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The effect of variation in light and nitrogen on growth and defence in young Sitka Spruce

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Summary

1. Young plants of a northern (Alaska) and southern (Oregon) provenance of Sitka Spruce, *Picea sitchensis*, were subject to high and low light and high and low nitrogen treatments in a polyhouse experiment. The effect of treatments on growth, needle and resin duct size, water content and concentration of quantitative defences (resin and polyphenols), sugars and nitrogen in needles, stems and roots was determined.
2. Concentrations of resin, polyphenols and carbohydrates were higher in low nitrogen treatments as predicted by resource-availability models of defence and the changes were similar in all parts of the trees including roots and in tissues formed prior to experimental treatments. Variation in the relative concentration of resin and polyphenols between tissues may indicate a defensive trade-off. The size of needle resin ducts was positively correlated with tree growth but no evidence for 'structural' limitation of resin concentration in needles was found.
3. Changes in concentration of quantitative defences did not appear to be the result of a direct trade-off with growth but reflected treatment-induced variation in the root/shoot ratio. Production of quantitative secondary chemicals may therefore be part of an integrated response of the trees to environmental stress.
4. Bioassays with *Elatobium abietinum, Gilpinia hercyniae* and the fungus *Phacidium coniferarum* showed that changes in needle size, the nutritional and water content of tissues and the balance between nutrients and secondary chemicals influenced performance of one or more of the organisms. Changes in the concentration of carbon-based secondary chemicals alone were, therefore, of only limited value in predicting susceptibility of Spruce to insects and fungi.

Key-words: Elatobium abietinum, Gilpinia hercyniae, Phacidium coniferarum, phenology, quantitative defence, resin ducts, root/shoot ratio

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Introduction

When the supply of mineral nutrients is limiting, application of fertilizer usually increases tree growth (Savill & Evans 1986). This 'carbon-demanding' response to fertilization can reduce resources allocated to carbon-based defences as predicted by resource-availability models of plant defence (Bryant, Chapin & Klein 1983; Bryant *et al*. 1985; Coley, Bryant & Chapin 1985; Lorio 1986; Herms & Mattson 1992). Thus, in general, while the nitrogen content of plants can increase following fertilization (Harrington & Wierman 1990; Crane & Banks 1992; McCullough, Swedenborg & Kulman 1993; McNulty & Aber 1993; Mugasha, Pluth & Hillman 1993), levels of defences may fall (Herms & Mattson 1992; Muzika & Pregitzer 1992). These changes are likely to increase vulnerability to herbivore attack.

Many of the studies on the apparent trade-off between growth and quantitative defence have been carried out on trees and other perennial plants (Bryant *et al*. 1985; Coley *et al*. 1985). This relationship is of particular interest in plantation forestry, one of the aims of which is to maximize yield by appropriate silvicultural techniques. The sites on which trees are planted can also influence their growth through, for example, differences in rainfall and fertility and, in addition, newly planted trees are increasingly likely to be the products of selection and breeding programmes designed to increase genetic gain in growth and form (Rook 1992).

Most studies on the effects of fertilization and other treatments on the nutritive and defensive status of trees have focused on one particular part of the plant, such as leaves (Larsson *et al*. 1986; Kainulainen *et al*. 1996) or stems (Bryant *et al*. 1987), rather than the plant as a whole and have not considered the effects on tissues formed prior to the application of experimental treatments. Another important factor is the

effect that environmentally induced changes in the nutritional and defensive status of plants has on their susceptibility to pests and diseases. In this paper, we report the results of a study on growth and defence in two provenances of Sitka Spruce [*Picea sitchensis* (Bong.) Carr]. Young Spruce were grown under two light and nitrogen regimes to determine effects on growth, quantitative defences and nutritional status of both above- and below-ground parts of the tree. The effects of some of the induced changes on an aphid, a sawfly and a facultatively pathogenic fungus were determined in a series of bioassays.

Materials and methods

ORIGIN OF PLANTS

Two-year-old Sitka Spruce transplants (17–38 cm in height) of Alaskan [IUFRO 81 (7987) 1] or Oregon provenance [IUFRO 85 (30) 500] grown at the Bush nursery, Edinburgh, were lifted in February–March in either 1992 or 1993 and stored in sealed plastic bags at 2·5 °C for 2–6 weeks. They were planted in a limed peat mix with no added nutrients in 3 litre pots supported above the ground on wire mesh to ensure free drainage and 'air-pruning' of roots within an unheated polythene-covered greenhouse (polyhouse).

EXPERIMENTAL LAYOUT, TREATMENT AND USE OF PLANTS

Trees of each provenance (64–80 plants) were used in three repetitions (A and B in 1992, C in 1993) of the same experimental layout. Plants from repetition A were used for estimates of growth. Bioassays with the fungus *Phacidium coniferarum* (Hahn) DiCosmo *et al*., the green spruce aphid *Elatobium abietinum* (Walker) and adult spruce sawfly *Gilpinia hercyniae* (Hartig) were carried out with plants from A and B while plants from C provided material for bioassays with *G. hercyniae* larvae. All trees were subject to two levels ['high' (h) and 'low' (1)] of light (L) and nitrogen (N) to give four factorial treatment combinations as follows: hLhN, hLlN, lLhN and lLlN. The treatments were arranged within a split-plot design of four blocks with light as the main plot and the two levels of nitrogen as subplots. The basic experimental unit consisted of plots of four or five trees of a single provenance subjected to the same experimental treatment. Trees in low light (lL) were shaded with a proprietary horticultural shade netting (green 'Rokolene') which reduced light levels to about 22% of ambient [ambient (hL)]. The nitrogen treatments were applied using a proprietary fertilizer (20% N, 7% P, 19% K) adjusted to 100 (hN) or 10 (lN) p.p.m. N and applied at a rate of 250 ml twice a week (once per week from late October to early November). Additional watering was carried out as necessary. The tunnel vents remained open throughout the experiment. Plants were sprayed during May–July with non-persistent pesticide (Rotenone) to control aphid or mite infestations.

