RESISTANCE BY SITKA SPRUCE TO THE WHITE PINE WEEVIL: CHEMOTYPING RESISTANT TREES

by

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ABSTRACT

A solvent extraction technique was developed to isolate the specific monoterpene fraction for analysis by gas chromatography of those monoterpenes in buds, foliage and bark of Sitka spruce, Picea sitchensis, (Bong.) Carr. Seasonal differences in the relative monoterpene content of developing buds and 1-year-old foliage were followed. To test the hypothesis that a resistant chemotype to the white pine weevil, Pissodes strobi, Peck could be identified, the relative composition of monoterpenes between weevil-susceptible and apparently resistant trees were compared. In second-year foliage of trees from the University of B.C. Research Forest, Maple Ridge, B.C., α-pinene and camphene levels increased in May and returned to their original levels by September. Myrcene, isopentenyl isovalerate and camphor levels decreased in May. Isopentenyl isovalerate and camphor levels increased over the summer but myrcene continued to decline, offsetting rising levels of 3-carene and β-pinene. To obtain representative monoterpene spectra, samples should be taken after September 30, when trees are dormant for the winter. There was significant developmental variation in buds from 20 trees from Sayward, B.C. α-Pinene and β-pinene were prominent initially and then declined, while myrcene increased to become the major volatile component of the elongating buds. β-Phellandrene levels declined and then increased as the season progressed. There was no significant difference in percent monoterpene composition between buds from resistant and

susceptible trees at Sayward. Foliar analysis of trees from Nootka Island, Sayward and the Nass River, B.C. revealed significant differences between 34 resistant and 55 susceptible trees. The resistant trees, as compared to susceptible trees, had significantly lower amounts of isoamyl isovalerate at all 3 sites and lower amounts of isopentenyl isovalerate at 1 site. Amounts of α-pinene, β-pinene, camphene and camphor were significantly higher in some resistant trees but these differences were not consistent between sites. The cortical monoterpene spectra of 4 resistant Sitka spruce from the Green Timbers Nursery, Surrey, B.C. were compared to those of 9 clones from their grafted scions at the B.C. Forest Service, North Road Laboratory, Victoria, B.C. 3-Carene and terpinolene were higher in the parent trees, while α-pinene and β-phellandrene-limonene were present in significantly higher amounts in the clones. These differences suggest that if breeding programs for resistance to the white pine weevil were initiated, a broader spectrum of resistant characteristics should be employed, than simply the monoterpene chemotype.

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INTRODUCTION

The white pine weevil, Pissodes strobi Peck is the most damaging insect pest of regenerating Sitka spruce, Picea sitchensis (Bong.) Carr. in the Pacific Northwest. Graham (1926), MacAloney (1930), Belyea and Sullivan (1956) and Silver (1968) have described the life history of the weevil. Adult weevils begin to feed on the cortex of laterals and 1-year-old leaders in late April through May, when temperatures are favorable. Copulation and oviposition occur on the leader. Females lay eggs in oviposition punctures chewed into the cortex below the terminal bud. The eggs hatch 2-3 weeks later into white larvae. They mine into the phloem-cambium layer and form a feeding ring which progresses downward, eventually girdling and killing the leader. The new shoot above the attacked leader wilts and becomes brown by mid-summer. Therefore, at least 2 years of height growth are killed in the attack, and if the weevil population is high, larvae may mine past the first whorl of branches, killing the 2-year-old growth of the stem.

The larvae undergo 5 instars and then pupate in 'chip cocoons' constructed from wood fibers. Adult eclosion occurs between early August and late September, 2 to 3 weeks after pupation. The entire life cycle takes 4 months and the species is generally univoltine. Adult weevils will feed for a short time on the laterals and stem before dropping off the trees to overwinter in the duff; they also overwinter on the trees in warmer climates. Adults may live for 3 to 4 years.

Weevil damage results in crooked stems and forked leaders, both of which inhibit height increment and volume increase. After repeated infestations, the tree is reduced to a bushy shrub and will never attain full height. In time, competition from the surrounding vegetation and other trees will decrease the likelihood of an attacked tree ever living to maturity.

In general, P. strobi infests plantations of young spruce and if attack intensity is high enough, entire plantations may be rendered non-merchantable by the weevil. It is this serious problem that many researchers are addressing. The infested leaders with cryptic larvae therein are such difficult targets that it has proven to be uneconomical or unfeasible to spray insecticides to control the weevil (Johnson and Zingg 1968). Physical maintenance of plantations by leader clipping is effective only at extremely low infestation rates [1] [2]. Breeding resistant trees is one of the remaining alternatives.

The white pine weevil is also a serious pest of eastern white pine, Pinus strobus L., although it rarely attacks its western counterpart, Pinus monticola Dougl. (Soles et al. 1970; VanderSar 1978; Wilkinson 1981). Eastern white pine is a valuable lumber species; hence there has been much pressure generated for the implementation of breeding programs for weevil-resistant trees (Wright and Gabriel; Gerhold 1966;

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[1] Michaelson, L., E. Jeklin, and T. Rushton. Internal report for the B.C. Ministry of Forests. 1981. Mechanical control of spruce terminal weevil on Nootka Island -1981.

[2] Wood,P., pers.comm. 1983. Pest Management Coordinator, Vancouver Region, B.C. Ministry of Forests.

Garrett 1970; Wilkinson 1983a). Little has actually been done in resistance breeding. Trees require such a long time to mature, that it is difficult to assess resistance to insects and growth potential in a reasonable time frame.

Various potential resistance mechanisms have been researched, such as the physical characteristics of the bark (Kriebel 1954; Wilkinson 1983b), the physiological characteristics of the tree (Stroh and Gerhold 1965; Hanover 1975; Smelyanets 1977) and growth form and chemical composition of the various tissues (Callaham 1966; Bridgen et al. 1979; Wilkinson 1979; Gollob 1980; Wilkinson 1983a) encountered by the attacking insect.

Phytophagous insects are known to be attracted by certain chemicals in their host plants (Dethier 1954; Thorsteinson 1960). Sitka spruce has an array of volatile monoterpenes in the resin of the needles and bark that weevils will encounter when attacking the host (Hrutfiord 1974; von Rudloff 1978). Many monoterpenes have been tested singly and in groups for their attractant and repellent qualities to P. strobi (Anderson and Fisher 1960; Alfaro et al. 1980; Alfaro et al. 1981). Alfaro et al. (1980) tested volatile monoterpenes with the non-volatile components in the bark of Sitka spruce and found that certain monoterpenes acted as synergists to the non-volatile chemicals in the bark to enhance feeding, while other compounds completely deterred feeding.

Biosystematic studies of many conifers have disclosed species-specific monoterpene spectra (von Rudloff 1964, 1975, 1977). According to Wilkinson et al. (1971), Squillace (1976) and Squillace et al. (1980), monoterpene biosynthesis is under direct genetic control and is unaffected by environmental factors. Therefore, the monoterpene spectrum of a species is potentially a good indicator of resistance if qualitative and/or quantitative differences are found between trees resistant and susceptible to the weevil. The problem of ascertaining which compounds or group of compounds confer or indicate resistance has been the subject of considerable research (Annila and Hiltunen 1977; VanderSar and Borden 1977; Wilkinson 1980; Harris et al. 1983).

Researchers have examined the monoterpene spectra of weevil-susceptible species and noted consistent differences in the spectrum between resistant or susceptible trees. For example, Wilkinson (1980) found that eastern white pines with high α-pinene and low limonene content were generally more resistant than ones with low α-pinene and high limonene. Harris et al. (1983) analyzed the cortical monoterpenes of 5 historically resistant Sitka spruce from Green Timbers Nursery, Surrey, B.C. These five trees had a characteristic monoterpene spectrum that differed from other susceptible trees on the same plantation and from susceptible trees on the U.B.C. Research Forest, Maple Ridge, B.C.

Based on these data it was hypothesized that a resistant chemotype might be definable for Sitka spruce. Testing of this hypothesis was the overall goal of my project. The specific objectives of the project were: 1) to develop an efficient chemical extraction technique for rapid gas-chromatographic analysis of monoterpenes; 2) to describe the monoterpene spectra in developing buds, and in one-year-old needle tissue throughout the year; 3) to examine differences in the monoterpene spectra of needles and cortex from numerous trees that appear resistant or susceptible in the field; and 4) to examine differences in the monoterpene spectra of clones and their parent trees.

The monoterpene spectrum of Sitka spruce may reflect a true resistance mechanism or it may act as an indicator of resistance. However, if consistent differences occur between resistant and susceptible trees, they could be used to identify candidates for breeding or clonal propagation programs to produce resistant Sitka spruce for future plantations.

MATERIALS AND METHODS

Collection and Maintenance of Sitka Spruce Samples

Samples from Sitka spruce were collected for analysis of monoterpenes at several sites throughout the coastal region of British Columbia (Fig. 1). Unless otherwise specified, 2 samples were taken from lateral branches on opposite sides of the second upper whorl. They were stored in plastic bags on ice in the field, and at 3-5°C in the laboratory until utilized. Most samples were analysed within 2 weeks of collection.

Solvent Extraction Procedure

Solvent extraction[3] of needles, cortex and buds was adopted as a standard method of extraction. Foliage was removed from twigs with scissors, cutting as closely as possible to the pedicel of each needle but avoiding the cortex. Bark was removed by shaving the twigs with a scalpel blade. Sapwood was not included in any sample. Whole buds were extracted directly because separation of the needle tissue from the stem tissue was not possible.

