DOI: 10.1111/j.1570-7458.2008.00693.x

Parasitoid load affects plant fitness in a tritrophic system

Renate C. Smallegange[1], Joop J.A. van Loon[1]*, Suzanne E. Blatt[1†], Jeff A. Harvey[2] & Marcel Dicke[1]

[1]*Laboratory of Entomology, Wageningen University, PO Box 8031, 6700 EH Wageningen, The Netherlands, and*
[2]*Department of Multitrophic Interactions, Netherlands Institute of Ecology, PO Box 40, 6666 ZG Heteren, The Netherlands*

Accepted: 5 February 2008

Key words: brood, clutch, mutualism, scramble competition, superparasitism, synomones, *Brassica nigra*, *Pieris brassicae*, *Cotesia glomerata*, Brassicaceae, Lepidoptera, Pieridae, Hymenoptera, Braconidae

Abstract

Plants attacked by herbivorous insects emit volatile compounds that attract predators or parasitoids of the herbivores. Plant fitness increases when these herbivorous insects are parasitized by solitary parasitoids, but whether gregarious koinobiont parasitoids also confer a benefit to plant fitness has been disputed. We investigated the relationship between parasitoid load of the gregarious *Cotesia glomerata* (L.) (Hymenoptera: Braconidae), food consumption by larvae of their host *Pieris brassicae* L. (Lepidoptera: Pieridae), and seed production in a host plant, *Brassica nigra* L. (Brassicaceae), in a greenhouse experiment. Plants damaged by caterpillars containing single parasitoid broods produced a similar amount of seeds as undamaged control plants and produced significantly more seeds than plants with unparasitized caterpillars feeding on them. Increasing the parasitoid load to levels likely resulting from superparasitization, feeding by parasitized caterpillars was significantly negatively correlated with plant seed production. Higher parasitoid brood sizes were negatively correlated with pupal weight of *Cotesia glomerata*, revealing scramble competition leading to a fitness trade-off for the parasitoid. Our results suggest that in this tritrophic system plant fitness is higher when the gregarious parasitoid deposits a single brood into its herbivorous host. A prediction following from these results is that plants benefit from recruiting parasitoids when superparasitization is prevented. This is supported by our previous results on down-regulation of synomone production when *Brassica oleracea* was fed on by parasitized caterpillars of *P. brassicae*. We conclude that variable parasitoid loads in gregarious koinobiont parasitoids largely explain existing controversies about the putative benefit of recruiting these parasitoids for plant reproduction.

Introduction

Plants attacked by herbivores emit a blend of volatiles that differs quantitatively and/or qualitatively from the blend emitted when intact or mechanically damaged (Turlings & Tumlinson, 1992; Paré & Tumlinson, 1999; Dicke & van Loon, 2000). Natural enemies, such as predators and parasitoids, use these volatiles to locate the herbivores (reviews by Vet & Dicke, 1992; Dicke, 1999; D'Alessandro & Turlings, 2006). The emission of herbivore-induced volatiles has been considered an adaptive trait for the plant as it recruits or 'enlists' natural enemies that attack the herbivore, thereby reducing the amount of damage to the plant. This synomone-mediated interaction between plant and natural enemy is supposedly benefiting plant fitness and in that sense mutualistic. The production of herbivore-induced plant volatiles has predominantly been studied from the perspective of the natural enemy in terms of signal reliability (Dicke & van Loon, 2000). In contrast, the relative costs and benefits of the synomones for plant fitness have been the subject of debate (van der Meijden & Klinkhamer, 2000). The discussion has mainly focussed on interactions between plants, herbivores, and koinobiont parasitoids. Koinobionts typically attack larval hosts that

---

*Correspondence: Joop J. A. van Loon, Laboratory of Entomology, Wageningen University, PO Box 8031, 6700 EH Wageningen, The Netherlands. E-mail: joop.vanloon@wur.nl
[†]Current address: Chemistry Department, Acadia University, 6 University Avenue, Wolfville, NS, Canada B4P 2R6.

continue to feed and grow for variable periods after parasitism, as opposed to idiobiont parasitoids, which attack non-growing host stages or paralyzed hosts (Mackauer & Sequeira, 1993).

