Chapter 2

Asymmetric plant-mediated cross-effects between a herbivorous insect and a phytopathogenic fungus

Abstract

1 Cross-effects between a herbivorous insect and a phytopathogenic fungus on their common host plant were examined. Specifically, we addressed the questions whether (i) infection of Chinese cabbage leaves by the fungus *Alternaria brassicae* affects the development and host selection behaviour of the leaf beetle *Phaedon cochleariae* and whether (ii) herbivory influences host suitability of Chinese cabbage for *A. brassicae*.

2 Feeding on fungus-infected leaves prolonged larval development and reduced pupal weight of *P. cochleariae*. Adult beetles avoided feeding and egg deposition on fungus-infected leaves. In contrast to these local effects, no systemic effect of phytopathogenic infection on the herbivore was detected.

3 Herbivory influenced fungal growth neither locally nor systemically.

4 Thus, our results demonstrate an asymmetric relationship between herbivore and fungus. While herbivory had no visible impact on fungal growth, infection of the plant induced local resistance against *P. cochleariae*.

Key words

Alternaria brassicae *Chinese cabbage* *cross-effects* *herbivory* *induced resistance* *pathogens* *Phaedon cochleariae* *three-way interactions*

INTRODUCTION

Presumably every plant species is exploited as a food source by a range of phytopathogenic fungi and herbivorous insects. Considering their abundance it is obvious that simultaneous or sequential occurrence of insects and pathogens on the same host plant must be assumed as a common ecological scenario (de Nooij *et al.*, 1992). Thus, plants often need to cope with both pathogens and herbivores.

Interactions between these plant antagonists can be direct or indirect, mutualistic or detrimental (Hatcher, 1995). Herbivores may act as vectors of fungal diaspores and facilitate penetration of mycelium into plant tissue by their feeding action (e.g. Dutch elm disease). Phytopathogenic fungi can be an additional source of sterols which are essential to virtually all insects (Bernays, 1992; Mondy & Corio-Costet, 2000). On the other hand, pathogens and herbivores may interact indirectly by influencing the suitability of their common host plant. From the plant's standpoint, both the attack by pathogens and herbivores are biotic stress factors that evoke a variety of complex changes in the plant's metabolism, including alterations of nutrient allocation patterns and the induction of defence-related compounds (Ayres, 1992; Baldwin & Preston, 1999; Hammerschmidt, 1999). In several studies the outcome of such plant-mediated interactions have been found to affect herbivores and pathogens detrimentally. Previous attack by fungi may induce resistance in plants against subsequent attack by herbivores (Karban *et al.*, 1987; Hatcher *et al.*, 1994b; 1995; Siemens & Mitchell-Olds, 1996), while herbivore-damaged plants may become less suitable for fungi (Karban *et al.*, 1987; Sipos & Sági, 1987; Hatcher *et al.*, 1994a; Russo *et al.*, 1997; Hatcher & Paul, 2000). However, several studies have failed to demonstrate cross-resistance between herbivores and plant pathogenic fungi (Apriyanto & Potter, 1990; Ajlan & Potter, 1991; Stout *et al.*, 1999). In some plants the induction of systemic aquired resistance (SAR) associated with pathogens even led to increased susceptibility towards herbivores (Moran, 1998).

Considerable progress has been made in elucidating the biochemical pathways involved in plant resistance against either herbivores or plant pathogens in recent years (Bostock, 1999; Hammerschmidt & Smith-Becker, 1999; Maleck & Dietrich, 1999; Staswick & Lehmann, 1999). The question of specificity in terms of inducing agents and organisms affected by induced plant responses against insects and pathogens has fuelled the interest in tripartite

interactions between plants, herbivores and phytopathogenic fungi (Karban & Kuc, 1999, Stout & Bostock, 1999, Paul *et al.*, 2000). Contrasting the number of molecular studies investigating specific or general responses of a plant towards pathogens and herbivores, there is a dearth of extended ecological studies as were carried out by Hatcher *et al.* (1994a, 1994b, 1995). Such ecological studies provide detailed information on cross-effects between plant pathogens and herbivores. The integration of molecular insights into the specificity of plant resistance and results from ecological studies may soon help to achieve a better understanding of these multitrophic interactions (Agrawal *et al.*, 1999; Felton & Korth, 2000; Paul *et al.*, 2000).

In this study we focused on possible cross-effects between a herbivorous and a fungal crucifer specialist on their common host plant, *Brassica rapa* (syn. *campestris*) ssp. *pekinensis* (Brassicaceae) (= Chinese cabbage).

The herbivore *Phaedon cochleariae* (Chrysomelidae) attacks many *Brassica* species and other cultivated or wild crucifers from May until September. Both larvae and adults of the so-called mustard leaf beetle feed on the leaves of their host plant and may occur in high population densities (Jones & Jones, 1966).

