The Effect of Variation in Light and Nitrogen on the Composition of Resin in Young Sitka Spruce

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Abstract

In a previous study of growth and defence in young Sitka spruce of Alaskan or Oregon provenance, 'high' and 'low' light and nitrogen treatments induced changes in quantitative defences that were broadly as predicted by resource availability models of defence. In the present study, resin extracts from leaves, stem and roots of these spruce trees were examined for changes in composition induced by the different light and nitrogen treatments. Canonical variate analysis of terpene log ratios revealed significant differences in resin composition between the roots, stems and needles. Treatments had no significant affect on resin composition of roots and stems, but both light and nitrogen treatments induced significant changes in the terpene composition of resin from needles, with evidence of variation in response between the two provenances. Induced changes in composition of quantitative defences should not be ignored in studies of environmental effects on plant resistance.

Key words: Light, monoterpenes, nitrogen, quantitative defence, resin, spruce.

Introduction

The way in which changes in the growth of plants influences carbon allocation to carbon-based secondary chemicals has been investigated in many different studies of the influence of the environment on plant defence. These studies have often demonstrated an apparent trade-off between allocation of carbon to growth versus defence and this is one of the central predictions of resource availability models of defence (BRYANT *et al.,* 1983, 1985; COLEY *et al.,* 1985; LORIO, 1986; HERMS and MATTSON, 1992). Thus when nutrient availability limits growth in a given light regime, the concentration of carbon-based 'quantitative' defences usually increases. Changes in the concentration of carbon-based secondary chemicals such as resin and polyphenols in response to variation in environmental conditions may also be accompanied by compositional changes, but this aspect has been much less studied. In this paper, we extend a previously reported study on quantitative defences in young Sitka spruce (WAINHOUSE *et al.,* 1998) to examine variation in the composition of resin induced by light and nutrient treatments.

Methods

The resin used for analysis was obtained from the needles, stems and roots of Sitka spruce (*Picea sitchensis*) of both Alaska and Oregon provenance. The young trees had been grown in different light or nitrogen regimes as part of a study of the relationship between growth and defence, experimental details of which are given in WAINHOUSE *et al.,* (1998). The following is a brief summary of the treatments applied.

Origin of plants and experimental treatments

Two-year old Sitka spruce transplants 17 cm to 38 cm in height of Alaskan [IUFRO 81 (7987) 1] and Oregon provenances [IUFRO 85 (30) 500] were planted in pots in 1992 within an unheated polythene covered greenhouse (polyhouse). Trees of each provenance were subject to two levels ['high' (h) and 'low' (l)] of light (L) and nitrogen (N) to give four factorial treatment combinations as follows: hLhN, hLlN, lLhN and lLlN. The experimental layout comprised a split-plot design of four blocks with light as the main plot and the two levels of nitrogen as subplots. The shading treatments (lL), using a proprietary horticultural shade netting, reduced light levels to about 22% of ambient levels (ambient = hL) within the polyhouse. The nitrogen treatments were applied at intervals using a proprietary fertilizer at 100 (hN) or 10 (lN) p.p.m. N. The trees were treated during a single growing season and harvested during December 1992 to January 1993 when needle, stem and shoot material was separated into current growth (1992) formed during experimental treatments and 'old' growth (1990+1991). Roots were not separated into different growth years. Prior to solvent extraction, samples of the five plant parts were stored at –50 °C.

Chemical analysis

Resin was extracted from the pooled material of the different parts of three plants from each subplot so that for each provenance, treatment and plant part there were four replicates, one from each block. In separate extractions for each plant part, 1 g of fresh material from each tree (0.1 g for current needles) was pooled and ground under liquid nitrogen. Approximately 0.3 g of the ground material was shaken in Teflon® centrifuge tubes with Analar® pentane in the ratio of 0.3 g/6 ml pentane for 15 min. Four mls of the pentane extract were filtered into a sample tube and held at –18 °C prior to analysis. Terpene analysis was carried out by capillary-GC in a Perkin Elmer 8700 chromatograph fitted with a fused silica SE-54 capillary column 25 m length, 0.32 mm I.D., linked to a PE Nelson 900 series analogue/digital interface. The temperature programme was: 50 °C isothermal for 1 min, increased to 210 °C at 10 °C/min, increased to 280 °C at 20 °C/min, held at 280 °C isothermal for 15 min. Peak integration and data analysis was carried out using PE Nelson 900 software.

