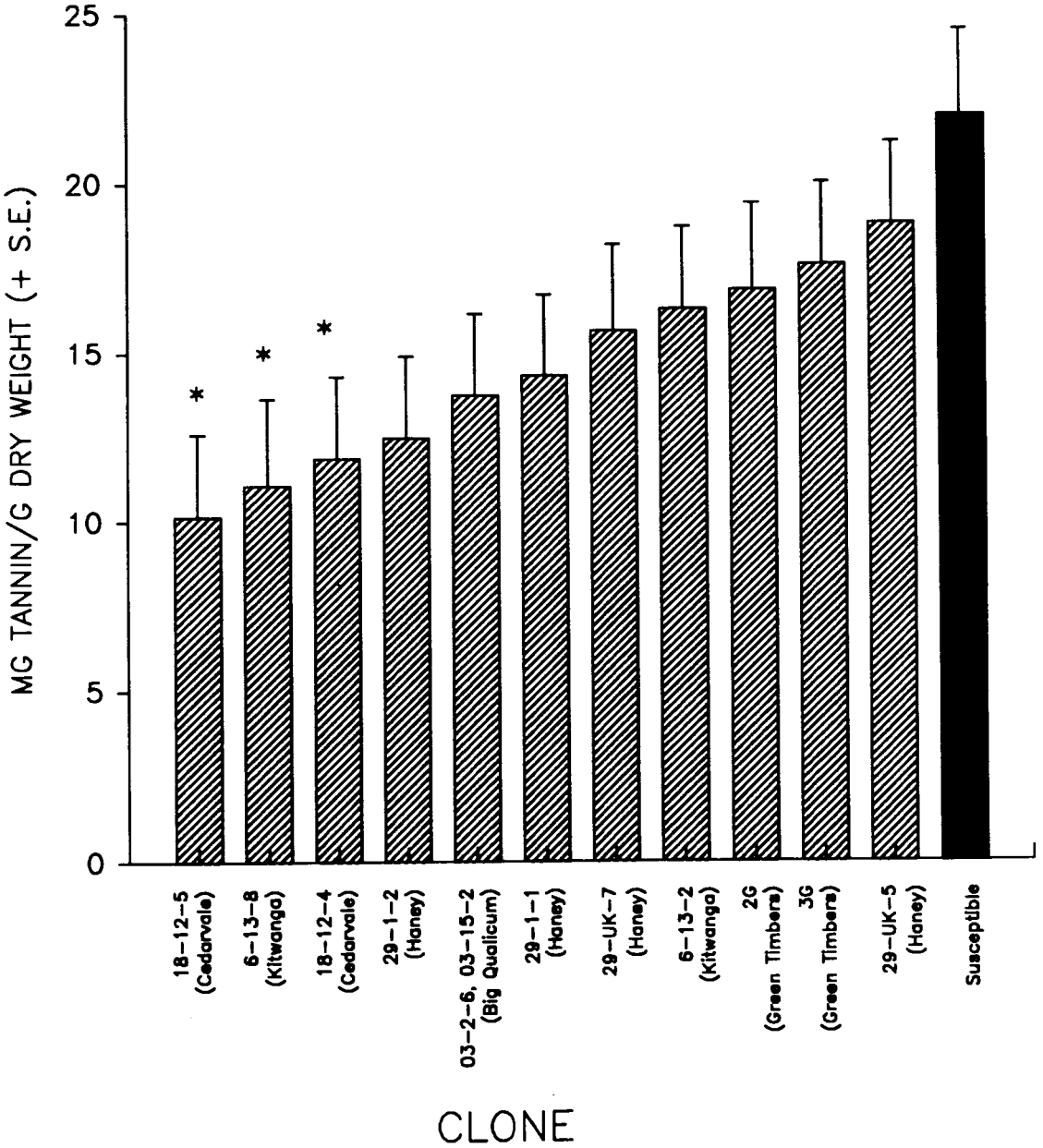
Analysis of CondensedTannins by Clone

The amount of condensed tannin differed significantly between clones at Fair Harbour (F=1.97; df=11,98; P=0.0389) (Fig 17), with the susceptible trees having the greatest amount. Three clones, 18-12-4, 18-12-5 (Cedarvale), and 6-13-8 (Kitwanga) had significantly lower amounts of condensed tannins than the susceptible trees.

DISCUSSION

Based on analysis of both provenances and clones, it is clear that Sitka spruce trees vary in both the quantity and quality of foliar terpenes. In the analysis of foliar terpenes by provenance (Table 8), resistant provenances, on average, had a lower terpene content than susceptible ones. Analysis by clone, however, revealed that one resistant clone (Haney, 29-1-2) had significantly more total foliar terpene content and several had significantly less content than the susceptible trees (Fig. 9). Trees with low amounts of foliar terpenes probably contained significantly fewer foliar resin ducts (White & Nilsson 1984). Trees with extremely high amounts of foliar terpenes (>40 mg per g dry weight) may be repellent to weevils, and may have a higher capacity for resinosis if the amount of foliar terpenes is correlated with the amount of cortical terpenes. Trees with very low amounts of foliar terpenes (<10 mg per g dry weight)

Figure 17. Ranked amounts of total condensed tannin in resistant clones and susceptible trees (black bar) (F=1.97; df:11,98; $P<0.0389$). Asterisks indicate clones that are significantly different from the susceptible trees, Dunnett's test, $P<0.05$.


MG TANNIN/G DRY WEIGHT (+ S.E.)
25
20
15
10
5
0
*
*
*
18-12-5 (Cedarvale)
6-13-8 (Kitwanga)
18-12-4 (Cedarvale)
29-1-2 (Haney)
03-2-6, 03-15-2 (Big Qualicum)
29-1-1 (Haney)
29-UK-7 (Haney)
6-13-2 (Kitwanga)
2G (Green Timbers)
3G (Green Timbers)
29-UK-5 (Haney)
Susceptible
CLONE

may lack apparency (Feeny 1975), and may not be easily located by weevils, if orientation to a 'spruce odour' is important in host selection. Sitka spruce generally grows in mixed stands, and the odour of low terpene-producing spruces may be masked by that of other conifers such as cedar and hemlock. Such a situation fulfills Fox's (1981) criteria for circumstances in which a long-lived plant may lack apparency. Based on the distribution of points in the principal components analysis of many different provenances, it would appear that it is most common for resistant trees to have the same amount or less foliar terpene content than susceptible trees, and uncommon for resistant trees to have high foliar terpene content.

The diterpene resin acid composition of clones varied significantly. The overall content, and to some extent the content of individual resin acids could be directly related to resistance or susceptibility of Sitka spruce to *P. strobi* (Fig. 15, Table 13), supporting the results obtained for provenances (Fig. 12). The high cortical resin acid content in resistant provenances (Fig. 12) and some resistant clones (Fig. 15) supports Hanover's (1975b) observation that eastern white pine had significantly less total resin acid than western white pine, a non-host for *P. strobi*. In addition, Smith (1975) related increased quantity of cortical resin with resistance of Ponderosa pine to the western pine beetle, *Dendroctonous brevicomis* LeConte. Large amounts of extractable resin acid may reflect greater resin-carrying capacity, resulting in a copious flow of primary resin, that may deter weevils from feeding or laying eggs. On average, eastern white pine (Stroh & Gerhold 1965) and Sitka spruce (Fig. 5, Table 7) that are resistant to *P. strobi* have more or larger cortical resin ducts than

susceptible trees. This resin flow hypothesis is supported in part by the relationship between total resin acids and cross-sectional area of resin ducts (Fig. 13); however, the low correlation (0.18) indicates that the volume of resin explains only a small amount of the variation observed. This agrees with my findings (Fig. 2) that only two resistant provenances of Sitka spruce had significantly more outer resin ducts than all other provenances. In addition, Moore & Hanover (1987) found that while there was a significant correlation between resin canal volume and extractable terpenes in needles, this correlation was weak in the bark. Large amounts of extractable resin acid are also due in part to trees having a high proportion of resin acid to terpene. Resistant provenances have resin containing proportionately more resin acid than susceptible ones, which may explain some of the variation in Fig. 13. It is noteworthy that lack of apparency may be achieved by low levels of volatile foliar terpenes in some resistant genotypes (Fig. 9), but levels of non-volatile cortical resin acids were never significantly lower in resistant than in susceptible trees. Sitka spruce genotypes having intermediate levels of foliar terpene content (Fig. 9) may rely on other types of resistance mechanisms such as high numbers of cortical resin ducts, dense spacing of cortical resin ducts (Stroh & Gerhold 1965), chemical-based feeding and oviposition deterrency (Alfaro *et al.* 1980; Section V), repression of reproductive maturation (Sahota *et al.* 1994), and the induced production of traumatic resin canals (Alfaro 1995).

Although the total amount of condensed tannin in the cortex varied significantly in Sitka spruce genotypes, only three clones had significantly less

condensed tannin than the susceptible trees (Fig. 17). The cost of producing tannins is about 120x (or more) in terms of metabolic energy than other types of secondary metabolites based on the energy cost per carbon atom, and the effective deterrent concentration required (Swain 1979). Variation in tannin content would thus be expected to serve some purpose. Tannins are most likely involved in defense against pathogens (Swain 1979), and could be involved in resistance or susceptibility of Sitka spruce to fungal pathogens. In addition, there may be multiple adaptive functions of secondary metabolites, including primary functions (Seigler & Price 1976). Thus tannins may be involved as intermediaries or regulators in a primary process, or in the storage of carbon (Seigler & Price 1976).

The data in Table 10 and Fig. 10 suggest that the volatile terpene complement of Sitka spruce could be useful for chemosystematic studies and identification of specific genotypes. However, such an analysis probably needs to be done at the clone, or individual level rather than the provenance or family level. A case in point is the large difference between clones and families in the Haney provenance (Fig. 10). So many variables are statistically important in distinguishing between genotypes (Table 10), that no single terpene is likely to be reliable in distinguishing between resistant and susceptible chemotypes. In addition, it is probable that there are several resistant chemotypes. Certain terpenes may infer resistance in one chemotype but not in another. This is reflected by the contradictions that exist between my provenance and clone results, and with the literature. Based on our analysis by provenance, resistant trees had lower amounts of most terpenes than did susceptible ones (Table 8).

However, the foliage of eastern white pine had more α-pinene and camphene and less β-pinene than the foliage of western white pine (Hanover 1975b), but unattacked eastern white pines contained more α-pinene than heavily attacked ones (Wilkinson 1980). Susceptible Sitka spruce leaders contained less myrcene than resistant ones (Hrutfiord & Gara 1989), but we observed the opposite trend in our analysis by provenance (Table 8). In a qualitative analysis, lower amounts of isoamyl and isopentenyl isovalerate were found consistently in foliage of resistant Sitka spruce when compared to susceptible provenances (Brooks *et al.* 1987a), which I confirmed in a quantitative assay (Table 8). In my analysis of genotypes, however, we found that the change in ratio of one isovalerate to the other may be most important in distinguishing between clones (Table 10). Figure 9 and Table 11 show that resistant trees can have terpene levels significantly higher or lower than susceptible trees, and Figure 10 shows that qualitatively, clones differ from each other in at least three dimensions. Thus a given group of resistant and susceptible trees may be distinguished from each other in a quite different way from another group of resistant and susceptible trees.

Clear differences in diterpene resin acid composition between clones revealed by canonical discriminant analysis (Fig. 16) may also be useful for chemotaxonomic purposes. The pronounced separation of the two Kitwanga clones and one clone from each of the Green Timbers and Cedarvale provenances may be particularly noteworthy in this regard. Kitwanga and Cedarvale trees probably contain white spruce genes resulting from natural hybridization of white and Sitka spruce in their transition zone

habitat (Ying 1991), and the Green Timbers trees actually originate from the Queen Charlotte Islands (Alfaro 1982) and would have been reproductively isolated from the mainland genotypes. As with foliar terpenes, analysis of cortical resin acids by provenance or family may be too variable to be useful for this type of taxonomic separation.

While there appears to be no clear relationship between volatile terpene profile and resistance or susceptibility, it should be noted that the resistant clones examined did not differ greatly in their degree of resistance (Table 3) (Ying 1991), and as discussed above, there may be a number of different resistant chemotypes. This may also be true in the case of cortical resin acids. The four most important variables (isopimaric, pimaric, neoabietic and palustric acid) selected by stepwise discriminant analysis as important in discrimination between clones (Table 14) only partially matched the resin acids that differed significantly based on univariate statistics between resistant clones and susceptible trees (Table 13). This supports the hypothesis that among resistant clones, different suites of resistance traits impart resistance. These four resin acids, however, were found by Hanover (1975b) to differ significantly between eastern and western white pine, respectively a host and non-host of *P. strobi.* In addition, the clones separated in a completely different way based on cortical resin acids than by volatile foliar terpenes, suggesting that chemotyping may be both tissue and/or chemical specific, further supporting the hypothesis that not all clones rely on the same resistance mechanism.

It has not been determined if the viscosity or rate of crystallization of resin

varies between Sitka spruce genotypes. It is possible that slowly crystallizing resi may be an important resistance mechanism in some clones, but not others, and that this property may be reflected by the complement of resin acids. Wilkinson (1979) was able to correlate several resin acids, both positively and negatively, with the rate of crystallization of resin in eastern white pine.

There is some evidence to suggest that the behaviour of *P. strobi* is affected by volatile terpenes. α-Pinene, along with β-pinene and myrcene synergized the positive effects of non-volatile feeding stimulants in an agar-disc feeding bioassay (Alfaro *et al.* 1980). In my analysis by provenance, I observed α-pinene to be positively associated with susceptibility (Table 8). α-Pinene was also the most volatile, and most repellent monoterpene to the fir engraver (Bordasch & Berryman 1977). It is possible that α-pinene, being so volatile is a primary host attractant for *P. strobi* in addition to other cues.

P. strobi behaviour may also be affected by diterpene resin acids, which may act as feeding deterrents to either adult or larval sawflies (Wagner *et al.* 1983, Björkman & Gref 1993), or as toxins to larvae or eggs. Larsson *et al.* (1986) correlated high resin acid content with slow development and high mortality of sawfly larvae, and Reid & Gates (1970) observed that direct contact with liquid resin greatly reduced the hatch of mountain pine beetle eggs.

I conclude that volatile foliar terpenes may be involved in resistance in a quantitative fashion, with resistant trees having extremely high or low amounts of foliar terpenes. The relationship between cortical resin acids and resistance or

susceptibility of Sitka spruce is both quantitative, with susceptible trees having significantly lower resin acid content than many resistant trees, and qualitative as clones differ with respect to which individual resin acids predominate. Both volatile foliar terpenes and cortical diterpene resin acids should be considered in developing a multicomponent resistance index, because in addition to varying quantitatively, their qualitative variation should be useful in chemotyping trees, regardless of whether they are involved directly or indirectly in resistance. In addition, the variation in resin acid composition may relate to the physical properties of the resin, which may be useful in clarifying other resistance mechanisms. Variation in cortical tannin content should not be incorporated into a multicomponent resistance index unless it is later correlated with some other desirable trait such as resistance to pathogenic fungi or other pests, or bark toughness.

V. CONSTITUTIVE DEFENSES: EFFECT ON ADULT BEHAVIOUR

INTRODUCTION

Mechanisms of resistance affecting host selection fall into the category of antixenosis (Kogan & Ortman 1978) or non-preference (Painter 1968). The process of host selection is characteristically assigned by researchers into distinct phases e.g. dispersal or orientation, landing, probing, feeding and oviposition (Thorsteinson 1960; Visser 1986). Thorsteinson (1960) suggests that foraging is probably random until the insect encounters the immediate vicinity of the host plant.

Host plant recognition and acceptance generally involves both physical and chemical factors (Thorsteinson 1960; Renwick 1983) which can be modified by various environmental factors (Renwick 1983). Physical factors may be visual or tactile, including variables such as colour, shape, presence of hairs or spines, surface texture and thigmotaxis (Renwick 1983). Chemical factors can affect the processes of orientation, feeding and oviposition. Volatile odours are generally involved in orientation of insects toward a food source (Visser 1986), and may function to inhibit locomotory movement and deter further dispersal (Thorsteinson 1960). Primary attraction of the bark weevil *Pissodes pini* L. by its host was demonstrated by Tunset *et al.* (1993) using window flight traps baited with freshly cut log billets of pine or spruce. The pales weevil, *Hylobius pales* (Herbst.) was attracted to the odour of gum turpentine of slash and longleaf pine, *Pinus elliotti* Engelm. and *Pinus palustris* Mill.,

and a synthetic mixture of monoterpenes similar in composition to turpentine (Siegfried 1987). Chemical stimuli for insects were defined by Dethier *et al.* (1960) in terms of the responses which they elicit. Repellents and attractants are defined as chemicals which cause insects to make oriented movements away from or towards the host. Feeding, mating, or oviposition stimulants or deterrents are defined as chemicals which elicit those behaviours or inhibit them when present in a place where these activities would normally occur.

The white pine weevil can be considered an oligophagous insect as it feeds on only a few species of spruces and pines. Oligophages are generally restricted to a few species within a family (bernays & Chapman 1994). An acceptable plant for an oligophagous insect is further defined by Hsiao (1969) as one that provides all of the positive chemical stimuli needed to fulfill sensory requirements of the insect while being devoid of negative chemical stimuli.

Host selection by *P. strobi* is hypothesized to involve visual cues (VanderSar & Borden 1977a) which probably operate at long range. Choice of oviposition sites is governed by positive phototaxis and negative geotaxis (VanderSar & Borden 1977b). Both of the above relate primarily to the vertical position and magnitude of the leaders of potential host trees. Host acceptance, however, is probably associated with thigmotaxis (Harris *et al.* 1990), as well as olfaction and contact chemoreception (Alfaro & Borden 1982; VanderSar & Borden 1977c). These may in turn be related to depth (Stroh & Gerhold 1965, Fig. 4) and number of cortical resin canals (Fig. 2).

VanderSar & Borden (1977c) compared preferences of weevils from coastal

British Columbia for freshly cut branches of hosts and non-hosts. Weevils fed on some non-hosts such as Douglas fir, *Pseudotsuga menziesii* (Mirb.) Franco, and western hemlock, *Tsuga heterophylla*, (Raf.), when given no choice, but if given Sitka spruce as a choice, preferentially fed on it. Alfaro *et al.* (1980) found that feeding stimulants were present on the bark and cuticle of Sitka spruce leaders. Stimulants were also found in the xylem of leaders only, and not in needles. Harris *et al.* (1990) observed that spruce needles actually contain feeding deterrents. Plank & Gerhold (1965) found that *P. strobi* was capable of distinguishing between eastern white pine, *Pinus strobus* L., Jack pine, *Pinus banksiana* Lamb., and red pine, *Pinus resinosa* Ait., with the preference in that order. Alfaro & Borden (1982) screened a variety of hosts and non-hosts using dried bark incorporated into an agar-disc bioassay for coastal British Columbian weevils. Feeding stimulants were present in almost all conifers tested but not in the non-conifers. Feeding responses were strongest in *Pinus* and *Picea spp.*, but optimal only in Sitka spruce. In addition, using the agar-disc bioassay, Alfaro (1980) compared feeding preferences for dried bark from heavily attacked and unattacked Sitka spruce trees from two plantations in the Malcolm Knapp Research Forest, Maple Ridge, B.C., as well as in three apparently resistant trees from the Green Timbers Nursery in Surrey, B.C. He observed feeding deterrency (or lack of attraction) to the bark of several unattacked trees in one plantation at the Research Forest, but not to any of the Green Timbers trees.

Soles *et al.* (1970) caged adult weevils collected from eastern white pine on leaders of acceptable and unacceptable hosts, respectively eastern and western white

pine, *Pinus monticola* Dougl. Almost all weevils survived, more feeding punctures were made on western than eastern white pine, and weevils produced broods with similar success on both hosts. They hypothesized that in nature, resistant western white pine must inhibit the arrival of weevils or induce them to leave, suggesting feeding or oviposition deterrency. This hypothesis was upheld by VanderSar (1978), who showed that when given a choice between western white pine and Engelmann spruce, *Picea engelmannii* Parry ex Engelm., overwintered weevils collected from Engelmann spruce would feed on both hosts but would oviposit only on the latter.

Utilizing the putatively resistant genotypes identified for Pacific coast weevils in British Columbia (Ying 1991), my objective was to test the hypothesis that feeding and oviposition deterrency, or repellency is a basis for resistance.

METHODS

All plant material was collected from the four provenance trials and clonal outplanting of Sitka spruce as previously described (Tables 2, 3). Resistant genotypes had an average of 1.00±0.10 attacks per tree and susceptible ones had an average of 3.30 ± 0.14 attacks (Table 3). Susceptible trees in the wild were at least 15 years old and had sustained at least two weevil infestations. In all cases, branches were collected from the upper crown of the tree, and transported in plastic bags. In the laboratory, the ends were re-cut and the branches placed in buckets of water outside for 24 h.

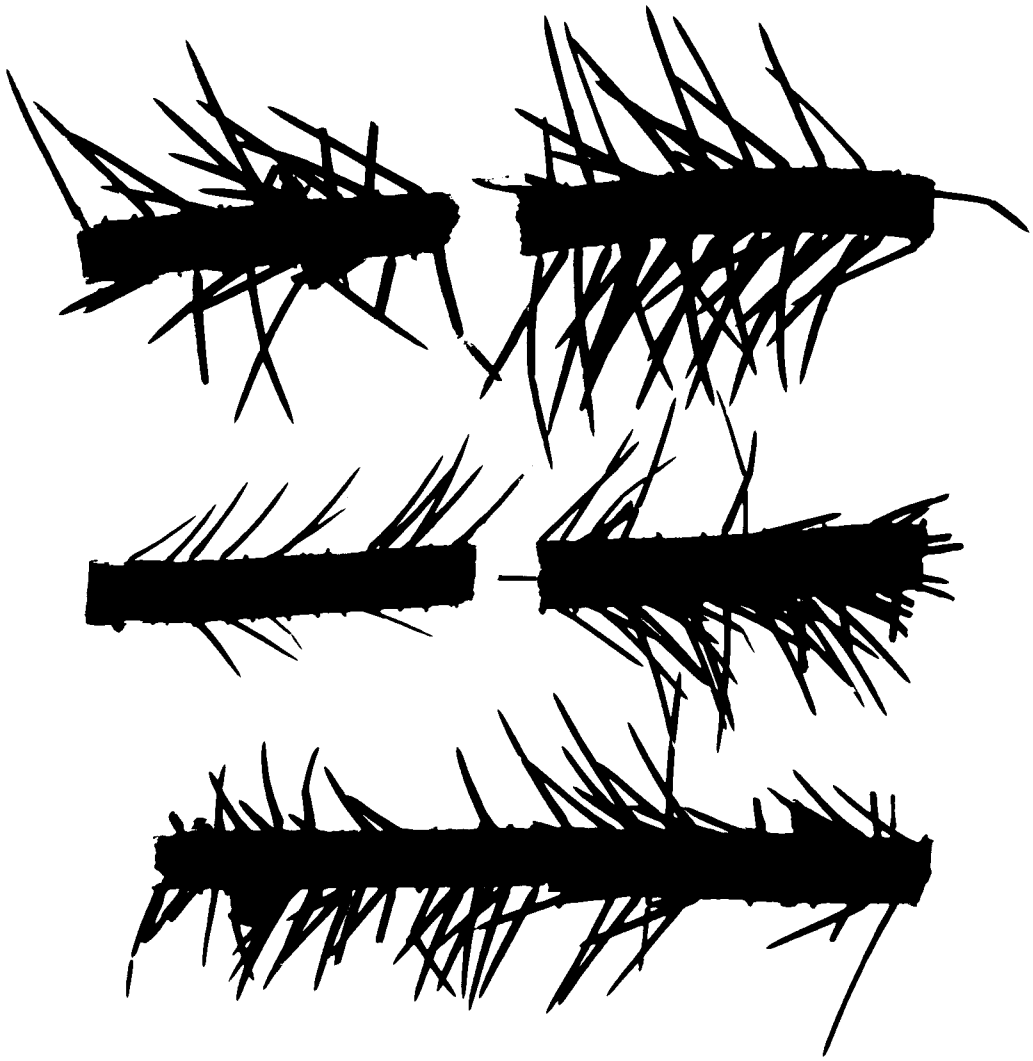
Paired-Twig Bioassay - Fall Weevils (September 1993)

Branches used in this experiment were collected from 10 replicates (trees) of 10 different resistant clones at Fair Harbour in September, 1993. In addition, 10 branches were collected from each of two unweevilled trees in the Big Qualicum provenance at the Sayward provenance trial. These trees had not been clonally propagated. Twelve branches were collected from each of 10 wild susceptible trees near the Fair Harbour trial.

