

The effect of environmentally induced changes in the bark of young conifers on feeding behaviour and reproductive development of adult *Hylobius abietis* (Coleoptera: Curculionidae)

D. Wainhouse*, J. Staley, J. Johnston and R. Boswell

Forest Research, Alice Holt Lodge, Wrecclesham, Farnham,
Surrey, GU10 4LH, UK

Abstract

Young plants of Sitka spruce, Scots and Corsican pine were subject to high and low light, and high and low nitrogen treatments in a polyhouse experiment. The effect of treatments on resin duct size and nitrogen concentration in stem bark was determined together with feeding by *Hylobius abietis* Linnaeus on the stems of 'intact' plants and on 'detached' stems cut from the plant. Resin duct size was largest on Corsican pine and smallest on Sitka spruce and inherent variation in duct size between the three conifer species appears to determine the pattern of weevil feeding between species. Resin ducts and the flow of resin from them protect the stems of young conifers from weevil feeding not by affecting the total amount of bark eaten but by limiting the depth of feeding and so protecting the inner phloem and cambium. Shallow feeding may increase the likelihood of effective wound repair. Duct size was positively related to plant growth and in particular increased with bark thickness. Overall, ducts were largest in the high light treatment although species differed in their response to the treatment. It is suggested that the effects of plant size, growing conditions and transplantation on susceptibility to attack by *H. abietis*, reported in various studies, may be due to underlying variation in resin duct size or flow rate. The effect on weevils of superficial feeding on stems is to increase the time for reproductive maturation by reducing consumption of the inner bark which has a higher nitrogen content.

Introduction

Environmental conditions that affect the growth of trees can alter the absolute and relative concentration of nutrients and carbon-based secondary chemicals within them and so have the potential to affect their defensive status. The relationship between growth and concentration of secondary chemicals is predicted in broad terms by 'resource-availability' models of defence that are discussed in detail by Herms & Mattson (1992). In essence, they predict that there can be a trade-off in the allocation of carbon to the

carbon-demanding processes of growth and synthesis of secondary chemicals. Thus, when nutrient deficiency or other environmental stresses restrict growth, without affecting photosynthesis, carbohydrates can accumulate and may be utilized in the production of secondary chemicals. When growth is restricted therefore, trees should, in general, be more resistant to attack by herbivores. The main experimental approach to verifying these predictions has usually been to manipulate the availability of light and nitrogen by respectively shading and fertilizing plants (Herms & Mattson, 1992). Although the predicted changes in concentration of secondary chemicals have been observed in a number of studies, it has proved difficult to generalize about their effects on the expression of resistance to particular pests and pathogens. Part of the reason for this is that the

*Fax: 01420 23653

E-mail: david.wainhouse@forestry.gsi.gov.uk

experimental treatments can cause confounding changes in concentrations of nutrients (nitrogen and carbohydrates) as well as secondary chemicals. In addition, there may be changes in physical attributes of the leaves or other plant parts and the significance of these changes for different organisms will depend on their particular adaptations (Smith *et al.*, 1977; DeAngelis *et al.*, 1986; Björkman *et al.*, 1991; Kainulainen *et al.*, 1996; Wainhouse *et al.*, 1998). The manipulation of plant growth to produce a range of phenotypes can, however, be a useful tool in the experimental analysis of polygenically controlled 'quantitative' resistance traits. In the present experiments, this experimental approach was adopted to identify key resistance traits that influence the amount of feeding by the pine weevil, *Hyllobius abietis* Linnaeus (Coleoptera: Curculionidae), on young conifers.

Hyllobius abietis is a major pest of conifer plantations in central and northern Europe where adult weevils feed on bark on the main stem of seedling conifers, often causing extensive mortality on newly planted areas (Leather *et al.*, 1999). Little is known about the expression of resistance to this insect but, from a practical point of view, traits that influence the amount and distribution of feeding are most likely to be of value. Several factors have been reported to influence the amount of feeding, including weevil size (Wainhouse *et al.*, 2004) and reproductive stage (Bylund *et al.*, 2004), plant size (Eidmann, 1969; Selander, 1993; Örlander & Nilsson, 1999; Thorsén *et al.*, 2001), method of nursery production (Selander *et al.*, 1990; Selander, 1993; von Sydow, 1997) and nitrogen concentration (Wainhouse *et al.*, 2004). Whether the different plant-related factors are linked by their common influence on the same resistance trait is unknown. It was hypothesized that quantitative resistance traits are likely to vary between different seedling species and to respond differentially to variation in growth conditions and so provide insights into the genotypic and phenotypic expression of resistance to *H. abietis*. In this paper therefore, the growth of three conifer species was manipulated using light and nitrogen treatments in order to produce a range of phenotypic effects in young conifers. The plants were then bioassayed with adult *H. abietis* and by studying the amount and distribution of weevil feeding damage on the young plants our aim was to obtain evidence for the existence and nature of underlying mechanisms of resistance.

Materials and methods

Origin of young plants and weevils

All plants were sourced from Forestry Commission nurseries and, prior to application of experimental treatments, were kept as follows. One-year-old transplants of Queen Charlotte Island provenance of Sitka spruce *Picea sitchensis* (Bong.) Carr. from Bush nursery, Edinburgh, and Corsican pine *Pinus nigra* ssp. *laricio* (Poirét) Maire from Delamere nursery, Cheshire were lifted in March 1997. After a few weeks storage at 2–3°C they were planted during April into a limed peat mix with no added nutrients in 31 containers and transferred to an unheated polythene covered greenhouse (polyhouse). Containers were supported above the ground on wire mesh to ensure free drainage and 'air-pruning' of roots. Plants were fed weekly during the growing season with a balanced liquid fertilizer (20% N, 7% P,

19% K) with additional watering as necessary. In April 1998, 2-year-old Scots pine *Pinus sylvestris* L. from Wykeham nursery, North Yorkshire were added to the plants in the polyhouse and all plants given experimental treatments during 1998 and 1999.

