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Insects as leaf engineers: can leaf-miners alter leaf structure for birch aphids?

S. N. JOHNSON,*†‡ P. J. MAYHEW,* A. E. DOUGLAS* and
S. E. HARTLEY‡§

**Department of Biology, University of York, PO Box 373, York YO10 5YW, UK and ‡Centre for Ecology & Hydrology, Hill of Brathens, Banchory AB31 4DA, UK*

Summary

1. This study examined the indirect impacts of leaf-mining insects, *Eriocrania* spp. Zeller (Lepidoptera: Eriocraniidae) on a phloem-feeding insect, *Euceraphis betulae* Koch (Homoptera: Drepanosiphinae). While many insect herbivores affect one another through changes to host plant chemical composition, *Eriocrania* also has the potential to affect *E. betulae* through structural modification of a shared leaf.

2. *Euceraphis betulae* mortality was higher when caged on leaves with *Eriocrania* leaf-miners. Mortality was not affected by the amount of leaf mined or elevated phenolic compound concentrations in mined leaves, but leaf-miner induced damage to the midrib was strongly correlated with poor aphid survival. In field surveys, *E. betulae* was significantly less abundant on mined leaves with midrib damage than on mined leaves with just lamina damage, or mine-free leaves.

3. Experiments simulating leaf-miner damage on *B. pendula* leaves pinpointed midrib damage as being associated with higher *E. betulae* mortality, whereas lamina damage had no effect on aphid mortality. Disruption of phloem hydraulics is proposed as the mechanism underpinning the negative impacts on the aphid.

4. *Eriocrania* larvae mining leaves with manually damaged midribs weighed more than those in which the midrib was intact. There was also a trend towards higher nitrogen concentrations in leaves in which *Eriocrania* had damaged the midrib. There could therefore be a selective advantage to leaf-miners that damage the midrib if severance improves leaf nutritional quality, in addition to rendering the leaves unsuitable to potential competitors.

Key-words: Aphid, midrib, phenolics, phloem

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Introduction

It is widely acknowledged that interspecies interactions comprise both direct pairwise interactions (e.g. exploitative competition and predation) and indirect interactions, mediated by a third species (Wootton 1994; Polis 1998; Fox & Olsen 2000). The importance of plant-mediated indirect interactions in shaping insect-herbivore communities in particular, is becoming widely recognized (Masters & Brown 1997) and illustrated by a growing catalogue of empirical studies (e.g. Denno *et al*. 2000; Fisher, Hartley & Young 2000; Petersen & Sandstrom 2001). Herbivore-induced changes to plant chemical composition frequently involve reductions in the availability of nitrogen compounds (Denno *et al*. 2000) and increases in secondary compound concentrations (Haukioja *et al*. 1990). Both of these changes have been associated with detrimental effects on other phytophagous insects and hence may underpin competitive indirect interactions between herbivores (Denno, McClure & Ott 1995). A third type of plant modification by insect herbivores, which is overlooked in comparison but could be equally important, is structural modification of plant tissues in a way that affects other phytophagous insects. This possibility has generally been disregarded (Masters & Brown 1997) or reported as idiosyncratic phenomena (e.g. Karban 1986; Mattson 1986), but the growing realization that many organisms may act as ‘ecosystem engineers’ by physically manipulating resource availability for other organisms (Jones, Lawton & Shachak 1994, 1997), has recently stimulated interest in how physically mediated interactions between insect herbivores could arise (e.g. Fukui 2001).

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†Author to whom correspondence should be addressed. E-mail: S.N.Johnson@reading.ac.uk
§Present address: School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 92G, UK.

Physical modification of a plant by an insect herbivore might be an incidental and inconsequential result of feeding, or it may ameliorate resource quality for itself or its offspring (Tuomi *et al*. 1994). Herbivore-induced disruption of the vasculature, for instance, is a common physical modification of plant tissues (Price & Louw 1996). Benefits derived from modifying vasculature include evasion of plant defence compounds (Carroll & Hoffman 1980; Dussourd & Eisner 1987; Dussourd & Denno 1994), predisposing the plant to subsequent attack (West 1947; Coutts & Dolezal 1966) and causing nutrient accumulation in disconnected tissues (Forcella 1982; White 1984). Gall-forming insects that improve plant nutritional quality by physically modifying vasculature are good examples of such ‘resource regulation’ (Price & Louw 1996; Hartley 1998; Wool *et al*. 1999). Gall-formers cause short-term structural modification of plant vessels, as well as longer-term changes in plant growth and architecture that could potentially affect other insect species feeding on shared leaves. Phloem-feeding insects might be particularly susceptible to vasculature manipulation by other insects, as they are reliant on the high flux of phloem sap (Raven 1983; Hartley & Jones 1997). While most attention to date has focused on the impacts of gall-forming insects on leaf vasculature, leaf-mining insects may also cause similar effects by virtue of their similar endophagous lifestyle (Connor & Taverner 1997).