Adult weevils used in this experiment emerged in June from branches that were infested in the laboratory by adult weevils collected near Terrace in May, 1993. All weevils were held on cut branches standing in water, with day and night temperature ranges of 28-32 and 18-20 °C, and a L:D photo regime of 15:9. Weevils were sexed 24 h prior to the start of the experiment (Harman & Kulman 1966), and held without food on moist filter paper at 4°C.

From each branch, two 5 cm long pieces of twig were cut, one for testing against male weevils and one for females. Each twig of a resistant clone was pinned end-to-end with a twig from a different susceptible tree with the needles pointing in the same direction to simulate a single branch (Brooks & Borden 1992) (Fig. 18). Twig pairs were randomized with respect to which end was resistant or susceptible. The exposed ends were dipped in melted paraffin wax to retard desiccation and impede release of volatiles from the cut surface. Each twig pair was placed in a 15.0 cm diam. Petri dish containing a 13.0 cm diam. piece of Whatman #1 filter paper. One weevil was placed at the junction of each twig pair and allowed to feed for four

Figure 18. Construction of twig pairs used in paired-twig feeding bioassays. Twigs are held together by a finishing nail with the head removed.



days. The experiment was arranged with 10 replicates per sex in a completely randomized block design on a laboratory bench. In addition to randomizing treatments within replicates, twig pairs were randomized within dishes so that they faced different directions. Blocks were separated by layers of Kraft paper and stacked vertically.

After four days, the feeding punctures were counted on each paired twig, and the frass pile under each weighed. The numbers were compared between resistant and susceptible twigs using pairwise t-tests by PROC UNIVARIATE which compares the average pairwise difference to zero (Schlotzhauer & Littell 1988). In addition, the weight of frass was regressed against the number of feeding punctures (PROC GLM, Schlotzhauer & Littell 1988).

Paired Twig Bioassay - Spring Weevils (May 1994)

Branches used in this experiment were collected in early May, 1994 from the same trees as the fall bioassay and in exactly the same way.

Weevils used in this experiment were hand-collected as adults near Terrace, B.C. in the last week of April, 1994. These weevils were held and prepared for the experiment as described above, and the experiment was assessed in the same manner as the paired-twig experiment with fall weevils. In addition to counting feeding punctures, the oviposition punctures and eggs laid were counted. The numbers of eggs were regressed against the number of oviposition punctures to determine if oviposition puncture counts reflected egg numbers, and the weight of frass was regressed against

the numbers of feeding punctures (PROC GLM, Schlotzhauer & Littell 1988). Mean differences in numbers of feeding punctures and frass weights between sexes and the two seasons were compared within clones using ANOVA (PROC GLM, SAS Institute 1988).

No-Choice Cut Branch Bioassay-Spring Weevils (April 1994)

Branches for this experiment were collected from Fair Harbour in early April, 1994 from five replicates (trees) of nine different clones. Only female weevils were used; they were collected as adults from Cowichan Lake, Vancouver Island in early April, 1994. The bottom end of each branch was re-cut and placed in a bucket of water. Two weevils were confined to the top 4 cm of branch in a fine cheesecloth cage. The 45 branches were distributed randomly between four buckets on a laboratory bench. Weevils were allowed to feed and oviposit for six days.

The feeding and oviposition punctures were counted on each branch and compared between resistant and susceptible clones using analysis of variance (PROC ANOVA, Schlotzhauer & Littell 1988). Multiple comparisons were made using Ryan's Q t-tests (PROC MEANS/REGWQ, SAS Institute 1988). Oviposition puncture data were transformed to square roots to normalize the variance.

Agar-Disc Bioassay - Fall Weevils (Late July 1993)

The weevils used in this experiment came from the same collection as for the September 1993 paired-twig bioassay.

Branches from trees in resistant provenances were collected in the fall of 1992 from Nass, Kitimat, Fair Harbour and Head Bay. A pooled sample of several trees represented each of five resistant provenances. Branches from susceptible trees were collected from a plantation near Terrace, B.C. Bark was stripped from the branches, oven dried, ground to a fine powder in a Wiley mill and refrigerated in glass jars.

Dried bark from each provenance was incorporated into an agar-based diet (Zerillo & Odell 1973) for testing in an agar-disc bioassay (Alfaro *et. al.* 1979). One disc of diet (1 cm diam. and 0.5 cm high) containing resistant bark, and one disc containing susceptible bark were placed in a 5.5 cm Petri dish. Each disc was covered with a piece of lens paper, and the dish was filled with paraffin wax to the level of the discs, embedding the edges of the lens paper. Three broken toothpicks were laid in the wax in order to give the weevils traction. Each treatment was replicated 10 times each for males and females. Two weevils were placed in the center of each Petri dish and allowed to feed freely. After 48 h, numbers of feeding punctures on each disc were counted, and the numbers on discs containing bark from resistant and susceptible provenances were compared using pairwise t-tests as above. It was previously determined by Alfaro *et al.* (1979) that the numbers of feeding punctures were correlated with the volume of diet ingested.

Agar Disc Bioassay - Spring Weevils (June 1993)

Weevils used in this experiment were collected near Terrace, B.C. in the last week of April, 1993. They were held and prepared for the experiment as described for

the paired-twig bioassays. In addition, a group of males collected in late May, 1993 on Jack pine in Ontario were tested.

The experiment was conducted as described for the late July 1993 agar-disc bioassay, except that bark from trees in the Big Qualicum provenance from only one site was tested, and each treatment was replicated five times in completely randomized block designs for females alone, one male plus one female, and Ontario males alone. Numbers of feeding punctures on discs containing bark from resistant and susceptible trees were compared using pairwise *t*-tests as above.

Agar Disc Bioassay - Tannins and Resin Acids (January 1995)

Weevils used in this experiment emerged in mid- to late summer from leaders collected in Terrace, and refrigerated after emergence until January 27. Weevils were held on cut spruce branches at 20°C for three days until the start of the experiment. Standard diets were made as previously described, using the susceptible bark mixture.

Unpurified quebracho tannin was dissolved in methanol at a rate of 0.0 (control), 0.01, 0.10 and 0.05 g per mL and 1 μL of a solution was applied to the surface of 1 cm^2 piece of the lens paper laid over the agar disc just before weevils were added. Each concentration was paired with a methanol control in a two-choice bioassay, and replicated 15 times each for males and females.

A stock solution of resin acids in methanol was prepared to simulate the composition of resin acids found in the bark of Kitwanga clones 6-13-2 and 6-13-8 (Table 15). The four treatment groups were a methanol control disc paired with

Table 15. Concentration of resin acids in a stock solution, formulated to mimic the concentration and composition of resin acids in the bark of Kitwanga clones 6-13-2 and 6-13-8.

Resin acid	Grams in 6 mL of methanol
Pimaric	0.06
Isopimaric	0.032
Levopimaric	0.025
Palustric	0.035
Dehydroabietic	0.010
Abietic	0.070
Neoabietic	0.035
Total	0.267

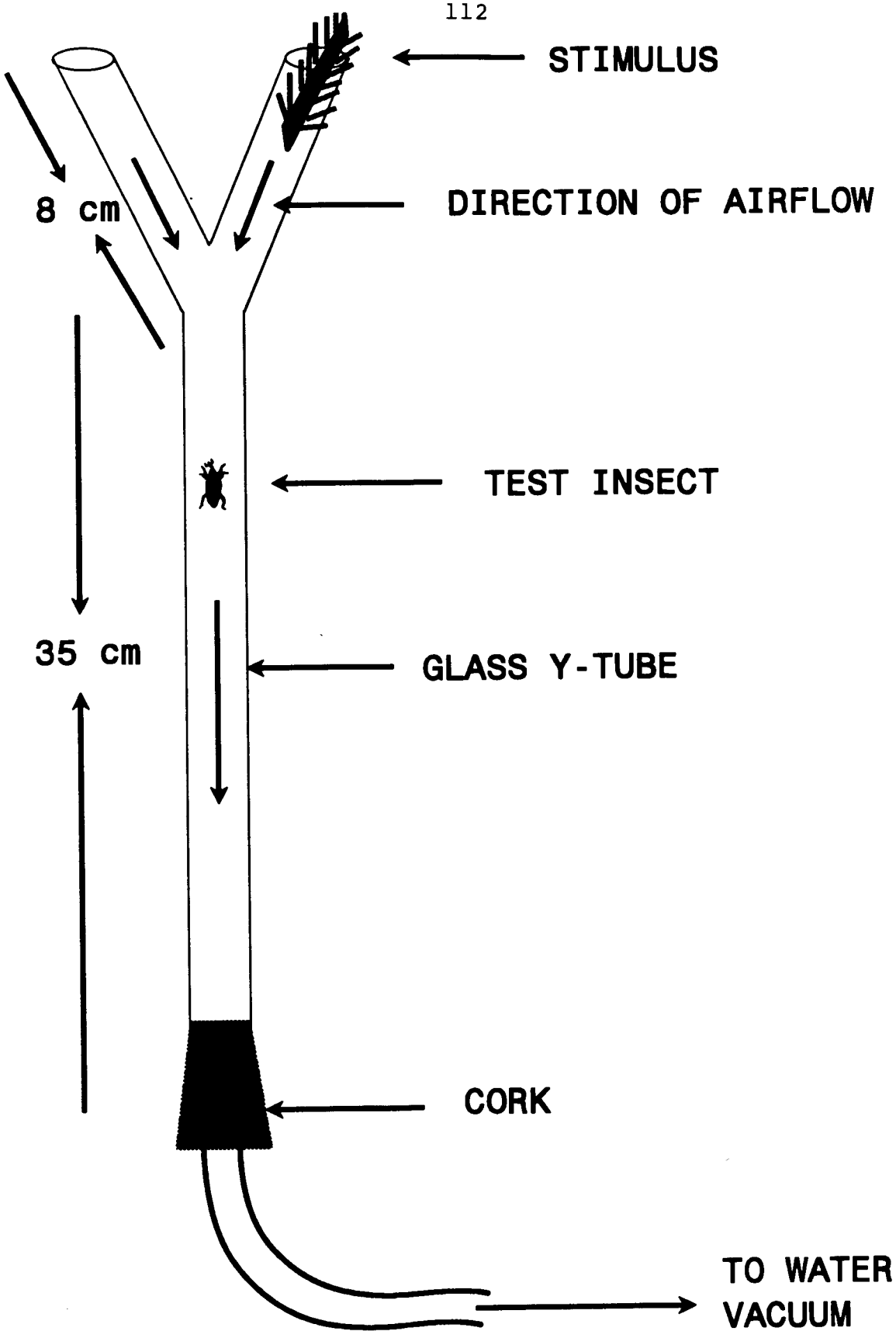
another control, undiluted stock solution, 10 or 1% dilutions of the resin acid stock solution. One μL of a control or treatment stimulus was applied to the lens paper covering the agar disc just before adding weevils. Each paired disc treatment was replicated 15 times each for males and females as above.

In each experiment, treatments were arranged in a completely randomized block design, and weevils were allowed to feed for five days. At the end of the experiment the number of holes were counted in each disc of diet and controls and treatments compared by pairwise *t*-tests as previously described, and the total amount of feeding on treated and untreated discs combined was compared by ANOVA (Proc GLM, SAS Institute 1988).

Olfactometer Bioassays

The responses of walking weevils to host odours were tested using a two-choice glass y-tube olfactometer (Fig. 19) held in a vertical position to exploit the weevils' negative geotaxis (VanderSar & Borden 1977b). Unfiltered air was drawn downward through the y-tube using a water vacuum at 1.18 L per min. Laminar flow was tested by observing titanium tetrachloride vapours in the y-tube. Bioassays conducted in the spring used weevils collected as adults in Terrace, B.C. in early May, 1995. Bioassays conducted in the fall used weevils emerged in the lab in July 1994 from branches infested by weevils under laboratory conditions. Only weevils that had six functional legs were used. At the start of each trial, a stimulus was placed at one arm of the y, and a single weevil was released at the bottom of the tube. Non-

Figure 19. Y-tube olfactometer used to test responses of *P. strobi* to volatile host stimuli.


STIMULUS
DIRECTION OF AIRFLOW
8 cm
TEST INSECT
35 cm
GLASS Y-TUBE
CORK
TO WATER
VACUUM

respondents did not move at all for 5 min. or did not reach the y junction within 15 min. Experiments were conducted at 24-27°C under flourescent lighting. Experimental and control arms were alternated, and a different weevil was used in each trial. Weevils were held separately by sex on cut branches at 4°C between experiments, on moist filter paper at 24-27°C during experiments. The time taken for a weevil to enter an arm of the y, and the y entered were recorded. For all olfactometer bioassays, the probability that the response to a given stimulus was different from random was tested by using an exact test based on the binomial distribution, comparing the observed data with an expected 50:50 distribution (Miller & Freund 1985).

Responses to Different Clones

The responses of weevils to 15 clones from Fair Harbour were tested in both the spring and the fall. In addition to the 12 resistant clones used previously in paired-twig bioassays, three susceptible clones 32-5-1 (Fair Harbour), 15-13-7 (Aberdeen) and 37-1-2 (Moresby Camp) were included. A twig from a particular clone was placed in one arm of the y-tube, and the other arm was unbaited. In each of two experiments, 15 males and 15 females were tested individually against 15 different twigs from each clone. Two clones were tested each day; twigs were cut at the beginning of the day and kept in sealed jars containing wet paper towel before use. In the spring, responses to twigs from all clones with newly flushed buds were tested. To test the hypothesis that bioactive volatiles are released from newly-flushed buds,

the responses to twigs with flushed buds were ranked separately for each sex, and every second clone bioassayed again using twigs with the flushing buds removed. In the fall, responses to all 15 clones were tested. In all cases, the wounds made by cutting twigs were not sealed.

Responses to Antennally-Active Compounds

The volatiles from two susceptible potted clones, Usk (1238), Hoquium (1050), and two resistant potted clones, Big Qualicum (881) and Haney (890), were collected. Volatiles from 250 g of twigs with current year's foliage were collected for 120 h by passing air over the foliage at 2 L per min. and trapping the volatiles on Porapak Q (Pierce *et al.* 1981). The volatiles were eluted from the Porapak Q with 200 mL pentane, and concentrated by distillation to 4.0 mL. Extracts were diluted so that between 1 and 300 ng of any given terpene were injected in 1 μL aliquots and analyzed splitless by coupled gas chromatographic-electroantennographic detection (GC-EAD) (Arn *et al.* 1975), performed by Regine Gries (Simon Fraser University, Burnaby, B.C., Chemical Ecology Research Group). Three male and five female antennae of fall weevils were tested.

The responses of both male and female fall weevils to three different doses (0.001 μl, 0.010 μl and 0.10 μl) of six identified antennally-active monoterpenes were tested using y-tube olfactometer bioassays. Each was diluted in pentane and the extract applied to filter paper inside a glass cylinder, 1 cm internal diameter; placed inside one arm of the olfactometer, and compared with a pentane control in the

opposite arm. Fifteen weevils of each sex were tested with each concentration.

RESULTS

Paired-Twig Bioassay - Fall Weevils

Several clones significantly deterred feeding by fall weevils, including Cedarvale (18-12-5), Haney (29-1-2), and Big Qualicum (03-02-6, 03-15-2) for males, and clones representing all provenances but Kitwanga for females (Fig. 20). There was a general trend to deterrency by all of the resistant clones. While results are discussed in terms of percent deterrency, this experiment does not distinguish between deterrency and absence of stimulation, thus clones that appear deterrent may actually lack feeding stimulants. The weight of frass was significantly correlated with the number of feeding punctures ($Y=0.04x+0.04$, $P<0.0001$, $r^2=0.44$). Thus the number of feeding punctures reflects the amount of tissue eaten.

Paired-Twig Bioassay - Spring Weevils

Significant feeding deterrency (or lack of stimulation) towards both males or females was expressed by clones from the Green Timbers (2G, 3G) and Big Qualicum (03-2-6, 03-15-2) provenances by feeding puncture counts and frass weights (Fig. 21), although there again was a general trend towards deterrency by all resistant clones, particularly against males. In contrast, significant oviposition deterrency was caused by only one Big Qualicum clone (03-02-6), and oviposition was highly stimulated by a

Figure 20. Results of paired-twig bioassay using newly emerged (fall) weevils. Bars indicate percent stimulation or deterrency expressed as a deviation from an expected 50:50 distribution by the formula [(n/2-a)/(n/2)]x100 where n= the total number of punctures or weight of frass and a=the number or weight on resistant twigs. Differences between resistant and susceptible twigs (paired t-test, $P<0.05$) indicated by *.

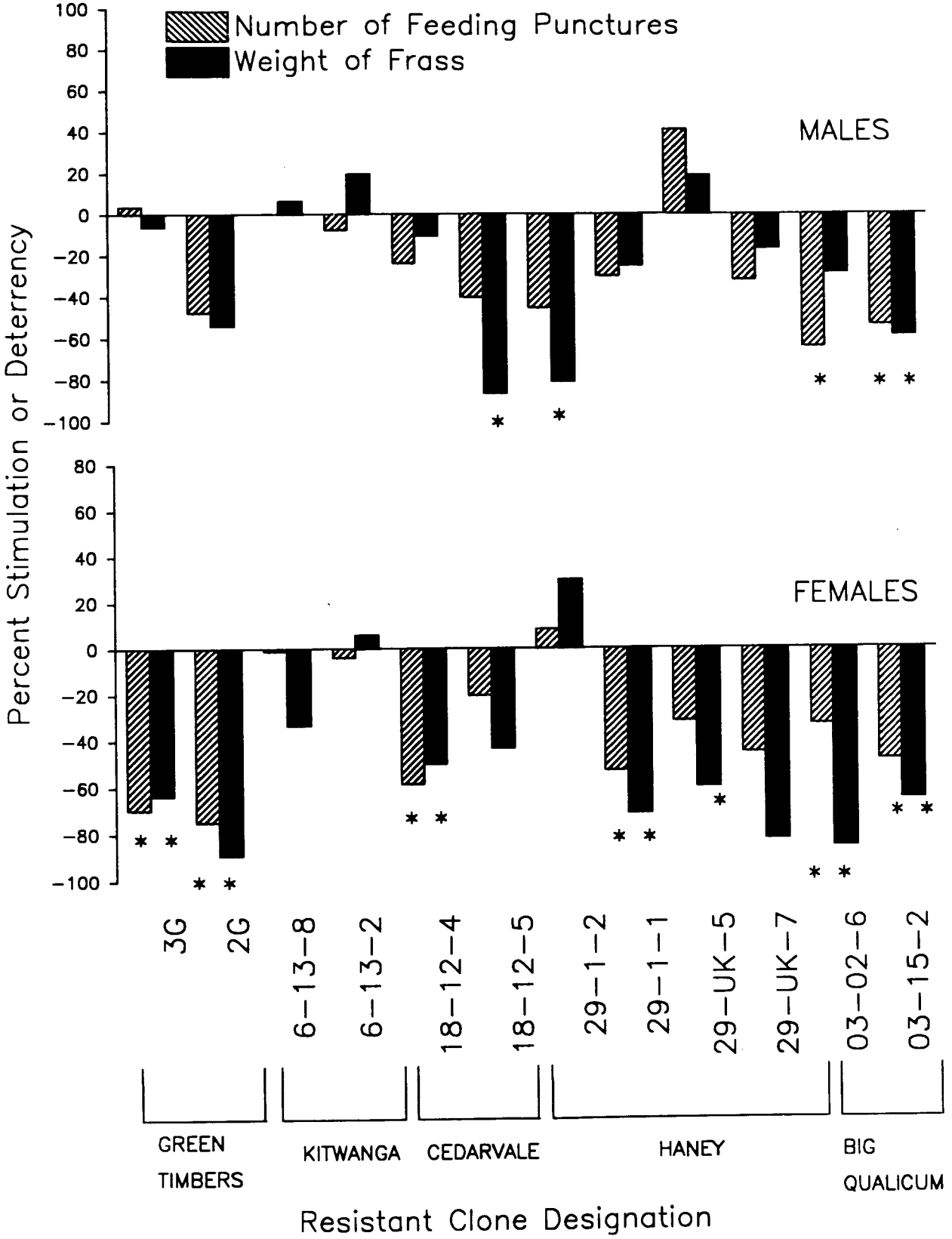
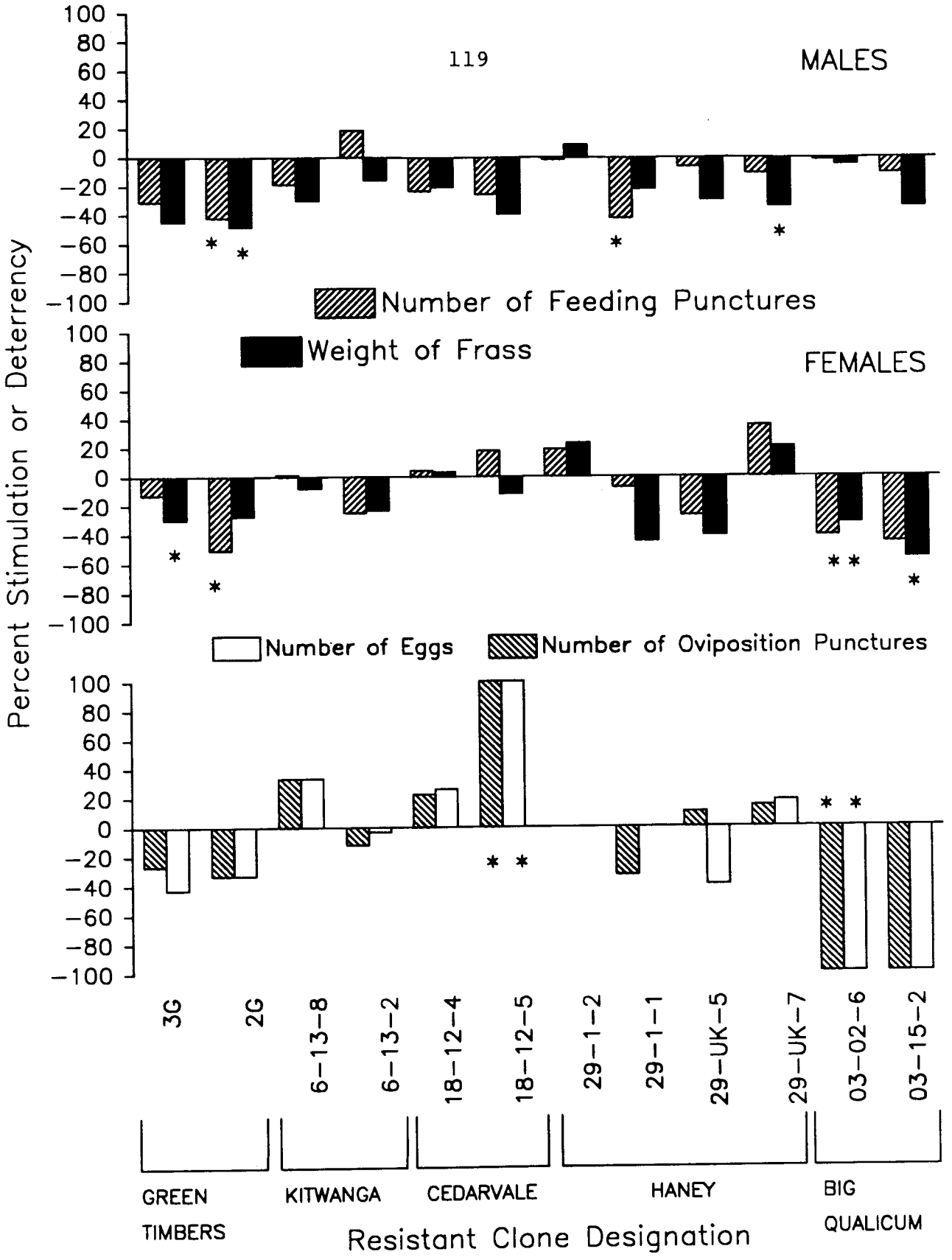

Number of Feeding Punctures
Weight of Frass
MALES
FEMALES
Percent Stimulation or Deterrency
100
80
60
40
20
0
-20
-40
-60
-80
-100
3G
2G
6-13-8
6-13-2
18-12-4
18-12-5
29-1-2
29-1-1
29-UK-5
29-UK-7
03-02-6
03-15-2
GREEN TIMBERS
KITWANGA
CEDARVALE
HANEY
BIG QUALICUM
Resistant Clone Designation

Figure 21. Results of paired-twig bioassay using spring weevils. Bars indicate percent stimulation or deterrency expressed as a deviation from an expected 50:50 distribution by the formula [(n/2-a)/(n/2)] x 100 where n= the total number of feeding or oviposition punctures, or weight of frass or number of eggs and a=the number or weight on resistant twigs. Differences between resistant and susceptible twigs (paired t-test, $P<0.05$) indicated by *. In the case of Big Qualicum clone 03-15-2, the 100% deterrency observed is not significant because there were very few oviposition punctures on the susceptible twigs


MALES
FEMALES
Number of Feeding Punctures
Weight of Frass
Number of Eggs
Number of Oviposition Punctures
Percent Stimulation or Deterrency
100
80
60
40
20
0
−20
−40
−60
−80
−100
3G
2G
6−13−8
6−13−2
18−12−4
18−12−5
29−1−2
29−1−1
29−UK−5
29−UK−7
03−02−6
03−15−2
GREEN TIMBERS
KITWANGA
CEDARVALE
HANEY
BIG QUALICUM
Resistant Clone Designation

Cedarvale clone (18-12-5). There was again a significant correlation between frass weight and number of punctures ($Y=0.04x+0.11$, $P<0.0001$, $r^2=0.31$), and between number of eggs laid and oviposition punctures ($Y=1.16x+0.04$, $P<0.0001$, $r^2=0.91$).