The fungus *Alternaria brassicae* (Deuteromycetes) is the causal agent of black spot, a widespread disease of crucifers (Smith *et al.*, 1988). All above ground parts including leaves, stems and the inflorescences of the plant can be affected by the necrotrophic fungus, reducing the photosynthetic area and accelerating senescence and defoliation. Optimum temperatures for sporulation are between 16 °C and 24 °C. Moisture in the presence of rain, dew, or high humidity is essential for infection, and a minimum of 9 – 18 h is required (Humpherson-Jones & Phelps, 1989).

The Chinese cabbage plant is known to respond to pathogen infection by producing phytoalexins (Takasugi *et al*., 1988; Pedras *et al*., 2000b), which have been shown to modify the behaviour of herbivores (Baur *et al*., 1998). Additionally, *Brassica* spp. may change the concentrations of their characteristic glucosinolates in response to herbivore and pathogen attack (Birch *et al*., 1996; Rask *et al*., 2000). However, the role of glucosinolates as a defence mechanism against specialists remains questionable (Bennett & Wallsgrove, 1994). To some degree *A. brassicae* appears to be adapted to the glucosinolate defence system and for *P. cochleariae* several glucosinolates may even act as feeding and oviposition stimulants (Doughty *et al.*, 1996; Koritsas *et al.*, 1991). Other physiological traits found to be inducible

in *Brassica* spp. by fungal infection involved changes in the allocation of sucrose, enhanced activities of defence-related enzymes and hypersensitive responses (Köhle, 1989; Gupta *et al.*, 1990). The formation of necrotic tissue is not only known as hypersensitive response towards pathogen infection, but also towards oviposition of a herbivorous insect (Shapiro & DeVay, 1987).

To our knowledge there is only one study available that examined interactions between plant pathogens and herbivores on *Brassica* spp. (Siemens & Mitchell-Olds, 1996). In this study the herbivore's performance and the impact of herbivory on fungal growth were not assessed. So, in spite of what is known about either pathogen- or herbivore-induced physiological changes in *Brassica* species, detailed information on possible ecological cross-effects of fungal and herbivore attack in Chinese cabbage is lacking. Our aims were to assess the type and quality of possible interactions between Chinese cabbage, the herbivore *P. cochleariae*, and the fungus *A. brassicae* with a focus on local and systemic cross-effects. The impact of fungal infection on the performance and host choice of larvae and adult beetles was investigated. Furthermore, we addressed the question whether herbivore damage had any plant-mediated effects on fungal growth.

MATERIALS AND METHODS

General

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 (20 °C – 25 °C). Standard potting soil (Einheitserde Typ P, Kausek GmbH and Co. KG, Mittenwalde, Germany) was used. A single dose of fertilizer was applied once 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 assigned to herbivore or fungus treatment when the 5th leaf (1st leaf = oldest leaf) had reached maximum size (i.e. 4 - 5 weeks after germination).

Strain BBA 64878 of *Alternaria brassicae* (Berk.) Sacc. was obtained from the Biologische Bundesanstalt (Berlin-Dahlem, Germany). The fungus was permanently cultured in Petri dishes on potato-dextrose-agar (PDA) at 20 °C without induction of sporulation. Five days before inoculation of the plants, small agar blocks containing mycelium were transferred to new Petri dishes.

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.

Fungal inoculations

The inoculation of Chinese cabbage leaves with *A. brassicae* was carried out as described by Gröntoft & O'Connor (1990). This method has the advantage of enabling the detection of small differences in fungal attack, which may not be visible when using the more standardized spray inoculation technique. Spraying leaves with a conidia suspension of the fungus may come closer to the actual infection process in the field. However, the high variability in lesion development associated with this method may conceal small but real effects. Number and age of the leaves inoculated in each experiment differed according to the questions studied (see below).

For inoculation, a small mycelium-containing agar disc (Ø 5 mm) was punched out from behind the growth front of a Petri dish culture, using a cork borer. The disc was laid upside down onto the adaxial face of the leaf bringing the mycelium in contact with the leaf surface. The disc was fixed between two blocks of firm foam material (10 x 10 x 7 mm) with the leaf being between the disc and the second foam material block on the abaxial side of the leaf. Finally, an insect needle (No. 00) was pierced through inoculum, leaf, and foam blocks. To control possible wound-induced effects by leaf piercing, experiments were conducted that examined whether mechanical damage of Chinese cabbage leaves affects the performance and host selection of *P. cochleariae*. No such effects were detected (Rostás & Hilker, unpublished results). Depending on the experiment, a leaf was inoculated with 2 or 4 agar discs (see below). Leaves of control plants were treated equally (control agar block, foam material, piercing), but the mycelium was omitted. Following inoculation, treated plants and controls

were placed under a tent made of thin transparent plastic foil in a completely randomised design. The tent ensured an atmosphere of 100 % relative humidity, thus enhancing fungal growth. In all experiments, leaves of infected plants were tested 5 days after inoculation.