The purpose of this study was to identify the processes by which two insect herbivores of different feeding guilds might interact via changes to their shared host plant, focusing on the physical changes to leaves caused by insects when feeding. Our research examined how a free-living aphid, *Euceraphis betulae* Koch (Homoptera: Drepanosiphinae), is affected by co-occurring leaf-mining moths of the genus *Eriocrania* Zeller (Lepidoptera: Eriocraniidae). The genus *Eriocrania* consists of six species in the UK, five of which are indistinguishable during larval stages, but whose identical life histories have led to them being treated as a single taxon in this and other research (e.g. Koricheva & Haukioja 1994; Fisher, Hartley & Young 1999, 2000). The aphid and leaf-miner feed on leaves of the deciduous tree *Betula pendula* Roth (Betulaceae) (Silver Birch). The two species are spatially separated and do not compete directly for the same resource; *Eriocrania* feeds internally between the upper and lower lamellae, whereas *E. betulae* feeds on phloem sap from the basal midrib and petiole (Hajek & Dahlsten 1986). *Eriocrania* have the potential to indirectly affect *E. betulae*, however, if their internalized feeding disrupts the vascular system.

The specific aims of this investigation were: (1) to establish whether there was a competitive interaction between the *Eriocrania* leaf-miner and the aphid *E. betulae* on shared *B. pendula* leaves; and (2) to determine the mechanistic basis for any competition observed, and its implications for both the aphid and leaf-miner. To achieve these aims, we measured aphid performance when reared with *Eriocrania*, and quantified the amount of leaf damaged by mining activity and its effect on foliar phenolic compound concentrations – the main group of secondary compounds in birch (Haukioja & Neuvonen 1985; Hartley & Firn 1989; Ossipov *et al*. 2001). We tested whether leaf-mining effects on aphid performance were the result of damage to the primary vasculature by comparing the effects of simulated leaf-miner damage to the leaf midrib and lamina on aphid survivorship. We also conducted surveys (a) to ascertain the frequency at which field populations of *Eriocrania* damaged the midrib and (b) to compare aphid occurrence on *Eriocrania* mined leaves with and without midrib damage. We examined whether midrib damage had beneficial effects on *Eriocrania* performance, for example by causing nitrogen compounds to accumulate in leaves.

Materials and methods

SITE DESCRIPTION AND STUDY SYSTEM

The study site, Dalhaikie Flat, Aberdeenshire, UK (57°075′ N, 2°582′ W; OS 3645 8992), comprised *c.* 3 ha of almost continuous birch thicket dominated by *Betula pendula* (with small numbers of *B. pubescens*) that had regenerated after mature trees were felled in 1989. All field investigations were conducted between May and July of 1999, 2000 and 2001. The aphid *E. betulae* lives and feeds entirely on birch, reproducing asexually from spring until autumn. Third instar aphid larvae (referred to as ‘young larvae’) and fourth instar larvae (‘old larvae’) were collected from foliage at the site for experiments. The adult *Eriocrania* moth oviposits under the epidermis of newly emerged birch buds during spring. The leaf-mining larvae emerge from the eggs 2–3 weeks later and feed internally, forming a blotch mine as they progress. Once larval development is complete, *Eriocrania* exits the leaf-mine and overwinters in the soil until the following spring whereupon the female oviposits as an adult (Heath 1976).

APHID PERFORMANCE ON *ERIOCRANIA* MINED LEAVES

In May 1999, 20 saplings with at least five mined leaves were labelled. On each tree, two of the mined leaves and two adjacent mine-free leaves were clip-caged. Pairs of either ‘young’ or ‘old’ aphid larvae were randomly assigned to each cage, so that each tree possessed: (a) a mined leaf with two young larvae; (b) a mined leaf with two old larvae; (c) a mine-free leaf with two young larvae; and (d) a mine-free leaf with two old larvae. After 7 days, aphid larvae were recovered with the leaf and surviving aphids scored.

Mined leaves were digitally scanned to measure mined and total leaf surface area using image analysis software (Scion Image Beta 4·02™, Scion Corp., USA). Leaf-mining damage to the midrib was recorded. All leaves (mine and mine-free) were frozen (−18 °C) within 3 h of recovery from the field, freeze-dried for 36 h, and assayed for total phenolic compound content using the Folin-Ciocalteau method outlined by Waterman & Mole (1994) and Kerslake, Woodin & Hartley (1998).

SIMULATED LEAF-MINER DAMAGE EXPERIMENT

To test whether leaf-miner damage to the midrib affected aphid survivorship, leaf-miner damage was simulated on birch leaves. In May 2000, six mine-free leaves from similar regions of the canopy of 20 birch saplings (1·7–2·0 m height) were selected. On each tree, two leaves had approximately 30 mm^2 of the abaxial lamina cauterized using an electronics soldering iron, two leaves had 30 mm^2 of the abaxial midrib region cauterized, and the remaining two leaves were undamaged. After 24 h, all leaves were clip-caged, and pairs of either young or old aphid larvae were assigned randomly to the leaves in a factorial manner (as above). After 7 days, aphids and leaves were recovered from the field. Leaves were stored and analysed for phenolic compound content (as above), and aphid survivorship and larval instar recorded for each leaf. Live aphids 2 were weighed (Cahn Instruments™, CA) to an accuracy of ±1 µg.

SURVEYS OF LEAF-MINING DAMAGE AND APHID ABUNDANCE ON MINED LEAVES

The closest 300 trees to a random co-ordinate at the 3 site were surveyed for *Eriocrania* mines (4 min per tree) between 6 and 8 June 2000. Patterns of leaf-miner damage were classified according to which zones of the leaf had been mined (Fig. 1). Leaf-mines were re-examined 3 weeks later and leaf-mining patterns were re-classified using the same protocol.

