PLANT-ANIMAL INTERACTIONS - ORIGINAL PAPER

Growth and defence in young pine and spruce and the expression of resistance to a stem-feeding weevil

D. Wainhouse · J. T. Staley · R. Jinks · G. Morgan

Abstract Defence in young trees has been much less studied than defence in older ones. In conifers, resin within ducts in bark is an important quantitative defence, but its expression in young trees may be influenced by developmental or physical constraints on the absolute size of the resin ducts as well as by differential allocation of resources to growth and resin synthesis. To examine these relationships, we used nitrogen fertilisation of 1- and 2-year-old pine and spruce to produce trees of different sizes and measured the effect on the number and size of resin ducts and the amount of resin they contained. All of these variables tended to increase with stem diameter, indicating a positive relationship between resin-based defence and growth of 1- and 2-year-old trees. In pine, however, the mass of resin flowing from severed ducts was much lower relative to duct area in 1- than in 2-year-old trees, suggesting that the older trees allocated a higher proportion of the carbon budget to resin synthesis. Resin-based defence in 1-year-old pines appears to be both positively related to growth and resource limited. In spruce, resin production was generally lower, and age-related differences were not observed, suggesting that resin-based defence is less important in this species. Bio-assays of 2-year-old trees with the pine weevil, *Hylobius abietis*, emphasised the importance of resin as a defence against this bark feeding insect. Nitrogen fertilisation had a limited influence on resistance expression. One-year-old trees remained susceptible because of their small size, low resin production and limited response to fertilisation. The strong growth response of 2-year-old trees to fertilisation increased resin-based defence, but most spruce trees remained susceptible, while most pines were resistant at all levels of fertilisation.

Keywords *Hylobius abietis* · Pine · Plant defence · Resin ducts · Spruce

Introduction

Defence against herbivory in relation to plant age or developmental stage has been observed in a number of tree-pest interactions (Boege and Marquis 2005; Bryant et al. 1994; Bryant and Julkunen-Tiitto 1995; Fritz et al. 2001; Goodger et al. 2004; Kearsley and Whitham 1989; Spiegel and Price 1996). The causes of this ontogenetic change are often not well understood and, in particular, defence has been much less studied in younger trees than in older ones (Boege and Marquis 2005; Hanley and Lamont 2002). Herbivory is a strong selective force on young plants (Watkinson 1997), but so is competitive ability and, as a result, there could be a trade-off between growth and expression of quantitative defences. Trade-offs between growth and defence are evident in some trees or for some carbon-based secondary chemicals (CBSC) but not others (Donaldson et al. 2006; Mutikainen et al. 2002; Osier and Lindroth 2006; Rosner and Hannrup 2004). These interactions are frequently discussed in the context of resource-availability models of defence (Herms and Mattson 1992). While such models can predict total allocation to CBSC (Koricheva et al. 1998), they provide an inadequate description of the underlying

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mechanism (Hamilton et al. 2001; Koricheva 2002; Nitao et al. 2002). Nevertheless, physiological trade-offs are a component of other models of phenotypic expression of defence (Herms and Mattson 1992; Nitao et al. 2002), and such interactions may be especially evident in young trees where growth during an early establishment phase is likely to be a particularly important component of competitive ability. There may, however, be additional developmental or physical constraints on levels of defence during early growth and establishment. Björkman et al. (1991), for example, suggested that when secondary chemicals are held in specialised structures, such as the resin ducts of conifers, there may be a positive relationship between growth and defence, with duct size (structural limitation) rather than availability of carbon (substrate limitation) constraining resin synthesis. The absolute size of the ducts is likely to be limited in very young trees, and a smaller carbon budget may result in insufficient resin to fill them, further limiting expression of 'containerised' resin-based defences (Wainhouse et al. 1998). So while the genetic, developmental and growth characteristics of young trees will determine the size and form of the resin duct system, allocation of carbon to growth and resin synthesis may still reflect trade-offs based on competing priorities. Understanding these relationships is important in assessing the extent to which the phenotypic expression of resistance to particular pests may be influenced by manipulating the growth of young trees.

The resistance of conifers to the stem bark feeding pine weevil, *Hylobius abietis*, provides a useful model system to examine these particular aspects of defence in young trees. Pine and spruce are most vulnerable to attack during the first few years of growth, and the principal defence is provided by resin ducts within the bark on which the weevils feed (Wainhouse et al. 2004, 2005). In the study reported here, we examined the physical and physiological constraints on the expression of resin-based defence in two conifer species widely planted in the UK—Sitka spruce [*Picea sitchensis* (Bong.) Carr.], which occurs naturally in coastal forests of the Pacific Northwest of North America (Harris 1990), and Corsican pine (*Pinus nigra* ssp. *laricio* Maire), which occurs in mountain regions of Corsica, Calabria and Sicily in the Mediterranean basin (Barbéro et al. 1998).

Methods

Experimental trees were grown under different nitrogen fertilisation treatments over 2 years to produce a range of phenotypes of 1- and 2-year-old trees in which traits were compared by physical- and bio-assay. The effect of nitrogen treatments on resistance expression was then used as a basis for assessing the extent to which it could be manipulated by altering the growing conditions of the trees.

Trees and experimental treatments

Seeds of Corsican pine were germinated in spring 2000 and those of a Sitka spruce family mixture germinated in spring 2001. They were repotted in early summer into 1-l pots containing a limed peat–vermiculite media with the amount of a balanced controlled release fertiliser (Osmocote $16 + 8 + 12 + TE$ 8–9 months) added depending on the experimental treatment. Trees were allocated at random to one of five nitrogen (N) treatments, N0.5, N1, N2, N4 and N8, corresponding to kilogrammes of nitrogen per cubic metre, in order to produce trees that varied in growth rate and size. They were arranged in single treatment blocks in a ventilated greenhouse in either southern Scotland (BNG. NT245635) (spruce) or southern England (BNG. SU803428) (pine). Pots were supported above the ground to ensure free drainage and 'air-pruning' of roots, and trees were watered regularly to maintain compost moisture levels. On several occasions, all trees were sprayed at the recommended rate with non-persistent 'Savona' insecticidal soap to control aphid infestations. After 1 year, trees were repotted in the spring into 3-l containers with the same treatment levels reapplied.