A. brassicae grows more or less concentrically from the spot of inoculation. As described by Gröntoft & O'Connor (1990), a chlorotic circle was frequently observed on the perimeter of the necrotic lesion. It has been assumed that in this zone, where fungal toxins were shown to be active (Bains & Tewari, 1987), fungal hyphae border intact leaf tissue. In fact, we observed that larvae of *P. cochleariae* did not feed in the immediate vicinity of the lesions, but kept a distance of 3 - 5 mm.

Herbivore treatment

Experimental plants were selected for equal size and arranged in a completely randomised design. One clip cage (∅ 21 mm, h: 18 mm) containing five second-instar larvae was attached to the 5th leaf of an experimental plant. After feeding for 24 h the larvae were either removed (24 h treatment) or confined to an undamaged part of the same leaf where they were left to feed for further 24 h before being moved to another undamaged part of this leaf for 24 h (72 h treatment). During one day of feeding the larvae removed about 3 % of the leaf area. Control plants received identical treatments with empty clip cages.

Effects of fungal infection on the development of *P. cochleariae*

The influence of fungal infection and leaf age on the development of *P. cochleariae* was assessed by feeding the insects with discs (∅ 10 mm) punched out from one of the following leaf types:

- old leaves infected by *A. brassicae* ('old infected leaves', 5th leaf)
- uninfected old leaves adjacent to 'old infected' leaves ('old systemic leaves', 6th leaf)
- old leaves of a healthy plant ('old control leaves', 5th leaf)
- young leaves of a healthy plant ('young control leaves', 8th-10th leaf)
- uninfected young leaves of an infected plant ('young systemic leaves', 8th-10th leaf).

Each infected leaf was inoculated with four fungal agar discs. Old leaves were fully unfolded. Young leaves are here referred to as leaves that had not yet been unfolded. 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 (∅ 55 mm) which were kept in transparent plastic boxes (20 x 20 x 5 cm) containing moist paper tissues. Each day larvae were supplied with one leaf disc (∅ 10 mm) that had been punched out with a cork borer from a plant belonging to one of the treatment groups mentioned above. Discs taken from an infected leaf excluded chlorotic and necrotic parts of the leaf. The leaves used for the experiments showed no signs of wilting. All experimental plants were used once only. Leaf discs were randomly sampled from four individual plants per day.

Fresh weight of larvae was measured 5 d after hatching and pupae were weighed 2 d after pupation had taken place. The amount of food consumed by second-instar larvae during 24 h was assessed by producing digitalised images of the leaf discs with a scanner connected to a computer. Total area of the feeding holes was measured to the nearest of 1 mm^2 by a self-written image analysis software program. Mortality rates until eclosion and duration of larval development were recorded. After eclosion male beetles were individually placed in Petri dishes containing a female from the same treatment group. Every day beetles were supplied with a leaf disc from a healthy plant. The number of eggs laid per female was counted for 3 d after the onset of oviposition. Within 3 d of oviposition gravid females have been observed to lay sufficient numbers of eggs for statistical analysis of differences in oviposition behaviour.

A second experiment was carried out to examine the effects of fungus infection on the leaf beetle performance under more natural temperature conditions as the ones described above**.** The same protocol was applied as described above with the exception of using exclusively old infected leaves and old control leaves. Both treatment groups were now no longer assigned to constant temperature, but to a temperature regime of 23 °C (16 h) and 12 °C (8 h), representing long-term mean maximum and minimum temperatures during August in Berlin, Germany (Institute for Meteorology, Freie Universität Berlin, Germany).

Effects of fungal infection on the host choice behaviour of *P. cochleariae*

A series of dual-choice assays was performed to study the effects of fungal infection of the plant on the feeding and oviposition preferences of adults and larvae of *P. cochleariae*. The 5th leaf of each experimental plant was inoculated symmetrically with four agar blocks as described above. Five days after inoculation the infected leaf was detached from the plant. This infected leaf and the 5th leaf of a control plant were placed into a transparent plastic box (20 x 20 x 5 cm). Either five larvae (L_2) or five gravid females (3 - 5 weeks old) of *P. cochleariae* were set into each box and left to feed and lay eggs, respectively. Twenty-four hours later the number of eggs laid was recorded and the leaf area consumed was measured using computer aided image analysis. Experiments conducted to determine systemic effects were carried out as described for local effects, using the uninfected 6th leaf of an infected plant instead.

