

# Metabolic Modifications of Birch Leaf Phenolics by an Herbivorous Insect: Detoxification of Flavonoid Aglycones via Glycosylation

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The metabolic modifications of birch (*Betula pubescens* Ehrh.) leaf phenolics in the digestive tract of its major defoliator, larvae of the autumnal moth *Epirrita autumnata*, were studied. The main phenolic acids of birch, *i.e.* chlorogenic and *p*-coumaroylquinic acids, were isomerised in the alkaline digestive tract. Moreover, only 16 to 92% of the ingested amounts of chlorogenic acid were found in the faeces of individual larvae; the missing portion is possibly being used in the formation of reactive *o*-quinones. Water-soluble flavonoid glycosides were mostly excreted unaltered. In contrast, lipophilic flavonoid aglycones were not excreted as such, but as glycosides after being detoxified by *E. autumnata* via glycosylation. When the larvae were fed with leaf-painted acacetin and kaempferide, *i.e.* two naturally occurring birch leaf flavonoid aglycones, acacetin-7-*O*-glucoside and kaempferide-3-*O*-glucoside appeared in larval faeces as major metabolites. However, the efficiency of aglycone glycosylation varied, ranging from 17 to 33%, depending on the aglycone and its dietary level. There was also large variation in the efficiency of glycosylation – from 2 to 57% – among individual larvae. These results demonstrate a compound-specific metabolism of phenolic compounds, leading to different phenolic profiles in the insect gut compared to its leaf diet.

**Key words:** Phenolic Metabolism, Flavonoid Aglycones, Glycosylation

## Introduction

Phenolic compounds have been considered for a long time to be important chemical defences of deciduous trees against insect herbivores. More than 30 years have now elapsed since Paul Feeny's pioneering work (Feeny and Bostock, 1968; Feeny, 1970) showed that the phenolic composition of oak leaf foliage correlated negatively with the performance of an oak leaf feeding herbivore. However, the rather crude chemical methods used in the sixties and seventies – *e.g.* measuring only total phenolics or total contents of phenolic subgroups like hydrolysable or condensed tannins – have only partially been replaced by more accurate measures like HPLC (*e.g.* Grayer *et al.*, 1994; Kause *et al.*, 1999; Ossipov *et al.*, 2001; Henriksson *et al.*, 2003; Tikkanen and Julkunen-Tiitto, 2003). But even when using more detailed chemical methods, often correlations between foliar phenolics and insect performance have been weak or inconsistent between different experiments (Haukioja, 2003). This may depend on a number of

reasons like, for instance, on interactions between phenolics and other, possibly even non-measured leaf constituents, or on variable fates of individual phenolics in digestive tracts of individual insects included in the correlative studies. Furthermore, the possibilities for good correlations may have been destroyed by pooling together individual compounds, which are processed differently by the insect (Salminen and Lempa, 2002).

Recently, we reported different fates of individual birch (*Betula pubescens*) leaf hydrolysable tannins in the guts of larvae of the geometrid moth *Epirrita autumnata*, the main defoliator of birch (Salminen and Lempa, 2002). The aim of this study was to investigate in detail how the rest of the main HPLC-detectable birch leaf phenolics – *i.e.* chlorogenic and *p*-coumaroylquinic acid, flavonoid glycosides and aglycones – are modified by *E. autumnata* larvae. To study the metabolism of phenolics would be relatively easy using artificial diets and commercially available standards (*e.g.* Martin *et al.*, 1987; Barbehenn and Martin, 1992, 1994; Barbehenn *et al.*, 1996; Zimmer, 1999), but

since these diets lack *e.g.* the foliar oxidants, surfactants and the other naturally occurring compounds, it would be difficult to estimate how well these kinds of “artificial” studies are able to reveal the “natural” metabolic modifications of phenolic compounds within insect guts. Therefore metabolic studies herein were done with natural leaf diets only. A particular interest was paid to the metabolism of flavonoid aglycones, a group of phenolics recently found on the lipophilic surface of birch leaves (Keinänen and Julkunen-Tiitto, 1998; Valkama *et al.*, 2003). By feeding *E. autumnata* with two leaf-painted flavonoid aglycones of birch, *i.e.* acacetin (5,7-dihydroxy-4'-methoxyflavone) and kaempferide (3,5,7-trihydroxy-4'-methoxyflavonol), we wanted to find out if even the difference of a single hydroxyl group between the two phenolics makes a difference to their metabolism.