Growth and resin-based defence in individual 1- and 2-year-old trees

Growth and defensive traits

Measurements on the trees were made prior to the onset of the growing season after the first (March 2001 and January 2002 for pine and spruce, respectively) and second year of growth (January 2002 or January 2003). At each assessment, ten trees were selected at random from each N treatment for each stem growth increment (GI), i.e. length of stem produced in a single year, to be measured. They were watered to container capacity approximately 24 h before assessment and, after about 9 h, transferred to a laboratory, rewatered and left to equilibrate to ambient temperature. The length and diameter of the stem for each GI (lower, first-year GI = GI 1; upper, current GI = GI 2) was measured for each tree. The mass of resin that flowed from cortical resin ducts was quantified by making a scalpel cut through the bark (collectively the vascular cambium, phloem, cortex and epidermis) to the xylem that encircled the main stem. This standardised wound was positioned near the middle of the appropriate GI, ignoring late season 'lammas' growth. Wounding was only done on one GI on an individual tree. Resin was collected at 10-min intervals in pre-weighed pipettes until the flow stopped, and total resin mass was then determined. A section of stem from the middle of the GI was removed and cut in half at the wounding site. On one of the cut ends,

bark thickness, stem diameter and the number and cross-section area of each resin duct was measured and total duct area determined.

After each assessment, the needles and remaining parts of individual trees, including roots, were dried at 100°C for 1 h and then at 70°C for approximately 47 h until constant mass.

Bio-assays and the expression of resistance

The GI of most 1-year-old trees was too small relative to the size of the assay cage and weevil for an effective bio-assay which was, therefore, only done on trees at the end of the second growing season. Five trees were selected at random from each N treatment for each GI to be assayed. Trees were watered and removed to laboratory conditions (approx. 20°C under artificial lighting ['Gro-lux' fluorescent tubes on for 16 h/day) with humidity uncontrolled]. Growth increments 1 and 2 were assayed on separate trees, and needles were clipped from the appropriate increment to accommodate an approximately 5-cm-diameter plastic mesh cage that enclosed a 3-cm length of stem. The cage was positioned near the middle of each GI, ignoring lammas growth, and GI length and diameter at the cage position were determined.

Weevils used in the bio-assays were newly emerged and reproductively immature when collected during July–September in 2001 and 2002 from net emergence traps secured over stumps of Corsican or Scots pine (*P. sylvestris*) at several locations in southern England. They were stored without food on moist paper at 2–3°C for up to 36 weeks during which time they were separated into single sex groups. Female weevils were removed from cold storage and kept individually on damp filter paper in small plastic boxes under ambient laboratory conditions for 24 h before being weighed and used in the bio-assays. The few unusually large or small individuals within the sample were disregarded, and a single female weevil was placed in each cage for 3 days. Trees were watered as necessary during the assay to maintain compost moisture levels. At the end of the assay, the weevils were transferred to plastic boxes for 48 h to void gut contents prior to re-weighing.

The amount of bark eaten by each weevil was measured as described by Wainhouse et al. (2005). Briefly, the stem section containing feeding damage was removed from the tree and immersed in pentane for 5 or 10 min to remove exuded resin at the feeding sites. Areas of feeding were traced onto transparent film, distinguishing 'shallow' feeding in the outer bark (i.e. up to a depth of 50% of bark thickness) from areas of 'deep' feeding where bark was consumed to a depth >50% of bark thickness. Measurements of bark thickness and density were used to determine

the dry mass of bark eaten. Note that estimates of the amount of deep feeding included all of the bark eaten, both inner and outer, whereas shallow feeding included only outer bark.

On the cut ends of the stem immediately above and below the bio-assayed area, bark thickness, stem diameter and the number and area of resin ducts was determined. For each stem, a mean for the two measurements was determined, rounding down to a whole number of resin ducts. Total duct area was determined for each GI assayed.

Effect of N treatments on growth and resistance expression in pine and spruce

The effects of N treatments on selected measurements made during the physical- and bio-assays were analysed to determine the extent to which the manipulation of growth of a population of trees influenced resin defence traits and the expression of resistance to *H. abietis*. The variables analysed were total biomass, number of resin ducts, resin duct area, resin mass and, from the bio-assays, the ratio of deep/shallow feeding.

Statistical analysis

Data for pine and spruce were analysed separately using the GenStat statistical package (Payne 2007). In analyses of phenotypic variation between individual trees and the effects of the five N treatments, particular emphasis was placed on measurements on parts of the tree associated with weevil feeding sites, such as stem diameter, the size and number of resin ducts and the mass of resin flowing from them. General linear models were used throughout, with the exception of the analysis of the influence of N treatments on individual duct area in GI 1 after the first and second year of growth, which was analysed in a REML mixed model, with tree as a random factor. Data were L_n transformed prior to analysis to normalise residuals. The models used are described in the appropriate figure captions or in Table 1. An all-regression approach was used to identify the significant models in Table 1. In linear regressions of the relationship between the number of resin ducts and stem diameter, duct area and stem diameter, and between resin mass and duct area, a common slope was fitted to the data where appropriate. The analysis of bio-assay data was based on an L_n transformed ratio of deep/shallow feeding (L_n ratio) (Wainhouse et al. 2005). Prior to analysis, seven observations of zero shallow feeding were given the value of 0.05 (minimum observed value = 0.06), and one observation with zero deep feeding was given the value of 1 (minimum observed value = 3).