There was significantly less deterrency in the spring than in the fall based on frass weights for both Big Qualicum clones, both Cedarvale clones, three Haney clones (29-1-1, 29-1-2, 29-UK-5) and one Green Timbers clone (2G), ($P<0.05$, ANOVA). In addition, there were significant differences between sexes with respect to deterrency. Females were more deterred by one Big Qualicum clone (03-02-6) in spring only, but slightly stimulated by a Haney clone (29-1-2) compared to males in both seasons ($P<0.05$, ANOVA). Females were more deterred than males in the fall only by one Haney clone (29-UK-5) ($P<0.05$, ANOVA).

No-Choice Cut Branch Bioassay - Spring Weevils

Based on analysis of variance, there were significant differences between the different clones tested in the numbers of feeding punctures ($F=2.43$; df=8, 35; $P=0.03$), and oviposition punctures ($F=3.17$; df=:8, 35; $P=0.0083$). There was a much stronger trend towards oviposition deterrency than feeding deterrency by resistant clones (Table 16). In contrast to the choice bioassay results (Figs. 20,21), Green Timbers (2G) was not particularly deterrent, and Kitwanga (6-13-8) was highly deterrent.

Table 16. Ranked means of feeding and oviposition punctures on different Sitka spruce clones by weevils given no choice. Weevils were collected in the spring.

Clone designation and resistant (R) or susceptible (S) classification[a]	Number of Feeding Punctures ($\bar{x}$ ± S.E.). N=5[a]	Clone designation and resistant (R) or susceptible (S) classification[b]	Number of Oviposition Punctures ($\bar{x}$ ± S.E.). N=5[a]
18-12-4 (R) (Cedarvale)	11.8 ±4.3 a	29-UK-7 (R) (Haney)	0.4±0.4 a
37-1-3 (S) (Moresby Camp)	12.8±1.9 a	6-13-8 (R) (Kitwanga)	0.8±0.8 a
6-13-8 (R) (Kitwanga)	14.0±4.5 a	18-12-4 (R) (Cedarvale)	1.2±1.0 a
29-UK-7 (R) (Haney)	14.8±2.1 a	30-3-7 (S) (Muir Cr., Sooke)	2.6±2.6 a
32-5-2 (S) (Fair Harbour)	16.6±5.2 a	2G (R) (Green Timbers)	3.2±1.9 a

Table 16 Continued

30-3-7 (S) (Muir Cr., Sooke)	19.0±2.0 a	32-5-2 (S) (Fair Harbour)	4.0±2.6 ab
2G (R) (Green Timbers)	23.4±2.0 a	36-2-4 (S) (Tasu Creek)	7.2±3.2 ab
15-13-7 (S) (Aberdeen)	26.8±3.4 a	15-13-7 (S) (Aberdeen)	11.0±4.7 ab
36-2-4 (S) (Tasu Creek)	27.0±5.5 a	37-1-3 (S) (Moresby Camp)	15.4±5.3 b

[a]Means followed by the same letter are not significantly different, Ryan's Q test, $P>0.05$.

[b] The name of the provenance to which each clone belongs is shown in brackets

Agar-Disc Bioassays - Fall and Spring Weevils

When incorporated into agar discs, the dried bark from trees in resistant provenances did not deter feeding by fall weevils, but feeding by spring weevils was deterred to some extent (Table 17). Differences were significant only for Cedarvale and Big Qualicum provenances probably because of a low number of replicates. Significance was approached in one or more treatments for Haney and Green Timbers provenances. Again the deterrency observed could be lack of stimulation.

Agar-Disc Bioassay - Tannins and Resin Acids

Forcing weevils to feed through lens paper treated with condensed tannins caused no difference in the total amount of diet eaten by either males (F=1.48; df=3,54; P=0.2302) or females (F=1.75; df=3,58; P=0.1667). There was also no effect of dose of tannins on the mean difference between treatment and control discs for either males (F=1.75; df=3,54; P=0.1680) or females (F=0.41; df=3,58; P=0.7840).

With one exception, resin acids had no effect on feeding by male or female weevils. There was no difference in feeding between treated and control discs at any concentration (F=0.81; df=3,61; P=0.4906) for females, and (F=1.62; df=3,51; P=0.1965) for males. There was no difference in the total amount eaten by females between treatments (F=0.15; df=3,61; P=0.9266), but for males there was a significant difference among all treatments in the total amount of feeding (F=3.48; df=3; P=0.0224). This was attributed to a 38 % higher number of feeding punctures made in the 1% resin acid treatment than in the next highest category, the 10% treatment. At

Table 17. Percent feeding stimulation by bark from trees in resistant provenances incorporated into an agar-disc bioassay for both fall and spring weevils. Probabilities (*P*) are given for pairwise t-test comparisons between numbers of feeding punctures on agar discs containing resistant or susceptible bark.

		Females alone		Males alone [a]		Males + Females	
Weevils and No. of Replicates	Provenance	Percent stimulation or deterrency	*t*-test *P*	Percent stimulation or deterrency	*t*-test *P*	Percent stimulation or deterrency	*t*-test *P*
Fall weevils	Cedarvale	-60.0	0.16	-60.0	0.63	-	-
N=10	Haney	8.6	0.88	-77.2	0.32	-	-
	Green Timbers	-44.8	0.28	11.1	0.86	-	-
	Big Qualicum-Head Bay	-15.2	0.73	-100.0	0.17	-	-
	Big Qualicum-Nass	-28.9	0.47	36.7	0.42	-	-
	Kitwanga	-9.4	0.86	24.6	0.52	-	-

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Table 17 continued

Spring weevils	Kitwanga	6.3	0.95	-56.8	0.14	-60.0	0.18
N=5	Cedarvale	-11.1	0.34	-47.6	0.05	-60.0	0.00
	Big Qualicum	-47.6	0.12	-63.9	0.00	-86.0	0.05
	Haney	-57.1	0.11	-91.0	0.07	-100.0	0.06
	Green Timbers	-84.0	0.07	-21.5	0.13	-33.0	0.52

[a] Males used in spring experiment were collected from Jack pine in Ontario.

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the 1% resin acid concentration, 68 % of the total number of feeding punctures were made on the resin acid discs.

Olfactometer Bioassays

Responses to different clones

Four resistant clones, 18-12-4 (Cedarvale), 2G, 3G (Green Timbers), and 29-1-1 (Haney), were significantly repellent to females in the spring, and none was significantly attractive (Fig. 22). Males were significantly repelled by three resistant clones, 3G (Green Timbers), 29-UK-7(Haney) and 18-12-5 (Cedarvale), and one susceptible clone, 37-1-2 (Moresby Camp), and significantly attracted by one resistant clone, 03-2-6 (Big Qualicum) (Fig. 22). The only repellent clones that males and females had in common were 18-12-5 (Cedarvale) and 3G (Green Timbers), two resistant clones.

In the fall females were repelled by eight resistant clones, 18-12-4 (Cedarvale), 6-13-2 (Kitwanga), 3G (Green Timbers), 03-2-6, 03-15-2 (Big Qualicum), and 29-1-1, 29-1-2, 29-UK-5 (Haney), and two susceptible clones 32-5-1 (Fair Harbour) and 15-13-7 (Aberdeen) (Fig. 23). The resistant clones 18-12-4 (Cedarvale), 3G (Green Timbers)and 29-1-1 (Haney) were also repellent in the spring (Fig. 22). Males were repelled by five resistant clones, 03-2-6 (Big Qualicum), 29-1-1, 29-1-2 (Haney), and 2G, 3G (Green Timbers), and one susceptible clone 15-13-7 (Aberdeen) (Fig. 23). The 3G clone (Green Timbers) was also repellent in the spring. Other than these few clones, the ranking between spring and fall preferences was different. No clone

Figure 22. Responses of male and female weevils in a y-tube olfactometer to cut twigs with intact buds in the spring. Bars indicate percent attraction or repellency expressed as a deviation from an expected 50:50 distribution by the formula [(n/2-a)/(n/2)] x 100 where n= the number of weevils entering the control arm of the y-tube, and a=the number entering the stimulus arm of the y-tube. Asterisks indicated clones that are significantly attractive or repellent by Fishers exact test ($P<0.05$).

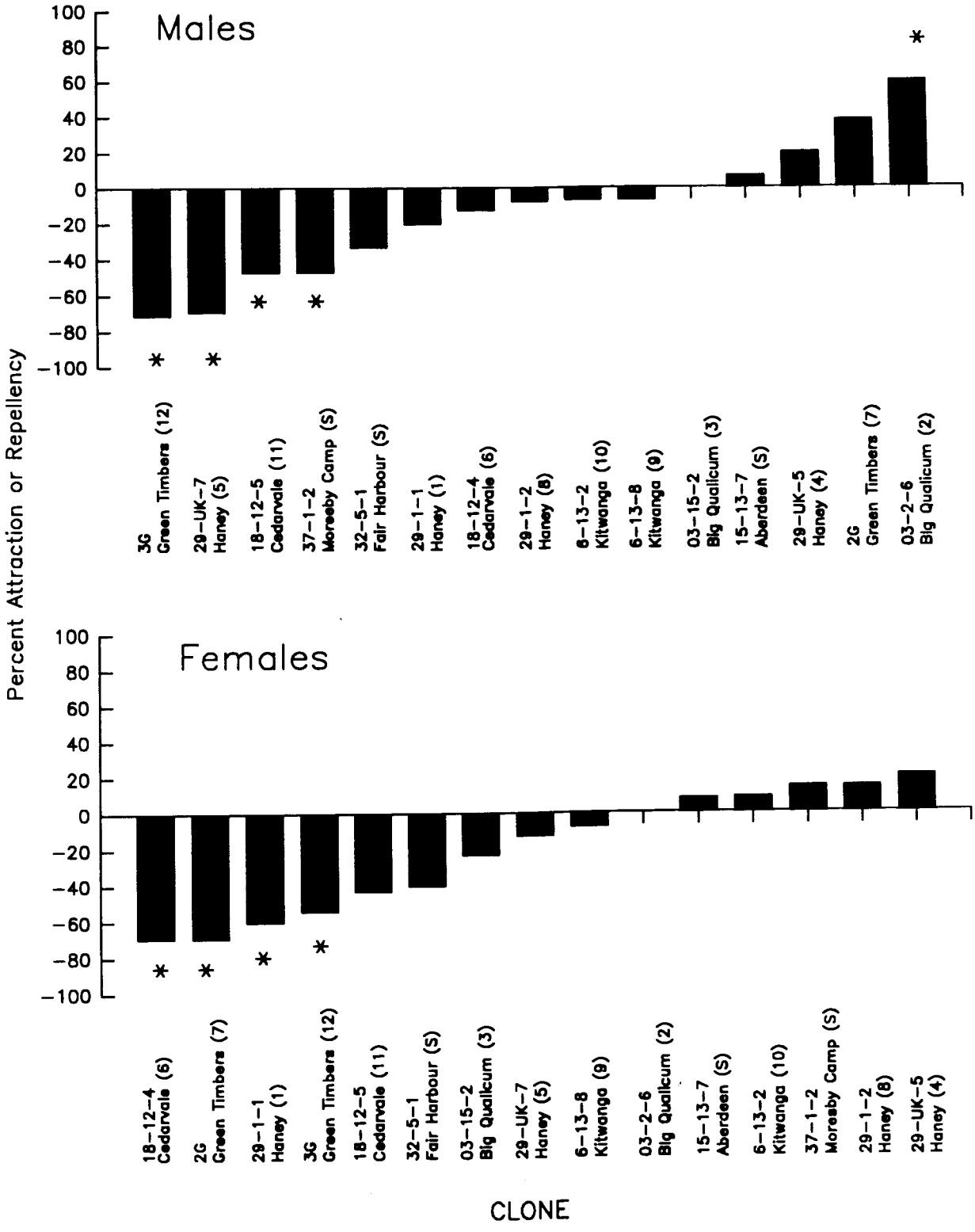

Males
Females
Percent Attraction or Repellency
100
80
60
40
20
0
-20
-40
-60
-80
-100
*
3G Green Timbers (12)
29-UK-7 Haney (5)
18-12-5 Cedarvale (11)
37-1-2 Moresby Camp (S)
32-5-1 Fair Harbour (S)
29-1-1 Haney (1)
18-12-4 Cedarvale (6)
29-1-2 Haney (8)
6-13-2 Kitwanga (10)
6-13-8 Kitwanga (9)
03-15-2 Big Qualicum (3)
15-13-7 Aberdeen (S)
29-UK-5 Haney (4)
2G Green Timbers (7)
03-2-6 Big Qualicum (2)
18-12-4 Cedarvale (6)
2G Green Timbers (7)
29-1-1 Haney (1)
3G Green Timbers (12)
18-12-5 Cedarvale (11)
32-5-1 Fair Harbour (S)
03-15-2 Big Qualicum (3)
29-UK-7 Haney (5)
6-13-8 Kitwanga (9)
03-2-6 Big Qualicum (2)
15-13-7 Aberdeen (S)
6-13-2 Kitwanga (10)
37-1-2 Moresby Camp (S)
29-1-2 Haney (8)
29-UK-5 Haney (4)
CLONE

Figure 23. Responses of male and female weevils in a y-tube olfactometer to cut twigs in the fall. Bars indicate percent attraction or repellency expressed as a deviation from an expected 50:50 distribution by the formula [(n/2-a)/(n/2)] x 100 where n= the number of weevils entering the control arm of the y-tube, and a=the number entering the stimulus arm of the y-tube. Asterisks indicated clones that are significantly attractive or repellent by Fishers exact test ($P<0.05$).

Males
100
80
60
40
20
0
−20
−40
−60
−80
−100
Percent Attraction or Repellency
03−2−6 Big Qualicum (2)
29−1−2 Haney (8)
3G Green Timbers (12)
29−1−1 Haney (1)
15−13−7 Aberdeen (S)
2G Green Timbers (7)
03−15−2 Big Qualicum (3)
32−5−1 Fair Harbour (S)
29−UK−5 Haney (4)
18−12−5 Cedarvale (11)
29−UK−7 Haney (5)
18−12−4 Cedarvale (6)
6−13−2 Kitwanga (10)
37−1−2 Moresby Camp (S)
6−13−8 Kitwanga (9)
Females
18−12−4 Cedarvale (6)
32−5−1 Fair Harbour (S)
6−13−2 Kitwanga (10)
3G Green Timbers (12)
03−2−6 Big Qualicum (2)
29−1−1 Haney (1)
15−13−7 Aberdeen (S)
03−15−2 Big Qualicum (3)
29−UK−5 Haney (4)
29−1−2 Haney (8)
18−12−5 Cedarvale (11)
2G Green Timbers (7)
37−1−2 Moresby Camp (S)
29−UK−7 Haney (5)
6−13−8 Kitwanga (9)
CLONE

attracted more than 50% of either male or female weevils; clones in the fall were generally more repellent than the same clones in the spring.

Removal of flushing buds from cut twigs in the spring caused almost a reversal in the ranking of repellency for both males and females (Figs. 24). With their buds removed, 18-12-4, 18-12-5 (Cedarvale), and 29-1-1(Haney) lost their repellency to females, and 37-1-2 (Moresby Camp) became repellent. 3G (Green Timbers) and 18-12-5 (Cedarvale) lost their repellency to males. Clone 03-2-6 (Big Qualicum) lost its attraction, and 32-5-1 (Fair Harbour) became attractive. Spring twigs with buds removed were still not as repellent as fall twigs (Figs. 23,24).

Responses to Antennally-Active Compounds

The excised antennae of weevils responded to nine host peaks which included α-pinene, myrcene, β-pinene, β-phellandrene, camphor, borneol, piperitone and two unknowns (Fig. 25). The response to β-pinene was small and inconsistent, and the strongest responses occurred to unknown #2 and camphor. α-Pinene, myrcene, β-phellandrene/limonene, camphor, borneol and piperitone were tested at three different doses in the olfactometer. Pure β-phellandrene was not available, but as β-phellandrene and limonene co-elute during GC analysis, the impure standard is more representative of what the antennae responded to than either of the pure compounds. α-Pinene was repellent to both sexes at 0.1 μL, and borneol and piperitone were repellent to females at 0.1 μl. Myrcene and β-phellandrene/limonene were attractive to males at 0.1 μL (Table 18). At lower doses no monoterpene was either attractive or repellent.

Figure 24. Responses of male and female weevils in a y-tube olfactometer to cut twigs in the spring with buds removed. Bars indicate percent attraction or repellency expressed as a deviation from an expected 50:50 distribution by the formula [(n/2-a)/(n/2)] x 100 where n= the number of weevils entering the control arm of the y-tube, and a=the number entering the stimulus arm of the y-tube. Asterisks indicated clones that are significantly attractive or repellent by Fishers exact test ($P<0.05$).

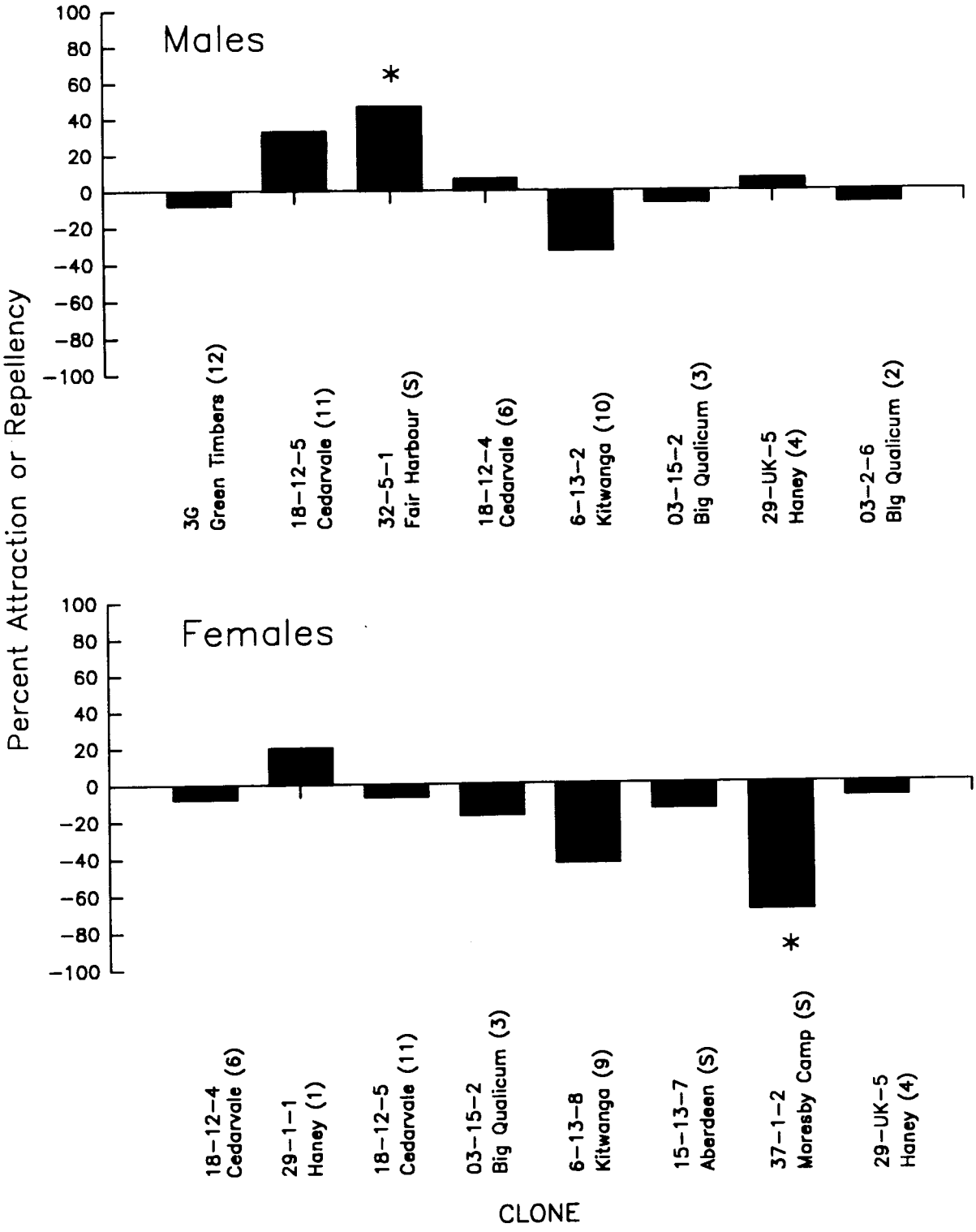
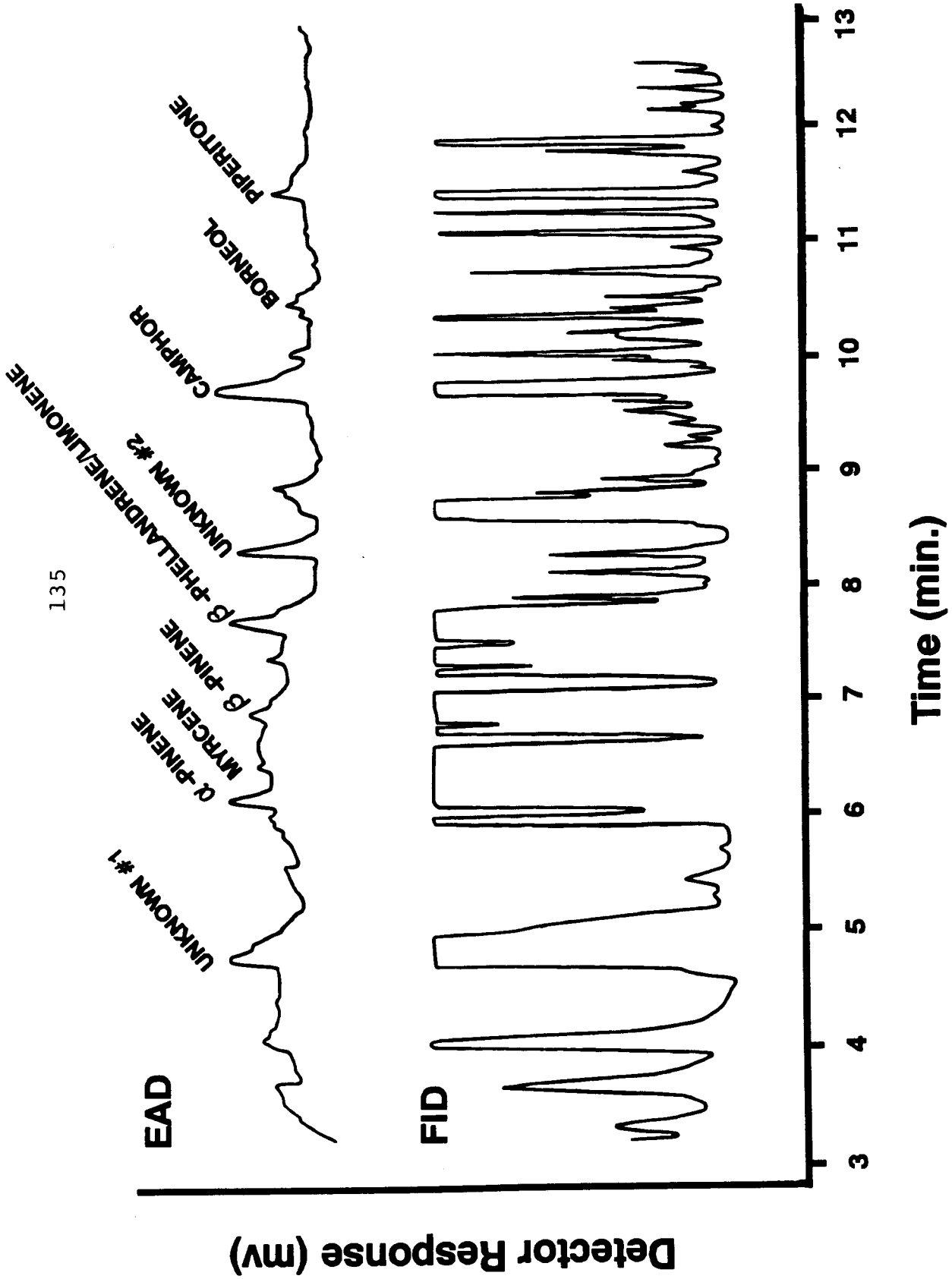

Males
Females
Percent Attraction or Repellency
100
80
60
40
20
0
−20
−40
−60
−80
−100
*
3G
Green Timbers (12)
18−12−5
Cedarvale (11)
32−5−1
Fair Harbour (S)
18−12−4
Cedarvale (6)
6−13−2
Kitwanga (10)
03−15−2
Big Qualicum (3)
29−UK−5
Haney (4)
03−2−6
Big Qualicum (2)
18−12−4
Cedarvale (6)
29−1−1
Haney (1)
18−12−5
Cedarvale (11)
03−15−2
Big Qualicum (3)
6−13−8
Kitwanga (9)
15−13−7
Aberdeen (S)
37−1−2
Moresby Camp (S)
29−UK−5
Haney (4)
CLONE

Figure 25. Representative simultaneous recordings from the flame ionization detector (FID) of the gas chromatograph and electroantennographic detector (EAD) (male *P. strobi* antenna) to aerated volatiles from one Kitwanga clone (6-13-8). Chromatography: splitless injection, injector temperature 240°C, FID temperature 240°C, HP Ultra 2 column, 1 min at 70°C, 10°C/min. to 280°C.


