

## Chapter 5

# Feeding damage by larvae of the mustard leaf beetle deters conspecific females from oviposition and feeding

*Key words:* Chinese cabbage, damage-induced response, host acceptance, larval frass, larval performance, larval secretion, oviposition behaviour, *Phaedon cochleariae*, regurgitant

### Abstract

Herbivorous insects may be informed about the presence of competitors on the same host plant by a variety of cues. These cues may derive from either the competitor itself or the damaged plant. In the mustard leaf beetle *Phaedon cochleariae* (Coleoptera, Chrysomelidae), adults are known to be deterred from feeding and oviposition by the exocrine glandular secretion of conspecific co-occurring larvae. We hypothesised that the exocrine larval secretion released by feeding larvae may adsorb to the surface of Chinese cabbage leaves, and thus, convey the information about their former or actual presence. Further experiments tested the influence of leaves damaged by conspecific larvae, mechanically damaged leaves, larval frass and regurgitant on the oviposition and feeding behaviour of *P. cochleariae*. Finally, the effect of previous conspecific herbivory on larval development and larval host selection was assessed.

Our results show that (epi)chrysomelidial, the major component of the exocrine secretion from *P. cochleariae* larvae, was detectable by GC-MS in surface extracts from leaves upon which larvae had fed. However, leaves exposed to volatiles of the larval secretion were not avoided by female *P. cochleariae* for feeding or oviposition. Thus, we conclude that secretion volatiles did not adsorb in sufficient amounts on the leaf surface to display deterrent activity towards adults. By contrast, gravid females avoided to feed and lay their eggs on leaves damaged by second-instar larvae for 3 d when compared to undamaged leaves. Mechanical

damage of leaves, treatment of artificially damaged leaves with larval frass or regurgitant did not affect oviposition and feeding of *P. cochleariae*. Since no adverse effects of previous herbivory on larval development were detected, we suggest that female *P. cochleariae* avoid Chinese cabbage leaves damaged by feeding larvae for other reasons than escape from competition or avoidance of direct negative effects that result from consuming induced plant material.

## **Introduction**

A variety of physical and chemical factors influence herbivorous insects in their decision to accept or reject a potential host plant for feeding and oviposition. Plant properties such as foliar form, colour, surface wax chemistry, and the composition of nutrients and secondary metabolites within the plant tissue, as well as plant volatiles inform herbivorous insects about the suitability of a plant (Bernays & Chapman, 1994; Schoonhoven et al., 1998). Additionally, it may be advantageous for herbivorous insects to recognise when host plants are already occupied by intra- and interspecific competitors (Prokopy & Roitberg, 2001). Avoidance of already occupied host plants may reduce competition. A wide variety of cues may inform herbivorous insects about previous attack of a plant by competitors. These signals may either be produced by the competitors or by the plant.

Cues from competitive herbivorous insects may already be released at the moment of egg laying. Such oviposition deterrents are known to inform both conspecific females (Nufio & Papaj, 2001; and references therein) and individuals of other species (e.g. Prokopy, 1981; Giga & Smith, 1985). However, not only the egg laying female is able to produce oviposition deterrents but also larvae that co-occur with gravid females (Corbet, 1973; Renwick & Radke, 1980; Hilker, 1989; Anderson & Löfquist, 1996). Larvae are known to contain oviposition deterrents in their frass (e.g. Dittrick et al., 1983; Hilker, 1985; Anderson et al., 1993), their regurgitant (e.g. Hilker & Weitzel, 1991), or their exocrine glandular secretions (e.g. Corbet, 1971).

Apart from insect-produced compounds, previous attack of a plant may also be recognised by changes in the plant's physiology. Feeding damage inflicted by larvae is known to alter the blend of volatiles released by the plant. The emitted volatiles can attract natural enemies of the herbivore, however, recent studies suggest that herbivorous insects may also avoid

induced plants for oviposition (DeMoraes et al., 2001; Kessler & Baldwin, 2001). Likewise, the induction of non-volatile plant compounds is regarded as a method of plant defence. Such plant responses induced by feeding damage often show a negative impact on larval development (Karban & Baldwin, 1997). Therefore, adults avoiding induced host plants for oviposition may help their offspring to start development at good conditions.

Among a wide range of herbivores (Karban & Baldwin, 1997) several leaf beetle species (Chrysomelidae) have been shown to avoid damaged host plants (e.g. Jeker, 1981; Baur et al., 1991; Raupp & Sadof, 1991; Palaniswamy & Lamb, 1993; Tschardt et al., 2001). Adults of the subfamily Chrysomelinae are able to recognise the presence of conspecific larvae on a host plant by the larval exocrine glandular secretion that is released when larvae are disturbed by either conspecifics (Hilker, 1989) or enemies (Pasteels et al., 1988). Laboratory bioassays proved that the exocrine glandular secretion of larvae of several chrysomeline species deters both feeding and oviposition of adults (Hilker, 1989). In the field, Chrysomelinae were shown to avoid plants that had already been attacked by conspecifics (e.g. Raupp & Sadof, 1991; Schindek & Hilker, 1996). However, these field experiments suggested that in addition to larval secretion other cues may also convey information about previous attack of the host plant. These suggestions are based on the results that (a) also artificially wounded plants (Raupp & Sadof, 1991), and (b) also plants that had already been abandoned by the larvae (Schindek & Hilker, 1996) were avoided.

