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A plant pathogen reduces the enemy-free space of an insect herbivore on a shared host plant

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An important mechanism in stabilizing tightly linked host–parasitoid and prey–predator interactions is the presence of refuges that protect organisms from their natural enemies. However, the presence and quality of refuges can be strongly affected by the environment. We show that infection of the host plant *Silene latifolia* by its specialist fungal plant pathogen *Microbotryum violaceum* dramatically alters the enemy-free space of a herbivore, the specialist noctuid seed predator *Hadena bicruris*, on their shared host plant. The pathogen arrests the development of seed capsules that serve as refuges for the herbivore's offspring against the specialist parasitoid *Microplitis tristis*, a major source of mortality of *H. bicruris* in the field. Pathogen infection resulted both in lower host-plant food quality, causing reduced adult emergence, and in two-fold higher rates of parasitism of the herbivore. We interpret the strong oviposition preference of *H. bicruris* for uninfected plants in the field as an adaptive response, positioning offspring on refuge-rich, high-quality hosts. To our knowledge, this is the first demonstration that plant-inhabiting micro-organisms can affect higher trophic interactions through alteration of host refuge quality. We speculate that such interference can potentially destabilize tightly linked multitrophic interactions.

Keywords: enemy-free space; multitrophic interactions; parasitoids; pathogens; refuges

1. INTRODUCTION

Plant-inhabiting micro-organisms can have effects that extend well beyond the direct effects that they exert on the physiology and growth of their host plants. Many plant pathogens alter the phenotype of their hosts in such a way that infected host plants show increased or decreased susceptibility to herbivores that exploit the same host plant as the pathogen (for reviews, see Jones 1984; De Nooij *et al.* 1992; Hatcher 1995; Hammerschmidt & Schultz 1996; Paul *et al.* 2000). Such alterations in susceptibility can be brought about by a wide variety of mechanisms, including changes in apparency (Gibbs 1980; Ajayi & Dewar 1983), nutritional quality (Paul & Ayres 1988; Karban & Baldwin 1997) and defence chemistry (Fischer *et al.* 1990; Krischik 1991; Karban & Kúc 1999; Stout & Bostock 2000) of the shared host. A virtually unexplored question is whether alterations of host-plant quality following microbial infection have consequences higher up the food chain, i.e. affect the strength of interactions at higher trophic levels (Dicke 1996). Since the seminal paper by Price *et al.* (1980), it has become increasingly clear that host-plant quality and defence chemistry affect not only plant–herbivore interactions, but also interactions at higher trophic levels. Many generalist insect herbivores show reduced growth rates on host plants with a low nutritional quality or a high level of defence chemicals, resulting in an increase in development time that prolongs their exposure time or 'window of vulnerability' to parasitoids or predators (the 'slow-growth–high-mortality hypothesis'; Benrey & Denno 1997; Williams 1999; Havill & Raffa 2000). However, many specialist herbivores that can cope with high levels of defence chemicals in their host plant sequester these compounds, resulting in reduced rates of parasitization by less well-adapted parasitoids (Bottrell *et al.* 1998). Recent studies suggest that the effects of host-plant quality may even extend to higher trophic levels, affecting parasitoid–hyperparasitoid interactions (J. A. Harvey *et al.*, unpublished data).

An elegant study by Omacini *et al.* (2001) has recently provided the first evidence that host-plant alterations caused by microbial infection can indeed affect interactions at higher trophic levels. The infection of a grass species by a fungal endophyte reduced not only the abundance of insect herbivores, but also the rates of parasitism of herbivores by their parasitoids, and decreased the dominance of the food web by a few trophic links. This finding implies that microbial infection can affect the pattern and strength of resource–consumer interactions at higher trophic levels and that such 'hidden' microbial infections can have community-wide effects. In the case of these mutualistic fungal endophytes, the effects at higher trophic level interactions are mediated by the toxic alkaloids produced by the fungus, which effectively limit energy transfer up the trophic cascade. We investigate the effects of microbial infection on herbivore–parasitoid interactions through a completely different mechanism, i.e. the alteration of the quality of host plants as a suitable refuge for herbivores.

