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Costs of herbivore resistance in clonal saplings of *Betula pendula*

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Abstract Several studies have found genetic variation in plant resistance to herbivory. One of the explanations suggested for the observed intermediate levels of resistance are the costs of resistance, i.e., negative genetic correlations between resistance and other fitness components that may constrain the evolution of resistance. We studied the cost of herbivore resistance by investigating the genetic correlations between resistance traits and plant growth traits, and between resistance to insect and mammalian herbivores in cloned saplings of silver birch, *Betula pendula*. We used the performance of a geometrid moth, *Epirrita autumnata*, as an indicator of insect resistance. The numbers of resin droplets at the base and at the tip of the saplings correlate with mammalian resistance, and were thus used here as indicators of vole and hare resistance, respectively. We have previously observed genetic variation in these resistance traits. Further, we examined the correlations between several groups of secondary chemicals and plant growth traits. Finally, to reveal the effect of environmental factors on the trade-offs mentioned above, we investigated the correlations in saplings that were grown at two nutrient lev-

els. We found significant negative correlations between indices of constitutive insect resistance and relative height growth in non-fertilized saplings, indicating cost of constitutive insect resistance. The two groups of secondary chemicals that have been shown to correlate strongly with constitutive insect resistance, i.e., condensed tannins and flavonol glycosides (especially myricetin glycosides), had different genetic correlations with plant traits; the concentration of condensed tannins did not correlate negatively with any of the plant traits, whereas the concentration of flavonol glycosides correlated negatively with plant height. Insect and mammalian resistance did not correlate negatively, indicating no ecological trade-offs.

Keywords Resistance to herbivory · Genetic correlations · Secondary chemicals · *Betula pendula* · *Epirrita autumnata*

Introduction

Since herbivory is detrimental to plant fitness it is reasonable to assume that selection has favored traits that decrease the probability and amount of herbivory (Janzen 1979; Futuyma 1983; Marquis 1992). By decreasing the damage caused by herbivores, resistance traits are beneficial to plants and most likely correlate with plant fitness. However, natural plant populations have genetic variation in resistance, and intermediate levels of resistance have been found in numerous studies (Hanover 1966; Berenbaum et al. 1986; Simms and Rausher 1989; Han and Lincoln 1994; Sagers and Coley 1995; Zangerl and Berenbaum 1997; Mutikainen et al. 2000; Underwood et al. 2000). One possible explanation for the variation observed in resistance lies in the genetic correlation structure: negative genetic correlations between resistance and other fitness components may constrain the evolution of resistance and thus maintain intermediate levels of resistance (Berenbaum et al. 1986; Simms and Rausher 1989; Rausher 1996). Negative correlation be-

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tween resistance and plant fitness, i.e., the costs of resistance, has been a central assumption in recent theories on the evolution of plant resistance (Feeny 1976; Rhoades and Cates 1976; Rhoades 1979; Mooney and Gulmon 1982; Coley et al. 1985). Previous studies on costs of herbivore resistance have obtained variable results. For example, Simms and Rausher (1987, 1989) did not detect any cost of resistance against insect herbivores whereas other workers have found costs in terms of plant fitness (e.g., Berenbaum et al. 1986; Han and Lincoln 1994; Sagers and Coley 1995; Zangerl and Berenbaum 1997; Baldwin 1998; Mauricio 1998).

Most plants are attacked by several herbivores simultaneously (e.g., Strong et al. 1984; Maddox and Root 1987, 1990; Senn et al. 1993; Roche and Fritz 1997). Consequently, some resistance traits may involve ecological trade-offs between resistance against multiple herbivores (Futyuma 1983; Gould 1983; Simms and Rausher 1987, 1989; Rausher 1996). Ecological trade-offs are expressed as negative correlations between traits that indicate resistance against these different herbivores. Again, results from previous studies are variable. In a few studies, increasing resistance to one herbivore decreased resistance against another herbivore species (Da Costa and Jones 1971; Maddox and Root 1990; Van Dam and Hare 1998; Agrawal et al. 1999). In other studies, ecological trade-offs among resistance to several herbivores were not observed (e.g., Fritz and Price 1988; Simms and Rausher 1989; Maddox and Root 1990; Orians and Fritz 1996).