In the mustard leaf beetle *Phaedon cochleariae* (Subfam. Chrysomelinae), it has been shown that gravid females are deterred from host plant leaves when secretion of conspecific larvae is directly applied onto these leaves (Hilker, 1989). The deterrent effect of the secretion is caused by its major volatile component, the cyclopentanoid monoterpene (epi)chrysomelidial (Pasteels et al. 1982; Gross & Hilker, 1995). In the present study we examined whether volatiles from this secretion may adsorb onto the leaf surface so that adult beetles are informed about the attack of the host plant by conspecific larvae even though they are not actually present anymore. Volatile pheromones may adsorb onto an apolar surface, like the epicuticular wax of a leaf, from where they may be re-released and perceived by conspecific insects. This has been demonstrated e.g. with the sex pheromone of the noctuid *Mamestra brassicae* (Noldus et al., 1991). The idea of herbivore-released volatiles adsorbing onto a leaf surface, led us to the question, whether secretion volatiles from *P. cochleariae*

larvae would also adsorb onto Chinese cabbage leaves, in amounts high enough to be an active deterrent for gravid female beetles.

To investigate whether factors other than larval secretion inform *P. cochleariae* about the attack of the plant by conspecifics, a series of further experiments assessed the role of larval feeding damage on the oviposition and feeding behaviour of adult *P. cochleariae*. When studying the effect of feeding damage, we differentiated between damage by feeding larvae, mechanical damage, and mechanical damage in conjunction with either larval regurgitant or larval frass. It has been demonstrated in numerous studies that damage of plants by herbivores may induce plant responses different from those induced by mechanical damage (De Moraes et al., 1998, Agrawal, 1998). Larval regurgitant may act as an elicitor of herbivore-induced changes in the plant. Several studies show that application of larval regurgitant onto a wounded plant mimicks the plant's response to herbivore feeding, while mechanical wounding *per se* does not (Felton & Eichenseer, 1999 and references therein). To date, larval frass has not been demonstrated to elicit inducible plant responses. However, since damaged plants are contaminated with the faeces of herbivores, we also examined the effect of wounded plants treated with larval frass on female *P. cochleariae*. At last, by observing the effect of previous herbivory on the performance and host choice of conspecific larvae we asked whether herbivory would result in induced plant resistance.

## **Materials and Methods**

*Plants and insects.* Chinese cabbage plants (*Brassica rapa* L. ssp. *pekinensis* cv. 'Kantonner') were grown from seed (obtained from Saatzucht Quedlinburg GmbH) in 7 x 8 x 7 cm plastic pots in a greenhouse at ca. 20 – 35 °C. Standard potting soil (Einheitserde Typ P, Kausek GmbH and Co. KG, Mittenwalde, Germany) was used and a single dose of fertilizer was applied three weeks after germination. Light from 400 W sodium vapour lamps supplemented natural daylight, providing an illumination of > 10,000 lx for 16 h a day. Plants were used when the 5<sup>th</sup> leaf (1<sup>st</sup> leaf = oldest leaf) had reached maximum size (4-5 weeks). A laboratory colony of *Phaedon cochleariae* (F.) was maintained in a climate chamber at 20°C, 70% r.h. and a photocycle of L18:D6. Larvae and adults were fed on Chinese cabbage leaves.

*Adsorption of (epi)chrysomelidial to leaf surface.* In order to analyse whether the major component of the larval secretion, (epi)chrysomelidial, adsorbs to the leaf surface, a clip cage

(Ø 21 mm, h: 18 mm, with gauze (mesh: 50 µm) covering bottom and top end) containing five larvae of *P. cochleariae* (second to third instar) was confined to a Chinese cabbage leaf (5<sup>th</sup> – 6<sup>th</sup> leaf) After a feeding period of 24 h the larvae were removed. The remaining leaf tissue within the boundaries of the clip cage was punched out with a cork-borer (Ø 21 mm). The surface of this leaf disk was extracted by dipping it into 10 ml dichloromethane (DCM) for 30 s. The extracts from 15 leaf disks were pooled, filtered (Schleicher & Schöll 589, Dassel, Germany) and then concentrated to 500 µl with N<sub>2</sub>. For control, an extract from 15 leaf disks was produced following the described procedure, however, clip cages contained no larvae.

Chemical analyses of the leaf extracts were performed by coupled gas chromatography-mass spectrometry (GC-MS) (GC: Fisons 8000 series, MS: MD800 quadrupole; Fisons Instruments, Mainz, Germany). Extracts (1 µl) were injected in a 30 s split mode on a 30 m x 0.32 mm i.d. RH5 ms fused silica column, film thickness 0.25 µm (J & W/Fischer Scientific, Wiesbaden, Germany), with helium as carrier gas (inlet pressure 10 kPa). The temperature program started at 40 °C and rose at 10 °C/min to 280 °C. The column effluent was ionised by electron impact ionisation (EI) at 70 eV. Mass range was 35 - 450 amu. (Epi)chrysolidial from the leaf extract was identified by comparing the mass spectra of the leaf extracts with the mass spectrum from pure larval secretion and with mass spectra from the institute library and from the literature (Blum et al., 1978; Meinwald et al., 1977). The secretion of *P. cochleariae* was obtained by disturbing three second-instar larvae with forceps. Small squares (1 mm<sup>2</sup>) of filter paper (Schleicher & Schöll 589, Dassel, Germany) were used to soak up the released secretion. The paper squares were immediately transferred into 300 µl DCM. The analysis was performed from three different samples.