Refuges play an important part in sustaining multitrophic interactions (Murdoch *et al.* 1987; Hassell & Pacala 1990; Hawkins *et al.* 1993; Hochberg & Holt 1995). They can enhance the persistence of multi-species interactions by preventing the over-exploitation of prey by their natural enemies, both in host–parasite (Begon *et al.* 1995; Lynch *et al.* 1998) and bacteria–phage interactions

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(Schrag & Mittler 1996). In the case of phytophagous insects, constitutive or induced host-plant structures such as fruits and galls often act as physical refuges, protecting them from attack by predators and parasitoids (Stiling & Rossi 1996; Udayagiri & Welter 2000). The quality of such structures as refuges can be strongly affected by the environment. For instance, nutrient conditions favouring high plant-nitrogen content enable some gall wasps to produce bigger galls that make them inaccessible to parasitism by their most abundant, small, parasitoids (Stiling & Rossi 1996), whereas the refuge function of small galls produced under low nutrient conditions is limited. We demonstrate the effects of a plant pathogen on the quality of its host plant as a refuge for a herbivore and its consequences for a herbivore–parasitoid interaction.

We used a tightly linked tri-trophic system of a (i) host plant; (ii) a specialist lepidopteran seed predator; and (iii) a specialist hymenopteran parasitoid. Most larval stages of the seed predator develop inside the closed seed capsules on this host plant, and we test whether these structures function as 'three-dimensional refuges', reducing the rates of parasitism by the parasitoid. Host plants are often infected by a fungal pathogen that prevents the development of seed capsules on the host plant. The seed predator shows an oviposition preference for uninfected host plants (see below). We tested whether we could interpret this oviposition preference as an adaptive response, i.e. whether it results in higher offspring fitness of the herbivore. We propose that pathogen infection reduces herbivore offspring survival: (i) by lowering the quality of host plants as a food source (due to the reduced availability of seeds as their primary food source); and (ii) by reducing the availability of refuges (seed capsules) that protect them from their parasitoids.

2. MATERIAL AND METHODS

(a) *Study system*

We studied the effects of the anther smut fungus *Microbotryum violaceum* [Pers.:Pers.] Deml & Oberw. (= *Ustilago violacea* [Pers.] Fuckel) (Ustilaginaceae), a plant pathogen that sterilizes its caryophyllaceous host plants (Thrall *et al.* 1993) on a tritrophic system of the dioecious, short-lived perennial host plant *Silene latifolia* Poiret (= *S. alba* [Miller] Krause, Caryophyllaceae), the specialist lepidopteran seed predator *Hadena bicruris* [Hufn.] (Noctuidae), and the specialist gregarious hymenopteran endoparasitic wasp *Microplitis tristis* [Nees] (Braconidae). Female *H. bicruris* (figure 1*a*) oviposit a single egg on the ovary of flowers on female host plants (figure 1*b*). The first instar caterpillar chews a small hole in the ovary wall (figure 1*b*), enters the ovary and develops within the expanding seed capsule during its first few instars, devouring all developing seeds. Seed development within these capsules is ensured by the pollination activity of females that precedes oviposition (Brantjes 1976). Caterpillars use the entrance hole to dispose of the frass that clogs the hole and visibly protrudes from it (figure 1*c*). During their fourth or fifth instar, when caterpillars have eaten the contents of the capsule on which they hatched, they leave this ('primary') seed capsule and complete their development by feeding externally on ripe seeds from additional ('secondary') capsules (Biere & Honders 1996) (figure 1*d*) before pupating in the soil. *Hadena bicruris* has a complex interaction with its host *S. latifolia*; it is an important pollinator, seed predator and vector of fungal spores of *M. violaceum* (see below). Brantjes (1976) has suggested that the positive effect of *H. bicruris* on *S. latifolia* as a pollinator is generally outweighed by its detrimental effect as a seed predator. The parasitoid *M. tristis* can attack all larval stages of *H. bicruris* in the laboratory (J. A. Elzinga, personal observation), but parasitism in the field is mainly restricted to the fourth and fifth instar of the host (see below), in which, on average, 18 eggs are deposited. Both herbivore and parasitoid are usually bivoltine and are common in host-plant populations in the Netherlands. In a survey of 62 Dutch *S. latifolia* populations along the river Waal in September 2001, *H. bicruris* was present in 92% of the populations. Of all caterpillars sampled from these populations, 46% of the fifth instar, 17% of the fourth instar and none of the smaller caterpillars (which are rarely encountered outside their seed capsules) were parasitized by *M. tristis* (figure 1*e*,*f*), indicating that this parasitoid is an important source of mortality in the field. In 32% of the populations, *S. latifolia* was also infected by the specialist pathogen *M. violaceum*. Population biology and genetics of this heterothallic fungus have been extensively studied (reviews in Day & Garber 1988; Alexander *et al.* 1996). Infection results in the early abortion of ovaries (and hence of seeds and seed capsules) and prevents pollen production in the anthers, in which the fungus produces teliospores that are florally transmitted by pollinating insects (Jennersten 1983). The most remarkable host alteration induced by the fungus is the 'masculinization' of female plants in dioecious host species such as *S. latifolia* (Scutt *et al.* 1997). Flowers on infected female plants undergo a morphological sex reversal. Their ovaries are aborted and, instead, they produce full-length stamens with spore-filled anthers (figure 1*g*), but no seed capsules or seeds. Infections are systemic, i.e. if the fungus reaches the rosette before the flower stalks die back at the end of the season, plants are usually completely sterile and produce only diseased flowers in succeeding years. Although *H. bicruris* visits both infected and uninfected host plants for nectar consumption, it shows a strong oviposition preference for healthy plants as compared with plants that are infected with *M. violaceum*. In a sample of single flowers that had opened the previous night, from a total of 1881 female *S. latifolia* plants, from eight populations in which both herbivore and pathogen were present, we found that the proportion of flowers that contained a *H. bicruris* egg was significantly higher on healthy (52.8%) than on diseased (9.0%) plants.