The expression of costs of resistance may be sensitive to environmental conditions (Coley et al. 1985; Bergelson 1994a, 1994b); usually allocation costs are predicted to be higher at low resource availability (Rhoades 1979; Zangerl and Bazzaz 1992; Bergelson and Purrington 1996). However, according to a recent meta-analysis by Koricheva (2002), phenotypic correlations between defence and fitness were negative only at high levels of nutrient availability. Studies on genetic correlations between herbivore resistance and plant fitness addressing the effects of resource levels are scarce (but see Orians and Fritz 1996). Such genotype-by-environment interactions indicate that costs of resistance may constrain the evolution of resistance in some environments but not in others.

We investigated the genetic correlations (1) between resistance traits and plant growth parameters, and (2) between insect and mammalian resistance in saplings of cloned silver birch, *Betula pendula*. We used the performance of an insect herbivore (a geometrid moth, *Epirrita autumnata*), number of resin droplets, and concentrations of secondary chemicals as indices of resistance. Slow relative growth rate indicates poor performance of *E. autumnata* (e.g., Haukioja et al. 1988; Ayres et al. 1987). The numbers of resin droplets at the tip and at the base of the stem of the sapling have previously been observed to correlate with resistance against hares and voles, respectively (Rousi et al. 1991, 1993). In previous studies, we have found significant among-clone variation in the

above-mentioned resistance traits (Keinänen et al. 1999; Mutikainen et al. 2000). Finally, to reveal the effect of environmental factors on the trade-offs mentioned above, we investigated the correlations in saplings that were grown at two nutrient levels.

Materials and methods

Betula pendula

We used commercially available *Betula pendula* plantlets (Hortus-Puutarha, Kaarina, Finland) that were cloned using a micropropagation technique during the spring of 1992. We used 15 clones originating from mother trees and crosses between trees growing in southern and central Finland. A more detailed description of the clones used is presented in Mutikainen et al. (2000). At the beginning of June 1992, the plantlets were individually transplanted to plastic trays into fertilized peat. For the first growing season, the saplings were grown in a greenhouse. On 15 May 1993, after overwintering at -4°C , the still leafless saplings were transplanted to the experimental field in Suonenjoki Research Station. The data for the present study were collected during the third growing season (1994).

Design of the experiment

The experimental field (54 m \times 140 m) was arranged according to a randomized block design and divided into two separate sub-areas; one sub-area was used for measurements of growth and biomass accumulation, and the other for bioassays with *Epirrita autumnata* and analyses of leaf secondary chemistry.

Both sub-areas were divided into ten blocks (9 m \times 24 m), each block thus constitutes one replicate. We planted 240 saplings to each block, i.e., 16 saplings from each of the 15 clones. The 16 saplings from each clone were randomly divided to four sub-groups. Each of the sub-groups was planted together to a randomly selected location within the block, each sapling approximately 1 m apart from other saplings. Within each block, two of the four sub-groups (consisting of the four saplings from the same clone) were randomly assigned to either control treatment (no fertilization) or fertilization treatment. The two remaining sub-groups were used for other purposes. Within each sub-group of the four saplings, one sapling was randomly assigned for the measurements conducted in the third growing season (1994). There were altogether 4,800 saplings in the experimental field.

For the saplings assigned to the fertilization treatment, we applied NPK (18:5:10) fertilizer twice during both growing seasons. The fertilizer was distributed around each sapling; the diameter of the fertilized area was 30 cm. The amount of fertilizer applied corresponds to 12 kg nitrogen/ha per year, 3.3 kg phosphorus/ha per year, and 6.7 kg potassium/ha per year.

Plant height and biomass

The height of the saplings was measured in the beginning and at the end of the 1994 growing season. The relative height growth during the growing season was calculated as $(X_2 - X_1)/X_1$, where X_1 = height at the beginning and X_2 = height at the end of growing season. At the end of the growing season, all above and below-ground biomass of the 600 saplings was collected. The plant parts were oven-dried (80 $^{\circ}\text{C}$, 48 h) and weighed.

