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# Multiplicity of biochemical factors determining quality of growing birch leaves

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**Abstract** Due to rapidly changing physical and biochemical characteristics of growing leaves, correlations between traits of foliage biochemistry and the performance indices of flush feeding herbivores may vary considerably following relatively minor changes in experimental conditions. We examined the effects of the seasonal and inter-tree variation of a comprehensive array of biochemical compounds on the success of an early season geometrid, *Epirrita autumnata*, feeding on maturing foliage of mountain birch, *Betula pubescens* ssp. *czerepanovii*. We monitored the concentrations of individual phenolics, sugars, total nitrogen, nitrogen of proteins, and nitrogen of soluble compounds, water and acetone-insoluble residue. Simultaneously we recorded larval consumption, physiological performance, growth, and pupal mass of *E. autumnata*. We found significant phenological changes in almost all leaf traits measured. In bioassays with half-grown leaves, leaf gallotannin concentrations showed a nonlinear effect: in trees with high foliar gallotannin concentrations (over 10 mg $g^{-1}$), physiological performance was strongly reduced by high gallotannin concentrations. In trees with lower gallotannin concentrations, on the other hand, larval growth was reduced by soluble proanthocyanidins, not gallotannins. Differences between high and low gallotannin trees largely depended on phenology, i.e., on the age of leaves. However, not all the differences in leaf traits between late (with high gallotannin concentrations at the time of the bioassay) and early flushing trees disappeared with leaf maturation, indicating that there is also phenology-independent variance in the tree population. In the full-grown leaves of all the study trees, low concentrations of water and of nitrogen of proteins (but not nitrogen of soluble compounds) were the main factors reducing pupal masses of *E. autumnata*, while neither gallotannin nor proanthocyanidins now played a significant role. The observed change in the factors underlying leaf quality (from gallotannins and proanthocyanidins to nitrogen and water) relate to the activity of the shikimate pathway and the formation of cell walls: gallotannins and proanthocyanidins are both produced in the pathway, and these tannins are assumed to contribute – via binding into cell walls – to tough and durable cell walls. Interestingly, low quality of leaves did not automatically translate into low foliar consumption (i.e., benefits to the tree). On the trees with young, high gallotannin leaves, larvae actually increased consumption on low quality foliage. In the group of trees with slightly more developed, low gallotannin leaves, the quality of leaves did not clearly modify amounts consumed. In full-grown leaves, low leaf quality strongly reduced leaf consumption. These results emphasize the strong influence of tree phenology on the relationships between biochemical compounds and the herbivore.

**Key words** Condensed and hydrolyzable tannins · Herbivory · Foraging behavior · Leaf quality · Plant phenology

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## Introduction

The leaf quality of plants for insect herbivores is often characterized by few and simple traits, such as leaf toughness or the concentrations of total foliar phenolics, water and nitrogen. However, especially in deciduous tree species, leaf quality may not be easily explained by any single leaf variable (Haukioja et al. 1978; Ayres and MacLean 1987; Scalbert and Haslam 1987; Rossiter et al. 1988; Matsuki and MacLean 1994; Suomela et al. 1995b). A potential explanation is that the traits con-

tributing to leaf quality vary in both time and space. This is a particularly noteworthy possibility when we try to correlate insect performance with the leaf traits of growing leaves; physical and biochemical characteristics of rapidly growing leaves display drastic seasonal changes (Feeny 1970; Rhoades and Cates 1976; Haukioja et al. 1978; Coleman 1986; Ayres and MacLean 1987; Baldwin et al. 1987; Coley and Barone 1996; Nurmi et al. 1996; Ossipov et al. 1997).

In our study species, the mountain birch, *Betula pubescens* ssp. *czerepanovii* (Orlova) Hämet-Ahti, concentrations of gallotannins (hydrolyzable tannins) decrease while those of proanthocyanidins (condensed tannins) increase during leaf maturation (Ossipov et al. 1997). This bimodality in seasonal patterns may generate different combinations of leaf quality traits with regard to flush-feeding insects. Traits determining the leaf quality of growing leaves are of particular interest; on the one hand many species of herbivores abound, and individuals grow well, on ephemeral young foliage (Feeny 1970; Niemelä and Haukioja 1982; Coley 1983; Raupp and Denno 1983; Price 1991; Coley and Barone 1996; Kaitaniemi et al. 1997), while on the other hand young leaves are assumed to be the most valuable ones for the plant (McKey 1979).

In this paper, we report the results of detailed analyses of biochemical traits contributing to the quality of growing leaves of the mountain birch for the larvae of a flush feeding geometrid, the autumnal moth, *Epirrita autumnata* (Borkhausen). We show the seasonally changing importance of the main nutritive compounds and allelochemicals for leaf quality. In addition to phenological changes in leaf characteristics (Haukioja et al. 1978; Ayres and MacLean 1987; Hanhimäki et al. 1995; Loponen et al. 1997; Nurmi et al. 1996; Ossipov et al. 1997), we also demonstrate large within-population variation in the leaf quality of trees for insect herbivores.

## Methods

### Study organisms

The autumnal moth is a univoltine, polyphagous geometrid whose populations reach outbreak densities in northwest Europe every 9 or 10 years, extensively defoliating mountain birch forests (Tenow 1972; Haukioja et al. 1988; Bylund 1995; Ruohomäki et al. 1997). The adult moths fly in the autumn, the eggs overwinter, and the larvae hatch in the spring simultaneously with the bud break of the mountain birch. In northernmost Europe, the larvae mainly consume the growing foliage of the mountain birch, whose biochemical and physical quality for *E. autumnata* declines rapidly during the larval period (Haukioja et al. 1978; Ayres and MacLean 1987; Hanhimäki et al. 1995; Nurmi et al. 1996; Loponen et al. 1997). Mountain birch forms almost single-species tree-line forests in northern Europe.

### Experimental trees and larvae

The study was conducted at the station of the Kevo Subarctic Research Institute of Turku University, in northern Finland (69°45′N, 27°01′E) in 1996. The mountain birch trees used for the biochemical analyses and bioassays were growing in a natural stand near the station. Since differences in leaf quality among individual mountain birch trees are consistent between successive years (Hanhimäki et al. 1995; Suomela et al. 1995b), large among-tree variation in leaf quality was obtained by choosing for the experiments 26 individual trees known from previous years' determinations to differ in leaf quality for *E. autumnata* (Nurmi et al. 1996; Ossipov et al. 1997).