*Effect of secretion volatiles adsorbed to host plant leaf surface on adult host choice.* The following experiment was conducted to examine whether volatiles of the larval secretion can adsorb in sufficient quantity to the leaf surface to affect the oviposition behaviour of *P. cochleariae*. Chinese cabbage plants were selected for equal size and arranged in a completely randomised design. Three square pieces of gauze (25 mm x 25 mm; mesh: 1 mm) were placed onto the 5<sup>th</sup> leaf in equal distance to each other. Five filter paper squares, each treated with the secretion of one second-instar larva, were placed upon one gauze. Thus, each Chinese cabbage leaf was exposed to the volatile constituents of the total secretion gained from 15 larvae. Direct contact of larval secretion with the leaf surface was prevented. A clip cage fixed

each gauze-filter paper arrangement. After one hour clip cages, gauze, and filter papers were removed. Treated and control leaves were excised and placed into a transparent plastic box (20 x 20 x 5 cm). Five gravid females (3 - 5 weeks old) of *P. cochleariae* were set into each box and left to feed and oviposit. The number of eggs laid on test and control leaves was counted in intervals of 1.5 h, 3 h, 6 h, 9 h, and 24 h (N = 20). Additionally, 10 leaf areas, exposed to volatile secretion as described above, were cut out and analysed by GC-MS at intervals of 1 h, 4 h and 24 h after the treatment.

*Effect of feeding damage of leaves on adult host choice.* Chinese cabbage plants were chosen and arranged as described above. A clip cage containing five second-instar larvae was attached to the 5<sup>th</sup> leaf of an experimental plant. After a period of 24 h and 48 h, respectively, the larvae were confined to an undamaged part of the leaf. The insects fed for 72 h in total, removing about 9 % of the leaf area. Control plants received identical treatment but clip cages contained no larvae.

The herbivore-damaged leaf and the 5<sup>th</sup> leaf of a control plant were placed into a transparent plastic box (20 x 20 x 5 cm). Five gravid females (3 - 5 weeks old) of *P. cochleariae* were set into each box and left to feed and lay eggs. Twenty-four hours later the number of eggs laid was recorded and the leaf area consumed was evaluated using computer-aided image analysis (N = 20). Experiments conducted to determine systemic effects of herbivore-damaged leaves (N = 20) were carried out as described for local effects with the exception of using the undamaged 6<sup>th</sup> leaf of a herbivore-damaged plant for the bioassays.

*Effect of mechanical damage of leaves on adult host choice.* The experiment was carried out the same way as the preceding adult choice tests, however, larval feeding was mimicked by mechanical damage. A hole of Ø 7 mm was punched into the 5<sup>th</sup> leaf of a test plant with a cork borer. After 3 h and 6 h, respectively, the hole was enlarged to 14 mm and a final 21 mm in diameter. This treatment was repeated on two consecutive days. Twenty-four hours after the last damage treatment, test leaves each with three holes of 21 mm diameter and undamaged control leaves were offered to gravid females (N = 20).

*Effect of mechanical damage of leaves and larval faeces on adult host choice.* The previous experiment was repeated but additionally ca. 4 mg (equals approx. fresh weight of one second-instar larva) of larval faeces were applied to the wound after each damage treatment using a paint brush. Faeces was gathered by keeping second-instar larvae (total fresh weight

ca. 6 g larvae) on a glass plate (20 cm x 20 cm) for approximately 1 h. The faeces was scraped off with a razor blade and diluted with demineralised water (1 : 4). Control plants were also damaged but wounds were treated with demineralised water (N = 20).

*Effect of mechanical damage of leaves and larval regurgitant on adult host choice.* Instead of faeces, in this experiment each wound was treated with the regurgitant of five second instar larvae. This was done by squeezing each larva with forceps. The ejected regurgitant was immediately applied to the wound. A single larva ejected in average 0.4 µl of regurgitant (N = 20).

*Effect of feeding damage of leaves on larval development.* The influence of herbivory on the development of *P. cochleariae* was assessed by feeding the insects with disks (Ø 10 mm) punched out from one of the following leaf types:

- leaves damaged by larvae of *P. cochleariae* ('damaged leaves', 5<sup>th</sup> leaf)
- undamaged leaves adjacent to 'damaged leaves' ('systemic leaves', 6<sup>th</sup> leaf)
- leaves of an undamaged plant ('control leaves', 5<sup>th</sup> leaf)

Damage treatment was performed as described above (see 'Effect of feeding damage of leaves on adult host choice'). The experiment was carried out in a climate chamber at a constant temperature of 25 °C, 70% r.h., and 18 h of light. Neonate larvae were placed individually into small Petri dishes (Ø 55 mm) which were kept in transparent plastic boxes (20 x 20 x 5 cm) with moist paper tissues. Each day larvae were supplied with one leaf disk (Ø 10 mm) that had been punched out with a cork borer from a plant belonging to one of the treatment groups mentioned above. All experimental plants were used once only. Leaf disks were sampled randomly from four individual plants per day. Development of N = 30 larvae was recorded per treatment.