Bioassays, resin droplets, and leaf chemistry

For both control and fertilized saplings, we measured the resistance levels of undamaged leaves in terms of the relative growth

rate (RGR) of the fourth-instar larvae of the generalist geometrid moth, *E. autumnata*. Prior to the experiment, the *E. autumnata* larvae were reared solitarily in plastic containers (48 ml) on leaves of mountain birch. The larvae were randomly assigned to the clones and the two fertilization treatments. For the bioassays, we collected two undamaged short-shoot leaves from the top of each sapling at the end of June. In the statistical analyses, the mean of these two leaves was used. Each leaf was placed to a rearing container with one weighed larva. An individual larva was used only once. After 24 h at 13°C, the larvae were weighed again. We calculated the RGR of the larva as weight gained (mg) / initial biomass (mg) per day. The fresh mass of the larva was transformed to dry mass using the equation: dry mass = exp [1.113×ln (fresh mass)–2.079]. We then continued rearing the larvae on the leaves of the same saplings to which they were assigned until they stopped feeding and began to pupate. Two days after pupation, the pupae were weighed. Altogether, we used 600 *E. autumnata* larvae in the bioassay.

We calculated the number of resin droplets with a stereomicroscope using 2–4 fold magnifications from a 1-cm-wide band around the stem between the third and fourth internode from the plant apex, and between the third and fourth internode from the base of the saplings. We collected two short-shoot leaves from the top of the saplings at the end of June for the analysis of secondary chemistry. Prior to the analysis, the leaves were air-dried. For the analyses of leaf chemistry, we used ten clones from eight experimental blocks and thus the clone means are based on eight replicates. We examined the correlations of the concentration of condensed tannins, total non-tannin phenolics, flavonol glycosides, flavone aglycones, and 3,4'-dihydroxypropiophenone 3-glucoside (DHPPG) with plant growth and biomass accumulation. Condensed tannins, total non-tannin phenolics, and flavonol glycosides were chosen because they are often considered to reduce herbivore performance (e.g., Feeny 1976; Scriber and Slansky 1981; Coley 1986; Dudt and Shure 1994; Ayres et al. 1997; Mutikainen et al. 2000). Flavone aglycones and DHPPG were chosen because their concentrations increased due to defoliation treatment (Keinänen et al. 1999). Further, the concentration of DHPPG correlated with the rapid induced resistance (RIR) measured as RGR of *E. autumnata* (Mutikainen et al. 2000). The secondary chemicals were extracted according to Keinänen and Julkunen-Tiitto (1996), and the amounts of non-tannin phenolics and DHPPG were analyzed by HPLC as in Keinänen and Julkunen-Tiitto

(1998). Condensed tannins were analyzed by a vanillin-HCl assay (Julkunen-Tiitto et al. 1996).

Statistical analysis

The relationships between plant biomass and relative height growth, and resistance against *E. autumnata* were analyzed with Pearson correlation coefficients using clone means. All data were normally distributed. The use of clone means allows us to draw conclusions of the evolutionary implications of these correlations (Simms 1992). We calculated the correlations separately for the non-fertilized and fertilized saplings ($n=15$, and for correlations of secondary chemicals $n=10$).

Results

Cost of resistance in terms of plant growth and biomass

In these data, a positive genetic correlation between the indices of constitutive insect resistance and plant growth or biomass indicates cost of resistance, since the higher the RGR or pupal mass of *Epirrita autumnata*, the lower the resistance. Both relative growth rate and pupal mass of *E. autumnata* correlated positively with the relative height growth of *Betula pendula* in the non-fertilized saplings, and pupal mass correlated positively with plant height growth in fertilized saplings (Table 1, Fig. 1).

There was no indication of costs of resistance against mammalian herbivores; instead, in fertilized saplings, both indicators of mammalian resistance (i.e., number of resin droplets) correlated positively with plant height (Table 1, Fig. 2). Further, the indicator of vole resistance, i.e., the number of resin droplets at the base of the sapling, correlated significantly positively with plant height also in non-fertilized saplings (Table 1, Fig. 2).

