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Cirsium arvense

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Indirect interaction between a fungal plant pathogen and a herbivorous beetle of the weed *Cirsium arvense*

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Abstract Interactions between plants and their natural enemies are well studied, but investigations on the indirect interactions between plant enemies that simultaneously exploit a host plant are rare. Yet these plant-mediated interactions are important because they may affect not only the impact of plant antagonists on plant survival but may also influence the performance of the other plant exploiters. This study focused on the indirect effects of a systemic infection of creeping thistle, [*irsium arvense* (L.) Scop., with the necrotrophic fungus *Phoma destructiva* (Plowr.) on the phytophagous leaf beetle *Cassida rubiginosa* Müller, by examining egg deposition, food plant choice, and larval and pupal performance of the beetle. Thus, the results give a broader view than most other studies of plant-mediated effects of a pathogen on a phytophagous insect. Since both the beetle and the fungus are considered as agents for the biological control of *C. arvense*, the results are also of interest for applied ecology. Potted plants of *C. arvense* were inoculated with a conidiospore suspension of *P. destructiva* to cause a systemic infection of the plants. In a cage experiment, ovipositing females of *C. rubiginosa* showed a significant preference for healthy thistles. In dual-choice tests, adults of *C. rubiginosa* preferred leaf discs from healthy thistles over those from *Phoma*-infected thistles. The beetles also consumed significantly more leaf tissue from healthy than from infected plants. Development time from freshly hatched larvae until pupation was significantly longer for larvae fed on infected leaves. The weight of last-instar larvae and pupae was lower, and larval and pupal mortality was higher when larvae had been fed with infected compared to healthy leaves. Thus, the combined use of both potential biological control agents may be of lowered efficiency because (1) *C. rubiginosa* avoided infected thistles for both egg deposition and adult feeding and (2) *Phoma* infection negatively affect-

ed larval development and increased larval and pupal mortality of the beetle.

Keywords Plant-mediated interactions · Insect performance · Herbivory · Pathogen · Biological control

Introduction

Phytophagous insects and plant pathogenic fungi are main components of global biodiversity (Hawksworth 1991). Since many of them often share the same host plant species, interactions between insects and pathogens are highly likely. In fact, such interactions are anything but rare. These interactions can be direct, e.g. when insects are consuming mycelia or spores, or when fungi are dispersed by insect vectors. Or they can be indirect, when alterations of the plant caused by one of its natural enemies affect another natural enemy (Hatcher and Ayres 1997). The probability and also the importance of those interactions should increase, the more species of phytophagous insects and pathogens are involved. As discussed by Barbosa (1991), the specific outcome of plant-pathogen-herbivore interactions will depend on the kind of plant, herbivore and pathogen involved in the interaction. The consequences for a herbivore feeding on a food plant infected by a pathogen can be beneficial, detrimental or neutral (Barbosa 1991) due to chemical or physical plant factors (Hatcher 1995). Although there are many separate studies on plant-pathogen and plant-herbivore interactions, only a few have brought both together and focused on indirect herbivore-pathogen interactions (for an overview see Barbosa 1991; Hatcher 1995; Hatcher and Ayres 1997).

Hatcher (1995) stated that combinations of plant antagonists can result in synergistic, additive, equivalent or inhibitory effects on plant performance, compared with the effect of each of the antagonists alone. Since combinations of plant natural enemies are also of interest use in the biological control of weeds (Hatcher 1995), the outcome of biological control success will strongly de-

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pend on the degree to which phytophagous insects and plant pathogenic fungi influence each other (Hammond and Hardy 1988; Hammerschmidt 1993; Moran 1998). Nevertheless, studies on the compatibility of insect and pathogen control agents are rare (Karban et al. 1987; Krischik 1991; Saner et al. 1994).

Creeping thistle, *Cirsium arvense* (L.) Scop., is a problematic weed on arable land, fallow and pastures (Hayden 1934). It causes high losses in crop yield (Donald 1994) and is difficult to control because it is capable of both sexual and vegetative propagation (Ang et al. 1994).

Several authors have studied the ability of phytophagous insects (Zwölfer and Harris 1966; Zwölfer et al. 1970; Peschken et al. 1982; Rees 1990; Ang et al. 1994, 1995; Wan et al. 1996a, 1996b; Peschken and Derby 1997) and plant pathogenic fungi (French and Lightfield 1990; Frantzen 1994; Völker and Boyle 1994; Guske et al. 1996; Spring and Kok 1997) to act as agents for the biocontrol of creeping thistle.

The leaf-feeding tortoise beetle, *Cassida rubiginosa* Müller, is one of the phytophagous insect species that has been favoured as a potential biocontrol agent (Ang et al. 1994; Spring and Kok 1997), because it is able to reduce thistle growth and biomass significantly (Ang et al. 1995).

The necrotrophic fungus *Phoma destructiva* (Plowr.), also suggested as a potential biocontrol agent, has only recently been investigated by Guske et al. (1996). It can be isolated from both asymptomatic and chlorotic thistle plants. Inoculation with conidiospores of *P. destructiva* can lead to a systemic infection of the thistles that may alter plant growth and C/N ratio (Huber 1998), as well as reduce flowering and seed set (A. Kruess, unpublished data).

