Changes in cortical and wood terpenes in Sitka spruce in response to wounding

J.R. Nault and Rene I. Alfaro

Abstract: Terpene levels were measured in bark and wood samples of Sitka spruce (*Picea sitchensis* (Bong.) Carrière) trees. The trees that had been selected as early or late flushing were subjected to artificial wounding to simulate attack by the white pine weevil, *Pissodes strobi* (Peck). Samples were taken at four times during the growing season: before wounding, shortly after wounding, and two times later in the season. Terpenes were extracted with hexane and quantified by capillary gas chromatography. There were no significant differences in total bark terpenes between early- and late-flushing trees or between control and wounded trees over all sampling times. There were no significant differences in total wood terpenes between early- and late-flushing trees or among sampling times, but a statistically significant difference was found between control and wounded trees. Eleven individual terpenes accounted for the majority of the terpenes in the extracts. Four bark terpenes showed significant differences with sampling time, two with flushing class, and none with treatment. Five wood terpenes showed significant differences with sampling time, two with flushing class, and nine with treatment. We concluded that flushing had only a minor effect on bark and wood terpene profiles and that wounding significantly increased terpene concentration in wood shortly after injury.

Résumé : Les niveaux de composés terpéniques ont été mesurés dans des échantillons d'écorce et de bois d'épinettes de Sitka (*Picea sitchensis* (Bong.) Carrière). Les arbres, sélectionnés en fonction de leur débourrement hâtif ou tardif, ont été artificiellement blessés de façon à simuler l'attaque du charançon du pin blanc, *Pissodes strobi* (Peck). Les échantillons ont été prélevés à quatre reprises pendant la saison de croissance : avant que les arbres soient blessés, peu après leur blessure et deux autres fois, plus tard au cours de la saison. Les composés terpéniques ont été extraits avec de l'hexane et quantifiés par chromatographie en phase gazeuse sur colonne capillaire. Au cours des quatre échantillonnages, aucune différence significative dans les terpènes totaux de l'écorce n'est apparue entre les arbres à débourrement hâtif et tardif ou entre les arbres blessés et les arbres témoins. Il n'y avait aucune différence significative entre les arbres à débourrement hâtif et tardif ou entre les dates d'échantillonnage dans les terpènes totaux du bois; cependant, une différence significative a été remarquée entre les arbres blessés et les arbres témoins. Les extraits étaient constitués en majeure partie de onze composés. Quatre terpènes de l'écorce présentaient des différences significatives en fonction de la date d'échantillonnage; deux, en fonction de la classe de débourrement; et aucun, en fonction du traitement. Cinq terpènes du bois présentaient des différences significative en fonction de la date d'échantillonnage; deux, en fonction de la classe de débourrement; et neuf, en fonction du traitement. Nous en avons conclu que le débourrement n'avait qu'un effet mineur sur les profils terpéniques de l'écorce et du bois et qu'une blessure augmentait significativement la concentration terpénique du bois peu de temps après être survenue.

[Traduit par la Rédaction]

Introduction

Terpenes are the main constituents of the essential oils of coniferous trees and have been extensively studied in attempts to understand tree physiology, phenology, defenses, and chemotaxonomy.

For Sitka spruce (*Picea sitchensis* (Bong.) Carrière), studies have examined seasonal variations in terpenes in cortical oleoresin exuded from wounds (Forrest 1980*a*) and in buds and needles (Brooks et al. 1987*a*). Geographic variations in terpenes have also been studied in needles (Von Rudloff 1978) and in cortical oleoresin exuded from wounds (Forrest 1980*b*). Within- and among-tree variations in terpenes have been examined in needles and cortex (Hrutfiord et al. 1974), in needles (Von Rudloff 1978), in cortical oleoresin exuded from wounds (Forrest 1980*b*), and in buds and needles (Brooks et al. 1987*a*). Attempts to correlate specific terpene patterns in needles and bark with insect resistance (Brooks et al. 1987*b*; Nault et al. 1999) have met with little or no success. While one study (Tomlin et al. 1997) found that trees which were susceptible to white pine weevil (*Pissodes strobi* (Peck)) attack had, on average, higher terpenes in foliage than trees that were resistant to attack, they concluded that "there may be too much variation within provenances to select trees on this basis." They also concluded that "there appears to be no clear relationship between terpene profile and resistance or susceptibility."