Table 1 Pearson correlation coefficients among resistance traits and relative height growth, height, and total biomass of *Betula pendula* analysed separately for non-fertilized and fertilized saplings. Number of resin droplets at the base and at the tip of the saplings indicates vole and hare resistance, respectively. Correla-

tions are based on clonal means ($n=15$ for insect and hare resistance and $n=10$ for secondary chemistry). Note that for constitutive insect resistance, positive correlations indicate cost of resistance, i.e., high relative growth rate (RGR) or pupal mass of *Epirrita autumnata* indicate low resistance

| | Non-fertilized | | | Fertilized | | |
|----------------------------|------------------------|--------|---------------|------------------------|--------|---------------|
| | Relative height growth | Height | Total biomass | Relative height growth | Height | Total biomass |
| Insect resistance | | | | | | |
| RGR | 0.58* | 0.18 | 0.09 | 0.06 | 0.15 | -0.02 |
| Pupal mass | 0.55* | 0.15 | 0.15 | 0.06 | 0.54* | 0.21 |
| Resin droplets | | | | | | |
| Vole resistance | 0.40 | 0.52* | 0.21 | 0.34 | 0.56* | 0.15 |
| Hare resistance | 0.29 | 0.36 | 0.49 | 0.31 | 0.52* | 0.13 |
| Secondary chemistry | | | | | | |
| Condensed tannins | -0.22 | -0.03 | -0.12 | 0.19 | -0.14 | -0.41 |
| Total non-tannin phenolics | 0.32 | -0.07 | 0.42 | -0.14 | -0.32 | 0.10 |
| Flavonol glycosides | 0.05 | -0.34 | 0.01 | -0.17 | -0.74* | -0.50 |
| Flavone aglycones | 0.40 | 0.48 | 0.48 | -0.36 | 0.06 | 0.15 |
| DHPPG | 0.22 | 0.45 | 0.62 | -0.78 ** | 0.16 | 0.56 |

*0.01 < P < 0.05, **0.001 < P < 0.01

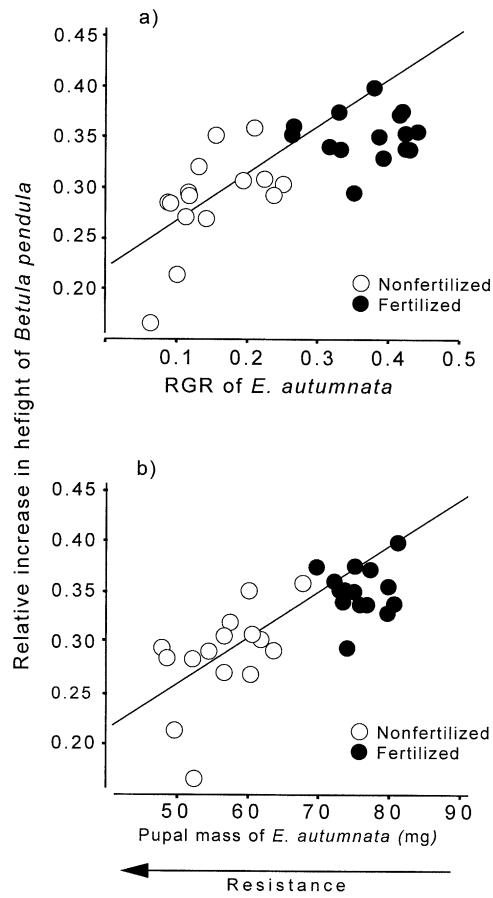


Fig. 1A,B Correlation between constitutive resistance against the autumnal moth (*Epirrita autumnata*) and relative height growth of 15 *Betula pendula* clones. Constitutive resistance was measured as the relative growth rate (RGR, **A**) and pupal mass (**B**) of *E. autumnata*. The line depicts the best fit for the non-fertilized saplings