Aphid abundance on mined leaves, with and without midrib damage, was measured on 63 trees (from the original 300) in similar regions of the canopy. From each tree, a mined leaf with midrib damage and one without, together with a neighbouring mine-free leaf were monitored. The number of aphids on each was scored on alternate days between 11 and 25 June 2000.

LEAF-MINER PERFORMANCE ON MIDRIB DAMAGED LEAVES

This experiment addressed how damage to the midrib might affect leaf-miner performance. The shortest distance between the mine and the midrib was measured on 100 leaves with recently initiated *Eriocrania* mines in May 2001. After 7 days, 15 trees bore at least four mined leaves with midrib damage and at least eight mined leaves with just lamina damage ('midrib intact'). On each of the 15 trees, one of 'midrib intact' mined leaves had the midrib manually severed using a mounted dissection pin, while the other two leaves were left unmodified. All three leaves were clip-caged and monitored until leaf-miner emergence from the mine, whereupon the leaf and the emergent larva were recovered from the field. The larva was weighed (Cahn Instruments™) to an accuracy of ±1 µg, and the leaf digitally scanned and measured as above. Leaf area was converted to leaf biomass using a regression equation (biomass (mg) = 17·05 × surface area (cm^2) − 3·9129) derived from leaf mass and surface area measurements from 100 leaves collected from the field ($r = 0{\cdot}966$).

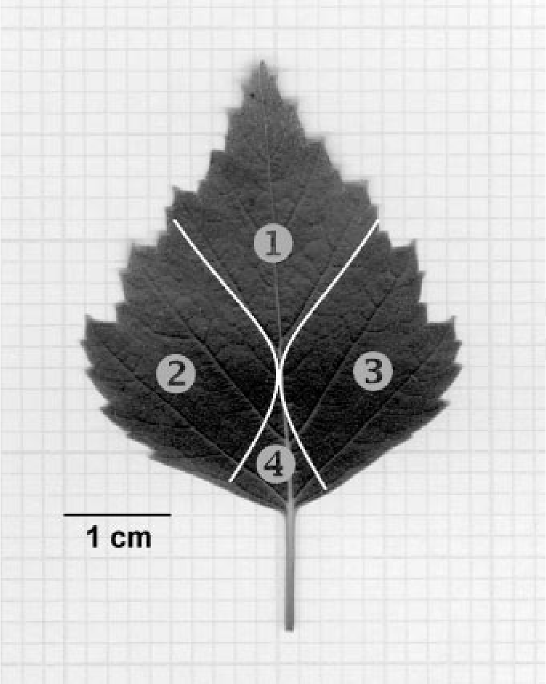

Fig. 1. Zones on *B. pendula* leaves used to assess *Eriocrania* spp. blotch mine development and orientation on the leaf (as viewed from base to apex).

LEAF CHEMISTRY

The 15 trees used in the leaf-miner performance experiment (see above) were also used for leaf chemical analysis. Mined leaves labelled in May 2001 that were not used in the leaf-miner performance experiment (above) were classified as either 'midrib damaged' or 'midrib intact'. Half of the 'midrib intact' leaves had the midrib manually severed, 48 h prior to collection from the field for C/N analysis while the leaf-miners were still feeding. At the end of the leaf-miner performance experiment (June 2001), five mine-free leaves that were adjacent the caged leaf-miner leaves were removed from each of the 15 trees for C/N analysis. All leaves were oven dried, milled to a fine powder, and *c.* 5 mg of leaf assayed for total carbon and nitrogen concentrations using a CHN combustion analyser (Model E1110, Carlo Erba Instruments, Milan, Italy).

STATISTICAL ANALYSIS

Statistical analysis was carried out in SAS version 8·12 (SAS Institute 1999). Aphid survivorship on mined and mine-free leaves was analysed using a generalized linear model with binomial distribution and logistic link function (PROC GENMOD) (SAS Institute 1999). Tree number, larval age ('young' or 'old'), and leaf-miner presence were analysed together and the least significant ($P > 0·10$) explanatory variable removed sequentially until all were significant. This procedure was also used to identify which aspect of leaf-mining affected aphid survivorship. Aphid survivorship and development on artificially damaged leaves were also analysed this way. *Post hoc* leaf type comparisons were made in which P values were calculated based on the asymptotic χ^2 distribution of the χ^2 statistic (SAS Institute 1999). Aphid mass conformed to normality and was compared using a general linear model (PROC GLM) (SAS Institute 1999), although low survivorship on midrib damaged leaves meant this category was excluded from the analysis. Aphid abundance on mined leaves, with and without midrib damage, and mine-free leaves was analysed using a generalized linear mixed model (GLIMMIX) (SAS Institute 1999) with Poisson distribution and log link function. Tree replicate was fitted as a random term in the model, and between leaf type comparisons made using differences in least squares means tests calculated within the macro. Degrees of freedom for fixed effects were adjusted using the Satterthwaite formulae (Littell *et al.* 1996). In the leaf-miner performance experiment, those response variables conforming to normality were analysed with a general linear model (PROC GLM) (SAS Institute 1999). Leaf-miner mass at emergence was analysed in the same way with amount of leaf eaten and nitrogen content of adjacent leaves fitted as covariates. Comparisons were made using Tukey's *post hoc* tests. Arcsine square root transformations of leaf C/N data were analysed using a general linear model (PROC GLM).