In 1999, newly emerged male and female *H. abietis* were collected during July–September from emergence traps placed over stumps of Scots pine in Hampshire, southern England. Weevils were stored without food on moist filter paper at 2–3°C for up to 8 weeks during which time they were separated into single-sex groups. Prior to their use in bioassays during October 1999, female weevils were kept individually with male weevils for 24 h at ambient laboratory temperature to allow mating and subsequently held at 2–3°C for 2 days before being weighed. A sample of up to 20 female weevils was dissected to confirm their immature reproductive state (Nordenhem, 1989).

Experimental treatment of plants

Treatments comprised two levels ('high' (h) and 'low' (l)) of light (L) and nitrogen (N) to give four factorial treatment combinations as follows: hLhN, hLiN, iLhN and iLiN. The treatments were arranged within a split-plot design of six blocks with light as the main plot and the two levels of nitrogen as subplots. The basic experimental unit consisted of plots of seven trees of a single species subjected to the same experimental treatment. Trees in low light (iL) were shaded from May 1998 to October 1999 with a proprietary horticultural shade netting (green 'Rokolene') which reduced light levels to about 22% of ambient (ambient = hL) (Wainhouse *et al.*, 1998). The nitrogen was applied at 100 (hN) or 10 (iN) mg l⁻¹ (with other nutrients pro rata in a balanced liquid fertilizer) at a rate of approx 250 ml per container usually applied twice a week from April/May to November in each year. Additional watering was done as necessary. On several occasions plants were sprayed with either non-persistent insecticide (rotenone) (1998) or 'Savona' insecticidal soap (1999) to control aphid infestations.

Treatment effects on plants

Attributes of plants likely to have been affected by the light and nitrogen treatments and which may influence weevil feeding were measured on experimental stems. These included stem diameter, bark thickness and size of resin ducts in bark. Stems that had been used in the bioassays (described in the following section) were cut above or below the area affected by weevil feeding. On these cross sections, bark thickness and stem diameter were measured, and on the first two stems, the size (radial and tangential diameters) of four resin ducts, one chosen at random and the remainder selected at 90° intervals. Mean duct cross-sectional area, calculated as the area of an ellipse, and the total number of ducts was used to determine total duct area across each stem section.

During selection of trees for bioassay (see below), the stems of 6–15 trees of similar size of each species and treatment were taken for analysis of nitrogen concentration and stored at –70°C. Bark was removed from appropriate growth years and samples amalgamated by species and treatment for analysis. Bark was dried at 100°C for 1 h and 70°C for a further 47 h and then ground in a rotor-speed mill (0.5 mm sieve perforations). Sub-samples of ground bark

were digested in sulphuric acid/hydrogen peroxide mixture to produce a clear colourless solution (Wolf, 1982). Nitrogen was determined colorimetrically as ammonia by the reaction with salicylate and dichloroisocyanurate using nitroprusside as catalyst and concentrations expressed as percentage of dry weight.

Bioassay of plants

For Corsican and Scots pine, the 1998 stem growth increment was used for bioassay but in Sitka spruce only the 1997 growth, which had elongated prior to application of experimental treatments, was of sufficient diameter for effective bioassay. Lengths of stem formed prior to the applications of treatments are known to respond in a similar way to those formed during treatment (Wainhouse *et al.*, 1998). Twenty-four hours prior to each bioassay with *H. abietis*, needles were removed from the appropriate main stem increment of the experimental plants. The six experimental blocks were paired at random to provide trees for three replicates of the bioassays. For each species and treatment combination, eight trees were selected at random for bioassay. On four trees, bioassays were done on the whole 'intact' potted plant and on the remaining four, appropriate increments were cut from the stem ('detached' stem). The diameter ranges for the experimental stem lengths were respectively: Sitka spruce (4.6–8.4 mm), Corsican (4.2–9.7 mm) and Scots pine (3.5–8.6 mm). Intact stems were bioassayed in ~5-cm diameter plastic mesh ('Tygan') cages, and detached ones in 12 × 8 × 2 cm ventilated plastic cages. Cages contained a single female weevil, moistened filter paper and either a 3-cm length of stem or 6-cm for stems ≤ 5 mm diam. During bioassays, which were done at ~20°C under artificial lighting (18 h day⁻¹, 'Gro-lux' fluorescent tubes), weevils were moved to new plants or given new stem sections of the same species and treatment at intervals of three days before bark had been fully consumed. The experiment was stopped after each weevil had fed on a total of four stem sections; this corresponded to the start of oviposition by some weevils.

For each stem, the amount of bark (referring collectively to vascular cambium, phloem, cortex and epidermis) eaten by each weevil was measured after removing surface resin as described below. Stems were wrapped in a single layer of transparent polyvinyl chloride film (Clingfilm[®]) and hot air used to shrink the film to achieve a close fit around the stem. Areas of feeding were traced onto the film, distinguishing 'shallow' feeding that occurred only in the outer part of the bark to a depth of up to 50% of bark thickness from 'deep' feeding where bark was consumed to a depth > 50% of bark thickness. Measurements of bark thickness and density were used to determine corresponding volumes and dry mass of bark eaten. Note that estimates of the amount of deep feeding included the 'core' of bark above the area of deep feeding, i.e. including both inner and outer bark, whereas shallow feeding included only outer bark.

Weevils were dissected to measure the size of the largest egg in each of the four ovarioles and to determine whether eggs had been laid as indicated by the presence of a corpus luteum (Nordenhem, 1989).

On intact stems, resin exudation had occurred at most of the feeding sites. To quantify this and facilitate measurement of feeding areas, stem sections were immersed in pentane for 5 or 10 min and pentane then evaporated under nitrogen

and the resin weighed. A correction was made for resin extracted from other parts of the stem, such as the cut ends and needle bases, by similar extraction from adjacent pieces of stem unaffected by weevil feeding. On detached stems only traces of resin were visible that were too small to distinguish from background levels using this method.