PLANT GROWTH

Estimates of growth were obtained from measurements on three trees selected at random from each plot in repetition A during December 1992–January 1993.

The trees were cut at the root collar and on individual plants all stem and shoot material separated into current (1992) and 'old' (1990 + 1991) growth and corresponding needles removed after immersion in liquid nitrogen. Current needles included those from leading and side shoots and any secondary growth while old needles included those from all the previous 2 years' growth. The fresh mass of needles and stem was determined, together with that of roots after they were washed and surface dried. A sample of each of the five plant parts (current and old needles, current and old stem, and roots), was dried at 70 °C to constant mass to determine dry mass and water content of tissues. The remaining fresh material was stored at –50 °C prior to extraction of resin and polyphenols.

PLANT PHENOLOGY

The remaining two trees in the plot were scored for bud development during April–May 1993 using the method outlined in Krutzsch (1973).

QUANTITATIVE DEFENCES

Resin ducts and resin

Prior to removal of needles for estimates of plant growth, five to 10 needles were taken from the primary growth on current and 1991 leaders to determine needle and resin duct size. There were usually two resin ducts in the basal portion of each needle (Fig. 6). After cutting 1 mm from the needle base, a 2 mm-long section was removed and the width and thickness of the needle, together with mean diameter of the resin ducts were measured on each cut end. Mean resin duct area (RDA), needle cross-section area (NA) and the percentage of needle cross-section area occupied by resin ducts (PRDA) were then calculated.

To illustrate variation in needle and resin duct size, a small sample of needles from leaders of experimental trees was sectioned on a freezing microtome, stained in safronin and light green (Gram & Jorgensen 1952) and photographed.

Resin content of the five plant parts was estimated gravimetrically and expressed as % composition on a dry mass basis. Equal masses of fresh material from the three trees sampled from each plot were pooled and 0·5–1 g ground under liquid nitrogen prior to homogenization in 'Teflon' centrifuge tubes with 10 ml of analar pentane for 1 min. The homogenate

was filtered and then rinsed through qualitative filter paper into preweighed tubes. The solvent was evaporated to dryness and resin mass determined.

Polyphenols

Polyphenol extraction was based on the method outlined in Sauvesty, Page & Huot (1992). Fresh material from the pooled samples was ground under liquid nitrogen and 0·2 g homogenized for 1 min in 20 ml 80% methanol. The homogenate was held at 40 °C in a water bath for 1 h. After centrifugation, extracts were divided and one sample filtered through Polyclar AT to allow correction for non-phenolics (Sauvesty *et al*. 1992). The concentration of polyphenols in the extracts was determined using the Folin–Ciocalteu colorimetric test (766 nm), based on the method given in Mole & Waterman (1987). Colorimeter readings were converted to mg equivalent of gallic acid using a calibration regression and results expressed as a percentage of dry mass.

CARBOHYDRATES AND NITROGEN

Dried samples of the five plant parts of each of the three sample trees were ground in a rotor-speed mill (0·5 mm sieve perforations). For each plant part, equal masses of material from the trees were pooled and analysed for total nitrogen. Material was digested in sulphuric acid/hydrogen peroxide mixture (Wolf 1982) to produce a clear colourless solution. Nitrogen was determined colorimetrically as ammonia by the reaction with salicylate and dichloroisocyanurate using nitroprusside as catalyst. The method employed eight calibration standards and two certified reference materials were run in every batch of analyses. Free sugar and starch content were determined by the method outlined in Ward & Deans (1993). Total carbohydrate concentration was expressed on a dry mass basis.

BIOASSAYS

The fungus Phacidium coniferarum

An isolate of *P. coniferarum*, a facultative pathogen on trees during the dormant season, was obtained from a bark lesion on Sitka Spruce and grown on Malt Agar (MA) at 20 °C. In February 1993, needles were clipped from around the mid-point of the stem of the 1991 leader of a single tree in each plot within the polyhouse. A core of bark, 5 mm in diameter or up to half the stem circumference on smaller stems, was removed to expose the cambium and a plug of fungus and MA taken from the edge of an actively growing colony was inserted with the mycelium facing the xylem. The plug was held in place by the excised bark and a wrapping of 'Parafilm'. Three additional control plants selected at random from different plots were inoculated with MA only. Stem diameter was measured at the inoculation point. After 38–39 days, any lesions that developed were exposed by removal of the outer bark and a tracing made of the extent of the discoloured inner bark. Lesion area was measured from the traced outline using a PC-based image analysis system.

The green spruce aphid Elatobium abietinum

Two trees from each plot were used for bioassays with aphids obtained from a non-clonal culture maintained on potted Sitka Spruce at 20 °C and 16 h light. Within the polyhouse during March 1993, two clear plastic insect-proof cages (7·8 cm × 4·5 cm × 2 cm) with net-covered ventilation holes were attached to each tree. The cages each enclosed the needles on approximately 7·8 cm of the tip of 1992 side-shoot foliage. Buds were removed from stems enclosed within the cages. A single gravid adult aphid was placed on the foliage in each cage and living aphids on the plant or cage were counted *in situ* at 2–7 day intervals. Dead aphids were also counted but were removed on each occasion and live aphids on the cage were repositioned on needles. Adults that had died were replaced with new ones until first generation adults were present so that, for each cage, the start of the experiment was taken to be the time of introduction of the first adult female to produce nymphs. The final assessment was made approximately 7 weeks later when cages and enclosed plant material were removed from plants. The numbers of 'early' (first to third instar nymphs) and 'late' instars (both alate and apterous fourth to fifth instars and adults) were counted. Apterous adults were dissected and the number of large (≥0·14 mm) embryos determined.

The spruce sawfly Gilpinia hercyniae

Sawflies were obtained from a culture established from laboratory-bred pupae (J. Lunderstadt, University of Gottingen, Germany) and maintained under long-day conditions (18 h light, 20 °C) on Sitka Spruce foliage.