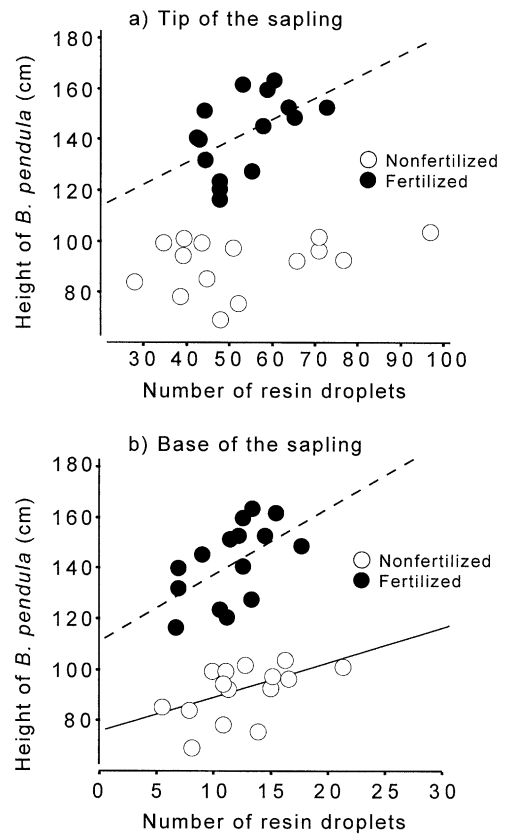


Fig. 2. Correlation between height of *Betula pendula* and number of resin droplets at the tip (**A**) and at the base (**B**) of saplings. Each dot represents one clone. The continuous line depicts the best fit for the non-fertilized saplings and the dashed line for the fertilized saplings

Table 2 Pearson correlation coefficients between indicators of insect and mammalian resistance

| | Non-fertilized | | Fertilized | |
|----------------------------|-----------------|-----------------|-----------------|-----------------|
| | Vole resistance | Hare resistance | Vole resistance | Hare resistance |
| Insect resistance | | | | |
| RGR | 0.14 | -0.00 | 0.04 | 0.22 |
| Pupal mass | -0.03 | 0.08 | 0.37 | 0.17 |
| Hare resistance | 0.42 | | 0.57* | |
| Secondary chemistry | | | | |
| Condensed tannins | 0.03 | -0.02 | 0.37 | 0.29 |
| Total non-tannin phenolics | -0.02 | -0.01 | -0.32 | -0.28 |
| Flavonol glycosides | -0.33 | -0.60 | -0.54 | -0.66* |
| Flavone aglycones | 0.59 | 0.19 | -0.13 | -0.25 |
| DHPPG | 0.71* | 0.66* | -0.35 | 0.11 |

*0.01 < P < 0.05

In fertilized saplings, the concentration of flavonol glycosides correlated negatively with plant height (Table 1, Fig. 3A), and the concentration of DHPPG correlated negatively with relative height growth of *B. pendula* (Table 1, Fig. 3B).

Ecological trade-offs

There were no significant negative genetic correlations among the indicators of insect, hare, and vole resistance (Table 2). The only significant correlation was found in

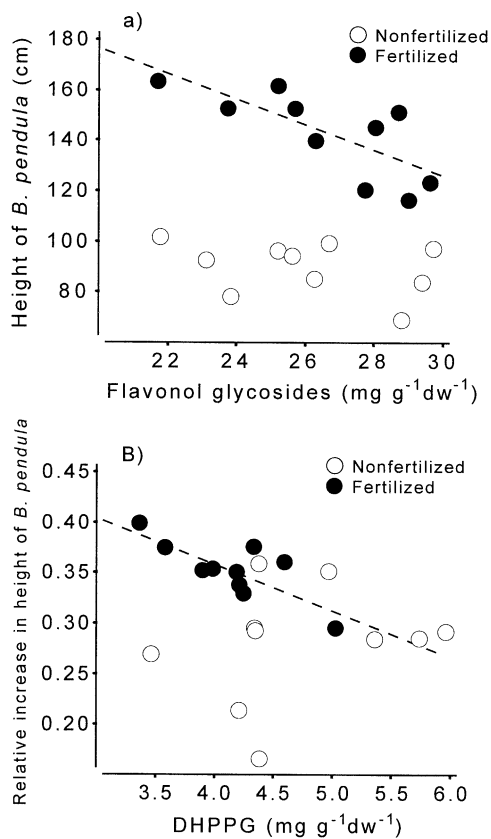


Fig. 3 **A** Correlation between height of *Betula pendula* and concentration of flavonol glycosides. **B** Correlation between relative height growth of *B. pendula* and concentration of DHPPG. Each dot represents one clone. The dashed line depicts the best fit for the fertilized saplings