Detector Response (mv)
EAD
FID
UNKNOWN #1
α-PINENE
MYRCENE
β-PINENE
β-PHELLANDRENE/LIMONENE
UNKNOWN #2
CAMPHOR
BORNEOL
PIPERITONE
3
4
5
6
7
8
9
10
11
12
13
Time (min.)

135

Table 18. Responses in a y-tube olfactometer of male and female weevils to six antennally-active host terpenes tested at three doses. Percent attraction or repellency is expressed as a deviation from an expected 50:50 distribution by the formula [(n/2-a)/(n/2)] x 100 where n= the total number of weevils tested (15 in each case) and a=the number responding positively to the stimulus.

		Percent attraction (+) or repellency (-)[a]	
Terpene	Dose (μL)	Males	Females
α-Pinene	0.001	20.0	-73.0*
	0.010	-20.0	-6.6
	0.100	-60.0*	-57.0*
Myrcene	0.001	20.0	7.6
	0.010	14.0	6.6
	0.100	60.0*	6.6
β-Phellandrene/limonene	0.001	7.6	0.0
	0.010	6.6	-14.0
	0.100	43.0*	7.6
Camphor	0.001	-6.6	14.0
	0.010	-6.6	6.6
	0.100	20.0	33.0
Borneol	0.001	20.0	-29.0
	0.010	6.6	-14.0
	0.100	0.0	-100.0*
Piperitone	0.001	-23.0	-17.0
	0.010	-29.0	6.6
	0.100	-20.0	-54.0*

[a] Asterisks indicated clones that are significantly attractive or repellent by Fishers exact test ($P<0.05$).

DISCUSSION

My results demonstrate that in three different types of bioassays consistent feeding deterrency, or lack of stimulation, is expressed by specific genotypes of Sitka spruce against the white pine weevil. In particular, Big Qualicum clones caused feeding deterrency for both spring and fall weevils in both paired-twig and agar-disc bioassays, and oviposition deterrency in the paired twig bioassay. Brooks & Borden (1992) also found that trees from the Big Qualicum provenance caused significant feeding deterrency.

Significant feeding deterrency was caused by both oven-dried bark in the agar-disc bioassays and as fresh twigs in the paired-twig bioassays, but to different extents. This suggests that both volatile and non-volatile chemicals play a role in host acceptance.

Several non-volatile compounds such as lignins, condensed tannins, stilbenes, and some coumarins (Norris 1986), and resin acids (Wagner *et. al.* 1983) are antifeedants for many insects. In this study, the total amount of condensed tannin varied among clones, and resin acids were found in significantly greater amounts in resistant trees than in susceptible ones (Figs. 15,17). However, based on the results of the agar-disc bioassays with tannins and diterpene resin acids, neither of these types of compounds appears to be involved in feeding deterrency against adults. In fact, similar to my results, Alfaro & Borden (1985) found a mixture of resin acids isolated from spruce bark to be highly stimulatory. Other phenolic compounds may act as feeding or oviposition deterrents, and the lack of sugars or other carbohydrates as

stimulants might affect feeding behaviour. Other terpenes such as sesquiterpenes and diterpenes (Norris 1986), and terpene alcohols may have been retained in the dried bark, and could have contributed to feeding deterrency. It is possible that tannins contribute to feeding deterrency in intact twigs if they increase the toughness of the bark.

Weevils showed antennal responses to three oxygenated terpenes, piperitone, borneol and an unknown terpene alcohol (Fig. 25), with the first two being repellent (Table 18). α-Pinene, the most volatile terpene bioassayed, was also repellent, which is similar to the results of Bordasch & Berryman (1977), who observed that repellency of monoterpenes to the fir engraver increases logarithmically with the boiling point, with the exception of α-pinene, which has a higher repellency rank than expected from that relationship. Alfaro *et al.* (1980) found that three volatile monoterpenes in Sitka spruce bark, (+)-camphor, limonene,and piperitone deterred feeding by *P. strobi*, and Anderson & Fisher (1960) found that pure α-pinene was repellent to weevils, but slightly less so than white pine bark oil. It should be noted that while most olfactory receptors are located on the antennae (Visser 1986), there is evidence that *P. strobi* is able to perceive host odours even with the antennae excised (Anderson & Fisher 1960); thus there may be other components of host odour to which weevils can respond that were not detected by the GC-EAD analysis.

It was clearly demonstrated in olfactometer bioassays that weevils respond to volatile host odours, with the clones 3G (Green Timbers) and 29-1-1 (Haney) being the most consistently repellent. Anderson & Fisher (1956), observed that the odour of

ground bark from lightly attacked eastern white pine trees was repellent to white pine weevils in an olfactometer, but ground bark from non-native spruces had no effect on behaviour.

In olfactometer bioassays, removal of buds from spring twigs eliminated any significant attraction or repulsion. Buds are composed primarily of the most volatile monoterpenes which include α-pinene, β-pinene, myrcene, β-phellandrene and limonene (Brooks *et al.* 1987b). These results support those of Hulme (1995) who observed that synchrony between weevil oviposition and host phenology can affect whether or not a leader is successfully attacked.

Given the differences between paired-twig and agar-disc bioassays, and that there is no consistency between olfactometer and feeding bioassays, it seems probable that volatile and non-volatile stimulants are affecting two separate phases of host selection. Volatile olfactory cues may determine which trees weevils actually choose to visit, and non-volatile, gustatory cues may be most important in determining whether feeding and/or oviposition occurs. This is not an unexpected result. Insects cannot engage in both dispersal and feeding simultaneously, and the two neuromuscular systems serving locomotion and ingestion are inversely, and in some cases antagonistically, related to central neuroregulatory centres (Thorsteinson 1960). Genetic recombination would result in trees with every possible combination of volatile attractants and repellents and non-volatile feeding stimulants and deterrents. There is likely, however, an interaction between these types of cues as Alfaro *et al.* (1980) observed that the volatiles α-pinene, β-pinene and myrcene synergized feeding

on the agar-based diet which contains primarily non-volatile feeding stimulants. In addition, as the paired-twig and agar-disc bioassays did not distinguish between deterrency and lack of stimulation, it is possible that some clones were deterrent and some simply lacked adequate stimulants, further complicating efforts to relate the different types of bioassays.

The criteria for acceptance of a host for oviposition appear to be more stringent than for feeding. For example, when female weevils were given no choice on cut branches, the highest number of feeding punctures was only 2.3 times greater than the lowest, but for oviposition punctures, there was a 38.5 fold difference (Table 16). Similarly, VanderSar (1978) found that when given no choice, female *P. strobi* would feed on western white pine, but would not oviposit on it. Trudel *et al.* (1994) found that weevils would feed on a diet containing 1 or 5% dried bark, but would not oviposit on it. Sahota *et al.* (1994) observed no difference in feeding rates between weevils caged on leaders of resistant or susceptible trees, but on resistant hosts, reduction in egg production and ovarian regression occurred. Feeding was observed for females on resistant trees, further supporting the hypothesis that feeding and oviposition stimulants differ. In addition, host acceptance may depend on whether weevils are given a choice. For example, clone 2G (Green Timbers) was deterrent to feeding by females in paired-twig bioassays (Figs. 20,21) but not in the no-choice assay (Table 16).

Trees from the Kitwanga provenance grow poorly and do not express strong resistance at Fair Harbour, where they are 'off-site' (Ying 1991), but are highly

resistant at the Nass River site. White spruce, *Picea glauca* (Moench Voss), exhibits a high degree of induced resinosis as a resistance mechanism against attack by *P. strobi* (Alfaro 1995). "Sitka" spruces from the Kitwanga provenance probably contain white spruce genes (Ying 1991), and when on-site may be able to resist attack through induced resinosis. Exudation of resin is frequently associated with resistance to bark beetles (Kozlowski 1969; Berryman & Ashraf 1970; Reid & Gates 1970; Hodges et al. 1979; Lorio 1986;), and successful attacks by bark beetles were related to low oleoresin exudation pressure (Vité 1961; Vité & Wood 1961). In turn exudation pressure is affected by differences in site and stand conditions and degree of stem hydration (Vité 1961; Vité & Wood 1961; Kozlowski 1969). Some degree of this type of resistance could have been expressed by cut branches actively growing in water (Table 16), but not by severed twigs in the choice bioassays (Figs. 20,21). Plank & Gerhold (1965) observed that resin did not exude from feeding punctures on cut branches to the same extent that it did on intact leaders in nature. Histological examination of the bark of Kitwanga trees (Section VI) suggests that the capacity for induced resinosis is present.

Male weevils collected from Ontario showed a greater degree of overall feeding in agar disc bioassays than weevils collected in B.C., and showed a greater degree of discrimination between resistant and susceptible bark (Table 17). Although these weevils are genetically distinct form those in B.C. (Lewis, 1995) it is probable that they respond to the same deterrent stimuli. VanderSar *et al.* (1977) observed that weevils reared from Sitka spruce were actually less selective than weevils reared from

Engelmann spruce or eastern white pine. Thus a resistance mechanism based on feeding and oviposition deterrency might be effective throughout the range of *P. strobi.*

There were seasonal differences in both feeding behaviour and olfactometer responses. In olfactometer bioassays, there was greater repellency to fall twigs by both males and females. In the paired-twig bioassays, males showed greater discrimination in the fall between resistant and susceptible twigs than did females, and did not discriminate in the agar-disc bioassay. These differences may be due to the seasonal change in terpene composition as previously discussed, or to differences in the weevils themselves. In the agar-disc bioassay, the same bark was used for both spring and fall weevils; thus the weevils themselves must be different. It seems possible that some host selection occurs in the fall. In captivity, fall weevils are very active upon emergence, spending a great deal of time on the walls and ceiling of the cage, suggesting a readiness to disperse. In contrast, Hamel *et al.* (1994) observed significant differences in host preferences of eastern populations of *P. strobi* in the spring, but not in the fall, using a no-choice bioassay on cut twigs; however only females were tested.

My results lead to the tentative conclusion that Sitka spruce clones from Big Qualicum and to a lesser extent other provenances are resistant, at least in part, due to feeding and oviposition deterrency or lack of stimulation. In addition, both gustatory and olfactory stimuli appear to be important in host selection, but the relative importance of each is still unclear. A measurement of both feeding and oviposition

deterrency, and repellency should be included in a multicomponent resistance index.

VI. INDUCED DEFENCES

INTRODUCTION

In conifers, exudation of constitutive resin as a primary defensive response is not always successful (Christiansen *et al.* 1987). The second line of defense in many cases is the hypersensitive or induced response. Induced resistance can be classified as premunity (non-specific, acquired immunity caused by an earlier inoculation) or hypersensitivity (Klement & Goodman 1967; Levin 1976). The predominant form of induced resistance in conifers to stem-invading insects appears to be the hypersensitive reaction, also known as induced resinosis. The hypersensitive reaction encompasses all morphological and histological changes that, when produced by an injurious agent, elicit premature dying, or necrosis, of the infected tissue, as well as inactivation and localization of the infectious agent (Fernandes 1990). Hypersensitivity is usually controlled by an individual gene, and rarely by a few (Fernandes 1990). In general, hypersensitive cell death is followed by synthesis of defensive chemicals and in many plants, lignification of tissues (Creasy 1985). The function of the hypersensitive reaction appears to be restoration of lateral meristems (phellogen and vascular cambium) and blocking of conductive sapwood (Mullick 1977), which inactivates and/or localizes the infectious agent (Klement & Goodman 1967; Russell & Berryman 1976; Fernandes 1990). In conifers, the hypersensitive reaction usually spreads in

advance of fungal invasion and if powerful enough, either prevents bark beetle attack or causes it to abort (Lieutier & Berryman 1988). Hypersensitive reaction, induced resinosis and secondary response are used interchangably in the literature, and the terminology used by different authors will be maintained in this text. The hypersensitive reaction occurs in response to inoculation by specific pathogens, but also occurs to a lesser extent in response to non-specific mechanical wounding. Kuć & Lisker (1978) observed that resin flow in white spruce lasts much longer after fungal infection than after mechanical wounding. Similarly, Raffa & Berryman (1982) observed that the defensive response of grand fir, *Abies grandis* (Douglas) Lindley, was less pronounced after mechanical injury than microbial insult. Contrary to Klement & Goodman (1967), Lewinsohn *et al.* (1991a) observed that monoterpene cyclase activity was proportional to the magnitude of injury in several conifers. The hypersensitive response can also be induced in conifers by chitosan, a mixture of β (1-4)-glucosamine polymers, which are constituents of arthropod integuments and fungal cell walls (Lieutier & Berryman 1988), and to a lesser extent by a proteinase inhibitor inducing factor (PIIF), which is derived from plant cell walls. Both Miller *et al.* (1986) and Croteau *et al.* (1987) observed the induction of the hypersensitive response in lodgepole pine, *Pinus contorta* Dougl. *var latifolia,* by chitosan and PIIF. In general, the concentration of inoculum does not appear to influence the formation of the hypersensitive reaction but affects the development of visible necrosis (Klement & Goodman 1967). The hypersensitive reaction tends to occur most quickly in young tissue and may be affected by environmental conditions (Klement & Goodman 1967).

Kemp & Burden (1986) observed that resinosis can also be induced by herbicide treatments, which may result in elevated metabolism, but Bergvinson & Borden (1992) found that the herbicide glyphosate caused an apparent inhibition of the secondary response of lodgepole pine to *Ophiostoma clavigerum* (Robinson-Jeffrey & Davidson).

Wounding or infection often disrupts normal patterns of terpenoid synthesis and degradation in plant cells. These changes may be related to wound repair or a disease resistance mechanism (Kuć & Lisker 1978). Accumulated terpenes can inhibit the growth of bacteria and fungi and in this case are classed as phytoalexins (Kuć & Lisker 1978). Dying parenchyma cells form fungistatic or fungitoxic compounds in the sapwood of trees after wounding or fungal attack (Kemp & Burden 1986). Klepzig *et al.* (1995) characterized the response of red pine, *Pinus resinosa* Ait., to artificial inoculation with a bark beetle-vectored fungus, *Leptographium tenebrantis* Barras and Perry. Artificially-inoculated trees contained high concentrations of phenolics and total monoterpenes, specifically α-pinene, β-pinene, 3-carene, limonene, camphene and myrcene. Concentrations increased with time after inoculation. Similarly, Shrimpton (1973) found that total terpene and phenolic levels increased in the sapwood of lodgepole pine in response to attack by the mountain pine beetle, *Dendroctonous ponderosae*, Hopkins, and its associated microorganisms, and that these extractives were not strikingly different in composition from compounds normally present in heartwood. The hypersensitive response occurs quickly, with a measurable increase in accumulation of extractives in lodgepole pine within three days after a single inoculation with *Europhium clavigerum* Robinson & Davidson (Raffa &

Berryman 1983). Russell & Berryman (1976) observed that the traumatic resin of grand fir contained higher concentrations of myrcene than did constitutive resin in 75% of trees sampled, and Raffa & Berryman (1982) found this increase to be exponential over time. Gref & Ericsson (1985) found that total resin acids, especially dehydroabietic acid, increased in the bark of Scots pine, *Pinus sylvestris* (L.), in response to mechanical wounding. Abietic and dehydroabietic acid are the major compounds in resin-soaked sapwood in white spruce, *Picea glauca* (Moench) Voss, and while pure resin acids are very toxic to some fungi, they lose their toxicity when mixed with oleoresin (Kuć & Lisker 1978). Chencilet (1987) observed that wounding did not affect the relative proportions of terpenes in maritime pine, *Pinus pinaster* Ait, but the ratio of resin acids to terpene hydrocarbons differed between primary and secondary resin. Stilbenes, the major form of condensed tannin in Sitka spruce (Forrest 1975; Woodward & Pearce 1988), derived from a phenylpropanoid precursor, were induced non-specifically in the living cells of red pine sapwood following injury or fungal attack (Kemp & Burden 1986). Phloem resinosis was accompanied by secretion of phenolic compounds in Ponderosa pine, *Pinus ponderosae* Lawson, lodgepole pine and western white pine, *P. monticola* Douglas (Lieutier & Berryman 1988). Lewinsohn *et al.* (1991b) found that in *Abies* and *Picea* species, the level of monoterpene cyclase activity increased five-to 15-fold, seven days after mechanical wounding. Croteau *et al.* (1987) also observed that elevated levels of monoterpenes and diterpene resin acids in wounded lodgepole pine were caused by a transient increase in the ability to biosynthesize cyclic monoterpenes and diterpene resin acids.

A number of histological changes can be observed after wounding. Barckhausen (1978) observed that specialized cells beneath a wound surface de-differentiate, lose their original functions, regain mitotic activity, and ultimately form meristematic tissues. These wound cambiums produce the wound periderms which are barrier zones of cells that are impervious to most bark-inhabiting fungi and bacteria (Shigo 1984). Tomiyama (1963) studied the process of infection of snowblight fungus, *Typhula incarnata* Lasch ex Fr., into wheat cells. They observed that protoplasmic granules gathered at the site of hyphal invasion, and that the nuclei also migrated towards the site of infection. Carroll (1966) observed the breakdown of chloroplasts as lesions developed in response to tobacco mosaic virus. Similarly, Goodman (1968) observed distortion of chloroplast conformation 6 h after infection of a tobacco leaf. This was attributed to the breakdown of the S-S bonds of the membrane proteins resulting in a change in host cell permeability. In an electron microscopic study of tobacco mosaic virus lesions, Weintraub & Ragetli (1964) observed that the size of starch grains in cells around the lesions increased within 8 h, and cytoplasm and chloroplast membranes ruptured. Within 24 h, an increase in the number of mitochondria was observed, and within 60 h some mitochondria became electron-opaque and some disintegrated. Eventually the cell contents became compressed into one area of the cell, which eventually collapsed away from the cell walls. The nuclei remained stable and intact.

Parenchyma cells live for many years and contain stored energy reserves which provide the substrate for synthesis of antimicrobial phytoalexins (Shigo 1984).

As the respiration rate, oxidase levels, and peroxidase levels increase in response to wounding or infection, so do numbers of mitochondria (Fernandes 1990). In grand fir, the formation of traumatic resin canals was observed at the cambium-sapwood interface after attack by *S. ventralis* (Berryman & Ashraf 1970). Also, the parenchyma cells surrounding the site of attack became resinous, increased in volume and eventually became resin cavities (Berryman 1969). Gallery excavation resulted in embolism in many tracheids (Berryman 1969). Synthesis of secondary resins was directly associated with parenchyma cells in the phloem and in the rays of both phloem and sapwood in three pines (Lieutier & Berryman 1988). They also noted that the tracheids that were impregnated with resin were not necessarily associated with longitudinal resin ducts (Lieutier & Berryman 1988). Chencilet (1987) observed that in wounded phloem of maritime pine, the neosynthesis of terpenes takes place in reactivated supernumary resin ducts which display structural features similar to those of active secretory cells in the normal condition. A reorganization of the epithelial cells lining the ducts took place.

The hypersensitive reaction appears to have an important role in conifer defense against bark-inhabiting insects. When a bark beetle excavates its gallery, it contaminates the phloem and cambial tissues with a number of microorganisms, including species-specific symbiotic fungi. Endogenous and exogenous elicitors diffuse from wounded cells and initiate the hypersensitive wound reaction (Raffa 1991). Necrotic cells form an elliptical lesion, devoid of nutrients and impregnated with resinous materials whose volatiles have fungistatic or toxic properties

(Christiansen & Bakke 1988; Raffa 1991). In addition, these tissues may be toxic to eggs and larvae, mechanically too hard to penetrate, and indigestible (Berryman 1972). As the number of infection sites increases, the defense may become exhausted. If so, the resin content of the individual reaction zones decreases, and the fungus and beetles eventually penetrate the wood (Christiansen & Bakke 1988). Necrosis generally spreads in advance of the fungus-beetle complex (Raffa 1991). Trees that are able to respond quickly and strongly can cause beetles to abort their attack (Berryman & Ashraf 1970; Lieutier & Berryman 1988). If egg galleries are flooded with resin the eggs do not survive, and if resinosis occurs after brood establishment, complete mortality occurs (Berryman & Ashraf 1970). The embryo either dies within the chorion, or the first instar larva dies. Berryman & Ashraf (1970) suggest that the secondary resins produced during the hypersensitive reaction are of greater importance than primary resin flowing from preformed resin canals in resistance to bark beetle attack. Berryman (1969) observed that the gallery establishment of *S. ventralis* was inversely related to the rapidity of the host's ability to produce secondary resin in the cells of the phloem parenchyma. Gallery elongation was determined by the speed of the host's response, as the beetles abandoned their galleries as soon as the resin flowed into it (Berryman & Ashraf 1970). Tomiyama (1963) observed that in general, the earlier the death of host cells, the more resistant is the plant. In many plants, the browning associated with the hypersensitive death of cells occurs most rapidly in resistant varieties, and the increase in phenolic compounds is also rapid (Tomiyama 1963). Fungal hyphae die some hours after the death of the host cell because they

are not able to grow through necrotic tissue (Tomiyama 1963). Bordasch & Berryman (1977) observed that the wound reaction vapours of grand fir contained higher concentrations of repellent monoterpenes than the constitutive cortical resin vapours. The increase in diterpene resin acids associated with the hypersensitive response of conifers may also affect the physiology of attacking insects. Björkman & Larsson (1991) found that European pine sawfly larvae, *Neodiprion sertifer* (Fourcroy), fed on high resin-acid needles produced larger defensive droplets than larvae fed on low resin acid needles, but at a cost of lower growth rate. Similarly, Larsson *et al.* (1986) observed longer development times for sawflies fed on high resin-acid diet, but no significant difference in cocoon weight. Larvae on high resin acid diet suffered greater mortality, especially in the first two instars. Wagner *et al.* (1983) found the diterpene resin acids, abietic acid, neoabietic acid, dehydroabietic acid, and isopimaric acid significantly reduced consumption rates, feeding efficiencies and growth rates of larch sawflies, *Pristophora erichsonii* (Hartig), when applied to needle tufts of tamarack, *Larix laricinia* (DuRoi) K. Kock.