A 1 g sample of tissue was placed in a test tube containing 4 ml of distilled hexane, 2 ml of methanol, and 1 ml of

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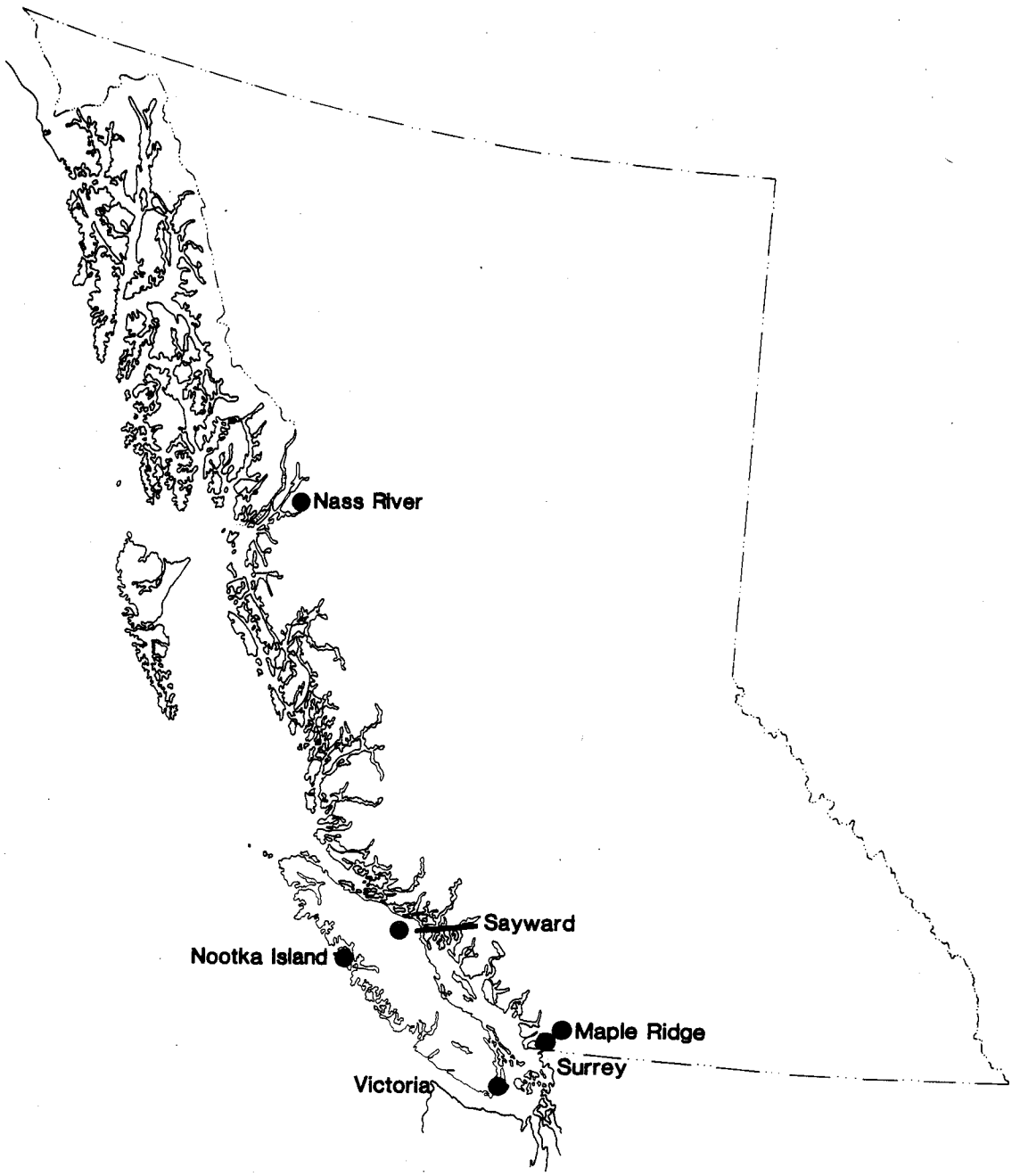
[3]Techniques used similar to methods employed by J.M. Manville(pers. comm.), Pacific Forest Research Centre, Victoria, B.C.

Figure 1. Location of sampling sites in British Columbia.


Nass River
Sayward
Nootka Island
Maple Ridge
Surrey
Victoria

distilled water. The tissue was finely ground using a Polytron tissue macerator (Brinkmann Instruments, Rexdale, Ontario), (Fig. 2), and the tube was then centrifuged at approximately 1000 rpm for 1 min. The top hexane layer was drawn off with a Pasteur pipet and forced through a filter pipet containing a 1:2 charcoal:Celite mixture (Fig. 3). The resultant clear extract was stored at -20°C in vials with Teflon-lined lids. With this procedure, 10 samples could be prepared for GC analysis in under 2 h. A drawback of the technique was the tendency for the charcoal filter to adsorb piperitone, altering its relative percent composition in the final monoterpene analysis. Because piperitone content was never different between resistant and susceptible trees, no corrections were made for this anomaly.

Gas Chromatographic Analysis

Analyses by gas chromatography(GC) were conducted on a Hewlett-Packard 5880A gas chromatograph equipped with a capillary unit system and a flame ionization detector. The temperatures of the injection port and flame ionization detector were 260°C and 270°C, respectively. Oven initial temperature was 50°C and was increased 6°C per min. to 180°C. Helium was the carrier gas. The monoterpenes were separated on a Durabond 1(DB-1) fused silica capillary column(15 m x 0.25 mm) (J. and W. Scientific, Rancho Cordova, CA). Peak areas were not corrected for differences in detector response. Each day, standard samples

Figure 2. Polytron used to macerate spruce tissue during the extraction process.