Results

Growth and resin-defence in individual 1- and 2-year-old trees

Growth and defensive traits

There was considerable overlap in tree size between the N treatments, and a preliminary analysis of the allometric relationship between stem diameter (GI 1) and biomass and between needle and non-needle biomass indicated that simple regressions best explained the consistent relationship between the variables both within and between treatments. The diameter of GI 1 in both species was positively related to the biomass of 1- and 2-year-old trees and so was a good index of tree growth and size (Fig. 1a). Diameter of GI 1 was therefore used, where appropriate, as the independent variable in further analyses. There was also a linear relationship between needle- and non-needle biomass which for each species was similar in both years (Fig. 1b). The approximate leaf area ratio (needle mass/total mass) for 1- and 2-year-old pines was 0.51 and 0.41, respectively, and for spruce, 0.31 and 0.33.

In pine, the number of resin ducts increased significantly with diameter (Fig. 2a). There were separate intercepts but a common slope for each year–GI combination corresponding to GI 1 in 1- and 2-year-old trees and GI 2. In 2-year-old pine, there were relatively more ducts in GI 2 than GI 1 (Fig. 2a). In spruce, a significant relationship between duct number and diameter was only evident in the current GI of 2-year-old trees (Fig. 2a). In both species, there was a significant positive relationship between duct area and diameter, with duct area relatively higher in pine than spruce (Fig. 2b). Separate intercepts corresponded to data for GI 1 (1- and 2-year-old trees) and GI 2 and indicated that in pine, ducts were relatively larger in GI 2 than GI 1, whereas in spruce, ducts were relatively smaller in GI 2 than GI 1 (Fig. 2b).

The factors that may have influenced the mass of resin flowing from ducts were analysed by linear regression. Three explanatory variables—resin duct area, number of resin ducts and stem diameter—were initially included in the analysis. All possible combinations were tested and, with one exception, only single explanatory variables for resin mass were significant. The fitted models are given in Table 1. In pines, duct area and the number of ducts explained similar amounts of variation. In spruce, duct area was the predominant explanatory variable for GI 1 in 1-year-old trees, but in 2-year-old trees, the best model included all three terms. For GI 2, all three single variables were significant. The relationship between resin mass and duct area for each species and GI was further explored in regression analysis (Fig. 3). In pine, separate intercepts corresponded to data for GI 1 (1-year-old trees) and GI
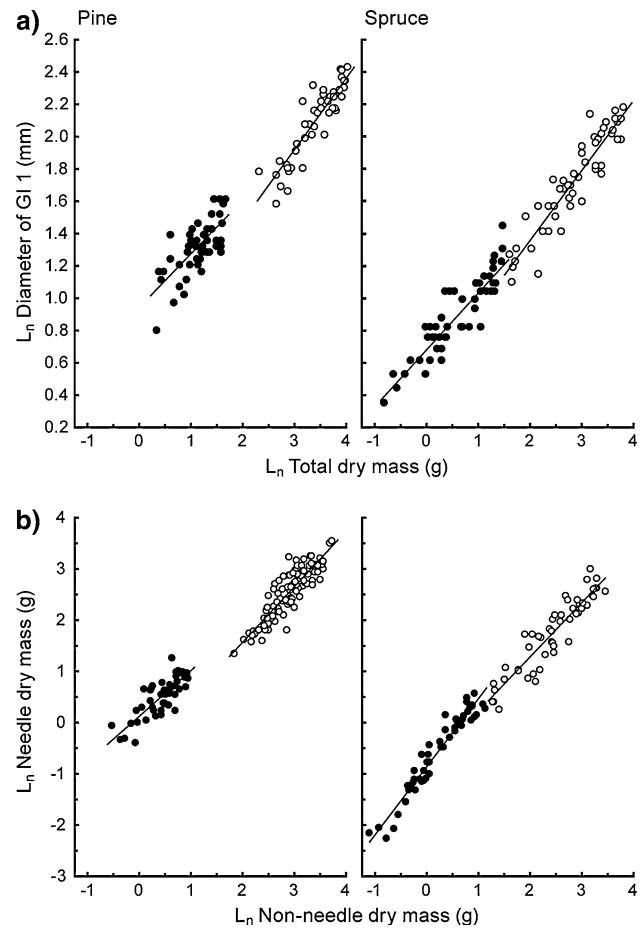

Fig. 1 a The relationship between stem diameter of first-year growth increment (GI 1) and dry mass of 1-year-old [$y = 0.93 + 0.33x$ ($R^2 = 50.6$, $P < 0.001$)] and 2-year-old pine [$y = 0.52 + 0.46x$ ($R^2 = 80.9$, $P < 0.001$)] and 1-year-old [$y = 0.68 + 0.35x$ ($R^2 = 80.1$, $P < 0.001$)] and 2-year-old spruce [$y = 0.50 + 0.43x$ ($R^2 = 85.8$, $P < 0.001$)]. **b** The relationship between needle and non-needle biomass of 1-year-old [$y = 0.12 + 0.85x$ ($R^2 = 62.9$, $P < 0.001$)] and 2-year-old pine [$y = -0.66 + 1.10x$ ($R^2 = 77.9$, $P < 0.001$)] and 1-year-old [$y = -0.88 + 1.34x$ ($R^2 = 93.3$, $P < 0.001$)] and 2-year-old spruce [$y = -0.89 + 1.07x$ ($R^2 = 85.7$, $P < 0.001$)]. *Filled circle* One-year-old trees, *open circle* 2-year-old trees

1 + GI 2 in 2-year-old trees, indicating that resin flow relative to duct area was much higher in 2- than in 1-year-old trees. In spruce, there were separate intercepts for each year–GI combination, but with no evidence of an overall increase in resin flow in 2-year-old-trees (Fig. 3). In fact, resin flow from GI 1 in the second year was slightly lower than that from the same increment in 1-year-old trees.