Effects of herbivory on fungal growth

Two sets of experiments were designed to test whether feeding damage induces local or systemic cross-effects that might influence the growth of *A. brassicae*. The 5th leaf of a Chinese cabbage plant was confined to herbivore damage treatment as detailed above. Larvae were allowed to feed for 24 h or 72 h, removing 3 % or 9 % of the leaf area. This induction time was chosen because comparable experiments were able to show induced plant resistance against subsequent fungal attack (Hatcher 1994a). At the end of a feeding induction period clip cages were removed. Damaged leaves (5th leaf) and adjacent systemic leaves (6th leaf) of a herbivore-treated plant, as well as the respective control leaves of a healthy plant, were each inoculated with two mycelium-containing agar discs. The agar discs were symmetrically fixed onto both leaf halves. Inoculations were carried out immediately (0 d) after the herbivores had been removed from the plant or after 3 d or 8 d, respectively. Areas of necrotised leaf tissue were measured from digitalised images 5 d after inoculation.

Statistics

Normality of data was verified using Shapiro Wilks' W test ($P > 0.05$). Larval performance parameters at constant temperature of 25 °C were analysed by one-way ANOVA and Scheffé

tests, except for survival rates which were analysed by a chi-square test. Student's *t*-test for independent samples examined the effects of fungal infection on larval performance at alternating temperatures and analysed the data of the no-choice consumption rate test. Data from feeding and oviposition dual-choice tests were analysed by Wilcoxon matched pairs tests. The effects of herbivory on fungal growth were assessed by two-way ANOVA with 'treatment' (control, locally damaged, systemic) and 'induction time' (0 d, 3 d, 8 d) as factors (compare Fig. 3). Post-hoc power analyses (software program 'G*Power', Erdfelder *et al*., 1996) ensured that the sample sizes used were large enough to detect a difference between treatments. The Statistica software package (StatSoft, Tulsa, OK, USA) was used for all analyses.

RESULTS

Effects of fungal infection on the development of *P. cochleariae*

At constant temperature the fresh weight of larvae reared on so-called 'old infected leaves' was significantly reduced on the 5^{th} day after hatching compared to all other treatment groups (one-way ANOVA, $df = 4$, $MS = 21.88$, $F = 13.87$, $P < 0.001$) (Table 1). Systemic effects of fungal infection on larval weight were not observed. Larvae feeding on old systemic leaves did not differ significantly in weight from individuals reared on old control leaves (Scheffé test: $P = 0.100$). The same applied to larvae reared on young systemic leaves and young control leaves, respectively (Scheffé test: $P = 0.545$). Equally, no effect of leaf age was found since individuals fed discs of young Chinese cabbage leaves (treatment or control plants) did not weigh significantly less than larvae kept on old control leaves (young control

Table 1 *Performance of* Phaedon cochleariae *reared on differently treated Chinese cabbage leaves at constant temperature (25°C). 'old infected leaves' = old leaves infected by* Alternaria brassicae*, 'old systemic leaves' = uninfected old leaves adjacent to 'old infected leaves', 'old control leaves' = old leaves of a healthy plant, 'young systemic leaves' = uninfected young leaves of an infected plant, 'young control leaves' = young leaves of a healthy plant. Data represent means ± standard deviation. n = 30. One-way ANOVA and Scheffé tests: ***P < 0.001. n.a. = not assessed.*

	Treatment									
	old infected leaves		old systemic leaves		old control leaves		young systemic leaves		young control leaves	
Larval performance (25°C)	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Larval weight after 5 d [mg]	2.8***	0.69	4.5	1.41	3.9	1.32	5.1	1.45	4.6	1.45
Duration of larval development [d]	13.2***	0.80	12.1	0.70	12.4	0.69	11.9	0.66	12.1	0.71
Pupal weight [mg]	8.4	1.47	8.6	1.83	8.5	1.63	8.1	1.05	8.8	1.41
Food consumed [mm^2] / L_2 / 24 h	23	10.7	n.a.		20	9.4	n.a.		n.a.	
Fecundity [no. eggs laid / 3 d]	49	9.2	49	10.0	51	20.0	51	16.2	53	8.6
Survival rate [%]	70		83		73		87		87	

leaves vs. old control leaves: Scheffé test: $P = 0.100$; and young systemic leaves vs. old control leaves: Scheffé test: $P = 0.794$). The duration of larval development was prolonged when insects fed on old leaves inoculated with *A. brassicae* compared to individuals reared on leaves of the other treatment groups ($df = 4$, $F = 13.15$, $P < 0.001$). Thus, the time until pupation commenced was increased by 9 %. However, pupae of *P. cochleariae* showed no difference in

Table 2 *Performance of* Phaedon cochleariae *reared on differently treated Chinese cabbage leaves at alternating temperatures (23 °C / 12 °C). 'old infected leaves' = old leaves infected by* Alternaria brassicae*, 'old control leaves' = old leaves of a healthy plant. Data represent means ± standard deviation. n = 30. Student's t-test for independent samples: ***P < 0.001, **P < 0.01. n.s. = not significant.*