Because both *C. rubiginosa* and *P. destructiva* are native species in central Europe, risks such as detrimental effects on non-target species (Simberloff and Stiling 1996; Louda et al. 1997) appear to be lower than for a classical biological control approach (Newmann et al. 1998). Combined use of the two biocontrol candidates requires comprehensive knowledge of their interactions. The effect of a fungal infection of *C. arvense* by *P. destructiva* on the beetle *C. rubiginosa* was investigated in this study.

The following questions were addressed:

1. What is the effect of *P. destructiva* on creeping thistle growth?
2. Are adult female beetles able to discriminate between healthy and infected thistles for oviposition?
3. Does a fungal infection of the thistle affect the feeding behaviour of *C. rubiginosa* adults?
4. Does a fungal infection of the thistle affect the performance of *C. rubiginosa*?

Materials and methods

Plant cultivation and infection

Roots of *C. arvense* were dug up from a 4-m² area on a 4-year-old fallow near Göttingen, Lower Saxony, Germany, in March 2000.

Roots of similar diameter (~5 mm) were cut into fragments 5 cm in length, and each fragment was placed in one of 140 pots (4 l) filled with standardized soil. The pots were watered regularly. The plants were allowed to grow for 4 weeks, resulting in 127 pots with healthy thistle plants. From these, two groups of 60 pots each were chosen randomly. The two plant groups were treated as follows. One treatment was sprayed with a suspension of conidiospores from *P. destructiva* in sterilized water and detergent (Tween® 40), at a concentration of 1.5 million spores/ml (for details see below). The second treatment (the control) was sprayed with only sterilized water and detergent. All plants were sprayed individually with water or spore solution until run-off. At the end of May, all plants were measured (shoot length, shoot basal diameter, number of leaves) and the inoculated thistles were examined for the occurrence of chlorotic leaves, indicating a successful infection. All thistles were equally and alternately used for obtaining leaf material necessary for the insect experiments. Thus, leaf loss should have been comparable for all plants. In the first week of August, thistle shoots from all pots were cut at soil surface level. Shoot length and basal shoot diameter were measured and the number of leaves per shoot was counted.

Cultivation of *P. destructiva*

Spores of *P. destructiva* were obtained from mycelium cultivations on malt-agar Petri dishes, after the growing mycelium had covered the entire Petri dish surface. The entire contents of all Petri dishes (both mycelium and medium) were homogenized in sterile water and vacuum-filtered. After centrifugation and resuspension, spore concentration was measured in a Thoma counting chamber (conidia were counted in 64×0.025 mm² squares, chamber height 0.1 mm), and then diluted with sterilized water to a concentration of 1.5 million spores/ml.

Sampling of adults and rearing of larvae from *C. rubiginosa*

Adult males and females of the beetle were collected in the field in May 2000. The beetles were caged and fed with leaves from healthy thistles. As soon as copulating adults were detected during regular surveys, they were separated, put in Petri dishes and fed with healthy thistle leaves. Fresh leaves were offered every 48 h. Old leaves were removed and screened for eggs, which were then stored in a refrigerator at 8°C until further use. After several hundred eggs had been collected from different females, eggs were exposed to room temperature in Petri dishes on slightly damp filter paper until larvae hatched.

Oviposition experiment

In a cage experiment, carried out at the end of May, adult female oviposition choice between healthy and infected thistles was analysed. In each of six gauze cages (area 2×1 m, height 1.5 m), six pots with thistles (three healthy thistles and three thistles with chlorotic leaves on the top) were arranged in a three by two block with alternation of healthy and infected thistle plants. Four adult *C. rubiginosa* females were released in the centre of each cage and allowed to oviposit for 48 h. Then, all beetles were collected, and all plants removed and inspected for eggs.

Dual-choice experiments

Two different experiments were conducted to investigate the feeding preferences of *C. rubiginosa* adults. Adult beetles collected from a fallow at the end of May were used for the experiments.

In the first experiment, choice of *C. rubiginosa* adults between leaf discs from healthy control thistles (Co) and infected thistles was investigated. This experiment was carried out twice, one with unchlorotic leaves from infected plants (Ph1) and the other with chlorotic leaves from infected plants (Ph2). In each case, 15 bee-

les fed with leaves from healthy thistles were left without food for 12 h before the experiment started. Two leaf discs (15 mm diameter), one from a healthy and one from either a chlorotic or non-chlorotic leaf, were placed in each of 15 Petri dishes (11 cm diameter). Then, one adult beetle was added to each Petri dish. After 30 min, the disc on which the beetle was feeding was recorded. The experiment was repeated seven times with each of the 15 beetles. Time between replicates was 24 h (12 h feeding, 12 h starvation). The frequencies of leaf disc choice were compared.