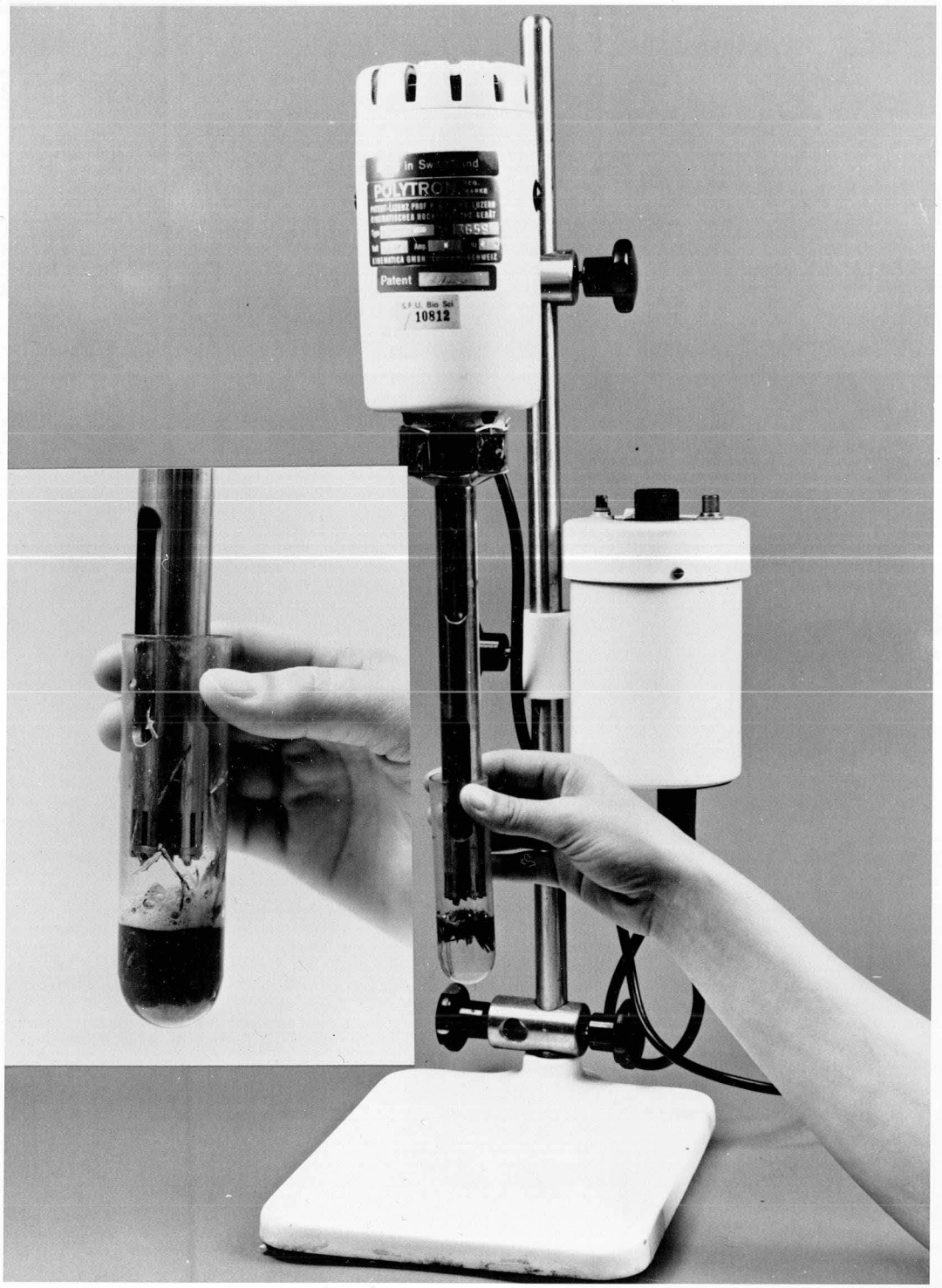

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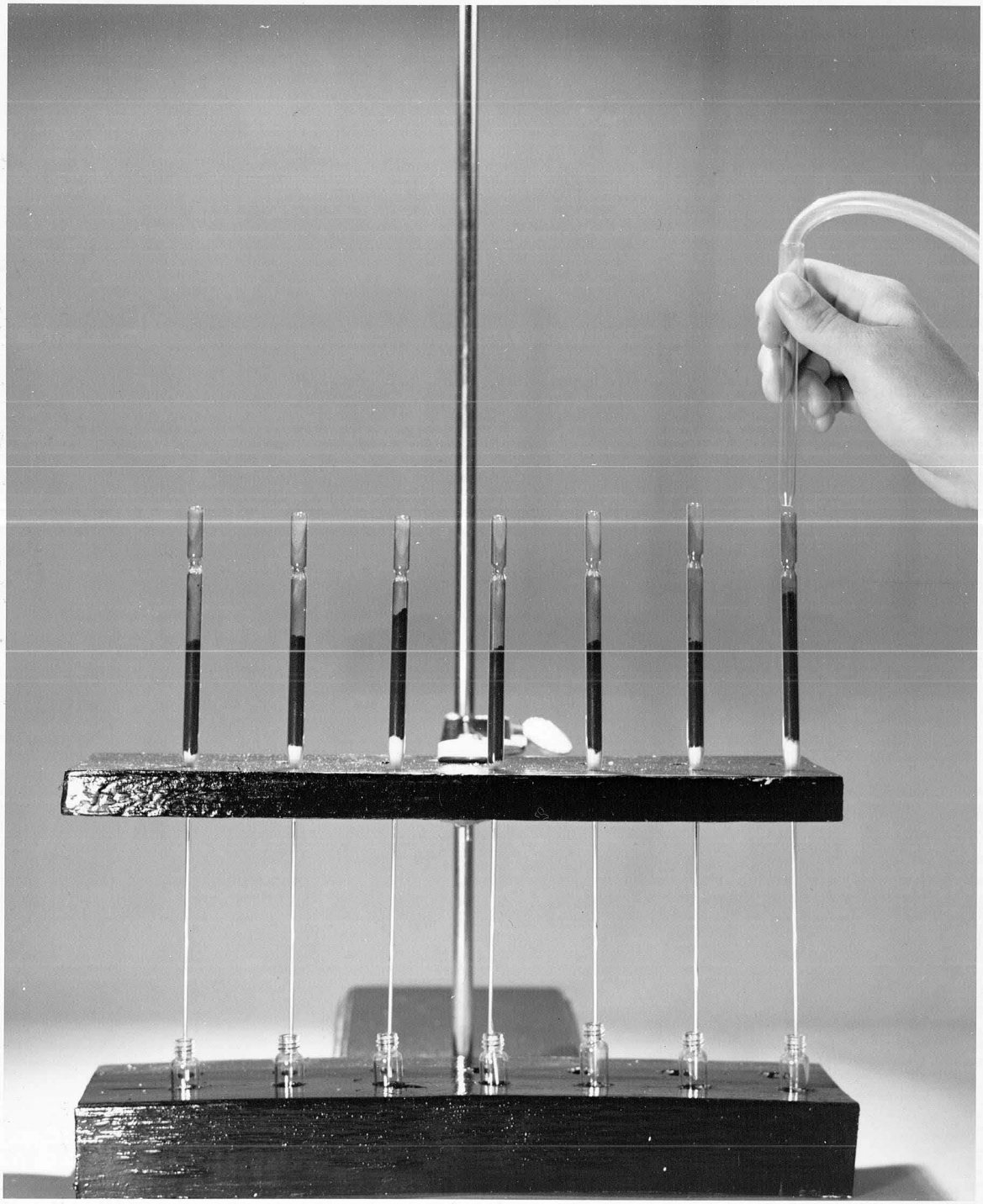
Figure 3. Apparatus for filtration of extracts showing Pasteur pipets containing activated charcoal; filtration is facilitated by forced air stream (far right).



consisting of 3 monoterpenes were analyzed for determination of absolute retention times.

Monoterpenes in the sample were identified by comparison of retention times to those of standard monoterpenes [4]. Compounds were also verified by co-injection of standard monoterpenes with the sample. Several compounds were identified by GC-mass spectrometry(GC-MS) on a HP5985A GC/MS/DS using a DB-1 column. Identification and quantitation of limonene and β-phellandrene were not always possible because they often co-eluted on the DB-1 column.

Sample Size Determination

Five g samples were compared to 1 g samples of spruce needles to assess the reliability of the smaller sample size. Samples were collected at all 4 cardinal directions from each of 5 trees at the UBC Research Forest on May 25, 1983. They were extracted by steam distillation and analyzed by GC.

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[4]Commonly occurring monoterpenes were obtained commercially,but isoamyl isovalerate and isopentenyl isovalerate were synthesized by H.D. Pierce, Jr., Dept. of Chemistry, Simon Fraser University, Burnaby, B.C.

Cardinal Direction Differences

To determine whether or not there were significant differences in the monoterpene profiles between foliage on branches at each of the 4 cardinal directions, 2 trees from the UBC Research Forest were sampled on February 20 and one tree on April 5, 1984. Five 1 g samples from each of the cardinal directions were taken for each tree. The samples were prepared by solvent extraction and analyzed on the GC.

Individual Tree Variation

The 1 g samples that were collected to determine the appropriate sample size were also used to assess individual tree variation by comparing the monoterpene spectra of the 5 sampled trees.

Seasonal and Developmental Variation in Sitka Spruce Foliage

Seasonal variation in the relative monoterpene content of foliage was followed over a 9 month period on 4 trees from the U.B.C. Research Forest. Five samples per tree were taken, generally from the top half of the crown on the following dates: Feb. 17, April 10, April 24, May 24, June 19, July 19 and Sept. 27, 1984. They were extracted by the solvent extraction method, and analyzed by GC.

Variation in the monoterpene profile of developing buds and new shoots was examined in 10 resistant and 10 susceptible trees from Sayward (Fig. 1). Buds were sampled in 1984 on April 20 before they flushed and on the following dates thereafter: April 27, May 4, May 11, May 18, May 25, June 13, and October 30. Two 1 g samples per tree were collected, solvent extracted and analyzed on the GC. Samples were collected from the tree crown and were generally consistent in size.

Comparison between Resistant and Susceptible Trees

Cortex and foliage samples of 40 resistant and 64 susceptible trees were collected from the following locations: Nootka Island (foliage samples only), Sayward, Nass River and the Green Timbers Nursery, Surrey, B.C. (Fig. 1). Two 1 g samples of each tissue per tree were obtained. The criteria for judging a tree as resistant were: no apparent weevil damage, good height growth and superior overall form. Trees that had been weeviled at least once were regarded as susceptible. All samples were solvent extracted prior to GC analysis. The monoterpene spectra of resistant and susceptible were then compared.

To assess the genetic consistency of monoterpenes, 5 resistant trees from the Green Timbers Nursery, Surrey, B.C. and the clones of 3 of the trees from the North Road Laboratory, Victoria, B.C. were sampled and their cortical monoterpene

profiles compared. Samples from the "parent" trees were taken from the lower third of the crown at approximately 15m and solvent extracted. The clones were sampled from the second whorl of laterals.

Data Analysis

The data were either analyzed by t tests or ANOVA, which (if $P \leq 0.05$) was followed by a Newman-Keuls test. Data were transformed by log10 (x+1) when the variances were unequal and the distributions were not normal.

RESULTS AND DISCUSSION

Characteristic Monoterpene Spectra of the Cortex and Foliage

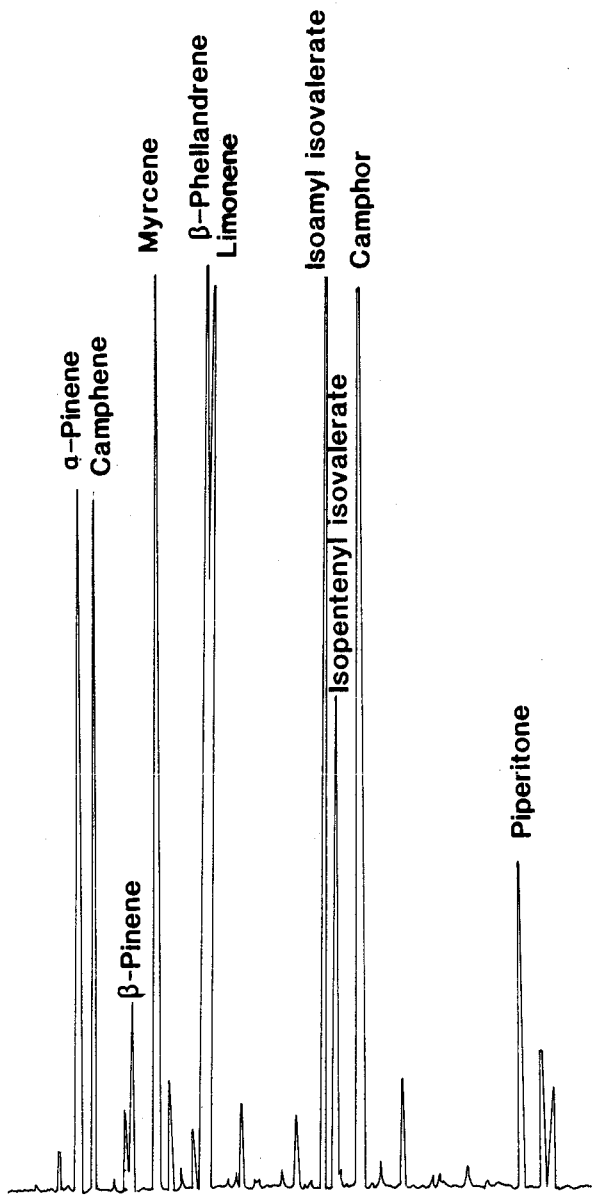
Figure 4 depicts the typical monoterpene spectra of Sitka spruce cortex and foliage. Qualitatively, the bark and foliage differ considerably. Therefore, separating the 2 tissues probably allowed the disclosure of significant differences that would otherwise have been obscured in whole branch samples.

Assessment of Sampling Methodology

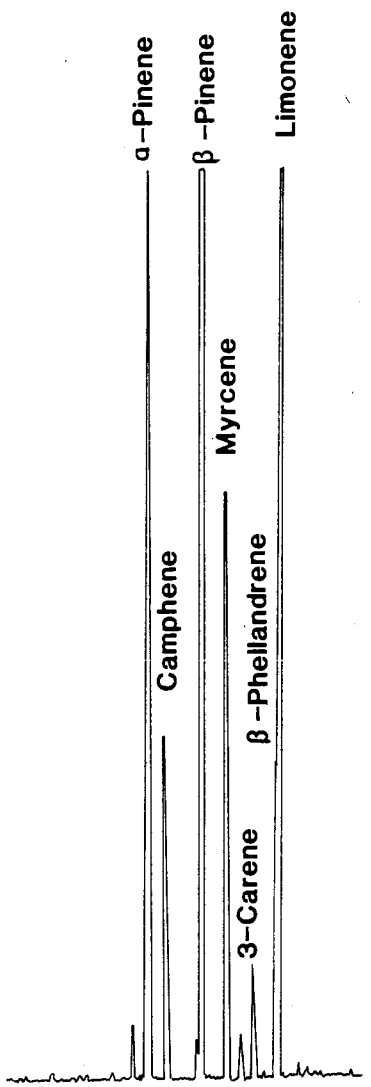
No major differences occurred in the monoterpene composition of 1 g and 5 g samples of one-year-old Sitka spruce foliage taken from 5 trees (Table 1). Only one 5 g sample was obtained per tree; therefore no statistical analysis was performed on the data. As 1 g samples were less destructive to the tree and easier to extract than 5 g samples, they were used for all further experiments.