All experimental larvae were treated identically before and during the experiment. The eggs overwintered in an underground cellar. In the spring the hatched larvae were individually reared in plastic vials outdoors and fed on fresh mountain birch leaves from trees other than those used in the experiments. The vials were randomly placed within and among vial frames. The larvae that were the first to start molting to the 4th-instar were moved to a temperature of 1°C for 1–3 days, until the rest of the larvae reached the same developmental stage. By this treatment we synchronized larval development at the beginning of the bioassay. This treatment is within the natural temperature range in the our subarctic study area, and has no detectable effects on the subsequent growth and development of larvae (M. Ayres, unpublished work).

### Growth experiment

From the beginning of the experiment to pupation, each larva was reared on leaves excised from a single tree. In nature, larvae are likely to spend the whole larval period consuming leaves of a single tree. A total of 28 larvae per tree (each larva from a different brood) were reared on leaves from each of the 26 experimental trees. The larvae were kept singly in 100-ml plastic vials.

To measure pre- and post-ingestive traits and the larval growth rate, a growth experiment was started in the laboratory with newly molted 4th-instar larvae (on 4 July). The larvae were offered leaves (usually three) attached to intact short shoots, picked from all over the tree canopy. The larvae were first weighed to the nearest 0.1 mg, then allowed to feed for 24 h at 12°C, and finally reweighed. The remains of the leaves were collected and pressed after the experiment. The leaf areas consumed were analyzed using an image analysis system (MCID, M4, Imaging Research Inc., Brock University, Ontario, Canada) and transformed into leaf masses consumed by calculating the fresh leaf biomass per area unit ($mg\ mm^{-1}$). For this purpose, five additional leaves per tree were collected, weighed fresh, and the leaf areas measured. For each larva, we recorded the relative consumption rate [RCR = Leaf mass fed × Initial larval $mass^{-1}$ × $Time^{-1}$ ($mg \times mg^{-1} \times day^{-1}$)], the efficiency of conversion of ingested food to larval biomass [ECI = Growth/Leaf mass fed ($mg\ mg^{-1}$)], and the relative growth rate {RGR = [ln(Final mass) − ln(Initial mass)] × $Time^{-1}$ ($mg \times mg^{-1} \times day^{-1}$)}; modified from Waldbauer (1968).

Since foliar biochemistry changes with leaf maturation and larval responses may change simultaneously, we recorded the leaf consumption of the larvae once more on 12 July, when the larvae had arrived at the middle of the last (5th) instar. Each larva was offered an intact short shoot from its experimental tree. The remains of the leaves were collected after 24 h feeding and treated as in the above experiment with the 4th-instar larvae.

Larvae cease feeding when they prepare for pupation. At that time larvae were checked twice a day to detect the cessation of feeding. They were allowed to pupate individually in moist moss. The pupae were weighed and sexed two weeks after pupation. Fresh larval masses (FM) were transformed to dry larval masses according to Neuvonen and Haukioja (1984); statistical analyses based on dry larval and dry leaf masses yielded results similar to those based on fresh masses and are not reported here.

### Leaf traits measured

For each tree, we recorded leaf flush phenology, average leaf area, and concentrations of several biochemical compounds.

In 1996, the development of short-shoot leaves in our study area took approximately 1 month. The buds of the mountain birch opened between 9 and 13 June and the leaves reached full size between 12 and 16 July (I. Saloniemi, unpublished work). The phenology of leaf flush in each of the experimental trees was recorded at the time of bud break, on 12 June, by estimating the ratio between leaf length and bud scale length in 20 buds all over the canopy (see Sulkinoja and Valanne 1987). A high leaf/bud scale length ratio describes advanced leaf growth and early leaf flush.

For the measurement of leaf area, foliar water concentration (on 4 and 12 July), and biochemical analyses (on 5 and 16 July), the leaves were sampled twice, matching the two larval food utilization trials. Leaf area and water concentration were recorded for five leaves collected from each experimental tree. The leaves were weighed fresh, pressed and dried, reweighed, and the leaf areas measured. For the biochemical analysis, a sample of 10–15 short shoot leaves was collected throughout the canopy of each experimental tree. The leaves were clipped (the petioles were cut off) and placed in sealed plastic vials, which were enclosed in an insulated box filled with ice and transported to the laboratory. The leaves were vacuum freeze-dried, homogenized to a powder and stored in plastic vials in the laboratory at −20°C. About 200 mg of the resulting powder was suspended in 10 ml of 70% aqueous acetone, allowed to stand for 1 h at room temperature with continuous stirring and centrifuged at 2500 *g* for 10 min. The pellet was re-extracted twice. The acetone extract was reduced to the aqueous phase by evaporation at room temperature and the resulting aqueous phase was frozen and lyophilized. The residue of extractive compounds was dissolved in 9 ml of water and centrifuged at 3000 *g* for 20 min. The acetone-insoluble residue of birch leaves (cellulose, hemicellulose, pectin, lignin, cell-wall bound phenolics, proteins, and starch) was collected, lyophilized, and weighed.

The following biochemical compounds were analyzed: individual phenolic compounds covering the main groups (such as gallotannins, proanthocyanidins, flavonoids; see Table 1), individual sugars (sucrose, fructose, glucose, galactose, and inositol), acetone-insoluble residue, total nitrogen, nitrogen of proteins and nitrogen of soluble compounds (mainly free amino acids). Soluble (PAS) and cell-wall bound (PAB) proanthocyanidins were analyzed by the method of Terrill et al. (1992), modified and optimized for birch leaf proanthocyanidins (V. Ossipov, S. Ossipova, E. Haukioja, K. Pihlaja, unpublished work). The quantitative analysis of gallic acid in the hydrolysate of extracts was used for the total determination of gallotannins (Ossipov et al. 1997). Low molecular mass phenolics (individual gallotannins, *p*-coumaroylquinic acid derivatives, flavanols, and flavonoid-glycosides) were analyzed by HPLC (Nurmi et al. 1996; Ossipov et al. 1995, 1996). The nitrogen concentrations of the plant samples were determined with a Perkin Elmer 2400 CHNS/O Elemental Analyzer. The dry leaf sample and the acetone-insoluble residue were used for the determination of total nitrogen and nitrogen in insoluble compounds (mainly proteins). Their difference was assumed to represent the nitrogen of soluble compounds (mainly free amino acids). Glucose, fructose, sucrose, galactose, and inositol were quantified using a gas chromatographic method (Kallio et al. 1985).