Fresh weight of larvae was measured 5 d after hatching and pupae were weighed 2 d after pupation had taken place. Duration of larval development and survival rates were recorded. After eclosion male beetles were placed individually in Petri dishes containing a female from the same treatment group. Every day beetles were supplied with a leaf disk from an untreated plant. The number of eggs laid per female was counted for 3 d after the onset of oviposition.

*Effect of feeding damage of leaves on larval host choice.* The experiments on the effect of feeding damage on *P. cochleariae* larvae were performed in the same manner as the adult host

choice tests (see above,  $N = 20$ ). The leaf area consumed by five second-instar larvae of *P. cochleariae* was recorded after 24 h. Leaf consumption of the larvae was calculated by measuring the total area of feeding holes to the nearest of  $1 \text{ mm}^2$  by a self-written image analysis software program.

*Statistics.* All choice tests were analysed by using the Wilcoxon matched pairs test. Treatment effects on larval performance were compared with one-way ANOVA, except for survival rate, which was assessed by a  $\text{Chi}^2$  test. For all analyses the Statistica software package (StatSoft, Tulsa, OK, USA) was used.

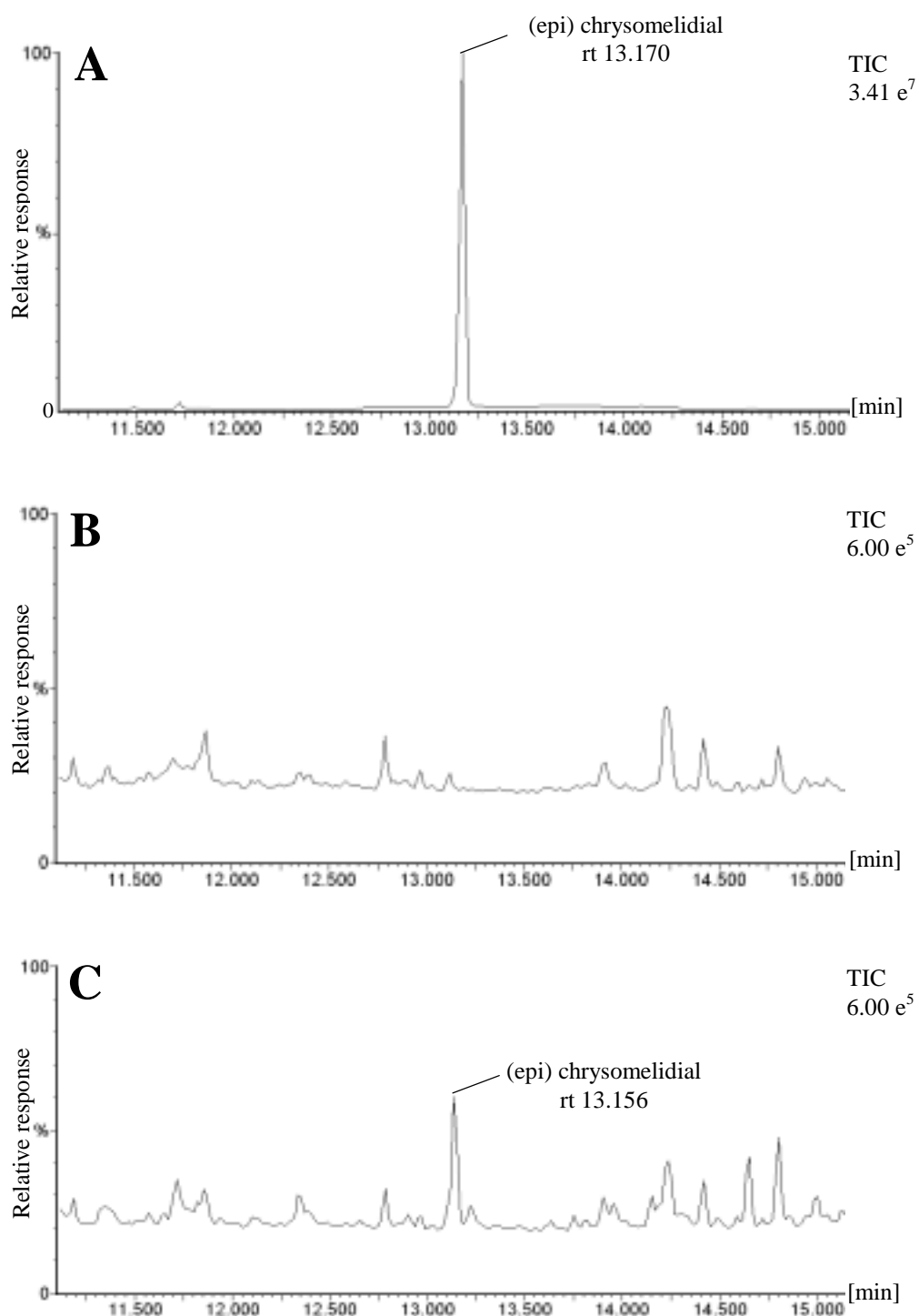
## **Results**

*Adsorption of (epi)chrysolidial to leaf surface.* (Epi)chrysolidial, the main component of the larval secretion of *P. cochleariae*, was identified in low quantities in the surface extracts of leaves on which beetle larvae had fed. No traces of (epi)chrysolidial were detected in the surface wash of control leaves (Fig. 1).