In the second experiment, the preference of *C. rubiginosa* adults for healthy or infected thistles was quantified by estimating the amount of consumed leaf tissue of simultaneously offered leaf cuttings. Two leaf cuttings of 1×2 cm, one from a healthy leaf (Co), the other from a non-chlorotic infected leaf (Ph1), were placed together in each of 30 Petri dishes. One adult beetle was added to each Petri dish and allowed to feed for 12 h. Then, the proportion of consumed leaf tissue was measured in intervals of 5% using a binocular microscope. The experiment was repeated after 24 h (12 h feeding, 12 h starving of the beetles) but with leaf cutting pairs from healthy (Co) and chlorotic (Ph2) leaves.

Performance experiment with *C. rubiginosa* larvae

The experiment was carried out in Petri dishes (11 cm diameter, with moist filter paper on the bottom). Three treatments with 30 replicates each were randomly arranged on a laboratory table: (1) control leaves from healthy thistles (Co); (2) non-chlorotic leaves from infected thistles (Ph1); (3) chlorotic leaves from infected thistles (Ph2). Larval weight (mg) and length (mm) of 90 freshly emerged *C. rubiginosa* larvae (1 day old) were assessed. Then, one larva was put on the leaf in each of the 90 Petri dishes. Every 48 h, leaves and filter papers were replaced by fresh ones, and each larva was weighed and measured. Time until pupation was recorded and fresh pupae were weighed. Additionally, the time until adults hatched was assessed and freshly hatched adults were weighed.

Statistical analyses

The effects of *Phoma* infection on plant growth and the results from the performance experiment were analysed using a one-way ANOVA. Results from the oviposition experiment and from the two dual-choice tests were compared using *t*-tests. Percentage data were arcsine $\sqrt{}$ -transformed. Other data that did not fit a normal distribution, were ln-transformed. Means and standard errors given in the text are from untransformed data.

Results

Growth of the thistles

Of the original 60 potted plants in each of the two treatments, some plants died during the first 3 weeks after treatment. These pots were removed. So finally, 50 plants from the control and 55 plants from the *Phoma* treatment were used for the experiments and for final plant growth assessment. By the end of August, 47 of 55 plants from the *Phoma* treatment showed the typical chlorosis, mostly on the upper leaves.

Growth differed significantly between healthy and infected thistles, and these results were consistent for data from May and August. In May, shoots of infected thistles were significantly thinner than shoots of healthy thistles ($F_{1,103}=4.78$, $P=0.03$, $n=105$), and the same effect was found in August, at the end of the experiment (Fig. 1A).

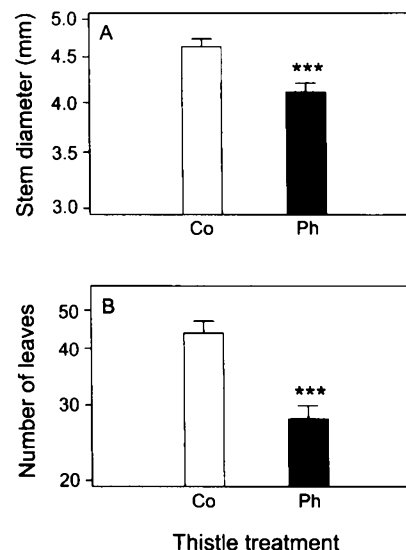


Fig. 1A, B The effect of fungal infection with *Phoma destructiva* on growth of potted creeping thistles (Co healthy plants, Ph infected plants). Bars show means \pm 1 SE. Results were tested with a simple ANOVA with lognormal-transformed data. **A** Basal stem diameter: $F_{1,103}=12.5$, $P<0.001$, $n=105$. **B** Number of leaves per shoot: $F_{1,103}=16.9$, $P<0.001$, $n=105$

Shoot length was not significantly different between the two treatments (May: $F_{1,103}=0.03$, $P=0.57$, $n=105$; August: $F_{1,103}=0.02$, $P=0.89$, $n=105$). Despite similar shoot length, the number of leaves per shoot was significantly higher on healthy than on infected shoots in May ($F_{1,103}=5.19$, $P=0.02$, $n=105$) and August (Fig. 1B).

Oviposition experiment

Adult females of *C. rubiginosa* showed a significant preference to oviposit on healthy plants. An average of 73.8% of the oothecae were laid on healthy thistles and 26.5% on *Phoma*-infected thistles (paired *t*-test: $t=3.48$, $P=0.02$, $n=6$). A mean of 3.4 ± 0.27 oothecae per plant were laid on healthy thistles compared to 1.3 ± 0.46 on infected thistles. The number of eggs per oothecum (egg batch size) was higher on infected thistles (8.7 ± 2.1) than on healthy thistles (5.8 ± 0.6) but this difference was not significant (paired *t*-test: $t=-1.39$, $P=0.22$, $n=6$). Therefore, the number of oothecae but not the number of eggs per plant (healthy thistles: 19.8 ± 2.7 , infected thistles: 9.9 ± 2.7 ; paired *t*-test: $t=2.50$, $P=0.06$, $n=6$) differed significantly between the treatments.