Results

APHID SURVIVORSHIP ON *ERIOCRANIA* MINED LEAVES

Euceraphis betulae survivorship was significantly depressed when caged in the presence of an *Eriocrania* leaf-miner for both 'young' and 'old' aphid larvae (Fig. 2 and Table 1a). Young larvae survivorship was lower than for their older counterparts when caged on leaves, but there was no interaction between the effects of the leaf-miner and aphid age on survivorship (Table 1a). Examining aphid survivorship on mined leaves alone (Table 1b) showed that the amount of leaf mined (both the absolute area and as a proportion of the whole leaf) and phenolic compound content had no significant effect on *E. betulae* survivorship. Leaf-mining damage to the midrib, in contrast, was strongly correlated with low aphid survivorship, with no significant difference between young and old aphid larvae (Table 1b).

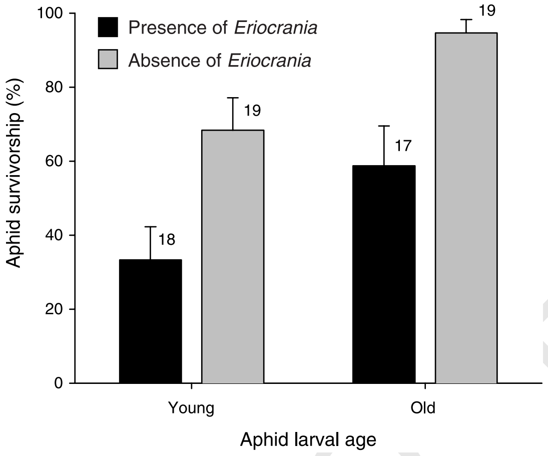

Fig. 2. *Euceraphis betulae* survivorship (mean % ± SE) when caged in the presence and absence of an *Eriocrania* leaf-miner. Number of replicates is shown in figure.

SIMULATED LEAF-MINER DAMAGE EXPERIMENT

Euceraphis betulae survivorship was significantly lower and its development retarded when reared on leaves in which the midrib had been damaged compared with those in which the lamina had been damaged or the leaf remained intact (Table 2). There was no significant difference in aphid survivorship and development on lamina damaged and undamaged leaves. There was also no significant difference in aphid mass upon reaching adulthood when reared on lamina damaged and undamaged leaves (1·33 and 1·35 mg, respectively) or the final larval instar 'old larvae' (1·26 and 1·24 mg, respectively) ($F_{1,83} = 0·07$, $P = 0·791$). As with the caging experiment with leaf-miners, young aphid larvae survived and developed less well than their older equivalents in clip-cages, but both age groups were equally affected by the type of damage imposed on the leaf (Table 2). Phenolic compound concentrations were significantly higher in cauterized leaves, whether the damage was imposed on the midrib (154 mg g^{-1} dry mass) or the lamina (152 mg g^{-1} dry mass) compared with undamaged leaves (134 mg g^{-1} dry mass) ($F_{2,57} = 3·94$, $P = 0·025$). Phenolic concentrations in cauterized leaves were statistically indistinguishable from each other ($t = -0·158$, $P = 0·986$).

SURVEYS OF LEAF-MINING DAMAGE AND APHID ABUNDANCE ON MINED LEAVES

There was no obvious pattern to how leaf-miners mined *B. pendula* leaves. Of the 221 mined leaves examined on 27–29 June 2000; 91 (41%) were oriented to the left of the leaf (viewed from base to apex), 77 (35%) to the right and 53 (24%) had no overall inclination to the

Table 1. Summary of generalized linear model results for *E. betulae* survivorship (a) when caged on *Eriocrania* mined and mine-free leaves and (b) on mined leaves alone, exploring effects of midrib damage, amount of leaf mined and foliar phenolics concentration on aphid survivorship. *F* and *P* values for non-significant terms were derived from individually adding these terms to the model containing all significant terms

Sources of variation	df	*F*	*P*
(a)			
Larvae age	1,70	8·40	<0·01
Leaf-miner presence	1,70	14·76	<0·001
Non-significant terms			
Tree number	19,51	0·71	0·796
Larvae age × leaf-miner presence	1,69	0·91	0·342
(b)			
Larvae age	1,32	3·85	0·058
Midrib damage	1,32	12·97	0·001
Non-significant terms			
Area mined (cm^2)	1,31	1·56	0·222
Area mined (% of leaf)	1,31	1·67	0·206
Phenolic concentration	1,31	0·08	0·781
Larvae age × midrib damage	1,31	<0·00	0·949

Table 2. *Euceraphis betulae* performance when reared on *B. pendula* leaves with simulated leaf-miner damage to the midrib and lamina and undamaged leaves. Lower-case superscripts indicate significant differences between leaf types. *F* and *P* values for non-significant terms calculated as Table 1

