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Parasitoid-plant mutualism: parasitoid attack of herbivore increases plant reproduction

Joop J.A. van Loon, Jetske G. de Boer & Marcel Dicke
Laboratory of Entomology, Wageningen University, P.O. Box 8031, 6700 EH Wageningen, The Netherlands (Fax: +31 317 484821; E-mail: joop.vanloon@users.ento.wag-ur.nl)

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Abstract

We tested whether a plant's life time seed production is increased by parasitization of herbivores in a tritrophic system, *Arabidopsis thaliana* (Brassicaceae) plants, *Pieris rapae* (Lepidoptera: Pieridae) caterpillars and the solitary endoparasitoid *Cotesia rubecula* (Hymenoptera: Braconidae). We established seed production for intact *A. thaliana* plants, plants that were mechanically damaged, plants fed upon by parasitized caterpillars and plants fed upon by unparasitized caterpillars. In the first experiment, with ecotype Landsberg (*erecta* mutant), herbivory by unparasitized *P. rapae* caterpillars resulted in a strongly reduced seed production compared to undamaged plants. In contrast, damage by *P. rapae* caterpillars that had been parasitized by *C. rubecula* did not result in a significant reduction in seed production. For the second experiment with the ecotype Columbia, the results were identical. Plants damaged by unparasitized caterpillars only produced seeds on regrown shoots. Seed production of plants that had been mechanically damaged was statistically similar to that of undamaged plants. Production of the first ripe siliques by plants fed upon by unparasitized caterpillars was delayed by 18–22 days for Landsberg and 9–10 days for Columbia. We conclude that parasitization of *P. rapae* by *C. rubecula* potentially confers a considerable fitness benefit for *A. thaliana* plants when compared to plants exposed to feeding damage by unparasitized *P. rapae* larvae. Plants that attract parasitoids and parasitoids that respond to herbivore-induced plant volatiles will both experience selective advantage, justifying the use of the term mutualism for this parasitoid-plant interaction. This type of mutualism is undoubtedly very common in nature.

Introduction

Plants are subject to damage by herbivorous insects and have evolved different chemical defense mechanisms to counteract this (Schoonhoven et al., 1998). Direct chemical defense involves constitutive or induced synthesis of secondary metabolites that (1) repel or deter herbivorous insects prior to or during ingestion or (2) exert post-ingestive toxic effects or (3) have both effects. Most (>80%) herbivorous insect species, however, are specialist feeders on a particular plant family and experience no detrimental effects of the secondary metabolites. A second defense mechanism has been termed indirect chemical defense or extrinsic defense (Price et al., 1980; Dicke, 1994, 1999; Turlings et al., 1995). This type of defense can operate via different mechanisms, one of which is the provision of foraging cues to natural enemies of the herbivores. Plants can produce volatile infochemicals (Dicke & Sabelis, 1988), often following induction by insect damage, that guide insect predators or parasitoids to the herbivore (Vet & Dicke, 1992; Dicke & Vet, 1999). Upon finding the herbivore, a predator kills it, thus preventing further damage to the plant that produces the infochemicals. In the case of parasitoids, the possible benefit to the plant is not as straightforward, because successful parasitoid development requires the survival of the host for some time. In the case of many koinobiont species of larval parasitoids, the host continues to feed during parasitoid development, whereas many idiobiont species paralyze their host thereby arresting its food ingestion. Several studies document that feeding rate and total amount of food consumed by parasitized caterpillars can be considerably reduced. This appears to be true for all species of solitary parasitoids of leaf-chewing herbivores studied thus far (e.g., Rahman, 1970; Guillot & Vinson, 1973; Brewer & King, 1978; Parkman & Shepard, 1981; Powell, 1989). Studies on gregarious parasitoids, on the other hand, show variable results and in some cases an increase in food consumption has been observed (Brewer & King, 1980; Slansky, 1978; Coleman et al., 1999). None of these studies, however, addressed the critical issue of whether plant reproduction was affected. Removal of vegetative tissue does not necessarily result in a decreased reproductive output, as plants are known to tolerate (sometimes considerable) damage to vegetative tissues without measurable loss of seed production (Brown et al., 1972; Hendrix, 1988 and references therein; Rosenthal & Kotanen, 1994; Baldwin & Preston, 1999). Seed production is crucial in the life cycle of an annual plant species such as *A. thaliana*.