Dual-choice tests

In the first feeding choice experiment, *C. rubiginosa* adults showed a significant preference for leaf discs from healthy thistles over those from *Phoma*-infected plants during the 30-min test intervals (Fig. 2A). In the choice test between healthy thistles and non-chlorotic infected

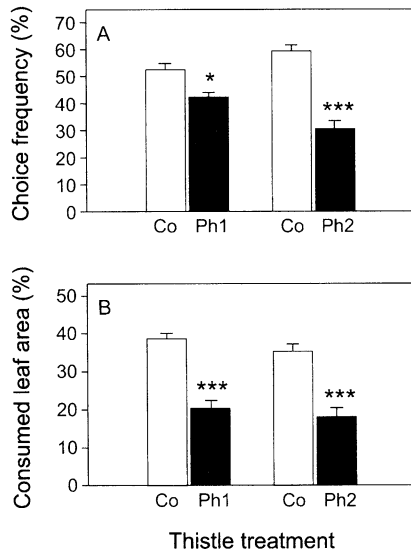


Fig. 2A, B Effect of host plant infection with *P. destructiva* on feeding preference of *Cassida rubiginosa* in dual-choice tests (Co leaves from healthy plants, Ph1 leaves without chloroses from *Phoma*-infected plants, Ph2 leaves with chloroses from *Phoma*-infected plants). Bars show means \pm 1 SE. Results were analysed with paired *t*-tests with arcsine $\sqrt{}$ -transformed data. **A** Choice frequency of leaf discs: Co vs Ph1: $t=2.74$, $P=0.016$, $n=15$; Co vs Ph2: $t=5.99$, $P<0.0001$, $n=15$. **B** Consumed leaf tissue (% area): Co vs Ph1: $t=6.29$, $P<0.0001$, $n=30$; Co vs Ph2: $t=5.17$, $P<0.0001$, $n=30$

thistles (Ph1), the choice frequency for leaf discs from healthy thistles was 52% compared to 42% for the Ph1 leaf discs, and 6% did not chose any disc. In the choice test between healthy thistles and chlorotic thistles (Ph2), the choice frequency was 59% for the healthy thistles, only 30% for the chlorotic leaves and 11% did not make any choice. The results of the two experiments did not differ significantly ($\chi^2=3.80$, $P=0.05$).

In the second feeding choice experiment, the beetles also showed a significant preference for healthy over infected plants, as can be seen in Fig. 2B. The mean percentage of leaf tissue consumed by each of the 30 *C. rubiginosa* adults was significantly higher for leaves from healthy plants (38.5%) compared to unchlorotic infected leaves (20.3%). In the second comparison, the beetles also showed a significantly higher consumption of leaves from healthy plants (35.2%) compared to chlorotic leaves from infected plants (17.5%).

Larval performance

Both development time and mortality of *Cassida* larvae differed between the three treatments. Larvae fed with leaves of healthy thistles developed better and pupated earlier than larvae fed with leaves from infected thistles (Fig. 3). Mean larval development time was significantly lower for larvae fed with healthy leaves (18.0 ± 0.42 days) than for larvae fed with either unchlorotic (20.4 \pm 0.43 days) or chlorotic leaves (20.2 \pm 0.57 days)

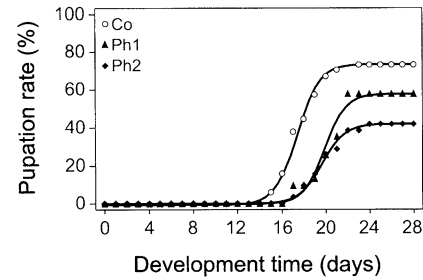


Fig. 3 Dependence of larval development and pupation rate of *C. rubiginosa* on host plants (*Cirsium arvense*) infected with the fungus *P. destructiva*. Of the 93 larvae used in the experiment, 39 died during early larval development and 54 pupated. Co: $y=\exp(-17.09+0.98x)/[1+\exp(-17.09+0.98x)]$, $r^2=0.99$, $P<0.001$; Ph1: $y=\exp(-20.57+1.03x)/[1+\exp(-20.57+1.03x)]$, $r^2=0.97$, $P<0.001$; Ph2: $y=\exp(-18.70+0.95x)/[1+\exp(-18.70+0.95x)]$, $r^2=0.99$, $P<0.001$. Results from logistic regression analyses

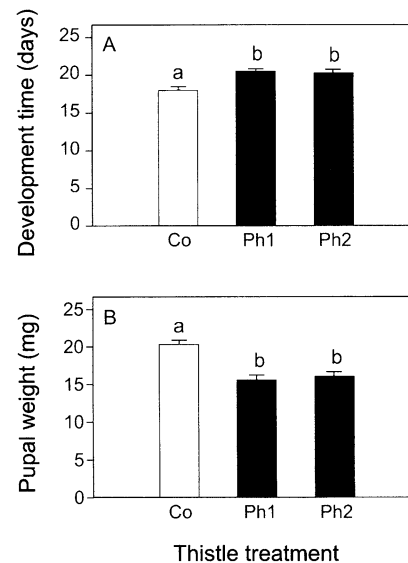


Fig. 4A, B Effect of host plant infection with *P. destructiva* on larval development time and pupal weight of *C. rubiginosa* (Co leaves from healthy plants, Ph1 leaves without chloroses from *Phoma*-infected plants, Ph2 leaves with chloroses from *Phoma*-infected plants). Bars show means \pm 1 SE. Results were analysed with a simple ANOVA. **A** Larval development time: $F_{2,51}=8.9$, $P<0.001$, $n=54$. **B** Pupal weight: $F_{2,51}=14.6$, $P<0.001$, $n=54$

from infected thistles, as shown in Fig. 4A. But there was no significant difference in development time between the two treatments with leaves from *Phoma*-infected thistles (Ph1 and Ph2).