In 2 of the 3 trees sampled, there were significant differences among cardinal directions in percent composition for 5 compounds: myrcene, β-phellandrene, isoamyl isovalerate, isopentenyl isovalerate and camphor (Table 2). However, the consistency in percent composition between the 5 samples within any one direction resulted in even slight differences between directions becoming apparent. The compounds that increased or

Figure 4. Characteristic monoterpene spectra of foliage and cortex from 2 representative samples from Sayward, B.C., prepared by solvent extraction.



Monoterpene spectrum of
Sitka spruce needle oil



Monoterpene spectrum of
Sitka spruce cortex oil

Table 1. Qualitative comparison of monoterpene composition in one-year-old foliage of Sitka spruce, using 1 g and 5 g samples. All samples normalized to 100%.

	Tree 1		Tree 2		Tree 3		Tree 4		Tree 5	
	1 g	5 g	1 g	5 g	1 g	5 g	1 g	5 g	1 g	5 g
Compound	$\bar{X}$(N=4)		$\bar{X}$(N=3)		$\bar{X}$(N=5)		$\bar{X}$(N=5)		$\bar{X}$(N=3)	
α-pinene	1.6	1.7	2.1	3.3	0.7	0.8	1.6	2.3	4.2	4.2
camphene	2.5	3.0	2.9	3.4	0.5	ND[1]	2.8	3.6	3.3	3.4
β-pinene	0.6	0.8	0.2	0.7	0.6	0.6	0.7	1.1	1.8	1.7
myrcene	46.6	44.8	56.6	56.4	59.3	56.1	46.2	52.5	52.8	58.5
β-phellan-drene-limonene	15.6	14.4	8.2	8.1	13.1	14.0	16.4	17.2	10.0	8.6
isoamyl isovalerate	4.6	5.2	4.6	4.8	8.9	9.9	7.2	5.3	6.0	4.8
isopentenyl isovalerate	3.5	3.4	2.6	2.7	3.5	4.1	2.5	2.0	3.0	2.2
camphor	2.9	3.6	12.5	11.8	ND	ND	0.2	0.6	8.1	7.3
piperitone	22.4	23.1	10.2	8.8	13.4	14.4	22.1	15.4	10.7	9.4

[1] ND= not detectable

Table 2. Ranked significant differences in monoterpene composition of foliage by cardinal direction for 2 trees sampled at the U.B.C. Research Forest in February, 1984. (No significant differences were found between cardinal directions for any compounds in a third tree sampled in April, 1984). N=5 samples, 1 g each, per cardinal direction.

	Percent composition ($\bar{X}$ ± SE)[1]				
Tree no.	myrcene	isoamyl isovalerate	isopentenyl isovalerate	β-phellandrene	camphor
1	45.7 ± 1.2(W)a	9.3 ± 0.4(S)a	4.5 ± 0.3(N)a		
	46.3 ± 0.6(E)a	9.7 ± 0.7(N)ab	4.5 ± 0.3(S)a		
	47.5 ± 0.9(N)ab	11.3 ± 0.6(E) b	5.8 ± 0.2(W) b		
	49.9 ± 0.7(S) b	11.5 ± 0.4(W) b	6.1 ± 0.4(E) b		
2		5.2 ± 0.3(N)a	2.6 ± 0.2(N)a	7.5 ± 0.1(E)a	9.3 ± 0.3(N)a
		5.6 ± 0.2(E)ab	2.9 ± 0.2(E)ab	7.6 ± 0.1(N)a	10.0 ± 0.5(S)ab
		6.1 ± 0.4(S)ab	3.2 ± 0.2(S)ab	8.0 ± 0.1(W) b	10.3 ± 0.2(E)ab
		6.7 ± 0.3(W) b	3.6 ± 0.2(W) b	8.1 ± 0.1(S) b	11.7 ± 0.7(W) b

[1]Letters in parentheses indicate cardinal direction. Means followed by same letter not significantly different, Newman-Keuls test, $P < 0.05$,

decreased significantly in the trees, differed in different trees. With the exception of a tendency for compounds from the north side of the tree to be present in lower amounts and compounds from the south and west sides to be present in greater amounts, there was little consistency in the ranked order of significant differences. Hanover (1966) also found similar trends in western white pine insofar as south-facing cortical tissues were higher in α-pinene and myrcene than any other cardinal direction. Overall, however there was a lack of consistent trends in relative amounts of compounds, and no subsequent effort was made to standardize sampling according to cardinal direction. However, when more than one sample was taken from a tree, they were taken from opposites sides of the same whorl.

The 5 even-aged trees from the U.B.C. Research Forest that were sampled holding sample height, needle age and time sampled identical, had many compounds that differed significantly between trees (Table 3). This inherent variability between normal weevil-susceptible trees supports von Rudloff's (1977) suggestion that at least 10 trees should be sampled to obtain a representative monoterpene profile for a population of trees.

It is important that foliage and branches of the same age are sampled to avoid additional variation between monoterpenes in tissues and branches of different ages (Hanover 1966; Hrutfiord et al. 1974). As well, von Rudloff (1982) stated that sampling during the winter is the only time to obtain a truly

Table 3. Comparison of the monoterpene composition between trees using 1 g foliage samples subjected to steam distillation and GC analysis. Samples are from 15-20 year-old weevil-susceptible trees, U.B.C. Research Forest, May 25, 1983. N= 4 samples/tree.

	Percent composition ($\bar{X}$± SE)									
Tree No.	α-pinene	camphene	β-pinene	myrcene	β-phellandrene	limonene	isoamyl isovalerate	isopentenyl isovalerate	camphor	piperitone
1	1.6± 0.2b	2.5± 0.2b	0.7± 0.1b	44.0± 2.6a	10.6± 0.6b	3.8± 0.3c	4.4± 0.5a	3.2± 0.4a	1.0± 0.3a	23.1± 2.4b
2	1.9± 0.4b	2.8± 0.2b	0.2± 0.3a	59.5± 6.2c	7.3± 1.6a	ND[1]a	3.5± 2.4a	2.0± 1.4a	11.8± 1.0c	10.6± 1.2a
3	0.7± 0.1a	NDa	0.5± 0.1b	58.2± 2.4c	13.3± 1.1c	NDa	9.0± 1.4b	3.6± 0.8a	NDa	13.3± 1.6a
4	1.5± 0.3b	2.7± 0.4b	0.6± 0.4b	43.8± 2.1a	15.5± 0.6d	NDa	6.9± 1.2ab	2.4± 0.5a	NDa	21.4± 3.1b
5	4.1± 0.4c	3.2± 0.2c	1.7± 0.0c	51.0± 4.4b	8.7± 1.4b	0.4± 0.8b	5.4± 1.3a	2.6± 0.8a	6.8± 1.3b	9.6± 0.9a

1 ND= not detectable. Means followed by same letter not significantly different, Newman-Keuls test, $P < 0.05$,

representative monoterpene spectrum of the foliage or cortex oil, and that large changes in bud oil may be occurring even when twig and foliage monoterpene composition are remaining constant.

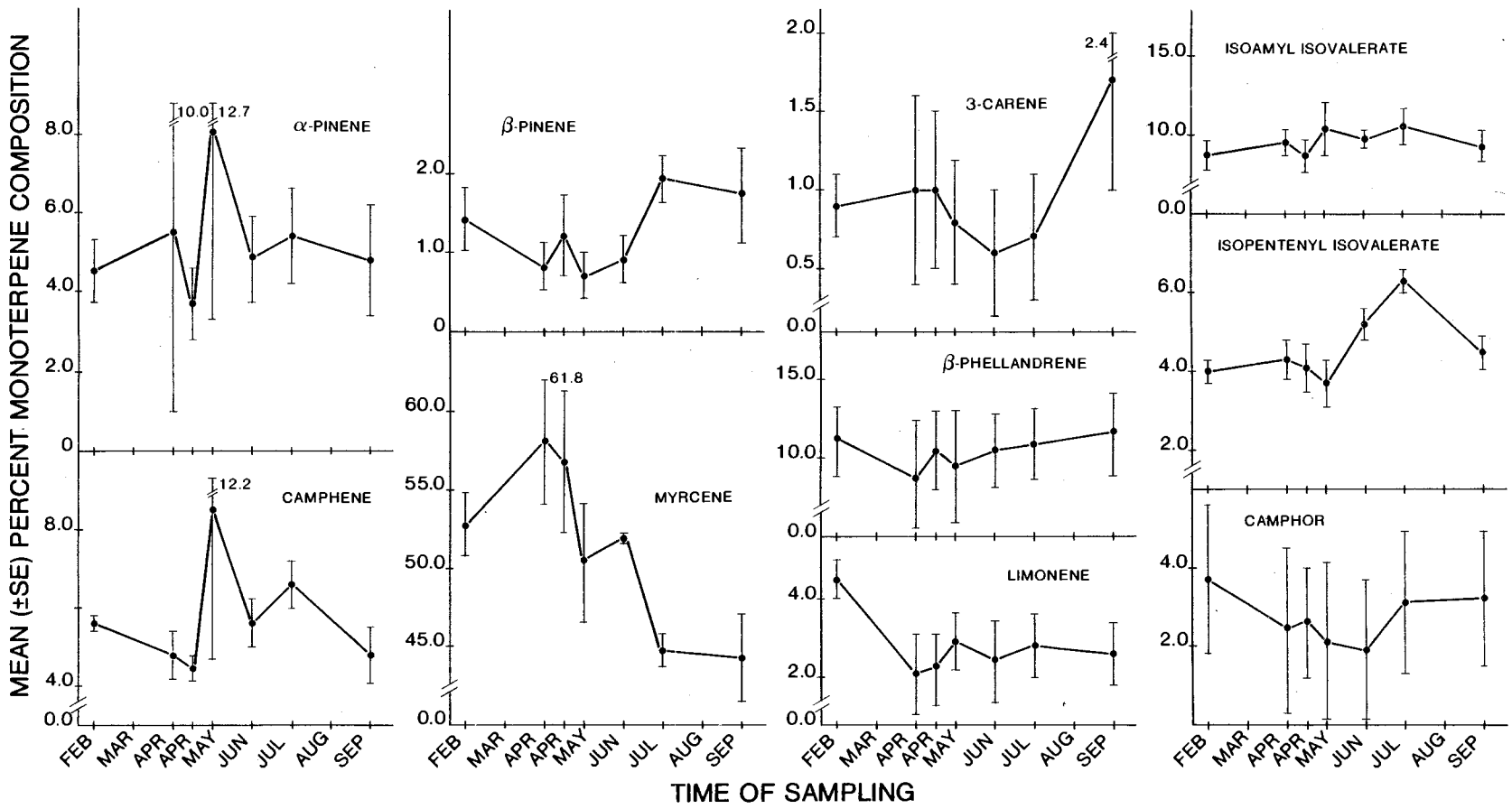
Because of these inherent sources of variation, the determination of a resistant chemotype would require large differences between resistant and susceptible trees.