*Effect of secretion volatiles adsorbed to host plant leaf surface on adult host choice.* Female *P. cochleariae* laid similar numbers of eggs on untreated leaves and leaves exposed to the volatile compounds of the secretion (Fig. 2). No significant differences were observed at any time interval (Wilcoxon matched pairs test;  $P > 0.05$ ). Adsorbed (epi)chrysolidial was detectable by GC-MS at 1 h and 4 h after the treatment. Twenty-four hours after volatile treatment the (epi)chrysolidial peak was no longer detectable.

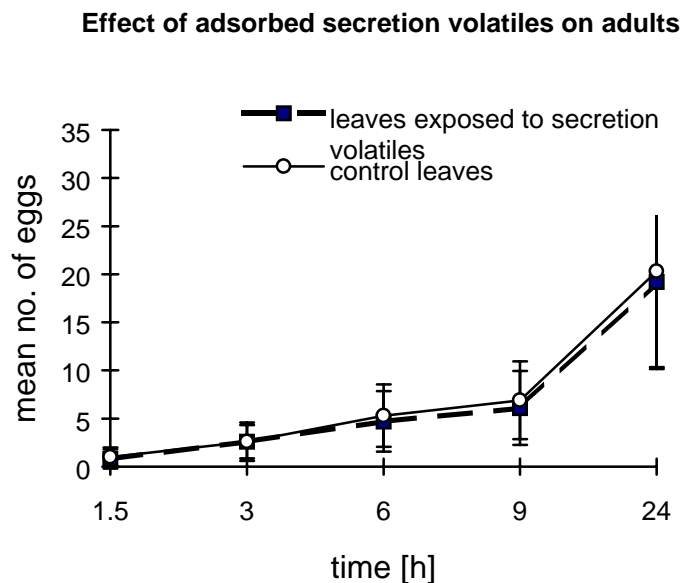
*Effect of feeding damage of leaves on adult host choice.* Gravid females of *P. cochleariae* significantly preferred undamaged Chinese cabbage leaves to leaves which had been fed on by conspecific larvae both for feeding and oviposition (Fig. 3). The beetles consumed more leaf tissue (Wilcoxon matched pairs test;  $P = 0.004$ ) and laid a higher number of eggs on undamaged controls (Wilcoxon matched pairs test;  $P = 0.006$ ) (Fig. 3). However, gravid females did not distinguish between systemic leaves and control leaves from an undamaged plant (Wilcoxon matched pairs test;  $P > 0.05$ ).





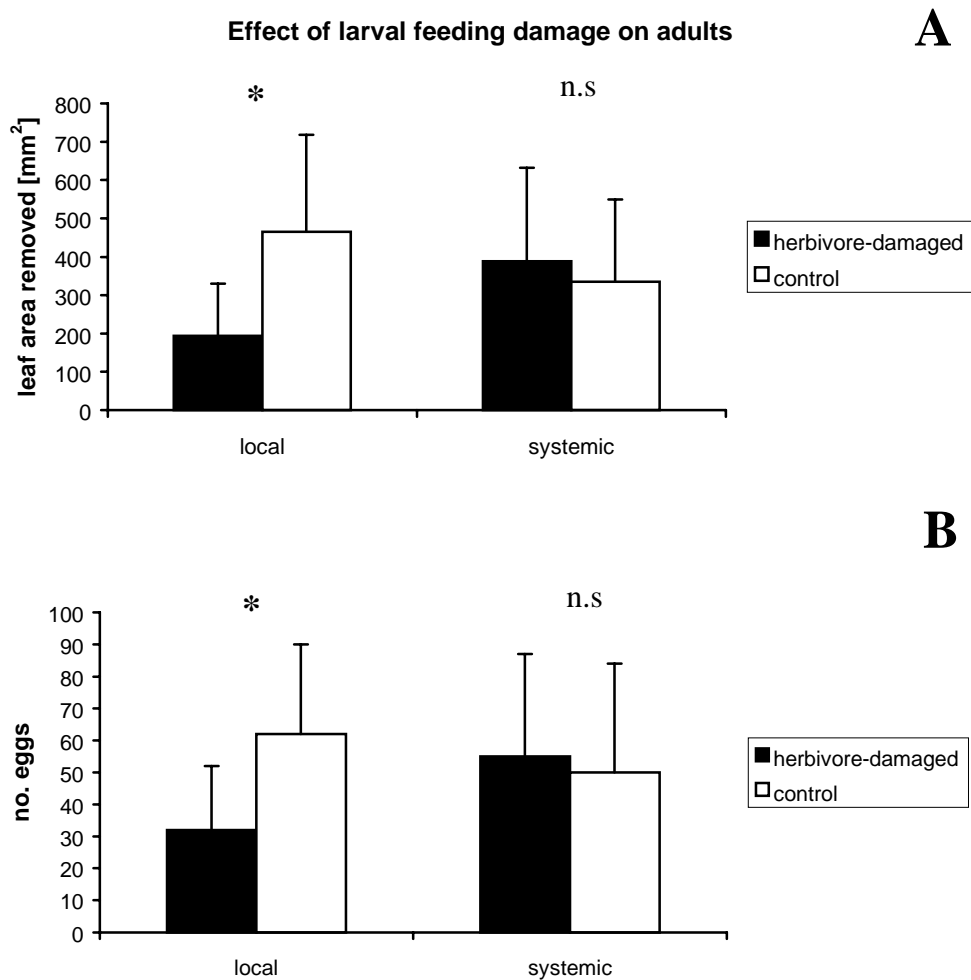
**Figure 1** Total ion current chromatograms obtained from (A) the dorsal exocrine secretion of a *Phaedon cochleariae* larva, (B) the surface extract of undamaged Chinese cabbage leaves, and (C) the surface extract of Chinese cabbage leaves fed upon by larvae of *P. cochleariae* for 24 h. rt = retention time.