Statistical analysis

Data were analysed by CSS (StatSoft, 1995) and Genstat statistical packages (Genstat 5 Committee, 1993) using generalized linear models (GLM). During analysis using GLM, a maximal model including all relevant terms and their interaction was initially fitted. The final model was derived by backwards elimination of clearly non-significant terms. In most cases using a general linear regression model, the inclusion of terms was tested using *F*-tests. For the proportion of females ovipositing, a binomial generalized linear model was used. Resin ducts were measured on only two of the four twigs used in the feeding experiments so only data from two twigs were used in analyses that included resin duct data. When analysing the ratio of deep to shallow feeding a value of 0.0001 (4 twigs) or 0.00001 (2 twigs) was added to a single zero value for shallow feeding.

Results

Treatment effects on plants

Bark thickness was an important variable used in later analyses and so analysis of the effects of species, light and nitrogen treatments on growth was confined to bark thickness because of its importance as an explanatory variable. Stem diameter and bark thickness were, however, significantly related ($R^2 = 0.58$, $P < 0.001$) (fig. 1). Bark thickness was influenced by a significant species × nitrogen interaction ($F_{2,56} 4.54$, $P = 0.014$) and a light × nitrogen interaction ($F_{1,56} 9.08$, $P = 0.004$). For all species, bark thickness was highest in the hLhN treatment.

A similar analysis of treatment effects on total resin duct cross-section area (square root transformed) showed that duct area was determined by a species × light interaction ($F_{2,119} 4.4$, $P < 0.05$), with the effect of light on duct size most

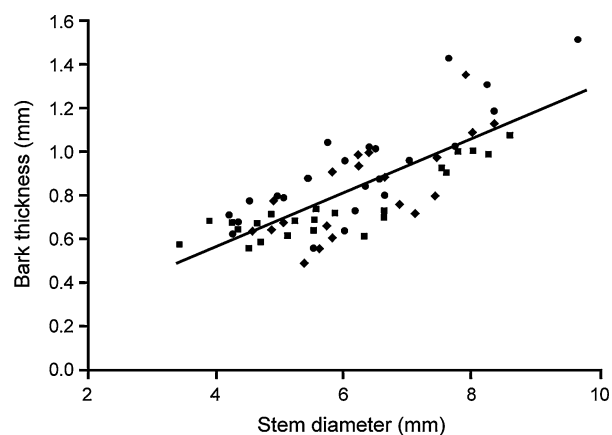


Fig. 1. Relationship between the bark thickness and the diameter of stems ($y = 0.08 + 0.12x$) used in bioassays of feeding by *Hylobius abietis*. ●, Corsican pine; ■, Scots pine; ◆, Sitka spruce.

evident in Corsican pine (fig. 2). The final model accounted for 31.7% of the variance.

The relationship between duct area and bark thickness was analysed by a linear regression model fitted with a common slope but with a separate intercept for each species (fig. 3). The final model, which accounted for 22.3% of the variance, included the terms bark thickness ($F_{1,121}$ 19.8, $P < 0.001$) and species ($F_{2,121}$ 9.4, $P < 0.001$). Resin duct size increased with bark thickness with ducts being largest in Corsican pine and smallest in Sitka spruce.

The nitrogen content of bark, based on a pooled analysis of plant material from several individual plants is shown in fig. 4. For all species, the highest nitrogen concentration occurred in plants from the lLhN treatment in which growth was limited by the low light levels.

Bioassay of plants

The bioassay data were analysed in two stages. The first part of the analysis was based on the designed experiment and used to determine the effects of species, light and nitrogen treatments on weevil feeding and reproduction. In the second stage, bark thickness and resin duct area were examined for their effects on weevil feeding.

The total dry mass of bark eaten by weevils was analysed using general linear regression which included the covariate weevil initial mass and the factors species, light, nitrogen and whether bioassays were on intact or detached stems. None of these factors significantly affected the total amount of bark eaten but some of them did influence the depth of feeding. For analysis of differences in feeding depth, the log ratio of deep (D) to shallow (S) feeding ($\ln(D/S)$) was used. The final model included terms for intact Corsican pine ($F_{1,63}$ 34.1, $P < 0.001$), intact/detached bioassays ($F_{1,63}$ 21.5, $P < 0.001$), light ($F_{1,63}$ 15.2, $P < 0.001$) and nitrogen ($F_{1,63}$ 7.1, $P = 0.01$). The model accounted for 52.4% of the variance. The effect of the light and nitrogen treatments was similar in both detached and intact stems (fig. 5a,b) with deep feeding generally highest in the lLhN treatments and lowest in the hLhN treatment. There was generally less deep feeding by weevils on intact than detached stems but the effect was much more pronounced on Corsican pine than on Scots pine and Sitka spruce (fig. 5c).

Analysis of the amount of bark eaten in relation to plant characteristics was affected by the high degree of correlation

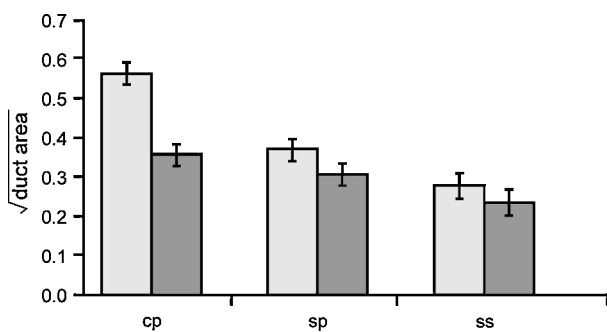


Fig. 2. The effect of light treatments on $\sqrt{\text{resin duct area}}$ in the bark of the main stem of conifers used in bioassays. cp, Corsican pine; sp, Scots pine; ss, Sitka spruce; \square , high light; \blacksquare , low light. Bar ± 1 SE.