	Treatment				
	old infected leaves		old control leaves		
Larval performance (23°C/12°C)	mean	SD	mean	SD	*P*-level
Larval weight after 5 d [mg]	0.9	0.30	1.3	0.41	**
Duration of larval development [d]	14.7	0.9	13.6	1.0	***
Pupal weight [mg]	7.1	0.78	8.2	0.89	***
Fecundity [no. eggs laid / 3 d]	54	5.6	57	7.1	n.s.
Survival rate [%]	92		96		n.s.

weight on the second day of their pupal stage, regardless of the food type they were reared on ($df = 4$, $F = 1.57$, $P = 0.190$). Neither the amount of food consumed by the larvae (Student's *t*-test for independent samples: $P = 0.424$) nor the fecundity of the resulting females ($df = 4$, $F = 0.44$, $P = 0.778$) nor the total survival rate (chi-square test, $df = 4$, *chi-square* = 6.73, $P = 0.151$) was significantly affected by the fungal infection of the plant.

When reared at a changing temperature regime of 23 °C / 12 °C, larvae feeding on infected leaves for 5 d also weighed less (Student´s *t*-test: $P < 0.001$) and larval development was prolonged by about 8 % compared to individuals feeding on infected leaves ($P < 0.001$) (Table 2). In contrast to the results obtained at constant temperature conditions, pupal weights of beetles reared on fungus-infected leaves were significantly decreased at changing temperatures in comparison to those reared on uninfected leaves. Still, the number of eggs laid

by females that had spent their larval life on infected leaves did not differ from the oviposition rate of females that were fed healthy leaves during their development ($P = 0.246$) (oviposition rate recorded for 3 days). The survival rate of both treatment groups remained equally unaffected ($df = 1$, *chi-square* $= 0.200$, $P = 0.655$).

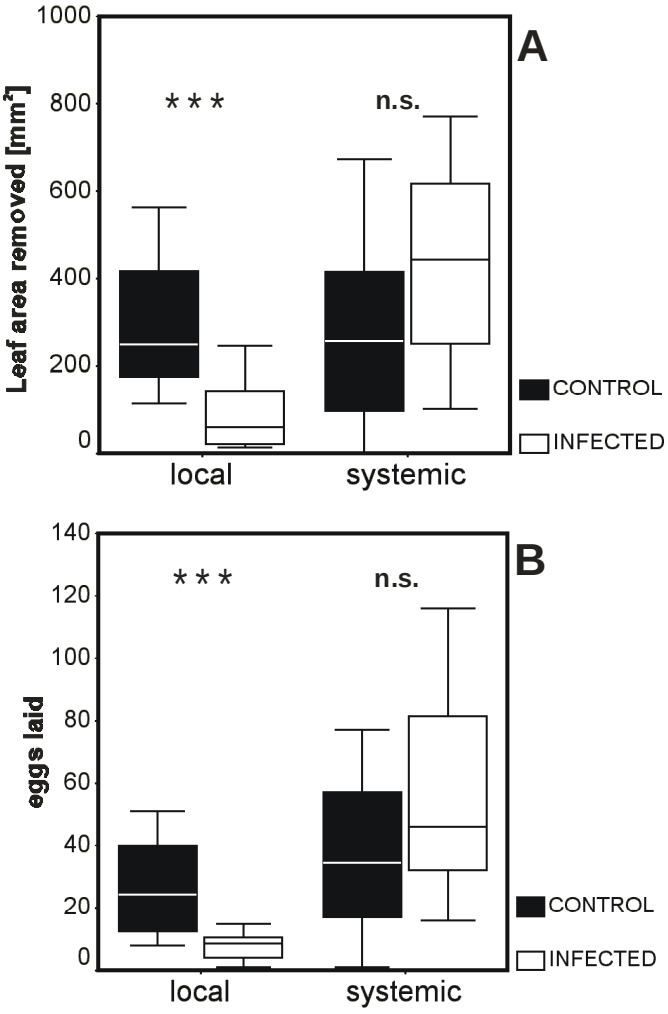

Figure 1 *Feeding* (**A**) *and oviposition* (**B**) *preference of female* Phaedon cochleariae *in dual choice tests on healthy control plants of Chinese cabbage (dark columns) and plants inoculated by the fungus* Alternaria brassicae *(white columns). 'local': infected leaves. 'systemic': uninfected leaves adjacent to infected ones. Boxes represent data between 25% quartile and 75% quartile. Median is depicted by a line dividing each box. 'Whiskers' show minimum and maximum extremes. n = 20. Asterisks denote significant differences. Wilcoxon matched pairs test: ***P < 0.001, n.s. = not significant.*

Effects of fungal infection on the host choice behaviour of *P. cochleariae*

Gravid female beetles preferred to feed on healthy leaves when offered in combination with leaves locally infected by *A. brassicae* (local; Wilcoxon matched pairs test, $P < 0.001$) (Fig. 1A). Likewise, significantly more eggs were laid on control leaves (local; $P = 0.003$) (Fig. 1B).