We investigated a tritrophic system well suited to address this issue. It consisted of *Arabidopsis thaliana* (L.) Heynh, *Pieris rapae* L. and *Cotesia rubecula* (Marshall). Interactions in the tritrophic system *P. rapae* and *C. rubecula* and another brassicaceous host plant, *Brassica oleracea* L., have been studied extensively (Agelopoulos & Keller, 1994; Blaakmeer et al., 1994; Geervliet et al., 1994, 1996). Several studies have demonstrated that *C. rubecula* is attracted to herbivore-induced plant volatiles released by *B. oleracea* plants when these are damaged by *Pieris rapae* caterpillars (Agelopoulos & Keller, 1994; Geervliet et al., 1994; Blaakmeer et al., 1994). Damage by *P. rapae* is the main factor affecting the functional response of *C. rubecula*, and even has a larger influence than host density (Nealis, 1990). *C. rubecula* females are able to discriminate between plant host complexes with a different profitability (host density) on the basis of volatile cues (Kaiser & Cardé, 1992; Geervliet, 1997; Geervliet et al., 1998a,b). Females land more often on host damaged leaves and on these they spend more time and move more slowly than on undamaged leaves (Nealis, 1986). It has been demonstrated that *C. rubecula* responds to the headspace of *A. thaliana* plants damaged by *P. rapae* caterpillars as observed in response to volatiles from *Pieris*-infested *B. oleracea* (van Poecke et al., unpubl.). In nature *P. rapae*, which readily lays on several small crucifer species (Bink, 1992), has been observed to use *A. thaliana* as a host plant (Geervliet, 1997; Yano & Ohsaki, 1993). In the field *P. rapae* populations are restrained by a number of natural enemies of which *C. (Apanteles) rubecula* is considered to be the most important (Sengonca & Peters, 1993). *A. thaliana* is common through Europe, Western Asia, North America and can also be found in North Africa and East Asia (Meyerowitz & Somerville, 1994; Bowman, 1994; Rédei, 1970). Thus, *P. rapae* and *A. thaliana* co-occur over large geographic areas, although this occurrence is not everywhere seasonally synchronized.

Here we tested the hypothesis that parasitization of the herbivore resulted in increased reproductive output of the plant when compared to the seed production of plants fed upon by unparasitized herbivores for two *A. thaliana* ecotypes. In the first experiment we simulated the extent of leaf herbivory by mechanical removal of leaf rosette tissue to test the hypothesis that herbivore damage can be simulated by leaf tissue removal.

Materials and methods

Insects. *Pieris rapae* eggs and butterflies were obtained from a laboratory strain reared on greenhouse grown Brussels sprouts, *Brassica oleracea* var. *gemmifera* cv Icarus. Caterpillars and butterflies were reared at 22 ± 1 °C, 50–60% r.h. and L16:D8. *Cotesia rubecula* females were obtained from an established laboratory strain reared on *P. rapae* as a host which was kept as described above. The *C. rubecula* colony was kept at 23 ± 2 °C, 60–70% r.h. and L16:D8 (details in Geervliet et al., 1994). Adult parasitoids had access to honey and water. A few times a week parasitoids were allowed to parasitize first instar *P. rapae* larvae feeding on excised Brussels sprouts leaves.