Bio-assays and the expression of resistance

For each species, the relationship between the ratio of deep to shallow feeding (L_n ratio) and resin duct area on bio-assayed trees was similar for the two GIs and, data from the separate increments was therefore pooled for regression analysis. The L_n ratio decreased as duct area increased in
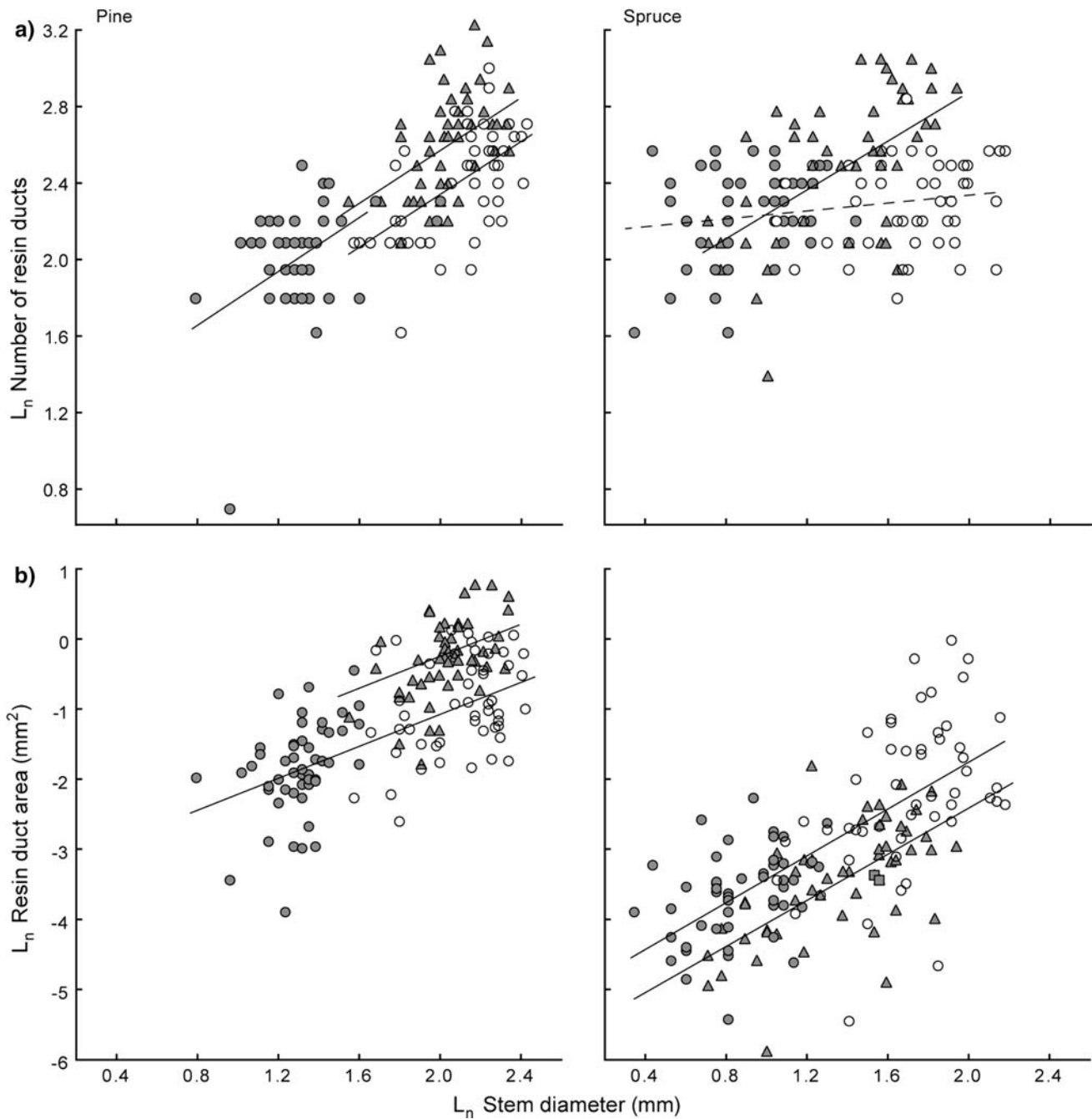
Fig. 2 The number of resin ducts (**a**) and resin duct area (**b**) in relation to stem diameter of GI 1 [1-year-old (*filled circle*) and 2-year-old trees (*open circle*)] and growth increment 2 (GI 2, *filled triangle*) on pine and spruce. **a** For pine, a common slope was fitted to the data, with separate intercepts for each year/GI combination (IY): $y = IY_i + 0.70x$ (overall $R^2 = 56.9$, $P < 0.001$). For spruce GI 1 (1- + 2-year-old trees), the regression (*dotted line*) was non-significant ($P = 0.053$); for GI 2,

$y = 1.56 + 0.57x$ ($R^2 = 29.5$, $P < 0.001$). **b** For pine, a common slope was fitted to the data for GI 1 (1- + 2-year-old trees): $y = -3.48 + 1.21x$ ($P < 0.001$); for GI 2, $y = -2.76 + 1.21x$ ($P < 0.001$) (overall $R^2 = 57.7$) Corresponding regressions for spruce were: $y = -5.07 + 1.64x$ ($P < 0.001$) and $y = -5.70 + 1.64x$ ($P < 0.001$) (overall $R^2 = 49.3$)

both pine and spruce, indicating that there was relatively less deep feeding as ducts increased in size (Fig. 4). In 2-year-old spruce, the small duct area in current growth (GI 2) resulted in relatively more deep feeding (high L_n ratio) than in GI 1, whereas in pine, ducts were relatively large in

current growth so that there was relatively less deep feeding in comparison to GI 1.