Given the choice between uninfected leaves that were adjacent to infected ones (systemic) and healthy leaves of uninfected control plants, females did not distinguish between these types of leaves concerning consumption and oviposition (systemic; leaf consumption: $P = 0.086$, oviposition: $P = 0.162$). In contrast to gravid females, second-instar larvae of *P. cochleariae* removed larger portions from locally infected leaves than from healthy control leaves (local; $P = 0.002$) (Fig. 2). More leaf tissue was also removed from uninfected systemic leaves than from leaves of healthy control plants. However, this difference was not significant (systemic; $P = 0.060$) (Fig. 2).

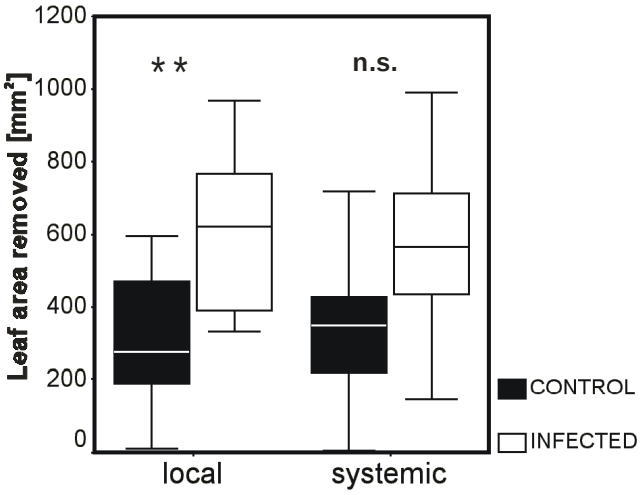

Figure 2 *Feeding preference of larvae (second instar) of* Phaedon cochleariae *in dual-choice tests on healthy control plants of Chinese cabbage (dark columns) and plants inoculated by the fungus* Alternaria brassicae *(white columns). 'local': infected leaves. 'systemic': uninfected leaves adjacent to infected ones. Boxes represent data between 25% quartile and 75% quartile. Median is depicted by a line dividing each box. 'Whiskers' show minimum and maximum extremes. n = 20. Asterisks denote significant differences. Wilcoxon matched pairs test: **P < 0.01, n.s. = not significant.*

