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RAPID HERBIVORE-INDUCED CHANGES IN MOUNTAIN BIRCH PHENOLICS AND NUTRITIVE COMPOUNDS AND THEIR EFFECTS ON PERFORMANCE OF THE MAJOR DEFOLIATOR, *Epirrita autumnata*

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Abstract—Insect damage changes plant physiology and chemistry, and such changes may influence the performance of herbivores. We introduced larvae of the autumnal moth (*Epirrita autumnata* Borkh.) on individual branches of its main host plant, mountain birch (*Betula pubescens* ssp. *czerepanovii* (Orlova) Hämet-Ahti) to examine rapid-induced plant responses, which may affect subsequent larval development. We measured systemic responses to herbivory by analyzing chemistry, photosynthesis, and leaf growth, as well as effects on larval growth and feeding, in undamaged branches of damaged and control trees. Larvae reared on leaves from intact branches of the herbivore-damaged trees grew faster than those reared on leaves of control trees, indicating systemic-induced susceptibility. Herbivore damage did not lead to systemic changes in levels of primary nutrients or phenolic compounds. The analyses of photosynthetic activity and individual hydrolyzable tannins revealed a reversal of leaf physiology-herbivore defense patterns. On control trees, consumption by *E. autumnata* larvae was positively correlated with photosynthetic activity; on damaged trees, this correlation was reversed, with consumption being negatively correlated with photosynthetic activity. A similar pattern was found in

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the relationship between monogalloylglucose, the most abundant hydrolyzable tannin of mountain birch, and leaf consumption. Among the control trees, consumption was positively correlated with concentrations of monogalloylglucose, whereas among herbivore-damaged trees, this correlation was reversed and became negative. Our results suggest that herbivore performance is related to both concentrations of phenolic compounds and photosynthetic activity in leaves. This linkage between herbivore performance, leaf chemistry, and physiology was sensitive to induced plant responses caused by slight herbivore damage.

Key Words—*Epirrita autumnata*, *Betula pubescens* ssp. *czerepanovii*, galloylglucoses, larval growth, leaf consumption, Lepidoptera, photosynthesis, rapid-induced responses.

INTRODUCTION

Herbivory modifies plant growth and physiology, including chemistry, which may change plant quality for subsequently attacking herbivores (induced resistance and susceptibility) (Karban and Baldwin, 1997). The bulk of current research in this area concentrates on induced resistance, in part because delayed forms of induced resistance (DIR), affecting the following generation of herbivores, offer a mechanism that may contribute to cycles in herbivore populations.

In the mountain birch (*Betula pubescens* ssp. *czerepanovii*) – autumnal moth system, herbivore-induced responses in host leaf quality are not restricted to DIR; intact birch leaves tend to be of lower quality to *E. autumnata* within days after manual damage to nearby leaves (i.e., rapid-induced resistance, RIR) (Haukioja and Hanhimäki, 1985; Hanhimäki and Senn, 1992). These rapid-induced changes also have the potential to influence population dynamics. For example, there are indications that damage to birch buds and shoots makes foliage more palatable for later herbivory (Haukioja et al., 1990; Danell et al., 1997). Such induced susceptibility is a potentially important phenomenon for population cycles because it might introduce positive feedback into the population dynamics of the herbivore, which could explain the elusive increase phase of outbreaking herbivores (Ruohomäki et al., 2000).

The mechanisms by which birch trees show induced resistance and susceptibility are extensively studied. Birch leaves have both physical (leaf toughness) and chemical traits that may serve defensively against insect herbivores (Ruohomäki et al., 1996; Kause et al., 1999; Ossipov et al., 2001). Levels of total nitrogen and total phenolics are known to change after defoliation (Neuvonen and Haukioja, 1984; Haukioja et al., 1985; Ruohomäki et al., 1996; Kaitaniemi et al., 1998), making a potential link between plant responses and resistance to herbivores. There is some data demonstrating local RIR on *E. autumnata* after manual damage to leaves, and also of systemic RIR after larval damage (Kaitaniemi and Ruohomäki, 2001). To further test for the existence and chemical correlates of possible systemically

induced effects after real herbivore damage on the performance of *E. autumnata* larvae, we conducted a field experiment by introducing larvae to trees in a natural population.

Recent investigations have revealed large differences in seasonal patterns among different groups of phenolic compounds and carbohydrates and amino acids, the main pool of nitrogen-rich compounds in birch (Kause et al., 1999; Riipi et al., 2002). Among phenolic compounds, proanthocyanidins increase during leaf expansion and maturation, whereas flavonoids and galloylglucoses, a group of hydrolyzable tannins, decrease with phenology (Salminen et al., 1999, 2001; Riipi et al., 2002). Thus, because our experiment followed the seasonal development of *E. autumnata* larvae on damaged and undamaged trees, we were able to examine seasonal patterns in the expression of plant resistance traits and how they were modified by herbivory.

Specifically, we addressed three main questions: (1) Do chemical, physical, and physiological traits of birch rapidly and systemically respond to herbivory? (2) Do responses in such traits affect consumption by or growth of *E. autumnata*? (3) Does herbivore damage to trees modify correlations among putatively defensive leaf traits, or between leaf traits and insect performance? To answer these questions we measured responses over the entire larval period of *E. autumnata* and measured larval performance during each instar.

METHODS AND MATERIAL

Study Organisms. Mountain birch (*Betula pubescens* spp. *czerepanovii*) is a northern European hardwood species forming uniform stands at the arctic tree line in northern Fennoscandia. There are large chemical changes in foliage during leaf unfolding and maturation (Ossipov et al., 1997, 2001; Riipi et al., 2002).

The autumnal moth (*Epirrita autumnata*) is a univoltine lepidopteran species and the main defoliator of mountain birch in NW Europe, with significantly cyclic regional peaks at 10-year intervals (Ruohomäki et al., 2000).