Adult oviposition. Oviposition experiments were carried out in July 1993 with 8–10 cm of 1992 side-shoot growth, obtained from two trees in each plot, and from which current (1993) growth had been removed. Each bioassay was carried out using a single female, up to 48 h old, using shoot material taken from the eight plots in a single block. A total of 12 females was used to give three separate assays of material from each block. Shoots were placed at random in holes (two rows of four holes 10 cm × 10 cm apart) in the central part of an arena (56 cm × 28 cm) with their basal ends submerged in water contained within a supporting tray. The arena, covered with a clear plastic dome, was maintained at approximately 20 °C under artificial lights. Females were maintained in the arena

for 24 h after which the number of eggs laid in the needles and the number of attempted ovipositions without eggs (oviposition scars) were counted on each shoot.

Larval feeding. Three replicates of larval bioassays were carried out between August and November 1993 with 2–9 cm of 1992 side-shoot growth, obtained from two trees in each plot and from which current (1993) growth had been removed. In each bioassay, a shoot from each plot was separately supported in a Teflon-coated funnel with the basal end in water and arranged at random within an arena at 20 °C under artificial lights. A single newly moulted fourth instar larva was starved for 24 h, weighed and placed on each shoot at the start of the experiment. Larvae were replaced on foliage as necessary during the experiment and mortality noted. On moulting to the fifth instar, larvae were removed, starved for 24 h and dried to constant mass. Initial larval dry mass was estimated from a wet–dry mass regression.

STATISTICAL ANALYSIS

Data were analysed by CSS and Genstat statistical packages using regression or analysis of variance (ANOVA). Analysis of bioassay results was carried out on pooled data for both provenances where preliminary analysis indicated no significant differences between them. Experiments with *E. abietinum* and *P. coniferarum* carried out in the polyhouse were analysed as a split-plot design while the laboratory-based bioassays with *G. hercyniae* were analysed as completely randomized designs.

Results

PLANT GROWTH

Although initially smaller, trees of Oregon provenance grew more than those from Alaska during the experimental period. For both provenances, growth was greatest in the hLhN treatment (Fig. 1) in which there was also limited secondary ('lammas') growth on some trees. The growth of plants, estimated by total dry mass, was influenced by significant interactions ($P \leq 0{\cdot}01$) between light (L), nitrogen (N) and provenance (Pr) as follows: L × N, L × Pr, N × Pr and L × N × Pr. The root/shoot ratio (RSR) (Fig. 2) tended to be higher in trees of the Alaskan provenance and in both provenances was highest in the hLlN treatment. Overall, the RSR was determined by the interaction between L and N ($P < 0{\cdot}05$).

Water content estimated for the whole tree (see quantitative defences) tended to be lower in hL (Fig. 7a), but overall was influenced by a significant N × Pr interaction ($P < 0{\cdot}05$).

PLANT PHENOLOGY

The effect of treatments on the timing of budburst was similar in the two provenances (Fig. 3), with a significant L × N interaction ($P < 0{\cdot}001$), evident as a proportionately greater delay in budburst in the lN treatments in high light.

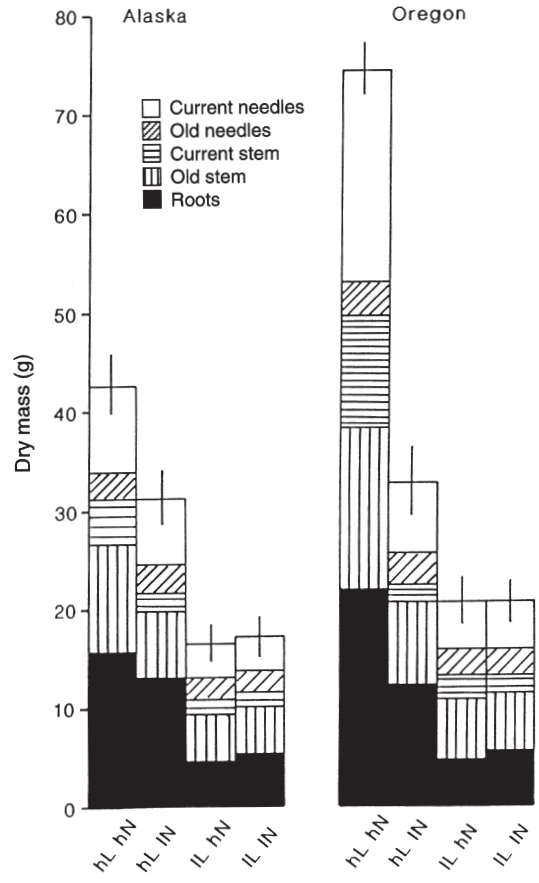


Fig. 1. Effect of light and nutrient treatments on growth of Alaska and Oregon provenances. Data are treatment means ± SE.

QUANTITATIVE DEFENCES

In general, the response of individual plant parts to the treatments was similar, so to simplify the presentation of results on resin and polyphenol concentration, as well as those for carbohydrates, nitrogen and water content, the main statistical analysis was carried out on the average concentration in whole plants. This was determined from concentrations in the different plant parts adjusted for their proportional contribution to total plant biomass. Concentrations in the different plant parts are shown in Tables 1 and 2 with the standard error of difference in each case being the largest obtained from the split-plot ANOVA.

Resin ducts and resin

Needle and resin duct size and tree growth. In current needles, the size of resin ducts was highly correlated with needle size (R^2 76·4%, $P < 0{\cdot}001$) and like needles themselves, their size was positively related to total dry mass of trees (Alaska, R^2 57·3%, $P < 0{\cdot}001$; Oregon, R^2

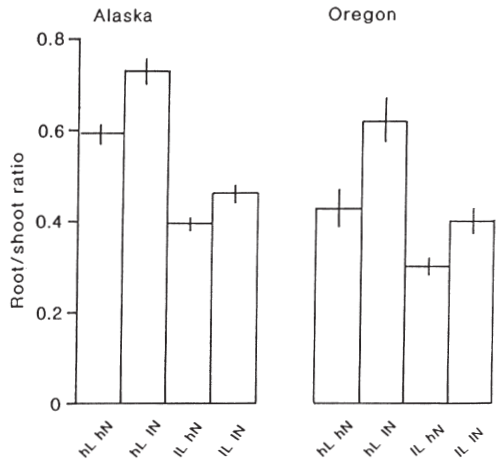


Fig. 2. Effect of light and nutrient treatments on root–shoot ratio of Alaska and Oregon provenances. Data are treatment means ± SE.