	Number of aphids surviving*		Number of aphids developing to next instar†	
Leaf damage type	Young	Old	Young	Old
Leaf midrib cauterized	7^a	11^c	0	2^f
Leaf lamina cauterized	35^b	38^d	11^e	32^g
Leaf undamaged	32^b	39^d	10^e	33^g
Significant terms				
Damage type	$F_{2,116}$ = 65·04,	P < 0·001	$F_{2,91}$ = 10·53,	P < 0·0001
Larvae age	$F_{1,116}$ = 7·90,	P = 0·005	$F_{1,91}$ = 39·82,	P < 0·0001
Non-significant terms				
Tree number	$F_{19,97}$ = 0·97,	P = 0·507	$F_{19,72}$ = 1·30,	P = 0·129
Larvae age × damage type	$F_{2,114}$ = 1·34,	P = 0·266	$F_{2,89}$ = 0·11,	P = 0·851
Phenolic concentration	$F_{1,115}$ = 0·66,	P = 0·41	$F_{1,90}$ = 0·45,	P = 0·507

*40 aphids initially caged.
†Of the surviving aphids.

left or right. Seventy-three (33%) leaf-miners damaged the midrib, although they seldom completely severed it (S. N. Johnson *et al.*, personal observation), whereas 148 (67%) left the midrib intact.

Aphids were significantly less abundant on mined leaves with midrib damage than either mined leaves with the midrib intact or mine-free leaves, for which aphid abundance was statistically indistinguishable (Fig. 3).

LEAF-MINER PERFORMANCE ON MIDRIB DAMAGED LEAVES

Mine initiation always occurred at similar distances to the midrib, regardless of whether the midrib was subsequently damaged (Table 3), and all juvenile mines touched the leaf perimeter at the beginning of mining activity (S. N. Johnson *et al.*, personal observation). There was no difference in the mass of mined leaves with and without midrib damage (Table 3), nor with the mine-free leaves collected to derive a regression equation relating leaf biomass and surface area ($F_{1,143}$ = 0·13, P = 0·72). The time taken until *Eriocrania* emerged from mines did not differ significantly between the three types of mine, nor did the amount of leaf eaten while mining the leaf (Table 3). *Eriocrania* larval mass at emergence from leaf-mines was, however, positively correlated with the amount of leaf eaten ($F_{1,40}$ = 82·52, P < 0·001) (Fig. 4), but there was no significant relationship with nitrogen concentrations of adjacent leaves ($F_{1,40}$ = 1·38, P = 0·247).

Eriocrania larval mass was significantly heavier when the midrib was damaged than when it was intact ($F_{2,40}$ = 4·22, P = 0·022). *Post hoc* analysis indicated that the difference was only significant between leaf-miners

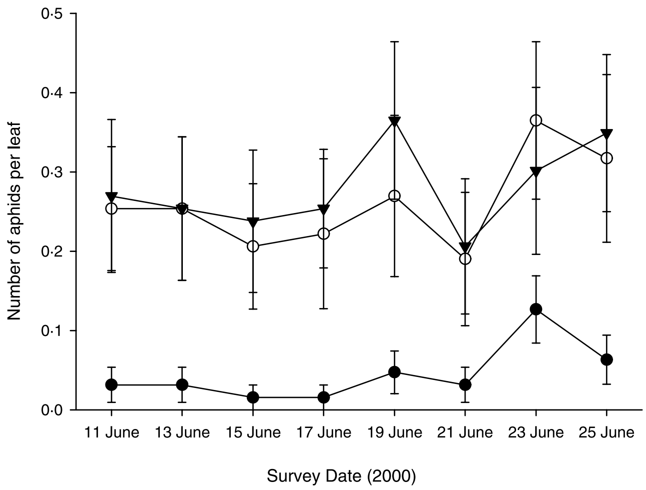

Fig. 3. Mean number of *E. betulae* aphids recorded per leaf examined (± standard error) on alternate days for 2 weeks during *Eriocrania* mine development. Leaf types were: mined leaves possessing midrib damage (●), mined leaves with the midrib intact (○) and mine-free leaves (▼). Survey date $F_{7,1427} = 1·73$, $P = 0·099$; leaf type $F_{2,1427} = 22·00$, $P < 0·0001$; Survey date × leaf type $F_{14,1427} = 0·41$, $P = 0·972$. Least square mean tests between leaf types: (● – ○) $t = −6.29$, $P < 0·001$; (● – ▼) $t = −6·57$, $P < 0·0001$ and (○ – ▼) $t = −0·55$, $P = 0·580$.

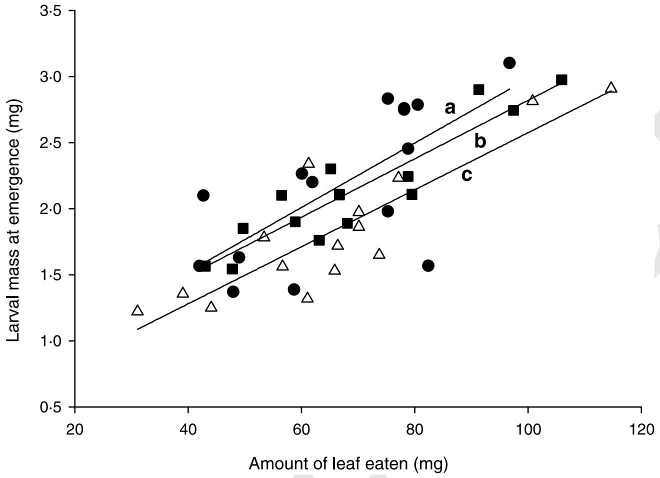

Fig. 4. Relationship between amount of *B. pendula* leaf eaten by *Eriocrania* larvae and larval mass at emergence from leaves in which the lamina was damaged by the larva and the midrib was manually damaged (●, line a); the lamina and the midrib were both damaged by the larva (■, line b) and the lamina alone was damaged by the larva (△, line c). Lines of best fit added for illustration.