Experimental Design. We haphazardly selected 24 trees at a site situated in a river valley close to the Kevo Subarctic Research Station (69°45′ N, 27°01′ E). Twelve trees were randomly assigned to the larval introduction (herbivory) treatment, while 12 were left as unmanipulated controls. We used large (height 3–5 m) and mature mountain birch containing at least five large ramets with well-developed canopy. In each experimental tree, we selected five large ramets and two branches per ramet. The branches in the treatment trees were bagged with large (30 × 80 cm) nylon mesh bags (10 per tree) before bud burst in late May 2000, and we introduced approximately 20 larvae per bag during the natural egg hatch at bud burst. The mesh bags prevented larval dispersal and excluded parasitoids. We did not find wild *Epirrita* in the study site, and in general, there was negligible amount

of background herbivory. Although control trees were not bagged, in a separate experiment, we demonstrated that bags did not change suitability of the control trees to *E. autumnata*. In a laboratory bioassay, the means for the fifth instar larvae RGR reared with leaves of unbagged and bagged (empty) trees were 0.294 and 0.272 mg/d, respectively; $F_{1,18} = 1.19$, $P = 0.29$).

Leaf phenology is an important covariate of leaf resistance and herbivore performance, since delayed budbreak has deleterious effects on herbivore performance (Kaitaniemi et al., 1997). Therefore, at an early phase during the bud burst period, we measured leaf phenology as the ratio between the visible part of leaf blade and bud scale for each tree (Sulkinoja and Valanne, 1987).

Bioassays. We conducted laboratory bioassays by employing 20 larvae for each of the 24 trees, (480 larvae in total). The larvae were reared under natural light and temperature conditions out of doors, individually in 48-ml plastic vials. Larvae were fed from hatch to pupation with short shoot leaves detached from the experimental trees. Leaves were harvested from the short shoots in the branches without mesh bags. The order of vials was randomized in trays. We conducted bioassays with the experimental larvae in the second through fifth instar; the first instar larvae is too small and delicate to be successfully used in a bioassay. To allow natural variation in development, we did not synchronize development of experimental larvae before the bioassays. Therefore, all larvae could not be used in a given bioassay because some were molting. The numbers of larvae in the second, third, fourth, and fifth instar bioassay trials were 383, 369, 355, and 350, respectively. Although there was some variation, most larvae were individually followed throughout their development and employed in four bioassays.

The timing of the bioassays was decided by the developmental stage of the defoliating larvae reared in the bags of the experimental trees in the field. As soon as the mode number of experimental larvae molted into the particular instar, we conducted the bioassay. A 48-hr bioassay was used for the second and the third instar larvae, while a 24-hr one was used for the more voracious fourth and fifth instar larvae. Leaves for the bioassays were picked from experimental trees and stored in vials in a cooler until used. Each larva and leaf was weighed before the experiment. During the bioassays, larvae were kept in a temperature-controlled room at 12°C and in continuous light (average conditions at our high latitude study site). At the end of the bioassay, larvae were reweighed and leaf remnants and frass were collected. We freeze-dried leaf remnants for 48 hr before weighing. Larval fresh weight was converted into dry weight with the equation dry weight = $0.125 \times (\text{fresh weight})^{1.113}$ (Neuvonen and Haukioja, 1984). For each larva, we computed dry weight in the beginning of the bioassay and the amount of leaf consumed on a dry weight basis. During the bioassay, we collected 10 leaves per tree, weighed them fresh, dried them in an oven at 60°C for 24 hr, and reweighed them dry. The tree-specific regression models between fresh and dry weight of individual leaves explained more than 99% of total variation. For each tree, the

regression equation derived from the tree-specific model was used to convert leaf fresh weight into dry weight.

Chemical Analyses. We sampled short shoot leaves from untreated branches of the ramets of each experimental tree for chemical analysis when we collected leaves for each of the bioassays. Leaves were placed in a cooler, transferred to the laboratory, and freeze-dried. Freeze-dried leaves were homogenized, and about 300 mg of the powder were suspended in 10 ml of 70% aqueous acetone, allowed to stand for 1 hr at room temperature with continuous stirring, and centrifuged for 10 min at 2500 *g*. The pellet was reextracted 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 lyophilized residue was redissolved in 6 ml of water and centrifuged for 20 min at 3000 *g*. This purified extract was used for the determination of soluble phenolics and carbohydrates. The acetone-insoluble residue was collected, lyophilized, and weighed, and was used for the determination of cell-wall-bound proanthocyanidins and protein amino acids.

Soluble and cell-wall-bound proanthocyanidins were analyzed by the method of Terrill et al. (1992), which was modified and optimized for proanthocyanidins from birch leaves (Ossipova et al., 2001). Low molecular mass phenolics (chlorogenic acid and flavonoid-glycosides) were analyzed with HPLC (Salminen et al., 1999).

Soluble carbohydrates (glucose, fructose, sucrose, and galactose) and inositol were quantified using a gas chromatographic method (Kallio et al., 1985). Proteins were hydrolyzed with 6 N HCl for 24 hr at 105°C, and protein amino acids were derivatized with 9-fluorenylmethyl chloroformate and analyzed by HPLC with a fluorescent detector (Bank et al., 1996). Detailed description of all methods used can be found in Salminen et al. (1999), Ossipov et al. (2001), and Ossipova et al. (2001).

We paid special attention to analysis of hydrolyzable tannins because they undergo rapid biosynthesis during *E. autumnata's* larval period. HPLC-ESI-MS analysis of individual galloylglucoses and ellagitannins was performed as in Salminen et al. (1999, 2001), except that differences in the ESI-MS performance between individual runs were standardized by using 6-bromo-2-naphthyl-β-D-glucopyranoside as an internal standard.

Leaf Biomass, Toughness, and Photosynthesis Measurements. We measured tree-specific fresh weights of separately collected short shoot leaves at the time of each of the bioassays (five leaves per tree). We also measured leaf toughness by using a force gauge penetrometer (Chatillon DFIS, Amtek Inc. Largo, FL). We took, two measurements per leaf, piercing only intervenal parts of the leaf blade. After the toughness measurements, leaves were dried in the oven at 60°C for 48 hr and reweighed to get leaf dry weight.

Photosynthetic activity changes rapidly during bud burst and subsequent leaf development (Valanne and Valanne, 1984; Larcher, 1995), and previous defoliation reduces photosynthetic activity in mountain birch (Hoogesteger and Karlsson, 1992). We measured photosynthesis (A_{net}, net assimilation rate) with a portable photosynthesis system (a closed model CIRAS-1, PPSystems, Hitchin, UK) for each tree twice, June 21 and 28, and once after the bioassays, on July 14. A tree-specific $A_{net}$is the mean of the three dates. The amount of photosynthetically active radiation (PAR) was controlled with an external light source (1000 mol/m^{-2}/s^{-2}), which is above the photon flux density of *Betula* (Ovaska et al., 1992; Oleksyn et al., 1998). To minimize environmental noise, we took five measurements per replicate tree. Moreover, we measured A_{net} in the morning hours when A_{net} is the most active (Larcher, 1995), and bioassay specific measurements were taken during two or more days.