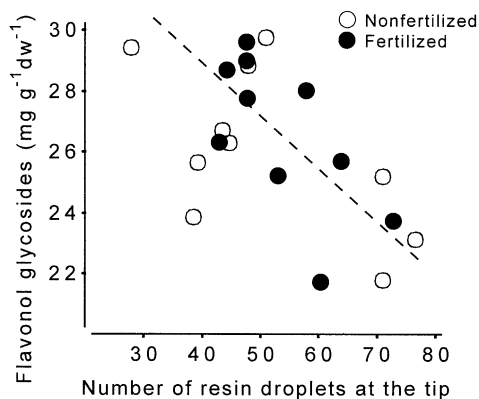


Fig. 4 Correlation between concentration of flavonol glycosides and the number of resin droplets at the tip of *B. pendula* saplings. Each dot represents one clone. The dashed line depicts the best fit for the fertilized saplings

fertilized saplings: the concentration of flavonol glycosides correlated negatively with hare resistance (i.e., number of resin droplets at the tip of the sapling; Table 2, Fig. 4). Further, the two indicators of resistance

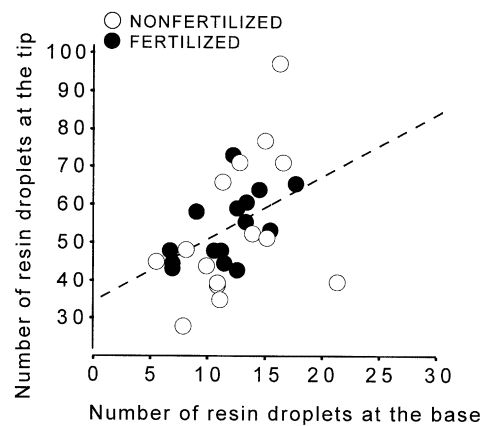


Fig. 5 Correlation between the number of resin droplets at the tip and at the base of *B. pendula* saplings. Each dot represents one clone. The dashed line depicts the best fit for the fertilized saplings

against mammalian herbivores correlated positively in fertilized saplings (Table 2, Fig. 5).

Discussion

The explanation of costs as a factor constraining the evolution of herbivore resistance is based on the premise that resistance is beneficial and ameliorates the negative fitness effects of herbivory. This assumption seems to be true in birch species. In *Betula pubescens* and *B. pendula* herbivore-induced resistance has been shown to decrease the leaf area consumed by herbivores (Hartley and Lawton 1987; Silkstone 1987; for a review see Karban and Baldwin 1997). Further, *B. pendula* saplings that have high levels of resin droplets on their stems are less palatable to voles and hares (Rousi et al. 1991, 1993).

Costs of insect resistance and secondary chemicals

Our results indicate costs of constitutive resistance against an insect herbivore, but only if costs were measured in terms of plant height growth. These results suggest that relative height growth and total biomass may not be correlated, which is the case here ($r=0.30$, $P=0.281$ for non-fertilized and $r=-0.20$, $P=0.478$ for fertilized saplings, $n=15$ clones for both). The lack of a negative genetic correlation between plant biomass accumulation and resistance traits might be explained by the age of the saplings. The saplings used in this experiment were only on their third growing season, and thus the negative effects of higher resistance level on relative height growth might not yet have been reflected in biomass accumulation since the absolute differences are expected to be minor. It is likely that juvenile saplings that have high resistance also end up accumulating less biomass later during their lifespan due to their slower growth rate (Bryant and Julkunen-Tiitto 1995).

Of the several secondary chemicals analyzed, only the production of flavonol glycosides and DHPPG seem to incur costs in terms of plant height or relative height growth, respectively. Interestingly, the concentration of DHPPG was positively correlated with the induced insect resistance in our previous study (Mutikainen et al. 2000) and here it correlated negatively with height growth. Thus, these results also suggest that induced insect resistance may be costly in terms of plant growth. In this case, the induced response is mainly determined by the constitutive level of the particular secondary chemical that causes the induced resistance (Mutikainen et al. 2000). Thus, our results suggest that even the maintenance and construction of the mechanisms of induced resistance against herbivores may be costly. At the mechanistic level, the synthesis, storage, transport, and maintenance of a high constitutive level of the inducible chemical form part of the costs (Gershenson 1994).