**Table 1** Concentrations of phenolic groups and individual compounds analyzed

| |
|---|
| Total gallotannins |
| Soluble proanthocyanidins (PAS) |
| Cell-wall bound proanthocyanidins (PAB) |
| *p*-Coumaroylquinic acid derivates: |
| *Trans*-5-caffeoylquinic acid |
| *Trans*-5-*p*-coumaroylquinic acid |
| Chlorogenic acid |
| *Trans*-3-*p*-coumaroylquinic acid |
| *Cis*-3-*p*-coumaroylquinic acid |
| Kaempferol-glycosides: |
| Quercetin-3-*O*-b-D-glucupyranoside |
| Kaempferol-3-*O*-a-L-rhamnopyranoside |
| Kaempferol-glycoside |
| Quercetin-glycosides: |
| Quercetin-3-*O*-b-D-glucuronopyranoside |
| Quercetin-3-*O*-b-D-galactopyranosidede |
| Quercetin-3-*O*-b-D-arabinofuranoside |
| Quercetin-glycoside (two compounds) |
| Quercetin-3-*O*-a-L-(4″-*O*-acetyl)-rhamnopyranoside |
| Myricetin-glycosides: |
| Myricetin-3-*O*-b-D-glucuronopyranoside |
| Myricetin-3-*O*-b -D-galactopyranoside |
| Myricetin-3-*O*-a-L-(acetyl)-rhamnopyranoside |
| Catechins: |
| (+)-Catechin |
| (+)-Epicatechin |

## Statistical analyses

Exploratory data analysis revealed strong nonlinear relationships among the biochemical compounds and the larval traits. For instance, nonlinear regressions (in the form $y = a + b_1x + b_2x^2$) calculated over all the trees between gallotannins and larval ECI ($a = 0.25$, $P = 0.0002$, $b_1 = 0.08$, $P = 0.03$, $b_2 = -0.013$, $P = 0.02$, $r^2 = 20.3$, $n = 26$) and RCR ($a = 2.1$, $P = 0.0001$, $b_1 = -0.27$, $P = 0.10$, $b_2 = 0.05$, $P = 0.067$, $r^2 = 17.8$), and between soluble proanthocyanidins and ECI ($a = 0.55$, $P = 0.0001$, $b_1 = -0.005$, $P = 0.04$, $b_2 = 2.70^{-5}$, $P = 0.06$, $r^2 = 21.3$) and RCR ($a = 0.93$, $P = 0.01$, $b_1 = 0.02$, $P = 0.02$, $b_2 = -0.0002$, $P = 0.02$, $r^2 = 22.3$) revealed that gallotannins and proanthocyanidins had opposite nonlinear effects on the larvae. In particular, low gallotannin concentrations did not predict variation in larval food utilization (Fig. 1). Instead, in trees with high gallotannin concentrations there was a linear negative relationship with the efficiency of conversion of ingested food to larval biomass (i.e., ECI) (Fig. 1). We interpret these results as indicating that gallotannins had a threshold-like effect on larval physiology, and that gallotannins were important only in trees with high gallotannin concentrations. In low gallotannin trees, other compounds presumably contributed to larval performance. Below (in Results) we show that among-tree differences in leaf age explained the nonlinear patterns observed to a considerable extent. Due to this nonlinearity, crude correlations between leaf and larval traits calculated over all trees, which might differ for environmental (e.g., phenological) or genetic reasons, would have been useless. Accordingly, for the first sampling date we conducted separate analyses of the biochemical traits determining the responses of 4th-instar larvae in experimental trees with high gallotannin con-

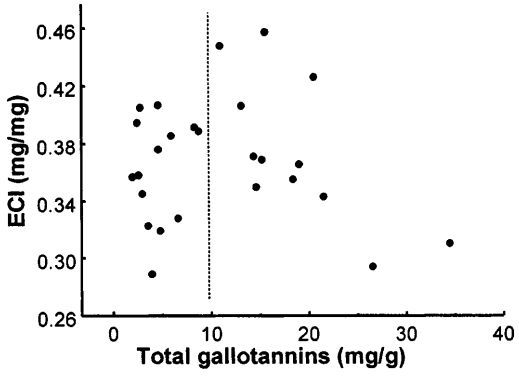


**Fig. 1** Relationship between total concentration of gallotannins and efficiency of conversion of ingested food to larval biomass (ECI). The trees were divided into two groups according to total foliar gallotannin concentrations: higher than 10 mg $g^{-1}$ and lower than 10 mg $g^{-1}$ (indicated by *dashed line*)

centrations (over 10 mg $g^{-1}$, Fig. 1) and those with low concentrations (the rest of the trees).

Before the statistical analyses, we calculated tree-specific means for each larval trait [the effect of larval sex was removed by the LSMEAN option of the GLM procedure (SAS Institute 1990)]. Correlations among the leaf variables (on 5 July) and ECI, RCR, and RGR were calculated separately for high and low gallotannin trees. To compute correlations between leaf traits and the pupal mass of *E. autumnata* and the leaf biomass consumption of the 5th-instar larvae, we first calculated for each foliar trait the tree-specific averages of the two samples (5 and 16 July; Fig. 2). The averaged leaf traits were used to take into account the cumulative effects on the larvae of changing leaf quality. Because of the linear relationships between the averaged leaf traits and pupal mass and 5th-instar consumption, we were now able to use all the trees simultaneously in computing the correlations. To normalize the distributions of the trait values, the concentrations of quercetin-glycosides and kaempferol-glycosides were $\log_e$-transformed and total gallotannins square-root-transformed.

To test for phenology-dependent and -independent between-tree group differences in the leaf traits, we performed two sets of parametric repeated ANOVAs (procedure GLM with type III sum of squares, SAS Institute 1990). The same trees were sampled twice and the sampling date was thus regarded as a within-subject variable. In the first set of ANOVA models the tree grouping based on the early concentration of gallotannins (trees with either high or low concentrations, see Fig. 1) and the sampling date were included and regarded as fixed independent effects. Hence the mean squares of the error variation were used as error terms (Zar 1984). In the second set of ANOVAs the same models were analyzed with data from which the influence of leaf flush phenology was removed by running a GLM model for each leaf trait with leaf/bud scale length ratio as a covariate, and by using the residuals of the models in the repeated ANOVAs. A phenology-dependent difference between the tree groups in a leaf trait is indicated when a statistically significant group main effect and/or a group-by-time interaction in the first ANOVA becomes non-significant in the second ANOVA (eliminating the effect of phenology). A consistent difference, independent of leaf phenology, is indicated in the second ANOVA by a significant main group effect and/or a significant group-by-time interaction. To meet the assumptions of parametric analyses, the leaf areas and concentrations of quercetin-glycosides, kaempferol-glycosides, sucrose and galactose were $\log_e$-transformed and concentrations of total gallotannins, *p*-coumaroylquinic acid derivates, catechin-based flavanoids, and all nitrogen measures were square-root-transformed. To avoid pseudoreplication in the analyses, we used the tree-specific means of leaf area and foliar water concentration. All figures are based on non-transformed data.