Seasonal Variation in the Monoterpene Composition of One-year-Old Foliage

Seasonal differences occurred in the relative monoterpene composition of 10 compounds in one-year-old foliage of 4 trees sampled at the U.B.C. Research Forest, from February to September, 1984 (Fig. 5). The pronounced inter-tree variation (as evidenced by large standard errors) that occurred in May, may be indicative of varying phenologies of individual trees, as they resumed active metabolism in the spring.

Levels of α-pinene and camphene increased substantially in May, generally following the same pattern of change throughout the season, and returned to their original levels by September. Myrcene, isopentenyl isovalerate and camphor decreased in May. Isopentenyl isovalerate and camphor levels increased over the summer, and returned to their original levels, but myrcene levels declined, offsetting rising levels of 3-carene and β-pinene. β-Phellandrene, limonene and isoamyl isovalerate

Figure 5. Seasonal variation in 10 compounds in one-year-old foliage subjected to solvent extraction and GC analysis. Samples from 4 15-20-year-old trees from the U.B.C. Research Forest, February to September, 1984. Means for each date from 5 pooled samples per tree.


MEAN (±SE) PERCENT MONOTERPENE COMPOSITION
α-PINENE
10.0
12.7
β-PINENE
3-CARENE
2.4
ISOAMYL ISOVALERATE
CAMPHENE
12.2
MYRCENE
61.8
β-PHELLANDRENE
ISOPENTENYL ISOVALERATE
LIMONENE
CAMPHOR
FEB
MAR
APR
APR
MAY
JUN
JUL
AUG
SEP
TIME OF SAMPLING

levels remained fairly constant throughout the season.

These data correspond fairly well with data obtained by Hrutfiord et al. (1974) and von Rudloff (1977). The September results suggests that the trees were not yet in a quiescent phase, supporting von Rudloff and Granat's (1982) contention that the time of sampling for chemosystematic studies should be well into the winter.

Forrest (1980) stressed the importance of remaining with the same individual Sitka spruce trees when examining seasonal variation, due to the large inter-tree variation. In support of Forrest's (1980) observation, there was relatively little fluctuation in between-tree variation for the 4 trees, although the levels of one compound, camphor, consistently fluctuated greatly between trees.

Seasonal Variation in the Monoterpene Composition of Developing Buds

There were striking changes in monoterpene composition in flushing buds and new shoots during the spring (Fig. 6, 7). α-Pinene and β-pinene were present initially at fairly high levels and then declined as the season progressed (Fig. 6). Myrcene was present at low levels prior to bud flush and increased to become the predominant monoterpene in the oil by May 18.

Figure 6. Comparison of the seasonal variation in 5 compounds in developing buds subjected to solvent extraction and GC analysis. Samples are from 10 resistant and 10 susceptible 10-year-old trees from the B.C. Forest Service, Sayward Provenance Trial, April 20 to October 30, 1984.

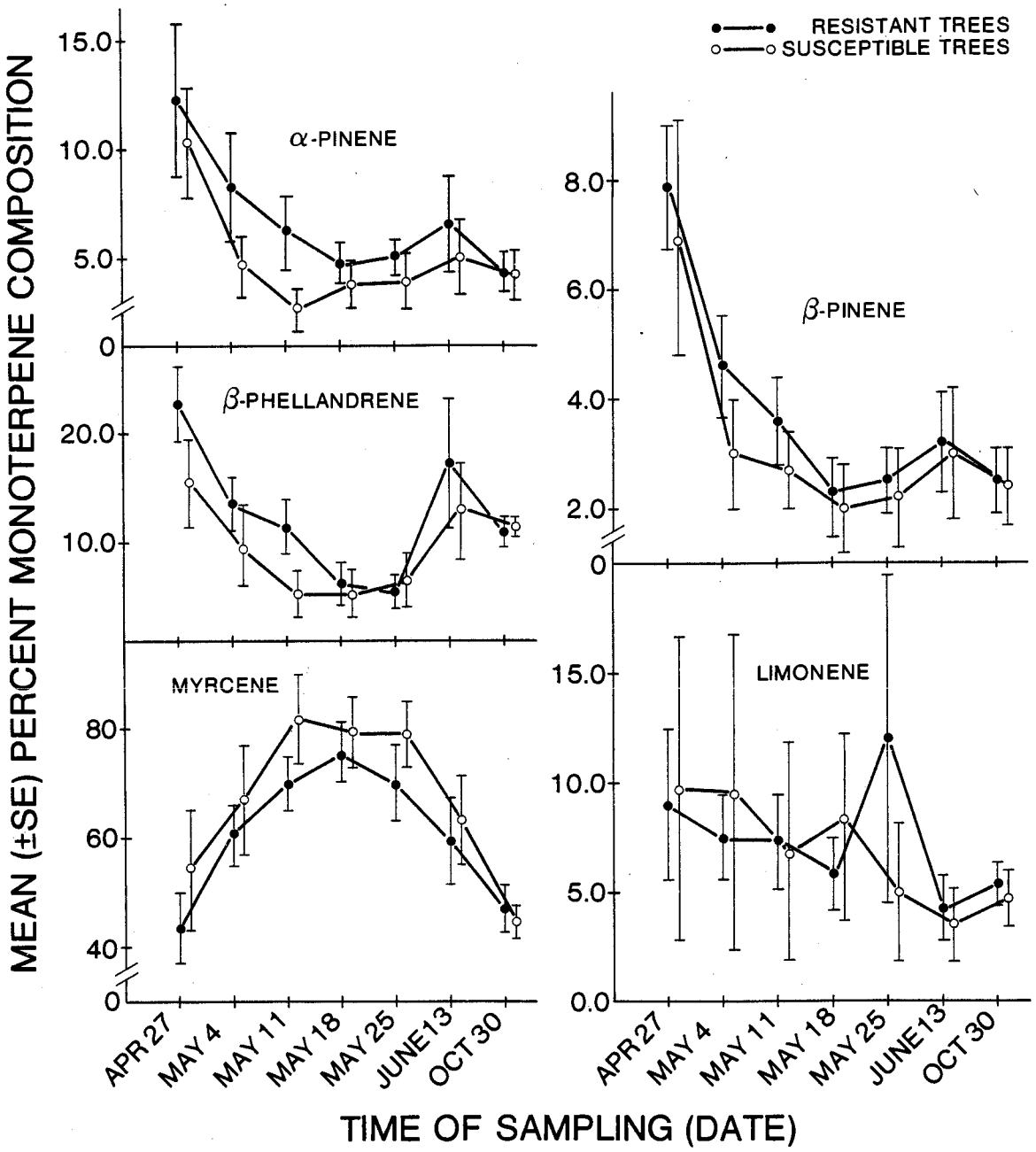

RESISTANT TREES
SUSCEPTIBLE TREES
α-PINENE
β-PINENE
β-PHELLANDRENE
MYRCENE
LIMONENE
MEAN (±SE) PERCENT MONOTERPENE COMPOSITION
TIME OF SAMPLING (DATE)
APR 27
MAY 4
MAY 11
MAY 18
MAY 25
JUNE 13
OCT 30

Figure 7. Seasonal variation in developing buds from one resistant tree from the B.C. Forest Service, Sayward Provenance Trial, April 20 to October 30, 1984.

APRIL 20
α-Pinene
β-Pinene
Myrcene
β-Phellandrene
Limonene
MAY 25
α-Pinene
β-Pinene
Myrcene
β-Phellandrene
Limonene
JUNE 13
α-Pinene
β-Pinene
Myrcene
β-Phellandrene
Limonene
OCTOBER 30
α-Pinene
Camphene
β-Pinene
Myrcene
β-Phellandrene
Limonene
Isoamyl Isovalerate
Isopentenyl Isovalerate
Piperitone

My results do not support those of Hrutfiord et al. (1974) who found that the oil of flushing buds consisted almost entirely of myrcene. Although myrcene was the major component at this stage, other compounds such as α-pinene, β-pinene, β-phellandrene, and limonene were present in measurable amounts (Fig. 6). Only rarely did myrcene comprise more than 90% of the volatile bud oil. The late season decline in myrcene levels and the accompanying increase in β-phellandrene content agree with Hrutfiord et al. (1974). Limonene levels were extremely variable between trees and no general trend was observed, except that by the end of the season, the relative percent composition of limonene was less than at the beginning (Fig. 6).