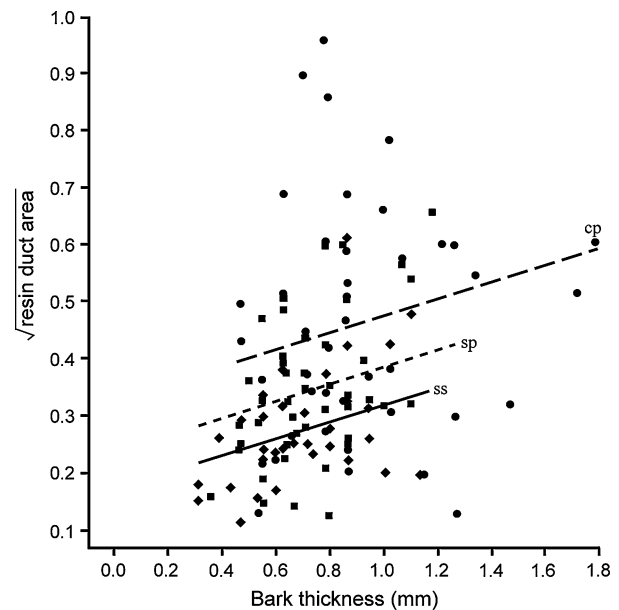


Fig. 3. Relationship between $\sqrt{\text{resin duct area}}$ and bark thickness. A common slope was fitted to the data in a regression model of the form $y = S_i + 0.15x$. \bullet , Corsican pine (cp); \blacksquare , Scots pine (sp); \blacklozenge , Sitka spruce (ss).

between variates such as stem diameter and bark thickness and their relationship with species (fig. 1). A linear regression model of the influence of bark thickness on the amount of bark eaten showed a significant relationship in Sitka spruce (fig. 6a). Since bark thickness could potentially influence depth of feeding, it was used in analyses rather than stem diameter. Analysis of the ratio of deep to shallow feeding showed that there was a similar negative relationship with bark thickness for all three species with a significant difference in the amount of deep feeding on intact and detached stems. The final model included terms for intact/detached bioassays ($F_{1,65}$ 38.7, $P < 0.001$) and bark thickness ($F_{1,65}$ 16.6, $P < 0.001$). A common slope was fitted to the data for intact and detached bioassays (fig. 6b).

The mass of resin extracted from the feeding area on intact stems was not related to the amount of bark eaten but there was, however, a significant relationship with total duct

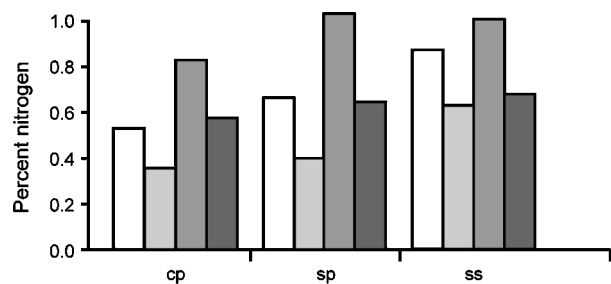


Fig. 4. Concentration of nitrogen in the bark of stems of conifers in the light and nitrogen treatments. cp, Corsican pine; sp, Scots pine; ss, Sitka spruce; \square , high light:high nitrogen; \square , high light:low nitrogen; \blacksquare , low light:high nitrogen; \square , low light:low nitrogen.

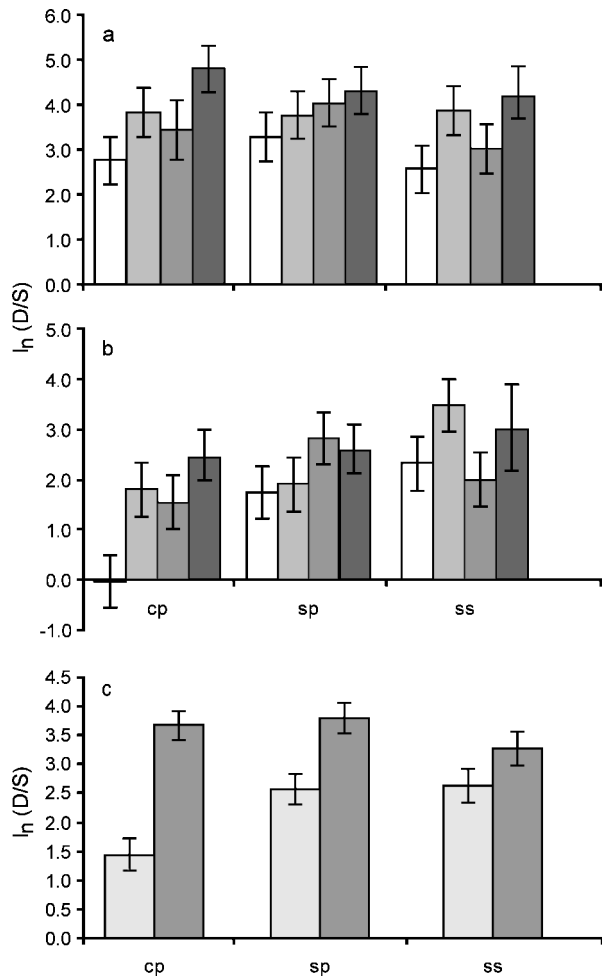


Fig. 5. The effect of light and nitrogen treatments on the ratio of deep to shallow feeding ($\ln(D/S)$) by *Hylobius abietis* on conifers in the detached (a) and intact (b) bioassays (see text). \square , high light:high nitrogen; \square , high light:low nitrogen; \blacksquare , low light:high nitrogen; \blacksquare , low light:low nitrogen. (c) The ratio of deep to shallow feeding on conifers in the intact (\square), and detached (\blacksquare) bioassays. cp, Corsican pine; sp, Scots pine; ss, Sitka spruce. Bar ± 1 SE.

area (square root transformed) on the intact stem (R^2 0.48, $P < 0.01$) (fig. 7). On detached stems so little resin flowed from ducts severed during feeding, that it could not be accurately measured by the gravimetric method used and was nominally set to zero in fig. 7.