## Results

### Leaf phenology and intrapopulation variation of trees

The foliar concentrations of practically all biochemical traits changed strongly with leaf development, as indicated by the significant time effects in the ANOVA models with the raw data (Table 2). Minor differences in leaf age had a significant effect on leaf traits, as demonstrated by the analysis of trees sampled at the same time (5 July): the leaf length/bud length ratio (low values indicate young leaves) at bud burst (12 June) correlated negatively with foliar concentrations of *p*-coumaroylquinic acid derivates, kaempferol-glycosides, nitrogen of proteins, and water (all $r$ from −0.40 to −0.47, $P = 0.05$–$0.015$, $n = 26$). In addition, correlations with the concentrations of glucose, fructose, and galactose were positive (all $r = 0.44$–$0.57$, $P = 0.02$–$0.002$).

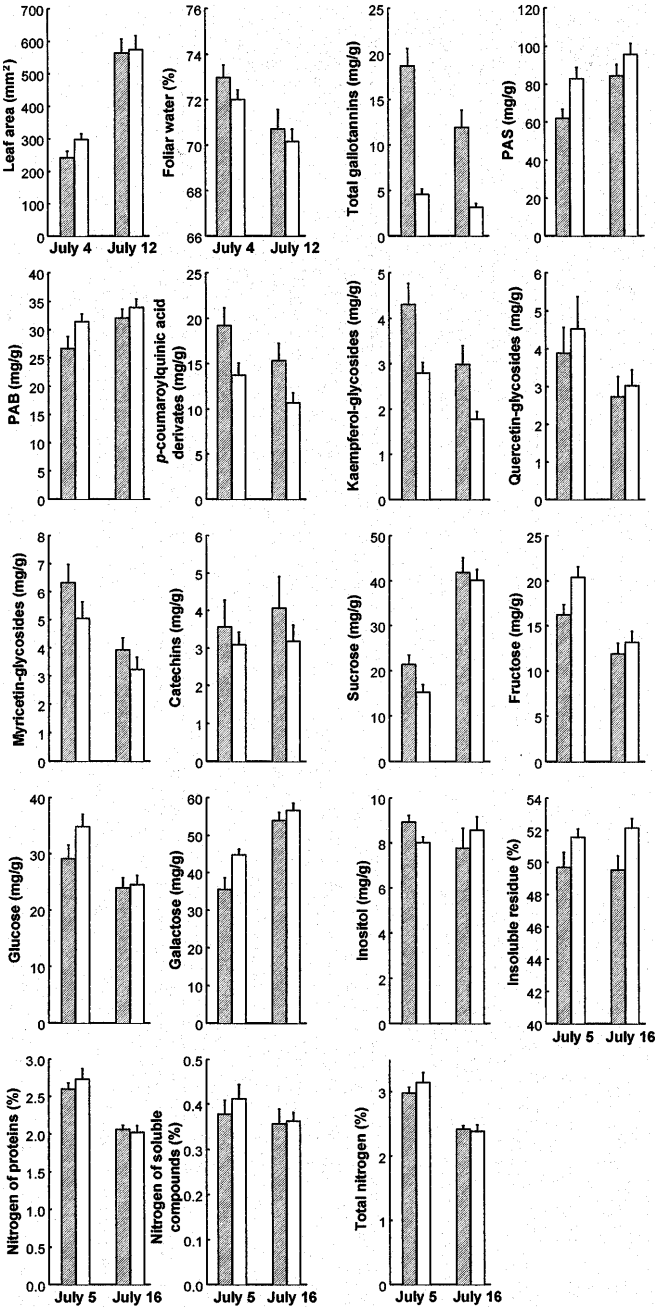


**Fig. 2** Foliar characteristics of groups of trees with early and late leaf flush during the two leaf samplings. Means and SEs are given [*filled bar* tree group with young leaves and initially high gallotannin concentrations (YHG trees, $n = 12$), *open bar* tree group with more mature leaves and low gallotannin concentrations (MLG trees, $n = 14$)]

Although the concentrations of total gallotannins, which were used as the criterion for dividing the trees into the two groups during the early leaf sampling, displayed only a nearly significant correlation with the leaf/bud length ratio ($r = -0.35$, $P = 0.08$), we next checked whether the differences between trees with high and low gallotannin concentrations could be explained by their phenology alone. Trees with high concentrations of

**Table 2** Results of repeated ANOVAs for differences in leaf traits between tree groups (early leafing vs. late leafing) and sampling dates