The maximum weight of larvae was negatively affected by *Phoma* infection of the host plant: weight of larvae before pupation was significantly higher ($F_{2,55}=5.63$, $P=0.006$, $n=58$) for those fed with healthy leaves (22.1 \pm 1.0 mg) than for larvae that fed on unchlorotic infected leaves (18.8 \pm 0.95 mg) and chlorotic infected leaves (17.8 \pm 0.81 mg). This reduction in larval weight led to a reduced weight of the pupae. As shown in Fig. 4B, mean pupal weight was significantly higher for larvae that developed on healthy leaves than for larvae

on either non-chlorotic or chlorotic leaves from infected thistles.

The pupation rate of *Cassida* larvae was also affected by fungal infection of the host plant, with increasing mortality of larvae that fed on leaves from *Phoma*-infected thistles (Fig. 3). The pupation rate declined from 74% on healthy thistles (Co) to 58% on unchlorotic leaves from infected thistles (Ph1), and was lowest at 42% on chlorotic leaves from infected thistles (Ph2). These differences in pupation rate were significant only between the Co and the Ph2 treatment ($\chi^2=5.37$, $P=0.01$).

The mortality rate of the pupae was significantly higher on the two *Phoma* treatments compared to the control (Co vs Ph1: $\chi^2=12.6$, $P=0.01$; Co vs Ph2: $\chi^2=10.0$, $P=0.01$). Only four adult beetles hatched from pupae of the Ph1 treatment (hatching rate: 22.2%), and only three beetles hatched in the Ph2 treatment (hatching rate: 23.1%). In contrast, hatching rate of the pupae from healthy thistles was 82.6%.

Discussion

The results showed that a single inoculation with conidiospores of *P. destructiva* significantly reduced the growth of *C. arvensis* plants. *Phoma*-infected thistles were characterized by alterations in plant growth, reduced aboveground biomass (measured as number of leaves) and chlorotic leaves. Such alterations in host plant quality are highly likely to cause responses of the phytophagous beetle *C. rubiginosa*, utilizing creeping thistle as its main host plant.

The results from the oviposition experiment showed that females of *C. rubiginosa* can clearly discriminate between healthy thistles and those infected with *P. destructiva* since they preferred healthy thistles over infected ones for oviposition. These findings are similar to results from S. Kluth (unpublished data) who found oothecae in the field on healthy shoots of *C. arvensis* but not on shoots infected with the rust *Puccinia punctiformis*. In contrast, Kok et al. (1996) found no effect of infection with the rust *Puccinia carduorum* on oviposition of *C. rubiginosa* and two curculionid beetles (*Rhinocyllus conicus*, *Trichosiromalus horridus*) on musk thistle (*Carduus thoermeri*).

Choice of food plant by *C. rubiginosa* adults was also negatively affected by an infection with *P. destructiva*, as shown by the dual-choice tests. Adult beetles preferred tissue from healthy plants for feeding, and avoided feeding on infected leaf tissue regardless of whether or not the infected leaves had chlorotic lesions. A feeding preference of *C. rubiginosa* for healthy thistle compared to rust-infected thistles was found by Kluth et al. (2001), studying the rust *P. punctiformis* on *C. arvensis*. They found that *C. rubiginosa* larvae chose leaf tissue from healthy thistles four times as often as rust-infected leaf tissue. Kok et al. (1996) found that rust infection of musk thistle did not affect larval feeding, but had a negative impact on adult feeding.

The results also showed that the fungus *P. destructiva* negatively affected larval development of *C. rubiginosa*. The period of larval development was significantly longer and growth of larvae (larval weight) as well as weight of pupae were reduced when larvae were fed with *P. destructiva*-infected instead of healthy leaves. These negative effects on preimaginal development apparently increased mortality during larval and pupal stages and resulted in a dramatic reduction in adult hatching success. Corresponding effects on the development of *C. rubiginosa* were found for an infection of thistles with the rust *P. punctiformis* (S. Kluth, A. Kruess, T. Tschartke, unpublished data). Other negative effects of fungi on leaf-chewing insects have previously been reported, such as those of the rust *Uromyces rumicis* on the chrysomelid beetle *Gastrophysa viridula* (Hatcher et al. 1994b) and of the wilt fungus *Verticillium albo-atrum* on the moth *Spodoptera eridania* (Kingsley et al. 1983). Generally, many insects appear to avoid fungus-infected plant tissue (Lewis 1979; Apriyanto and Potter 1990; Hatcher et al. 1994a, 1995; Hoy et al. 1998; Moran 1998). Negative effects on growth and reproduction of other insects due to host plant fungal infection are also known, including the biotrophic rust *U. viciae-fabae* (Zebitz and Kehlenbeck 1991) and the necrotrophic fungus *Phytophthora erythroseptica* (Pratt et al. 1982; Ellsbury et al. 1985). More generally, plant infections by biotrophic pathogens are more likely to cause negative effects on insect herbivores than are plant infections by necrotrophic fungi (Hatcher 1995).