Plants. *Arabidopsis thaliana* seed of two ecotypes, Landsberg (*erecta* mutant) and Columbia, was provided by Dr M. Koornneef, Department of Genetics, Wageningen University and used in two separate experiments. Seeds were placed on wet filterpaper in a Petri dish (diam. 9 cm), which was incubated in a refrigerator at 5 °C in the dark for 3 to 5 days to vernalize. The Petri dish was subsequently transferred to a second incubator where it was incubated at 23 ± 0.5 °C for 2 days under a L16:D8 regime to induce germination. Two or three germinated seeds were then sown together in one pot by placing them on top of the soil with a small paintbrush. Plastic pots of 5×5×4.5 cm were used. A soil mixture was used that consisted of one part of sterilized river sand, 4 parts of steamed soil (Lentse potgrond) and 1 part of vermiculite. Pots were placed in plastic trays of 45×30×7.5 cm. During the first 3 days after sowing, water was misted on top of the pots twice a day. Water was subsequently provided on the bottom of the tray 2–3 times per week as judged necessary upon inspection of humidity of the top layer of the potting soil. About 2 weeks after sowing, the pots were checked for number of growing plants and if necessary the number was reduced to one plant per pot. Germinating seeds and plants of the Landsberg ecotype were reared in a greenhouse compartment at 21 ± 1 °C during the photophase (16 h) and 18 ± 1 °C during the scotophase (8 h) from sowing onwards. The Columbia plants used in the second experiment had been grown for 28 days since sowing under a L10:D14 regime to stimulate vegetative growth and prevent flowering and were then transferred to the same conditions under which the Landsberg plants had been grown. Light was provided by 4 SON-T (Philips, Eindhoven, the Netherlands) greenhouse lights of 400 W each, which were suspended 1 m above pot level. In case flowering stems tended to lodge, these were supported by a metal coil.

Measurement of seed production. The experiments were carried out in the greenhouse compartment in which the plants were grown prior to inoculation with first instar larvae of *P. rapae*. One month old *A. thaliana* plants of ecotype Landsberg were divided into four groups as follows: (A) Each plant inoculated with one *P. rapae* first instar larva; (B) Each plant inoculated with one *P. rapae* first instar larva parasitized by *C. rubecula*; (C) Control plants, not inoculated; (D) mechanically damaged plants. Parasitization of the larvae in group B occurred by individually exposing first instar larvae 1–2 days after egg hatch to 5–6 days old *C. rubecula* females taken from the laboratory colony. The occurrence of oviposition was verified by observation. Group D-plants were mechanically damaged with a sharp needle (first 3–4 days) and subsequently with a razor blade. Damaging was initiated on the day the *P. rapae* eggs hatched in groups A and B and terminated when all the leaves were removed from the plant (9–10 days later). The amount of plant material artificially removed each day was approximately similar to the amount removed by the caterpillar on the basis of visual estimates. For the experiment with ecotype Columbia treatment D was omitted. The two experiments were carried out with an interval of 9 months. Each day the plants were checked for the presence of the introduced larvae. In both experiments most of the group A-larvae experienced food shortage during their final instar and to allow normal pupation, additional ca. 40 day-old *A. thaliana* plants were placed next to completely defoliated plants. Fifth instar caterpillars could move freely between the original food plant and the extra food plant. At that time, a water layer of approximately 1 cm was put in the trays to confine wandering-phase larvae that searched for a place to pupate, thus forcing them to pupate on plant remains or pot. The same watering regime was applied to B-, C- and D-group plants. Water was again provided in the normal regime when all caterpillars had pupated. There was a 5–6-day difference in entering the prepupal phase between the first and last caterpillar. After pupation of all *P. rapae* caterpillars and emergence of all *C. rubecula* cocoons the ripening seeds were collected. Ripe siliques were cut from the plant using scissors. The siliques were sieved by a metal tea strainer to separate seed from silique material. Seed collected from each individual plant was stored in 2 ml glass vials and capped. Plants were checked for ripe siliques 3 times a week. Collecting of the seeds stopped when the plants were just over 100 days old since sowing at which time senescence was considered complete. Two weeks before the last seed was collected, greenhouse temperature was raised to 25 °C and one week later plants were no longer provided with water to speed up seed ripening. The total amount of seed was weighed for each individual plant on a Cahn microgram balance to an accuracy of 1 μg. Subsequently the weight of approximately 100 seeds was determined for each plant. After weighing these ca. 100 seeds, the precise number of seeds in the weighed aliquot was counted. The calculated weight per seed served to estimate the total number of seeds produced by dividing total seed weight produced by an individual plant by the average weight per seed, as determined for that particular plant. The non-parametric Kruskal–Wallis analysis of variance of ranks was used to compare seed production between treatment groups.

Time course in silique production. To monitor the time course of seed production more precisely, in the Columbia-experiment the time course of silique production was monitored every other day by harvesting siliques that were ripe as judged by their yellow-brown colour and the visible contours of ripened seeds inside.