The effects of duct area and bioassay method (intact or detached) on the ratio of deep to shallow feeding was analysed by general linear regression. The negative relationship between the ratio of deep to shallow feeding and duct area was similar for all three species, the final model including terms for intact/detached bioassays ($F_{1,65}$ 25.9, $P < 0.001$) and duct area ($F_{1,65}$ 14.6, $P < 0.001$) (fig. 8). The model accounted for 36.5% of the variance.

Examples of deep and shallow feeding on stems of Corsican pine are shown in fig. 9. Resin ducts are visible on the stem on which weevil feeding was only superficial (fig. 9a).

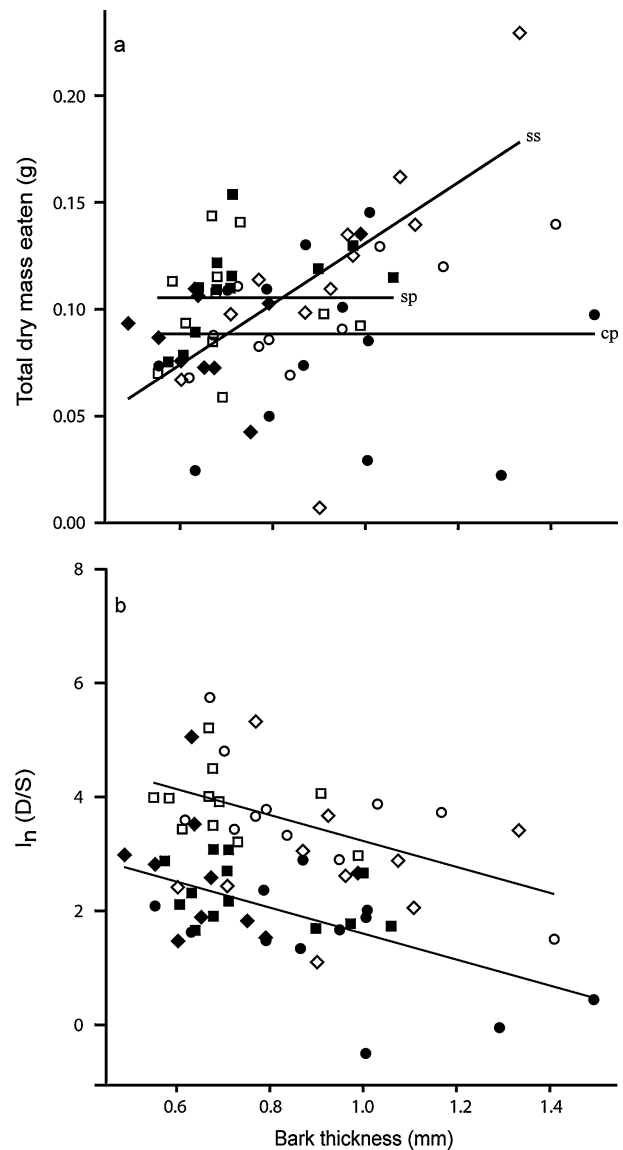


Fig. 6. (a) The relationship between dry mass of bark eaten by *Hylobius abietis* and bark thickness for the different conifer species used in bioassays. There was a significant relationship only for Sitka spruce ($y = -0.0114 + 0.14x$). (b) The relationship between the ratio of deep to shallow feeding ($\ln(D/S)$) and bark thickness. A common slope was fitted to the data for intact (I) and detached stems (D) ($y = ID_i - 2.28x$). There were no significant differences between species. Closed symbols, intact stems; open symbols, detached stems; \bullet , \circ , Corsican pine; \blacksquare , \square , Scots pine; \blacklozenge , \diamond , Sitka spruce.

When weevils were dissected at the end of the bioassay, most were reproductively immature with relatively small developing eggs. Females that were reproductively mature usually contained large eggs with the presence of a corpus luteum indicating previous oviposition (Nordenhem, 1989) (table 1). Oviposition was not inhibited in the current or similar experiments where only young conifers were available to reproductively mature females (Wainhouse *et al.*, 2001), so that the proportion of females ovipositing can be

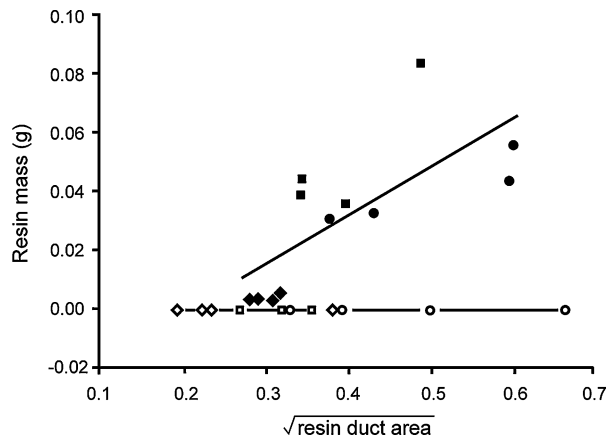


Fig. 7. The relationship between the mass of resin extracted from the feeding area and $\sqrt{\text{resin duct area}}$ on intact stems ($y = -0.034 + 0.164x$). On detached stems, the amount of resin was too small to quantify by the extraction method used and nominally set to zero. Closed symbols, intact stems; open symbols, detached stems; ●○, Corsican pine; ■□, Scots pine; ◆◇, Sitka spruce.

taken as an index of the rate of reproductive development (table 2). An analysis of all treatment effects on the proportion of females ovipositing was not possible because of the low number of egg-laying females in some treatments. Preliminary analysis suggested that the most important factors were nitrogen treatment and whether weevils were feeding on intact or detached stems. These two terms, together with species, were included in the final model (GLM with