In this study, all experiments showed consistently that a pathogen infection of *C. arvensis* with *P. destructiva* negatively affects the beetle *C. rubiginosa* both in preference and performance. Numerous other studies have found contrasting results between insect feeding on the one hand and reproduction on the other. Moran (1998), who summarized results from studies on cross-effects of plant infection on herbivores, stated that the use of insect feeding as an indicator of cross-effects of infection is popular but may often be misleading. For example, Hatcher et al. (1994a, 1994b, 1995) found that consumption of leaf beetles on rust-infected *Rumex* plants was higher, but their larval growth and adult reproduction were reduced. The increase in feeding may arise from compensatory feeding due to changes in carbohydrate and nitrogen content. Such alterations in plant nutrient quality induced by pathogen infection (Hatcher and Ayres 1997) may be more important for indirect interactions between plant pathogens and phytophagous insects than the induction of peroxidases and phenolics (Moran 1998).

The slower larval development and an increase of mortality of *C. rubiginosa* feeding on *Phoma*-infected thistle leaves cannot easily be attributed to a specific mechanism. Due to tally effects of the biotrophic rust *P. punctiformis* on the development of *C. rubiginosa* (S. Kluth, et al., unpublished data), the underlying mechanism seems to be independent of the pathogen species involved. The pathogen infection likely caused systemic

effects on carbohydrate and nitrogen content (Hatcher 1995). These effects could not be locally restricted to the necrotic lesions since results were similar for both chlorotic and unchlorotic leaves. But *C. rubiginosa* possibly also responded negatively to plant resistance pathways induced by the pathogen (Moran 1998; Felton and Korth 2000; Paul et al. 2000). Inhibitory effects can also be caused by the induction of peroxidases or other pathogenesis-related enzymes responsible for both changes in leaf toughness and the production of oxidized metabolites and free radicals (Duffey and Felton 1991). The production of toxins by the fungus could explain both the high mortality of larvae and pupae and the behavioural responses of the beetles to avoid infected thistles. Avoidance of toxic plants by insects is known from many studies (Rowan and Latch 1994; Hoy et al. 1998), and the production of toxins, e.g. alkaloids, is common for pathogenic and endophytic fungi (Clay 1997). As mentioned by Raps and Vidal (1998), phytosterol metabolism in plants can also affect larval growth and mortality. This can be due to reduced cholesterol uptake caused by pathogen metabolites (Bernays 1993) or to competition between the pathogen and the insect for the sterol resource of the shared host plant (Hendrix 1970).

There is evidence that indirect interactions between thistle pathogens and thistle herbivores are idiosyncratic and strongly depend on the herbivore species involved because responses on the same thistle pathogen differed between herbivore species. In contrast to *C. rubiginosa*, leaf feeding by slugs was higher on thistles infected with *P. destructiva* than on healthy thistles (A. Kruess, unpublished data), and stem-boring weevils preferred shoots of rust-infected thistles but stem-boring and gall-inducing fly species preferred healthy thistles (Kluth et al. 2001). Therefore, further investigations on how an infection with *P. destructiva* can change thistle physiology are necessary.

Both the beetle and the fungus are considered as possible biological control agents. Therefore, the result that (1) adults of *C. rubiginosa* avoided plants infected with *P. destructiva* for oviposition and feeding and (2) larval performance and survival of the beetle were reduced on infected thistles may indicate that in a combination of both agents, synergistic effects are unlikely. However, these results from laboratory and cage experiments cannot be simply extrapolated to the field situation. For example, a combination in sequence, with the beetle first, i.e. inoculating with the fungus only after hatching of *C. rubiginosa* larvae, may increase the impact of *P. destructiva* due to a facilitation of fungal infection rate via leaf damage, as shown by Kluth et al. (2001) for the rust *P. punctiformis* on *C. arvense*. In addition, even the deterrent effect of *P. destructiva* on *C. rubiginosa* oviposition and feeding on infected plants may lead to a synergistic effect in suppressing thistle growth at the population level: the concentration of *Cassida* attack on healthy thistles may increase the mortality of healthy thistles, especially when a critical damage threshold is exceeded.