Two-year-old pines appeared to be relatively resistant to weevils, with a L_n ratio that was often close to or below zero, indicating that there was relatively more shallow than

Table 1 Regression models of the mass of resin flowing from different growth increments of 1- and 2-year-old pine and spruce

Pine		Spruce	
Growth increment 1			
One-year-old ($n = 50$)	RM = $-5.45 + 0.50$DA, $R^2 = 8.3\%$, $P < 0.05$ RM = $-9.10 + 1.36$DN, $R^2 = 9.4\%$, $P < 0.05$	One-year-old ($n = 50$)	RM = $-4.42 + 0.58$DA, $R^2 = 13.6\%$, $P < 0.01$
Two-year-old ($n = 50$)	RM = $-2.88 + 0.35$DA, $R^2 = 9.2\%$, $P < 0.05$ RM = $-4.95 + 0.72$DN, $R^2 = 6.7\%$, $P < 0.05$	Two-year-old ($n = 47$)	RM = $-11.4 + 0.50$DA + 1.86DN + 1.25D, $R^2 = 59\%$, $P < 0.01$
Growth increment 2			
Two-year-old ($n = 50$)	RM = $-2.75 + 0.29$DA, $R^2 = 9.1\%$, $P < 0.05$ RM = $-4.39 + 0.60$DN, $R^2 = 8.2\%$, $P < 0.05$	Two-year-old ($n = 49$)	RM = $-3.0 + 0.75$DA, $R^2 = 21.8\%$, $P < 0.001$ RM = $-9.91 + 1.74$DN, $R^2 = 24.1\%$, $P < 0.001$ RM = $-7.76 + 1.60$D, $R^2 = 18.0\%$, $P < 0.001$

Data were log transformed prior to analysis. RM = L_n resin mass, DA = L_n duct area, DN = L_n no. of ducts, D = L_n stem diameter
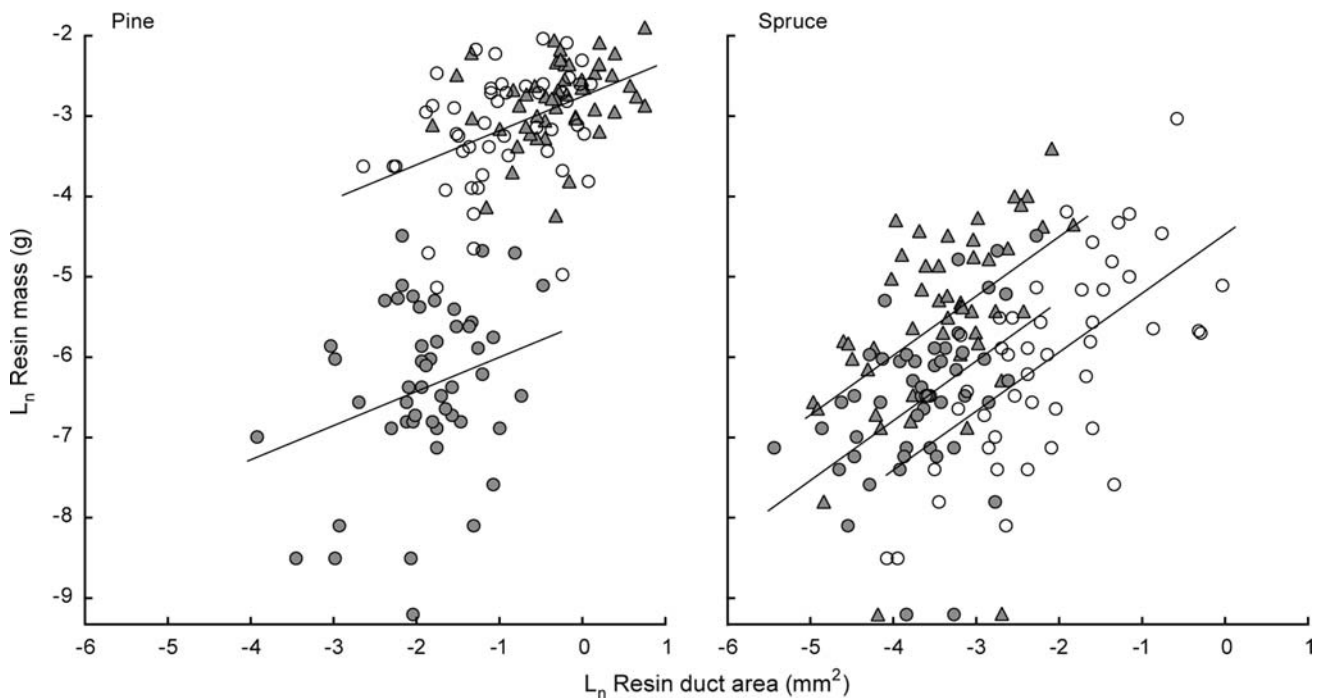
Fig. 3 The amount of resin flowing from a standardised wound in relation to resin duct area in GI 1 [1-year-old (*filled circle*) and 2-year-old trees (*open circle*)] and GI 2 (*filled triangle*) on pine and spruce. For pine, a common slope was fitted to the data for 1-year-old trees (GI 1), $y = -5.6 + 0.42x$ ($P < 0.001$), and 2-year-old trees (GI 1 + GI 2), $y = -2.81 + 0.42x$ ($P < 0.001$) (overall $R^2 = 77.6$). For spruce, a common slope was fitted to the data, with separate intercepts for each year/GI combination (IY): $y = IY_i + 0.74x$ ($P < 0.001$) (overall $R^2 = 35.5$)

deep feeding (Fig. 4). In both species, therefore, GIs were defined as 'resistant' when the L_n ratio ≤ 0. Because shallow feeding includes only outer bark but deep feeding includes approximately equal amounts of inner and outer bark, when the L_n ratio = 0 (i.e. equal mass of deep and shallow feeding), only around 25% of total feeding would damage the vulnerable inner bark on resistant trees.