To date, attempts to relate terpenes in Sitka spruce to geography have been unsuccessful using needle terpenes (Von Rudloff 1978) or terpenes from cortical oleoresin exuded

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from wounds (Forrest 1980*b*). Relating specific tree characteristics to needle terpenes (Von Rudloff 1978) and terpenes in buds and needles (Brooks et al. 1987*a*) has also not been successful. Within-tree variation in terpene composition has been demonstrated to be small for needle terpenes (Von Rudloff 1978), while among-tree variation in terpenes has been demonstrated to be large for needles (Von Rudloff 1978), needles and bark (Nault et al. 1999), needles and buds (Brooks et al. 1987*a*), and cortical oleoresin exuded from wounds (Forrest 1980*a*, 1980*b*). Seasonal variations in terpene composition are large for buds and needles in the first year of growth and small for mature needles and cortical tissue (Forrest 1980*a*; Brooks et al. 1987*a*).

Terpene composition has also been extensively studied in other species of spruce. Black spruce (*Picea mariana* (Mill.) BSP) foliage showed major changes in terpene composition just after bud burst and little seasonal variation in mature foliage (Von Rudloff 1975). White spruce (*Picea glauca* (Moench) Voss) and Engelmann spruce (*Picea engelmanii* Parry ex Engelm.) terpene composition of needles and bark was demonstrated to be very similar among the ramets of a clone, while those of open-pollinated progeny varied considerably (Nault et al. 1999). Drought is known to affect the needle terpenes in Norway spruce (*Picea abies* (L.) Karst) (Kainulainen et al. 1992), while wounding has been demonstrated to significantly increase terpene cyclase activity in the wood and bark of species of spruce and true firs (*Abies* spp.) (Lewinsohn et al. 1991). Terpene and diterpene resin acid contents in white spruce wood were found to be elevated at the wound site after artificial wounding with a drill (Tomlin et al. 2000).

The white pine weevil is the most damaging insect pest of young spruce and pine trees in North America. The main hosts in British Columbia are Sitka spruce, white spruce, and Engelmann spruce. In early spring (late March, April) adult weevils emerge after overwintering in duff to feed on the phloem of lower internodes. Later, after mating, they oviposit in the upper section of the previous year's leader. After hatching, the larvae mine downwards, consuming phloem and subsequently girdling and killing the leader. Larvae pupate in chambers excavated in the xylem. Adults emerge from the leaders from late July to September, and when the temperature drops and the photoperiod shortens they hibernate in duff (Silver 1968).

The weevil excavates characteristic feeding and oviposition punctures with its elongated mouth parts. Resin is often seen flowing from feeding punctures. This resin originates from constitutive resin ducts severed during feeding or is produced by traumatic resin ducts, which form from the cambium in response to wounding (Alfaro 1995). This induced resin production is a characteristic response of many conifers to wounding and insect or pathogen attack (Safranyik et al. 1974; Berryman 1972, 1988).

The amount of resin production varies seasonally. When wounding spruce trees at monthly intervals, Safranyik et al. (1983) found low resin flow early in the season and suggested that it would be advantageous for spruce beetles (*Dendroctonus rufipennis* Kirby) to colonize trees at this time. Studying weevil attack rates on Sitka spruce, Hulme (1995) noted that the least damaged trees started apical-bud development earlier in the season relative to susceptible clones, suggesting earlier resin production.

The objectives of this study were to determine if measurable changes in terpene composition could be detected in the bark and wood of young Sitka spruce after a mechanical wounding treatment that simulated attack by the white pine weevil. The availability of early- and late-flushing (as measured by apical-bud development) Sitka spruce families allowed us to test for a possible influence of flushing date on the terpene profiles of wounded and unwounded trees.

Material and methods

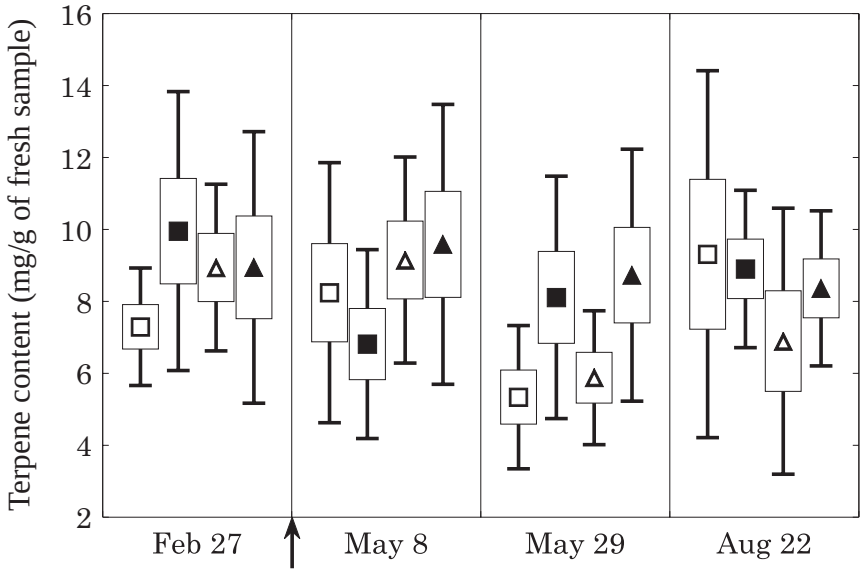
Open-pollinated seeds were collected from 120 Sitka spruce parent trees in coastal British Columbia (B.C.). The geographic source of the parent trees ranged from coastal B.C. to coastal Washington and Oregon. Seeds were germinated, and the resulting seedlings were planted in the spring of 1993 at the Western Forest Products seed orchard in Saanichton, B.C. on Vancouver Island. The progeny trial consisted of 120 families with four replicates of seven trees per family (28 seedlings/family). The trial was surrounded by a row of Sitka spruce buffer trees.