| Leaf variable | Source | *df* | MS | *F* | *P* |
|---|---|---|---|---|---|
| Leaf area | Group | 1 | 0.1896 | 2.11 | 0.16 |
| | Error (Between) | 24 | 0.0898 | | |
| | Time | 1 | 7.2745 | 235.51 | 0.0001 |
| | Time × Group | 1 | 0.1436 | 4.65 | 0.04 |
| | Error (Within) | 24 | 0.0309 | | |
| Water percent | Group | 1 | 7.3325 | 0.95 | 0.34 |
| | Error (Between) | 24 | 7.7489 | | |
| | Time | 1 | 55.06 | 39.77 | 0.0001 |
| | Time × Group | 1 | 0.5853 | 0.42 | 0.52 |
| | Error (Within) | 24 | 1.3846 | | |
| Total gallotannins | Group | 1 | 46.56 | 66.16 | 0.0001 |
| | Error (Between) | 24 | 0.7038 | | |
| | Time | 1 | 5.1241 | 87.99 | 0.0001 |
| | Time × Group | 1 | 1.0488 | 18.01 | 0.0003 |
| | Error (Within) | 24 | 0.0582 | | |
| PAS | Group | 1 | 10.61 | 5.10 | 0.03 |
| | Error (Between) | 24 | 2.0821 | | |
| | Time | 1 | 13.36 | 26.40 | 0.0001 |
| | Time × Group | 1 | 1.2842 | 2.54 | 0.12 |
| | Error (Within) | 24 | 0.5061 | | |
| PAB | Group | 1 | 140.43 | 2.67 | 0.12 |
| | Error (Between) | 24 | 52.51 | | |
| | Time | 1 | 200.50 | 14.74 | 0.0008 |
| | Time × Group | 1 | 26.89 | 1.98 | 0.17 |
| | Error (Within) | 24 | 13.61 | | |
| p-Coumaroylquinic acid derivates | Group | 1 | 5.3891 | 5.45 | 0.028 |
| | Error (Between) | 24 | 0.9897 | | |
| | Time | 1 | 2.5942 | 26.43 | 0.0001 |
| | Time × Group | 1 | 0.0131 | 0.13 | 0.72 |
| | Error (Within) | 24 | 0.0981 | | |
| Kaempferol-glycosides | Group | 1 | 2.6221 | 10.99 | 0.0029 |
| | Error (Between) | 24 | 0.2386 | | |
| | Time | 1 | 2.3158 | 141.67 | 0.0001 |
| | Time × Group | 1 | 0.0187 | 1.14 | 0.30 |
| | Error (Within) | 24 | 0.0163 | | |
| Quercetin-glycosides | Group | 1 | 0.2418 | 0.36 | 0.55 |
| | Error (Between) | 24 | 0.6709 | | |
| | Time | 1 | 1.5435 | 13.02 | 0.0014 |
| | Time × Group | 1 | 0.0059 | 0.05 | 0.83 |
| | Error (Within) | 24 | 0.1186 | | |
| Myricetin-glycosides | Group | 1 | 12.63 | 1.96 | 0.17 |
| | Error (Between) | 24 | 6.4388 | | |
| | Time | 1 | 57.19 | 78.98 | 0.0001 |
| | Time × Group | 1 | 1.1005 | 1.52 | 0.23 |
| | Error (Within) | 24 | 0.7240 | | |
| Catechins | Group | 1 | 0.2782 | 0.98 | 0.33 |
| | Error (Between) | 24 | 0.2837 | | |
| | Time | 1 | 0.0596 | 0.33 | 0.57 |
| | Time × Group | 1 | 0.0327 | 0.18 | 0.67 |
| | Error (Within) | 24 | 0.1813 | | |
| Sucrose | Group | 1 | 0.4894 | 3.54 | 0.07 |
| | Error (Between) | 24 | 0.1382 | | |
| | Time | 1 | 9.2587 | 238.38 | 0.0001 |
| | Time × Group | 1 | 0.3517 | 9.06 | 0.0061 |
| | Error (Within) | 24 | 0.0388 | | |
| Fructose | Group | 1 | 97.87 | 3.30 | 0.08 |
| | Error (Between) | 24 | 29.63 | | |
| | Time | 1 | 429.97 | 61.88 | 0.0001 |
| | Time × Group | 1 | 27.68 | 3.98 | 0.057 |
| | Error (Within) | 24 | 6.9489 | | |
| Glucose | Group | 1 | 129.45 | 1.75 | 0.20 |
| | Error (Between) | 24 | 74.16 | | |
| | Time | 1 | 783.07 | 25.08 | 0.0001 |
| | Time × Group | 1 | 89.16 | 2.86 | 0.10 |
| | Error (Within) | 24 | 31.22 | | |
| Galactose | Group | 1 | 3.0166 | 6.75 | 0.015 |
| | Error (Between) | 24 | 0.4470 | | |

**Table 2** contd.

| Leaf variable | Source | *df* | MS | *F* | *P* |
|---|---|---|---|---|---|
| | Time | 1 | 16.93 | 56.76 | 0.0001 |
| | Time × Group | 1 | 1.1682 | 3.92 | 0.059 |
| | Error (Within) | 24 | 0.2983 | | |
| Inositol | Group | 1 | 0.0334 | 0.04 | 0.85 |
| | Error (Between) | 24 | 0.9512 | | |
| | Time | 1 | 1.1890 | 1.01 | 0.32 |
| | Time × Group | 1 | 9.6975 | 8.26 | 0.0084 |
| | Error (Within) | 24 | 1.1747 | | |
| Acetone-insoluble residue | Group | 1 | 66.72 | 6.07 | 0.02 |
| | Error (Between) | 24 | 10.99 | | |
| | Time | 1 | 0.4676 | 0.17 | 0.68 |
| | Time × Group | 1 | 1.7277 | 0.62 | 0.44 |
| | Error (Within) | 24 | 2.7705 | | |
| Nitrogen of insoluble compounds (protein) | Group | 1 | 0.0019 | 0.10 | 0.76 |
| | Error (Between) | 24 | 0.0190 | | |
| | Time | 1 | 0.5261 | 252.44 | 0.0001 |
| | Time × Group | 1 | 0.0086 | 4.15 | 0.052 |
| | Error (Within) | 24 | 0.0021 | | |
| Nitrogen of soluble compounds | Group | 1 | 0.0048 | 0.54 | 0.47 |
| | Error (Between) | 24 | 0.0088 | | |
| | Time | 1 | 0.0099 | 1.71 | 0.20 |
| | Time × Group | 1 | 0.0011 | 0.20 | 0.66 |
| | Error (Within) | 24 | 0.0058 | | |
| Total nitrogen | Group | 1 | 0.0035 | 0.17 | 0.68 |
| | Error (Between) | 24 | 0.0206 | | |
| | Time | 1 | 0.5047 | 259.93 | 0.0001 |
| | Time × Group | 1 | 0.0103 | 5.31 | 0.03 |
| | Error (Within) | 24 | 0.0019 | | |

foliar gallotannins on 5 July had a marginally later leaf flush (and therefore younger leaves in July) than the rest of the trees: on 12 June their leaf/bud scale length ratios were almost significantly lower than those found in trees with low gallotannin concentrations (mean ratio: 1.94 ± 0.19 (SE) and 2.42 ± 0.14 in the high and low gallotannin trees, respectively; one-way parametric ANOVA: $F_{1,24} = 3.98$, $P = 0.058$). Importantly, the leaves of the high gallotannin trees displayed delayed growth compared to those of the low gallotannin trees; at the time of our first leaf area recording on 4 July, the former had smaller leaves than the latter. This difference disappeared when the leaves achieved their final size (second sampling date, 12 July), as indicated by the significant group-by-time interaction in leaf area (Table 2, Fig. 2). Accordingly, in the following we refer to the two groups of birches as "trees with young leaves and high gallotannin concentrations" (YHG) and "trees with more mature leaves and low gallotannin concentrations" (MLG).

