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

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-
ttributing 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. czepepanovii (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 re-weighed. 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⁻²). 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 \( RCR = \frac{\text{Leaf mass fed} \times \text{Initial larval mass}^{\ln \text{day}^{-1}}} {\text{Time} \times (\text{mg} \times \text{mg}^{-1} \times \text{day}^{-1})} \), the efficiency of conversion of ingested food to larval biomass \( ECI = \frac{\text{Growth/Larval mass fed} \times \text{mg} \times \text{mg}^{-1}} {\text{mg} \times \text{mg}^{-1} \times \text{day}^{-1}} \), and the relative growth rate \( \text{RGR} = \frac{\ln (\text{Final mass}) - \ln (\text{Initial mass})}{\text{Time} \times (\text{mg} \times \text{mg}^{-1} \times \text{day}^{-1})} \); 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.