Statistical Analysis. We calculated tree-specific means by date for leaf consumption, larval growth, leaf phenology, toughness, and photosynthesis. Moreover, we calculated the sum of the concentrations of the individual compounds for galloylglucoses, ellagitannins, flavonoids, proanthocyanidins, carbohydrates, and protein-bound amino acids. We used concentrations (mg/g) of compounds instead of total amounts, since concentration of a compound indicates the quality of a plant as a food for herbivores (Koricheva, 1999). We performed a set of repeated measures MANCOVAs (proc GLM, SAS, 1996) to study effects of larval feeding on birch foliage: chemistry, leaf growth, leaf toughness, *E. autumnata* growth, and consumption. The sampling date was regarded as a within-subject variable. The models tested statistical significance of larval introductions (herbivory, main effect), leaf and larval growth, as well as changes in biochemical and physical variables through the season (date, main effect) and effects of defoliation on seasonal change (interaction of herbivory and date). The leaf phenology index at bud burst was employed as a covariate. We used the Pearson product moment correlation coefficient (proc CORR, SAS, 1996) to analyze relationships between biochemical and physical variables with larval traits. To meet the assumptions of analysis of variance, the values for concentrations of ellagitannins and proanthocyanidins were square-root transformed.

RESULTS

Seasonal Changes and Effects of Leaf Chewing on Leaf Chemistry. Herbivory by *E. autumnata* larvae in the trees did not change concentrations of any of the measured phenolic traits: hydrolyzable tannins or their subgroups (galloylglucoses and ellagitannins), flavonoids, proanthocyanidins (condensed tannins), chlorogenic acid, soluble carbohydrates (sugars), or protein-bound amino acids (in MANCOVAs $P > 0.25$) . There were significant seasonal development changes

in the concentrations of all compounds except total ellagitannins (Figure 1). Total concentrations of galloylglucoses, as well as monogalloylglucose, the most abundant galloylglucose, increased between June 8 and 16 and declined between June 16 and 28. Among ellagitannins, a decline in concentrations of tellimagrandin I and II, casuarictin, and potentillin was detected between June 21 and 28. Among major groups of low-molecular weight phenolic compounds, concentrations of flavonoids declined during leaf development. Eight individual flavonoid compounds out of nine followed the same pattern (data not shown). Concentrations of proanthocyanidins and chlorogenic acid, the most abundant phenolic compound in mountain birch, displayed a steady increase during the experimental period. Among major primary metabolites, total concentration of carbohydrates increased during leaf development, but defoliation did not modify concentrations ($P = 0.39$; Figure 2). This pattern was consistently found among four individual carbohydrates, except sucrose, which peaked during the third instar of *E. autumnata* (data not shown). Total concentration of protein-bound amino acids decreased during leaf development, but defoliation did not change concentrations ($P = 0.78$; Figure 2).

Effects of Defoliation on Leaf Growth, Photosynthesis, and Toughness. Leaf biomass, photosynthetic activity, and leaf toughness all increased during the experimental period. Defoliation, however, did not affect these traits, suggesting that mountain birch did not respond systemically to the relatively small amount of damage imposed (Figure 3).

Effects of Rapid-Induced Responses on Larval Growth and Leaf Consumption. The presence of experimentally imposed herbivory by *E. autumnata* larvae systemically modified the growth of bioassay larvae during leaf development. The larvae reared on leaves from defoliated trees developed 16% faster than those on leaves from control trees. The growth curves set apart during the fourth instar, as indicated by a significant time $\times$ defoliation interaction (Figure 4, Table 1). Interestingly, defoliation did not affect pupal mass ($F_{1,\ 22} = 0.16$, $P = 0.69$), indicating that the slower growing larvae in control trees could feed longer, thus compensating for the difference in growth rates. Leaf consumption by *E. autumnata* naturally increased during larval development, but defoliation did not modify consumption patterns during leaf development (Table 1, Figure 4).

Chemical and Physiological Correlates of Larval Growth and Feeding. We conducted correlational analyses to reveal how the chemical and physiological quality of leaves was related to growth of, and consumption by *E. autumnata*. The main chemical compounds correlating with moth performance were concentrations of flavonoids, chlorogenic acid, and protein-bound amino acids. Flavonoids and chlorogenic acid were positively correlated with leaf consumption, whereas amount of protein-bound amino acids was positively related to pupal mass (Table 2).

Effects of Rapid-Induced Responses on Relationships Between Putative Defenses and Insect Performance. To understand how defoliation may affect