Germination. In the Columbia-experiment the aliquots used to determine the average weight per seed were transferred to filter paper in 5 cm diam. glass petri dishes, vernalized (see above) and incubated at 22 ±0.5 °C to determine germination percentage. Germination was evaluated 3 days after the start of incubation.

Results

Total seed production per plant

Landsberg-experiment. Plants damaged by unparasitized caterpillars (group A) initially did not produce any seed at the time caterpillars pupated (15–20 days since introduction of first instar larvae) because the caterpillars consumed all developing siliques and most of the flowering stem(s). However, ca. 20–22 days after pupation of the caterpillars, 85% of these plants showed regrowth; new shoots (1–4 per plant) were formed at the basis of the main stem and these flowered and produced siliques. Sampling of the seeds was terminated after all the seeds from these regrowth shoots of *P. rapae*-inoculated plants had ripened. Group B, C and D plants already yielded ripe seeds at the time when the unparasitized caterpillars pupated. After this primary seed had ripened they began to produce new branches on the stems which produced some more siliques. The number of replicates was only seven for plants exposed to parasitized *P. rapae* larvae. This low replicate number was mainly due to a low percentage of successful parasitization. Table 1 presents the results for total seed weight per plant for the groups A–D. Plants fed upon by unparasitized *Pieris rapae* (A) produced significantly less total seed weight than plants fed upon by parasitized *P. rapae* larvae, control plants and mechanically damaged plants. Total seed weight did not differ significantly between plants from groups B, C and D.

Columbia-experiment. Results for this ecotype were essentially similar to those for Landsberg. Production of ripe siliques on regrown flowering stems by plants of ecotype Columbia severely defoliated by *P. rapae* caterpillars occurred 9–10 days later than the production of the first ripe siliques by intact plants. Plants fed upon by parasitized *P. rapae* produced such siliques only 1–2 days later than intact plants (Figure 1). Total seed weight was significantly lower for plants fed upon by unparasitized *P. rapae* than for either plants fed upon by parasitized *P. rapae* larvae or intact plants (Table 1).

Weight per seed

Landsberg-experiment. Weight per seed of Landsberg plants as determined from samples of ca. 100 seeds was highest (21.0 ± 1.9 μg/seed) for the plants that were fed upon by *P. rapae* caterpillars until pupation (Table 1). The mean weight/seed for this treatment was significantly higher than for seeds produced by intact plants or plants fed upon by parasitized *P. rapae* which had similar values (Table 1).

Columbia-experiment. Individual seed weight ranged between 16 and 17.5 μg/seed for Columbia plants and was similar for the three groups.

Estimated number of seeds per plant

Table 1 presents the number of seeds per plant for groups A–D. Plants fed upon by unparasitized *P. rapae* (A) produced a significantly lower estimated number of seeds in both experiments. Plants fed upon by parasitized *P. rapae* (B) produced seeds in numbers similar to those observed for intact plants or mechanically damaged plants (D, Landsberg-experiment).

Seed germination

Percentage germination was uniformly high in all three groups of the Columbia-experiment (87.6–96%, Table 1).

Discussion

Delay in reproduction by P. rapae *– damaged* A. thaliana. *Arabidopsis thaliana* plants of ecotype Landsberg displayed a remarkable ability to recover from almost complete defoliation by *P. rapae* by producing regrowth shoots 18–22 days after pupation of the unparasitized caterpillars. These shoots, formed at the basis of the main stem, produced buds and siliques and these gave rise to seed production, albeit with considerable delay. Plants fed upon by *P. rapae* caterpillars parasitized by *C. rubecula,* intact plants and mechanically damaged plants had produced all their primary seed at the time when the plants exposed to feeding damage by unparasitized *P. rapae* started seed production. When the latter plants showed regrowth, they only produced few very small leaves

Table 1. Total weight of seeds produced, weight of individual seeds and estimated number of seeds (mean ± SD) produced by *A. thaliana* plants of ecotypes Landsberg (*erecta* mutant) and Columbia, subjected to four different treatments (A–D)