Statistical analysis

Data on terpene concentration estimated from peak areas was analysed by canonical variate analysis (CVA) using Genstat 5 (Genstat 5 Committee, 1993). Prior to analysis, data were transformed to log ratios $y_{ij} = \log_e (area_i/area_j)$, $i \neq j$ to remove constraints associated with analysis of proportional data (BIRKS and KANOWSKI, 1993). This procedure restricted analysis to subsets of terpenes having non-zero values in all the tissues included in the analysis. Some of the datasets contained a missing value so that in some of the analyses, only 3 non-zero peak areas were included for some of the plant parts. Terpene 4 (myrcene) was selected as the divisor for the ratio since it had fewest non-zero values in the overall dataset. The significance of axes in group separation was indicated in tests for dimensionality. To increase reliability of tests, some datasets were amalgamated to ensure that the number of observa-

tions (n) minus the number of treatment groups (g) was large compared to the number of terpene variates (v) in the analysis.

Results

On the chromatograms, a total of 35 peaks could be distinguished representing all of the quantitatively important monoterpenes, sesquiterpenes and diterpenes that were present in some or all of the five plant parts (*Fig. 1*). Some of the monoterpenes present were identified by comparison with pure samples and by GC/MS identification using a similar capillary column in an analogous system (G.I. Forrest, unpublished data) and these are shown in *table 1.* There were clear differences in both composition and concentration between the different parts of the plant. Some terpenes e.g. 9 to 14, 21, 24 and 26 were largely absent from stem and root material whereas camphene (terpene 2), was absent from stems but always present in needles and about half the root samples. In stem material, the concentrations of α- and β-pinene (terpenes 1 and 3) were higher than those in needles but in contrast, the concentration of myrcene (terpene 4) was much higher in the needles. For most terpenes concentration tended to be higher in the Oregon provenance.

For each provenance, the mean peak area for all 35 terpenes for each plant part was calculated for the four experimental treatments to provide an estimate of the overall concentration of 'resin' in the tissues (*Fig. 2*). Resin concentration was generally higher in the Oregon provenance and for both provenances, tended to be higher in high compared to the low light treatment. The highest concentrations of resin occurred in the hLlN treatments.

Table 1. – Monoterpenes identified by comparison with pure samples and GC/MS (G.I. Forrest, unpublished).

Terpene number	Retention time (mins)	Monoterpene
1	5.30	α-pinene
2	5.54	camphene
3	6.00	β-pinene
4	6.19	myrcene
5	6.55	3-carene
6	6.87	β-phellandrene
7	7.89	terpinolene
8	8.13	methyl butyl-methyl butanoate
9	8.32	methyl butenyl-methyl butanoate
10	8.85	camphor
14	10.67	piperitone
15	11.16	bornyl acetate

Variation in composition between different parts of the tree

Only five terpenes 1, 3, 4, 6 and 29 (v=4) were present in all of the plant parts (g=5), giving only four terpenes for comparison of log ratios. Preliminary analysis suggested similar variation between plant parts in both Alaska and Oregon provenances so data were pooled for this analysis, giving a total of 154 observations (n). Significant differences were found between the different plant parts with significant separation along both the first and second canonical axes (P<0.001) (*Table 2, Fig. 3*). In the analysis, 91.8% of the variance in the data was accounted for in the first axis which separates stem