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References

- Ang BN, Kok LT, Holtzmann GI, Wolf DD (1994) Competitive growth of Canada thistle, tall fescue, and crownvetch in the presence of a thistle defoliator, *Cassida rubiginosa* (Coleoptera: Chrysomelidae). *Biol Control* 4:277–284
- Ang BN, Kok, LT, Holtzmann GI, Wolf DD (1995) Canada thistle (*Cirsium arvense* (L.) Scop.) response to density of *Cassida rubiginosa* Müller (Coleoptera: Chrysomelidae) and plant competition. *Biol Control* 5:31–38
- Apriyanto D, Potter DA (1990) Pathogen-activated induced resistance of cucumber: response of arthropod herbivores to systemically protected leaves. *Oecologia* 85:25–31
- Barbosa P (1991) Plant pathogens and non-vector herbivores. In: Barbosa P, Krischik VA, Jones CG (eds) *Microbial mediation of plant-herbivore interactions*. Wiley, New York, pp 341–382
- Bernays EA (1993) Plant sterols and host-plant affiliations of herbivores. In: Bernays EA (ed) *Insect-plant interactions*, vol IV. CRC, Boca Raton, Fla, pp 45–57
- Clay K (1997) Fungal endophytes, herbivores and the structure of grassland communities. In: Gange AC, Brown VK (eds) *Multitrophic interactions in terrestrial systems*. 36th Symposium of the British Ecological Society. Blackwell, London, pp 153–169
- Donald WW (1994) The biology of Canada thistle (*Cirsium arvense*). *Rev Weed Sci* 6:77–101
- Duffey SS, Felton GW (1991) Enzymatic antinutritive defenses of the tomato plant against insects. In: Hedin PA (ed) *Naturally occurring pest bioregulators*. American Chemical Society, Washington, DC, pp 166–197
- Ellsbury MM, Pratt RG, Knight WE (1985) Effects of single and combined infection of arrowleaf clover with bean yellow mosaic virus and a *Phytophthora* sp on reproduction and colonization by pea aphids (Homoptera: Aphididae). *Environ Entomol* 14:356–359
- Felton GW, Korth KL (2000) Trade-offs between pathogen and herbivore resistance. *Curr Opin Plant Biol* 3:309–314
- Frantzen J (1994) An epidemiological study of *Puccinia punctiformis* Str. (Röhl) as a stepping-stone to the biological control of *Cirsium arvense* (L.) Scop. *New Phytol* 127:147–154
- French RC, Lightfield AR (1990) Induction of systemic aerial infection in Canada thistle (*Cirsium arvense*) by teliospores of *Puccinia punctiformis*. *Phytopathology* 73:1196–1199
- Guske S, Boyle C, Schultz B (1996) New aspects concerning biological control of *Cirsium arvense*. *IOBW WPRS Bull* 19: 281–290
- Hammerschmidt R (1993) The nature and generation of systemic signals induced by pathogens, arthropod herbivores, and wounds. *Adv Plant Pathol* 10:307–337
- Hammond AM, Hardy TN (1988) Quality of diseased plants as hosts for insects. In: Heinrichs EA (ed) *Plant stress-insect interactions*. Wiley, New York, pp 381–432
- Hatcher PE (1995) Three-way interactions between plant pathogenic fungi, herbivorous insects and their host plants. *Biol Rev* 70:639–694
- Hatcher PE, Ayres PG (1997) Indirect interactions between insect herbivores and pathogenic fungi on leaves. In: Gange AC, Brown VK (eds) *Multitrophic interactions in terrestrial systems*. 36th Symposium of the British Ecological Society. Blackwell, London, pp 133–149
- Hatcher PE, Paul ND, Ayres PD, Whittaker JB (1994a) The effect of an insect herbivore and a rust fungus individually, and combined in sequence, on the growth of two *Rumex* species. *New Phytol* 20:71–78