*Effect of mechanical damage alone or combined with larval faeces or regurgitant on adult host choice.* Female mustard leaf beetles consumed neither less nor did they lay fewer eggs on mechanically damaged than on healthy Chinese cabbage leaves (Wilcoxon matched pairs test;  $P > 0.05$ ). Likewise, no effects on adult host choice behaviour were observed when larval faeces (Wilcoxon matched pairs test;  $P > 0.05$ ) or regurgitant (Wilcoxon matched pairs test;  $P > 0.05$ ) was applied to the wounds (Table 1).



**Figure 2** Effect of secretion volatiles adsorbed to leaf surface on oviposition of *Phaedon cochleariae* in a dual-choice assay. Beetles were allowed to choose between untreated Chinese cabbage leaves (control) and leaves exposed to volatiles from the larval secretion of conspecifics. Numbers of eggs were counted after indicated periods of time. Wilcoxon matched pairs tests. n.s. = not significant.  $n = 20$ .

*Effect of feeding damage of leaves on larval development.* Previous herbivory by conspecific larvae had no detectable effects on the performance of *P. cochleariae* larvae (Table 2). No significant differences between treatments were found in larval weight (one-way ANOVA;  $F = 2.60$ ,  $df = 2$ ,  $P > 0.05$ ), pupal weight (one-way ANOVA;  $F = 0.24$ ,  $df = 2$ ,  $P > 0.05$ ), survival rate ( $\text{Chi}^2 = 0.04$ ,  $P > 0.05$ ), developmental time (one-way ANOVA;  $F = 2.60$ ,  $df = 2$ ,  $P > 0.05$ ) or eggs laid as adults (one-way ANOVA,  $F = 1.68$ ,  $df = 2$ ,  $P > 0.05$ ).



**Figure 3** Effect of larval feeding damage on feeding (**A**) and oviposition (**B**) of adult (female) *Phaedon cochleariae* in dual-choice assays. Beetles chose between Chinese cabbage leaves damaged by conspecific larvae (dark columns) and undamaged control leaves (white columns). ‘local’: damaged leaves. ‘systemic’: undamaged leaves adjacent to damaged ones. Asterisks denote significant differences. Wilcoxon matched pairs test.  $**P < 0.01$ , n.s. = not significant.  $n = 20$ .

Effect of feeding damage of leaves on larval host choice. Second-instars of *P. cochleariae* did not remove differing areas of leaf tissue from undamaged Chinese cabbage leaves or from leaves on which conspecific larvae had previously grazed on (Wilcoxon matched pairs test;  $P > 0.05$ ) (Fig. 4). Likewise, larvae did not choose between the undamaged leaf adjacent to a herbivore-damaged leaf and a control leaf from an undamaged plant (Wilcoxon matched pairs test;  $P > 0.05$ ).

**Table 1** Effect of mechanical leaf damage, alone or in conjunction with larval faeces or regurgitant, on feeding and oviposition of adult (female) *Phaedon cochleariae* in dual-choice assays. Beetles were allowed to choose between treated and control Chinese cabbage leaves for 24 h. Values represent means and standard deviations (in parenthesis). Wilcoxon matched pairs tests. n.s. = not significant. n = 20.

Treatment	No. eggs			Leaf area removed [mm <sup>2</sup> ]		
	Test	Control	P-level	Test	Control	P-level
Mechanically damaged	37 (17)	48 (18)	n.s.	175 (85)	226 (85)	n.s.
Mechanically damaged + larval faeces	45 (19)	47 (19)	n.s.	226 (101)	204 (79)	n.s.
Mechanically damaged + regurgitant	6 (3.5)	5 (2.6)	n.s.	126 (57.9)	97 (52.3)	n.s.