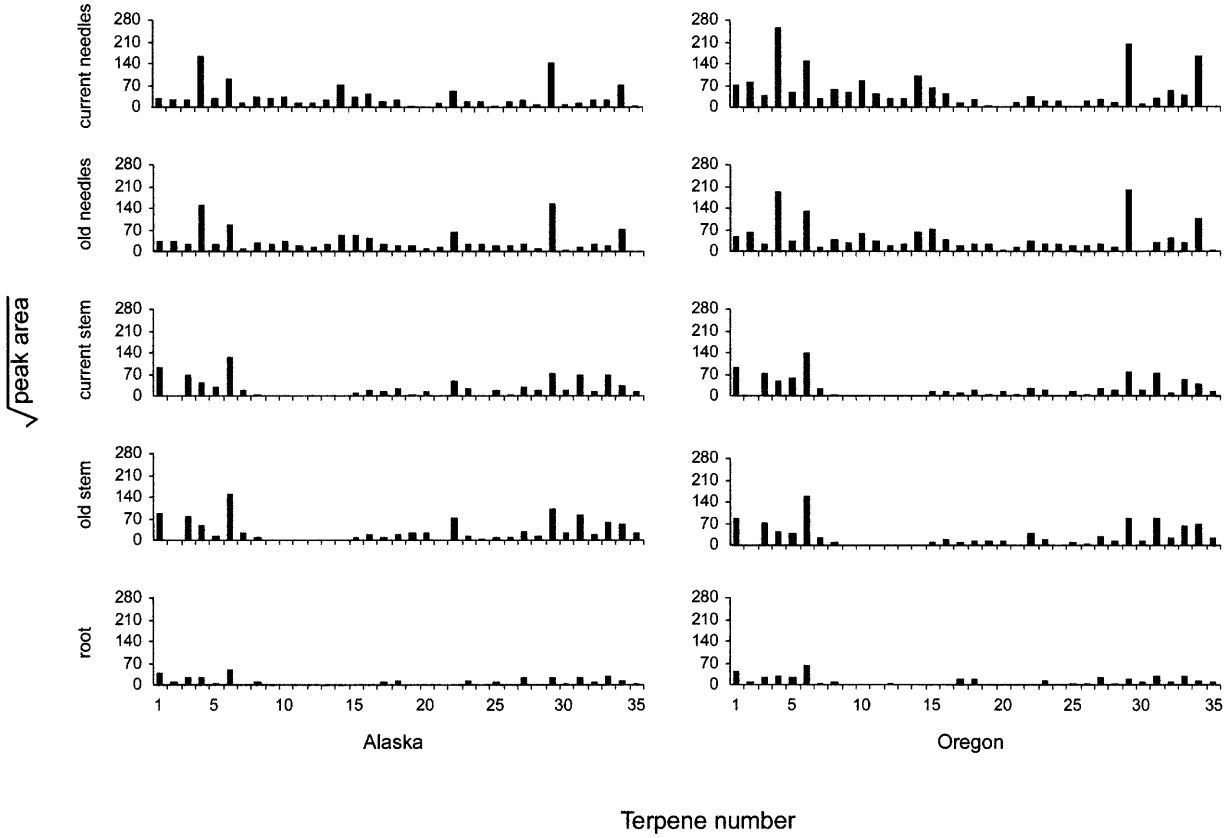

Figure 1. – Concentration (√peak area) of 35 terpenes extracted from Sitka spruce plants subjected to light and nitrogen treatments (see text).