There is evidence that *P. strobi* is affected by the induced hypersensitive response of its host. Overhulser & Gara (1981a) found that the effects of Sitka spruce resin on brood survival between the egg stage and the fourth instar were the major cause of mortality, with most occurring earlier than later in development. Sullivan (1960) observed in eastern white pine, *Pinus strobus* L., that a high percentage of mortality occurs during early larval development when competition is high and low temperatures slow larval movement, permitting pitch drowning.

Mortality due to pitch flow is greatest early in the season before the leader is girdled (Silver 1968). Overhulser & Gara (1981b) discovered in Sitka spruce that occluded resin canals often bordered egg chambers of *P. strobi*. These occlusions resembled tyloses that develop in the non-functional resin canals found in the heartwood of some conifers and might be associated with the process of phellogen restoration after wounding. Although some bark beetles are tolerant of resin from primary resin canals, in trees with well-developed resin canals, galleries are often oriented vertically, rather than horizontally, presumably to avoid them (Berryman 1972). In *Abies spp.*, beetles rarely contact preformed resin which is in cortical pitch blisters (Russell & Berryman 1976). The occlusion of resin canals observed by Overhulser & Gara (1981b) may prevent the flow of primary resin into oviposition cavities. Santamour (1965) observed that crushed heads of *P. strobi* larvae induced differing extents of crystallization of resin in several species of pines and their hybrids, with shoot resin having a stronger tendency to crystallize than wood resin. van Buijtenen & Santamour (1972) correlated non-crystallizing resin with a low incidence of weevil attack in eastern white pine. Alfaro (1995) observed that traumatic resin canals form in the xylem of interior spruce, (the complex of white spruce, *Picea glauca* (Moench) Voss, Engelmann spruce, *Picea engelmannii* Parry, and their hybrids) (Kiss & Yanchuk 1991), in response to injury by the white pine weevil. Resin from these canals can flow into oviposition cavities, presumably killing eggs and larvae. In a stand of Sitka spruce in southwestern Washington, Overhulser *et al.* (1972) found that 70% of the emergent weevil population was produced by trees that were attacked for the first

time. It is possible that induction of the hypersensitive response in Sitka spruce provides protection to trees for some period of time.

Assuming that Sitka spruce is capable of a hypersensitive response, and that infestation of a leader by *P. strobi* is analogous to infestation of a bole by bark beetles, variation in the speed or intensity of that response between clones might be a basis for resistance against *P. strobi*. Histological examination of different clones may reveal differences in the size of lesions, rapidity of lesion development, induction of resin canals in the phloem and xylem, occlusion of resin canals, and visible changes in parenchyma tissue, such as accumulation of phenolics and other materials, or cell death. In addition, if monoterpenes and resin acids were shown to have a negative effect on eggs or larvae, the hypothesis that induction of the hypersensitive response is a resistance mechanism in Sitka spruce would be further supported.

The objectives of this study were: 1. to use histological techniques to compare the hypersensitive reaction in the bark and xylem of different genotypes of Sitka spruce to determine if either the intensity or speed of reaction varies, and 2. to determine the effect of volatile terpenes and resin acids on the egg hatch and larval development of *P. strobi*. Much of the reported research is preliminary and exploratory in nature.

METHODS

Histology of Wound Reaction in Bark

Samples of uninfested bark, and bark on which weevils had been allowed to feed and oviposit in the laboratory were collected from branches of the same clones used in the no-choice feeding and oviposition bioassay described in (Section V). Six days after feeding and oviposition punctures were formed, the punctures and enough surrounding tissue to include the entire reaction lesion were excised. The maximum length and width of one feeding and one oviposition lesion in the cortex from each branch were measured immediately using an ocular micrometer on a dissecting microscope. Lengths and widths were compared by ANOVA followed by Ryan's Q tests.

Two 1 mm lengths of lesion from a feeding puncture on each branch were excised. For examination of general cell morphology, tissue was fixed for 16 h in 3% glutaraldehyde in 0.04 M phosphate buffer at pH 6.8. For examination of the distribution of tannins (and possibly other phenolics), the other portion was fixed for 48 h in aqueous ferrous sulphate and glutaraldehyde (Johansen 1940). Fixed samples were dehydrated in ethanol to 95%, and embedded in JB-4 resin (J.B. EM Services Inc., Dorval, Quebec). Blocks were sectioned using glass knives on an ultramicrotome to a thickness of 1.5 μm, and sections were stained for 1 min. in methylene blue (Johansen 1940), and mounted on glass slides with Permount.

Three characteristics that could be quantified between clones under examination by microscopy were assigned scores for low, medium and high prevalence (Table 19).

The proportion of observations falling into each category were then calculated for each clone (all replicates pooled), further quantified by multiplying the proportion of observations by 1, 2 or 3, and summing these to produce a single score for each clone, for each of the three categories of observation. The mean value of scores, averaged across clones, for unwounded tissue, and tissue adjacent to feeding punctures were then compared by a Wilcoxon two-sample test (NPAR1WAY, SAS Institute 1988).

Progression of Lesion Development After Artificial Wounding

The rate of lesion growth was examined in 40 potted four year-old seedlings from the same unknown susceptible seed source. The trees were divided into four groups of 10. Half of the trees in each group were designated as the controls. On day zero, weevil attacks were simulated on the other five trees in each group by making 10, 1 mm diam. drill holes through the bark without penetrating the xylem, starting from the top of the leader and working down, with holes 1 cm apart. The trees were arranged in a completely randomized design in an outdoor enclosure, and watered regularly. On day two, 10 trees were removed from the experiment (five treatment and five controls) and the length of five lesions from each treatment tree measured as described previously. Regression analysis was used to quantify the change in lesion length over time (PROC GLM, SAS Institute 1988). In addition, a sample of tissue was excised from each of the treatment and control trees and fixed for histological examination of general cell morphology as described above. This sampling procedure was repeated on days four, eight and 15.

Table 19. Criteria used to compare histological changes in bark tissue between damaged and undamaged clones.

Characteristic	Class (score)	Criterion
Degree to which the cells lining resin ducts stained darkly	High (3)	All or most cells filled
	Medium (2)	All cells partially filled or at least 50% cells completely full
	Low (1)	Most empty or < 50% of the cells filled
Frequency of very dark-staining parenchyma cells	High (3)	≥ 50% dark
	Medium (2)	25% < 50% dark
	Low (1)	< 25%
Frequency of clear parenchyma cells containing granular inclusions.	High (3)	Many
	Medium (2)	Few
	Low (1)	Very few or none

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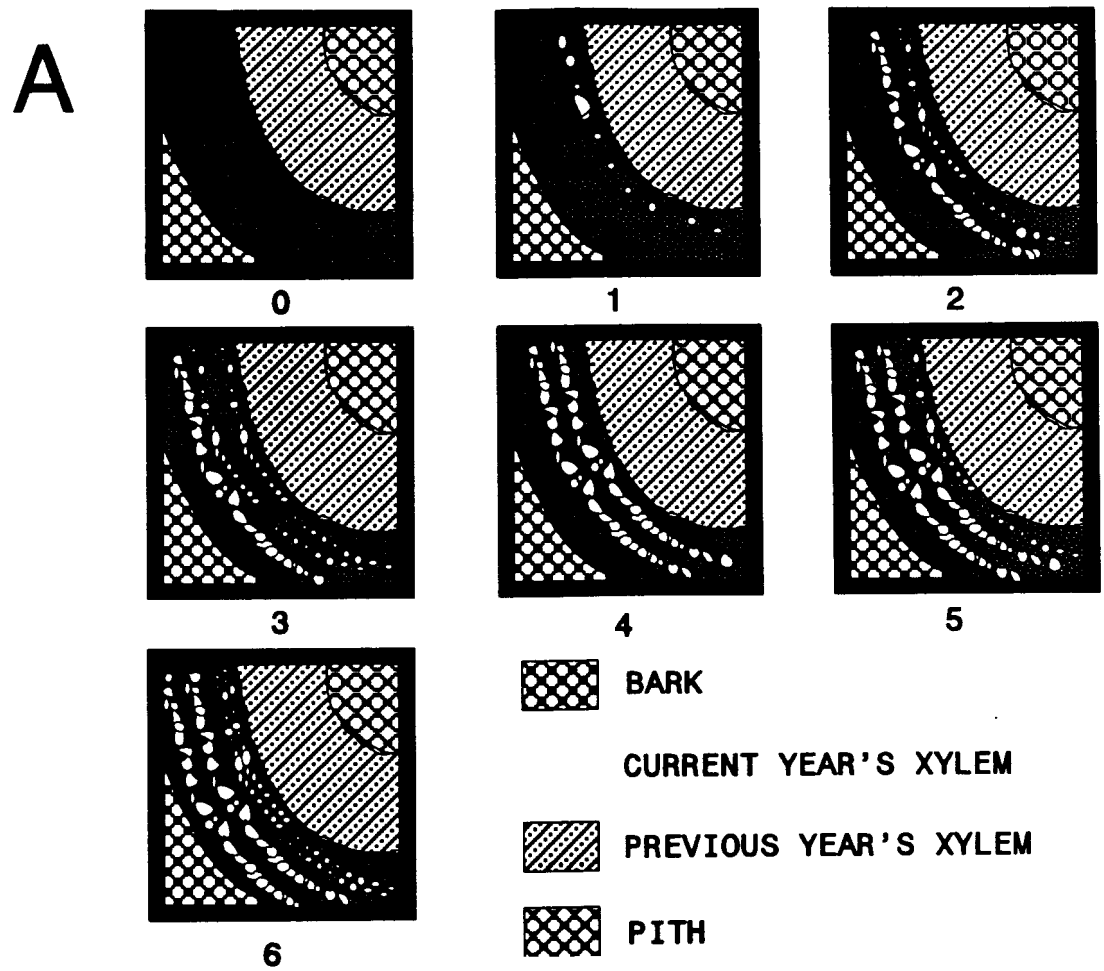
Induction of Traumatic Resin Ducts in Xylem

To determine if, like interior spruce (Alfaro 1995), Sitka spruce produces traumatic resin canals in the xylem in response to weevil attack, samples were collected from replicates of four resistant, and one susceptible clone at Fair Harbour. These trees had been assessed in 1990 (G. Brown, Canadian Forest Service, Pacific Forestry Centre, Victoria, B.C., *pers. comm.*) as to whether their leaders had feeding punctures only, feeding and oviposition punctures, killed, or were completely asymptomatic. In October, 1994, 5 mm diam. core samples were taken from the 1990 internode (ie. the ex-leaders) of 12, 22, 21 and 23 trees in each respective damage category including five different clones, 29-1-3, 29-UK-7 (Haney), 2G (Green Timbers), 18-12-5 (Cedarvale) and 32-5-2 (Fair Harbour). There were 2-4 replicates of each clone within each damage category. The cores penetrated to the pith, ensuring that the 1990 xylem was removed. They were fixed in formalin-acetic acid-alcohol (FAA) (Johansen 1940) for at least 24 h and then transferred to 70% ethanol. Sections were cut (60 μm thick) with a sliding microtome, stained in 1% aqueous safranin, and mounted in Gurr's medium on glass slides. A scoring system for rating resin duct induction in interior spruce (R.I. Alfaro, Pacific Forestry Centre, Victoria, B.C., *pers. comm.*) was adapted for use on Sitka spruce in which there was a very low level of resin duct induction (Fig. 26). Differences in score between clones were compared separately for each category of damage by a Kruskal-Wallis test (NPAR1WAY, SAS Institute 1988).

Figure 26. Scoring system to assess induced resinosis in the xylem of a) white spruce (Alfaro 1995, pers. comm.) and b) Sitka spruce. Traumatic ducts are indicated by white circles, and score for each level of damage is indicated below each box.


A
0
1
2
3
4
5
6
BARK
CURRENT YEAR'S XYLEM
PREVIOUS YEAR'S XYLEM
PITH
B
0.1
0.3
0.5
0.8
1
1.5

Effects of Resin Constituents on Egg Hatch and Larval Development

The effect of resin constituents on egg hatch and larval development were assessed in two types of experiments.

Egg Hatch

Sitka spruce leaders were collected in Terrace in early May 1995. They were stored at 4°C until May 21, when *P. strobi* eggs were excised and rinsed in zephiran chloride (Zerillo & Odell 1973). In each of these experiments, Petri dishes were lined with moist filter paper. The paper was treated with a stimulus or control solution, and a group of eggs was placed on the treated substrate.

For analysis of the effect of resin acids, 12 dishes were split into four treatment groups: methanol control, undiluted resin acid stock solution, and 10 or 1% dilutions thereof (Table 15). Twenty μl of each experimental or control treatment was applied to the filter paper, then 9-12 eggs were placed in each dish, and the dishes were held on a laboratory bench under ambient light conditions and a temperature of about 25°C. Each treatment was replicated three times. The number of eggs that hatched were recorded daily until all had either hatched or died. The filter papers were misted daily with distilled water to prevent desiccation.

Differences between the number of eggs hatched on each day were compared using analysis of variance (PROC ANOVA, SAS Institute 1988).

For determination of the effect of monoterpenes, one Petri dish, prepared and maintained as above, was treated with 50 μl of hexane, and a second dish was treated with 50 μl of 50% distillate of Sitka spruce needle oil. Bark oil was not available;

however bark and needles have at least 8 monoterpenes in common (Brooks *et al.* 1987a). Twelve eggs were placed in each dish, and the numbers that hatched were recorded daily until all had either hatched or died. Differences between numbers of eggs hatched in treatment and control were compared on each day by a chi-square test (Zar 1984).

For the third experiment, 0.28 g of bark resin from susceptible Sitka spruce branches, was bled directly from cortical resin ducts, and dissolved in 0.5 ml hexane. The filter paper lining these Petri dishes for each treatment was treated with 50 μL of hexane or resin solutions at concentration of 0.14, 0.07, 0.035 g per mL. Each treatment dish was replicated three times. Ten *P. strobi* eggs were added to each Petri dish and the number of eggs that hatched recorded daily for eight days. Dishes were maintained as above.

Differences between the number of eggs hatched on each day were compared using analysis of variance (PROC ANOVA, SAS Institute 1988).

Larval Development

The effect of bark resin constituents on *P. strobi* larvae were investigated. Only one experiment could be conducted because few insects were available. Resin acids were chosen because the amount and composition in the bark are known, and there is a clear relationship between amount and resistance (Fig. 15.) To make a resin acid-treated diet, 0.75 ml of the resin acid stock solution (Table 15) was added to 225 ml of diet (Section V), simulating the bark composition of Kitwanga trees. The

control diet had 0.75 ml of methanol added to the same amount of diet. The two treatments were replicated 10 times each, in 18 mL wells containing 5 mL of diet arranged in a completely randomized block design in a plastic tray. After 11 and 18 days, larvae were transferred to fresh diet that had been stored at 4°C. These transfers avoided contamination by microorganisms and prevented the larvae from running out of food. Five *P. strobi* eggs from the same source, and treated in the same manner as in the egg hatch experiment were buried in the diet in each well so that larvae would not desiccate after hatching; the tray was covered with a plastic lid with 0.5 mm holes punched in the top with a sharp probe. At 11, 18, 30, 33, 41, 46 and 54 days after the experiment was set up, the numbers of larvae, pupae or adults were counted, and the head capsule diameters of larvae were measured using an ocular micrometer on a dissecting microscope.

RESULTS AND DISCUSSION

Histology of Wound Reaction in Bark

There was no difference in the width of the lesions at either feeding punctures (F=1.60; df=8,32; P=0.1639) or oviposition punctures (F=0.78; df=8,12; P=0.6267) among clones. Lesion lengths caused by feeding punctures did not differ between clones (F=1.11; df: 8,32; P=0.3830), but lesion lengths caused by oviposition punctures varied significantly (F=3.19; df=8,12; P=0.0347) (Table 20). Although the treatments were not found to be different by a Ryan's Q multiple range test, Haney and Cedarvale, two resistant clones, had the longest lesion lengths. The variation in

Table 20. Ranked means of oviposition puncture lesion lengths for cut branches in water of resistant and susceptible clones from Fair Harbour.

Clone and resistant (R) or susceptible (S) classification	Lesion lengths (Mean ± S.E.)[a]	N
29-UK-7 (R) (Haney)	0.90±0.13a	1
18-12-4 (R) (Cedarvale)	0.90±0.13a	1
36-2-4 (S) (Tasu Cr.)	0.88±0.07a	3
15-13-3 (S) (Aberdeen)	0.82±0.07a	3
2G (R) (Green Timbers)	0.80±0.13a	1
6-13-8 (R) (Kitwanga)	0.69±0.09a	2
32-5-2 (S) (Fair Harbour)	0.58±0.07a	3
30-3-7 (S) (Muir Cr., Sooke)	0.56±0.07a	3
37-1-3 (S) (Moresby Camp)	0.56±0.06a	4

[a]Means followed by the same letter are not significantly different ($P<0.05$, Ryans Q test). Means and standard errors were computed by the least squares methods to allow analysis of variance on unequal sample sizes. In the cases where N=1, means and standard errors are estimated by PROC GLM (SAS Institute 1988).

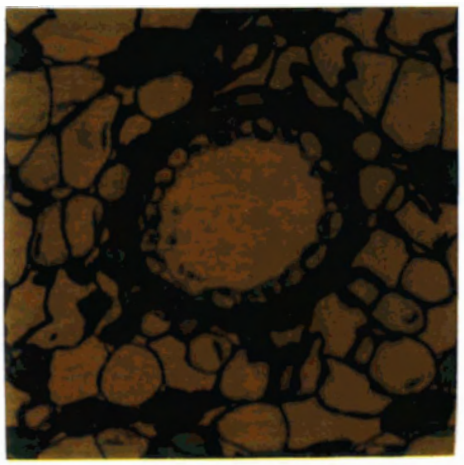
lesion length in response only to oviposition suggests that the trees might be responding to a substance on the surface of the egg, or possibly to a microorganism introduced on the egg or in the oviposition plug.

When examined for both general morphological changes and the prevalence and location of tannins, significant changes were observed in tissue surrounding feeding punctures (Fig. 27). In tissue surrounding feeding punctures the cells lining the lumen of resin ducts tended to stain more darkly than in unwounded tissue (Fig. 27a). There was a greater frequency of darkly-staining parenchyma cells, and clear parenchyma cells containing granular inclusions in wounded tissue (Fig. 27b). In sections stained for tannins, the darkly stained portion of parenchyma cells was restricted to an area close to the cell walls. This may be an artifact of the fixation process, as a buffer was not used, or it may reflect close association of condensed tannins with the cell walls. Underwood & Pearce (1991) observed that deposits of stilbenes, the only form of condensed tannin in Sitka spruce, were localized in the lumen of parenchyma cells, and parenchyma cells adjacent to the periderm in white pine trees were observed to be filled with tannin (Struckmeyer & Riker 1951). Parham & Kaustinen (1977) observed that in cell suspension cultures of Douglas fir, *Pseudotsuga menziessii* (Mirb.) Franco, and loblolly pine, *Pinus taeda* L., that tannins were actually synthesized in rough endoplasmic reticulum that gave rise to small tannin-containing vacuoles. It is possible that these vacuoles were disrupted during fixation, resulting in the pattern of tannin deposits observed. There is no mention in the literature of tannin-containing cells surrounding the lumen of resin ducts. There

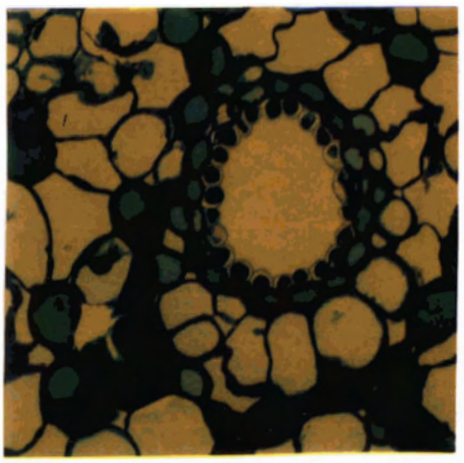
Figure 27. Histological characteristics scored in comparison of wounded and unwounded bark tissue in Sitka spruce. A shows difference in staining characteristics of cells surrounding the lumen of a resin duct. B shows staining characteristics of parenchyma cells.

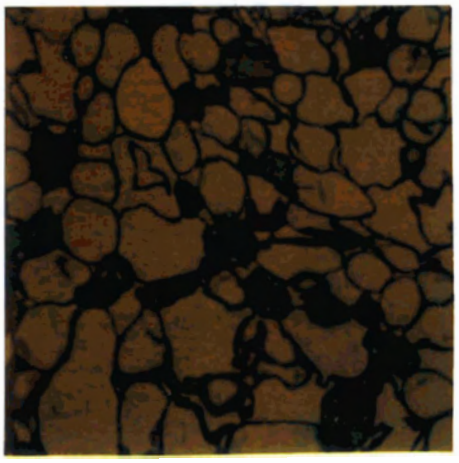
NO WEEVIL FEEDING

WEEVIL FEEDING

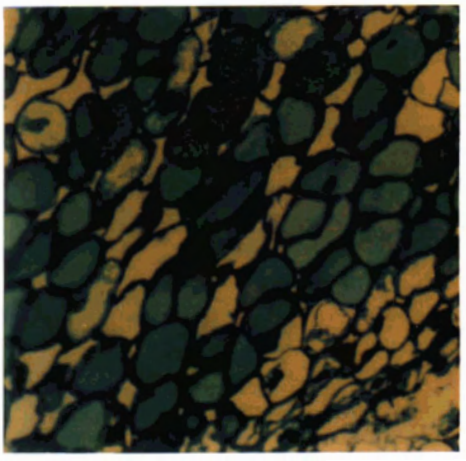


A





B



was no evidence of occluded resin canals in the phloem as observed by Overhulser & Gara (1981b), nor was there evidence of induced resin ducts in the bark. Chencilet (1987) observed that induced resin ducts appeared in the phloem of maritime pine within four days of wounding, so presumably adequate time for reaction had passed.

For sections stained for general morphological observations, the most significant difference between damaged and undamaged tissue was an increase in the prevalence of darkly staining cells lining resin ducts after wounding (Fig. 27a, Table 21). All clones expressed such an increase except those from Green Timbers. These results suggest an increase in the amounts of terpenes or phenolics being synthesized within these cells for secretion into resin ducts in response to wounding. There was a greater frequency of darkly staining parenchyma cells in tissues surrounding feeding punctures than in unwounded tissue (Fig. 27b, Table 21). However, in two clones, Aberdeen (15-13-3)and Cedarvale (18-12-4), the frequency decreased. The increase in darkly staining parenchyma might indicate a change in metabolism in these cells, resulting in *de novo* synthesis of terpenes and phenolics. Using aqueous cupric acetate to stain the resin-soaked tissue, Lieutier & Berryman (1988) observed that the synthesis of secondary resins was directly associated with phloem parenchyma cells surrounding an injury in three different species of pines. On average, there was no significant increase in the frequency of clear parenchyma cells containing inclusions (Table 21).

In sections stained for tannins, the proportion of darkly-staining cells lining resin ducts increased after weevil feeding (Table 21), again suggesting an increase in

Table. 21 Difference in scores (Table 19) between unwounded tissue and tissue adjacent to feeding puncture fixed and stained for observation of general morphology or the prevalence of tannins, averaged across clones.