### Materials and Methods

#### *Monitoring metabolic modifications of birch phenolics by Epirrita autumnata larvae*

10 larvae of the autumnal moth, *E. autumnata*, from three different broods of southern Finnish origin were randomly allocated to control leaves originated from a single mature white birch (*Betula pubescens* Ehrh.) growing in the botanical garden of the University of Turku (the same tree as used by Salminen and Lempa, 2002). The larvae were reared under a regime of ambient temperature (approx. 0 °C to 23 °C) and light (approx. 16 h light/8 h dark), and the faeces of individual larvae were collected on a daily basis during the 4<sup>th</sup> and 5<sup>th</sup> larval instars (21<sup>st</sup> May–4<sup>th</sup> June 2000). The combined faeces produced by each individual larva was freeze-dried and extracted with 70% acetone (containing 0.1% ascorbic acid). The phenolic composition of the faeces extracts was compared to that extracted from the foliage of the experimental tree. For further details, see Salminen and Lempa (2002).

#### *The 72-h bioassay with leaf-painted flavonoid aglycones and Epirrita autumnata larvae*

To study in more detail the fates of individual flavonoid aglycones of birch leaf surface in the digestive tract of *E. autumnata*, we conducted an additional 72-h bioassay with the most voracious 5<sup>th</sup> instar *E. autumnata* larvae (27<sup>th</sup>–30<sup>th</sup> May 2002). The larvae were fed with commercially available

acacetin and kaempferide at two levels, 5 and 10 mg/g leaf dry weight. These levels were chosen since they are close to the average contents of acacetin and kaempferide on the surface of young white birch leaves (Valkama *et al.*, 2003; kaempferide preliminarily identified as flavonol methyl ether). The compounds were dissolved into 90% acetone and known volumes were painted onto the surface of fresh birch leaves (originated from two mature *Betula pubescens* trees growing in the botanical garden of the University of Turku) to approximate the desired content. Details of the painting procedure have been described earlier by Salminen and Lempa (2002). The only exception to their method was that before painting the leaves were washed to remove the flavonoids naturally occurring on the birch leaf surface. Washing was conducted by immersing the leaves in 50 ml of 95% ethanol for 10 s and subsequently in 50 ml of water for 5 s to remove the traces of ethanol. The excess water was gently removed by keeping the leaf between two pieces of filter paper. This procedure removed the lipophilic substances – like flavonoid aglycones – dissolved in the sticky surface of leaves, as noticed by the lack of stickiness in the washed leaves compared to unwashed ones. Three kinds of leaves were used as controls: (1) non-washed and non-painted, (2) washed but non-painted, and (3) washed and painted with 90% acetone. The bioassay included 84 larvae of *E. autumnata*, from four different broods of southern Finnish origin. 12 larvae were randomly allocated to each level of painted compounds and each type of controls. Larvae and leaves were placed in 20-ml vials at + 12 °C and approx. 16 h light/8 h dark. The bioassay was a no-choice experiment: the larvae were placed individually in vials and received only one type of food. Leaves were replaced with fresh ones after 24 and 48 h. At the same time, and after 72 h, the faeces of individual larvae was collected, freeze-dried and weighed. Larval leaf consumption during the experiment was measured as described by Salminen and Lempa (2002).