(b) *Pathogen effects on host food quality*

(i) *Experiment 1*

The effects of pathogen infection of the shared host plant on the performance of the herbivore in the absence of parasitoids were studied using 45 female host plants, 27 uninfected and 18 infected with the anther smut fungus following seedling inoculation (Alexander & Maltby 1990). The plants were grown in 17 cm diameter pots filled with standard potting soil in an unheated greenhouse (16 h light) during late summer in 1996. Infected plants were all systemically diseased, producing only flowers containing fungal spores. Fungal infection induces flower production in the host, thus increasing the number of anthers available for fungal reproduction (Alexander & Maltby 1990), and diseased plants, on average, produced more flowers than healthy plants during the experiment (40.1 ± 5.5 versus 10.1 ± 0.9; log-transformed data: $F_{1,43} = 7.49$, $p < 0.001$). Freshly field-collected *H. bicruris* eggs (326 in total) were hand deposited on an equal number of ovaries the morning after the flower had opened, using a toothpick. A range of densities of

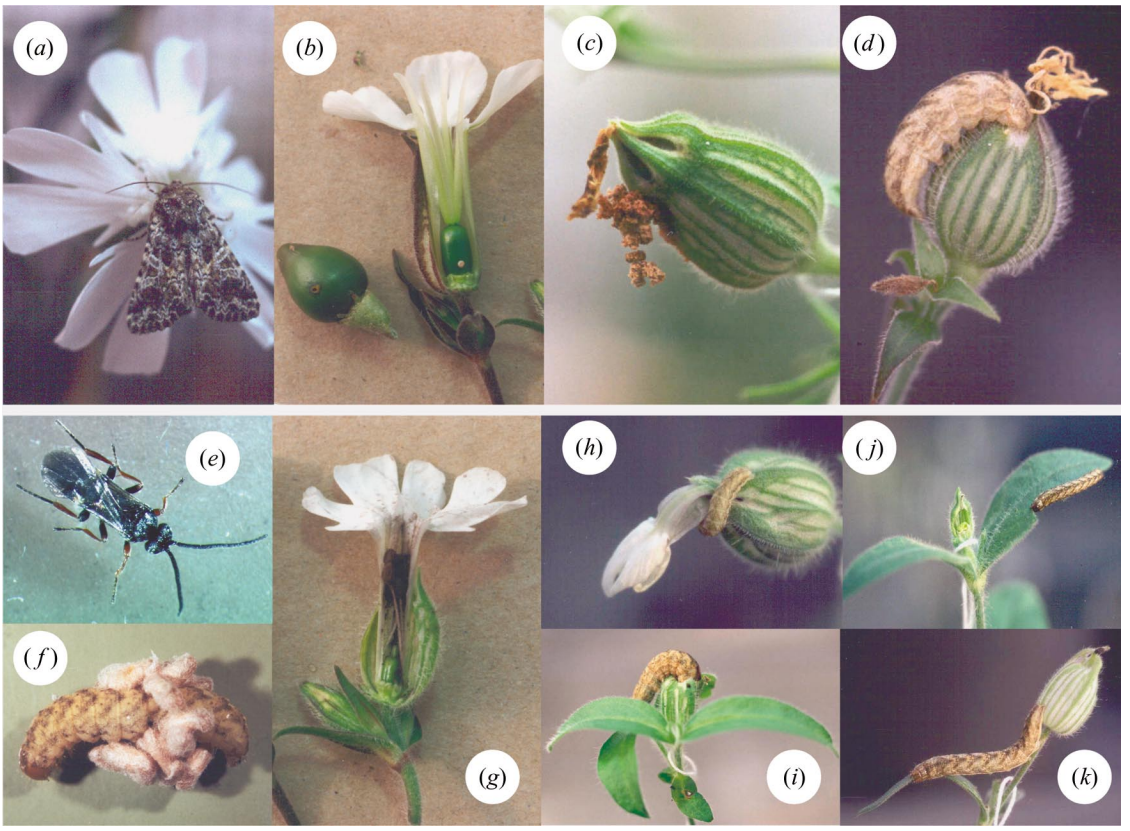

Figure 1. Study system to investigate the impact of fungal infection on a tri-trophic interaction. The noctuid *Hadena bicruris* pollinates (*a*) and oviposits (*b*) on the ovary of the flowers of *Silene latifolia*. Larvae develop within the seed capsule during their first instars (*c*) and later feed externally on additional seed capsules (*d*). Capsules serve as refuges from the braconid parasitoid *Microplitis tristis*: (*e*) adult, and (*f*) pupae emerging from *H. bicruris*. Infection of female plants by the anther-smut fungus *Microbotryum violaceum* leads to abortion of the ovary and production of spore-filled anthers (*g*). Larvae of *H. bicruris* then feed on alternative, low-quality tissues (*h–k*), leading to reduced pupal mass, and are deprived of refuges, leading to higher rates of parasitism.

caterpillars per host plant was created by dividing both healthy and diseased host plants into three equal groups and depositing eggs on every fourth (25%), every second (50%) and every (100%) flower produced by the host. The range of densities was applied because oviposition rates and hence caterpillar densities in the field differ between healthy and diseased plants, and this set-up allows us to compare the effects of disease status on caterpillar success, both at fixed caterpillar density and at natural densities. All flowers on healthy host plants were hand pollinated using a large source of concurrently grown male host plants. Pots were placed in 60 cm diameter containers, open at the top, which successfully prevented among-plant migration of caterpillars. Egg hatching, caterpillar survival and pupal weight of caterpillars were recorded on each plant. Pupae were overwintered at 4 °C in separate sand-filled vials and adult emergence recorded in the following spring.