Preparation	Characteristics	Unwounded tissue	Tissue adjacent to feeding puncture	*P* value
Fixed for general morphological observation	Proportion of dark cells around resin ducts	1.27±0.13	2.51±0.13	0.0001
	Frequency of dark parenchyma	1.61±0.12	2.10±0.12	0.0109
	Frequency of parenchyma with inclusions	1.72±0.16	2.07±0.16	0.1349
Fixed for observation of tannins	Proportion of dark cells around resin ducts	1.28±0.17	2.41±0.17	0.0002
	Frequency of dark parenchyma	1.54±0.15	1.65±0.15	0.6282
	Frequency of parenchyma with inclusions	1.67±0.19	2.04±0.19	0.2099

amounts of phenolic material. In contrast to sections stained for general morphology, the frequency of darkly-staining cells after wounding did not increase (Table 21). There was also no increase in the number of clear parenchyma cells with inclusions (Table 21). As with sections stained for general morpholgy, clones varied in their degree of response, but there was no relationship with resistance status.

Progression of Lesion Development After Artificial Wounding

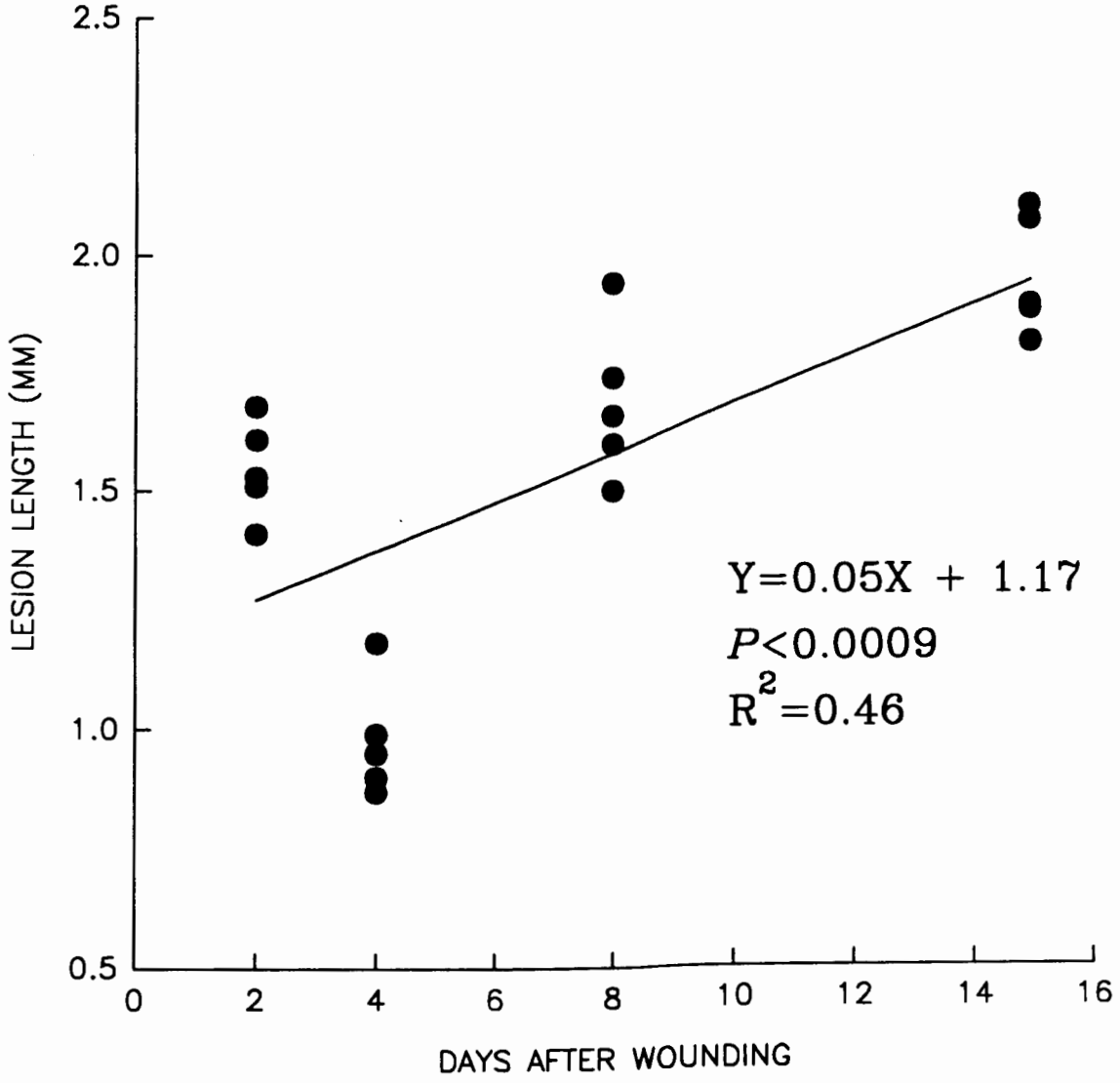
The lengths of lesions caused by drilling increased linearly over time (Fig. 28). In contrast to changes in the phloem tissue caused by weevil feeding (Fig. 27), there was no observable change in tissue characteristics after drilling. The experiment was thus not repeated using different clones. Sitka spruce bark clearly does not respond to mechanical wounding as strongly as it does to either weevil feeding or oviposition. This result suggests that weevil activity could be associated with a microorganism that stimulates the hypersensitive reaction of the tree, or that some component of weevil saliva causes a reaction in bark tissue.

Induction of Traumatic Resin Ducts in Xylem

Most of the induced resin ducts observed were in the latest portion of the 1990 xylem, where the cells become smaller and denser than in the springwood. This might be a result of Sitka spruce having a slower response time than interior spruce or, a result of significant lateral growth occurring before weevil oviposition.

There was no difference in induced resistance scores between clones for any

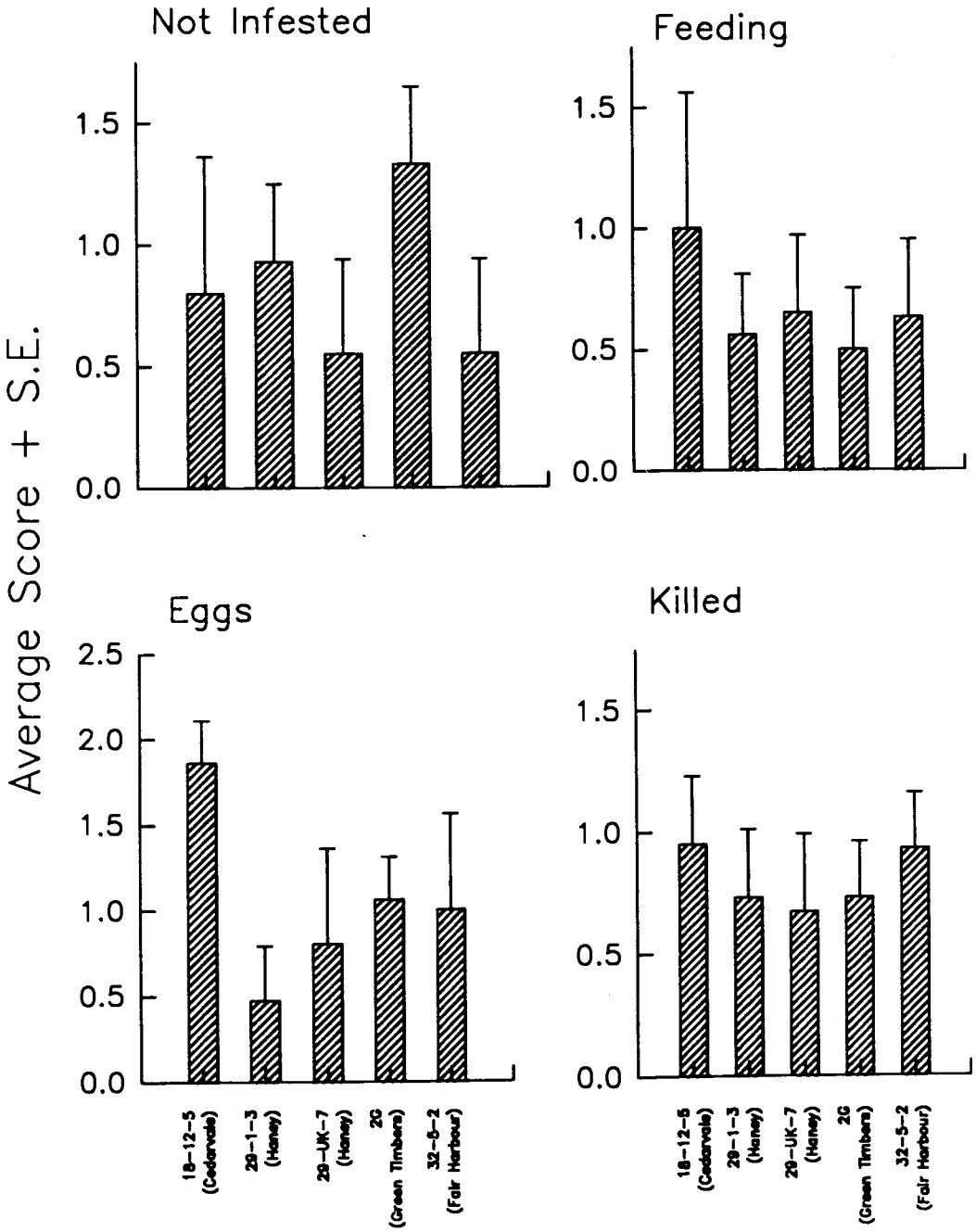
Figure 28. Relationship between lesion lengths and duration after artificially wounding susceptible Sitka spruce leaders with a 1 mm drill bit.


2.5
2.0
1.5
1.0
0.5
LESION LENGTH (MM)
0
2
4
6
8
10
12
14
16
DAYS AFTER WOUNDING
Y=0.05X + 1.17
P<0.0009
R2=0.46

category of damage (Fig. 29).

Sitka spruce evidently does not produce traumatic resin ducts to the same degree as interior spruce (Alfaro 1995) (Figs 26, 29). I hypothesize that Sitka spruce relies more heavily on the cortical resin canal system than does interior spruce. Weevils are probably active for a greater part of the year on the coast than in the interior because of the lower elevation and milder winters (Hulme 1990). Therefore Sitka spruce would be vulnerable to feeding damage for prolonged durations, and would be more 'apparent' (Feeny 1975) than interior spruces. Matson & Hain (1983) hypothesized that the degree to which the preformed resin canal system is developed depends on how frequently trees are attacked. By surveying the literature, they concluded that species subject to many asynchronous generations of bark beetles every year were most likely to rely on a constitutive resin canal system, and species that harboured very few synchronous generations per year were most likely to rely heavily on induced resinosis. They suggested that pre-formed resin would be energetically less expensive than a hypersensitive response that is continually induced. It would likely be metabolically less expensive for coastal spruces to maintain a strong constitutive defensive system, and for interior spruces, which may be under attack for a shorter period of time to rely more on induced than constitutive mechanisms.

Figure 29. Comparison of induced resistance scores (based on Fig. 26) in the 1990 sapwood clones from Fair Harbour that were diagnosed as not infested, feeding punctures or eggs, or having been killed by weevil attack.


Not Infested
Feeding
Eggs
Killed
Average Score + S.E.
0.0
0.5
1.0
1.5
2.0
2.5
18-12-5 (Cedarvale)
29-1-3 (Haney)
29-UK-7 (Haney)
2G (Green Timbers)
32-5-2 (Fair Harbour)

Effects of Resin Constituents on Egg Hatch and Larval Development

Egg Hatch

Exposure to the stock solution of resin acids significantly accelerated hatching of most eggs by one day, but this increase was only significant on day 7 (F=7.19; df=3,18; P=0.0117) (Fig. 30). No other treatment had any effect. Exposure to Sitka spruce needle oil significantly reduced the number of eggs hatched on day 3 (χ^2=4.28; df=1; P<0.05) and day 5 (χ^2=4.44; df=1; P<0.05) (Fig. 31). Sitka spruce cortical resin did not significantly affect the number of exposed eggs that hatched on days 1-6, but numbers were significantly different on days seven (F=7.78; df= 3,8; P=0.0124) and day eight (F=3.81; df= 3,8; P=0.0516) (Fig. 32). These differences were probably caused by exposure to volatile terpenes at the two highest concentrations.

Based on these preliminary experiments, resin acids alone, or volatile terpenes alone appear to affect the hatching of *P. strobi* eggs in offsetting ways, causing exposure to constitutive resin to have relatively little effect. If traumatic resin induced by weevil feeding or oviposition differs in resin acid or monoterpene composition, this may have a significant effect on the survival and hatching of weevil eggs. In these experiments, eggs were not coated with resin constituents, so the possible physical effects of pitch were not tested.

A high ratio of volatile terpenes to resin acids could result in reduced hatching, and death of larvae and unhatched eggs if a feeding ring is not formed rapidly. Traumatic resin in the xylem of interior spruce contains proportionately more volatile terpenes than constitutive resin (E. Conner, Dept. Biol. Sci. , Simon Fraser University,

Figure 30. Effects of exposure to a concentration series of resin acids on the rate of hatching of *P. strobi* eggs. There was a significant difference between treatments in the number of eggs hatched on day 7 (ANOVA, P=0.0117).

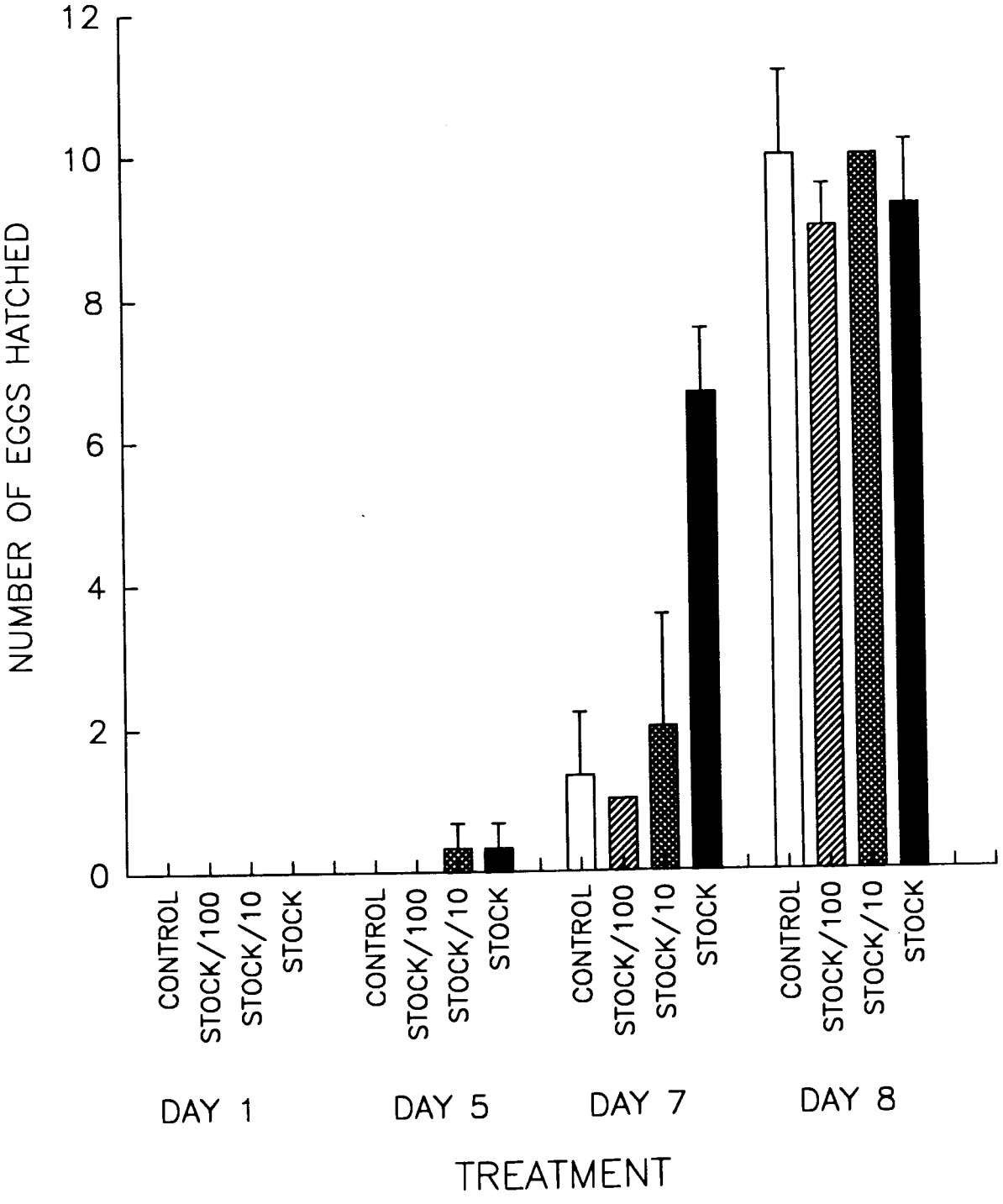

12
10
8
6
4
2
0
NUMBER OF EGGS HATCHED
CONTROL
STOCK/100
STOCK/10
STOCK
CONTROL
STOCK/100
STOCK/10
STOCK
CONTROL
STOCK/100
STOCK/10
STOCK
CONTROL
STOCK/100
STOCK/10
STOCK
DAY 1
DAY 5
DAY 7
DAY 8
TREATMENT

Figure 31. Effect of exposure to Sitka spruce needle oil on the rate of hatching of *P. strobi* eggs. Hatching from day 3 and 5 on was significantly reduced by needle oil treatment, χ^2 test, $P<0.05$.

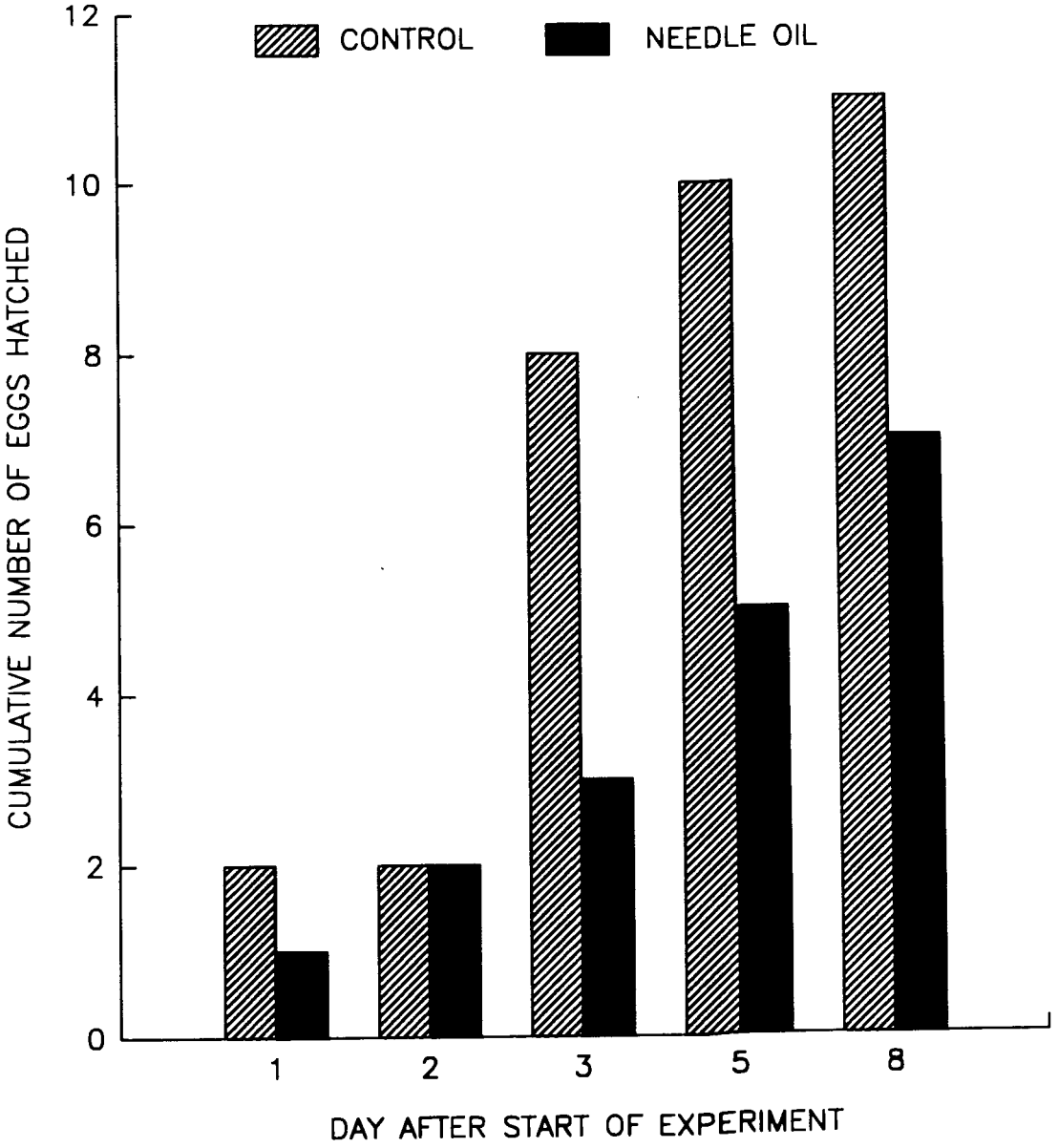
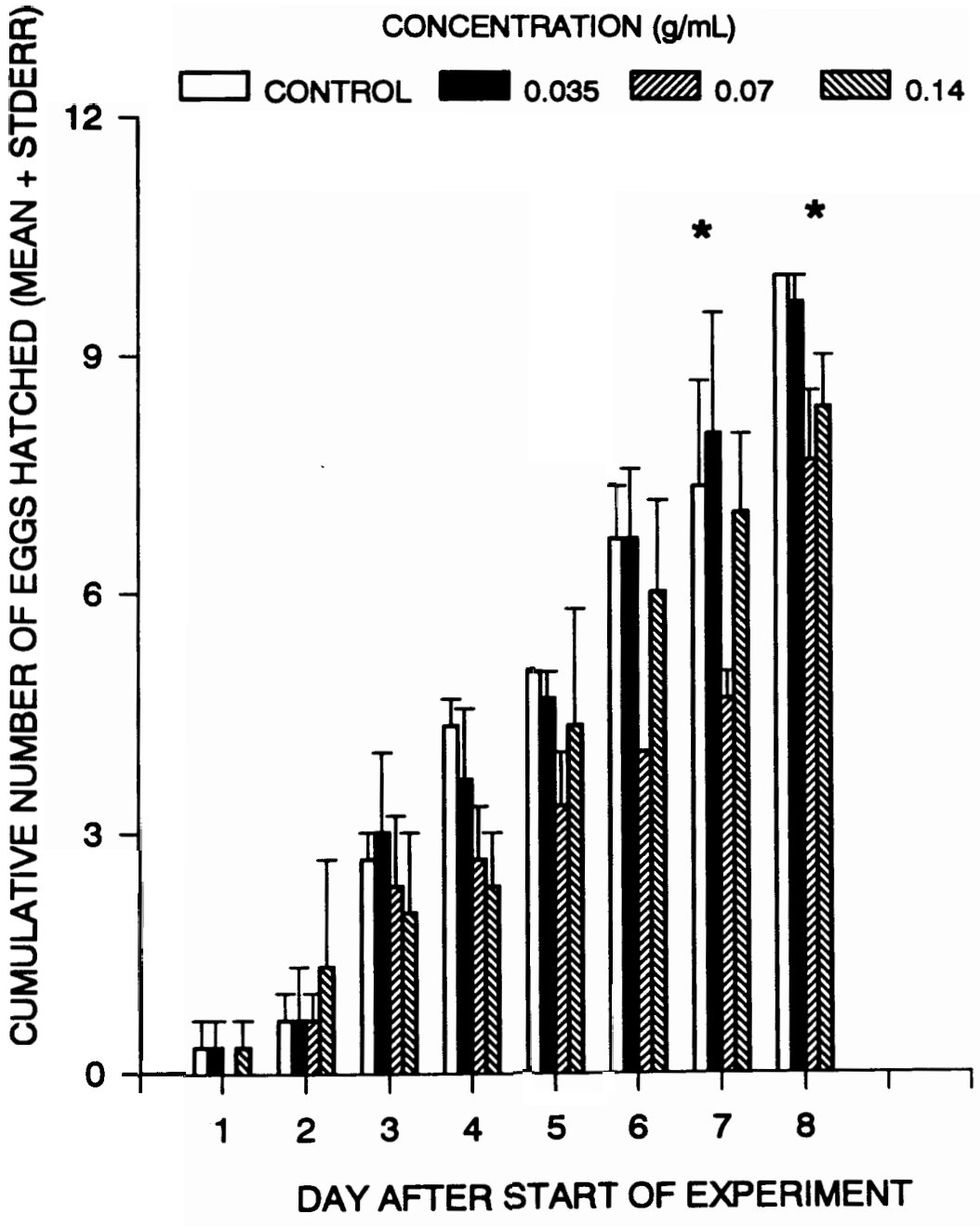

CONTROL
NEEDLE OIL
CUMULATIVE NUMBER OF EGGS HATCHED
0
2
4
6
8
10
12
1
2
3
5
8
DAY AFTER START OF EXPERIMENT

Figure 32. Effects of exposure to a concentration series of Sitka spruce cortical resin on the rate of hatching of *P. strobi* eggs. Asterisks indicate significant differences in egg hatch between resin doses (ANOVA, $P<0.05$).