Effect of N treatments on growth and resistance expression

The N treatments affected the growth of trees in both the first and second year, with significant effects on total biomass in both pine (year 1, $F_{4,45} = 8.6$, $P < 0.001$; year 2,

$F_{4,94} = 55.6$, $P < 0.001$) and spruce ($F_{4,45} = 23.6$, $P < 0.001$ and $F_{4,45} = 22.1$, $P < 0.001$, respectively) (Fig. 5). Dry mass increased with nitrogen treatment up to N4 with no further increase at N8.

For the analysis of the effects of N treatments on resin duct number and area and the mass of resin flowing from ducts, the data for each species were separated into three groups, corresponding to GI 1 in 1- and in 2-year-old trees and GI 2 in 2-year-old trees. For graphical presentation, mean values were backtransformed, and approximate standard errors determined.

In pine, the number of resin ducts varied significantly between groups ($F_{2,135} = 67.1$, $P < 0.001$) and in response
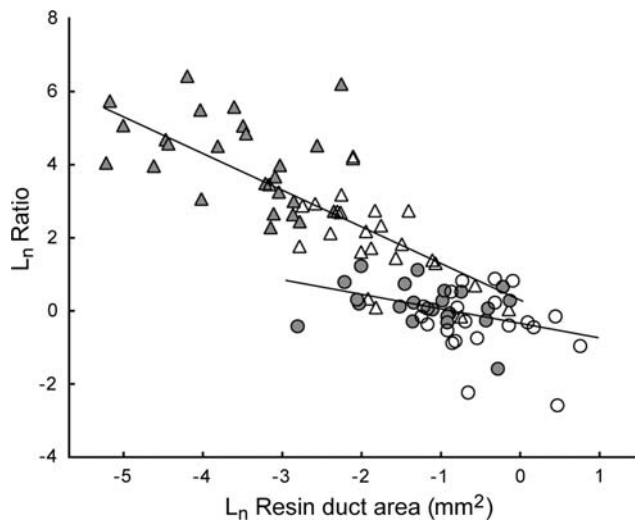
Fig. 4 The ratio of the amount of deep to shallow feeding in bark (L_n ratio) in relation to resin duct area in 2-year-old pine and spruce. Pine [$y = -0.41 - 0.39x$ ($R^2 = 11.5$, $P < 0.05$)]: *open circle* GI 1, *filled circle* GI 2. Spruce [$y = 0.22 - 1.08x$ ($R^2 = 54.8$, $P < 0.001$)]: *open triangle* GI 1, *filled triangle* GI 2

to N treatment ($F_{4,135} = 4.9$, $P < 0.001$) (Fig. 6a). The number of resin ducts was higher in 2-year-old than in 1-year-old trees and increased in response to increasing N. In spruce, there were also significant differences between groups ($F_{2,134} = 12.3$, $P < 0.001$) and N treatment ($F_{4,134} = 3.9$, $P < 0.01$), but there was in addition a significant interaction ($F_{8,134} = 2.9$, $P < 0.01$), with only the ducts in the current GI (GI 2) responding to treatment. The number of ducts in GI 1 did not increase during the second year of growth as they did in pine.
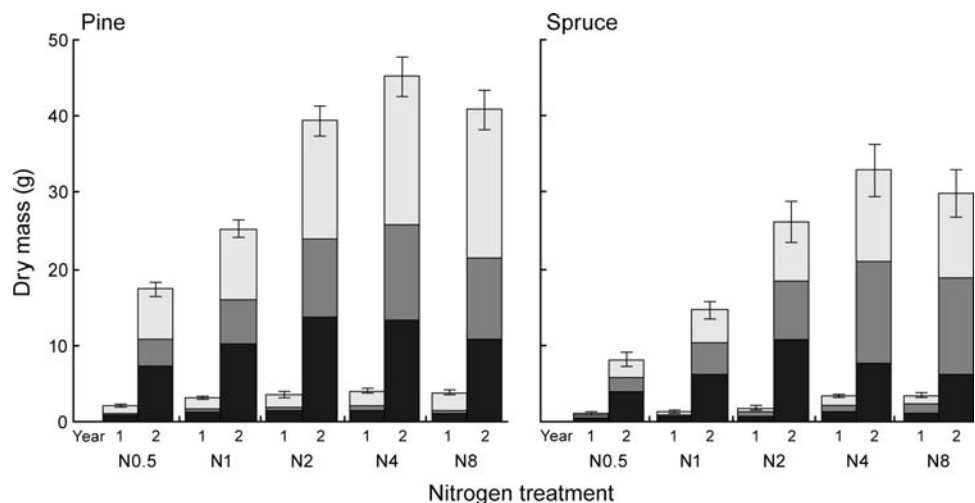
The area of ducts in pine varied between groups ($F_{2,135} = 75.5$, $P < 0.001$) and with N treatment ($F_{4,135} = 3.1$, $P < 0.05$) (Fig. 6b). Duct area was much higher in 2-year-old than in 1-year-old trees and was highest in the N4 treatment, with evidence of a decrease at the