Cyclic oxygenated monoterpenes such as camphor and piperitone did not appear in the new shoots until after mid-June. (Fig. 7). These data are in general agreement with results for Sitka spruce (Hrutfiord et al. 1974; Forrest 1980) and balsam fir, Abies balsamea (L) Mill. (von Rudloff 1974; von Rudloff and Granat 1982). In contrast to the sequence of development in Sitka spruce (Fig.6), Maarse and Hepner (1970) found cyclic oxygenated monoterpenes to be present immediately in the newly flushed foliage of Douglas-fir, Pseudotsuga menziesii (Mirb.) Franco, while acyclic oxygenated monoterpenes such as citronellal, geranyl acetate, and linalool, did not occur until the leaves were maturing. They hypothesized that the neryl pyrophosphate mechanism accounted for the early biosynthesis of the cyclic oxygenated monoterpenes and that

another precursor, geranyl pyrophosphate, increased in importance for the biosynthesis of the acyclic oxygenated monoterpenes later in the season. Neryl pyrophosphate is also important in the biosynthesis of monoterpene hydrocarbons such as myrcene, α-pinene, and β-pinene (Zavarin 1970: Osvaldo 1983). Since neryl pyrophosphate is thought to be a precursor for many of the monoterpenes found in Sitka spruce, perhaps the enzymes responsible for production of piperitone and camphor, both cyclic oxygenated monoterpenes, are not synthesized until later in the season.

The characteristic isovalerates commonly present in Sitka spruce foliage (von Rudloff 1977) were not present at all in the developing bud tissue, even on June 13 when the new shoots had elongated. By October, both esters were present in their usual amounts and the monoterpene spectrum of the new foliage closely resembled that of one-year-old needles (Fig.4, 7). Until this time, sampling from new tissue would probably not give a representative spectrum for chemosystematic studies. There were no significant differences in monoterpene composition between buds from resistant and susceptible trees (Fig. 6). Generally, the variation in percent composition of monoterpenes in resistant and susceptible buds followed the same developmental trends. Therefore, it is unlikely that resistance resides in qualitative differences in bud monoterpenes. It is possible however, that the phenologies of the 2 tree types differ, or that there are differences in total amount of needle oil, that

could affect the host selection behavior of the weevils.

Comparison of Resistant and Susceptible Trees

There were several significant differences in relative percent monoterpene composition between resistant and susceptible trees in all 3 locations. Foliar analysis of the trees from Nootka Island demonstrated that isoamyl isovalerate and isopentenyl isovalerate were present in significantly greater amounts in susceptible than resistant trees, while camphene and myrcene were higher in the foliage of resistant trees (Table 4). Isoamyl isovalerate was higher in the foliage of susceptible trees from Sayward (Table 5). No significant differences appeared between resistant and susceptible bark tissue of the Sayward trees (Table 6).

In the Nass River Provenance Trial, the differences in monoterpene composition, between resistant and susceptible trees within 2 provenances, Kitwanga and Big Qualicum were explored (Tables 7-10).

There were no significant differences in the cortex or the foliage of resistant and susceptible trees from the Big Qualicum provenance (Tables 7,8). Camphene was significantly higher in the susceptible than the resistant foliage from the Kitwanga provenance (Table 9). Susceptible trees from the Kitwanga provenance had higher cortical myrcene levels than the resistant trees (Table 10).

Table 4. Comparison of percent monoterpene composition between resistant and susceptible trees, using 1 g foliage samples subjected to solvent extraction and GC analysis. Samples from 12 year-old trees, Nootka Island, B.C., August, 1983.

	Resistant (N=3)			Susceptible (N=4)[1]		
Compound	No. trees with compound	Range	$\bar{X}$ ± SE	No. trees with compound	Range	$\bar{X}$ ± SE
α-pinene	3	4.4 - 8.9	6.6 ± 1.3	4	3.0 - 8.0	4.8 ± 1.1
camphene	3	4.9 - 6.8	5.9 ± 0.6	4	1.0 - 5.2	3.0 ± 1.9*
β-pinene	1	ND[2] - 5.6	1.9 ± 1.9	4	1.4 - 5.8	3.0 ± 1.0
myrcene	3	43.9 - 63.9	56.7 ± 6.4	4	38.2 - 50.3	42.7 ± 2.9[3]
β-phellandrene	3	9.8 - 15.1	12.1 ± 1.6	4	12.3 - 16.0	14.1 ± 1.0
limonene	3	3.2 - 8.3	6.6 ± 1.7	4	3.6 - 13.7	8.4 ± 2.2
isoamyl isovalerate	3	4.0 - 6.6	5.4 ± 0.8	4	7.2 - 14.8	10.8 ± 1.6*
isopentenyl isovalerate	1	ND - 2.6	0.9 ± 0.9	4	2.0 - 6.1	3.6 ± 0.9[3]
camphor	1	ND - 0.5	0.2 ± 0.2	3	ND - 3.8	1.8 ± 0.9
piperitone	0	-	-	0	-	-

[1]Significant difference indicated by *, t test, $P < 0.05$.

[2]ND=not detectable.

[3]t test $P < 0.07$ for myrcene, and $P < 0.9$ for isopentenyl isovalerate.

Table 5. Comparison of percent monoterpene composition between resistant and susceptible trees, using 1 g foliage samples subjected to solvent extraction and GC analysis. Samples from 10 year-old trees, Sayward Provenance Trial, August, 1983.

	Resistant (N=13)			Susceptible (N=13)[1]		
Compound	No. trees with compound	Range	$\bar{X} \pm SE$	No. trees with compound	Range	$\bar{X} \pm SE$
α-pinene	13	1.9 - 15.3	6.1 ± 1.2	13	0.9 - 8.4	4.2 ± 0.7
camphene	8	ND[2] - 16.4	4.1 ± 1.5	8	ND - 8.0	1.6 ± 0.6
β-pinene	12	ND - 11.9	2.9 ± 0.8	13	1.0 - 7.2	2.7 ± 0.6
myrcene	13	26.9 - 84.9	52.9 ± 4.7	13	49.8 - 70.5	56.9 ± 2.0
β-phellandrene	12	ND - 19.9	9.8 ± 1.7	13	3.9 - 18.3	11.8 ± 1.1
limonene	13	1.7 - 18.6	6.3 ± 1.3	13	1.5 - 12.2	4.6 ± 0.9
isoamyl isovalerate	10	ND - 8.3	3.0 ± 0.8	13	0.2 - 17.8	6.6 ± 1.3*
isopentenyl isovalerate	7	ND - 3.7	1.2 ± 0.4	10	ND - 5.4	2.4 ± 0.5
camphor	7	ND - 25.4	4.8 ± 2.1	8	ND - 8.8	2.3 ± 0.8
piperitone	6	ND - 3.7	0.8 ± 0.3	4	ND - 6.2	1.0 ± 0.5

[1]Significant difference indicated by *, t test, $P < 0.05$.

[2]ND=not detectable.

Table 6. Comparison of percent monoterpene composition between resistant and susceptible trees, using 1 g cortex samples subjected to solvent extraction and GC analysis. Samples from 10 year-old trees, Sayward Provenance Trial, B.C. Forest Service, August, 1983.

Compound	Resistant (N=15)			Susceptible (N=15)[1]		
	No. trees with compound	Range	$\bar{X}$ ± SE	No. trees with compound	Range	$\bar{X}$ ± SE
α-pinene	15	11.5 - 47.9	29.8 ± 3.0	15	18.7 - 52.1	35.7 ± 2.7
camphene	12	ND[2] - 1.4	0.4 ± 0.1	14	ND - 1.2	0.5 ± 0.1
β-pinene	15	8.1 - 48.1	21.4 ± 2.7	15	12.3 - 29.4	19.8 ± 1.1
myrcene	15	0.9 - 25.1	5.2 ± 1.5	14	ND - 6.6	3.1 ± 0.5
3-carene	10	ND - 40.6	8.8 ± 3.2	12	ND - 20.9	5.9 ± 2.0
β-phellandrene-limonene	15	15.3 - 56.7	30.5 ± 3.5	15	13.3 - 54.7	30.8 ± 2.9

[1]No significant differences detected, t test, $P > 0.05$.

[2]ND=not detectable.

Table 7. Comparison of percent monoterpene composition between resistant and susceptible trees, using 1 g foliage samples subjected to solvent extraction and GC analysis. Samples were taken in October, 1983 from 10 year-old trees, provenance, Big Qualicum, in a B.C. Forest Service Provenance Trial, Nass River, B.C.

	Resistant (N=8)			Susceptible (N=6)[1]		
Compound	No. trees with compound	Range	$\bar{X} \pm SE$	No. trees with compound	Range	$\bar{X} \pm SE$
α-pinene	8	1.9 - 8.0	4.9 ± 0.9	6	1.6 - 8.0	3.8 ± 1.1
camphene	7	ND[2] - 8.4	3.8 ± 1.3	3	ND - 9.3	2.7 ± 1.6
β-pinene	8	0.9 - 3.0	2.0 ± 0.3	6	1.1 - 2.9	1.8 ± 0.3
myrcene	8	36.0 - 53.1	46.9 ± 2.4	6	38.4 - 59.2	50.7 ± 3.0
β-phellandrene-limonene	8	12.9 - 21.8	17.8 ± 1.0	6	14.6 - 23.8	18.7 ± 1.4
isoamyl isovalerate	8	4.7 - 16.2	9.1 ± 1.3	6	4.9 - 11.3	8.1 ± 1.0
isopentenyl isovalerate	8	2.0 - 6.6	3.9 ± 0.5	6	2.0 - 5.1	3.7 ± 0.5
camphor	7	ND - 12.2	4.7 ± 1.7	3	ND - 13.3	4.1 ± 2.4

[1] No significant differences detected, t test, $P > 0.05$.

[2] ND=not detectable.

Table 8. Comparison of percent monoterpene composition between resistant and susceptible trees, using 1 g cortex samples subjected to solvent extraction and GC analysis. Samples were taken in October, 1983 from 10 year-old trees, provenance Big Qualicum, in the B.C. Forest Service Provenance Trial, Nass River, B.C.