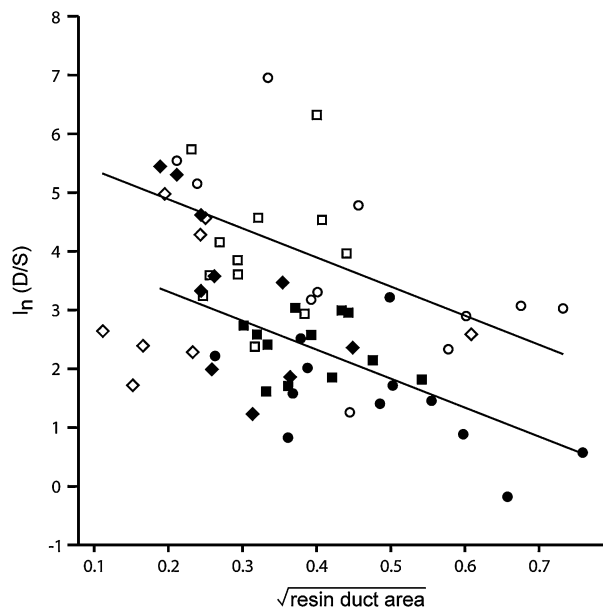


Fig. 8. The relationship between the ratio of deep to shallow feeding ($\ln(D/S)$) by *Hylobius abietis* and $\sqrt{\text{resin duct area}}$. A common slope was fitted to the data for intact (I) and detached (D) stems in a regression model of the form $y = ID_i - 4.95x$. Closed symbols, intact stems; open symbols, detached stems; ●○, Corsican pine; ■□, Scots pine; ◆◇, Sitka spruce.

binomial error distribution) as follows, nitrogen (mean deviance_{1,58} 17.99 $P < 0.001$), intact/detached bioassays (mean deviance_{1,58} 15.66 $P < 0.001$) and species (mean deviance_{2,58} 2.57 $P = 0.077$) (fig. 10). More females laid eggs in the high nitrogen treatments on detached than intact stems.

Discussion

This study provides several lines of evidence that the expression of resistance in the bark of young conifers to feeding by adult *H. abietis* is determined largely by the presence of resin ducts and the flow of resin from them when they are damaged by the weevils. The negative relationship between depth of feeding (strictly $\ln(D/S)$) and duct area suggests that resin ducts protect the stems of young plants not by affecting the total amount of bark eaten but by limiting the depth of feeding and so reduce damage to the inner phloem and cambium. On detached stems, there was more deep feeding than on intact ones, but a similar negative relationship to duct area suggests that the presence of the ducts themselves has some effect on weevil feeding. Supporting evidence for this comes from visual examination of feeding areas which shows that weevils sometimes feed around resin ducts without puncturing them. The importance of resin flow is demonstrated by the effect of detaching stems from the plant. On intact plants, the amount of resin that flows into the area of bark damaged by feeding is proportional to resin duct size but on similar detached plants where there is more deep feeding, resin is virtually absent from the feeding sites. As well as protecting vulnerable parts of the plant, the presence of resin ducts adversely affects weevil reproductive development. The position of resin ducts within the bark allows superficial feeding by weevils, most evident in the intact plants, but this results in a slower rate of reproductive development as indicated by the proportion of females that have oviposited. Given that the rate of reproductive development was fastest in weevils feeding on plants from the high nitrogen treatment (see also Wainhouse *et al.*, 2004), the clear inference is that shallow feeding in intact plants restricts nitrogen intake. As there were no overall differences in the total amount of bark eaten on experimental plants, this suggests that the nitrogen content of inner bark, which incorporates the phloem, is higher than that of outer bark. This was not measured on experimental plants but on similar Corsican pines, the nitrogen concentration in the outer half of bark was found to be ~78% of that of inner bark (unpublished data).

Pines have a better developed and interconnected network of resin ducts than spruce (Lewinsohn *et al.*, 1991; Wu & Hu, 1997; Phillips & Croteau, 1999). The differences in resin duct size observed in the young conifers support these observations and suggest that relative resistance to *H. abietis*, increases in the order, Sitka spruce, Scots and Corsican pine.

Environmental factors strongly influence resin duct size and so have considerable potential to affect relative resistance of the different species. The size of resin ducts was correlated with bark thickness (and therefore stem diameter), demonstrating a positive relationship between plant growth and duct size as reported in other studies (Björkman *et al.*, 1991; Wainhouse *et al.*, 1998). In all three species, resin ducts were larger in the high light treatment but the small response to increased light observed in Sitka spruce may be a reflection of the different growth year studied.