with manually severed midribs and leaf-miners which mined the lamina ($t = −2·83$, $P = 0·0193$), and not between leaf-miners from leaves with naturally damaged midribs ($t = −0·81$, $P = 0·70$). There was no significant difference between the masses of leaf-miners emerging from leaves with naturally damaged midribs and leaves with intact midribs ($t = −2·01$, $P = 0·122$).

LEAF CHEMISTRY IN MINED AND MINE-FREE LEAVES

The total carbon and nitrogen concentrations, and the C/N ratio in mined and mine-free leaves were not significantly different (Fig. 5). However, there was a trend

Table 3. Characteristics of leaves and leaf-mining activity in which *Eriocrania* larvae mined leaves and damaged the midrib, mined the lamina alone (midrib intact) and mined the lamina alone, but the midrib was manually damaged. Data displayed as mean ± SE, 15 replicates

	Leaf characteristics		Leaf-mining characteristics	
Mined leaf type	Initial distance of mine from midrib (mm)	Initial leaf mass (mg)	Time from caging to larval emergence (days)	Leaf eaten (mg)
Midrib damaged by larvae	7·7 ± 0·30	81·9 ± 6·15	9·93 ± 0·35	69·20 ± 4·73
Lamina damaged by larvae (midrib intact)	8·5 ± 0·31	85·4 ± 6·92	10·13 ± 0·32	65·69 ± 5·57
Lamina damage by larvae (midrib manually damaged)	7·7 ± 0·21	88·2 ± 6·10	10·27 ± 0·42	67·17 ± 4·29
	$F_{2,42} = 2·59$, $P = 0·087$	$F_{2,42} = 0·25$, $P = 0·782$	$F_{2,42} = 0·21$, $P = 0·81$	$F_{2,42} = 0·13$, $P = 0·88$

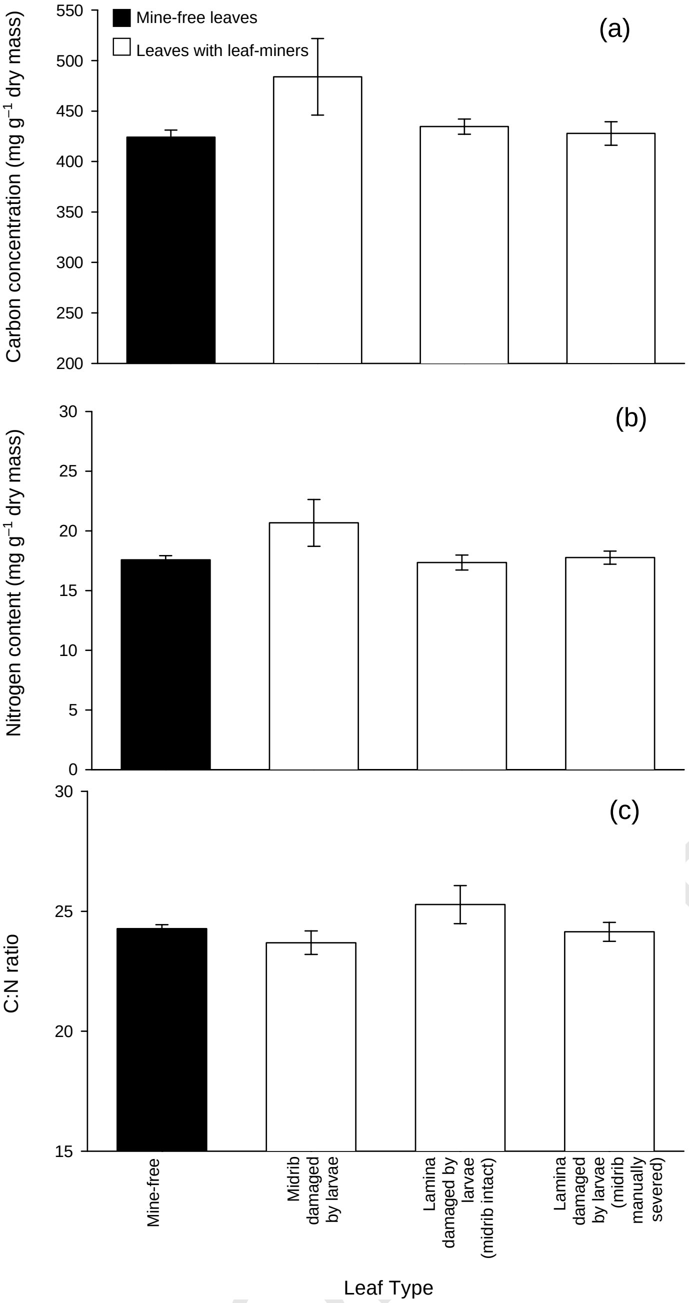

Fig. 5. Leaf chemistry of mine-free *B. pendula* leaves and leaves bearing *Eriocrania* leaf-miners with the midrib intact and with (natural and manual) midrib damage: (a) total carbon concentration ($F_{3,98} = 1{\cdot}74$, $P = 0{\cdot}164$); (b) total nitrogen concentration ($F_{3,98} = 2{\cdot}41$, $P = 0{\cdot}072$) and (c) C:N ratio ($F_{3,98} = 1{\cdot}82$, $P = 0{\cdot}148$).