The YHG and MLG groups of trees displayed phenology-dependent differences in *p*-coumaroylquinic acid derivates, fructose, galactose, acetone-insoluble residue, and measures of nitrogen. This was indicated by the loss of statistical significance: effects which were significant in the ANOVAs with the raw data (Table 2) became non-significant when the effect of phenology was removed from the data (main effects: *p*-coumaroylquinic acid: $F_{1,24} = 2.34$, $P = 0.14$, galactose: $F_{1,24} = 2.79$, $P = 0.11$, insoluble residue: $F_{1,24} = 2.89$, $P = 0.11$ and group-by-time interaction effects: fructose: $F_{1,24} = 2.07$, $P = 0.16$, galactose: $F_{1,24} = 1.13$, $P = 0.30$, nitrogen of proteins: $F_{1,24} = 1.23$, $P = 0.28$, total nitrogen: $F_{1,24} = 2.14$, $P = 0.16$).

However, phenology alone did not totally explain the differences between the two tree groups, indicating an innate heterogeneity in the biochemical traits of the tree population during the early season. This was displayed in the ANOVA models from which the influence of leaf flush phenology was removed, by the significant tree group effects in the concentrations of soluble proanthocyanidins ($F_{1,24} = 4.62$, $P = 0.04$), kaempferol-glycosides ($F_{1,24} = 5.58$, $P = 0.03$), and by the significant group-by-time interactions in gallotannins ($F_{1,24} = 12.74$, $P = 0.0015$), sucrose ($F_{1,24} = 9.82$, $P = 0.0045$), and inositol ($F_{1,24} = 4.19$, $P = 0.05$). Accordingly, at the time of the second sampling date the late-flushing YHG trees had not reached the trait values characterizing the MLG trees, in spite of the ample accumulation of degree-days (degree-days over 2°C, dd2) between the first sampling (265 dd2) and the second one (357 dd2).

## Biochemical traits underlying leaf quality

In the YHG trees, high gallotannin and low inositol concentrations and low acetone-insoluble residue ex-

plained the reduced physiological ability of larvae to process ingested biomass (ECI) in the first leaf sampling (5 July) (Table 3). Acetone-insoluble residue largely consists of cell wall polysaccharides and displayed a strong negative correlation with gallotannins ($r = -0.69$, $P = 0.01$, $n = 12$), again implying that the trees with the highest gallotannin concentrations had the least developed cell walls (Ossipov et al. 1997). Interestingly, the larvae compensated for the reduced ECI in the low-quality YHG trees by compensatory consumption, as shown by the significant positive correlation between gallotannin concentration and larval RCR in these trees (Table 3). As a consequence, in the YHG trees low ECI led to increased leaf consumption.

In the group of MLG trees (with more advanced, low gallotannin leaves on 5 July), gallotannins did not determine leaf quality. Instead, high concentrations of soluble proanthocyanidins predicted low leaf quality (Table 4). Interestingly, on MLG trees the larvae did not display effective compensatory feeding: both larval ECI and RCR were weakly reduced by high concentrations of soluble proanthocyanidins, thus contributing to a marginally significant reduction in RGR (Table 4). However, high concentrations of myricetin-glycosides (a group of flavanoids) resulted in a low consumption rate. Altogether, unlike the YHG trees, the level of RGR in the MLG trees was not directly connected to the level of leaf damage experience by the trees.

**Table 3** Correlations among leaf and larval traits for trees with young leaves and high gallotannin concentrations (5 July; $n = 12$) (*RCR* relative consumption rate, *ECI* efficiency of conversion of ingested food, *RGR* relative growth rate)

| | RCR | | ECI | | RGR | |
|---|---|---|---|---|---|---|
| | *r* | *P* | *r* | *P* | *r* | *P* |
| Total gallotannins | **0.66** | **0.02** | **−0.69** | **0.01** | −0.52 | 0.08 |
| Soluble proanthocyanids | 0.34 | 0.29 | −0.41 | 0.19 | −0.21 | 0.52 |
| Cell-wall bound proanthocyanids | −0.05 | 0.87 | −0.13 | 0.69 | −0.16 | 0.61 |
| *p*-Coumaroylquinic acid derivates | 0.21 | 0.51 | −0.25 | 0.43 | −0.39 | 0.20 |
| Kaempferol-glycosides | −0.05 | 0.88 | 0.12 | 0.71 | −0.11 | 0.74 |
| Quercetin-glycosides | 0.13 | 0.70 | −0.11 | 0.74 | 0.31 | 0.32 |
| Myricetin-glycosides | 0.06 | 0.84 | −0.01 | 0.98 | −0.15 | 0.63 |
| Catechins | 0.30 | 0.34 | −0.35 | 0.26 | 0.08 | 0.80 |
| Sucrose | −0.34 | 0.28 | 0.33 | 0.30 | 0.13 | 0.70 |
| Fructose | 0.24 | 0.45 | −0.19 | 0.56 | 0.31 | 0.33 |
| Glucose | 0.46 | 0.13 | −0.38 | 0.23 | 0.42 | 0.18 |
| Galactose | 0.36 | 0.25 | −0.36 | 0.25 | 0.25 | 0.43 |
| Inositol | −0.56 | 0.06 | **0.59** | **0.04** | 0.31 | 0.33 |
| Acetone-insoluble residue | **−0.69** | **0.01** | **0.68** | **0.01** | 0.14 | 0.66 |
| Water percent | −0.31 | 0.33 | 0.43 | 0.16 | −0.11 | 0.75 |
| Nitrogen of insoluble compounds (protein) | −0.37 | 0.23 | 0.47 | 0.12 | −0.03 | 0.93 |
| Nitrogen of soluble compounds | −0.39 | 0.21 | 0.37 | 0.24 | −0.12 | 0.71 |
| Total nitrogen | −0.41 | 0.19 | 0.50 | 0.10 | −0.05 | 0.88 |