#### *Sample preparation and HPLC-DAD/HPLC-ESI-MS analysis*

Leaf samples were collected in the beginning of each bioassay to monitor the foliar phenolic composition of the experimental trees. Leaf sample collection, sample preparation and extraction fol-

lowed Salminen *et al.* (2001). In short, freeze-dried leaves were extracted with 70% acetone (containing 0.1% ascorbic acid). After freeze-drying of the extracts, water-soluble phenolics were dissolved into  $3 \times 2$  ml water; the lipophilic residues of the extracts were then dissolved into  $3 \times 1$  ml ethanol. The combined faeces produced by each individual larva were freeze-dried and extracted in the same way as the birch leaves. Leaf and faeces extracts were filtered through  $0.45\text{-}\mu\text{m}$  PTFE filters and analysed with HPLC-DAD at 280 nm and 349 nm. The HPLC system (Merck-Hitachi, Tokyo, Japan) consisted of a pump L-7100, a diode array detector L-7455, a programmable autosampler L-7250, and an interface D-7000. Column and chromatographic conditions were as described earlier (Salminen *et al.*, 1999), except that  $0.1\text{ M H}_3\text{PO}_4$  was replaced with  $0.05\text{ M H}_3\text{PO}_4$ . A selected set of samples was analysed also with HPLC-ESI-MS as described by Salminen *et al.* (1999). Phenolic compounds were identified on the basis of their UV and mass spectra and retention times reported in the literature (Ossipov *et al.*, 1995, 1996; Salminen *et al.*, 1999, 2001; Valkama *et al.*, 2003). Phenolic acids were quantified as gallic, chlorogenic and *p*-coumaric acid, myricetin glycosides as myricetins, quercetin glycosides as quercetins, kaempferol glycosides as kaempferols and flavonoid aglycones as acacetins.

#### *Isolation and identification of the metabolic products of acacetin and kaempferide*

To produce enough larval metabolites of acacetin and kaempferide for their isolation and structure elucidation, two separate sets of 5<sup>th</sup> instar *E. autumnata* were fed with washed birch leaves artificially enriched with high levels ( $> 20$  mg/g dry weight) of acacetin or kaempferide only. After all leaf-painted compounds had been consumed, faeces of the two sets of larvae were freeze-dried and extracted, and one major metabolite detected in the extract of both sets. The two metabolites were isolated from the water-soluble fractions of the faeces extracts with Merck LiChroprep RP-18 column ( $44 \times 3.7$  cm in diameter,  $40\text{--}63\text{ }\mu\text{m}$ ; Darmstadt, Germany) using different gradients of  $\text{H}_2\text{O}$  in  $\text{CH}_3\text{CN}$ . The metabolites were analysed with HPLC-ESI-MS, and  $^1\text{H}$  NMR spectra were recorded on a Jeol Lambda 400 MHz series spectrometer (Tokyo, Japan) and a Bruker routine AM-200 NMR spectrometer (Karlsruhe,

Germany) using  $\text{DMSO-d}_6$  as a solvent. To confirm the identities of the sugar moieties of the metabolites, they were hydrolysed according to Chin *et al.* (2000) and analysed by GC-MS (Perkin-Elmer Autosystem XL/TurboMass Gold, Norwalk, USA) after trimethylsilylation.

## Results and Discussion

Comparing the composition and content of phenolic compounds in the leaf diet and in the faeces of each individual larva, we were able to elucidate the metabolic modifications of different compounds in the larval digestive tract. The HPLC-DAD chromatograms of the water-soluble and lipophilic fractions of the birch leaf diet and the corresponding fractions of larval faeces revealed significant differences in the composition of phenolic compounds (quantitative results shown in Table I).

#### *The appearance of uric acid in larval faeces*

The water-soluble fraction of larval faeces contained a non-dietary compound as one of the major metabolites (compound 23 in Table I). On the basis of its UV spectrum ( $\lambda_{\text{max}}$  230 and 283 nm), molar mass (168 g/mol) and mass spectral fragmentation ( $m/z$  of the main fragment was 124) in ESI-MS and co-elution with a commercially available standard, this nitrogen-containing metabolite was identified as uric acid. It was surprising to find such a major nitrogenous waste product in the larval faeces of *E. autumnata*, an herbivore whose growth is supposed to be limited by the nitrogen intake from the diet (*e.g.* Ruohomäki *et al.*, 1996; Kaitaniemi *et al.*, 1998; Kause *et al.*, 1999). However, due to practical reasons we had sampled the faeces once a day only; this might have enabled post-intestinal modification of the larval metabolites and the formation of artefacts in the faeces. To rule out this possibility, we did an additional experiment with fifteen 5<sup>th</sup> instar *E. autumnata* larvae. Larvae were fed with birch (*Betula pubescens*) leaves for 9 h and during that time every second drop of faeces produced by individual larvae was collected directly into 70% acetone, *i.e.* the extraction solvent. The other drops of faeces were collected in a separate vial and left to stand at room temperature for 24 h; after that time both sets of faeces were extracted with 70% acetone. Interestingly, the only difference between the HPLC traces of the two differently collected faeces samples was the absence of uric acid in the