During the growing season of 1996, apical-bud development in all the families was monitored by visually comparing the appearance of the apical bud with a chart describing eight observable bud stages for Sitka spruce (Alfaro et al. 2000). The first and last five families to reach bud phenology stage 4 were classified as early- and late-flushing families, respectively. To reach bud development stage 4, early families required a mean accumulation of 240 degree-days (threshold temperature, 5°C), counted from April 1, 1996, while late-flushing families required a mean of 324 degree-days.

From each of the early- and late-flushing classes, 56 trees were randomly selected and randomly assigned to control (nonwounded) and wounded groups. Four sets of samples were taken from February 27 to August 22, 1997, and each time, seven trees were randomly sampled from each flushing class and treatment ($7 \times 2 \times 2$). Wounding was conducted on April 23 by drilling twenty-four 1 mm diameter holes with a portable drill. Wound holes were arranged in three vertical rows (eight in each row) on three sides of the leader, just below the apical bud. Within the row, holes were approximately 1 cm apart. Sampling times were February 27 (before wounding, buds at rest), May 8, May 29, and August 22.

For sampling, leaders were cut from the selected trees and stored in a dark cold room at –5°C. A section about 5 cm long was cut from each leader about 2 cm below the wounding site. Needles were removed from the leader and then the bark was peeled from the wood. This bark contained both outer and inner bark (phloem). To aid the extraction process, the wood and bark samples were cut into smaller pieces. Wood samples were also ground with a mortar and pestle with liquid nitrogen. Wood or bark samples were then placed in a test tube containing cold methanol : water (2:1, 6 mL), hexane (6 mL), and internal standard (4 mg/mL methyl palmitate, in 2 mL isooctane). With the test tubes immersed in ice water, the samples were rapidly homogenized by means of a Polytron (FT 2000) homogenizer equipped with a modified cutting head to aid with disintegrating the material. The solvent mixture was centrifuged, the hexane (upper) layer (known to contain terpenes, chlorophyll, and other lipophilic constituents) was filtered through surgical grade cotton wool, and the aliquots were stored in vials for analysis. The polar compounds were partitioned into the methanol–water fraction and discarded. For each set of samples a solvent blank was also prepared using exactly the same procedure to ensure solvent purity.

Fig. 1. Total bark terpenes (mg/g fresh sample). Symbols for means are as follows: □, early-flushing control; ■, late-flushing control; △, early-flushing wounded; ▲, late-flushing wounded. Boxes are ±SE, whiskers are ±SD. ↑, date of wounding (April 23).



All terpene analyses were made using a Hewlett-Packard (HP) 5890 gas chromatograph equipped with a HP 7673A autosampler and autoinjector. The injector temperature was kept at 180°C, and the flame ionization detector (FID) detector was held at 295°C. The capillary column was 25 m × 0.20 mm i.d. with 0.33 µm HP Ultra-2 (5% diphenyl – 95% dimethyl siloxane copolymer) coating. The following temperature program was used: 60°C for 1 min, then increased to 275°C at a rate of 7°C/min, and held at 275°C for 10 min. The detector output was captured by Chemstation software (HP).

Terpene levels were calculated as milligrams of terpene per gram of fresh sample, using the following formula:

$$T = \frac{(P/I)CE}{S}$$

where T is the terpene level (mg/g) in the fresh sample, P is the terpene peak area from the chromatogram, I is the internal standard peak area from the chromatogram, C is the concentration of internal standard (mg/mL) in the extraction volume, E is the volume (mL) of extraction solvent used, and S is the fresh sample mass (g). This formula assumes that the FID response is equal for all terpenes and the internal standard. This approximation is justified in that we are looking at relative levels among groups and not necessarily absolute levels.