**Table 4** Correlations among leaf and larval traits for trees with developed leaves and low gallotannin concentrations (5 July; $n = 14$)

| | RCR | | ECI | | RGR | |
|---|---|---|---|---|---|---|
| | *r* | *P* | *r* | *P* | *r* | *P* |
| Total gallotannins | 0.06 | 0.84 | 0.13 | 0.67 | 0.14 | 0.63 |
| Soluble proanthocyanids | −0.25 | 0.39 | −0.14 | 0.64 | **−0.52** | **0.056** |
| Cell-wall bound proanthocyanids | −0.12 | 0.68 | −0.04 | 0.90 | −0.20 | 0.50 |
| *p*-Coumaroylquinic acid derivates | 0.04 | 0.88 | 0.20 | 0.50 | 0.17 | 0.56 |
| Kaempferol-glycosides | 0.49 | 0.08 | −0.18 | 0.53 | 0.50 | 0.07 |
| Quercetin-glycosides | 0.29 | 0.32 | 0.02 | 0.96 | 0.36 | 0.21 |
| Myricetin-glycosides | **−0.53** | **0.05** | 0.21 | 0.47 | −0.44 | 0.11 |
| Catechins | 0.18 | 0.53 | −0.15 | 0.60 | 0.06 | 0.83 |
| Sucrose | −0.05 | 0.87 | −0.05 | 0.86 | −0.01 | 0.97 |
| Fructose | 0.33 | 0.24 | −0.22 | 0.44 | 0.09 | 0.75 |
| Glucose | 0.46 | 0.10 | −0.31 | 0.28 | 0.13 | 0.67 |
| Galactose | 0.14 | 0.63 | −0.31 | 0.28 | −0.30 | 0.29 |
| Inositol | −0.12 | 0.68 | −0.00 | 0.99 | −0.04 | 0.88 |
| Acetone-insoluble residue | 0.11 | 0.70 | 0.04 | 0.89 | 0.30 | 0.30 |
| Water percent | 0.31 | 0.29 | 0.08 | 0.80 | 0.49 | 0.08 |
| Nitrogen of insoluble compounds (protein) | 0.18 | 0.54 | −0.03 | 0.92 | 0.24 | 0.41 |
| Nitrogen of soluble compounds | 0.10 | 0.73 | −0.11 | 0.70 | 0.13 | 0.65 |
| Total nitrogen | 0.20 | 0.50 | −0.06 | 0.83 | 0.26 | 0.37 |

At the later sampling date (16 July), neither gallotannins nor proanthocyanidins were still correlated with larval performance or consumption. At this time the leaves had already attained their full size, and the important traits determining both pupal mass and leaf biomass consumed by the 5th-instar larvae were the concentrations of water, of nitrogen in proteins, and of kaempferol-glycosides (Table 5). At this time, trees supporting low pupal mass of *E. autumnata* experienced low leaf damage levels. Low pupal masses correlated significantly with low concentrations of foliar water, with nitrogen of proteins (but not soluble nitrogen), and with kaempferol-glycosides. The water concentration correlated strongly with the concentrations of nitrogen of proteins and kaempferol-glycosides ($r = 0.74$, $P = 0.0001$, $n = 26$ and $r = 0.49$, $P = 0.01$, respectively). In addition to these leaf traits, low consumption also correlated with a low inositol concentration, a trait independent of leaf water concentration ($r = 0.26$, $P = 0.20$).

## Discussion

Our results explain why correlations between traits of foliage biochemistry and the performance indices of flush-feeding herbivores may vary considerably following relatively minor changes in experimental conditions. The significant biochemical leaf traits changed completely within 2 weeks during leaf maturation, and we found no single leaf trait which was significant for the performance of both penultimate and ultimate instar larvae. Gallotannins, proanthocyanidins, foliar water, nitrogen concentrations, and compounds correlated with them, all contributed to the success of *E. autumnata* larvae and the leaf consumption levels of mountain birch leaves. Among-tree variation in biochemical characteristics resulted both from phenological differences in leaf maturation and from among-tree heterogeneity in the seasonal behaviors of phenolics. Similar features may also characterize other tree species, as gallotannins, proanthocyanidins, and other products of the shikimate pathway are typical in the foliage of deciduous tree species. Moreover, tannins in other tree species display seasonal trends similar to the mountain birch (e.g., Feeny and Bostock 1968; Baldwin et al. 1987; Herms and Mattson 1992). The advantage of the present study over the most of previous ones is that a comprehensive array of possible biochemicals was analyzed. In addition, there is a depth of biochemical and ecological background to which our results can be related.

### Biochemical traits underlying leaf quality

The young leaves of the mountain birch are typically rich in gallotannins. Gallotannins are responsible for over 90% of the protein precipitation capacity of mountain birch leaf extracts (Ossipov et al. 1997; Kaitaniemi et al. 1998). Accordingly, the leaf quality in the YHG trees (with young leaves) was determined by foliar gallotannin concentrations, which reduced the efficiency of conversion of ingested food to larval biomass (i.e., ECI) (Table 3). A decrease in leaf quality was also connected with decreasing concentrations of inositol and acetone-insoluble residue. Despite this reduction in ECI, leaves from high gallotannin birches within the group of YHG trees were consumed more, not less, due to compensatory consumption (for a review see Slansky 1993).

During leaf maturation, the concentrations of proanthocyanidins increase and they represent the major phenolic compounds in mature birch leaves (Ossipov et al. 1997; our Fig. 2). In the MLG trees, with lower gallotannin concentrations and more developed leaves,

**Table 5** Correlations among leaf and larval traits for mature leaves (July 16; $n = 26$)

| | Pupal mass | | Leaf mass removed by larvae in 24 h during 5th instar | |
|---|---|---|---|---|
| | *r* | *P* | *r* | *P* |
| Total gallotannins | 0.08 | 0.69 | −0.07 | 0.72 |
| Soluble proanthocyanids | −0.34 | 0.09 | −0.29 | 0.15 |
| Cell-wall bound proanthocyanids | −0.20 | 0.32 | −0.17 | 0.40 |
| *p*-Coumaroylquinic acid derivatives | −0.03 | 0.88 | −0.21 | 0.30 |
| Kaempferol-glycosides | **0.56** | **0.003** | **0.47** | **0.02** |
| Quercetin-glycosides | −0.34 | 0.09 | −0.03 | 0.89 |
| Myricetin-glycosides | 0.13 | 0.52 | −0.19 | 0.34 |
| Catechins | −0.07 | 0.72 | −0.07 | 0.72 |
| Sucrose | 0.22 | 0.28 | −0.15 | 0.46 |
| Fructose | −0.05 | 0.82 | 0.25 | 0.21 |
| Glucose | 0.03 | 0.89 | 0.35 | 0.08 |
| Galactose | −0.28 | 0.17 | 0.09 | 0.65 |
| Inositol | 0.22 | 0.27 | **0.39** | **0.05** |
| Acetone-insoluble residue | 0.25 | 0.22 | 0.22 | 0.28 |
| Water percent | **0.55** | **0.004** | **0.61** | **0.001** |
| Nitrogen of insoluble compounds (protein) | **0.61** | **0.0009** | **0.57** | **0.002** |
| Nitrogen of soluble compounds | 0.13 | 0.54 | 0.07 | 0.73 |
| Total nitrogen | **0.57** | **0.002** | **0.53** | **0.006** |

soluble proanthocyanidins, not gallotannins, reduced larval relative growth rate (Table 4). In contrast to the YHG trees, *E. autumnata* larvae did not display effective compensatory consumption on low quality leaves with high levels of soluble proanthocyanidins. Thus the reduction of larval growth rate on the MLG trees was not clearly connected to the level of leaf material consumed by larvae.