Table I. Distribution of the water-soluble and lipophilic phenolic compounds (mg/g dry weight) in the leaf diet (*Betula pubescens*) and faeces of the 5<sup>th</sup> instar *Epirrita autumnata* larvae.

Class	No	Compound	Leaf diet		Faeces	
			Water-soluble fraction	Lipophilic fraction	Water-soluble fraction <sup>a</sup>	Lipophilic fraction <sup>a</sup>
Vacuolar birch leaf phenolics	1	Chlorogenic acid	6.85	–	4.42 <sup>b</sup>	–
	2	<i>p</i> -Coumaroylquinic acid	0.52	–	1.67 <sup>b</sup>	–
	3	Myricetin-3- <i>O</i> -galactopyranoside	1.78	–	0.68	–
	4	Quercetin-3- <i>O</i> -galactopyranoside	2.34	–	2.05	–
	5	Quercetin-3- <i>O</i> -glucuronopyranoside	8.80	–	8.46	–
	6	Quercetin-3- <i>O</i> -arabinofuranoside	1.10	–	n.q.	–
	7	Kaempferol-3- <i>O</i> -glucopyranoside	0.99	–	n.q.	–
	8	Kaempferol glycoside	2.26	–	1.98	–
	9	Kaempferol glycoside	1.06	–	0.82	–
	10	Kaempferol-3- <i>O</i> -rhamnopyranoside	0.65	–	n.q.	–
Cuticular birch leaf phenolics	11	Naringenin	–	0.47	–	–
	12	Apigenin	–	0.92	–	–
	13	Kaempferol	–	1.86	–	–
	14	Flavonol methyl ether	–	2.02	–	–
	15	Flavanone methyl ether	–	7.09	–	–
	16	Acacetin	–	2.72	–	–
	17	Kaempferide	–	7.73	–	traces
	18	Flavonol dimethyl ether	–	3.84	–	–
	19	Pentahydroxyflavone trimethyl ether	–	1.31	–	–
	20	Flavanone	–	2.01	–	traces
	21	Kaempferol derivative	–	1.97	–	traces
	22	Apigenin derivative	–	1.53	–	–
Additional faecal metabolites	23	Uric acid	–	–	7.41	–
	24	Flavonoid glycosides (aglycones glycosylated by <i>E. autumnata</i> larvae) <sup>c</sup>	–	–	18.83	–

<sup>a</sup> Mean value of ten larvae.

<sup>b</sup> A sum of four isomers.

<sup>c</sup> A sum of eleven flavonoid glycosides.

n.q. = not quantified (peaks overlapped by compounds 24).

extract of 70% acetone collection (data not shown). This clearly suggested that rather than a larval waste product, uric acid actually was an artefact caused by post-intestinal modification of larval metabolites. This finding was further supported when the uric acid free extract was left to stand at room temperature; the composition of the phenolic metabolites was not altered but increasing amounts of uric acid appeared in the extract as a function of time. Thus it is presumable that uric acid is a catabolic product of some water-soluble, non-phenolic and nitrogenous metabolites that as such could not be detected by UV spectroscopy. Nevertheless, this result questions whether the previous studies reporting the presence of uric acid in insect faeces have been dealing just with an artefact, and not with a real product of insect metabolism as such (*e.g.* Bursell, 1965; Ritter, 1996).