	A Plants damaged by *P. rapae* until pupation		B Plants damaged by *P. rapae* parasitized by *C. rubecula*		C Intact Plants		D Mechanically damaged plants	
Parameter								
Total weight of seed produced per plant (mg)								
		N		N		N		N
Landsberg	15.6 ± 11.66 a[1]	23	57.6 ± 9.26 b	7	77.2 ± 28.11 b	28	61.0 ± 11.60 b	28
Columbia	26.5 ± 15.57 a	30	79.4 ± 49.63 b	19	106 ± 49.99 b	25	nd[2]	
Average weight per seed (μg)								
Landsberg	21.0 ± 1.94 a	23	18.8 ± 0.87 b	7	18.1 ± 1.24 b	28	19.1 ± 0.90 b	28
Columbia	16.0 ± 1.80 a	30	17.2 ± 1.12 a	19	17.5 ± 1.27 a	25	nd	
Estimated number of seeds per plant								
Landsberg	876 ± 484 a	23	3066 ± 550 b	7	4257 ± 1409 b	28	3148 ± 582 b	28
Columbia	1665 ± 953 a	30	4569 ± 2650 b	19	6065 ± 2856 b	25	nd	
Germination (%)								
Columbia	93.5 ± 7.0 a	23[3]	87.6 ± 20.2 a	16	95.7 ± 3.5 a	26	nd	

[1] Parameter values that are followed by different letters are significantly different between treatments according to Kruskal–Wallis analysis ($P<0.05$), followed by three pair-wise comparisons using the Mann–Whitney U-test with Bonferroni correction ($P<0.017$).

[2] Not done.

[3] Based on 90–140 seeds per plant for groups A–C. N = number of plants.

and started producing regrowth shoots almost at the same time. One week later, however, the other three groups of plants also started producing new branches on the secondary shoots which produced some extra siliques and seed. Faecal pellets of the caterpillars had dropped onto the potting soil and most probably have contributed to partial recycling of mineral plant nutrients (Lovett & Ruesink, 1995). Seed sampling was terminated when the plants fed upon by unparasitized *P. rapae* had produced their regrowth seeds, which was at the time that plants from the other groups were producing seed from their secondary growth. It is unknown to which extent such secondary seed production occurs in the field. Regrowth occurred faster and delay in seed production was shorter in the experiment with the Columbia ecotype. These plants had been grown under short-day conditions for 3 weeks to stimulate vegetative growth and postpone generative development, before they were placed under long-day conditions. Therefore, the difference cannot be ascribed to the (genetic) difference between ecotypes only. Harper (1977) stresses that a delay in plant reproduction caused by defoliation might sometimes even be more important than a reduced seed yield. Life time viable seed production as measured here is not equivalent to plant fitness (Strauss, 1997), but it is reasonable to assume that it is strongly correlated with fitness in the proper sense (e.g., Baldwin, 1998). Translated to the field situation, the delay in reproduction might not have an important influence on *A. thaliana* fitness in temperate areas because seeds are released in spring and they germinate only in autumn, staying dormant during summertime. Unless phenological escape from seed predators or herbivores would select for early seed production, the effect of staying dormant 10–20 days shorter might not affect fitness. On the other hand, because *A. thaliana* plants reproduce early in spring, plants that are damaged severely might not have the possibility to recover from this damage as neighbouring conspecifics might outcompete them. Avoidance of competition and herbivory would lead to selection for early reproduction, which in fact is a feature typical for *A. thaliana.*

Plant reproduction as affected by vegetative or generative tissue removal. Our results demonstrate that parasitization of *P. rapae* by *C. rubecula* neutralizes loss of fitness (here measured as life time seed pro-