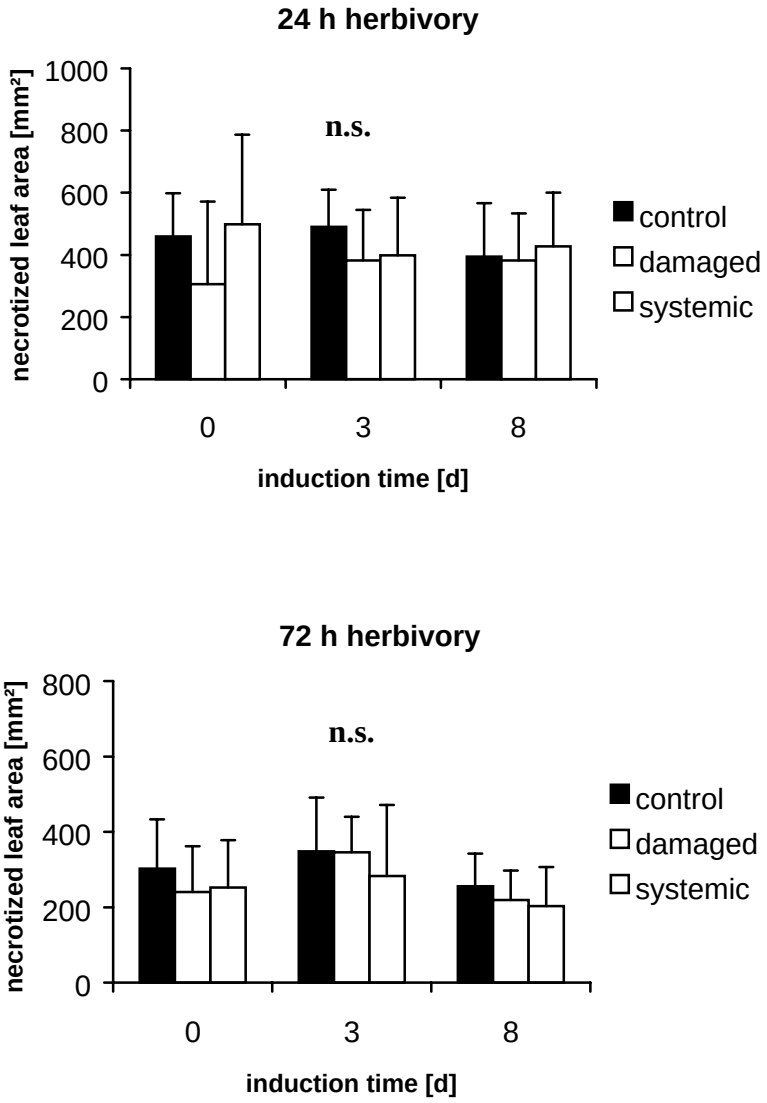

Figure 3 *Mean (± SD) necrotised area on Chinese cabbage leaves caused by* Alternaria brassicae *infection. Duration of feeding by larvae of* Phaedon cochleariae *was 24 h* (**A**) *and 72 h* (**B**). *Inoculations were conducted at the same day when feeding damage was stopped (0 d), or 3 d, or 8 d after this (= induction time). Control: undamaged leaves (dark bars). Damaged: leaves that had been fed on (white bars). Systemic: undamaged leaves adjacent to herbivore-damaged ones (hatched bars). n = 15 for each treatment group. Student's t-test for independent samples: n.s.= not significant.*

Effects of herbivory on fungal growth

On Chinese cabbage leaves the extent of herbivore damage (24 h or 72 h feeding) had no detectable effect on fungal growth measured as necrotised leaf area (Fig. 3A, B). The only difference was found in the second experiment (72 h feeding),

where leaves inoculated 3 d after herbivore treatment had significantly larger lesions than leaves inoculated 8 d after herbivory had ceased (Scheffé test: $P = 0.001$). However, neither a

treatment effect (control, locally damaged, systemic) nor interactions between the treatment and induction time could be observed (24 h herbivory: two-way ANOVA, treatment: $df = 2$, $F = 2.88$, $P = 0.060$, induction time: $df = 2$, $F = 0.16$, $P = 0.847$, treatment x induction time: $df = 4$, $F = 1.19$, $P = 0.320$; 72 h herbivory: two-way ANOVA, treatment: $df = 2$, $F = 1.70$, $P = 0.189$, induction time: $df = 2$, $F = 5.47$, $P = 0.006$, treatment x induction time: $df = 4$, $F = 0.17$, $P = 0.954$).

DISCUSSION

This study demonstrated that infection of Chinese cabbage plants by the fungus *A. brassicae* had significant effects on the performance and behaviour of *P. cochleariae* in the laboratory. In contrast, herbivory of this leaf beetle had no detectable effect on fungal growth.

Local but no systemic effects of fungal plant infection on the leaf beetle's performance and behaviour were found. Only symptom-free parts of infected leaves were fed to the larvae when studying local effects of the fungal infection on herbivore performance. Microscopic studies of stained infected leaf material (staining agent**:** lactophenol aniline blue) revealed hyphae exclusively in parts where symptoms were also visible macroscopically (Rostás & Hilker, unpublished results). Thus, two explanations may be given for reduced larval and pupal weights and prolonged larval development (Table 1, Table 2) after feeding on diseased leaves: (1) the fungus releases noxious components that spread within the infected leaf as e.g. destruxins, a family of cyclic peptide toxins (Pedras *et al.*, 2000a). Destruxins with insecticidal properties are also produced by the entomopathogenic fungus *Metarhizium anisopliae* (Kershaw *et al.*, 1999); (2) the fungus induces, significant metabolic changes in the infected leaf that may have a negative impact on *P. cochleariae* (Köhle, 1989; Gupta *et al.*, 1990; Doughty *et al.*, 1996). Further research will have to elucidate the physiological changes within fungus-infected Chinese cabbage leaves that influence performance and behaviour of the herbivore.

The impact of phytopathogenic fungus infection on the performance of *P. cochleariae*, i.e. the prolongation of larval development on infected leaves, may have significant ecological consequences. Slow growing larvae may suffer greater mortality caused by parasitoids,

predators or entomopathogens as predicted by the 'slow-growth, high-mortality' hypothesis (Feeny, 1976). Since few empirical tests of this hypothesis with inconsistent results are available (Benrey & Denno, 1997; Williams, 1999), our system could provide an interesting model. Comparing the beetle's performance at constant temperature conditions with results obtained from using a changing temperature regime that mimicked natural conditions, we found the pupal weight to be significantly reduced in the latter case, only**.** The differing results concerning the pupal weights in both performance experiments of our study demonstrate the importance of abiotic factors, suggesting that optimal abiotic conditions, such as high constant temperature might compensate deleterious effects of fungal infection on the herbivore. It may be speculated that suboptimal food in combination with suboptimal temperature leads to a trade-off: instead of extending developmental time until maximum body mass is reached, it might be more advantageous to pupate earlier, taking into account a lower body mass. This strategy makes sense if e.g. slow growth means higher vulnerability to natural enemies (Feeny, 1976).