highest N level (N8). In spruce, duct area also varied between groups ($F_{2,134} = 41.7$, $P < 0.001$) and in response to N treatment ($F_{4,134} = 5.9$, $P < 0.001$). In 2-year-old trees, duct area was highest in the N4 treatment and, in contrast to pine, much higher in GI 1 than GI 2. A REML analysis of individual duct area data for GI 1 after the first and second year of growth showed that there was a significant increase in the area of individual ducts from the first to the second year in both pine ($F_{1,88} = 8.8$, $P < 0.01$) and spruce ($F_{1,140} = 77.3$, $P < 0.001$).
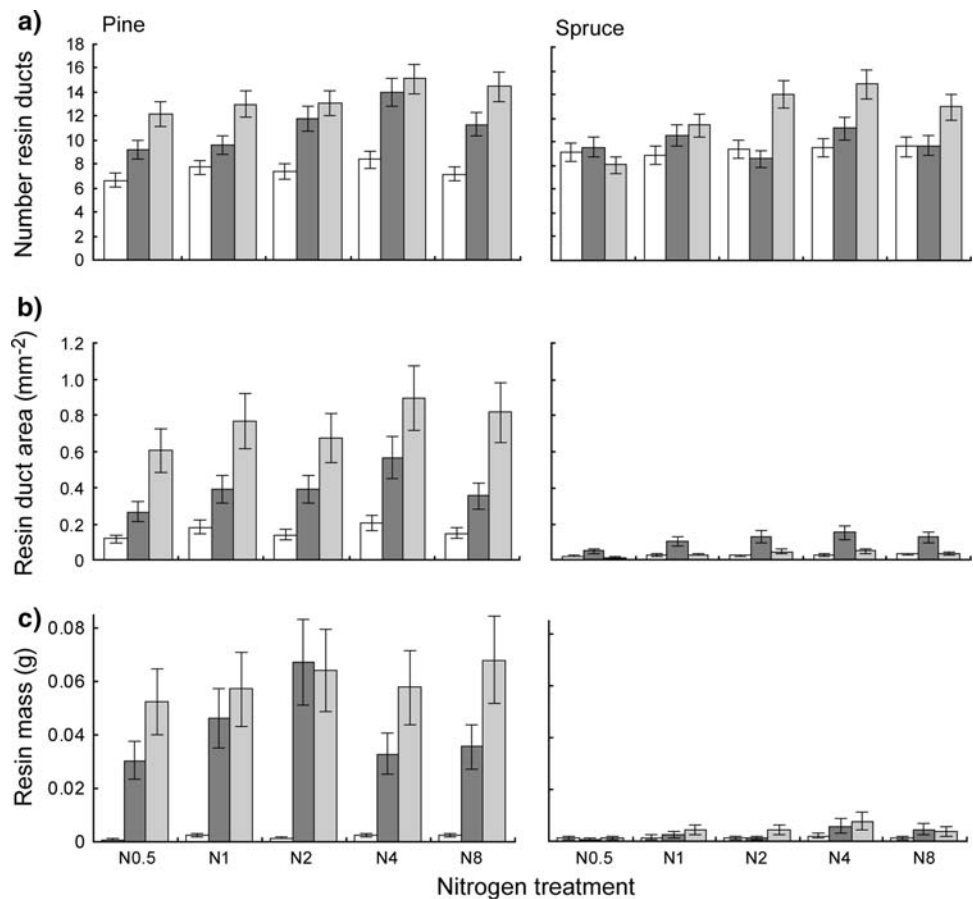
The mass of resin flowing from ducts in pine varied significantly between groups ($F_{2,135} = 329.1$, $P < 0.01$) and in response to treatment ($F_{4,135} = 2.6$, $P < 0.05$), with a significant interaction ($F_{8,135} = 2.0$, $P < 0.05$) that was due to the very small amount of resin from trees in the low N treatment in the first year (Fig. 6c). Resin mass was much higher in 2-year-old than in 1-year-old trees, with evidence, at least for GI 1, of a decrease at the two highest N treatments. In comparison with pine, overall resin mass was very low in spruce, even in 2-year-old trees, but appeared to be highest in the N4 treatment. There were no interaction effects in spruce where resin mass was influenced by significant group ($F_{2,131} = 11.2$, $P < 0.01$) and treatment effects ($F_{4,131} = 6.7$, $P < 0.01$).

The bio-assay data for pine and spruce were grouped separately by GI for analysis. There were significant GI and treatment effects on the L_n ratio in both pine (GI $F_{1,34} = 8.2$, $P < 0.01$, treatment $F_{4,34} = 5.0$, $P < 0.01$) and spruce ($F_{1,40} = 53.1$, $P < 0.001$, and $F_{4,40} = 6.0$, $P < 0.001$, respectively) with no significant interaction (Fig. 7). In pine, the L_n ratio was close to or below zero for both GIs, which were therefore predicted to be resistant. In spruce, both GIs were susceptible (L_n ratio > 0) in all treatments, although susceptibility tended to decrease with increasing N. Growth increment 2 in spruce remained particularly susceptible (Fig. 7).
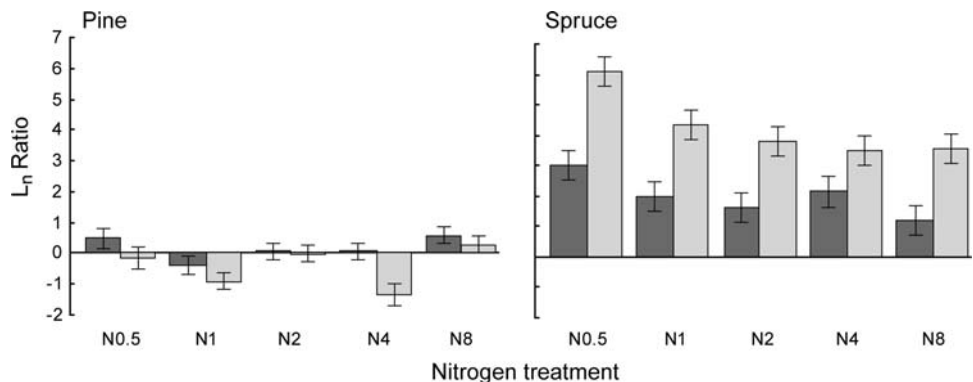
Fig. 5 Dry mass of 1- and 2-year-old pine and spruce in relation to nitrogen fertilisation treatment ($N0.5$, $N1$, $N2$, $N4$, $N8$; in kilogrammes of nitrogen per cubic metre). *Black area of bar* root, *dark-grey (shaded) area of bar* woody shoot (stem, branches and buds), *light-grey (shaded) area of bar* needles . *Vertical line* standard error (SE) for total biomass