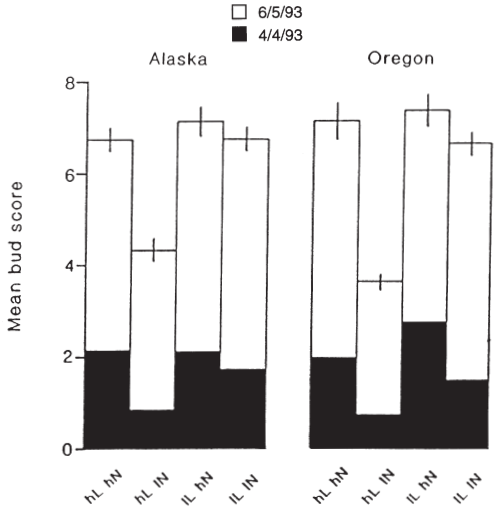


Fig. 3. Effect of light and nutrient treatments on bud development stage (see text) for Alaska and Oregon provenances. Data are treatment means ± SE.

68·5%, $P < 0·001$) (Fig. 4). Resin ducts in the Oregon provenance were about three times larger than those from Alaska. For the Oregon provenance, % resin duct area (PRDA) and needle cross-section area (NA) were also positively correlated (R^2 47·4%, $P < 0·01$) (Fig. 5) indicating that resin ducts occupied a larger proportion of the cross-sectional area of large needles. A similar but non-significant trend was evident for the Alaskan provenance. No relationship was found between growth and resin duct size in needles formed prior to treatment.

Representative sections of needles from trees of Alaskan and Oregon provenances in the hLhN and lLlN treatments are shown in Fig. 6.

Resin. The highest concentrations of resin in both Alaska and Oregon provenances occurred in stems and needles, with the lowest concentration in roots (Table 1). For whole plants, the resin concentration was influenced by a significant L × N ($P < 0·001$) and Pr × N interaction ($P < 0·05$) and for both provenances the highest concentration occurred in the hLlN treatment (Fig. 7b).

Polyphenols

There was considerable variation in polyphenol concentration among the different tissues (Table 1). For both provenances, old needles had the highest concentration while that in roots and current needles was also relatively high. The lowest concentration occurred in stem material.

For whole plants, the highest polyphenol concentration occurred in the hLlN treatment (Fig. 7c) and like resin, was affected by a significant L × N interaction ($P < 0·001$). Overall, polyphenol concentration tended to be higher in the Alaska provenance.

CARBOHYDRATES AND NITROGEN

The concentration of total carbohydrate was highest in needles and roots and lowest in stem material

Table 1. Effect of light and nutrient treatments on dry mass percentage concentration of resin and polyphenols in needles, stem and roots of Alaska and Oregon provenances. See methods for light and nutrient treatments: SED, largest standard error of difference for comparing any treatment of each plant part

Provenance	Alaska					Oregon					
treatment	hLhN	hLlN	lLhN	lLlN	Mean	hLhN	hLlN	lLhN	lLlN	Mean	SED
RESIN											
Current needles	4·4	4·2	3·1	3·1	3·7	3·7	4·8	3·5	4·2	4·1	0·26
Old needles	3·8	4·2	2·3	3·0	3·3	3·9	4·7	3·0	3·5	3·8	0·27
Current stem	4·2	5·6	2·9	3·6	4·1	3·8	6·0	2·6	2·8	3·8	0·44
Old stem	3·9	5·1	3·1	2·9	3·8	2·4	4·8	2·5	2·7	3·1	0·24
Roots	1·7	2·9	1·8	2·1	2·1	1·8	2·5	1·4	1·8	1·9	0·14
POLYPHENOLS											
Current needles	4·3	10·7	2·4	3·2	5·2	4·0	8·7	1·9	3·0	4·4	0·55
Old needles	7·5	11·7	9·3	9·8	9·8	7·5	12·1	7·6	8·8	9·0	0·99
Current stem	2·1	4·1	1·6	2·2	2·5	1·8	4·0	1·2	1·7	2·2	0·27
Old stem	3·0	4·1	2·2	2·4	2·9	2·3	3·5	1·4	2·3	2·4	0·28
Roots	2·7	6·4	2·5	4·2	4·0	3·4	6·5	2·0	3·9	4·0	0·44

Table 2. Effect of light and nutrient treatments on dry mass percentage concentration of total carbohydrate and nitrogen and on percentage water content in needles, stem and roots of Alaska and Oregon provenances. See methods for light and nutrient treatments: SED, largest standard error of difference for comparing any treatment of each plant part