- Hatcher PE, Paul ND, Ayres PD, Whittaker JB (1994b) The effect of a foliar disease (rust) on the development of *Gastrophysa viridula* (Coleoptera: Chrysomelidae). *Ecol Entomol* 19:349–360
- Hatcher PE, Paul ND, Ayres PD, Whittaker JB (1995) Interactions between *Rumex* spp. herbivores and a rust fungus: the effect of *Uromyces* infection on leaf nutritional quality. *Funct Ecol* 9:97–105
- Hawksworth DL (1991) The fungal dimension of biodiversity: magnitude, significance, and conservation. *Mycol Res* 95:641–655
- Hayden A (1934) Distribution and reproduction of Canada thistle in Iowa. *Am J Bot* 21:142–144
- Hendrix JW (1970) Sterols in growth and reproduction of fungi. *Annu Rev Phytopathol* 8:111–130
- Hoy CW, Head GP, Hall, FR (1998) Spatial heterogeneity and insect adaptations to toxins. *Annu Rev Entomol* 43: 571–594
- Huber H (1998) Konkurrenten, Herbivore, Pathogene und Mahd als Stressoren der Acker-Kratzdistel. Diploma thesis, Agroecology, Göttingen University
- Karban R, Adamchak R, Schnathorst WC (1987) Induced resistance and interspecific competition between spider mites and a vascular wilt fungus. *Science* 235:678–680
- Kingsley P, Scriber JM, Grau CR, Delwiche PA (1983) Feeding and growth performance of *Spodoptera eridania* (Noctuidae: Lepidoptera) on “vernal” alfalfa, as influenced by *Verticillium* wilt. *Protect Ecol* 5:127–134
- Kluth S, Kruess A, Tschamtko T (2001) Interactions between the rust fungus *Puccinia punctiformis* and ectophagous and endophagous insects on creeping thistle. *J Appl Ecol* 38:548–556
- Kok LT, Abad RG, Baudoin ABAM (1996) Effects of *Puccinia carduorum* on musk thistle herbivores. *Biol Contr* 6:123–129
- Krischik VA (1991) Specific or generalized plant defense: reciprocal interactions between herbivores and pathogens. In: Barbosa P, Krischik VA, Jones CG (eds) *Microbial mediation of plant-herbivore interactions*. Wiley, New York, pp 309–340
- Lewis AC (1979) Feeding preference for diseased and wilted sunflower in the grasshopper, *Melanoplus differentialis*. *Entomol Exp Appl*. 26:202–207
- Louda S, Kendall D, Connor J, Simberloff D (1997) Ecological effects of an insect introduced for the biological control of weeds. *Science* 277:1088–1090
- Moran PJ (1998) Plant-mediated interactions between insects and a fungal plant pathogen and the role of plant chemical responses to infection. *Oecologia* 115:523–530
- Newmann R, Thompson D, Richmann D (1998) Conservation strategies for the biological control of weeds. In: Barbosa P (ed) *Conservation biological control*. Academic Press, San Diego, pp 371–396
- Paul ND, Hatcher PE, Taylor JE (2000) Coping with multiple enemies: an integration of molecular and ecological perspectives. *Trends Plant Sci* 5:220–225
- Peschken DP, Derby JL (1997) Establishment of *Urophora cardui* (Diptera: Tephritidae) on Canada thistle, *Cirsium arvense* (Asteraceae), and colony development in relation to habitat and parasitoids in Canada. In: Dettner K, Baur G, Völkl W (eds) *Vertical food web interactions*. Springer, Berlin Heidelberg New York, pp 53–66
- Peschken DP, Finnamore DB, Watson AK (1982) Biocontrol of the weed Canada thistle (*Cirsium arvense*): releases and development of the gall fly *Urophora cardui* (Diptera, Tephritidae) in Canada. *Can Entomol* 114:349–357
- Pratt RG, Ellsbury MM, Barnett OW, Knight WE (1982) Interactions of bean yellow mosaic virus and an aphid vector with *Phytophthora* root diseases in arrowleaf clover. *Phytopathology* 72:1189–1192
- Raps A, Vidal S (1998) Indirect effects of an unspecialized endophytic fungus on specialized plant-herbivorous insect interactions. *Oecologia* 114:541–547
- Rees NE (1990) Establishment, dispersal, and influence of *Ceutorhynchus litura* on Canada thistle (*Cirsium arvense*) in the Gallatin Valley of Montana. *Weed Sci* 38:198–200
- Rowan DD, Latch GCM (1994) Utilization of endophyte-infected perennial ryegrasses for increased insect resistance. In: Bacon W, White JF (eds) *Biotechnology of endophytic fungi of grasses*. CRC, Boca Raton, Fla, pp 169–183
- Saner M, Jeanneret P, Müller-Schärer H (1994) Interaction among two biological control agents and the developmental stage of their target weed, Dalmatian toadflax, *Linaria dalmatica* (L.) Mill. (Scrophulariaceae). *Biocontrol Sci Technol* 4:215–222
- Simberloff D, Stiling P (1996) How risky is biological control? *Ecology* 77:1965–1974
- Spring A, Kok LT (1997) Field studies on the reproductive capacity and development of *Cassida rubiginosa* (Coleoptera: Chrysomelidae) on musk and Canada thistle. *Environ Entomol* 26:876–881
- Völker K, Boyle C (1994) Bean rust as a model system to evaluate efficiency of teliospore induction, especially in the potential mycoherbicide *Puccinia punctiformis*. *Weed Res* 34:275–281
- Wan FH, Harris P, Cai LM, Zhang MX (1996a) Biology and ecology of *Altica carduorum* (Chrysomelidae: Coleoptera), a defoliator of *Cirsium arvense* (L.) Scop. (Asteraceae) from north-western China. *Biocontrol Sci Technol* 6:521–530
- Wan FH, Harris P, Cai LM, Zhang MX (1996b) Host specificity of *Altica carduorum* Guer (Chrysomelidae: Coleoptera) from north-western China: a potential biocontrol agent for *Cirsium arvense* (Asteraceae) in Canada. *Biocontrol Sci Technol* 6:509–519.
- Zebitz CPW, Kehlenbeck H (1991) Performance of *Aphis fabae* on chocolate spot disease-infected faba bean plants. *Phytoparasitica* 19:113–119
- Zwölfer H, Harris P (1966) *Ceutorhynchus litura* (F.) (Col. Curculionidae), a potential insect for the control of thistle, *Cirsium arvense* (L.) Scop. in Canada. *Can J Zool* 44:22–38
- Zwölfer H, Englert W, Pattulo W (1970) Investigations on the biology, populations, ecology and the distribution of *Urophora cardui* L. Weed projects for Canada, progress report No XXVII, Nov 1970, CIBC