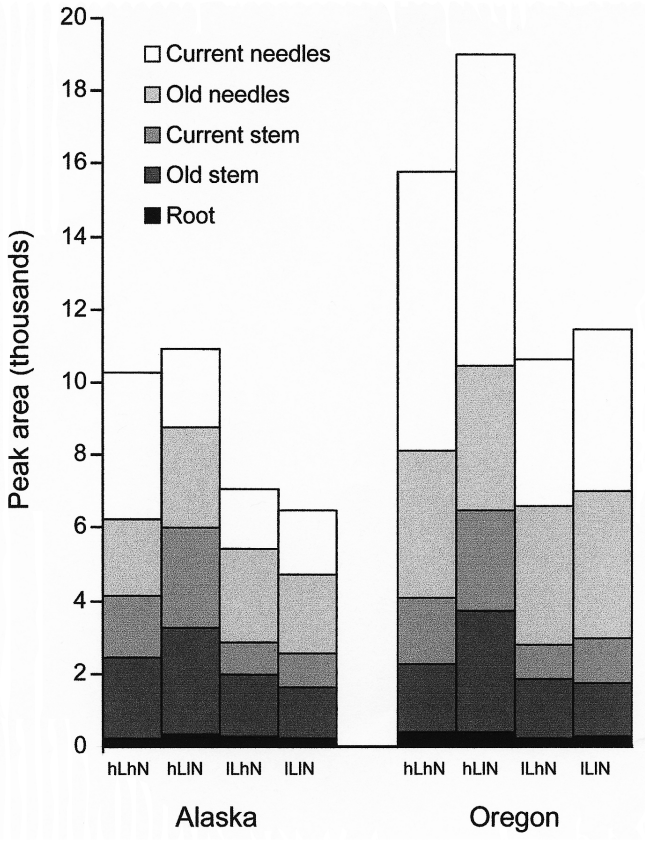

Figure 2. – Mean peak area for terpenes 1 to 35 in Sitka spruce of Alaska and Oregon provenance in 4 light and nitrogen treatments (see text).

and root from needles and was largely due to the log-ratio of ß-pinene (terpene 3). Most of the remaining variance was accounted for by the second axis separating roots from stems largely as a result of the contrast between the log-ratio of α-pinene (terpene 1) and of terpene 29.

Table 2. – Significance test for dimensionality for variation in terpene composition between plant parts. Data were pooled for provenance and light and nitrogen treatments. n = no. of observations, g = no. of groups (plant parts), v = no. of variates (terpenes) (see text)

	K	χ^2	df	P
Plant part	0	759.0	16	<0.001
n 154, g 5, v 4	1	218.2	9	<0.001
	2	7.7	4	0.104

Effects of light and nitrogen treatments on terpene composition of needles

In a preliminary analysis of the effect of treatments on terpene composition, we found no consistent evidence for an effect of treatments on resin composition in stems and roots so these data were not considered further. Data for the 17 terpenes (v=16) common to current and old needles were analysed separately and in a pooled analysis in which data were grouped by provenance and either light or nitrogen treatment (g=4) with either 32 observations (n) or 64 for the pooled data. Tests for dimensionality for each analysis are given in *table 3.* Results for current and old needles were similar but with clearer separation of groups for current needles in both light and nitrogen. In the combined analysis the first axis separated provenances based largely on the contrasts between the log-ratio of ß phellandrene (terpene 6) and of terpenes 18 and 29 for both light and nitrogen treatments (*Fig. 4*). Differences in terpene composition resulting from nitrogen treatments (*Fig. 4a*) were partly characterised by the contrast between the log-ratio of methyl butyl-methyl butanoate (terpene 8) and of terpenes 14 and 34 and were greatest for the Alaskan provenance. However, the effects of light on terpene composition were greatest in the Oregon provenance (*Fig. 4b*), with differences between light levels largely characterised by the contrast between the log-ratios of terpenes 23 and 17.

Discussion

In many conifer species, there is usually considerable variation between individual trees in the terpene composition of resin (Tobolski and Hanover, 1971; Zavarin *et al.,* 1971; Sturgeon, 1979; Forrest, 1980a; Adams and Edmunds, 1989; Jactel *et al.,* 1996; Katoh and Croteau, 1998). But there may