Peaks were identified by comparing their retention times with those of compounds in standard samples. Terpene identifications were verified for selected samples by using gas chromatography (GC) – mass spectronomy (MS) and GC – Fourier transform infrared spectroscopy (FTIR) experiments. Columns and conditions for the GC–MS and GC–FTIR were identical to the method described above, except that a slightly larger capacity column was used for the GC–FTIR (25 m × 0.32 mm i.d. with 0.17 µm HP Ultra-2), as required by the detector. After verifying correct peak assignment and integration, the data were analyzed with SAS® software (SAS Institute Inc. 1990) using analysis of variance (ANOVA; PROC GLM) and Duncan's multiple means test. Differences among flushing classes, sampling times, and treatments (wound and control) were considered significantly different at $P < 0.05$.

Table 1. Results from the analysis of variance of total bark terpenes of early- and late-flushing Sitka spruce trees before and after mechanical wounding.

	$P > F$
F	0.086
S	0.172
T	0.602
F × S	0.257
F × T	0.810
S × T	0.283
F × S × T	0.508

Note: Samples were collected at four times throughout the growing season, once before wounding, and three times after wounding. F, flushing (56 trees × 2 classes); S, sampling time (28 trees × 4 times); T, treatment (56 trees × 2 treatments).

Results

Total terpenes

For bark terpenes variability was high within each treatment and flushing class (Fig. 1). Using ANOVA, no significant effects or interactions were found for flushing class, sampling time, or treatment (Table 1).

For wood terpenes variability was also high for each treatment and flushing class (Fig. 2). Using ANOVA, no significant effects were found for flushing class or sampling time (Table 2). Treatment was found to have a significant effect, with wounded samples (1.51 ± 0.67 mg/g, mean ± SD) having higher terpene levels than control samples (1.16 ± 0.49 mg/g). No significant interactions were found.

Fig. 2. Total wood terpenes (mg/g fresh sample). Symbols for means are as follows: □, early-flushing control; ■, late-flushing control; △, early-flushing wounded; ▲, late-flushing wounded. Boxes are ±SE, whiskers are ±SD. ↑, date of wounding (April 23).

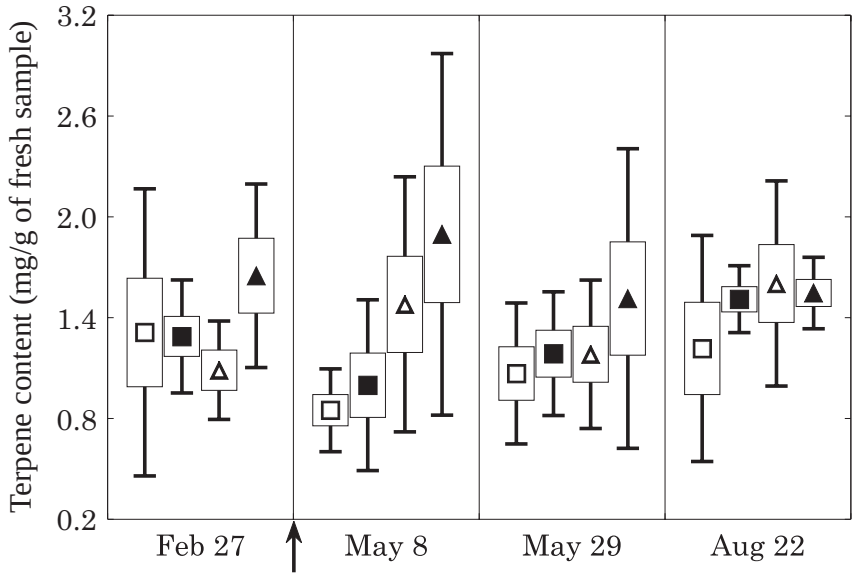


Table 2. Results from the analysis of variance of total wood terpenes of early- and late-flushing Sitka spruce trees before and after mechanical wounding.

	P >*F*
F	0.053
S	0.514
T	0.003
F × S	0.960
F × T	0.329
S × T	0.172
F × S × T	0.400

Note: Samples were collected at four times throughout the growing season, once before wounding, and three times after wounding. F, flushing (56 trees × 2 classes); S, sampling time (28 trees × 4 times); T, treatment (56 trees × 2 treatments).

Table 3. Individual bark and wood terpene retention times and percent contribution to total bark and wood terpenes in the Sitka spruce trees used in this study (*n* = 112).

Terpene	Relative retention time (vs IS)*	Mean % contribution to total terpenes	
		Bark	Wood
Terpene 1	0.157	4.90	29.44
α-pinene	0.275	19.62	10.90
Sabinene	0.307	2.04	1.98
β-pinene	0.312	10.96	5.74
Myrcene	0.317	4.75	2.19
△-3-carene	0.339	3.31	1.84
Limonene	0.355	6.32	2.41
β-phellandrene	0.356	28.78	14.35
Terpinene-4-ol	0.798	2.88	1.52
Terpene 10	1.097	2.90	2.10
Terpene 11	1.199	0.43	1.11
Total		86.89	73.58

*IS, internal standard (methyl palmitate).