Provenance treatment	Alaska					Oregon					SED
	hLhN	hLlN	lLhN	lLlN	Mean	hLhN	hLlN	lLhN	lLlN	Mean	
TOTAL CARBOHYDRATE											
Current needles	11·4	14·3	11·8	11·7	12·3	11·4	13·4	8·1	9·7	10·7	0·69
Old needles	11·0	13·1	9·6	10·5	11·1	9·2	12·5	7·1	8·3	9·3	0·58
Current stem	8·4	7·7	6·1	6·0	7·1	6·8	6·3	4·4	4·6	5·5	0·43
Old stem	7·0	6·7	4·7	4·5	5·7	5·1	5·8	3·2	3·5	4·4	0·46
Roots	10·5	12·0	6·0	7·4	9·0	8·5	12·2	4·1	5·9	7·7	0·63
NITROGEN											
Current needles	2·6	0·8	2·4	1·7	1·9	1·9	0·7	2·1	1·5	1·6	0·11
Old needles	2·0	0·8	1·5	0·9	1·3	1·5	0·7	1·5	1·1	1·2	0·11
Current stem	1·7	0·5	1·4	0·8	1·1	1·0	0·4	1·1	0·6	0·7	0·10
Old stem	0·9	0·3	0·6	0·4	0·6	0·5	0·3	0·5	0·3	0·4	0·06
Roots	1·6	0·6	1·3	0·6	1·0	1·1	0·6	1·2	0·7	0·9	0·12
WATER											
Current needles	56·6	58·0	63·3	64·3	60·6	62·7	59·1	66·7	65·6	63·5	0·82
Old needles	53·0	54·8	57·2	58·7	55·9	56·6	54·3	59·6	59·8	57·6	0·45
Current stem	52·2	47·7	54·0	54·3	52·1	53·4	47·0	56·5	52·2	52·3	0·83
Old stem	49·5	47·3	50·8	49·1	49·2	51·6	47·4	54·0	51·4	51·1	0·89
Roots	65·4	62·8	67·6	66·2	65·5	67·9	65·4	70·3	69·4	68·3	1·33

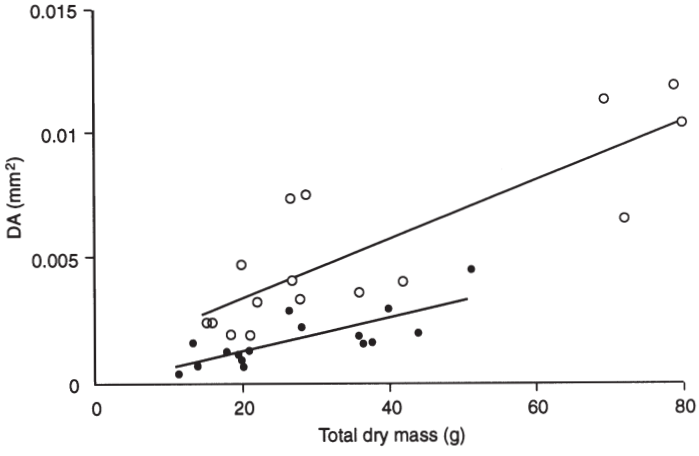


Fig. 4. Relationship between resin duct area (DA) in current needles and biomass of Alaska (●) ($y = -0{\cdot}0002 + 0{\cdot}00007x$) and Oregon (○) provenance ($y = 0{\cdot}00076 + 0{\cdot}00012x$).

(Table 2). For the whole plant, the highest concentration occurred in the hLlN treatment in both provenances although overall, concentrations tended to be higher in the Alaskan provenance (Fig. 7d). There was a significant L×N ($P < 0{\cdot}01$) and L×Pr interaction ($P < 0{\cdot}05$).

For nitrogen, the highest concentration occurred in needles, with lower concentrations in stems and roots (Table 2). Overall, nitrogen concentration was influenced by a significant L×N ($P < 0{\cdot}001$), L×Pr ($P < 0{\cdot}05$) and N×Pr interaction ($P < 0{\cdot}01$) (Fig. 7e).

BIOASSAYS

The fungus Phacidium coniferarum

Lesions were only formed on trees inoculated with the fungus and were observed within the bark of 23 of the 32 inoculated trees. There was considerable variation in the lesion area produced in response to *P. coniferarum* inoculation both within and between treatments. Stem diameter, which was negatively related to lesion size, was included as a covariate ($P < 0{\cdot}05$) in the ANOVA of square-root transformed lesion area. Only the light treatment had a significant effect on lesion size, with larger lesions formed in the hL treatment ($P < 0{\cdot}05$) (Fig. 8).

The green spruce aphid Elatobium abietinum

Adults that died during the early part of the experiment before nymphs were deposited were replaced and this happened more frequently for aphids on the Alaskan provenance ($P < 0{\cdot}05$). Once established, however, population growth of aphids within cages was similar for both provenances. Only the nitrogen treatment significantly affected the number of early ($P < 0{\cdot}01$) and late instars ($P < 0{\cdot}01$) (Fig. 9). The number of large embryos within adult aphids was also significantly affected by nitrogen fertilization ($P < 0{\cdot}001$).

The spruce sawfly Gilpinia hercyniae

Adult oviposition. Separate analysis of the number of eggs laid and the number of oviposition scars revealed

a similar response to treatment. Data were therefore combined in the final analysis of the untransformed female means. Only the light treatment significantly affected oviposition ($P = 0{\cdot}001$) (Fig. 10), with approximately four times as many scars and eggs on needles from the high-light treatment.

On some needles, eggs (approximate width 0·6 mm) protruded from the oviposition slits, most obviously on needles from lL which were narrower (0·77 ± 0·02 mm) than those from hL (0·87 ± 0·02 mm). A weighted regression analysis of the mean number of eggs plus scars (with weighting of $1/\sigma^2$ estimated for each observation) on needle width (Fig. 11), revealed a significant positive relationship ($P < 0{\cdot}001$) accounting for 37·5% of the variance.

Larval feeding. Many larvae failed to complete development to the fifth instar. Although there were no differences in survival between provenances, significantly more larvae survived in the hLhN treatment (Fig. 12) ($\chi^2 = 8{\cdot}08$, $P = 0{\cdot}044$). To allow for variation in the number of surviving larvae, data on relative growth rate (RGR) were analysed as a randomized block design using a REML procedure, pooling data from the three replicates. There were no significant differences in RGR between provenances or between light and nitrogen treatment.