The indices based on herbivore performance indicate costs of constitutive insect resistance. Interestingly, the two groups of secondary chemicals that correlated strongly with constitutive insect resistance, i.e., condensed tannins and flavonol glycosides (especially myricetin glycosides; Mutikainen et al. 2000), show different genetic correlations with plant traits; the concentration of flavonol glycosides, contrary to that of condensed tannins, correlated negatively with plant height. Thus the role of any single group of secondary chemicals as an explanation for constitutive resistance is not straightforward. Furthermore, the fact that the negative correlation between insect performance and plant growth traits is not explained by a corresponding correlation between the secondary chemicals and plant growth traits may also indicate that other plant traits than those measured here may play a significant role in determining insect resistance. For example, leaf toughness and plant nutrient concentrations are important determinants of resistance (e.g., Hartley and Jones 1997).

In a recent meta-analysis on fitness costs of herbivore resistance, Koricheva (2002) found that both phenotypic and genetic correlations showed higher costs of resistance when resistance was measured as the inverse of herbivore density or damage than when it was measured in terms of specific secondary chemicals. This seems to be the case in the present study, but only in non-fertilized saplings. In fertilized saplings, the correlations of flavonol glycosides and DHPPG with plant growth traits were stronger than those with herbivore performance. When using insect performance as a measure of resistance we have assumed that this measure correlates negatively with the level of damage the plant experiences. This assumption would not be fulfilled if the insects compensated for low-quality leaves by consuming higher quantities of leaves or by moving to better-quality leaves. Unfortunately, we did not measure food consumption of the *E. autumnata* larvae in this experiment, and thus our results need to be interpreted with some caution.

Mammalian resistance and ecological trade-offs

Contrary to insect resistance, the resistance to the two mammalian herbivores does not seem to incur costs in terms of plant growth or biomass accumulation; actually plants that had a high number of resin droplets also grew taller at the end of the growing season. These results are in accordance with previous results of Rousi et al. (1991, 1993) who found that height at the end the growing season correlated positively with vole resistance in *B. pendula*.

The concentration of flavonol glycosides correlated negatively with resistance to hares. Apart from this correlation, we did not observe any other negative correlations among the indices of resistance to insect and mammalian herbivores. This result is in accordance with the results of a previous study on *B. pendula*, where the palatability of seedlings to voles, hares, and weevils (*Phyllotribus argentatus* and *P. oblongus*) did not have significant correlations (Rousi et al. 1997). In other systems, positive genetic correlations among resistance to several herbivore species are more often observed than negative correlations (for a review, Fritz 1992). In addition, there are several cases where non-significant correlations have been found (Fritz and Price 1988; Simms and Rausher 1989; Maddox and Root 1990). In general, non-significant correlations among resistances to several herbivores suggest that joint selection for increased resistance by several herbivore species is not likely. Thus, the resistance to different herbivores can evolve independently (Fritz 1992).

The independence of insect and mammalian resistance in *B. pendula* is not surprising in the present case, since the mechanisms of resistance are clearly different. The resistance to insect herbivores is mainly determined by factors that affect leaf quality, whereas the vole and hare resistance is mainly based on shoot quality of the saplings, mainly papyriferic acids in resin glands (Reichart et al. 1984) and platyphylloside in shoot tissue (Palo 1987). Correspondingly, it is not surprising that vole and hare resistances correlate positively, since the mechanism is similar. In the case of positive correlations between resistances to two herbivores, resistance should evolve more rapidly since at least some resistance genes confer resistance to both herbivores (Fritz 1992). In these saplings, genotype explained a higher proportion of variation in number of resin droplets at the top of the stem than in the indices of insect resistance (32% vs 9–13%, Mutikainen et al. 2000). This suggests that the broad-sense heritability of vole and hare resistance is higher than that of insect resistance.

Costs of resistance and the maintenance of genetic variation in resistance

In this study, the costs of resistance were highly dependent on the fertilization level of the saplings. Similarly, in *Salix sericea*, the costs of resistance against leaf min-

ers (assessed as genetic correlations) were higher at high nutrient levels (Orians and Fritz 1996). Studies using phenotypic correlations show a similar pattern, i.e., negative correlations are more likely to be detected at high levels of nutrient availability (Koricheva 2002). Taken together, these results thus suggest that plant-environment interactions may be significant both for the existence and assessment of costs of herbivore resistance in plants.

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