Individual terpenes

To determine if individual terpenes varied among flushing classes, sampling times, or treatments, the 11 terpenes with the largest mean contributions to wood and bark terpene totals were studied in more detail. These 11 terpenes (Table 3) represented 87% of the total bark terpenes and 74% of the total wood terpenes.

Individual bark terpenes

For virtually all terpenes, variability within each flushing class and treatment was large compared with differences among groups (Table 4). ANOVA (Table 5) showed that although individual terpene levels were generally higher in late-flushing trees (8 out of 11 terpenes) (Table 4), this difference was significant only for myrcene and terpene 10 (not identified). Sampling time had a significant effect on terpene 1 (not identified), sabinene, terpinene-4-ol, terpene 10, and terpene 11 (not identified). Treatment did not have a significant effect on any of the individual terpenes. The only significant interaction found was sampling time × treatment for terpene 1.

Individual wood terpenes

For virtually all wood terpenes, variability within each flushing class and treatment was large compared with differences among groups (Table 6). ANOVA (Table 7) showed that although individual terpene levels were generally higher in late-flushing trees (9 out of 11 terpenes) (Table 6), this difference was significant only for sabinene, myrcene, and terpene 10. Sampling time had a significant effect on terpinene-4-ol, terpene 10, and terpene 11. Treatment had a significant effect on all terpenes except terpene 1 and sabinene, with the wounded trees having higher terpene lev-

Table 4. Individual bark terpene statistical analysis.

	Flushing class		Sampling time				Treatment	
Terpene	Early (n = 56)	Late (n = 56)	Feb. 27 (n = 28)	May 8 (n = 28)	May 22 (n = 28)	Aug. 22 (n = 28)	Control (n = 56)	Wounded (n = 56)
Total	7.6 (3.2)*a*	8.4 (3.2)*a*	8.8 (3.0)*a*	8.5 (3.3)*a*	7.0 (3.0)*a*	8.3 (3.4)*a*	8.0 (3.3)*a*	8.3 (3.1)*a*
Terpene 1	0.34 (0.11)*a*	0.34 (0.08)*a*	0.38 (0.15)*a*	0.29 (0.08)*b*	0.34 (0.05)*a*	0.37 (0.04)*a*	0.34 (0.11)*a*	0.34 (0.08)*a*
α-pinene	1.5 (0.8)*a*	1.6 (0.9)*a*	2.0 (1.1)*a*	1.5 (0.6)*b*	1.4 (0.7)*b*	1.6 (0.8)*ab*	1.6 (0.9)*a*	1.6 (0.8)*a*
Sabinene	0.14 (0.19)*a*	0.24 (0.21)*a*	0.09 (0.08)*c*	0.19 (0.20)*ab*	0.14 (0.16)*bc*	0.26 (0.27)*b*	0.15 (0.22)*a*	0.19 (0.18)*a*
β-pinene	0.89 (0.62)*a*	0.94 (0.46)*a*	0.89 (0.34)*ab*	1.1 (0.7)*a*	0.75 (0.41)*b*	0.94 (0.58)*ab*	0.92 (0.64)*a*	0.90 (0.45)*a*
Myrcene	0.34 (0.17)*b*	0.42 (0.21)*a*	0.42 (0.18)*a*	0.41 (0.18)*a*	0.34 (0.24)*a*	0.38 (0.17)*a*	0.40 (0.22)*a*	0.38 (0.16)*a*
△-3-carene	0.30 (0.37)*a*	0.27 (0.36)*a*	0.32 (0.31)*a*	0.38 (0.46)*a*	0.18 (0.22)*a*	0.30 (0.40)*a*	0.27 (0.32)*a*	0.31 (0.41)*a*
Limonene	0.46 (0.62)*a*	0.51 (0.78)*a*	0.74 (0.81)*a*	0.55 (0.58)*a*	0.42 (0.53)*a*	0.53 (0.87)*a*	0.56 (0.75)*a*	0.56 (0.67)*a*
β-phellandrene	2.2 (1.0)*a*	2.5 (1.0)*a*	2.4 (1.0)*a*	2.4 (0.9)*a*	2.1 (1.0)*a*	2.4 (1.1)*a*	2.3 (1.0)*a*	2.4 (1.0)*a*
Terpinene-4-ol	0.22 (0.18)*a*	0.26 (0.18)*a*	0.16 (0.15)*b*	0.34 (0.21)*a*	0.26 (0.15)*a*	0.14 (0.10)*b*	0.21 (0.15)*a*	0.24 (0.19)*a*
Terpene 10	0.18 (0.14)*b*	0.26 (0.20)*a*	0.31 (0.19)*a*	0.31 (0.22)*a*	0.17 (0.13)*b*	0.14 (0.12)*b*	0.22 (0.14)*a*	0.26 (0.22)*a*
Terpene 11	0.03 (0.03)*a*	0.03 (0.05)*a*	0.07 (0.06)*a*	0.03 (0.03)*b*	0.03 (0.02)*b*	0.02 (0.01)*b*	0.04 (0.04)*a*	0.04 (0.04)*a*