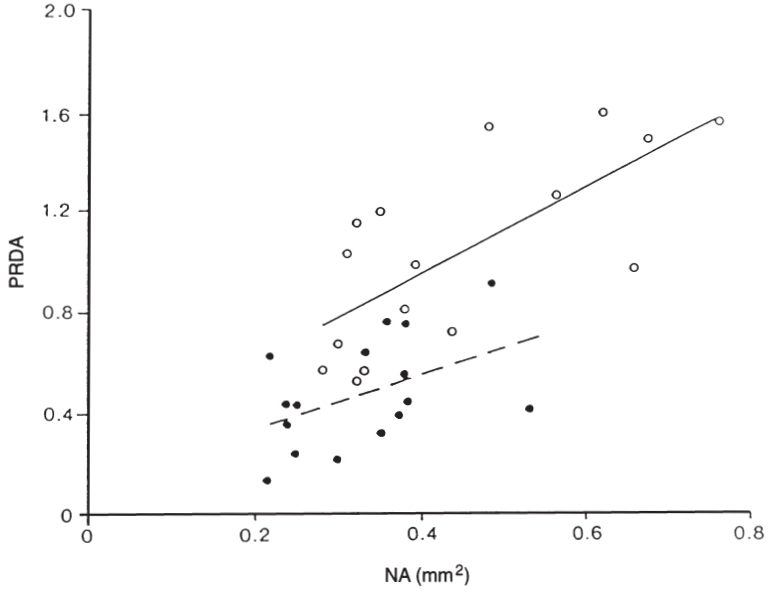


Fig. 5. Relationship between % resin duct area (PRDA) of current needles and needle cross-section area (NA) for Alaska (●) no significant regression and Oregon provenance (○) $y = 0{\cdot}276 + 1{\cdot}688x$.

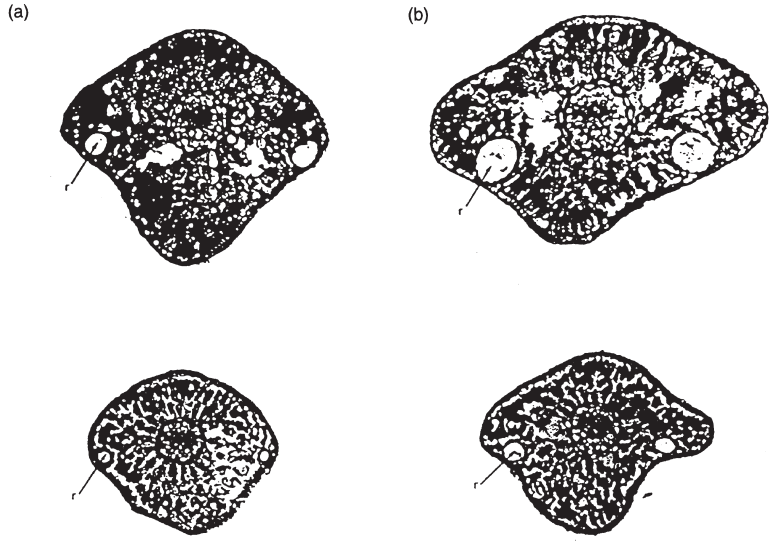
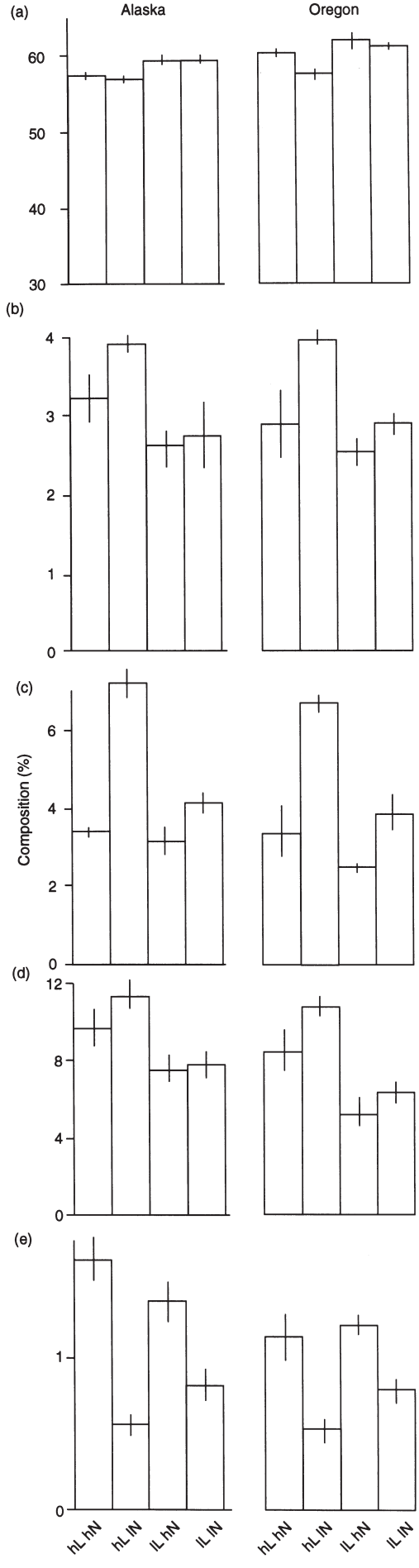


Fig. 6. Representative cross-sections of needles of (a) Alaska and (b) Oregon provenance. Needles are from primary growth of 1992 leaders formed during experimental treatments: top, hLhN; bottom, lLlN; r, resin duct.

Discussion

For both provenances of Sitka Spruce, the different light and nitrogen treatments affected the concentration of resin, polyphenols and total carbohydrate in ways predicted by resource-availability models of defence (Bryant *et al*. 1983, 1985; Coley *et al*. 1985; Lorio 1986; Herms & Mattson 1992). Thus concentrations of quantitative defences and carbohydrate were higher in the low nitrogen treatment that limited growth under both high and low light intensities. These results were based on measurements on needles, stems and roots and show that, in general, all parts of the plant were affected by the treatments, even though there were obvious differences in concentration between them (Table 1). The effect of treatments also extended to parts, such as needles, formed prior to the experiment. There were, nevertheless, some differences between particular parts in response to the treatments. Light, for example, did not affect the amount of polyphenols in old needles, concentrations of which were high in all treatments. This may in part be a carry over from earlier growth in the open nursery where light intensity was higher than in the polyhouse (Waterman, Ross & McKey 1984; Bryant *et al*. 1987; Mole, Ross & Waterman 1988; Dudt & Shurer 1994). The apparent persistence of high concentrations of polyphenols in old needles suggests that there was limited turnover in the plant during the following growing season. Variation in the relative concentration of resin and polyphenols in the different plant tissues, for example, between stem and root (Table 1), may indicate a defensive trade-off such as that suggested to occur between resin and lignin in bark of older spruce trees (Wainhouse, Rose & Peace 1997).