## Discussion

Our experiments show that (epi)chrysomelidial, the major volatile component of the larval secretion of *P. cochleariae*, can adsorb to the surface of host plant leaves. Chinese cabbage leaves exposed to the volatiles from secretion of 15 second-instar larvae for 1 h adsorbed (epi)chrysomelidial in trace amounts that were detectable by GC-MS in leaf extracts up to 4 h after exposure. However, within this time we could not detect any effect of the adsorbed secretion volatiles on oviposition of *P. cochleariae*. In contrast, when larval secretion from 3 larvae (second to third instar) of *Gastrophysa viridula* was directly applied onto a small piece of a host plant leaf, oviposition was significantly avoided on these leaves for 19 h (Hilker, 1989). The major volatile component of the secretion of *G. viridula* larvae is also (epi)chrysomelidial (Pasteels et al., 1982). Most likely, in our experiment with *P. cochleariae* the concentration of the adsorbed main component (epi)chrysomelidial, released by undisturbed larvae, was too low to show any effect on adult beetles. Presumably, to be deterred, a gravid female has to disturb a grazing larva, provoking it to discharge its secretion and thus, to perceive high concentrations of (epi)chrysomelidial.

However, female *P. cochleariae* are able to recognise herbivore attack of their host plant also by other cues than the presence of secretion emitted by conspecific larvae. Female beetles that were given the choice between a Chinese cabbage leaf damaged by conspecific larvae for

3 d and an undamaged leaf, significantly preferred to feed and oviposit on the undamaged leaf. This effect was not systemic

**Table 2** Performance of *Phaedon cochleariae* reared on differently treated Chinese cabbage leaves. ‘Damaged leaves’ = leaves exposed to feeding larvae for 72 h, ‘Systemic leaves’ = leaves adjacent to damaged leaves, ‘Control leaves’ = leaves from undamaged plants. Values represent means and standard deviations. One-way ANOVA and Chi-square test (survival rate). n.s. = not significant. n = 30.

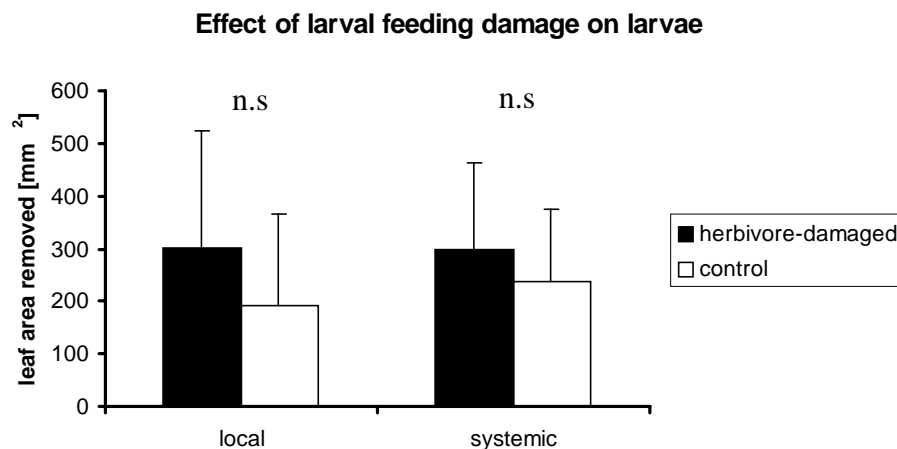
Parameters	Treatment						P-level
	Damaged leaves		Systemic leaves		Control leaves		
	mean	SD	mean	SD	mean	SD	
Larval weight [mg]	3.6	1.11	3.9	1.95	3.3	1.07	n.s.
Pupal weight [mg]	7.3	1.27	7.1	1.17	7.1	1.17	n.s.
Survival rate [%]	80		83		83		n.s.
Duration of larval development [d]	12.9	1.01	12.3	0.88	12.6	0.86	n.s.
Fecundity [No. eggs laid / 3 d]	50	9	49	6	55	5	n.s.

as the insects did not differentiate between the leaf adjacent to the damaged leaf and a control leaf from an uninfested plant. We hypothesise that gravid *P. cochleariae* respond to changes in host plant constituents induced by previous larval feeding. Indeed, an earlier study had demonstrated a lower sucrose content and higher concentrations of indole glucosinolates and anthocyanins in Chinese cabbage leaves in response to damage by larvae of *P. cochleariae* (Rostás et al., submitted). Sucrose is well known as a feeding stimulant in several herbivorous insects (Bernays & Chapman, 1994)

Mechanical damage of leaves that mimicked larval feeding did not affect beetle behaviour. Females laid similar numbers of eggs and fed as much plant tissue on artificially damaged leaves as on control leaves. This result shows that the altered physical appearance of herbivore-damaged leaves is not likely to have caused the deterrent effect we observed, since identical areas of leaf tissue were removed in both experiments testing the effects of herbivory and mechanical damage, respectively. Furthermore, this result shows that mechanical damage

obviously did not induce the same plant responses as herbivory. This is in accordance with a number of other studies demonstrating different plant responses to herbivory and mechanical damage (Karban & Baldwin, 1997).

A further experiment assessed the role of combined mechanical damage and larval frass on the oviposition and feeding behaviour of *P. cochleariae*. Female beetles did not distinguish between leaves that were treated with faeces of second-instar larvae and controls which were merely mechanically damaged. Thus, the results show that larval frass had no direct deterrent effect on female *P. cochleariae*. This contrasts with several other studies which demonstrated that faeces of herbivore larvae may have a direct effect on the oviposition behaviour of conspecifics (e.g. Renwick & Radke, 1980; Hilker & Klein, 1989; Anderson et al., 1993). Furthermore, the results of this study here show that treatment of wounded Chinese cabbage leaves with faeces did not induce plant responses which deter the beetles from feeding and egg laying. This is in accordance with the outcome of other studies on the inductive ability of herbivore faeces. For example, corn seedlings when placed with their stem into extracts of faeces from larvae of



**Figure 4** Effect of larval feeding damage on feeding of second-instar larvae *Phaedon cochleariae* in dual-choice assays. Larvae chose between Chinese cabbage leaves damaged by conspecific larvae (dark columns) and undamaged control leaves (white columns). 'local': damaged leaves. 'systemic': undamaged leaves adjacent to damaged ones. Wilcoxon matched pairs test. n.s. = not significant. n = 20.