Discussion

Resin contained within ducts is an important quantitative defence in many conifers. In some species there is a positive relationship between tree growth and resin duct size (Björkman et al. 1991, 1998; Wainhouse et al. 1998) though the relationship between growth and defence would depend on the extent to which ducts were filled with resin (Wainhouse et al. 1998; Lombardero et al. 2000). In other words, the physical size of ducts may set the upper limit to resin content, but the amount of carbon allocated to resin synthesis may determine a lower limit. In this study on

young pine and spruce, the relationship between tree growth, duct size and the mass of resin that flows from them demonstrates a positive relationship between growth and resin-based defence. The small amount of resin produced during the first year of growth is partly a reflection of the absolute size of ducts in small trees, but at least in pine, these ducts appeared to be only partially filled with resin. This is suggested by the observation that resin mass relative to duct area was much lower in 1-year-old than in 2-year-old pines (Fig. 3). This age difference in resin content was not evident in spruce, and one possible explanation is that there were differences in the internal movement of resin

rather than an actual difference in resin production. In pine, some ducts appear to traverse nodes between GIs (DW, unpublished observations), whereas in spruce, ducts are shorter, often branched, and do not appear to cross nodes. The greater 'connectedness' of the duct system in pine (Lewinsohn et al. 1991; Phillips and Croteau 1999; Wu and Hu 1997) suggests that some resin could flow between GIs. A predominantly downward movement of resin in two-year-old pines should increase flow in the lower GI (GI 1) relative to the upper, current growth (GI 2). In fact, resin mass relative to duct area was similar in both GI 2 and GI 1 (Fig. 3) so that the increased resin in 2-year-old pines was not explained by movement from other parts of the tree.

Allometric relationships provide further insight into the interaction between growth and resin-based defence. In both pine and spruce, there was about a tenfold increase in biomass from the first to the second year of growth, but resin flow from GI 1 in pine increased by about 20-fold, while that from spruce increased only twofold. The leaf area ratio was higher in pine than spruce, but for each species, needle biomass relative to that of the rest of the tree was similar in 1- and 2-year-old trees (Fig. 1b).

Our results therefore provide strong circumstantial evidence that in 1-year-old pines, resin production was limited not by a trade-off with growth or the size of resin ducts but by a generally lower proportional allocation of the carbon budget to resin production in 1-year-old than in 2-year-old trees. Age-related increases in resin production were not evident in spruce, although in this species, differences would be more difficult to detect given that resin flow from 2-year-old spruce was only about 10% of that of pine. These, presumably genetically based, species differences in resin synthesis per unit duct area are likely to reflect the relative importance of resin-based defence in pine and spruce. Certainly in older Sitka spruce, other quantitative defences in bark, such as stone cells, may be as significant a defence against bark beetles and fungi as resin flow (Wainhouse et al. 1990, 1997; Wainhouse and Ashburner 1996).

How the observed age difference in allocation in pine relates to the overall defence 'budget' cannot be assessed directly because resin represents only part, albeit an important one, of the tree's investment in quantitative defence. While an increase in resin synthesis could result in a decrease in other carbon-based defences, it is worth noting that in the 2-year-old trees, the total polyphenol content of bark was about 5% (unpublished), a concentration similar to that in the bark of other young conifers (Wainhouse et al. 2004).

Nitrogen fertilisation clearly modified the levels of resin-based defence in pine and spruce, but its influence on the expression of resistance to *H. abietis* was relatively limited (Fig. 7). This is partly a reflection of the small size of trees and the limited extent to which they responded to nitrogen

fertilisation in the first year when all trees were predicted to be susceptible. During the second year of growth, trees responded strongly to fertilisation. This clearly increased levels of resin-based defence in both species, but effects on resistance expression were dominated by species differences in resources allocated to resin synthesis. As a consequence, most spruce trees remained susceptible despite a general increase in duct size and resin mass in response to treatment, with current growth (GI 2) being particularly vulnerable to weevil feeding (Fig. 7). Most of the pines were, in contrast, relatively resistant, producing much more resin than 1-year-old trees whatever the fertilisation level.

From a practical point of view, measuring duct area or resin mass is destructive, and an indirect method would be needed to predict the relative resistance of trees. The relationship between L_n ratio and stem diameter provides the best estimate of the size of 2-year-old trees likely to be resistant—i.e. diameter of trees when L_n ratio = 0. A significant regression for Sitka spruce ($R^2 = 58.0$, $P < 0.001$) gave a predicted minimum diameter for resistant GIs of 10.3 mm. For Corsican pine, the regression was not significant, but the data suggested that trees with a GI diameter > 7 mm would be relatively resistant.

In summary, the results of this study on 'containerised' resin-based defence in the bark of young pine and spruce emphasise that growth and quantitative defence in very young trees can be positively related. However, a lower proportion of the carbon budget allocated to resin synthesis in 1-year-old than in 2-year-old pines indicates that it can also be 'carbon limited'. The small size of 1-year-old trees and, therefore, resin ducts, and age or species differences in allocation of resources to resin synthesis constrain the expression of resistance to *H. abietis*. The extent to which resistance expression can be manipulated by fertilising trees to increase growth was limited by the relatively small response to fertilisation in the first year. In the second year, most pines were resistant and most spruce susceptible. Species differences in the size and content of resin ducts are assumed to reflect the relative importance of this quantitative defence.

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