#### *The metabolic modifications of chlorogenic and p-coumaroylquinic acid*

The HPLC-DAD and HPLC-ESI-MS analysis of the faeces showed that the main phenolic acids of the leaf diet – having a difference of only one hydroxyl group *i.e.* chlorogenic and *p*-coumaroylquinic acid (compounds 1 and 2 in Table I) – were isomerised in the larval digestive tract. Such isomerisation has been observed to happen to chlorogenic acid *in vitro* under basic conditions (Nagels *et al.*, 1980), suggesting that basic conditions would prevail also in the gut of *E. autumnata*. To ensure this the gut pH of some 5<sup>th</sup> instar larvae was measured and found to be close to 9, *i.e.* strongly basic just like in most lepidopteran species (Martin and Martin, 1983; Appel, 1993). Interestingly, these kinds of basic conditions may promote the auto-oxidation of phenolic compounds

even in the absence of polyphenol oxidases (Appel, 1993). Moreover, the oxidation of phenolics into quinones, especially *o*-quinones, is thought to be one of the main mechanisms in turning otherwise harmless compounds into reactive and harmful ones, at least from the insect's point of view (Felton *et al.*, 1989; Appel, 1993). Chlorogenic acid, one of the main phenolics of birch leaves (see Table I), would be an ideal substrate for the production of *o*-quinones due to its benzene ring located *o*-dihydroxyl group. Interestingly, on average 63% of the ingested chlorogenic acid was missing from the faeces of the larvae (when calculated as a sum of all isomers), possibly as a result of the transformation of chlorogenic acid (or its isomers) into *o*-quinones in the digestive tract of *E. autumnata*.

#### *The metabolic modifications of flavonoid glycosides and aglycones*

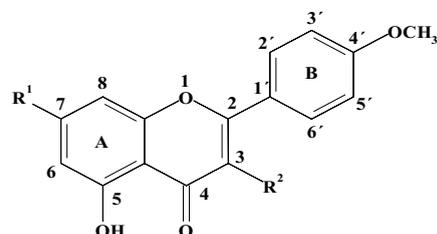
Flavonoid glycosides of the birch leaf diet were the only group of compounds that were mostly excreted unchanged (*e.g.* compounds 4, 5, 8 and 9 in Table I), although there were compound-specific differences in their metabolism as well. To pinpoint the differences in larval metabolism even within one phenolic subgroup, *i.e.* the flavonoids, the most easily observed changes in the composition of phenolics between the leaf diet and faeces were related to flavonoid aglycones (compounds 11–22 in Table I). The lipophilic aglycones were almost totally absent in the lipophilic fraction of the faeces, while several metabolites having UV spectra similar to the flavonoid aglycones appeared into the water-soluble fraction (compounds 24 in Table I). However, the retention times or molar masses of these metabolites did not match with the flavonoid aglycones or glycosides present in the larval diet (Ossipov *et al.*, 1995, 1996; Valkama *et al.*, 2003). In contrast, the mass spectral characteristics of the metabolites suggested them to be flavonoid glycosides, of which the aglycone parts had similar molar masses as the aglycones found on birch leaf surface (Valkama *et al.*, 2003). Moreover, the level of these glycosides was highly suppressed when *E. autumnata* larvae were fed with leaves from which most of the leaf surface aglycones were removed by washing with 95% ethanol. Therefore the new faecal flavonoid glycosides were preliminarily classified as glycosylation products of flavonoid aglycones by *E. autumnata*

larvae. The aglycones as such are lipophilic but become water-soluble once glycosylated; this explains the almost total absence of flavonoid aglycones in the lipophilic fraction of faeces and the presence of the additional flavonoid glycosides in the water-soluble fraction (see Table I).

#### *Ensuring the glycosylation of flavonoid aglycones by *Epirrita autumnata* larvae*

Since flavonoid aglycones were the group of phenolics that were most modified by *E. autumnata*, more attention was paid to their metabolic fate in the larval digestive tract. To prove the above mechanism of aglycone glycosylation, two separate sets of 5<sup>th</sup> instar *E. autumnata* were fed with washed birch leaves that were artificially enriched with high levels (> 20 mg/g dry weight) of acacetin (compound 16 in Table I) or kaempferide (compound 17 in Table I) only. When all leaf-painted compounds had been consumed by larvae, the faeces of the two sets of larvae were freeze-dried and extracted, and one major metabolite was detected in the extract of both sets. The *m/z* values from negative ion ESI-MS for the metabolite of acacetin were 445, 891 and 283, and for the metabolite of kaempferide 461, 923 and 299. These values corresponded to  $[M-H]^-$ ,  $[2M-H]^-$  and  $[aglycone-H]^-$ , respectively. As the  $[M-H]^-$  value for acacetin is 283 and for kaempferide 299, the metabolites were tentatively identified as acacetin and kaempferide glycosides. After GC-MS analysis of the products of hydrolysis of these compounds, the sugar moiety of both of the flavonoids was confirmed to be glucose.