(ii) *Experiment 2*

We tested whether the differences in performance of caterpillars on healthy and diseased hosts could be explained by the main types of food that they consumed on these hosts. We raised 38 caterpillars individually in small containers in a growth cabinet (16 : 8 h; 25 : 15 °C, day : night) on non-limiting, daily refreshed amounts of either seed capsules or leaves, the main types of biomass consumed on healthy and diseased hosts, respectively. The development time (days from egg hatching to pupation) and pupal mass were recorded for each caterpillar.

(c) *Pathogen effects on host refuge quality*

(i) *Experiment 3*

A cage experiment, replicated eight times in the course of the season, was performed in 1998 to study parasitism rates of caterpillars as a function of their location inside or outside seed capsules on healthy and diseased plants. We used two-year-old plants grown from seeds that were either uninfected or systemically diseased following seedling inoculation, and planted in the experimental garden of the Netherlands Institute of Ecology, Heteren, the Netherlands, in 1997. Each replicate comprised 3–4 flowering shoots from uninfected female hosts, with a total of *ca.* 30 seed capsules, and an equal number of flowering shoots from diseased female hosts, that supported *ca.* 80 flowers. These were transplanted into two 14 cm pots (one for each disease status) and placed in a Perspex cage (30 cm × 40 cm × 60 cm) with 0.52 mm mesh gauze on top. Both healthy and diseased plants had been naturally colonized by *H. bicruris*, but colonization rates of diseased plants were low. To increase sample sizes of caterpillars on diseased plants, additional caterpillars of different sizes were collected from diseased plants in the experimental garden and placed inside the corolla of diseased flowers (where naturally colonized caterpillars on diseased plants are primarily located until flowers are consumed) such that their numbers and size distribution approximately matched those on healthy plants. Caterpillars were allowed to distribute within flowering stalks for 24 h prior to the release of an average of 24 newly emerged parasitoids with an average sex ratio of *ca.* 40% females in the