Note: Values are means ± SE in mg/g. For each terpene, means that are significantly different (Duncan's multiple means test, $P < 0.05$) for flushing class, sampling time, and treatment are followed by different letters (column-wise comparisons within flushing class, sampling time, and treatment).

els in all cases. The only significant interactions found were flushing class × treatment for terpene 10 and sampling time × treatment for terpene 11.

Discussion

In general, the results of this research are in agreement with the works of others on Sitka spruce. Terpenes have been found to be highly variable among trees in needles (Von Rudloff 1978), cortical oleoresin from wounds (Forrest 1980*a*, 1980*b*), and for buds, needles, and cortical tissue (Brooks et al. 1987*a*, 1987*b*), with tree-to-tree variability being much greater than within-tree or among-site variability. The types of terpenes found in bark and wood and their relative amounts all agree with previously published data.

Bark

For the bark terpenes, total terpene content was not influenced by flushing class, time of sampling, or wounding treatment. Early- and late-flushing plants exhibited similar terpene profiles. The lack of response of bark terpenes to wounding suggests that there is little terpene synthesis in the bark in response to injury. Since the wood terpenes were found to be significantly increased by wounding, we can also surmise that there is little transport of the induced terpenes from the wood to the bark. This is consistent with published observations that resin is produced from traumatic resin canals produced from the cambium and extending into the xylem (Alfaro 1995; Tomlin et al. 1998; Christiansen et al. 1999). It would thus appear that total bark terpenes would be of no use as indicators of early or late flushing or attack.

Myrcene and terpene 10 were found to be influenced by flushing class. Both of these terpenes are relatively minor constituents, accounting for 4.8 and 2.9% of total bark terpenes, respectively. While the mean levels of these terpenes differed significantly between the early- and late-flushing classes, the large tree-to-tree variability again makes these terpenes of little use in predicting flushing class.

Terpene 1, sabinene, terpinene-4-ol, terpene 10, and terpene 11 were all found to vary significantly with time. These results disagree with Forrest (1980*a*), who found that variations in cortical oleoresin of Sitka spruce over the course of a year were insignificant. Each of these terpenes exhibited a different pattern of increase and decrease with large tree-to-tree variability. The tree-to-tree variability precludes the use of these terpenes as indicators of individual-tree phenology.

Wood

Wounding was found to have a significant effect on wood terpene levels, with wounded trees having higher total terpene levels, on average, than control trees. Flushing class or time of sampling did not influence total terpene content. If the data for early- and late-flushing trees are pooled, the effects of wounding can be clearly seen (Fig. 3). At time 1 (before wounding), terpene levels in control and wounded groups are essentially the same. At time 2 (15 days after wounding), there is a large and significant increase in the total terpene level for wounded trees. This difference is smaller at time 3 and time 4, and not statistically significant.

Table 5. Results from the analysis of variance of individual bark terpenes of early- and late-flushing Sitka spruce trees before and after mechanical wounding.

	P >*F*						
Terpene	F	S	T	F × S	F × T	S × T	F × S × T
Terpene 1	0.968	0.000	0.653	0.327	0.059	0.030	0.162
α-pinene	0.153	0.055	0.995	0.845	0.506	0.408	0.770
Sabinene	0.083	0.005	0.357	0.614	0.474	0.120	0.752
β-pinene	0.689	0.181	0.839	0.460	0.415	0.244	0.339
Myrcene	0.013	0.407	0.645	0.132	0.655	0.569	0.465
△-3-carene	0.959	0.231	0.568	0.097	0.709	0.561	0.781
Limonene	0.199	0.457	0.973	0.163	0.256	0.592	0.881
β-phellandrene	0.225	0.556	0.598	0.195	0.517	0.334	0.391
Terpinene-4-ol	0.915	0.000	0.262	0.542	0.318	0.874	0.338
Terpene 10	0.001	0.000	0.174	0.758	0.223	0.815	0.300
Terpene 11	0.156	0.000	0.915	0.630	0.427	0.160	0.684

Note: Samples were collected at four times throughout the growing season, once before wounding, and three times after wounding. F, flushing (56 trees × 2 classes); S, sampling time (28 trees × 4 times); T, treatment (56 trees × 2 treatments).

Table 6. Individual wood terpene statistical analysis.