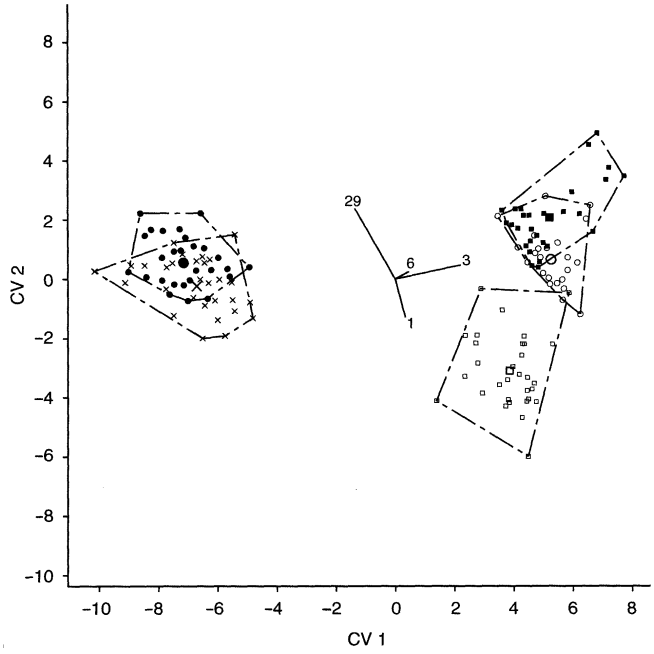

Figure 3. – Canonical variate analysis of 4 terpene log-ratios for current (X) and old needles (●), current (O) and old stem (■) and roots (□) of Sitka spruce (see text). Terpenes included in analysis shown for vectors. Dotted line is convex hull for each group. Large symbols are corresponding group means. Some points have been omitted for clarity.

also be considerable variation within-trees and in the young Sitka spruce used in our experiments, the needles, stems and roots differed in both the absolute and relative concentration of terpenes. Similar variation has been reported in a number of different conifer species (Roberts, 1970; Hrutfiord *et al.,* 1974; Forrest, 1980b; Hafizoglu and Reunanen, 1994; Gallis *et al.,* 1998) although the biological significance of these differences is often not clear.

Despite the considerable within- and between-tree variation in resin composition, standardised sampling of particular tis-

Table 3. – Significance test for dimensionality for variation in terpene composition in needles in response to provenance and nitrogen and light treatments. n = no. of observations, g = no. of groups (provenance and nitrogen or light treatments), v = no. of variates (see text).

	Provenance x nitrogen					Provenance x light				
	K	χ^2	df	P	% variance accounted for	K	χ^2	df	P	% variance accounted for
Current needles	0	116.9	48	<0.001	76.3	0	111.0	48	<0.001	80.7
n 32, g 4, v 16	1	54.3	30	<0.01	17.7	1	48.2	30	<0.05	13.0
	2	19.1	14	0.162	6.0	2	19.0	14	0.166	6.3
Old needles	0	77.7	48	<0.01	84.9	0	91.2	48	<0.001	75.6
n 32, g 4, v 16	1	26.6	30	<0.05	10.9	1	40.5	30	0.095	14.1
	2	8.7	14	0.851	4.2	2	18.2	14	0.197	10.3
All needles	0	175.6	48	<0.001	83.7	0	171.4	48	<0.001	85.4
n 64, g 4, v 16	1	58.9	30	<0.01	11.9	1	53.4	30	<0.01	11.6
	2	18.7	14	0.178	4.4	2	13.6	14	0.478	3.0

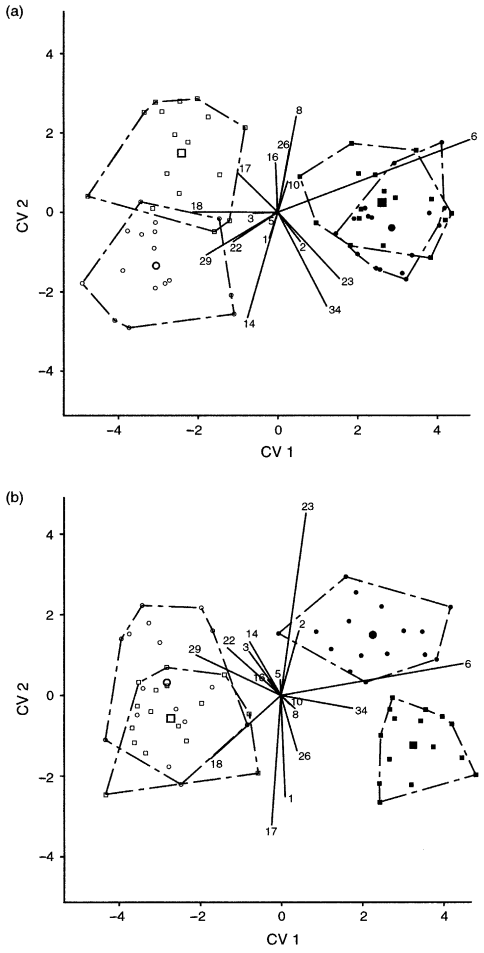

Figure 4. – Canonical variate analysis of 16 terpene log-ratios for combined data for current and old needles in (a) high (□■) and low (○●) nitrogen treatment and (b) hight (□■) and low (○●) light levels. Terpenes included in analysis shown for vectors. Dotted lines are convex hulls for treatment groups of Alaska (open symbols) and Oregon provenance (filled symbols). Large symbols are corresponding group means.