cage. All parasitoids originated from naturally parasitized caterpillars collected from a single plant population earlier in the year and subsequently raised on a seed capsule diet in a growth cabinet. Ninety-six hours after parasitoid release, caterpillars were collected from the cages, their position (on a healthy or diseased stalk, in or outside a seed capsule) and fresh weight were recorded, and they were grown in individual containers on a diet of seed capsules that was refreshed every second day until pupation or emergence of parasitoid larvae. Because caterpillar fresh weights were recorded 96 h after parasitoid release, this procedure slightly overestimates their true size at parazitation. For caterpillars that were still feeding within their primary seed capsule at the time of exposure to the parasitoids, we measured the diameter of the hole that they had created in their seed capsule when entering the ovary. Higher parasitation rates of caterpillars of a given size within capsules with larger hole diameters could indicate that the refuge function of capsules is disrupted by large-sized holes. The holes grow in size as the capsule expands. *M. tristis* can cross gauzes with mesh sizes of *ca.* 0.7 mm, and a range of hole diameters from 0.38 to 4.17 mm was observed in the experiment. None out of the 83 caterpillars sampled over the season from the experimental garden were parasitized by *M. tristis*, suggesting that background parasitism levels in the experiment were low. Laboratory studies have shown that encapsulation rates of parasitoid eggs in caterpillars stung by *M. tristis* are extremely low even when fed an optimal diet (J. A. Elzinga, unpublished results). This suggests that the observed parasitation rates of *H. bicruris* in the experiment mainly reflect rates of successful contacts without confounding effects of differences in resistance among caterpillars. Complete data were obtained for 189 caterpillars.

(d) *Statistical analyses*

(i) *Experiment 1*

The effect of plant infection status (healthy versus diseased) on the percentage of egg hatching, survival to the pupal stage, and proportion of pupae emerging as adults per plant was analysed using a generalized linear model (Procedure GENMOD in SAS v. 6.12, SAS Institute, Cary, NC) with a binomial response variable and a logit-link function. The resulting *F*-values are based on mean deviance terms (twice the log-likelihood ratio divided by the number of degrees of freedom) (Schmid & Dolt 1994). The effects of plant infection status (class variable) and caterpillar density at eclosion (log-transformed numbers of caterpillars per flower, regression variable) on mean pupal weight were analysed using the same procedure, specifying a normal distribution for the response variable and an identity link function.

(ii) *Experiment 2*

The effects of diet (leaves versus seed capsules) on time from egg hatching to pupation and pupal weight, were analysed by one-way ANOVA.

(iii) *Experiment 3*

The effects of caterpillar fresh weight, replicate and plant infection status on caterpillar parasitism rates were analysed using a generalized linear model with a binomial response variable and a logit-link function. Replicate and plant infection status were specified as class variables. Similar analyses were performed to test the effects of caterpillar fresh weight, replicate and feeding location of caterpillars (inside or outside primary capsules) on parasitism rates. Two separate contrasts for the factor feeding location were tested: (i) inside versus outside capsules on healthy plants; and (ii) outside capsules on healthy versus diseased plants. Caterpillar fresh weight was log-transformed before analysis and specified as a continuous variable.

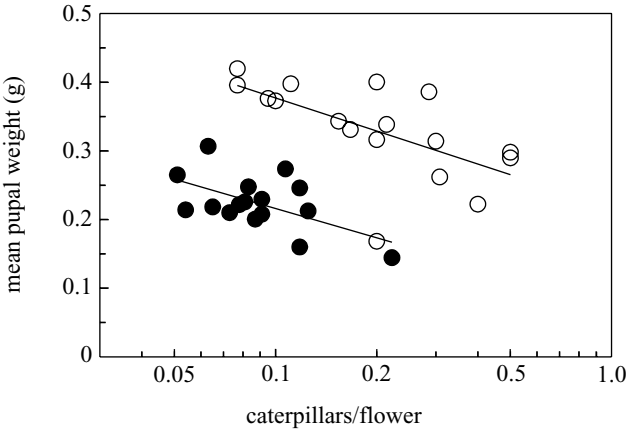

Figure 2. Mean pupal weight of the lepidopteran seed predator *Hadena bicruris* raised on host plants of *Silene latifolia* that were infected by the anther-smut fungus *Microbotryum violaceum* (filled circles) and on uninfected plants (open circles), as a function of the density of caterpillars at eclosion.