	Flushing class		Sampling time				Treatment	
Terpene	Early (*n* = 56)	Late (*n* = 56)	Feb. 27 (*n* = 28)	May 8 (*n* = 28)	May 22 (*n* = 28)	Aug. 22 (*n* = 28)	Control (*n* = 56)	Wounded (*n* = 56)
Total	1.2 (0.6)*a*	1.4 (0.6)*a*	1.3 (0.6)*a*	1.3 (0.8)*a*	1.2 (0.6)*a*	1.5 (0.5)*a*	1.2 (0.5)*a*	1.5 (0.7)*b*
Terpene 1	0.35 (0.18)*a*	0.35 (0.14)*a*	0.39 (0.25)*a*	0.31 (0.14)*a*	0.36 (0.11)*a*	0.35 (0.08)*a*	0.35 (0.18)*a*	0.36 (0.13)*a*
α-pinene	0.14 (0.11)*a*	0.17 (0.15)*a*	0.17 (0.12)*a*	0.18 (0.18)*a*	0.14 (0.13)*a*	0.15 (0.10)*a*	0.13 (0.09)*b*	0.19 (0.16)*a*
Sabinene	0.02 (0.03)*a*	0.04 (0.05)*a*	0.01 (0.01)*b*	0.02 (0.03)*b*	0.03 (0.04)*b*	0.06 (0.05)*a*	0.02 (0.04)*a*	0.04 (0.04)*a*
β-pinene	0.07 (0.05)*a*	0.10 (0.09)*a*	0.07 (0.04)*a*	0.11 (0.11)*a*	0.07 (0.07)*a*	0.08 (0.05)*a*	0.07 (0.05)*b*	0.10 (0.09)*a*
Myrcene	0.03 (0.02)*b*	0.04 (0.03)*a*	0.03 (0.02)*a*	0.04 (0.04)*a*	0.03 (0.03)*a*	0.04 (0.02)*a*	0.02 (0.02)*b*	0.04 (0.03)*a*
△-3-carene	0.02 (0.04)*a*	0.02 (0.05)*a*	0.03 (0.05)*ab*	0.04 (0.05)*a*	0.01 (0.01)*b*	0.02 (0.04)*ab*	0.02 (0.02)*b*	0.04 (0.06)*a*
Limonene	0.03 (0.04)*a*	0.03 (0.06)*a*	0.05 (0.07)*a*	0.03 (0.04)*a*	0.02 (0.04)*a*	0.03 (0.04)*a*	0.02 (0.03)*b*	0.04 (0.06)*a*
β-phellandrene	0.18 (0.16)*a*	0.25 (0.21)*a*	0.18 (0.13)*a*	0.24 (0.25)*a*	0.20 (0.21)*a*	0.23 (0.13)*a*	0.16 (0.12)*b*	0.26 (0.22)*a*
Terpinene-4-ol	0.02 (0.02)*a*	0.02 (0.02)*a*	0.02 (0.01)*b*	0.03 (0.02)*a*	0.02 (0.01)*b*	0.01 (0.01)*b*	0.02 (0.02)*b*	0.02 (0.02)*a*
Terpene 10	0.02 (0.02)*b*	0.03 (0.03)*a*	0.04 (0.04)*a*	0.04 (0.03)*a*	0.02 (0.02)*b*	0.01 (0.01)*b*	0.02 (0.01)*b*	0.04 (0.04)*a*
Terpene 11	0.01 (0.01)*a*	0.02 (0.01)*a*	0.02 (0.01)*a*	0.02 (0.02)*a*	0.02 (0.01)*a*	0.01 (0.01)*b*	0.01 (0.01)*b*	0.02 (0.02)*a*

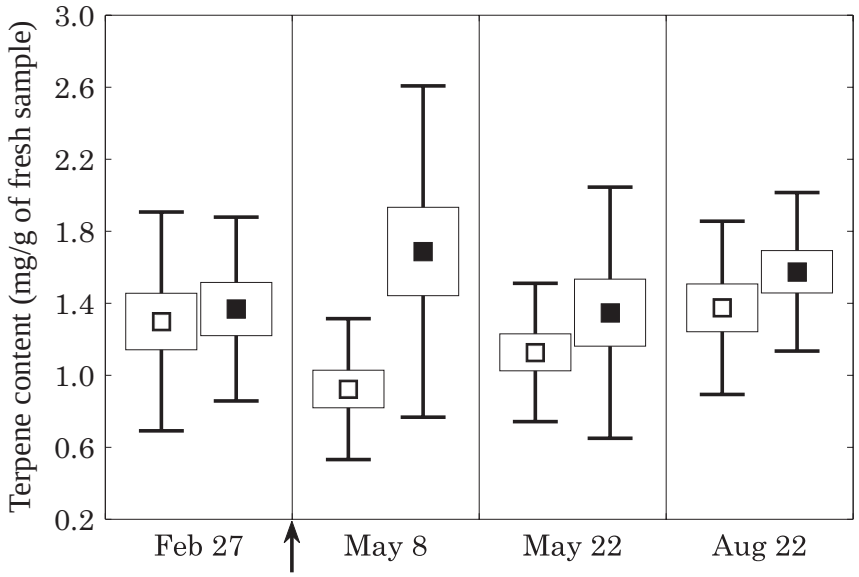
Note: Values are means ± SE in mg/g. For each terpene, means that are significantly different (Duncan's multiple means test, $P < 0.05$) for flushing class, sampling time, and treatment are followed by different letters (column-wise comparisons within flushing class, sampling time, and treatment).