sues often shows that the terpene composition of resin is sufficiently distinctive to be of value in chemotaxonomy of conifers and sometimes also in characterising trees of different seed or even clonal origin within a single species (FORREST, 1980b; LOCKHART, 1990; BURDON *et al.,* 1992; LANG, 1994; CANARD *et al.,* 1997). These differences in terpene composition appear to be largely genetic in origin, and monoterpenes in particular have been reported to be under 'strong' genetic control (HANOVER, 1966a and b, 1971; SQUILLACE, 1971; WILKINSON *et al.,* 1971; ROCKWOOD, 1973; BARADAT *et al.,* 1975, HILTUNEN, 1976; MEIER and GOGGANS, 1978; FORREST and SAMUEL, 1988; BURDON *et al.,* 1992) and are considered to be relatively little affected by environmental factors.

In contrast, variation in terpene concentration often occurs in response to environmental factors (SCHÖNWITZ *et al.,* 1990; MCCULLOUGH *et al.,* 1993; HELLER *et al.,* 1990; KAINULAINEN *et al.,* 1992; LERDAU *et al.,* 1995; WAINHOUSE *et al.,* 1998). From the GC analysis in our study, the total peak area of terpenes from the different plant parts gives an approximate quantitative estimate of terpene concentration in plants growing in the different light and nitrogen treatments. This method of calculating terpene concentration gave results similar to those obtained by gravimetric estimation of resin concentration from the same plant material (WAINHOUSE *et al.,* 1998, *Fig. 7b*) showing that the quantitative response to light and nitrogen treatments was broadly in line with that predicted by resource availability models of plant defence (BRYANT *et al.,* 1983, 1985; COLEY *et al.,* 1985; LORIO, 1986; HERMS and MATTSON, 1992).

In our study, however, the light and nitrogen treatments have clearly affected the composition as well as the concentration of resin in needles lending support to previous studies in which changes in concentration of individual terpenes in response to environmental factors have been observed. For example, the relative concentration of monoterpenes appears to be altered by the effects of fertilisation on grand fir (MUZIKA *et al.,* 1989), of ozone pollution (HELLER *et al.,* 1990) and drought (KAINULAINEN *et al.,* 1992) on spruce, of soil type and light intensity on cypress (SCHILLER, 1993), and of drought (HODGES and LORIO, 1975; GILMORE, 1997) and fertilisation (KAINULAINEN *et al.,* 1996) on pines. The response of the two Sitka spruce provenances to the light and nutrient treatments appeared to differ in degree and similar interactions have been noted in *Cupressus sempervirens* with the relative effect of light intensity and soil type on terpene composition differing between two varieties (SCHILLER, 1993).

Determining the biological significance of these environmentally induced changes in terpene composition against a background of associated quantitative changes in resin will not be straightforward. However, when larvae of *Gilpinia hercyniae* were fed on needles of the experimental trees, survival was not related to the concentration of quantitative defences but appears likely to have been affected by nutritional factors

(WAINHOUSE *et al.,* 1998). Because survival was low in all treatments involving either low nitrogen or low light levels, the observed changes in terpene composition may have had an influence on sawfly survival. The composition of terpenes has been shown in a number of studies to influence insect-plant interactions (ANNILA and HILTUNEN, 1977; VISSER, 1986; ROSELAND *et al.,* 1992; NORDLANDER *et al.,* 1986) and our results suggest that in studies of the effects of environment on quantitative changes in defences in plants, compositional changes could also have an important influence on resistance.

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