3. RESULTS

(a) *Pathogen effects on the herbivore through alteration of host food quality*

Despite the absence of developing seeds on diseased host plants, the proportion of *H. bicruris* caterpillars that successfully completed their development until the pupal stage was no lower on diseased plants (69.4%) than on healthy plants (60.0%) ($F_{1,43} = 0.94$, $p = 0.33$) in the absence of parasitoids. On diseased plants, caterpillars consumed the ovary on which they had hatched, and then continued feeding on alternative tissues in a distinct hierarchical order. First, the remaining flower parts were consumed (figure 1*h*), except for the hairy calyx, followed by flower buds (figure 1*i*), leaves (figure 1*j*) and finally calyces (figure 1*k*). Feeding on these alternative tissues resulted in a significantly lower pupal mass at any given caterpillar density (figure 2; $F_{1,29} = 60.0$, $p < 0.001$). On each type of host, average pupal weight also decreased with caterpillar density estimated at eclosion ($F_{1,29} = 16.8$, $p < 0.001$). The extent of this decrease was independent of plant infection status (no significant interaction; $F_{1,29} = 0.0$, $p = 0.84$). The lower pupal weight on diseased plants was accompanied by a lower probability of adult emergence (69.8%) compared with pupae of caterpillars raised on healthy host plants (95.6%; $F_{1,43} = 7.67$, $p < 0.01$). The lower pupal weight of caterpillars on diseased plants could at least partly be explained by differences in food quality between leaves and seeds, the two main types of plant biomass that caterpillars consumed on diseased and healthy plants, respectively. An experiment in which caterpillars were raised on these types of diet revealed that pupal weight (mean ± s.e.) of caterpillars was significantly lower on a leaf diet (296.3 ± 7.5 mg) than on a seed diet (327.7 ± 8.9 mg; $F_{1,36} = 6.72$,

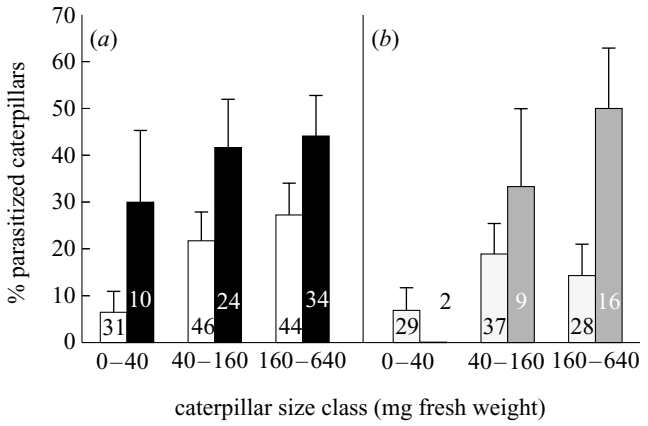

Figure 3. (*a*) Rates (%) of parasitism (means ± s.e.) of *Hadena bicruris* by the parasitoid *Microplitis tristis* on pathogen-infected host plants (black bars) and on uninfected plants (open bars) for three caterpillar size classes (roughly corresponding to instars 1–3, 4 and 5, respectively). (*b*) Rates of parasitism on uninfected plants are divided into caterpillars inside (light grey bars) and outside (dark grey bars) primary capsules. Numbers within bars indicate sample sizes.

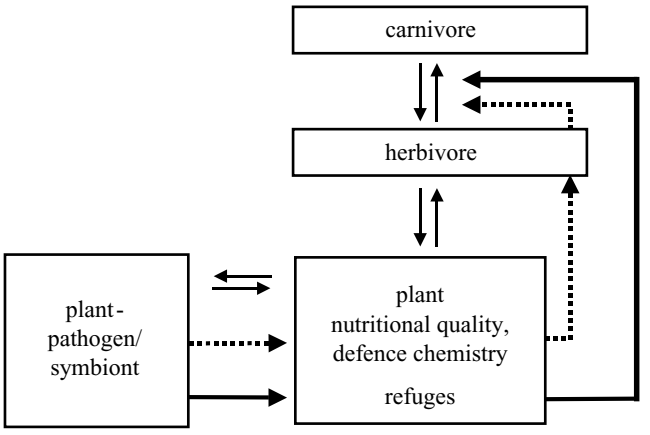

Figure 4. A generalized scheme of documented pathways mediating effects of plant-inhabiting micro-organisms on higher trophic levels (herbivore–parasitoid or herbivore–predator interactions) on a shared host. Dotted line: the effects on host-plant quality are 'transferred' to higher levels within the food web, altering the energy flow up the trophic cascade (e.g. Omacini *et al.* 2001). Solid line: the effects on the structural attributes of host plants affect the 'refuge quality' of hosts for herbivores, altering the extent to which they can be over-exploited by their natural enemies.