In full-grown birch leaves, low foliar water, nitrogen of proteins, and kaempferol-glycoside concentrations were the main correlates of low leaf quality (Table 5). In full-grown leaves, low pupal mass was related to low leaf damage levels. Nitrogen apparently plays an important part in determining the leaf quality of mature leaves; we have found it to have a strong effect on pupal mass in field experiments as well (Kaitaniemi et al. 1998). Leaf toughness is also often interpreted as the main determinant of leaf quality in mature leaves (e.g., Coley 1983; Coley and Barone 1996). In the current experiment we did not measure leaf toughness, but the toughness of mature mountain birch leaves has been found to correlate with low water concentrations ($r = -0.33$, $P = 0.07$, $n = 30$ trees, K. Lempa, unpublished work). Accordingly, low foliar water concentrations and high leaf toughness are parallel symptoms of mature leaves (Haukioja et al. 1978; Ayres and MacLean 1987). Leaf toughness together with nitrogen and water concentrations, may determine the quality of mature leaves for *E. autumnata*. Although the change in the factors underlying leaf quality (from gallotannins and proanthocyanidins to nitrogen and water) seems to be closely related to leaf maturation, we cannot exclude possible instar-specific changes in the nutritional requirements of larvae. For instance, early-instar larvae may have a greater sensitivity to allelochemicals than late-instar larvae (e.g., Gould 1984).

The degree of foliage maturation largely contributed to the high among-tree variation in early leaf characteristics, but our data also indicate innate intrapopulation heterogeneity in the seasonal behaviors of foliage phenolics. Possible polymorphism within a mountain birch population may result from hybridization and introgression of birch species (Kallio et al. 1983; Hanhimäki et al. 1994) and/or heterogeneity in soil and microclimate (Senn et al. 1992).

The ability of mountain birch to combine the production of gallotannins with active leaf growth presumably relates to the carbon and energy efficiency of gallotannin synthesis. The synthesis of gallotannins does not proceed via the amino acid phenylalanine but via an intermediate compound of the shikimate pathway, dehydroshikimic acid (Gross 1992; Ossipov et al. 1997). In contrast, the synthesis of proanthocyanidins competes directly with synthesis of proteins with aromatic amino acids, due to phenylalanine as the common precursor (Shen et al. 1986; Nurmi et al. 1996). This produces a typical Y-shaped resource based trade-off (de Jong and van Noordwijk 1992) between the synthesis of such proteins and proanthocyanidins but not between gallotannins and proteins containing aromatic amino acids (Haukioja et al. 1998; Koricheva et al. 1998). Furthermore, since gallotannins are probably incorporated into primary cell walls during the early phases of active leaf growth (Ossipov et al. 1997), their active synthesis simultaneously with leaf growth is a necessity.

We found that the pooled values of hydrolyzable and condensed tannins were well suited to describe leaf quality for *E. autumnata*. However, the concentrations of some minor groups of phenolics (myricetin and kaempferol-glycosides) as well as of inositol also displayed significant correlations with larval traits, emphasizing the need for a more detailed analysis.

Our results reflect that the biochemical and physical properties of leaves have different importance for the plant and for a foliage-consuming herbivore. When biochemical compounds reduced larval growth rate or ECI, this did not necessarily lead to the reduction of leaf damage level experienced by a tree. We suggest that this difference can be described in terms of two related concepts: leaf suitability and leaf resistance. Suitability is the outcome of those leaf properties which are important for the herbivore: whether and how the leaves support herbivore success and ultimately fitness on the host (e.g., growth of the feeding life stage, final size achieved, fecundity, and survival). On the other hand, resistance is a characteristic relating to plant fitness, and is defined as a property of the plant which deters herbivores or reduces the damage (Kennedy and Barbour 1992). Although resistance may be determined by the same traits as suitability, in our data low suitability (reduced RGR, ECI, or pupal mass) did not automatically contribute to a high level of resistance (reduced consumption). We reserve the term defense for those cases in which high resistance has been selected for by natural selection. This requires a hereditary basis for the traits, and assumes that herbivores are agents of natural selection modifying plant fitness for resistance (Bernays and Graham 1988). We usually do not know whether this is the case, or whether plant resistance results from normal non-defensive plant functions (Honkanen and Haukioja 1998).

## Strategies of insects to circumvent rapidly changing leaf quality

The larvae of the autumnal moth have several ways of coping with low leaf quality. These means are fundamental in maintaining relatively rapid larval growth rates on high and low quality leaves alike (Kause et al. 1999b). Rapid growth is vital for flush- feeding autumnal moth, because rapid larval growth results in high pupal mass (Tammaru 1998; Kause et al. 1999b), a reliable index of fecundity (Haukioja and Neuvonen 1985; Tammaru et al. 1996a, b), and in high survival (Haukioja et al. 1988). First, *E. autumnata* larvae prefer feeding on young and soft leaves (Niemelä and Haukioja 1982) within heterogeneous birch canopies (Suomela et al. 1995a, b). Second, unlike the larvae of early season

sawflies (Kause et al. 1999a), *E. autumnata* larvae are able to increase their consumption on the young leaves of low quality trees and maintain relatively equal growth rates over gradients of resource quality (Suomela et al. 1995a; this study). Third, when compared to late instars, early instar larvae grow faster on young leaves, while last instar larvae are least sensitive to the seasonal deterioration in leaf quality (Ayres and MacLean 1987).

In conclusion, mountain birch leaves show a multitude of biochemical traits which determine the quality of leaves for insect herbivores. The results suggest two alternative and/or seasonally very rapidly changing determinants of leaf quality during early leaf development (gallotannins and proanthocyanidins). Furthermore, nitrogen and water explained herbivory-related traits after the completion of leaf growth. Interestingly, this change in the factors underlying leaf quality (from gallotannins and proanthocyanidins to nitrogen and water) can be traced back to the activity of the shikimate pathway and the formation of cell walls; gallotannins and proanthocyanidins are products of the shikimate pathway, and the transformation of soluble gallotannins and proanthocyanidins into insoluble cell-wall bound constituents presumably contributes to leaf toughness (Nurmi et al. 1996; Ossipov et al. 1997).

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