($P = 0{\cdot}072$) towards higher nitrogen concentrations in leaves in which *Eriocrania* had damaged the midrib.

Discussion

The aim of this study was to investigate the plant-mediated indirect effects of a leaf-mining moth, *Eriocrania*, on a phloem-feeding aphid, *Euceraphis betulae*, on birch. This study focused on the physical changes that occur during leaf-mining activity. *Euceraphis betulae* was negatively affected by *Eriocrania* when the leaf-miner damaged the primary vasculature, and *E. betulae* was less abundant on mined leaves with this type of damage in the field. Structural damage to the leaf midrib was not a prerequisite of *Eriocrania* leaf-mining, but leaf-miner performance was positively affected when the midrib was manually damaged.

MECHANISTIC BASIS FOR THE INTERACTION

The negative effect of one insect herbivore (*Eriocrania*) on the survivorship of another of a different feeding guild (*E. betulae*) is usually considered in terms of nutritional or allelochemical changes induced by one that subsequently affects the other (Masters & Brown 1997). *Eriocrania* was associated with higher concentrations of foliar phenolic compounds, the main group of antiherbivore allelochemicals in birch (Hartley & Lawton 1991; Ossipov *et al.* 2001), but this, together with the actual amount of leaf mined, was unrelated to *E. betulae* survivorship. Birch aphid performance has been shown to be unaffected when reared on birch leaves with elevated phenolic compound concentrations in the past (Martin, Cappuccino & Ducharme 1994), perhaps because of the low phenolic concentrations in phloem sap (Raven 1983; Karban & Myers 1989).

The actual region of the leaf mined rather than leaf-miner presence *per se* was strongly correlated with *E. betulae* survivorship. Specifically, when *Eriocrania* mines impinged on the leaf midrib, aphid survivorship was significantly lower than when leaf-mining was restricted to the lamina. Similarly, when *E. betulae* was reared on leaves with simulated leaf-miner damage, aphid performance was significantly lower on leaves with midrib damage compared with either lamina damaged or undamaged leaves. Aphid survivorship, development and mass were statistically indistinguishable when reared on lamina damaged and undamaged leaves, despite lamina-damaged leaves having higher phenolic compound concentrations. This provides further support for elevated phenolic compound levels not being responsible for the depressed *E. betulae* performance. Artificial damage is known to produce slightly different effects from those of natural herbivory (Baldwin 1990), but these manipulations allowed potentially confounding effects to be uncoupled from damage type and permitted standardization of leaf damage.

Euceraphis betulae were significantly less abundant on mined leaves with midrib damage compared with mined leaves with an intact midrib or mine-free leaves. That *E. betulae* were equally abundant on leaves with damaged and undamaged lamina reinforces the suggestion that it is a particular consequence of leaf-mining, namely damage to the primary vasculature, that makes a leaf unsuitable as a resource to the aphid, rather than leaf-miner presence as such.

IMPLICATIONS OF LEAF MIDRIB DAMAGE FOR THE APHID

It is unlikely that *Eriocrania* caused changes in the nutritional chemistry of phloem sap only in leaves with midrib damage and not in lamina-damaged leaves. Although not an ideal indication of phloem sap quality, the slightly higher nitrogen concentrations of mined leaves with midrib damage also make this possibility look doubtful. A more likely explanation for the negative effects on the aphid, is that damage to the midrib disrupts phloem hydraulics on which phloem feeders are dependent (Dixon 1998). A comparable situation arose when an aphid gall (*Forda formicaria*) was situated on the midrib of a *Pistachia* leaf, thereby diverting nutrients away from a second aphid gall (*Geioica* sp.) (Inbar, Eshel & Wool 1995).

Euceraphis betulae might be particularly susceptible to interference with phloem hydraulics because of its specific feeding sites on the larger primary veins, namely the basal midrib and petiole. The phloem is more deeply embedded in primary veins than elsewhere on the leaf but since *E. betulae* possesses a particularly long stylet (0·4–0·5 mm at adulthood) it can tap into them successfully (Hajek & Dahlsten 1986). While the flow of phloem sap is greater in the larger veins, the concentration and quality tend to be lower than in neighbouring smaller vessels (e.g. Fellows & Geiger 1974), perhaps suggesting that *E. betulae* is adapted to feeding on lower-quality sap but at higher flux rates. Any interference with the phloem hydraulics might then be especially detrimental to *E. betulae* compared with an aphid that is adapted to feed on vessels with a lower flow of phloem sap. Indeed, Prestidge & McNeil (1982) have suggested that the availability of phloem sap has selected for two discrepant life-history styles in phloem-feeding insects. Those phloem feeders which have highly specific demands for phloem sap (e.g. high flux) are highly mobile so as to meet those demands, whereas phloem feeders that are more tolerant of fluctuating phloem sap availability tend to be less mobile. Under such circumstances, vasculature damage by leaf-mining insects might disproportionately affect large and mobile aphids such as *E. betulae*.