$p < 0.05$). Time to pupation (36.1 ± 0.5 versus 35.1 ± 0.6 days) was not significantly affected by diet ($F_{1,36} = 1.82$, $p = 0.19$).

(b) *Pathogen effects on the herbivore through alteration of host refuge quality*

Caterpillars feeding on infected host plants suffered, on average, two-fold higher rates of parasitism by *M. tristis* than similarly sized caterpillars on uninfected host plants (figure 3*a*). The effect was highly significant ($F_{1,157} = 7.4$, $p < 0.01$). Parasitization rates increased with caterpillar size (figure 3*a*; $F_{1,157} = 7.0$, $p < 0.01$) and varied among replicates of the experiment (8–57%, $F_{7,157} = 2.9$, $p < 0.01$), but there were no significant interactions between plant disease status and caterpillar size ($p = 0.54$) or replicate ($p = 0.31$), indicating that differences in parasitism rate between healthy and diseased hosts were consistent across size classes and replicates. We argue that it is the protection of caterpillars within capsules, rather than some general attribute associated with healthy plants, that is responsible for the lower rates of parasitism of caterpillars on healthy plants (figure 3*b*). Caterpillars on healthy plants that had left their primary capsule and that were found feeding externally on secondary capsules (figure 3*b*, dark grey bars, representing 6.5, 19.6 and 36.4% of small, intermediately sized and large caterpillars, respectively) had significantly higher parasitation rates than similarly sized caterpillars that were still in their primary capsule (figure 3*b*, light grey bars; $F_{1,92} = 15.7$, $p < 0.001$; interactions between feeding location and size or replicate n.s., $p = 0.50$ and $p = 0.08$), whereas their parasitation rates were equally high as those of (externally feeding) caterpillars on diseased plants (figure 3*a*, black bars; $F_{1,66} = 0.0$, $p = 0.98$). This suggests that the lower parasitation rates of caterpillars on healthy plants can be largely explained by their location within capsules, rather than being on a healthy plant *per se*. Parasitization rates of caterpillars within capsules did not increase with the diameter of the primary hole created by their entry into the ovary (generalized linear model, $F_{1,104} = 1.29$, $p = 0.26$).

4. DISCUSSION

(a) *Structural plant attributes as herbivore refuges*

Refuges play an important part in sustaining multitrophic interactions by preventing the over-exploitation of prey by their natural enemies (Murdoch *et al.* 1987; Hassell & Pacala 1990; Hawkins *et al.* 1993; Begon *et al.* 1995; Hochberg & Holt 1995; Schrag & Mittler 1996; Lynch *et al.* 1998). There are several types of refuges, that confer spatial (Begon *et al.* 1995; Schrag & Mittler 1996), temporal (Murdoch *et al.* 1987) or chemical (Vos *et al.* 2001) evasion from natural enemies. Our results clearly show that the seed capsule of *S. latifolia* is an example of a structural plant attribute that functions as a refuge for its specialist seed predator *H. bicruris*, protecting its first larval stages from parasitism by *M. tristis*. Initially, the strong barrier function of seed capsules to parasitism was a surprise. Although the thick fruit wall turns into a woody tissue during maturation, and is probably too tough for *M. tristis* to penetrate, one could expect that the holes in the capsules that are created by the first instar caterpillars to gain entrance to the ovary (figure 1*b*) could be used by the parasitoids, either to insert their ovipositor and probe for the caterpillar locked inside, or to actually enter the seed capsule, as the hole quickly grows beyond the critical diameter that physically limits entry by *M. tristis* when the seed capsule expands and matures. In addition, a specialist braconid parasitoid could evolve the ability to use the faeces protruding from infested seed capsules as a visual or chemical cue to locate the caterpillars. Both in the field and on healthy plants in the experiment, female *M. tristis* were observed spending considerable amounts of time near such holes. However, because parasitization rates of caterpillars within capsules did not increase with the diameter of the primary hole, even though the maximum hole

diameter in the experiment exceeded the critical threshold size that can be passed by *M. tristis* by a factor of six, it seems unlikely that hole size *per se* prevents them from entering the seed capsule. We speculate that the failure of *M. tristis* to parasitize the caterpillars efficiently within this refuge is due to: (i) their short ovipositor length, preventing them from reaching caterpillars within their refuge from outside the capsule; and (ii) the toughness of the caterpillar's faecal plug (figure 1*c*), which clogs the hole and prevents them from entering the seed capsule through the entrance hole made by the caterpillar.