	Resistant (N=7)			Susceptible (N=7)[1]		
Compound	No. trees with compound	Range	$\bar{X}$ ± SE	No. trees with compound	Range	$\bar{X}$ ± SE
α-pinene	7	23.8 - 67.3	35.2 ± 5.7	7	33.9 - 56.3	42.9 ± 3.2
β-pinene	7	11.0 - 55.5	24.7 ± 5.5	7	12.0 - 43.3	20.3 ± 4.0
myrcene	4	ND[2] - 5.3	1.3 ± 0.7	5	ND - 2.8	1.7 ± 0.5
3-carene	6	ND - 36.1	12.6 ± 5.0	5	ND - 19.6	6.6 ± 2.5
β-phellandrene-limonene	7	2.1 - 37.9	21.9 ± 5.0	7	18.2 - 37.8	25.0 ± 2.6

[1]No significant differences detected, t test, $P > 0.05$.

[2]ND=not detectable.

Table 9. Comparison of percent monoterpene composition between resistant and susceptible trees, using 1 g foliage samples subjected to solvent extraction and GC analysis. Samples were taken in October, 1983 from 10 year-old trees, provenance, Kitwanga, in the B.C. Forest Service Provenance Trial, Nass River, B.C.

	Resistant (N=10)			Susceptible (N=11)[1]		
Compound	No. trees with compound	Range	$\bar{X} \pm SE$	No. trees with compound	Range	$\bar{X} \pm SE$
α-pinene	10	3.9 - 15.1	7.4 ± 1.0	11	1.9 - 19.3	10.8 ± 1.7
camphene	9	ND[2] - 17.1	6.2 ± 1.6	10	ND - 23.8	12.7 ± 2.6*
β-pinene	10	1.0 - 6.8	3.1 ± 0.6	11	1.2 - 5.8	3.3 ± 0.5
myrcene	10	31.0 - 64.8	52.3 ± 3.5	11	17.9 - 71.2	42.1 ± 5.3
β-phellandrene-limonene	10	5.5 - 38.9	19.0 ± 3.6	11	4.3 - 37.9	14.4 ± 3.0
isoamyl isovalerate	5	ND - 1.0	0.3 ± 0.1	8	ND - 1.3	0.5 ± 0.1
isopentenyl isovalerate	2	ND - 0.2	-	3	ND - 0.2	0.1 ± 0.0
camphor	9	ND - 24.2	8.2 ± 2.8	9	ND - 28.3	11.8 ± 3.1

[1] significant differences indicated by *, t test, $P < 0.05$.

[2] ND=not detectable.

Table 10. Comparison of percent monoterpene composition between resistant and susceptible trees, using 1 g cortex samples subjected to solvent extraction and GC analysis. Samples were taken in October, 1983 from 10 year-old trees, provenance, Kitwanga, in the B.C. Forest Service Provenance Trial, Nass River, B.C.

	Resistant (N-10)			Susceptible (N=10)[1]		
Compound	No. trees with compound	Range	$\bar{X}$ ± SE	No. trees with compound	Range	$\bar{X}$ ± SE
α-pinene	10	20.1 - 67.5	40.4 ± 4.3	10	19.7 - 48.5	34.2 ± 3.3
β-pinene	10	17.6 - 37.3	26.2 ± 2.4	10	11.1 - 44.4	26.9 ± 3.7
myrcene	4	ND[2] - 2.9	0.7 ± 0.4	9	ND - 14.7	4.8 ± 1.5*
3-carene	8	ND - 25.4	10.5 ± 3.0	7	ND - 16.6	6.3 ± 2.0
β-phellandrene-limonene	8	ND - 31.0	16.7 ± 3.7	10	2.9 - 35.3	22.7 ± 3.7

[1]Significant difference indicated by *, t test, $P < 0.05$.

[2]ND=not detectable.

The monoterpene spectra of these 2 provenances were also compared to those of 24 susceptible trees from 3 other provenances: Holberg, B.C., Tahsis Inlet B.C. and Necanicum, Oregon (Tables 11-14). Isopentenyl isovalerate was significantly higher in the needle tissue of the susceptible trees than in the Big Qualicum needle tissue (Table 11). No significant differences existed between cortical monoterpenes of the Big Qualicum provenance and those of the susceptible trees (Table 12). Isoamyl isovalerate and isopentenyl isovalerate were significantly higher in the needle tissue of the 3 susceptible provenances (Table 13). α-Pinene, β-pinene and camphor were significantly higher in the foliage of resistant trees from the Kitwanga provenance than that of the susceptible provenances (Table 13). Myrcene and β-phellandrene-limonene levels were significantly higher in the cortex samples of the susceptible provenances than the resistant Kitwanga trees (Table 14).

There were relatively few intra-provenance differences in monoterpene composition in the Big Qualicum and Kitwanga provenances.

The Big Qualicum provenance also had a similar spectrum to that of the 3 other provenances sampled. Only the level of isopentenyl isovalerate was significantly different between resistant and susceptible trees. This result stands in direct contrast to the resistant Kitwanga trees which exhibited many differences between resistant and susceptible trees (Table 13, 14). Two different types of resistance may be involved in the 2

Table 11. Comparison of percent monoterpene composition between resistant and susceptible trees, using 1 g foliage samples subjected to solvent extraction and GC analysis. Samples were obtained in October, 1983, from 10 year-old trees from the B.C. Forest Service Provenance Trial, Nass River, B.C. Samples from resistant trees are from the provenance Big Qualicum and those from susceptible trees are from the provenances Holberg, B.C., Tahsis Inlet, B.C. and Necanicum, Oregon.

	Resistant (N=8)			Susceptible (N=21)[1]		
Compound	No. trees with compound	Range	$\bar{X}$ ± SE	No. trees with compound	Range	$\bar{X}$ ± SE
α-pinene	8	1.9 - 8.0	4.9 ± 0.9	21	1.3 - 9.4	4.5 ± 0.5
camphene	7	ND[2] - 8.4	3.8 ± 1.3	18	ND - 9.0	4.0 ± 0.7
β-pinene	8	0.9 - 3.0	2.0 ± 0.3	21	1.1 - 3.0	2.0 ± 0.1
myrcene	8	36.0 - 53.1	46.9 ± 2.4	21	31.8 - 62.6	46.3 ± 2.1
β-phellandrene-limonene	8	12.9 - 21.8	17.2 ± 1.0	21	9.2 - 33.0	17.8 ± 1.4
isoamyl isovalerate	8	4.7 - 16.2	9.1 ± 1.3	21	4.4 - 19.0	11.2 ± 0.8
isopentenyl isovalerate	8	2.0 - 6.6	3.9 ± 0.5	21	1.4 - 9.5	5.7 ± 0.5*
camphor	7	ND - 12.2	4.7 ± 1.7	15	ND - 13.9	3.4 ± 0.8

[1]Significant difference indicated by *, t test, $P < 0.05$.

[2]ND=not detectable.

Table 12. Comparison of percent monoterpene composition between resistant and susceptible trees, using 1 g cortex samples subjected to solvent extraction and GC analysis. Samples were obtained in October, 1983, from 10 year-old trees from the B.C. Forest Service Provenance Trial, Nass River, B.C. Samples from resistant trees are from the provenance Big Qualicum and those from susceptible trees are from the provenances Holberg, B.C., Tahsis Inlet, B.C. and Necanicum, Oregon.

	Resistant (N=7)			Susceptible (N=21)[1]		
Compound	No. trees with compound	Range	$\bar{X}$ ± SE	No. trees with compound	Range	$\bar{X}$ ± SE
α-pinene	7	23.8 - 67.3	35.2 ± 5.7	21	15.2 - 52.2	33.3 ± 2.2
β-pinene	7	11.0 - 55.5	24.7 ± 5.5	21	14.2 - 41.2	20.7 ± 1.5
myrcene	4	ND[2] - 5.3	1.3 ± 0.7	14	ND - 7.4	2.7 ± 0.5
3-carene	6	ND - 36.1	12.6 ± 5.0	19	ND - 26.6	7.8 ± 1.6
β-phellandrene-limonene	7	2.1 - 37.9	21.9 ± 5.0	21	10.5 - 47.8	29.9 ± 2.6

[1]No significant differences detected, t test, $P > 0.05$.

[2]ND=not detectable.

Table 13. Comparison of percent monoterpene composition between resistant and susceptible trees, using 1 g foliage samples subjected to solvent extraction and GC analysis. Samples were obtained in October, 1983 from 10 year-old trees from the B.C. Forest Service Provenance Trial, Nass River, B.C. Samples from resistant trees are from the provenance Kitwanga, and those from susceptible trees are from the provenances Holberg, B.C., Tahsis Inlet, B.C. and Necanicum, Oregon.

	Resistant (N=10)			Susceptible (N=21)[1]		
Compound	No. trees with compound	Range	$\bar{X} \pm SE$	No. trees with compound	Range	$\bar{X} \pm SE$
α-pinene	10	3.9 - 15.1	7.4 ± 1.0	21	1.3 - 9.4	4.5 ± 0.5*
camphene	9	ND[2] - 17.1	6.2 ± 1.6	18	ND - 9.0	4.0 ± 0.7
β-pinene	10	1.0 - 6.8	3.1 ± 0.6	21	1.1 - 3.0	2.0 ± 0.1*
myrcene	10	31.0 - 64.8	52.3 ± 3.5	21	31.8 - 62.6	46.3 ± 2.1
β-phellandrene-limonene	10	5.5 - 38.9	19.0 ± 3.6	21	9.2 - 33.0	17.8 ± 1.4
isoamyl isovalerate	5	ND - 1.0	0.3 ± 0.1	21	4.4 - 19.0	11.2 ± 0.8*
isopentenyl isovalerate	2	ND - 0.2	-	21	1.4 - 9.5	5.7 ± 0.5*
camphor	9	ND - 24.2	8.2 ± 2.8	15	ND - 13.9	3.4 ± 0.8*

[1]Significant difference indicated by *, t test, $P < 0.05$.

[2]ND=not detectable.

Table 14. Comparison of percent monoterpene composition between resistant and susceptible trees, using 1 g cortex samples subjected to solvent extraction and GC analysis. Samples were obtained in October, 1983 from 10 year-old trees from the B.C. Forest Service Provenance Trial, Nass River, B.C. Samples from resistant trees are from the provenance Kitwanga, and those from susceptible trees are from the provenance Holberg, B.C., Tahsis Inlet, B.C. and Necanicum, Oregon.