IMPLICATIONS OF LEAF MIDRIB DAMAGE FOR THE LEAF-MINER

Most leaf-mining insects avoid mining major veins (Kimmerer & Potter 1987; Scheirs, Vandevyvere & DeBruyn 1997; Scheirs, De Bruyn & Verhagen 2001 and references therein) because they are nutritionally unfavourable (Scheirs *et al.* 2001) and frequently the toughest tissues in the leaf (Choong 1996). Such avoidance results in distinctively shaped mines between the veins (Stiling, Simberloff & Anderson 1987; Scheirs *et al.* 1997). *Eriocrania,* in contrast, apparently did not avoid large leaf veins, since there was no obvious pattern to how the leaf was mined and the midrib was actually mined in a third of the leaf-mines examined. As the majority of *Eriocrania* left the midrib intact, mining the midrib cannot be considered to be an essential part of their feeding, unlike the Holly Leaf-miner, *Phytomyza ilicis*, which must initially mine the midrib to enter the laminal parenchyma (Valladares & Lawton 1991).

There was a strong positive correlation between larval mass of leaf-miners at emergence from their mines and the amount of leaf they had consumed. Perhaps more interesting is that leaf-miners that fed on leaves where the midrib was artificially damaged performed disproportionately well compared with leaf-miners from leaves with intact midribs, when the amount of leaf eaten was taken into account. In terms of dietary nutrition, most phytophagous insects are nitrogen limited (Waring & Cobb 1992) and damaging the midrib might increase the nutritional value of the leaf for *Eriocrania*. In addition to restricting the supply of water to leaves via the xylem, damaging the midrib could also curb the export of nutrients via the phloem. Accelerated protein degradation as a result of water stress could cause more readily digestible nitrogen compounds to accumulate in leaves (White 1984). This phenomenon is known to be made use of by a range of phytophagous insects including the sawfly *Eriocampa ovata* (MacKay & Wellington 1977); the lepidopterans *Danaus plexippus* (Brewer 1977; Rothschild 1977) and *Manduca sexta* (Heinrich 1971); and the coleopteran *Oncideres cingulata* (Forcella 1982). Phloem feeders such as *E. betulae* would not be able to take advantage of this nutritional improvement, since they are reliant on the flux of amino acids rather than the total amount *per se* (Dixon 1998). There was a trend towards higher nitrogen concentrations in mined leaves with miner-induced midrib damage, which may have reflected an accumulation of nitrogenous compounds in leaves with damaged midribs. This may ultimately benefit the leaf-miner, but as Choong (1996) demonstrated, the severance of the tough midrib is energetically demanding and nutritionally unfavourable.

It is also possible that damaging the midrib is an incidental effect of leaf-mining by *Eriocrania*, and may even be disadvantageous for the leaf-miner in some cases. Preszler & Price (1993), for instance, showed that rapid leaf-miner development on young willow leaves caused early leaf abscission by the plant, resulting in high leaf-miner mortality. Whether birch leaves are abscised in direct response to *Eriocrania* mining is unknown but damaging the midrib may reduce the structural integrity of the leaf, since the midrib contains high levels of structural compounds such as lignin (Choong 1996), possibly making such leaves prone to dislodgement. Alternatively, damage to the midrib may actually impede the plant chemical processes that cause leaf abscission in response to environmental stress (Taylor & Whitelaw 2001), thereby prolonging leaf attachment to the parent plant.

INSECT COMPETITION THROUGH PHYSICAL MODIFICATION OF LEAVES

Regardless of whether damaging the midrib is advantageous to *Eriocrania*, these findings demonstrate that if the midrib is damaged, the leaf is rendered unsuitable for *E. betulae* to feed upon. Mining part of the midrib does not deprive *E. betulae* of its feeding site in the strictest sense, but it could disrupt phloem turgor on which aphids are at least partially dependent (Raven 1983). In contrast to *E. betulae*, all *Eriocrania* larvae were alive at the end of the caging experiments, suggesting that *E. betulae* has little discernible impact on *Eriocrania*. This supports the findings of Fisher *et al.* (2000), who also found that birch aphids had no impact on *Eriocrania* performance in field trials. The competitive interaction between *Eriocrania* and *E. betulae*, like many direct (Lawton & Hassell 1981) and indirect (Bonsall & Hassell 1997) interactions between insects, is very probably asymmetric. The asymmetric indirect effect of *Eriocrania* on *E. betulae* is likely to be fundamentally more stable than a symmetric interaction between the two insects, since there is no mechanism for a positive feedback on *Eriocrania* that would tend towards the exclusion of *E. betulae* (Lawton & Hassell 1981).

To our knowledge, this is the first time that the indirect effects of a leaf-miner on a phloem feeder via structural changes to the vasculature of a shared host plant have been reported. We suggest that anatomical modification of a shared resource could be more widespread than previously reported. If so, the extent of interspecific competition between insect herbivores may have been underestimated, particularly since most studies of competitive interactions between insect herbivores focus on changes in plant chemical composition rather than plant structure.

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