As well as affecting the ratio of carbon-based defences to nitrogen, the light and nitrogen treatments caused changes in the absolute and relative sizes of different parts of the trees through their effects on growth. There was, for example, a marked effect on both the absolute and relative size of needle resin ducts. The size or frequency of resin ducts can influence resin concentration in needles (White & Nilsson 1984; Moore & Hanover 1987). Within the woody tissue of conifers, increases in the size or number of



resin ducts have been linked to fertilization or other environmental factors (Smith, Wellwood & Elliot 1977; DeAngelis, Nebeker & Hodges 1986; Kainulainen *et al*. 1996), sometimes resulting in an increase in resin flow (Chudnyi 1974) and sometimes not (Hodges, Elam & Bluhm 1981). Bjorkman, Larsson & Gref (1991) provided circumstantial evidence that the increase in both the nitrogen and the resin acid concentration of pine needles following fertilization arose from the positive association between growth, formation of resin ducts and subsequent resin-acid production. They suggested that size of the specialized resin secreting ducts (structural limitation) rather than availability of carbon (substrate limitation) constrains resin-acid synthesis. In the present study, resin concentration was higher in needles in the low-nitrogen treatments in which growth was limited and was not positively related to growth and therefore duct size. Therefore, results from young Sitka Spruce plants suggest a complex relationship between resin duct size and resin concentration. Movement of resin within plants (Croteau 1988) that left some resin ducts partly empty, or the occurrence of intracellular resin (Kramer & Kozlowski 1979), would obviously affect the relationship between resin duct size and resin concentration in needles. These alternative stores of resin appear to be important in Sitka Spruce.

Although treatments caused large differences in overall growth of the young trees, results were not entirely consistent with a trade-off between growth and defence because in the low light (lL) treatment, growth was unaffected by nitrogen fertilization (Fig. 1) while the concentration of quantitative defences and total carbohydrate (Fig. 7) decreased. Although fertilization did not affect overall growth in low light, it did influence proportional allocation to root and shoot growth resulting in a lower root/shoot ratio (RSR) (Fig. 2) as found in many other plants (Bloom, Chapin & Mooney 1985; Chapin *et al*. 1987; Mooney & Winner 1991). There is, therefore, a striking correspondence between RSR (Fig. 2) and concentration of resin, polyphenols and total carbohydrate (Fig. 7). In the absence of a trade-off with growth in the lL treatment, our results suggest the possibility that the regulatory mechanism controlling the dynamic balance between root and shoot (Larcher 1995) in response to resource availability may also influence the production of quantitative defences as part of an integrated response to environmental stress.

The wide-ranging effects of the light and nitrogen treatments on the growth and physiology of young Spruce trees are reflected in the complex picture that

Fig. 7. Effect of light and nutrient treatments on mean % (a) water, (b) resin, (c) polyphenols, (d) total carbohydrate and (e) nitrogen, calculated from concentrations in leaves, stems and roots and their proportional contribution to total plant biomass (see text). Data are treatment means ± SE expressed for b–e on a dry mass basis.

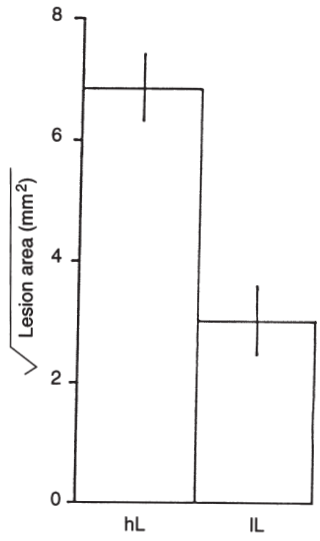


Fig. 8. Area of lesions formed by *Phacidium coniferarum* in stem bark of spruce in high and low light treatments. The areas, which were adjusted for stem diameter covariate, were estimated from pooled data for Alaska and Oregon provenances. Vertical lines are ± SE from ANOVA.

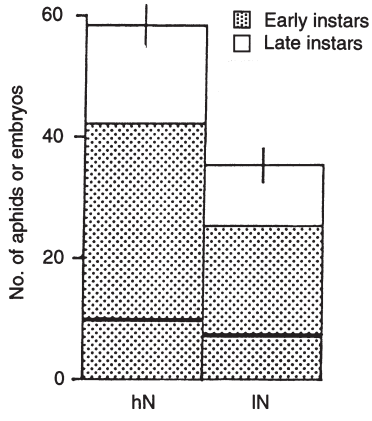


Fig. 9. Mean number of early or late instars per cage (see text) or mean large embryos per adult (bold line) in high and low nitrogen treatments. ± SE derived from ANOVA of total aphids per cage.

emerges of changes in susceptibility to the insects and fungus used in the bioassays. The lesions that developed in bark in response to inoculation with *P. coniferarum* resulted from both the growth of the fungus through the phloem (Wong & Berryman 1977; Christiansen & Horntvedt 1983; Raffa & Berryman 1987; Ross, Fenn & Stephen 1992) and a localized induced defensive reaction, the dynamic wound response which contains the infection (Berryman 1972; Wong & Berryman 1977). In our experiments, bark formed a high proportion of the whole-stem material used for chemical analysis, results of which showed that the highest concentration of both carbohydrates and secondary chemicals occurred in bark from the hL treatment in which the largest lesions were formed. Although carbohydrates are an important source of energy for the dynamic wound response (Reid, Whitney & Watson 1967; Christiansen & Ericsson 1986), abundant carbohydrate in tissues can also increase the energy available to invading fungi (Entry *et al*. 1991, 1992) and this may have affected growth of *P. coniferarum*, resulting in larger lesions. The lower water content of bark in high light may also have increased susceptibility to *P. coniferarum* as has been observed with other fungi (Crist & Schoeneweiss 1975; Lindberg 1991; Vannini & Mugnozza 1991; Gao & Shain 1995). In contrast, secondary chemical concentration does not appear to have inhibited the growth of *P. coniferarum* and restricted lesion size.