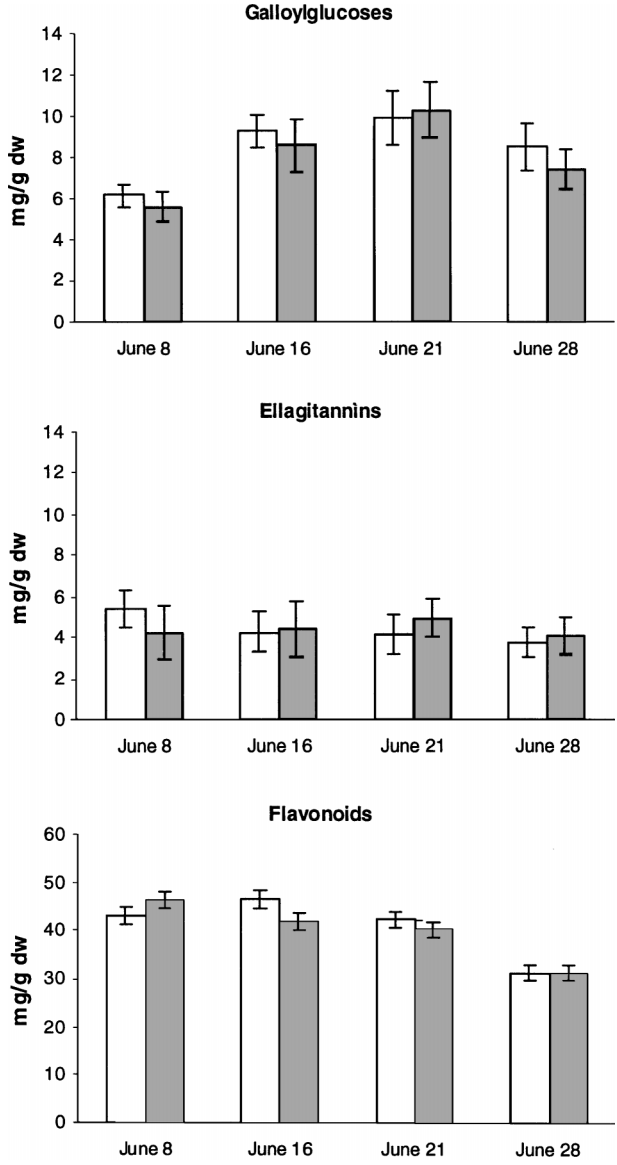

FIG. 1. Contents of ellagitannins, galloylglucoses, flavonoids, proanthocyanidins, and chlorogenic acid during the early phases of birch leaf development. The clear bars represent controls, and grey bars represent trees with experimentally imposed herbivory. Means and their standard errors are shown. Seasonal changes are statistically significant ($P < 0.01$) except for total ellagitannins ($P = 0.19$).

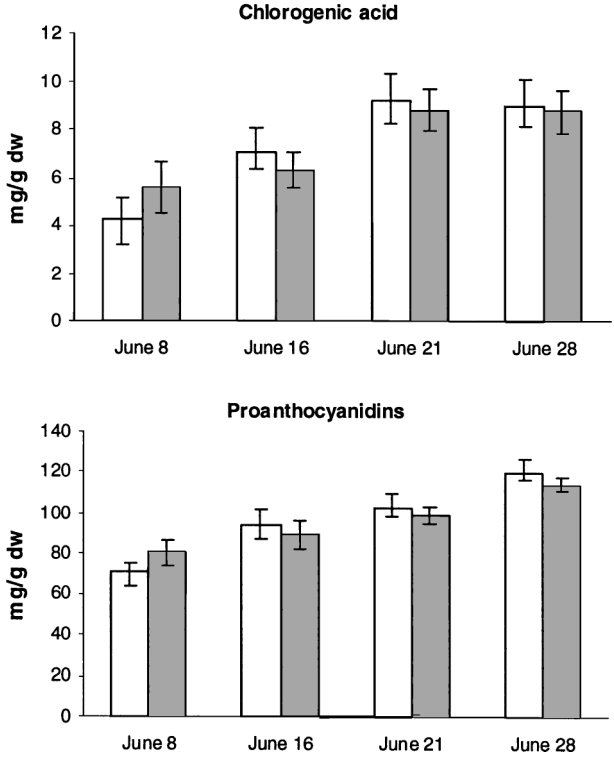

FIG. 1. CONTINUED.

relationships between foliar quality and herbivore performance, we conducted many ANCOVAs to explain herbivore growth and leaf consumption. As explanatory factors, we used herbivory treatment, foliar chemistry/physiology, and their interactions; leaf phenology was used as a covariate. A significant interaction term suggests that herbivory not only modified leaf suitability for larvae, but also the relationship between chemical and physiological quality and herbivore performance. After a sequential Bonferroni correction, we discovered two significant interactions: relationships of leaf consumption between photosynthesis and the simplest galloylglucose, monogalloylglucose, were different between control trees and herbivore-damaged trees. In the control trees, leaf consumption was positively correlated with photosynthetic activity, whereas in the defoliated trees, leaf consumption was negatively correlated (interaction term in ANCOVA: $F_{1,19} = 7.04$, $P = 0.015$, Figure 5). A similar pattern was found between monogalloylglucoses and leaf consumption (interaction term in ANCOVA: $F_{1,19} = 9.29$, $P = 0.006$, Figure 5). Such reversals of the correlations indicate a

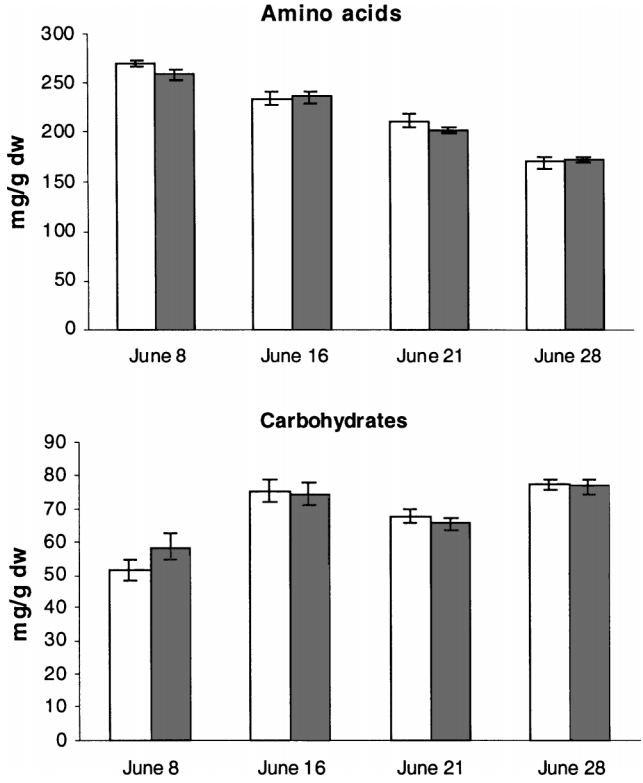

FIG. 2. Contents of amino acids and carbohydrates during the early phases of birch leaf development. The clear bars represent controls, and grey bars represent trees with experimentally imposed herbivory. Means and their standard errors are shown. Seasonal changes are statistically significant ($P < 0.01$).

fundamental change in photosynthetic activity, physiological basis of growth, and mode of action of monogalloylglucose, a putative defensive compound of mountain birch.