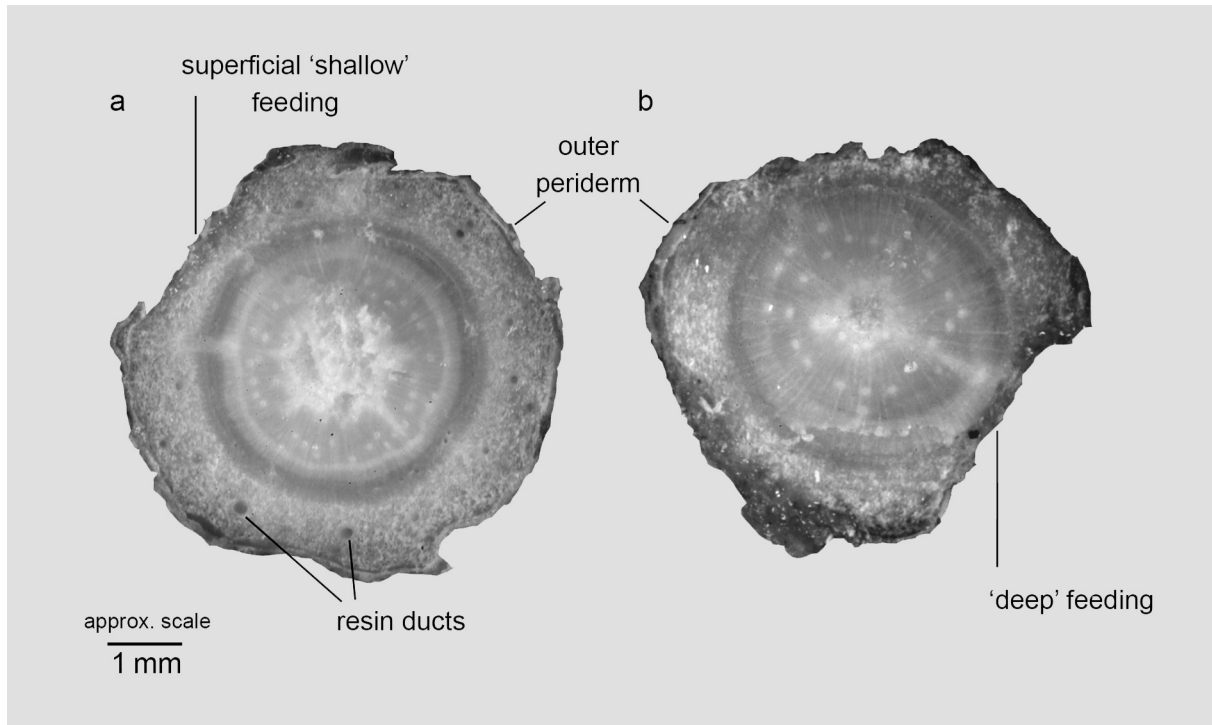


Fig. 9. Cross-sections of the stems of Corsican pine after feeding by *Hylobius abietis*. (a) Superficial feeding on stems on which resin flows from severed resin ducts. (b) Stems with only small resin ducts in the bark and limited resin flow sustain extensive, often deep feeding penetrating to the xylem.

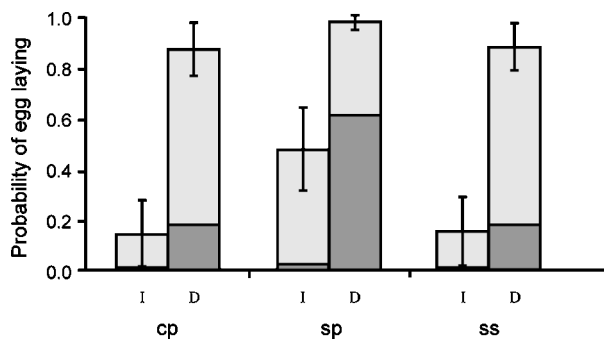


Fig. 10. Predicted probability of female *Hylobius abietis* laying eggs when feeding on the bark of intact and detached conifer stems from the high \square , and low \blacksquare , nitrogen treatments. cp, Corsican pine; sp, Scots pine; ss, Sitka spruce; I, intact stems; D, detached stems. Bar \pm 1 SE.

Evidence of resistance in the field is difficult to discern from the literature. Several studies have related transplant survival to methods of commercial production of seedlings such as whether they are containerized or bare-rooted. In general, mortality caused by *H. abietis* tends to be higher in containerized plants than bare-rooted ones (Selander *et al.*, 1990; Selander, 1993; von Sydow, 1997) and decreases with increasing stem diameter in both spruce (Örlander & Nilsson, 1999; Thorsén *et al.*, 2001) and pine (Selander, 1993). Survival of larger plants appears to be due mainly to the

Table 1. The mean volume of the four largest eggs, one from each ovariole, in female *Hylobius abietis* after feeding in bioassays of intact and detached stems.

Treatment	Corpus luteum	Egg volume mm ³	No. females
Intact	Absent	0.13 \pm 0.034	22
Intact	Present	0.52 \pm 0.035	6
Detached	Absent	0.11 \pm 0.040	13
Detached	Present	0.40 \pm 0.035	20

Table 2. Number of female *Hylobius abietis* ovipositing on the three conifers in the detached and intact bioassays.

Species	Detached stems		Intact stems	
	No. weevils	No. oviposited	No. weevils	No. oviposited
Corsican pine	11	6	11	1
Scots pine	11	9	11	3
Sitka spruce	11	5	8	2

reduced probability of girdling of the stem. While this may be partly attributed to the physical effect of size, the positive relationship between stem diameter and duct size observed in the present study suggests an important role for resistance, with largely superficial feeding on large plants less likely to destroy cambium. Feeding damage in these field studies was usually scored as a proportion of the stem

damaged, with no distinction between superficial and deep feeding, so that variation in the amount of damage on sublethally affected trees cannot be related to possible variation in resistance.

An important question related to between- and within-species variation in plant resistance is whether the effect of resin ducts in restricting some weevil feeding damage to the outer bark increases plant survival. Superficial feeding damage is likely to reduce the risk of girdling, and wound repair by callus formation or development of secondary periderm (Mullick, 1977; Biggs *et al.*, 1984; Biggs, 1985) is likely to be more effective where only a limited amount of feeding damage penetrates to the xylem. There is no direct evidence for this in the present study but in other experiments on young conifers from which the outer stem bark was physically removed, plants were able to repair the damage effectively (J. Johnston, unpublished data). However, additional experiments with insect-induced damage are needed to confirm this.

In summary, resin ducts in the bark of young conifers provide an important defence against *H. abietis* by reducing the amount of deep feeding likely to penetrate to the xylem. It is suggested that shallow feeding increases the likelihood of effective wound repair. Inherent variation in duct size between the three conifer species appears to determine their relative susceptibility. There were also strong environmental effects on resin duct size and, within species, duct size was positively related to growth. It is predicted that the effects of plant size, growing conditions and transplantation on susceptibility to attack by *H. abietis*, reported in various studies, are likely to be due principally to inherent differences in resin duct size between species or to the effects of plant growth and possibly transplantation on duct size and/or resin flow. The effect on weevils of superficial feeding on the stems is to increase the time for reproductive maturation by reducing nitrogen consumption. The removal of comparatively small amounts of bark by these large weevils can be lethal to young unprotected transplants. Their vulnerability, even when exposed to relatively low density populations of weevils, is a key factor in the prophylactic use of insecticide to control this pest. Identifying resistance mechanisms that reduce the amount of feeding and increase the probability of survival of transplants could therefore be of considerable value in integrated pest management, especially since even relatively low levels of resistance can significantly reduce the amount of insecticide needed to protect plants when used in combination with other methods of population reduction (van Emden, 1991). Further studies are needed to determine the importance of the effects described here in determining resistance in the field.