Table 7. Results from the analysis of variance of individual wood terpenes of early- and late-flushing Sitka spruce trees before and after mechanical wounding.

	P >*F*						
Terpene	F	S	T	F × S	F × T	S × T	F × S × T
Terpene 1	0.820	0.431	0.808	0.892	0.855	0.748	0.217
α-pinene	0.117	0.707	0.007	0.329	0.361	0.448	0.751
Sabinene	0.038	0.000	0.095	0.514	0.410	0.296	0.827
β-pinene	0.085	0.200	0.019	0.481	0.246	0.370	0.813
Myrcene	0.038	0.544	0.005	0.760	0.255	0.327	0.708
△-3-carene	0.451	0.094	0.006	0.191	0.539	0.243	0.356
Limonene	0.127	0.245	0.024	0.324	0.206	0.921	0.288
β-phellandrene	0.120	0.670	0.009	0.864	0.308	0.208	0.778
Terpinene-4-ol	0.982	0.000	0.018	0.692	0.323	0.573	0.406
Terpene 10	0.011	0.000	0.002	0.267	0.037	0.508	0.132
Terpene 11	0.713	0.014	0.020	0.589	0.549	0.001	0.712

Note: Samples were collected at four times throughout the growing season, once before wounding, and three times after wounding. F, flushing (56 trees × 2 classes); S, sampling time (28 trees × 4 times); T, treatment (56 trees × 2 treatments).

Fig. 3. Total wood terpenes (mg/g fresh sample, flushing classes pooled). Symbols for means are as follows: □, control; ■, wounded. Boxes are ±SE, whiskers are ±SD. ↑, date of wounding (April 23).



Large tree-to-tree variability in wood terpene content was found. This result is consistent with findings by Lewinsohn et al. (1991) that terpene synthase (cyclase) activity in wood and bark increases more than 10-fold nine days after wounding, followed by a decline. They also found this effect to be localized to the vicinity of the wound and dependent upon the severity of the wound. Activation of genes coding for terpene synthases has been demonstrated as early as 4 days after artificial wounding of Sitka spruce (S.X. Wang, W. Hunter, and A.I. Plant, unpublished paper).

Sabinene, myrcene, and terpene 10 were found to have higher concentrations in the wood of late- flushing trees versus early-flushing trees. These terpenes are relatively minor constituents, accounting for 2.0, 2.2, and 2.1% of total wood terpenes, respectively. While the mean levels of these terpenes differed significantly between the early- and late-flushing classes, the large tree-to-tree variability again makes these terpenes of little use in predicting flushing class.

Sabinene, terpinene-4-ol, terpene 10, and terpene 11 in wood were all found to vary significantly with time. Each of these terpenes exhibited a different pattern of increase and decrease, with large tree-to-tree variability; again, this variability makes terpene profiles of little use as indicators of individual tree phenology. All terpenes, except terpene 1 and sabinene, were found to be influenced by wounding, with terpene levels in wounded trees being higher in all cases.

Overall, these results demonstrate that the wood of Sitka spruce trees responds to artificial wounding by accelerating terpene synthesis at the site of injury. This is the first finding of such terpenoid enhancement in Sitka spruce caused by artificial wounding, and it agrees with similar findings by Tomlin et al. (2000) for white spruce. Berryman (1972) concluded that this response was part of a generic reaction of conifers to injury. In this study, this response was greatest at the first measurement after wounding (time 2) and diminished as time progressed. The induced response observed here was triggered by a single wounding episode. Wounding inflicted by the feeding and ovipostion of adult white pine weevils is more complex in nature with hundreds of feeding punctures throughout the plant occurring over the entire season. Additional wounding occurs as the larvae hatch and consume the phloem tissues of the apical shoot. With this continual wounding, it is likely that the production of elevated terpene levels in wood occurs over a much longer time period than the one that was detected in this study.

In addition to changes in terpene profiles, it is likely that wounding may accelerate synthesis of other defensive chemicals. Elucidation of the array of chemicals produced in response to herbivores is important for the understanding of host–insect interactions.

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