DISCUSSION

Our results show that *E. autumnata* grew better on leaves from trees damaged by *E. autumnata* larvae than on control trees, indicating rapid-induced susceptibility. Our goal was to create moderate levels of herbivory that would simulate the types of damage during the increase phase of an outbreak. The positive effect of the presence of damaging larvae (350 out of 480 larvae lived up to fifth instar)

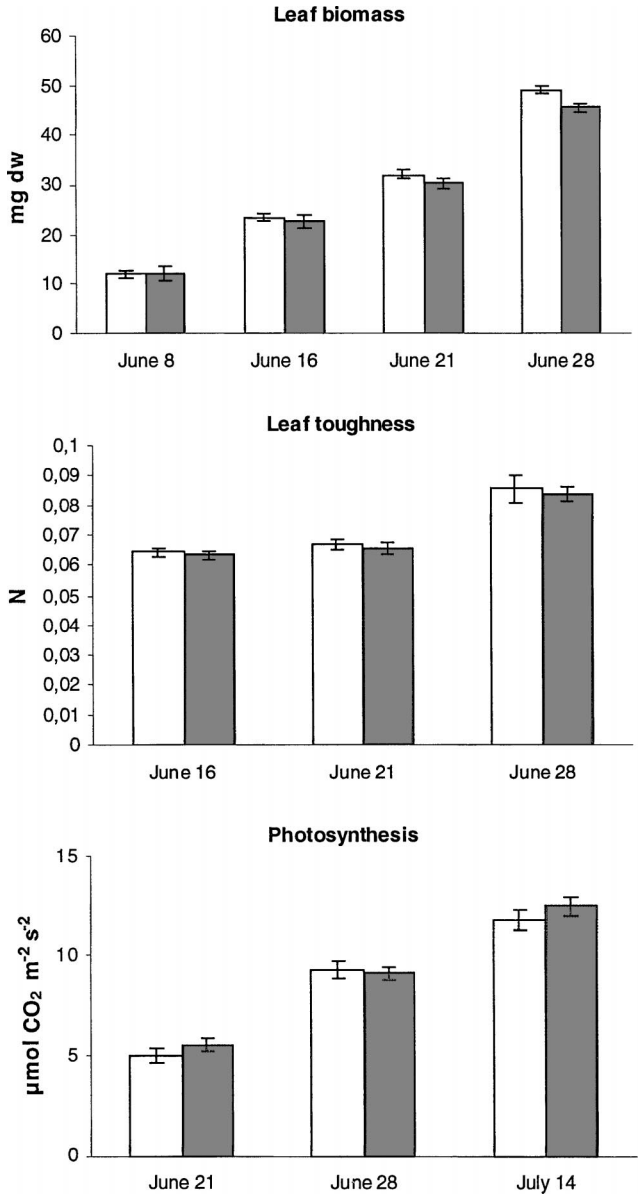

FIG. 3. Changes in leaf biomass, photosynthetic activity, and leaf toughness during birch leaf development. The clear bars represent controls, and grey bars represent trees with experimentally imposed herbivory. Means and their standard errors are shown. Seasonal changes are statistically significant ($P < 0.01$).

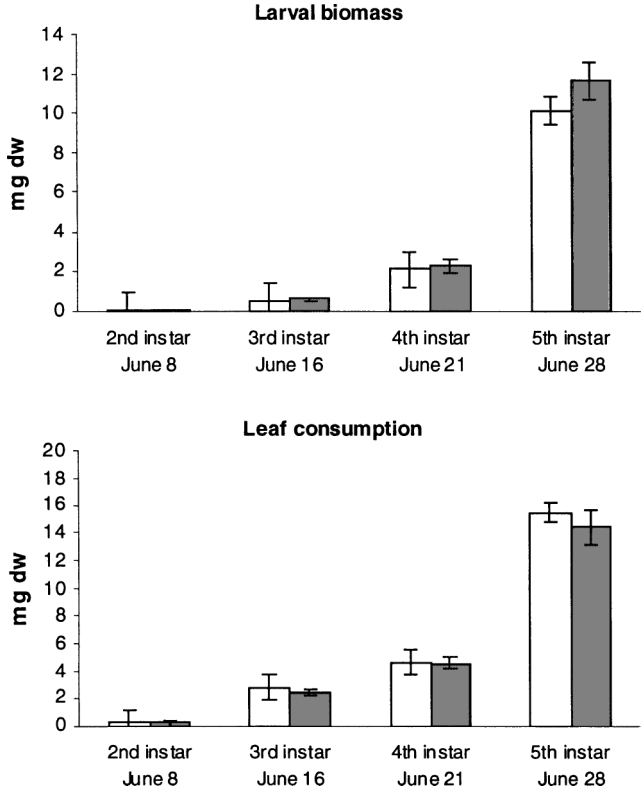

FIG. 4. Leaf consumption by and biomass of *E. autumnata* larvae during early phases of birch leaf development. The clear bars represent controls, and grey bars represent trees with experimentally imposed herbivory. Means and their standard errors are shown.

developed gradually in the course of larval growth, and became significant between the fourth and fifth instars. However, we did not detect effects on pupal mass or leaf consumption, suggesting that *E. autumnata* damage in other parts of the tree may benefit the larvae in terms of growth. This did not increase the biomass losses in mountain birch. Nevertheless, larvae may benefit from a higher growth rate, since large larvae are less susceptible to parasitoids than young ones (Kaitaniemi and Ruohomäki, 1999).

Previous studies of the mountain birch system have reported variable strengths of rapid-induced responses to manual defoliation (Haukioja and Hanhimäki, 1985; Hanhimäki and Senn, 1992). Other studies of birch, also using short pulses of manual defoliation, have demonstrated main effects of defoliation on primary metabolites (protein-bound amino acids, carbohydrates) and on secondary metabolites

TABLE 1. RESULTS FOR REPEATED MEASURES MANCOVA OF *E. autumnata* GROWTH AND LEAF CONSUMPTION

Source of variation	Defoliation	Time	Defoliation[a] time	Leaf phenology
E. autumnata growth	Wilks' lambda = 0.565	Wilks' lambda = 0.029	Wilks' lambda = 0.615	Wilks' lambda = 0.506
	$F_{4,18} = 3.46$, $P = 0.029$	$F_{3,19} = 213.07$, $P < 0.001$	$F_{3,19} = 3.97$, $P = 0.024$	$F_{4,18} = 4.39$, $P = 0.012$
Leaf consumption by *E. autumnata*	Wilks' lambda = 0.888	Wilks' lambda = 0.416	Wilks' lambda = 0.931	Wilks' lambda = 0.578
	$F_{4,18} = 0.57$, $P = 0.69$	$F_{3,19} = 8.90$, $P < 0.001$	$F_{3,19} = 0.47$, $P = 0.71$	$F_{4,18} = 3.28$, $P = 0.034$

Note. Leaf Phenology was used as a covariate.