Acknowledgements

The authors thank Nina Morgan for technical assistance and Geoff Morgan for statistical advice. Richard Jinks gave much helpful advice on the experimental manipulation of plants and made perceptive comments on the final draft of the manuscript.

References

- Biggs, A.R. (1985) Suberized boundary zones and the chronology of wound response in tree bark. *Phytopathology* **75**, 1191–1195.
- Biggs, A.R., Merrill, W. & Davis, D.D. (1984) Discussion: response of bark tissues to injury and infection. *Canadian Journal of Forest Research* **14**, 351–356.
- Björkman, C., Larsson, S. & Gref, R. (1991) Effects of nitrogen fertilisation on pine needle chemistry and sawfly performance. *Oecologia* **86**, 202–209.
- Bylund, H., Nordlander, G. & Nordenhem, H. (2004) Feeding and oviposition rates in the pine weevil *Hylobius abietis* (Coleoptera: Curculionidae). *Bulletin of Entomological Research* **94**, 307–317.
- DeAngelis, J.D., Nebeker, T.E. & Hodges, J.D. (1986) Influence of tree age and growth rate on the radial resin duct system in loblolly pine (*Pinus taeda*). *Canadian Journal of Botany* **64**, 1046–1049.
- Eidmann, H.H. (1969) Rüsselkäferschäden an verschiedenen Nahrungspflanzen. *Anzeiger für Schädlingskunde und Pflanzenschutz* **42**, 22–26.
- Genstat 5 Committee of the Statistics Department, Rothamsted Experimental Station (1993) *Genstat 5 Release 3 Reference manual*. Oxford, Clarendon Press.
- Herns, D.A. & Mattson, W.J. (1992) The dilemma of plants: to grow or defend. *Quarterly Review of Biology* **67**, 283–335.
- Kainulainen, P., Holopainen, J., Palomaki, V. & Holopainen, T. (1996) Effects of nitrogen fertilisation on secondary chemistry and ectomycorrhizal state of Scots pine seedlings and on growth of grey pine aphid. *Journal of Chemical Ecology* **22**, 617–636.
- Leather, S.R., Day, K.R. & Salisbury, A.N. (1999) The biology and ecology of the large pine weevil, *Hylobius abietis* (Coleoptera: Curculionidae): a problem of dispersal? *Bulletin of Entomological Research* **89**, 3–16.
- Lewinsohn, E., Gijzen, M. & Croteau, R. (1991) Defense mechanisms of conifers: differences in constitutive and wound-induced monoterpene biosynthesis among species. *Plant Physiology* **96**, 44–49.
- Mullick, D.B. (1977) The non-specific nature of defence in bark and wood during wounding, insect and pathogen attack. *Recent Advances in Phytochemistry* **11**, 395–441.
- Nordenhem, H. (1989) Age, sexual development, and seasonal occurrence of the pine weevil *Hylobius abietis* (L.). *Journal of Applied Entomology* **108**, 260–270.
- Örlander, G. & Nilsson, U. (1999) Effect of reforestation methods on pine weevil (*Hylobius abietis*) damage and seedling survival. *Scandinavian Journal of Forest Research* **14**, 341–354.
- Phillips, M.A. & Croteau, R.B. (1999) Resin-based defences in conifers. *Trends in Plant Science* **4**, 184–190.
- Selander, J. (1993) Survival model for *Pinus sylvestris* seedlings at risk from *Hylobius abietis*. *Scandinavian Journal of Forest Research* **8**, 66–72.
- Selander, J., Immonen, A. & Raukko, P. (1990) Resistance of naturally regenerated and nursery-raised Scots pine seedlings to the large pine weevil, *Hylobius abietis* (Coleoptera, Curculionidae). *Folia Forestalia* **766**, 1–19.
- Smith, C.J., Wellwood, R.W. & Elliott, G.K. (1977) Effects of nitrogen fertilizer and current climate on wood properties of Corsican pine (*Pinus nigra* var *maritima* (Ait.) Melv.). *Forestry* **50**, 117–138.
- Thorsén, Å., Mattsson, S. & Weslien, J. (2001) Influence of stem diameter on the survival and growth of containerised Norway spruce seedlings attacked by pine weevils (*Hylobius* spp.). *Scandinavian Journal of Forest Research* **16**, 54–66.

- van Emden, H.F.** (1991) The role of the host plant in insect pest mis-management. *Bulletin of Entomological Research* **81**, 123–126.
- von Sydow, F.** (1997) Abundance of pine weevils (*Hylobius abietis*) and damage to conifer seedlings in relation to silvicultural practices. *Scandinavian Journal of Forest Research* **12**, 157–167.
- Wainhouse, D., Ashburner, R., Ward, E. & Rose, J.** (1998) The effect of variation in light and nitrogen on growth and defence in young Sitka spruce. *Functional Ecology* **12**, 561–572.
- Wainhouse, D., Ashburner, R. & Boswell, R.** (2001) Reproductive development and maternal effects in the pine weevil *Hylobius abietis*. *Ecological Entomology* **26**, 655–661.
- Wainhouse, D., Boswell, R. & Ashburner, R.** (2004) Maturation feeding and reproductive development in adult pine weevil, *Hylobius abietis* (Coleoptera: Curculionidae). *Bulletin of Entomological Research* **94**, 81–87.
- Wolf, B.** (1982) A comprehensive system of leaf analysis and its use for diagnosing crop nutrient status. *Communications in Soil Science and Plant Analysis* **13**, 1035–1059.
- Wu, H. & Hu, Z.-H.** (1997) Comparative anatomy of resin ducts of the Pinaceae. *Trees* **11**, 135–143.

(Accepted 20 November 2004)
© British Crown Copyright, 2005