(b) *Plant pathogen effects on plant refuge quality*

Recent studies show that species can affect the refuge space for other, unrelated species. An elegant example of how species can increase refuge space is provided by Pallini *et al.* (1998). Thrips larvae suffer lower predation from a phytoseiid mite by using webs produced by the spider mite *Tetranychus urticae.* By contrast, our study suggests that the presence of the anther smut fungus *M. violaceum* on its host *S. latifolia* reduces the refuge space for a co-occurring herbivore. Infection by the pathogen halts the development of seed capsules and seeds. Caterpillars of the seed predator *H. bicruris* appeared to be quite plastic in their feeding behaviour and switched to feeding on alternative, more exposed tissues on diseased plants. However, this change in behaviour had two important fitness consequences. First, they had reduced pupal weight and concomitant lower survival to the adult stage. Second, there were significantly higher rates of parasitism in the presence of the specialist parasitoid. The magnitude of the reduction in pupal weight on diseased plants ('food quality effect') was relatively constant for any caterpillar density (*ca*. 150 mg; figure 2), but most probably reflects an upper estimate. Given the six-fold lower oviposition rates of *H. bicruris* on diseased as compared with healthy flowers in the field, we would (by extrapolation of figure 2) expect a density-corrected difference of *ca*. 50 mg. The destruction of seed capsule development by the pathogen ('refuge loss effect') basically increases the 'window of susceptibility' of caterpillars on diseased plants. Laboratory trials indicate that *M. tristis* readily oviposits and successfully develops in all larval stages of *H. bicruris*. On healthy plants, the actual realized window of susceptibility of caterpillars is predominantly shifted to the late, externally feeding instars because of the structural refuge provided by the seed capsule during early growth. By contrast, on diseased plants, caterpillars experience the full window of susceptibility, because potential refuges are destroyed by the fungus. Given the field data, indicating that *M. tristis* is a significant source of mortality of *H. bicruris* in the field, we conclude that oviposition on diseased plants could significantly decrease the offspring survival of *H. bicruris* in the field. The strong oviposition preference of *H. bicruris* for healthy plants can thus be interpreted as an adaptive response, resulting in the positioning of offspring on uninfected plants with their superior nutritional quality and enemy-free space. The latter would support recent findings that enemy-free space can affect host and feeding site selection in phytophagous insects (Brown *et al.* 1995; Feder 1995; Sait *et al.* 1997; Ballabeni *et al.* 2001), although, evidently, we have no way of knowing whether the oviposition preference in this system has indeed evolved as an evolutionary response to these presumed selective forces.

Our data do not support the proposition that, in addition to the effects of changes in refuge quality, pathogen infection in this system could also increase the window of vulnerability of the herbivore as a result of alterations in host diet quality. Reductions in the suitability of host plants caused by changes in their nutritional quality or defence chemistry often result in increased rates of parasitism of herbivores due to their slower development and increased exposure time to predators or parasitoids (Benrey & Denno 1997; Williams 1999). However, the results of the feeding experiment show that the switch in feeding behaviour of *H. bicruris* caterpillars (from seeds to leaves) following pathogen infection did not affect their development time.

(c) *Effects of plant-inhabiting micro-organisms on higher trophic interactions*

The most important result of this study is that plant pathogens can reduce the 'refuge space' of a herbivore and hence could facilitate the over-exploitation of one trophic level by another level, potentially destabilizing a tightly linked multi-species interaction. Such a process can lead to local-scale extinction of one or more component species (Begon *et al.* 1995). Whether such destabilization is likely to occur in the described system is difficult to determine without additional information on the effects of pathogen infection on the abundance and dynamics of the species within the trophic system. However, our results clearly show that there is the potential for an additional mechanism by which plant-inhabiting micro-organisms can affect herbivore–parasitoid interactions (figure 4). The first mechanism, recently shown for fungal endophytes by Omacini *et al.* (2001) (figure 4, dotted line), is through the disruption of energy flow up the trophic cascade. Disruption of host refuge quality (figure 4, solid line) can be considered as a second mechanism. These results emphasize that, in spite of their insignificant contribution to the flow of matter and energy, microbial parasites and symbionts can strongly affect community structure (Minchella & Scott 1991; Van der Putten *et al.* 1993; Dobson & Crawley 1994; Clay & Holah 1999), and support recent pleas to incorporate their effects in food webs (Marcogliese & Cone 1997).

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