CONCENTRATION (g/mL)
CONTROL
0.035
0.07
0.14
CUMULATIVE NUMBER OF EGGS HATCHED (MEAN + STDERR)
12
9
6
3
0
*
*
1
2
3
4
5
6
7
8
DAY AFTER START OF EXPERIMENT

pers. comm.), supporting this hypothesis. Premature hatching of eggs caused by excessive amounts of resin acids might result in increased susceptibility to predators and parasites that feed on both eggs and larvae (Stevenson 1967; Silver 1968; Wallace & Sullivan 1985; Hulme 1990; Hulme 1994). Plants can have both direct and indirect effects on natural enemies of herbivores (Price *et al.* 1980; Foster *et al.* 1992). Fritz (1995) showed that the impact of natural enemies on several willow herbivores varied among plant genotypes, in part because the host density varied among resistant and susceptible genotypes, and rate of parasitism was affected by host density. In addition, herbivore position may affect natural enemy search patterns (Price *et al.* 1980), and the hatching of weevil eggs in some leaders earlier than in others might make them more susceptible to predation or parasitism, if larval frass acts as an olfactory cue for natural enemies.

Larval Development

Small larvae were very difficult to find in the diet and were recorded as dead only if the body was actually recovered. Statistical analyses were not performed on these data because there were so many missing observations. Head capsules were measured until July 11, after which time there were too few larvae left to make comparisons. Head capsule diameters did not differ between larvae in the resin acid-treated diet and the controls (Table 22), indicating no effect of resin acids on growth rate. This is in contrast to results found with sawflies (Wagner *et al.* 1983; Larsson *et al.* 1986; Björkman & Larsson 1991) which had reduced growth on high resin acid

Table 22. Average head capsule diameters and numbers of dead larvae recovered in resin acid-treated and untreated diet.

	Head capsule diameter (mean ± S.E.[a])		Number dead (mean ± S.E.[a])	
Day	Control diet	Resin acid diet	Control diet	Resin acid diet
11	0.48±0.04	0.49±0.02	0.10±0.10	0.20±0.10
18	0.89±0.09	0.88±0.05	0.75±0.25	1.10±0.23
30	0.99±0.07	0.91±0.08	0.00±0.00	0.43±0.17
33	1.14±0.07	0.95	0.33±0.21	0.20

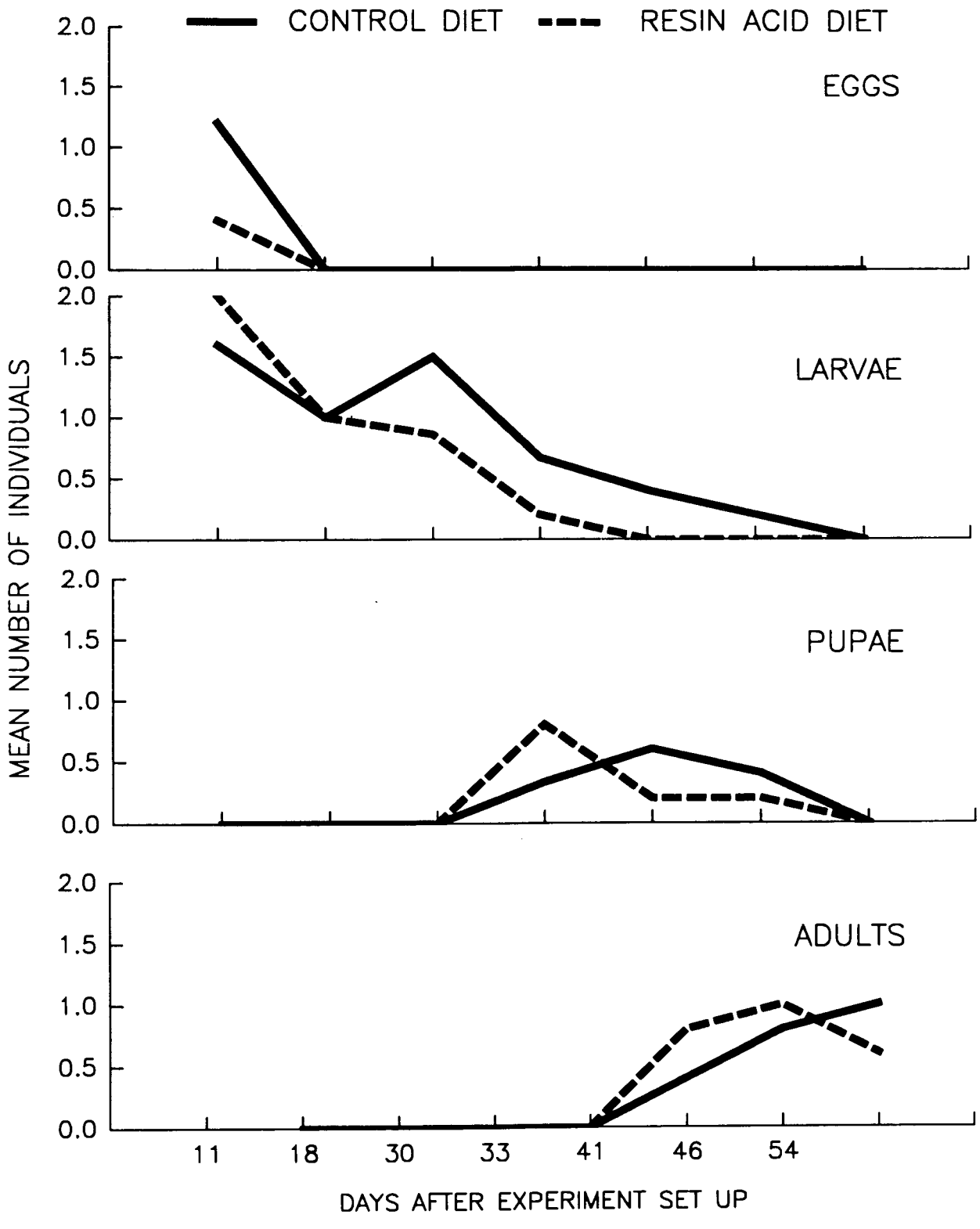
[a]If N=1 then no standard error was reported.

diets. As in Fig. 30, egg hatch occurred more quickly in the resin acid diet than in the control (Fig. 33). There was a corresponding lag in larval development and onset of pupation observed in the control diet, but there was also 39% less mortality in the control than the resin acid diet (Table 22). Adults emerged earlier in the resin acid diet than in the control diet, but also suffered earlier mortality. This result agrees with those of Larsson *et al.* (1986) who found that mortality of larval sawflies was greatest in the first two instars in diets that contained high amounts of resin acid.

CONCLUDING DISCUSSION

My results, although preliminary, indicate that there is a difference in the capacity of different clones to exhibit an induced or hypersensitive response. In addition, Sitka spruce probably relies less on induced resistance than other spruces or pines. There is circumstantial evidence for a microorganism associated with oviposition, and to a lesser degree, feeding. As for bark beetles (Raffa & Berryman 1983), multiple oviposition sites on a leader may be necessary to introduce a microorganism at a density high enough to predispose the leader to successful larval invasion. It is possible that the oviposition plug inserted by the female into feeding punctures after laying eggs is the most effective route for microbial inoculation. Preliminary microbial isolations (unpublished) from feeding and oviposition punctures, oviposition plugs, adult mouthparts and larval frass, suggest that a yeast could be associated with weevil activity.

Figure 33. Comparative growth and development of *P. strobi* in normal agar-based diet, and in diet into which a blend of seven resin acids had been incorporated


CONTROL DIET
RESIN ACID DIET
EGGS
LARVAE
PUPAE
ADULTS
MEAN NUMBER OF INDIVIDUALS
2.0
1.5
1.0
0.5
0.0
11
18
30
33
41
46
54
DAYS AFTER EXPERIMENT SET UP

Further research is necessary before the role of induced resistance in Sitka spruce is clear. Analysis of the monoterpene and resin acid composition of traumatic and constitutive resin should be performed to determine if traumatic resin is different from preformed resin, and if the ability to produce more toxic or repellent resin differs between clones. In addition, the condensed tannin content and composition in damaged tissue should be examined, as it may change in response to injury. Examination of the relationship between the preformed resin duct system and the capacity to produce traumatic resin ducts for both coastal and interior spruce may provide information about the relative importance of these two defense systems. A more thorough analysis of the physiological effects of traumatic resin on *P. strobi* needs to be performed. Dissections of leaders of different clones at regular intervals may provide information about when mortality occurs and why. Studies performed on live tissue are more realistic than on artificial diet because the effect of allelochemicals observed in diets may be reduced or absent in the host plant because of interaction with other chemicals (Myers 1988). Finally, further attempts to determine if there are microorganisms consistently associated with *P. strobi* may provide insight into the induced resistance mechanism. If a microbe is necessary to overcome host defenses, then resistance could be based, in part, on the ability of the fungus to survive in a particular host; thus the effects on *P. strobi* would actually be indirect. The ability of Sitka spruce clones to exhibit a hypersensitive response may be an important future addition to a multicomponent resistance index.

VII. MULTICOMPONENT RESISTANCE INDEX

INTRODUCTION

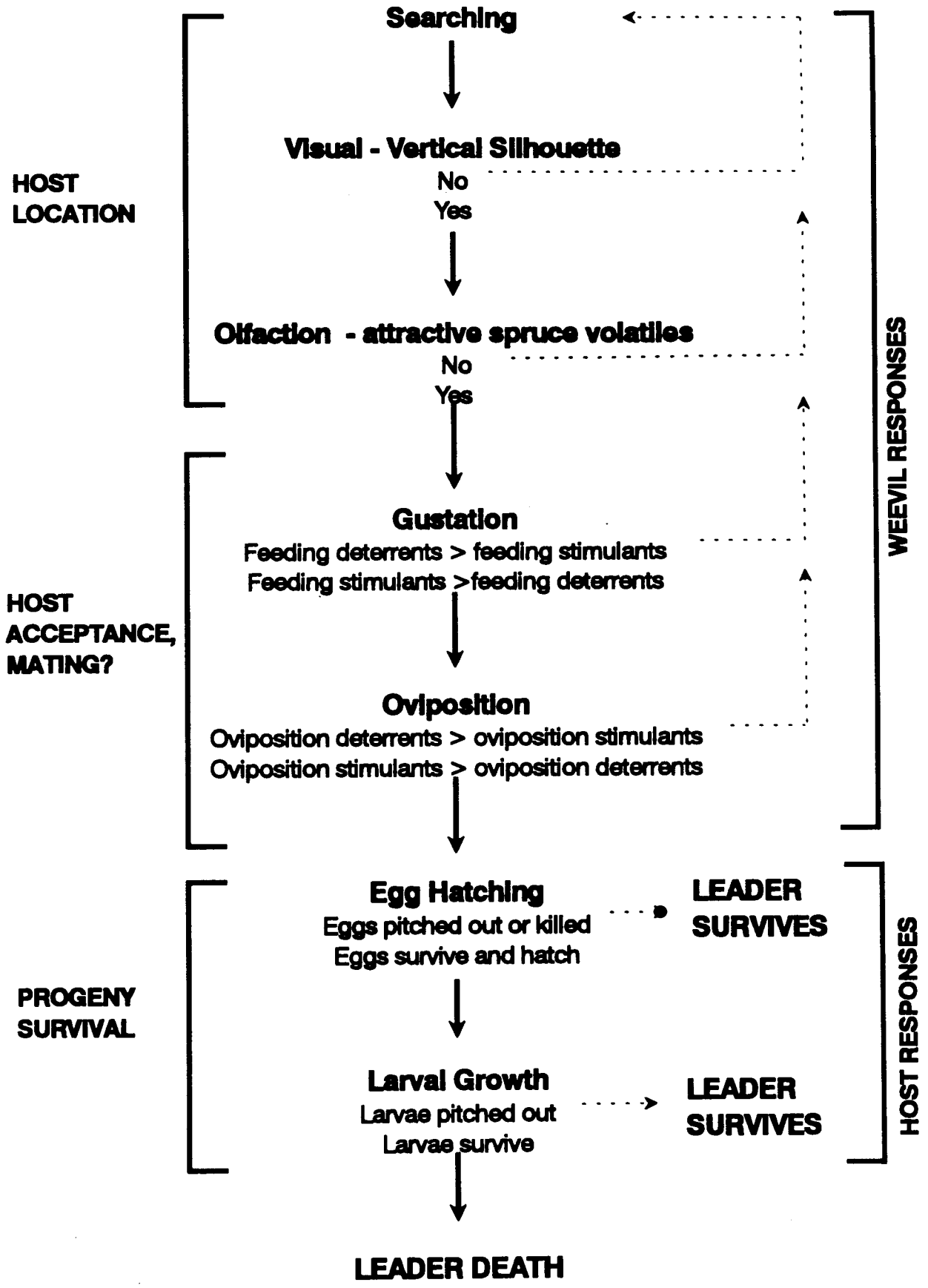
The overall objective of this research was to produce a multicomponent resistance index that could be used to select Sitka spruce for a resistance breeding program, and to screen progeny for resistance characteristics, as initially proposed by Brooks & Borden (1992). As discussed in the introduction, the aim of a breeding program should be to include as many resistance traits as possible in order to achieve stable, polygenetic resistance. Ideally, a resistance index should be able to quantify all of the resistance traits, assuming that they are identified.

A number of potential resistance mechanisms have been identified in the preceding chapters which include traits that may affect host location, host acceptance and progeny survival. The objective of this section, was to combine as many of these traits as possible into a resistance index that reflects the pattern of weevil attack observed on Sitka spruce in nature.

METHODS

In order to determine which terms should be included in the resistance index, and how much importance should be attributed to each, a flow chart was created showing the hypothesized process of host selection by *P. strobi* (Fig. 34). Leader death is the result of a series of steps which can be broken down into three separate processes: host location, host acceptance and progeny survival. The first two

Figure 34. Flow chart showing proposed sequence of behaviours by *P. strobi* and responses by Sitka spruce that determine whether or not a leader is killed.


Searching
Visual - Vertical Silhouette
No
Yes
HOST LOCATION
Olfaction - attractive spruce volatiles
No
Yes
Gustation
Feeding deterrents > feeding stimulants
Feeding stimulants >feeding deterrents
HOST ACCEPTANCE, MATING?
Oviposition
Oviposition deterrents > oviposition stimulants
Oviposition stimulants > oviposition deterrents
WEEVIL RESPONSES
Egg Hatching
Eggs pitched out or killed
Eggs survive and hatch
LEADER SURVIVES
PROGENY SURVIVAL
Larval Growth
Larvae pitched out
Larvae survive
LEADER SURVIVES
HOST RESPONSES
LEADER DEATH

processes involve responses by the weevil to the host, whereas the final process involves responses by the host to the weevil. Within each process are a series of steps which must be successfully completed in sequence for a leader to be ultimately killed. Failure of any one of these steps results in leader survival.

The three major processes outlined were each assigned a score of three, six and six which were divided between the steps within each process, based on their hypothesized importance. The score was broken down further to account for response variables within each step and levels of response within each variable (Table 23). Levels of response within each variable were determined by inspecting graphed data and levels of significance, within preceding sections. The maximum possible score that can be achieved by any tree is 15. Visual cues were omitted from the index at this point because of insufficient data, and the fact that leader length and width would need to be measured on trees in the absence of weevil attacks. Because scores for feeding and oviposition deterrency, and repellency were not available for all individual trees, an average score for the clone or provenance was used for each variable, and those averages summed to achieve the final score, rather than the final score being calculated for each individual tree, and then averaged by clone. Feeding deterrency, oviposition deterrency, and repellency were based on spring bioassays, and assessed for either males or females. It was assumed that the susceptible trees were not deterrent or repellent as they had sustained at least two weevil attacks. In the case of repellency, there were three susceptible clones used in the olfactometer bioassays. The score for susceptible trees was based on the number of susceptible clones that were

Table 23. Scoring system used to calculate multicomponent resistance index for clones sampled at Fair Harbour. Numbers in brackets indicate the maximum score assigned to each step or process.

Major Process	Step	Variable	Level of Variable	Score
Host Location (3)	Visual cues (1)	Leader length and width (not included in index, requires assessment in absence of weevil attacks) (1)		
	Olfaction (2)	Amount of foliar terpenes (1)	High (> 35 mg/g dry wt.)	1
			Medium (10-34.9mg/g dry wt.)	0
			Low (<9.9 mg/g dry wt.)	1
		Repellency in spring (1)	Yes	1
			No	0
Host Acceptance (6)	Gustation (1)	Feeding deterrency (1)	Yes	1
			No	0
	Morphology (3)	Density of constitutive resin ducts (2)	High (> 20 outer ducts/cm)	1.5
			Medium (10-19.9 outer ducts/cm)	0.5
			Low (< 9.9 outer ducts/cm)	0
		Bark thickness (1)	Thick (>1.0 mm)	0
			Thin (<1.0 mm)	1

192

Table 23 continued

	Oviposition (2)	Oviposition deterrency (2)	Yes	2
			No	0
Progeny Survival (6)	Resinosis (6)	Induced resinosis (4) (Insufficient data for inclusion in index at present)		
		Amount of constitutive cortical resin acid (2)	High (>35 mg/g dry wt.)	1.5
			Medium (20-34.9)	0.5
			Low (<19.9)	0
			Sum	**11**

repellent. For example if one clone of the three was repellent, the score would be 0.33. Bark thickness was included in the host acceptance section because of its strong relationship with depth of cortical resin canals (Fig. 5). Thick leaders may be attractive to weevils (Sullivan 1961; Stroh & Gerhold 1965), and thin leaders have shallow resin ducts; thus clones with thin bark were given the highest score. There were insufficient data to assess clones on the basis of induced resinosis; with this term omitted, the maximum possible score is 11. The resistance index for each clone and the susceptible trees was plotted along with the average number of weevil attacks, and the Pearson rank correlation coefficient between the two determined (PROC CORR, SAS Institute 1988). The number of weevil attacks was estimated as 2.5 for the susceptible trees, as the crowns were too bushy to count numbers of attacks accurately. All of the susceptible trees, however, sustained at least two-three attacks. To test the importance of each resistance factor to the index, correlations were computed, omitting different factors singly, in pairs, or triplets.

RESULTS AND DISCUSSION

Average resistance index scores for each variable are shown for the 11 clones and susceptible trees in Fig. 35. No single trait varied in a completely consistent manner with their overall index, each accounting for only part of the trend observed. Clones clearly differed from each other with respect to their complement of 'resistance' traits (Fig. 35).

There was a negative relationship between the resistance index with no variables omitted and number of weevil attacks (P=0.0324, R=-0.62) (Table 24). On

Figure 35. Comparison of resistance index scores for clones sampled at Fair Harbour separated into individual components of the index: bark thickness, number of outer resin ducts/cm, total amount of volatile foliar terpenes, total amount of cortical diterpene resin acids, feeding deterrency, oviposition deterrency, repellency, and the multicomponent resistance index based on the sum of scores for each component.

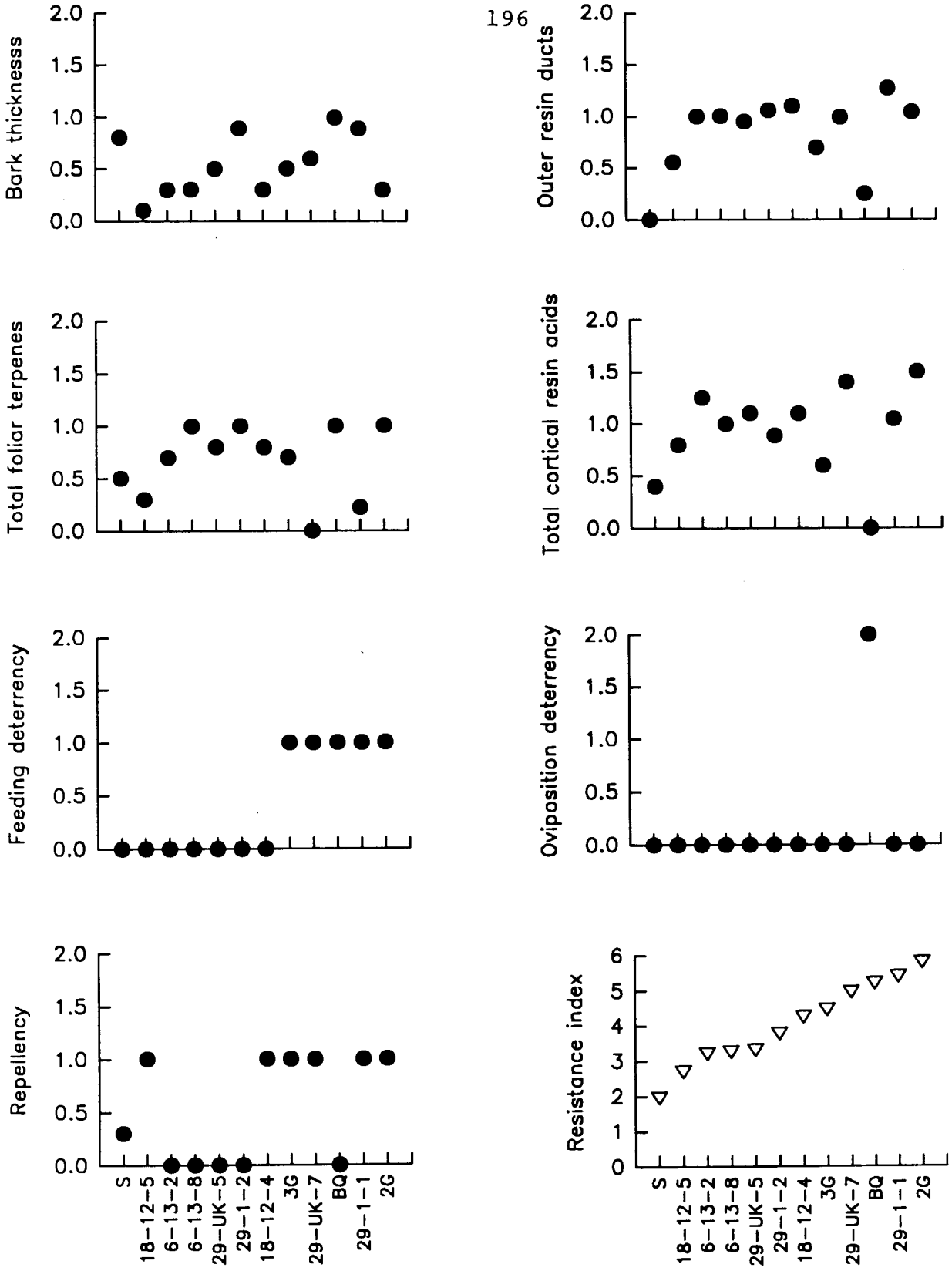

Bark thickness
Outer resin ducts
Total foliar terpenes
Total cortical resin acids
Feeding deterrency
Oviposition deterrency
Repellency
Resistance index
2.0
1.5
1.0
0.5
0.0
6
5
4
3
2
1
0
S
18–12–5
6–13–2
6–13–8
29–UK–5
29–1–2
18–12–4
3G
29–UK–7
BQ
29–1–1
2G
CLONE

Table 24. Pearson rank correlation coefficients, and probabilities that coefficient is equal to zero, for resistance index with different components removed.