[a] A defoliation-induced difference in larval mass between the fourth and fifth instar was statistically significant as revealed by analysis of variance contrast (interaction of fourth to fifth instar and treatment, $F_{1,21} = 5.81$, $P = 0.028$).

TABLE 2. PEARSON PRODUCT MOMENT CORRELATION COEFFICIENTS DESCRIBING THE RELATIONSHIPS OF *E. autumnata* PERFORMANCE TRAITS AND BIRCH LEAF PHENOLICS, NUTRITIVE COMPOUNDS, AND WATER[a]

	Initial weight of fifth instar larvae	Leaf consumption by fifth instar larvae	Pupal mass
Total hydrolyzable tannins	0.115	−0.020	0.061
Total galloylglucoses	0.171	−0.113	0.024
Total ellagitannins	0.123	0.009	0.046
Chlorogenic acid	−0.230	0.437*	0.391
Total flavonoids	−0.359	0.551*	0.404
Total proanthocyanidins	−0.107	0.081	−0.062
Total carbohydrates	−0.129	−0.004	0.079
Total protein-bound amino acids	−0.022	0.456*	0.558*
Photosynthesis	0.032	−0.176	0.040
Leaf toughness	−0.201	−0.185	−0.129
Leaf biomass	−0.211	−0.310	0.024

[a] Coefficients with "*" have $P < 0.05$. $N = 24$.

(flavonoids, chlorogenic acid, proanthocyanidins, and hydrolyzable tannins) (Hartley and Lawton, 1987; Keinänen et al., 1999). One possible reason for the discrepancy between these studies and ours may be the type of cue, since most other studies have used heavy manual defoliation, which may produce spurious results with respect to the more natural cues imposed by real herbivores (Karban and Baldwin, 1997). Both artificial and natural defoliation may induce production of systemic wound signals, but the production is more active in natural than in artificial defoliation (Schmelz et al., 2003), and only natural defoliation has a potential for herbivore-specific production of signals, i.e., elicitors (Kessler and Baldwin, 2002).

There were four major differences between our experiment and that of Kaitaniemi and Ruohomäki (2001), which recently reported rapid-induced resistance. First, they used smaller trees and few freely moving larvae to damage trees. Second, they fed larvae with leaves from experimental trees only during bioassays. Third, they found the negative effect in the third instar. Fourth, they point out that free-moving larvae showed the strongest responses to previous herbivory. It is not clear which of the factors are responsible for the difference between our study and Kaitaniemi and Ruohomäki (2001).

We conducted correlational analyses between tree-specific means of putative defensive compounds and tree-specific means of insect performance and consumption to reveal possible mechanisms of plant resistance. Our novel finding is that defoliation modified the relationships between photosynthetic rate and insect consumption. In the control trees, photosynthetic activity and concentrations of monogalloylglucose were positively correlated with leaf consumption. However,

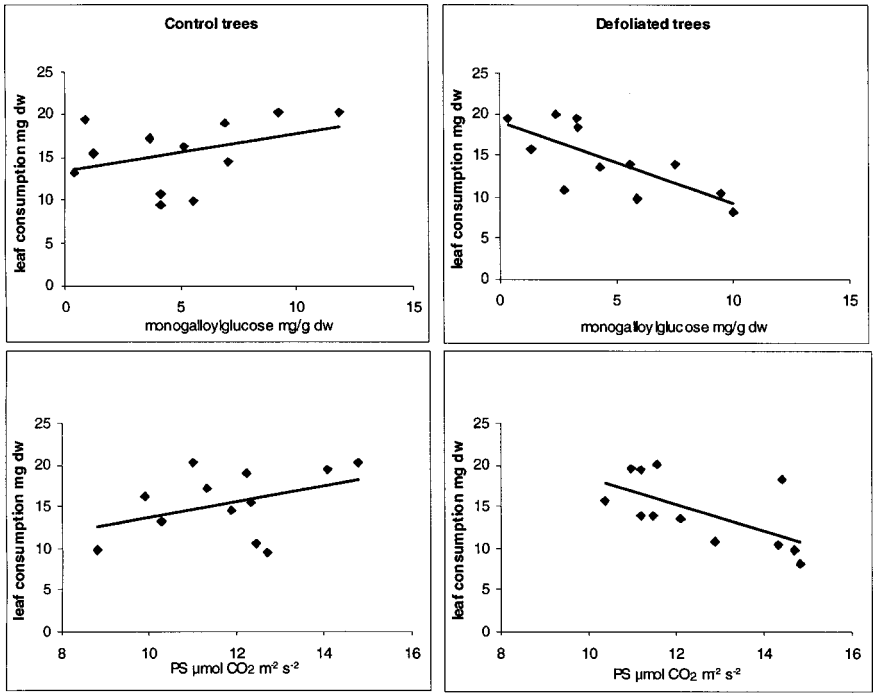

FIG. 5. Scatterplots describing relationships of leaf consumption by fifth instar *E. autumnata* larvae with birch photosynthesis and foliar content of monogalloylglucose in the control and defoliated trees.

in the defoliated trees, photosynthetic activity and monogalloylglucose correlated negatively with leaf consumption. Our results suggest that although *E. autumnata* feeding did not systemically change the levels of measured phenolic compounds, it modified the context in which these compounds act. The effects of monogalloylglucose, and hydrolyzable tannins in general, on herbivore performance are known to range from stimulatory to deterrent (Bernays et al., 1989). Presumably, monogalloylglucose stimulates *E. autumnata* feeding in the absence of previous damage, whereas in defoliated trees the same compound reduces leaf consumption. The physiological effects of hydrolyzable tannins are connected to their fate in the larval digestive tract, i.e., hydrolysis and oxidation, and their ability to precipitate proteins. These processes may have variable effects on herbivore performance (Bi and Felton, 1995; Johnson and Felton, 1996), and damage-induced changes may further modify their effects on herbivores.