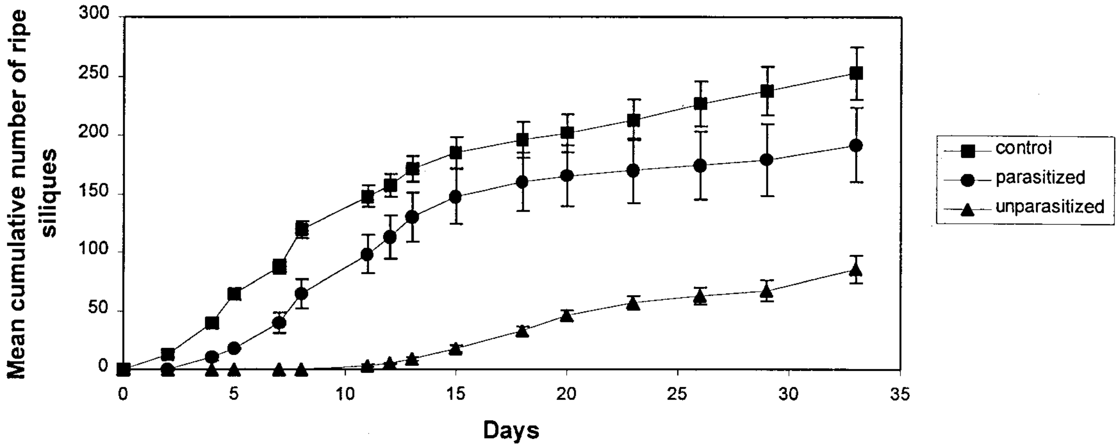

Figure 1. Time course in cumulative production of ripe siliques of *A. thaliana* plants, ecotype Columbia, that were either intact (control), fed upon by unparasitized caterpillars of *P. rapae* (unparasitized) or fed upon by parasitized caterpillars of *P. rapae.* Error bars represent SEM. Numbers of plants in each group are given in Table 1. Time axis starts at first day of silique production.

duction) of *A. thaliana* fed upon by unparasitized caterpillars. At present, no other studies that have addressed this issue in the context of signalling between plants and parasitoids are known to us. Indeed, studies that attempted to establish relationships between mechanical removal of vegetative tissue and seed production gave variable results (reviewed by Hendrix, 1988; Baldwin, 1990). Whereas in some plant species leaf tissue removal led to significantly lower seed production, in others defoliation resulted in higher seed production, due to compensatory growth (Trumble et al., 1993). Several factors have been invoked to explain this variation between plant species and between different experiments on the same species. Important among these are the life history of the plant species (perennial vs. annual), use of either stored or current photosynthate for growth, the age of tissues removed and the timing of removal. For example, removal of leaf tissue at the time seeds are filling is more detrimental to total seed production than defoliation prior to flowering (Coggin & Dively, 1980). For cauliflower, a domesticated plant bred for high vegetative and reproductive rate, a reduction in seed yield was found when plants were damaged by *P. brassicae* at bolt formation but not when many more larvae were feeding on the plant at the curd formation stage (Sood et al., 1993). In our experiments defoliation started in the pre-flowering phase, the criterion being that sufficient leaf biomass was present to sustain larval feeding until pupation. Caterpillars in the later instars, however, fed on generative tissues as well (see below).

Within a plant species considerable variation for regrowth can be present, a phenomenon that has been used in agriculture to breed for tolerance to herbivore-inflicted leaf damage (Crookston & Hicks, 1978; Hendrix, 1988; Rosenthal & Kotanen, 1994). We used two ecotypes of *A. thaliana* which genetically differed in vegetative biomass, augmented by the exposure to a short photoperiod of the larger ecotype during the early growth phase. Nevertheless, parasitization of the caterpillars neutralized loss of seed production established for plants fed upon by unparasitized caterpillars for both ecotypes, lending strong support for our first hypothesis.

Seed size may also have relevance for plant fitness. In our experiments, seeds from regrown Landsberg plants were bigger than seeds in the other groups. However, in view of published results on the relationship between seed size and seedling survival of *A. thaliana*, the small (though significant) difference found is not expected to result in significantly higher seedling survival rates (Krannitz et al., 1991). To establish whether the difference in seed size affected germination, this parameter was evaluated in the Columbia experiment. However, no effect on seed size was observed and germination was uniformly high for seeds collected from the control and treatment groups in this experiment. In contrast, *Senecio jacobaea* plants defoliated by cinnabar moth, *Tyria jacobeae* caterpillars, produced regrowth shoots after complete defoliation but the weight of individual seeds was only half that of the primary seeds from the control plants (Crawley & Nachapong, 1985). Regrowth seeds were expected to produce seedlings of lower competitive ability than the primary seeds of the control plants, and would therefore only increase fitness of *S. jacobaea* plants in places or years with competition-free microsites. In many plant species, seed and seedling mortality is high because of intra- and interspecific competition leading to microsite limitation of recruitment (Harper, 1977). The reduction in seed number due to herbivory can therefore be assumed to affect recruitment of a next plant generation significantly only when it reaches a certain threshold. In our experiments, seed production/plant was reduced by 75–80% for the plants fed upon by unparasitized *P. rapae*, a degree of reduction which might be assumed to exceed such a threshold, although field data are not yet available. In *A. thaliana*, an annual plant of disturbed habitats that only propagates by seeds and forms transient seed banks, microsite-limitation might not play a dominant role in determining offspring recruitment.