Similar, but more pronounced effects of foliar disease on leaf beetle development have been reported from a natural system including the host plants *Rumex obtusifolius* and *R. crispus*, a rust fungus frequently infecting *Rumex*, and the chrysomelid beetle *Gastrophysa viridula* (Hatcher *et al*., 1994b). Consistent with our results the developmental time of the herbivore on infected leaves was prolonged by 3 % (*R. crispus*) and 11 % (*R. obtusifolius*), respectively. Likewise pupal weight was reduced in spite of greater food consumption and female beetles previously reared on infected leaves laid equal numbers of eggs on healthy and diseased leaves. Systemic effects were not observed. However, larval mortality was higher in individuals feeding on infected leaves. The impact of rust disease on herbivore performance was also investigated by Tinney *et al.* (1998) who showed that fungal infection of *Senecio jacobaea*, *S. vulgaris*, and *Tussilago farfara* had varying effects on larvae of *Tyria jacobaea* (Lep., Arctiidae). Larvae performed worse when reared on infected leaves of *T. farfara*, a host plant of limited suitability for this species. On preferred *Senecio* leaves the development of *T. jacobaea* was not impaired.

Our experiments analysing the effect of fungus infection on the feeding and oviposition behaviour of adult leaf beetles (Fig. 1) revealed that locally infected leaves were avoided, while systemic leaves were not. Since feeding on infected leaves negatively affected larval

weight and developmental time (Table 1, Table 2), females could be expected to prefer healthy over infected leaves as oviposition sites in order to provide optimal conditions for their offspring. However, sometimes also the contrary has been observed (Courtney & Kibota, 1990). Other studies regarding the effects of phytopathogenic infection on the feeding behaviour of adult herbivores revealed results that contrast with our findings. Spotted cucumber beetles (Chrysomelidae) fed more on leaf discs showing necrotic symptoms caused by the cucurbit scab fungus, *Cladosporium cucumerinum*, but did not remove a larger area from systemic leaves (Moran, 1998). In a field choice test, the leaf beetle *Gastrophysa viridula* preferred rust infected leaves for oviposition (Hatcher *et al.*, 1994b).

Host choice of larvae and adults of *P. cochleariae* was found to be differentially affected by fungal infection of Chinese cabbage (Fig. 1, Fig. 2). Despite the adverse effect of fungal infection on the herbivore's development, larvae removed more leaf area from symptom-bearing leaves, whereas adults avoided infected leaves. Obviously, adults and larvae do not respond the same way to fungally induced changes of the plant's quality. Also larvae of another crucifer specialist, *Plutella xylostella* (Lep., Plutellidae), fed more on fungus-inoculated seedlings of *B. rapa* than on uninfected ones (Siemens & Mitchell-Olds, 1996). This effect was dependent on the glucosinolate level of the cotyledons and only at high levels were pathogen-inoculated plants more resistant to herbivory than controls. Siemens & Mitchell-Olds showed that herbivory by crucifer specialists varied curvilinearly with levels of glucosinolates. Maximum feeding damage occurred at intermediate concentrations. Further studies need to examine whether changes in glucosinolate concentrations induced by the fungus had also caused the preference for diseased leaves in larvae of *P. cochleariae*. However, other factors than glucosinolates may be important for such a change of feeding preferences (Bartlet *et al.*, 1999).

In our experiments herbivory on foliage of Chinese cabbage neither facilitated nor restricted fungal growth. This was consistent over almost all variations of extent of leaf damage (24 h and 72 h) and time given for inducing plant responses (0, 3, and 8 d) (Fig. 3). However, there was one exception: surprisingly, after 72 h of herbivory the mean necrotic area was larger on leaves that had been inoculated 3 d after the feeding treatment compared to leaves which had been inoculated 8 d after the herbivore treatment. Since neither treatment effects nor effects of interaction between treatment and induction time were detected, this

observation must be attributed to some unknown factor. In contrast to comparable studies, we used fungal mycelia instead of spores to inoculate experimental plants. Due to this procedure possible resistance effects against germinating spores might have been overlooked. Indeed, Hatcher *et al.* (1995) showed that simulated herbivory reduced the proportion of *Uromyces rumicis* spores that produced appressoria and penetration hyphae in *Rumex* species. On the other hand, the method we used had proved sensitive enough to detect small differences in constitutive resistance of over 40 varieties of Brassicaceae against *A. brassicae* (Gröntoft & O'Connor, 1990). Therefore, this method is able to detect resistance factors that affect growth of intercellular hyphae.

In summary, we demonstrated asymmetrical interactions in the laboratory between a herbivorous insect and a plant pathogenic fungus when occurring on the same host plant. Insect feeding damage failed to induce local or systemic cross-resistance against *A. brassicae* in our experiments, while fungal infection detrimentally affected larval performance and influenced the host selection behaviour of *P. cochleariae*. The asymmetric nature of this relationship adds to the diversity of tripartite interactions already described. Further studies will have to elucidate whether this asymmetric interaction also operates in natural populations, in particular since we were able to demonstrate that abiotic factors may have a modulatory effect.

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