*Spodoptera exigua* emitted much lower amounts of volatiles than seedlings supplied with herbivore regurgitant (Turlings et al., 1993).

The regurgitant from herbivorous insects (mostly lepidopteran larvae) applied to mechanical wounds has been shown to elicit defensive responses in numerous plant species

(Felton & Eichenseer, 1999). Most studies on the effect of herbivore regurgitant application onto wounded plants demonstrated the induction of volatiles that attract predators and parasitoids of the herbivore (Turlings et al., 1993; 1998; Mattiacci et al., 1995). Effects on non-volatile plant chemistry have been reported less frequently but regurgitant applied to wounds may elicit a higher activity of phenylalanine ammonia lyase in birch trees or the induction of proteinase inhibitor transcripts in potato (e.g. Hartley & Lawton, 1987; Korth & Dixon, 1997). In our study the application of regurgitant from mustard leaf beetle larvae on Chinese cabbage leaves did not evoke any significant effects on the host acceptance behaviour of gravid females. The following explanations are possible: i) the regurgitant did not induce any responses in the plant, since no eliciting components are present in the regurgitant, ii) the regurgitant did not induce the same responses as larval feeding. The eliciting compound is expected to be a component of the herbivore saliva released from glands opening into oral cavity or near the mouth (Felton & Eichenseer, 1999; Turlings et al., 2000). Regurgitant, however, is collected by squeezing the insect with forceps. The obtained oral fluid may or may not contain components of the herbivore's saliva (Felton & Eichenseer, 1999).

While herbivore-induced changes to Chinese cabbage plants deterred female leaf beetles, no such effects were found on the larvae. Second-instars of *P. cochleariae* did not distinguish between previously damaged leaves and undamaged controls. Likewise, no systemic effects were observed. Larval development was equally good on herbivore-damaged leaves, on systemic leaves and on controls from undamaged plants. Similar results were reported by Anderson and Alborn (1999) who investigated the effect of plant damage on the oviposition behaviour of the noctuid moth *Spodoptera littoralis* and the performance of its larvae. Moth females also avoided to oviposit on cotton plants previously damaged by conspecific larvae while artificial damage failed to produce any deterrent effects. As in our study, larval development was not detrimentally affected by herbivory. However, in our experiments the leaf material offered were leaf disks. Thus, we cannot exclude that the effects of this mechanical damage necessary to obtain the leaf disks may have masked possible differences in behaviour and performance of larvae that might have been detectable if they would have been offered entire plants.

In our experiments, *P. cochleariae* could respond both to contact and olfactory cues from the leaves. Several studies are available that examined exclusively the response of leaf beetles towards volatiles from damaged and undamaged plants. In contrast to our findings, adults of

the leaf beetle *Oreina cacaliae* (Kalberer et al., 2001) and the Colorado potato leaf beetle *Leptinotarsa decemlineata* (Bolter et al., 1997; Schütz et al., 1997) were attracted towards volatiles from damaged plants regardless whether the damage was artificial or conducted by conspecifics. Adults of the chrysomelid species *Phyllotreta cruciferae*, another crucifer specialist, were attracted towards host plants upon which conspecifics were feeding. However, this attraction was found to be due to an aggregation pheromone released by the beetles feeding on the plant (Peng & Weiss, 1992). The benefits of attraction of *O. cacaliae* and *L. decemlineata* to volatiles from damaged plants have also been discussed in the context of benefits of aggregation and mate finding (Bolter et al., 1997; Schütz et al., 1997; Kalberer et al., 2001).

The results of the experiments presented here and of a previous study (Rostás et al., submitted) indicate that herbivore attack induces physiological changes in Chinese cabbage plants. These changes are used by female mustard leaf beetles to recognise attacked plants and to avoid feeding and egg deposition on them. However, why are they doing so? Our results give some indication that larval performance on damaged and undamaged Chinese cabbage does not differ. If not escape from unsuitable food and intraspecific competition is the driving force for female *P. cochleariae* to avoid feeding and oviposition on damaged plants, another factor might be important. Feeding damage of *P. cochleariae* larvae on Chinese cabbage leaves may induce the emission of volatiles that attract predators or parasitoids of the larvae. The induction of synomones by herbivore damage is well known in *Brassica* (e.g. Agelopoulos & Keller, 1994; Blaakmeer et al., 1994; Mattiacci et al., 1994; Geervliet et al. 1994, 1998). *P. cochleariae* could benefit from avoiding damaged Chinese cabbage leaves because this behaviour may reduce the risk of being exposed to enemies. Future studies are needed to examine whether feeding activity of *P. cochleariae* larvae induces the emission of synomones in Chinese cabbage.

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