By comparing the <sup>1</sup>H NMR spectra of acacetin and kaempferide glucosides to those measured separately for acacetin and kaempferide, and to the matching spectra found in the literature (Sharaf *et al.*, 1997; Nielsen *et al.*, 1998; Curir *et al.*, 2001), the two metabolites were finally identified as acacetin-7-*O*-glucoside and kaempferide-3-*O*-glucoside, respectively (see Fig. 1). The <sup>1</sup>H NMR data for the acacetin-7-*O*-glucoside are as follows: 8.00 (2H, d, *J* = 8.8 Hz, H-2' and H-6'), 7.10 (2H, d, *J* = 8.8 Hz, H-3' and H-5'), 6.75 (1H, d, *J* = 4.8 Hz, H-8), 6.73 (1H, s, H-3), 6.70 (1H, d, *J* = 4.8 Hz, H-6), 5.69 (1H, d, *J* = 4.0 Hz, H-1 of glucose), 3.85 (3H, s, H-4', OMe), 3.15–3.50 (sugar protons). The <sup>1</sup>H NMR data for the kaempferide-3-*O*-glucoside are as follows: 12.45 (1H, s, H-5, OH), 8.09 (2H, d, *J* = 8.96 Hz, H-2' and H-6'), 7.05



	R <sup>1</sup>	R <sup>2</sup>
Acacetin	OH	H
Kaempferide	OH	OH
Acacetin-7-O-glucoside	O-glucose	H
Kaempferide-3-O-glucoside	OH	O-glucose

Fig. 1. Structures of the flavonoid aglycones (acacetin and kaempferide) fed to the 5<sup>th</sup> instar *Epirrita autumnata* larvae, and the corresponding glucosides (acacetin-7-O-glucoside and kaempferide-3-O-glucoside) found in larval faeces after detoxification of the aglycones by *Epirrita autumnata* via glycosylation.

(2H, d,  $J = 8.96$  Hz, H-3' and H-5'), 6.18 (1H, s, H-8), 5.98 (1H, s, H-6), 5.40 (1H, d,  $J = 7.90$  Hz, H-1 of glucose), 3.83 (3H, s, H-4', OMe), 3.10–3.60 (sugar protons).

#### The levels of glycosylation of flavonoid aglycones by 5<sup>th</sup> instar *Epirrita autumnata* larvae

To study the levels of glycosylation of acacetin and kaempferide by 5<sup>th</sup> instar *Epirrita autumnata*, we analysed the faeces produced by individual lar-

vae during the 72-h bioassay. As a further proof for the observed detoxification mechanism of flavonoid aglycones, the amounts of faecal acacetin-7-O-glucoside and kaempferide-3-O-glucoside correlated positively with the ingested amounts of acacetin and kaempferide, regardless of the dietary levels of the ingested aglycones (see Fig. 2). However, the efficiency of aglycone glycosylation was not even close to complete; its average level varied from  $17 \pm 2\%$  to  $33 \pm 4\%$  (mean  $\pm$  SE) depending on the aglycone and its dietary level. Interestingly, as the overall glycosylation efficiency by the larvae was  $22 \pm 2\%$  (mean  $\pm$  SE), within individual larvae the efficiency varied even from 2 to 57%. Moreover, the capacity of *E. autumnata* to glycosylate acacetin seemed to be overloaded already at the 5 mg/g level; contents of acacetin-7-O-glucoside did not any more increase in the faeces when the absolute intake of acacetin was experimentally doubled (compare Fig. 2A, B). On the contrary, although kaempferide was glycosylated less efficiently than acacetin at the 5 mg/g dose (compare Fig. 2A, C), the yield of faecal kaempferide-3-O-glucoside was almost doubled with the doubled dietary intake at the 10 mg/g level (compare Fig. 2C, D). These findings suggest that even a difference of one hydroxyl group between the flavonoids makes a clear difference to their metabolism. Moreover, also traces of non-transformed acacetin and kaempferide were found in the faeces (0.5–1.3% of the ingested amounts;