Artificial versus herbivore damage. Studies that used artificial removal of plant tissues have limited value for predicting and understanding the effects of natural herbivory (Hendrix, 1988; Baldwin, 1990). The reasons are that herbivore feeding is selective and the time pattern of feeding is difficult to simulate. Moreover, herbivores may secrete substances onto the damaged plant tissue that may affect plant growth negatively (phytotoxic compounds) or positively (Detling & Dyer, 1981). The artificial damage we applied in the Landsberg experiment was mimicking the time pattern of damage caused by feeding caterpillars as closely as possible, but a major difference with actual caterpillar feeding behaviour was that artificial removal was confined to leaf material. In contrast, the larvae also fed on the flowering stem, buds and siliques, thereby unexpectedly, but effectively acting as seed predators. Parasitized caterpillars had a distinctly lower tendency to climb the flowering stem, possibly an effect of biochemically mediated manipulation of host behaviour by the parasitoid. Crypsis of the host, by minimizing the optical contrast between the larval body (green in *P. rapae*) and the rosette leaves strongly reduces optical apparency to visually hunting predators as compared to a caterpillar present on a slender vertical structure like the flowering stem. The hypothesis that mechanical damage to the leaf rosette could be used to simulate similar degrees of herbivore feeding must therefore be rejected.

The issue of plant reproduction addressed here with a leaf feeding insect that also fed upon reproductive tissues approaches the situation documented for specialized seed-feeding herbivores (Gómez & Zamora, 1994). In a study on the seed feeding plume moth, *Amblyptilia pica*, Furbish's Lousewort plants, *Pedicularis furbishia*, fed upon by plume moth caterpillars parasitized by an ichneumonoid wasp produced more seeds than plants with unparasitized caterpillars, ca. 15% of the seed loss due to seed feeding being saved by the parasitoid (Menges et al., 1986). In our study parasitization of *P. rapae* by *C. rubecula* effectively neutralized the seed loss of *A. thaliana* plants caused by feeding of unparasitized *P. rapae.* In view of the demonstrated response of the parasitoids to volatiles emitted by the herbivore-damaged plant (van Poecke et al., unpbl.), our study has relevance for understanding the signalling relationship between plant and parasitoid and thereby implies a fitness benefit of the production of such volatiles by the plant.

Parasitoid-plant mutualism

In the tritrophic system investigated here, the plant benefits the parasitoids by producing volatiles in response to *P. rapae* damage. These volatiles are in turn used by *C. rubecula* to increase searching efficiency, which contributes to parasitoid fitness (Agelopoulos & Keller, 1994; Blaakmeer et al., 1994; Geervliet et al., 1994; 1996; van Poecke et al., 2000). At present it is not possible to generalize on mutualism in parasitoid-plant relationships as mediated by volatile plant infochemicals. Nevertheless, the large majority of published cases of the effect of solitary endoparasitoids on food consumption of their hosts suggest that these species drastically decrease food consumption and growth (e.g., Rahman, 1970; Guillot & Vinson, 1973; Harvey et al., 1999; Turlings & Fritzsche, 1999; but see Cloutier & Mackauer, 1979). Alternatively or in combination, other forms of behavioural manipulation such as confining the consumption by the host to vegetative tissues can be hypothesized to incur a selective advantage to both plant and parasitoid. In view of species numbers of solitary parasitoids that exploit herbivorous insects, plant-parasitoid mutualism is expected to be a common phenomenon in nature. We intend to corroborate the findings from the greenhouse study reported here by field studies, as the latter are indispensable to judge their evolutionary relevance.

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