Resin and polyphenol concentration appears to have played a relatively minor role in the performance of *E. abietinum*. The aphids are sensitive to secondary chemicals, however, because they avoid young needles by responding to monoterpenes and other volatiles present in epicuticular wax (Jackson & Dixon 1996). Needles of the Alaskan provenance contained a higher concentration of resin than those from Oregon and this may explain why aphids were more restless (i.e. walking from needles on to the cage) on trees of this provenance. Once aphids began feeding, however, there appeared to be no difference in the quality of the two provenances, with aphid fecundity apparently independent of secondary chemical concentration but strongly influenced by nitrogen as found in previous studies (Parry 1974).

The growth or survival of larvae of *G. hercyniae* has been related to a complex interaction between nutrients and phenolic compounds within spruce needles (Schopf 1986) and to the concentration of carbohydrates (Jensen 1988). In our experiments, larval survival was highest on needles from the hLhN treatment (Fig. 12) in which the concentration of polyphenols was relatively low and that of carbohydrates relatively high, factors that have been linked with low

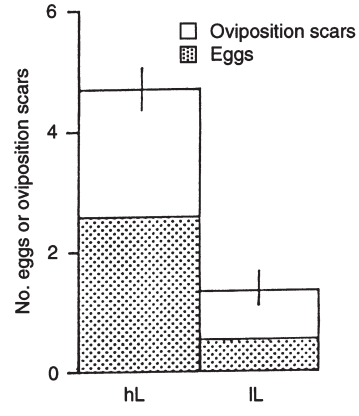


Fig. 10. Mean number of oviposition scars or eggs laid by *Gilpinia hercyniae* in needles from high or low light treatment. ±SE derived from ANOVA of total eggs and oviposition scars.

mortality of *G. hercyniae* larvae (Schopf 1986). We were unable to detect any effects of treatment on the growth rate of fourth instars, possibly because the larvae are relatively unresponsive to the nitrogen content of needles (Jensen 1988).

Oviposition by *G. hercyniae* appeared to be independent of both the secondary chemical and nutritional content of needles. There was no evidence that oviposition slits, that lacked eggs, represented 'probes' to determine needle quality as appears to be the case for other sawflies (Wilkinson & Popp 1989). Needle width, however, which was strongly influenced by the light treatments, was an important factor in successful oviposition (Fig. 11) Needle size has previously been recognized as an important influence on sawfly oviposition, in part because of the stereotyped needle gripping behaviour of ovipositing females (Ghent 1959; Wilkinson & Popp 1989).

Although not assessed in our experiments, the delay in budburst caused by the hLhN treatment (Fig. 3) could affect synchrony of insect attack (Hunter 1993; Quiring 1993, 1994; Mopper & Simberloff 1995).

In summary, our results emphasize the complexity of environmental effects on trees. The light and nitrogen treatments affected the concentration of quantitative secondary chemicals, carbohydrate and nitrogen in ways predicted by resource-availability models of defence. However, the water content of tissues, the size of needles and the absolute and relative sizes of resin ducts within them were also affected. For the insects and fungus we studied, changes in needle size, the nutritional and water content of tissues and the balance between nutrients and secondary chemicals all seemed to influence performance of one or more of the organisms. Thus, changes in the concentration of carbon-based secondary chemicals alone were of only limited value in predicting susceptibility and a much wider perspective of environmentally induced changes in plants is necessary for a proper understanding of effects on pests and pathogens. Changes in secondary chemical composition corresponded closely to changes in RSR and so may be a result of an integrated response of the trees to stress.

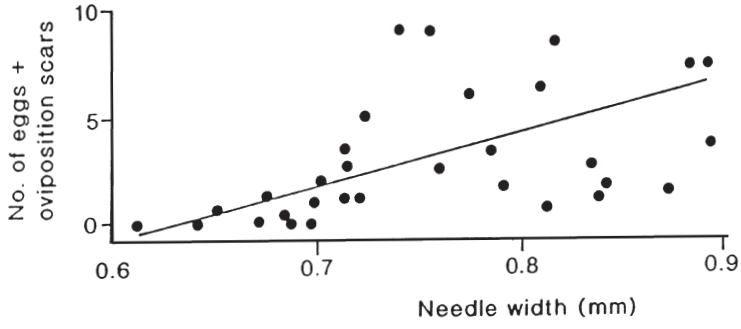


Fig. 11. Mean number of oviposition scars and eggs laid in spruce needles by *Gilpinia hercyniae* in relation to width of needles from the light and nutrient treatments (see text) ($y = -15{\cdot}54 + 24{\cdot}8x$).

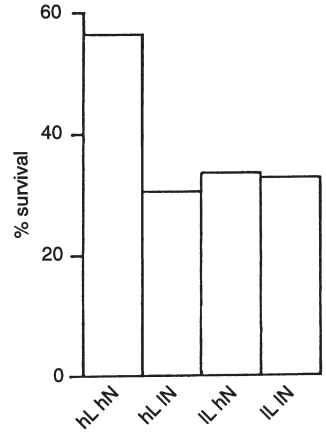


Fig. 12. Mean percentage survival of fourth instar *Gilpinia hercyniae*. Pooled data for Alaska and Oregon provenance ($\chi^2 = 8{\cdot}08$, $P = 0{\cdot}044$).

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