The connection between leaf consumption and photosynthetic activity suggests that individual mountain birch trees may meet quite different challenges

during an *E. autumnata* attack. Our data indicate that birch trees with high photosynthetic activity will both encounter lower leaf losses and have higher potential for compensation of biomass losses (by photosynthesis) than trees with low photosynthetic activity. Photosynthetic activity may link herbivore resistance and tolerance in mountain birch because monogalloylglucose is an early product following photosynthesis. The extreme phenotypes with high A_{net} will be able to capture and store more carbon resources during slight defoliation that is typical for early phases of *E. autumnata* outbreaks. This, in turn, may improve their ability to recover after more severe defoliation that is found during the peak phase of *E. autumnata* outbreaks. Extreme trees with low A_{net} may compensate for biomass losses by higher A_{net}, but only partially (Hoogesteger and Karlsson, 1992), indicating that these trees will have a lower capacity to recover. This may partially explain amongsite variation in recovery of birch after the severe defoliation in 1960s (Lehtonen and Heikkinen, 1995).

Our study indicates that only one or two of the measured leaf traits were involved in the rapidly induced responses. However, A_{net} determines the amount of carbon available for plant functions, and monogalloylglucose is the first compound in the pathway of hydrolyzable tannins. The close positive connection between A_{net} and monogalloylglucose suggests plant defense by chemical means may not always be mutually exclusive of plant compensation by growth (Mauricio et al., 1997; Strauss and Agrawal, 1999) contrary to the general assumption (Herms and Mattson, 1992; Haukioja et al., 1998; Koricheva et al., 1998). Rather, the highest concentrations of galloylglucoses are found in the early stages of leaf development (Salminen et al., 2001), and they are part of the hydrolyzable tannin pathway that contributes to cell-wall formation (Grundhoefer and Gross, 2001), indicating a close integration of the hydrolyzable tannin pathway to plant growth.

In summary, limited foliar damage on birch by *E. autumnata* changed the relationships between birch photosynthesis and *E. autumnata* consumption. A similar pattern was found between the most abundant hydrolyzable tannin in birch, monogalloylglucose, and leaf consumption by *E. autumnata*. In contrast, slight defoliation did not change the levels of major primary and secondary metabolites, nor their relationship with larval performance. Slight defoliation of mountain birch that was concentrated in pockets over the whole tree did, however, cause rapidly induced changes that improved larval growth and reduced leaf biomass losses for trees with high photosynthetic activity. The birch preferred by herbivores have the highest potential for successful recovery after herbivore damage.

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REFERENCES

BANK, R. A., JANSEN, E. J., BEEKMAN, B., and KOPPELE, J. M. T. 1996. Amino acid analysis by reverse-phase high-performance liquid chromatography: Improved derivatization and detection conditions with 9-fluorenylmethyl chloroformate. *Anal. Biochem.* 240:167–176.

BERNAYS, E. A., COOPER DRIVER, G., and BILGENER, M. 1989. Herbivores and plant tannins. *Adv. Ecol. Res.* 19:263–302.

BI, J. L. and FELTON, G. W. 1995. Foliar oxidative stress and insect herbivory: Primary compounds, secondary metabolites, and reactive oxygen species as components of induced resistance. *J. Chem. Ecol.* 21:1511–1530.

DANELL, K., HAUKIOJA, E., and HUSS-DANELL, K. 1997. Morphological and chemical responses of mountain birch leaves and shoots to winter browsing along a gradient of plant productivity. *Ecoscience* 4:296–303.

GRUNDHOEFER, P. and GROSS, G. G. 2001. Immunocytochemical studies on the origin and deposition sites of hydrolyzable tannins. *Plant Sci.* 160:987–995.

HANHIMÄKI, S. and SENN, J. 1992. Sources of variation in rapidly inducible responses to leaf damage in the mountain birch–insect herbivore system. *Oecologia* 91:318–331.

HARTLEY, S. E. and LAWTON, J. H. 1987. Effects of different types of damage on the chemistry of birch foliage, and the responses of birch feeding insects. *Oecologia* 74:432–437.

HAUKIOJA, E. and HANHIMÄKI, S. 1985. Rapid wound-induced resistance in white birch (*Betula pubescens*) foliage to the geometrid (*Epirrita autumnata*): A comparison of trees and moths within and outside the outbreak range of the moth. *Oecologia* 65:223–228.

HAUKIOJA, E., NIEMELÄ, P., and SIRÉN, S. 1985. Foliage phenols and nitrogen in relation to growth, insect damage, and ability to recover after defoliation, in the mountain birch *Betula pubescens* ssp. *tortuosa*. *Oecologia* 65:214–222.

HAUKIOJA, E., OSSIPOV, V., KORICHEVA, J., HONKANEN, T., LARSSON, S., and LEMPA, K. 1998. Biosynthetic origin of carbon-based secondary compounds: Cause of variable responses of woody plants to fertilization. *Chemoecology* 8:133–139.

HAUKIOJA, E., RUOHOMÄKI, K., SENN, J., SUOMELA, J., and WALLS, M. 1990. Consequences of herbivory in the mountain birch (*Betula pubescens* ssp. *tortuosa*): Importance of the functional organization of the tree. *Oecologia* 82:238–247.

HERMS, D. A. and MATTSON, W. J. 1992. The dilemma of plants: To grow or defend. *Q. Rev. Biol.* 67:283–335.

HOOGESTEGER, J. and KARLSSON, P. S. 1992. Effects of defoliation on radial stem growth and photosynthesis in the mountain birch (*Betula pubescens* ssp. *tortuosa*). *Funct. Ecol.* 6:317–323.

JOHNSON, K. S. and FELTON, G. W. 1996. Physiological and dietary influences on midgut redox conditions in generalist lepidopteran larvae. *J. Insect Physiol.* 42:191–198.

KAITANIEMI, P. and RUOHOMÄKI, K. 1999. Effects of autumn temperature and oviposition date on timing of larval development and risk of parasitism in a spring folivore. *Oikos* 84:435–442.

KAITANIEMI, P. and RUOHOMÄKI, K. 2001. Sources of variability in plant resistance against insects: Free caterpillars show strongest effects. *Oikos* 95:461–470.

KAITANIEMI, P., RUOHOMÄKI, K., and HAUKIOJA, E. 1997. Consumption of apical buds as a mechanism of alleviating host plant resistance for *Epirrita autumnata* larvae. *Oikos* 78:230–238.

KAITANIEMI, P., RUOHOMÄKI, K., OSSIPOV, V., HAUKIOJA, E., and PIHLAJA, K. 1998. Delayed induced changes in the biochemical composition of host plant leaves during an insect outbreak. *Oecologia* 116:182–190.