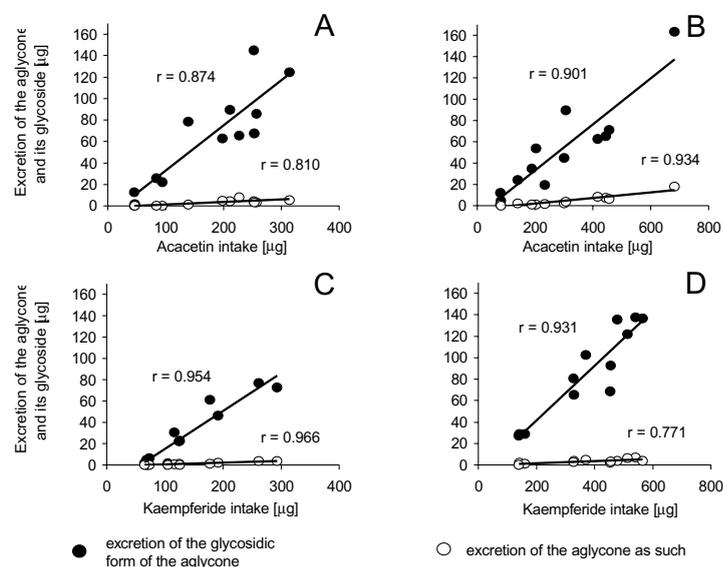


Fig. 2. The amounts of ingested acacetin (A: dietary level 5 mg/g; B: dietary level 10 mg/g) and kaempferide (C: dietary level 5 mg/g; D: dietary level 10 mg/g) by the 5<sup>th</sup> instar *Epirrita autumnata* larvae vs. the excreted amounts of these aglycones together with their glycosidic metabolites. In all,  $n = 12$  larvae.

see Fig. 2). While 65–83% of the ingested aglycones remained undetected in the faeces (as aglycones or glycosides), their ultimate fate represents an open question; presumably the undetected portion was either oxidised in the larval digestive tract, or due to the lipophilic nature bound to the fat body or peritrophic membranes of the insects (Barbehenn, 2001).

*Ecological implications of the observed patterns of phenolic metabolism in Epirrita autumnata*

Previously we showed that birch leaf hydrolysable tannins are partially hydrolysed by *E. autumnata* larvae (Salminen and Lempa, 2002). The present study further highlights the fact that individual phenolics face highly differential fates in the digestive tract of a lepidopteran herbivore. Chlorogenic and *p*-coumaroylquinic acid were isomerised, flavonoid glycosides excreted without visible metabolic modifications, whereas flavonoid aglycones were partially detoxified *via* glycosylation. These patterns strongly indicate that the phenolic profiles in the insect gut are very much different from those in the leaf diet. Further, the efficiency of processing of even a single phenolic compound was highly variable among individual insects (*e.g.* 8–84% of the ingested chlorogenic acid was recovered from the faeces of larvae eating foliage of the same tree). The reasons behind

such a variation are largely unknown, but at any case it is hardly surprising that the results of ecological studies trying to correlate insect performance directly with the phenolic composition of leaves have been variable and hard to repeat (Haukioja, 2003). We propose that finding the true effects of particular phenolic compounds on insect performance demands knowledge of the ways and levels of phenolic metabolism in the digestive tract of individual insects. For instance, instead of using the foliar content of chlorogenic acid in the correlations it could be more useful to correlate insect performance with the level of chlorogenic acid not recovered from the faeces; this level presumably being closer to the portion of chlorogenic acid biologically active against that particular insect. To conclude, in the absence of detailed studies on the metabolism of plant phenolics in the digestive tracts of different species of herbivorous insects, it is likely that many of the mechanisms of action – or even the lacks of long-proposed actions – of these compounds are still to be discovered.

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