Variable removed	Correlation coefficient	*P* value
None	-0.62	0.0324
1. Bark thickness	-0.57	0.0451
2. Number of outer resin ducts	-0.48	0.1145
3. Total foliar terpenes	-0.56	0.0609
4. Total amount of cortical resin acid	-0.51	0.0924
5. Feeding deterrency	-0.76	0.0042
6. Oviposition deterrency	-0.49	0.1004
7. Repellency	-0.71	0.0094
1+2	-0.45	0.1381
1+3	-0.52	0.0811
1+4	-0.49	0.0993
1+5	-0.67	0.0163
1+6	-0.43	0.1606
1+7	-0.71	0.0093
2+3	-0.41	0.1803
2+4	-0.32	0.3107
2+5	-0.61	0.0368
2+6	-0.38	0.2201
2+7	-0.55	0.0632
3+4	-0.45	0.1369
3+5	-0.69	0.0138
3+6	-0.42	0.1767
3+7	-0.69	0.0124
4+5	-0.61	0.0348
4+6	-0.44	0.1478

Table 24 continued

4+7	-0.56	0.0589
5+6	-0.51	0.0917
5+7	-0.81	0.0017
6+7	-0.65	0.0232
1+5+7	-0.66	0.0189
2+5+7	-0.65	0.0226
3+5+7	-0.72	0.0086
4+5+7	-0.59	0.0429
6+5+7	-0.64	0.0269

average, resistant trees scored higher for the resistance index than susceptible trees (Fig. 36). However, one clone, 3G (Green Timbers) had a higher number of weevil attacks than would be predicted by its resistance index score (Fig. 37a). From Table 24 it can be seen that omission of feeding deterrency and repellency, alone or combined, from the resistance index improved the correlation with number of weevil attacks. The highest correlation (-0.81) (Table 24) is achieved when both of these traits are omitted from the index (Fig. 37b). Omission of variables other than feeding deterrency or repellency weakened the correlation (Table 24). Feeding deterrency and repellency account for 44% of the total resistance index score for clone 3G compared with 20% for 29-UK-7 (Haney), the clone with the next highest score. If another trait such as induced resinosis were included in the index, 3G might fit the correlation better if it scored relatively lower for this trait than other clones. In the absence of data for traits such as induced resinosis, it is impossible to determine the relative importance of feeding deterrency or repellency compared with other components of the resistance index. It is also possible that repellency, in particular might not be an important resistance trait in fairly dense plantings of Sitka spruce such as at Fair Harbour. Under these conditions, host odours from different trees may blend together too much to be distinguished from each other.

In section III, I hypothesized that the Big Qualicum clone, which had very few outer resin ducts, should possess other resistance traits. Such traits are clearly evident in the high scores for other traits, particularly oviposition deterrency (Fig. 3), resulting in it achieving a high resistance score (Fig. 37).

Figure 36. Comparison of average resistance index with no variable omitted, and cumulative numbers of weevil attacks for resistant and susceptible trees at Fair Harbour.

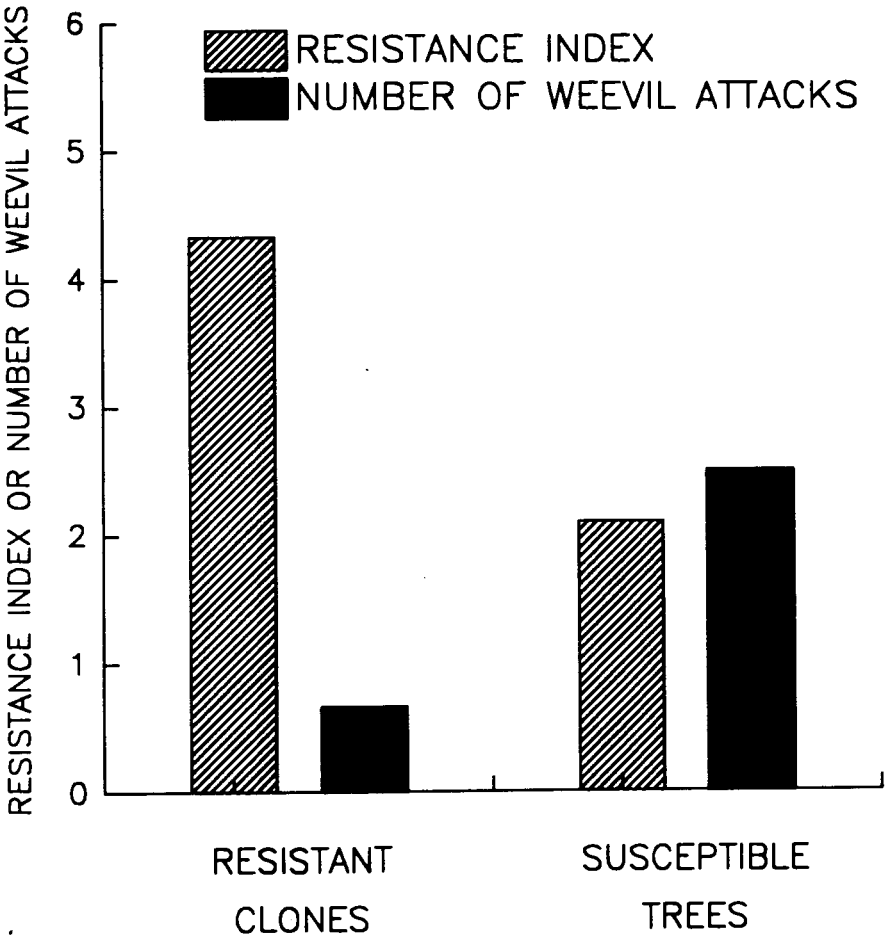
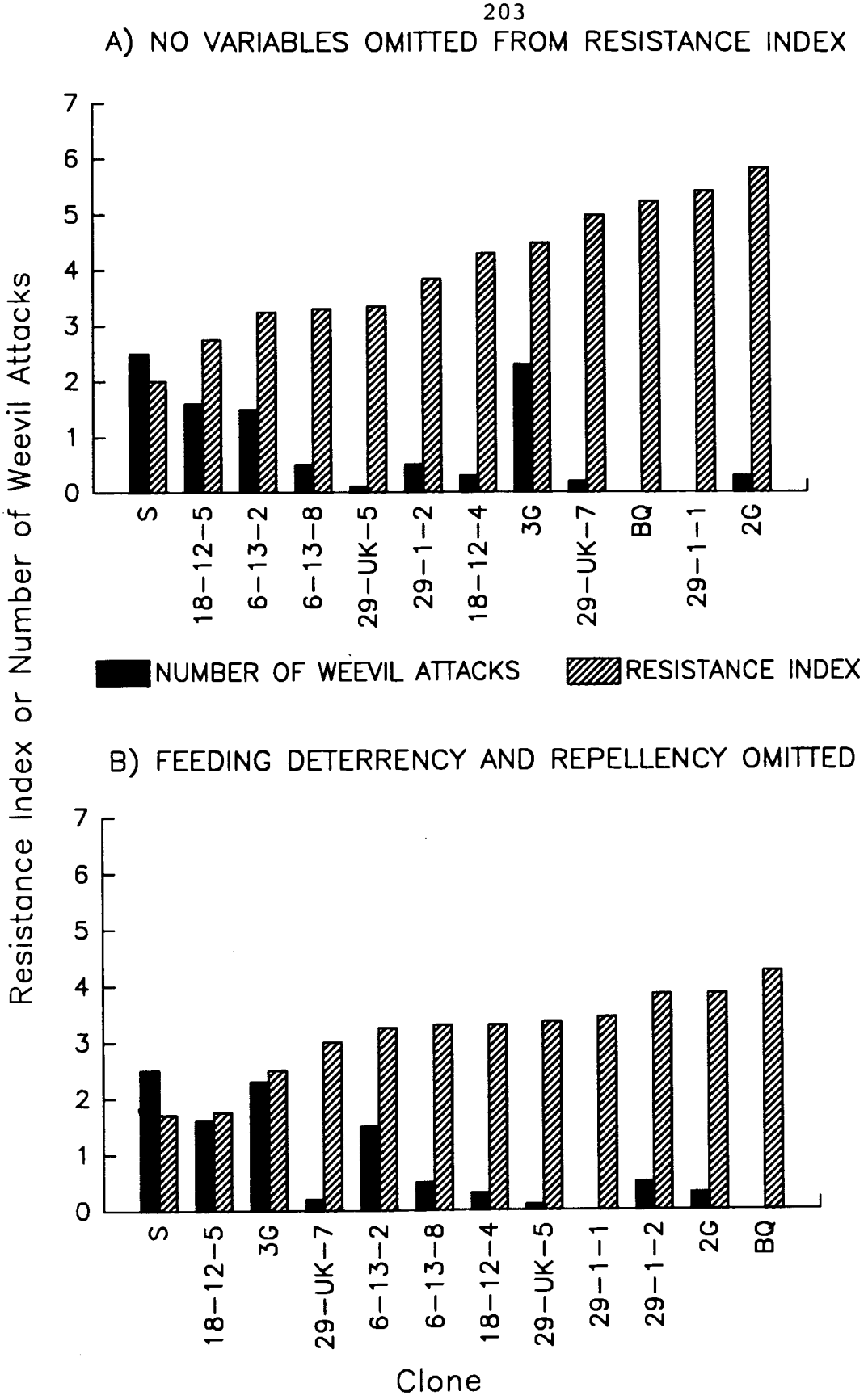

RESISTANCE INDEX OR NUMBER OF WEEVIL ATTACKS
0
1
2
3
4
5
6
RESISTANCE INDEX
NUMBER OF WEEVIL ATTACKS
RESISTANT CLONES
SUSCEPTIBLE TREES

Figure 37. Ranking of clones at Fair Harbour in order of ascending multicomponent resistance index with no variable omitted (maximum=11) (A), or feeding deterrency and repellency omitted (maximum=9) (B), compared with average cumulative number of weevil attacks on all the trees within a clone.


A) NO VARIABLES OMITTED FROM RESISTANCE INDEX
Resistance Index or Number of Weevil Attacks
0
1
2
3
4
5
6
7
S
18-12-5
6-13-2
6-13-8
29-UK-5
29-1-2
18-12-4
3G
29-UK-7
BQ
29-1-1
2G
NUMBER OF WEEVIL ATTACKS
RESISTANCE INDEX
B) FEEDING DETERRENCY AND REPELLENCY OMITTED
S
18-12-5
3G
29-UK-7
6-13-2
6-13-8
18-12-4
29-UK-5
29-1-1
29-1-2
2G
BQ
Clone

All of the clones used in developing the resistance index were resistant, and the magnitude of the resistance index differed only by a factor of two between the highest and lowest scoring clones in the best-fit relationship (Fig 37b). Had the index been applied to a large population of Sitka spruce, with many susceptible genotypes, the inverse relationship between resistance index and number of weevil attacks might be even more pronounced. Cost-benefit theory of plant defense predicts that optimal values are obtained by a trade-off between fitness benefits and associated costs Rhoades 1979; Simms 1990). A particular trait may be positively associated with resistance to one pest, and negatively assoiated with resistance to another pest resulting in stabilizing selection which maintains intermediate equilibrium values for particular traits (Simms 1990). The metabolic cost of defenses to a plant are difficult to measure (Fox 1981), but some traits must be energetically more expensive than others. Based on optimal defense theory (Rhoades 1979) organisms allocate defenses in a way that maximizes individual inclusive fitness, so very expensive, yet effective defenses might not be employed against a non-lethal pest. Thus it would be predicted that resistance to weevils would be maintained at an intermediate level, perhaps using less costly defenses, and a few successful attacks would be expected, even on clones with a high degree of resistance (Fig. 37).

Variation in resistance traits may be related to defense against organisms other than the weevil, resulting in less than 100% correlation with number of weevil attacks (Table 24). Diffuse coevolution occurs when the evolution of a particular trait in one or more species occurs in response to a trait, or suite of traits, in several other species

(Janzen 1980; Futuyma & Slatkin 1983). In large, persistent plants such as trees, each individual is attacked by a variety of different herbivores and pathogens, some of which are generalists and some specialists (Fox 1981). In addition, plant-herbivore interactions may be complicated by interactions with a third trophic level (Price *et al.* 1980, Barbosa & Saunders 1984). A broad array of responses by both the tree and the herbivores probably occurs, resulting in conflicting defensive demands; thus simple stepwise coevolution would be highly unlikely (Fox 1981; Myers 1988; Simms 1990). Furthermore, because trees undergo complete regeneration of their buds every year, somatic mutation can cause variation within an individual tree or clone, resulting in genetic mosaics that may embody new adaptive or disadaptive traits (Whitham & Slobodchikoff 1981).

Improvement of the resistance index could probably be achieved in a number of ways. Including a term describing induced resinosis is necessary to assess trees on the basis of their effect on progeny survival. Fine tuning of the index might be achieved if the specific chemicals responsible for feeding and oviposition deterrency, and repellency were known, and if the relative importance of mechanisms affecting host location, host acceptance and progeny survival could be determined. In addition it would be useful to assess the importance of other herbivores and pathogens to the growth and survival of Sitka spruce, as resistance or susceptibility to other pests might interact with resistance to *P. strobi.*

VIII. GENERAL CONCLUSIONS AND IMPLICATIONS FOR MANAGEMENT OF *PISSODES STROBI*

A number of specific traits were associated with resistance. Resistance in different genotypes appears to depend on different combinations of traits, suggesting that multiple resistant genotypes exist. The relationships between these traits are unclear and probably complex, a predictable conundrum given the longevity of trees and the variety of herbivores and pathogens that utilize them.

Specific relationships between host traits and resistance that were observed are as follows,

1. Resistance appears to be associated with a high density of outer resin ducts combined with thin bark.

2. Resistant trees tend to have extremely high or low levels of volatile foliar terpenes, and high levels of cortical diterpene resin acids

3. The significance of qualitative variation in foliar and cortical terpene complements in relation to resistance is unclear, but may be useful in 'chemotyping' trees

4. Both feeding and oviposition deterrency (or lack of attraction) are expressed by a few resistant genotypes, and these two activities appear to be controlled by different stimuli.

6. Resistant clones differ in their degree of repellency to weevils.

7. There is no clear relationship between repellency of volatiles from cut twigs

and feeding and oviposition deterrency of cut twigs, suggesting that host location, and host acceptance are controlled by different stimuli.

8. Induced resistance may be associated with feeding, but appears to be more strongly associated with the presence of eggs or the oviposition plug.

9. The capacity for induced resinosis appears to vary by clone, and this mechanism of resistance may be less important in Sitka than interior spruce.

10. Resin acids stimulate egg hatch, but may increase larval mortality

11. Volatile terpenes reduce egg hatch.

12. Whole resin had no effect on egg hatch.

13. The multicomponent resistance index with all factors included, predicted the resistance of all clones except for one (3G, Green Timbers), and the best relationship with resistance was with feeding deterrency and repellency omitted.

There are a number of important questions that still need to be answered before mechanisms of resistance of Sitka spruce to *P. strobi* can be clearly understood.

The role of volatile and non-volatile chemicals in the different phases of host selection behaviour needs to be clarified. Experiments must be designed which allow volatile and non-volatile chemicals to be tested both individually and together against weevils at different stages of host selection. Non-volatile feeding and oviposition stimulants must still be identified, and volatile ones confirmed. In addition, it must be determined if resistant clones contain feeding deterrents, or lack stimulants.

The relative importance of induced and constitutive resinosis needs to be

clarified. This will involve aseptic and microbial-associated wounding of trees under field conditions, and correlating preformed resin ducts with the capacity for induced resinosis. Identification of any microorganism associated with weevil activity would facilitate further studies of the wound response.

After inclusion of a term describing induced resinosis, the reliability and accuracy of the multicomponent resistance index needs to be tested on a completely separate population of resistant and susceptible Sitka spruce, with differing degrees of weevil attacks. In addition, the heritability of both host and weevil traits should be determined in order to predict how stable resistance will be in the future, and how resistant trees should be deployed. It must also be determined if there are environmental effects on expression of resistance traits, as these will also affect how resistance is managed.

Use of Resistant Trees in Management of *Pissodes strobi*

Sitka spruce, being a long lived perennial, has evolved chemical and physical defenses in response to a variety of insects, pathogens and possibly competitors, many of which may no longer be present. *P. strobi* is probably only generally adapted to Sitka spruce, rather than highly coevolved with it, especially given that its host range includes eastern white pine, *Pinus strobus* L., and other spruces, *Picea spp.* (Alfaro & Borden 1985), and that its ancestral host is hypothesized to be eastern white pine (VanderSar *et al.* 1977). *P. strobi* probably has a limited ability to detoxify highly toxic chemicals because it is not adapted to unapparent plants with qualitative

defenses. Edmunds & Alstad (1978) suggest that pests would theoretically drop superfluous detoxification mechanisms after many generations on a host where they were not needed. However, despite this, there still exists the potential for *P. strobi* to overcome the defenses of resistant Sitka spruce. It has been shown that repeated, long-term assault of resistant genotypes by pathogens allows ample opportunities for virulent recombinants or mutants to occur (Bingham *et al.* 1971)

The use of resistant trees in forest plantations could be considered analogous to the use of chemical insecticides in the sense that one places selection pressure on insects to detoxify or otherwise adapt to these defenses. Resistance of insect populations to pest management tactics is the most important problem in pest management (Pedigo 1989). It is most commonly associated with insecticides, but can occur with any factor that causes high mortality. Types of resistance that insects employ include biochemical, physiological and behavioral tactics. In addition to high mortality, the rate of development of resistance depends on genetic factors such as how many mutations are required for the insect to overcome resistance (Pedigo 1989), and whether there are any genetic constraints involved (Slatkin 1983). Genetic constraints such as pleiotropy, gene linkage, functional constraints and high gene flow may stop genetic evolution in a particular direction (Roughgarden 1983; Slatkin 1983).

Based on experiences in agriculture, several factors could promote the development of resistance (Pedigo 1989). These include prolonged and widespread exposure of an insect to a single insecticide, exposure of every generation of the insect to the insecticide, high mortality, lack of functional refugia to maintain susceptible

individuals in the population, spraying of a large geographic area, exposure occurring prior to mating, and the insecticide being closely related to one used earlier. Prolonged exposure of weevils to resistant Sitka spruce cannot be avoided, nor can placing selection pressure on every generation of weevils be avoided because trees are long-lived.

The best way of preventing herbivores from overcoming defenses is to use a variety of different tactics (Pedigo 1989). This is analogous to using several classes of insecticides in agriculture. A number of different resistance mechanisms should be incorporated into a Sitka spruce breeding program. Resistance mechanisms should represent the three major types of resistance, and there should probably be variability incorporated both within and between trees. That is, not only should single trees possess more than one defense mechanism, but trees should differ from each other in their suite of defensive tactics. This is an especially important consideration when asexual plant propagation techniques are used (Edmunds & Alstad 1978).

Resistance factors that kill eggs, larvae or pupae should be included because eggs would be 'wasted'. Mortality of immature insects is one of the most important limiting factors of insect populations (Wiseman 1994). Resistance mechanisms that kill eggs would be favoured operationally over a latent effect that kills larvae, because eggs do no serious damage to a tree.

In addition to maintaining genetic and spatial heterogeneity of resistance traits (Shultz 1983), it will also be necessary to provide refugia for weevils in order to maintain susceptible insects in the population, and maintain a population of natural

enemies. *P. strobi* is heavily preyed upon by two main dipteran predators and a parasite (Stevenson 1967; Hulme 1990), and in the absence of these predators, populations could be extremely high. The use of tolerant trees that can sustain an infestation of weevils, yet remain free of major defects would provide a source of natural enemies and susceptible weevils. However, it would be easier and probably as effective to interplant resistant and susceptible trees allowing the weevils to select and infest the susceptible trees, which would fail to compete for dominance with the resistant trees, and would eventually be suppressed in a natural thinning process. Such a tactic would support the contention of Bingham *et al.* (1971) that the essential features of coexistence and balance are allowing pathogens to reproduce, and accepting diseases and some tree losses. Leader clipping of susceptible or tolerant trees, while killing weevils and maintaining natural enemies (Rankin & Lewis 1994), may have the adverse effect of preventing reproduction by weevils that are not adapted to survive on resistant trees.

Raffa (1989) warns of the risk of biotype evolution if widespread, uninterrupted deployment of novel genes is carried out. For example, the winter moth, *Operophthera brumata* L., apparently developed a Sitka spruce biotype when faced with unbroken monocultures of this species in Scotland (Stoakly 1985). Thus in addition to interplanting with susceptible genotypes, resistant Sitka spruce trees should ideally be planted in small, well-separated plantations on high sites where resistance is most likely to 'pay off'. This would leave areas of natural Sitka spruce in between the resistant plantations readily accessible to the weevils, thus reducing the selection

pressure on the resistant plantations by making the resistant trees no more accessible than the wild, susceptible ones. Most plant feeding insects can find their host efficiently when there are no other species present to interfere (Strong *et al.* 1984), but *P. strobi* finds its host efficiently in mixed species forests. Nonetheless, interplanting resistant Sitka spruce that lack apparency (Fig. 9) with non-host species such as hemlock or western red cedar may further reduce the apparency of the host tree. The benefits of interplanting could be offset in part by extending the dispersal of weevils from the distance between trees (Harman 1975) to distance approaching their 1.2 km limit (McMullen & Condrashoff 1973).

Despite the above recommendations, there are still several potential difficulties associated with the use of resistant strains of Sitka spruce.

Host plant selection by *P. strobi* is clearly a complicated series of behaviours and genetic variability exists in host choice (Via 1990). This suggests that the potential exists for host shifts to occur (Via 1990), and studies of the feeding behaviour of *P. strobi* do not rule out this possibility. In a no-choice situation, *P. strobi* will feed on numerous conifers (VanderSar & Borden 1977c; Alfaro & Borden 1982). Alfaro & Borden (1982) found that feeding stimulants were present in almost all of 33 native and exotic conifers tested in a single-stimulus feeding bioassay. While Sitka spruce was the preferred host, other conifers elicited a strong feeding response, suggesting that the genetic potential for a host shift exists. In addition, it has been shown that there is considerable genetic variation between populations of weevils in Ontario and British Columbia, and within British Columbia (Lewis 1995). In nature,

P. strobi commonly infests three species of spruce in western North America and one species of spruce and two species of pines in eastern North America. It also adapts readily to exotic species like Norway spruce. Lodgepole pine, *Pinus contorta* Dougl. ex. Loud is closely related to, and readily hybridizes with, jack pine, *Pinus banksiana* Lamb., (Farrar 1995) an eastern host of *P. strobi.* As lodgepole pine is abundant in the range of *P. strobi,* I hypothesize that there is great potential for it to shift to pines as hosts in the west. In fact, Humble *et al.* (1994) found that in B.C., 2.7% of white pine weevil damage collections by the Forest Insect and Disease Survey (Canadian Forest Service) were on lodgepole pine. Studies of the genetic variability within and between populations could elucidate the evolutionary possibilities or constraints that might exist and may aid in predicting how a host shift might proceed (Via 1990).

Site factors might affect the expression of resistance in specific genotypes. Environmental stress has been shown to induce physiological variation among plants which may affect herbivory (Louda & Collingue 1992). For example, coastal spruces experience lower vapour deficit regimes, and consequently less stress, than inland spruces on the Olympic peninsula of Washington State, and the coastal spruces are also associated with the lowest levels of weevil attack (Warkentin *et al.* 1992).

Resistance characteristics may not be compatible with silvicultural goals. For example production of defensive chemicals may require twice as much energy as wood production (Loehle & Namkoong 1987), and historically, most gains in yield due to plant breeding involve a switch in the energy allocation pattern. Thus breeding for certain resistance mechanisms might divert energy from primary production into

defense. van Emden (1991) suggests that breeding for partial resistance is desirable because in addition to reducing selection pressure on the herbivore, there is less sacrifice of yield.

Resistant trees may have some adverse effect on entomophagous organisms (Price *et al.* 1980). For example, if resistant trees contain precocene-like compounds, they would likely have similar effects on natural enemies as on weevils, or a very strong wound reaction by the tree may make it unsuitable for natural enemies. Also if repellency is incorporated as a resistance mechanism, it would be adaptive for natural enemies also to be repelled from these trees, eliminating them as biological control agents if the weevil overcame resistance.

In conclusion, despite these potential problems, with continued research, and careful management, it should be possible to establish resistant Sitka spruce in plantations and to maintain resistance for a long duration, if not indefinitely.

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