KALLIO, H., AHTONEN, S., RAULO, J., and LINKO, R. 1985. Identification of the sugars and acids in birch sap. *J. Food Sci.* 50:266–269.

KARBAN, R. and BALDWIN, I. T. 1997. Induced Responses to Herbivory. University of Chicago Press, Chicago.

KAUSE, A., OSSIPOV, V., HAUKIOJA, E., LEMPA, K., HANHIMÄKI, S., and OSSIPOVA, S. 1999. Multiplicity of biochemical factors determining the quality of growing birch leaves. *Oecologia* 120:102–112.

KEINÄNEN, M., JULKUNEN-TIITTO, R., MUTIKAINEN, P., WALLS, M., OVASKA, J., and VAPAAVUORI, E. 1999. Trade-offs in phenolic metabolism of silver birch: Effect of fertilization, defoliation and genotype. *Ecology* 80:1970–1986.

KESSLER, A. and BALDWIN, I. T. 2002. Plant responses to insect herbivory: The emerging molecular analysis. *Annu. Rev. Plant Biol.* 53:299–328.

KORICHEVA, J. 1999. Interpreting phenotypic variation in plant allelochemistry: Problems with the use of concentrations. *Oecologia* 119:467–473.

KORICHEVA, J., LARSSON, S., HAUKIOJA, E., and KEINÄNEN, M. 1998. Regulation of woody plant secondary metabolism by resource availability: Hypothesis testing by means of meta-analysis. *Oikos* 83:212–226.

LARCHER, W. 1995. Physiological Plant Ecology, 3rd edn. Springer-Verlag, Berlin.

LEHTONEN, J. and HEIKKINEN, R. K. 1995. On the recovery of mountain birch after *Epirrita* damage in Finnish Lapland, with a particular emphasis on reindeer grazing. *Ecoscience* 2:349–356.

MAURICIO, R., RAUSHER, M. D., and BURDICK, D. S. 1997. Variation in defence strategies of plants: Are resistance and tolerance mutually exclusive? *Ecology* 78:1301–1311.

NEUVONEN, S. and HAUKIOJA, E. 1984. Low nutritive quality as defence against herbivores: Induced responses in birch. *Oecologia* 63:71–74.

OLEKSYN, J., KAROLEWSKI, P., GIERTYCH, M. J., ZYTKOWIAK, R., REICH, P. B., and TJOELKER, M. G. 1998. Primary and secondary host plants differ in leaf-level photosynthetic response to herbivory: Evidence from *Alnus* and *Betula* grazed by the alder beetle, *Agelastica alni*. *New Phytol.* 140:239–249.

OSSIPOVA S., OSSIPOV, V., HAUKIOJA, E., LOPONEN, J., and PIHLAJA, K. 2001. Proanthocyanidins from mountain birch leaves: Quantitation and properties. *Phytochem. Anal.* 12:128–133.

OSSIPOV, V., HAUKIOJA, E., OSSIPOVA, S., HANHIMAKI, S., and PIHLAJA, K. 2001. Phenolic and phenolic related factors as determinants of suitability of mountain birch leaves to an herbivorous insect. *Biochem. Syst. Ecol.* 29:223–240.

OSSIPOV, V., LOPONEN, J., OSSIPOVA, S., HAUKIOJA, E., and PIHLAJA, K. 1997. Gallotannins of birch *Betula pubescens* leaves: HPLC separation and quantification. *Biochem. Syst. Ecol.* 25:493–504.

OVASKA, J., WALLS, M., and MUTIKAINEN, P. 1992. Changes in leaf gas exchange properties of cloned *Betula pendula* saplings after partial defoliation. *J. Exp. Bot.* 43:1301–1307.

RIIPI, M., OSSIPOV, V., LEMPA, K., HAUKIOJA, E., and KORICHEVA, J. 2002. Seasonal dynamics of mountain birch foliar phenolics, sugars, and amino acids: Implications for plant–herbivore interactions. *Oecologia* 130:380–390.

RUOHOMÄKI, K., CHAPIN, F. S., III., HAUKIOJA, E., NEUVONEN, S., and SUOMELA, J. 1996. Delayed inducible resistance in mountain birch in response to fertilization and shade. *Ecology* 77:2302–2311.

RUOHOMÄKI, K., TANHUANPÄÄ, M., AYRES, M. P., KAITANIEMI, P., TAMMARU, T., and HAUKIOJA, E. 2000. Causes of cyclicity of *Epirrita autumnata* (Lepidoptera, Geometridae): Grandiose theory and tedious practice. *Popul. Ecol.* 42:211–223.

SALMINEN, J.-P., OSSIPOV, V., HAUKIOJA, E., and PIHLAJA, K. 2001. Seasonal variation in the content of hydrolyzable tannins in leaves of *Betula pubescens*. *Phytochemistry* 57:15–22.

SALMINEN, J.-P., OSSIPOV, V., LOPONEN, J., HAUKIOJA, E., and PIHLAJA, K. 1999. Characterisation of hydrolyzable tannins from leaves of *Betula pubescens* by high-performance liquid chromatography-mass spectrometry. *J. Chromatogr.* 864:283–291.

SAS INSTITUTE INC. 1996. SAS User's Guide: Statistics, Version 6.12. SAS Institute, Gary, NC.

SCHMELZ, E. A., ALBORN, H. T., and TUMLINSON, J. H. 2003. Synergistic interactions between volicitin, jasmonic acid and ethylene mediate insect-induced volatile emission in Zea mays. *Physiol. Plant* 117:403–412.

STRAUSS, S. Y. and AGRAWAL, A. A. 1999. The ecology and evolution of plant tolerance to herbivory. *TREE* 14:179–185.

SULKINOJA, M. and VALANNE, T. 1987. Leafing and bud size in *Betula* provenances of different latitudes and altitudes. *Rep. Kevo Subarctic Res. Stat.* 20:27–33.

TERRILL, T. H., ROWAN, A. M., DOUGLAS, G. B., and BARRY, T. N. 1992. Determination of extractable and bound condensed tannin concentrations in forage plants, protein-concentrate meals and cereal-grains. *J. Sci. Food Agric.* 58:321–329.

VALANNE, N. and VALANNE, T. 1984. The development of the photosynthetic apparatus during bud burst and leaf opening in two subspecies of *Betula pubescens* Ehrh. Rep. *Kevo Subarctic Res. Stat.* 19:1–10.