	Resistant (N=10)			Susceptible (N=21)[1]		
Compound	No. trees with compound	Range	$\bar{X}$ ± SE	No. trees with compound	Range	$\bar{X}$ ± SE
α-pinene	10	20.1 - 67.5	40.4 ± 4.3	21	15.2 - 52.2	33.3 ± 2.2
β-pinene	10	17.6 - 37.3	26.2 ± 2.4	21	14.2 - 41.2	20.7 ± 1.5
myrcene	4	ND[2] - 2.9	0.7 ± 0.4	14	ND - 7.4	2.7 ± 0.5*
3-carene	8	ND - 25.4	10.5 ± 3.0	19	ND - 26.6	7.8 ± 1.6
β-phellandrene-limonene	8	ND - 31.0	16.7 ± 3.7	21	10.5 - 47.8	29.9 ± 2.6*

[1]Significant differences indicated by *, t test, $P < 0.05$.

[2]ND=not detectable.

provenances, assuming that monoterpenes act as a defense mechanism for the tree. The Big Qualicum trees were frequently weeviled, but were tolerant to attack, because they recovered well from weeviling, suffering few bole deformities or forked leaders, whereas the Kitwanga trees simply were not attacked at the same rate as other trees. Thus, the possession of a monoterpene chemotype that is different from susceptible provenances may reflect a biochemically-based resistance in the Kitwanga trees.

The cortical monoterpene spectra of the resistant and susceptible trees from Green Timbers were similar to the spectra obtained by Harris et al. (1983) (Table 15). Very few monoterpenes were present in the foliage of the resistant Green Timbers trees. The foliage appeared chlorotic and perhaps did not have an extensive resin canal system within the needles. 3-Carene and terpinolene were higher in the resistant trees, while α-pinene, myrcene and β-phellandrene-limonene were present in greater quantities in the susceptible trees. Moreover, the monoterpene spectra of the cortex of both the Kitwanga and the Big Qualicum provenances did not resemble that of the resistant Green Timbers trees.

In support of reports that high myrcene levels generally coincide with resistance of conifers to insects (Gollob 1980), there was a slight (but not significant) trend for myrcene

Table 15. Comparison of percent monoterpene composition between resistant and susceptible trees, using 1 g cortex samples subjected to solvent extraction and GC analysis. Samples were obtained from the Green Timbers Nursery, B.C. Forest Service, Surrey, B.C., in March 1984.

	Resistant (N=4)			Susceptible (N=5)[1]		
Compound	No. trees with compound	Range	$\bar{X} \pm SE$	No. trees with compound	Range	$\bar{X} \pm SE$
α-pinene	4	10.2 - 20.3	14.7 ± 2.4	5	23.1 - 29.5	26.5 ± 1.1*
β-pinene	4	15.7 - 28.0	23.1 ± 2.7	5	16.1 - 21.5	18.6 ± 0.9
myrcene	4	2.5 - 3.9	3.1 ± 0.3	5	3.6 - 6.3	5.6 ± 0.5*
3-carene	4	20.0 - 50.8	37.7 ± 7.4	5	0.7 - 17.5	4.4 ± 3.3*
β-phellandrene-limonene	4	6.5 - 22.6	14.9 ± 3.5	5	32.5 - 46.6	42.7 ± 2.7*
terpinolene	4	2.4 - 5.2	3.8 ± 0.8	5	0.3 - 1.2	0.7 ± 0.2*

[1]Significant differences indicated by *, t test, $P < 0.05$.

levels in the foliage to be higher in the resistant trees [5] [6]. However, from both Sayward and the Nass River, there were significant differences in percent monoterpene composition only for compounds such as α-pinene, β-pinene, camphene and camphor.

Two foliar compounds, isoamyl isovalerate and isopentenyl isovalerate emerge as the most consistently different between susceptible and resistant trees. Both are usually present in much higher relative amounts in the susceptible trees than the resistant trees. Therefore, it is possible that resistant trees may in part lack a characteristic spruce odor that is imparted by these compounds. Few significant differences were found between the cortical monoterpenes of resistant and susceptible trees, despite the fact that weevils feed on the bark rather than the needle tissue. Myrcene,however, was significantly higher in susceptible trees than resistant ones at the Nass River site.

Comparison between Resistant Parents and their Grafted Scions

As for the resistant Green Timbers trees, their clones at the North Road Laboratory, Victoria, B.C. also had very few monoterpenes present in their foliage. Surprisingly, the

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[5]Hrutfiord, B. F., D. L. Warkentin, and R. I. Gara. 1983. Terpene complement of slow and fast growing Sitka spruce terminals as related to *Pissodes strobi* host selection behavior. Unpub. MS, College of Forest Resources, University of Washington, Seattle.

[6]Carlson, R. L. 1971 PhD. thesis, College of Forest Resources, University of Washington, Seattle.

cortical monoterpene spectra of the cloned trees differed from that of the 'parent' trees (Table 16). The relative levels of terpinolene and 3-carene were higher in the parent trees, while levels of α-pinene, and the β-phellandrene-limonene complex were higher in the clones. In fact, the monoterpene profile of the clones was more similar in composition to the susceptible trees sampled at the Green Timbers site (Table 15) than to their resistant parents.

This difference between grafted scions and their parents was unexpected as rootstocks have not generally been shown to influence the oleoresin monoterpene composition in scions (Schmidtling 1974; Kossuth et al. 1981). However , Kossuth et al. (1981) found that scions of slash pine, Pinus elliotii, Engelm. and sand pine, P. clausa var. immuginata, D. B. Ward altered the monoterpene composition of slash pine rootstock. They also suggested that there might be a slight effect of the rootstock on the scion. Possibly, this effect occurred with the grafted scions from the North Road Laboratory.

According to Kossuth et al. (1981) resin flow between scion and rootstock or vice versa should not occur across a new graft union until new phloem and xylem transport systems are established. Only then would new oleoresin be synthesized or old oleoresin show interconversions. The practice of using rootstocks to confer resistance against insects or disease or to favorably induce the processes of flowering and fruiting have long been employed (Schmidtling 1983). Depending on the type of

Table 16. Comparison of percent monoterpene composition between resistant parent trees and their grafted scions, using 1 g cortex samples subjected to solvent extraction and GC analysis. Samples were obtained from the Green Timbers Nursery, B.C. Forest Service, Surrey, B.C. and the B.C. Forest Service, North Road Laboratory, Victoria, B.C. in March and April 1984, respectively.

	Resistant parent trees (N=4)			Clones (N=9)[1]		
Compound	No. trees with compound	Range	$\bar{X}$ ± SE	No. trees with compound	Range	$\bar{X}$ ± SE
α-pinene	4	10.2 - 20.3	14.7 ± 2.4	9	11.1 - 38.6	29.6 ± 2.8*
β-pinene	4	15.7 - 28.0	23.1 ± 2.7	9	17.4 - 40.9	25.8 ± 3.0
myrcene	4	2.5 - 3.9	3.1 ± 0.3	9	1.1 - 5.6	3.4 ± 0.4
3-carene	4	20.0 - 50.8	37.7 ± 7.4	9	0.3 - 42.9	7.7 ± 4.6*
β-phellandrene-limonene	4	6.5 - 22.6	14.9 ± 3.5	9	11.4 - 43.2	30.1 ± 3.9*
terpinolene	4	2.4 - 5.2	3.8 ± 0.8	8	ND[2] - 4.8	1.0 ± 0.5*

[1]Significant differences indicated by *, t test, $P < 0.05$.

[2]ND=not detectable.

rootstock used, Chang and Philogene (1976) found that resistance to the pear psylla, Psylla pyricola L., was increased. Although it is unknown what confers resistance, resistance is imparted by some mechanism or factor that is transmitted to the susceptible grafted scion.

CONCLUSIONS

Several conlusions can be drawn from this study. Firstly, there is a great deal of between-tree variation as well as within-tree variation. Therefore, differences in monoterpene composition between resistant and susceptible trees would have to be very large to be evident.

Secondly, there is considerable seasonal variation in one-year-old foliage, and developing buds, especially in May when the trees are again becoming metabolically active. To achieve consistent results it is best to sample the mature foliage from different trees at the same time, holding as many parameters constant as possible. Current year foliage is not quiescent before October and should only be sampled from then on to obtain representative monoterpene profiles.

The two esters, isoamyl isovalerate and isopentenyl isovalerate differ significantly between resistant and susceptible trees. However, there is a wide range in the mean levels of these compounds in trees from the 3 principal sites; e.g. isoamyl isovalerate varied from 0.3-9.1% in resistant trees and from 0.5-11.2% in susceptible trees. With so much overlap it would be difficult to define accurately a resistant chemotype based solely upon these 2 compounds.

Wilkinson (unpub.)[7] found that the monoterpene spectra of resistant western white pine closely resembled that of the most

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[7]Wilkinson, R. C. unpublished data. U.S. Dept. Agric., Forest Service, Northeastern Forest Experiment Station, Durham, New Hampshire.

susceptible eastern white pines. Therefore, monoterpenes alone may not prove to be adequate in determining a resistant chemotype. As well, the significant differences in monoterpene composition that existed between the resistant Green Timbers trees and their grafted scions from the North Road Laboratory were unexpected. Further research should proceed to examine the influence of rootstock monoterpenes on the resin canal system of the grafted scions. If these differences are consistent, then it is unlikely that breeding programs using grafted scions could be maintained solely by the occurrence of a resistant chemotype. Rather, parent trees and their progeny should be selected on the basis of many alternative characteristics including low levels of the isovalerates in their monoterpene spectra. These other resistant characteristics might include morphological features such as size and depth of resin canals, the occurrence of chemical attractants, repellents, feeding stimulants and deterrents and the antibiotic effects of traumatic resin and other constituents. The ability of certain infested trees to tolerate infestation by allowing only one lateral branch to assume apical dominance, and the characteristics of these replacement leaders should also be considered. Long-term breeding programs for resistance against the white pine weevil are unlikely. However, should one ever be implemented for Sitka spruce, a broad spectrum of resistant characteristics should be used.

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