The amount of feeding damage by parasitized hosts and their subsequent growth during parasitism, relative to healthy individuals, frequently depends on certain eco-physiological characteristics of the host, relative to the parasitoid attacking it (Harvey & Strand, 2002; Harvey, 2005). For example, solitary koinobionts attacking comparatively large hosts frequently reduce host growth (Jones & Lewis, 1971; Vinson, 1972; Harvey et al., 1999). Moreover, herbivores parasitized by solitary koinobiont parasitoids usually consume significantly less plant biomass than unparasitized ones (Rahman, 1970; Parker & Pinnell, 1973; Slansky, 1986; Gomez & Zamora, 1994). Parasitism by three solitary endoparasitoid species has also been shown to result in a fitness benefit both for small *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) plants and large corn plants, *Zea mays* L. (Poaceae), that were under attack from their herbivores (van Loon et al., 2000; Fritzsche-Hoballah & Turlings, 2001).

On the other hand, no study is known to us that examined the effect of parasitism of an herbivorous insect by a gregarious koinobiont on plant fitness. Leaf feeding damage inflicted by herbivorous hosts that were parasitized by gregarious koinobionts was found to be similar to or even higher than that caused by unparasitized larvae (Rahman, 1970; Parker & Pinnell, 1973; Smith & Smilowitz, 1976; Slansky, 1978, 1986; Coleman et al., 1999). Other studies reported a decrease in food consumption after parasitism, concomitant with an increase in food consumption in hosts with larger parasitoid loads (Führer & Keja, 1976; Karowe & Schoonhoven, 1992; Harvey, 2000; Elzinga et al., 2003). However, leaf consumption cannot be simply translated into effects on plant reproduction due to mechanisms that plants possess to compensate for leaf tissue loss (Trumble et al., 1993; Dicke & van Loon, 2000; Blatt et al., 2008).

To address this apparent conflict, we studied a tritrophic system consisting of black mustard plants, *Brassica nigra* L. (Brassicaceae), the large cabbage white butterfly, *Pieris brassicae* L. (Lepidoptera: Pieridae), a common herbivore of this plant, and their gregarious koinobiont endoparasitoid, *Cotesia glomerata* (L.) (Hymenoptera: Braconidae). We recently documented that in the last three instars, *P. brassicae* caterpillars prefer to consume flowers over leaf tissues of *B. nigra* plants, making this herbivore especially suitable for plant fitness studies (Smallegange et al., 2007). *Cotesia glomerata* habitually parasitizes several species in the Pieridae, but prefers to attack first and second instars (hereafter L1 and L2) of *P. brassicae* (Mattiacci & Dicke, 1995; Geervliet et al., 2000). Here, we manipulated parasitoid loads in *P. brassicae* caterpillars, and explored the following questions: (i) what is the effect of parasitism by *C. glomerata* on caterpillar food consumption and growth of *P. brassicae*? and (ii) what is the effect of parasitism on seed production in *B. nigra*, as a proxy of plant fitness? We discuss the broader importance of possible evolutionary conflicts between koinobiont parasitoids and plants.

Materials and methods

Plants

Seedlings of black mustard *Brassica nigra* L. Koch var. *abyssinica* A. Braun Junius CGN06618 (seeds provided by the Centre for Genetic Resources, Wageningen, The Netherlands) were individually transplanted to 1.5-l pots filled with 'Lentse potgrond nr 4' (Lentse Potgrond, Lent, The Netherlands). The plants were maintained at 23 ± 5 °C with a r.h. of 50–70% during the photophase (16 h; Philips SON-T Agro 400 Watt sodium lamps; Philips, Eindhoven, The Netherlands) and scotophase (8 h). Once a week, plants were sprayed with Euparene Multi (Bayer, Monheim am Rhein, Germany) to control mildew. A small hive of honeybees (*Apis mellifera* L.) was placed in the compartment to pollinate the plants.

Insects

Pieris brassicae caterpillars were obtained from a laboratory strain reared on greenhouse-grown Brussels sprouts (*Brassica oleracea* var. *gemmifera* cv. Cyrus). The colony was maintained at 22 ± 2 °C, L16:D8, with a r.h. of 40–60%. *Cotesia glomerata* females were obtained from an established laboratory colony reared on *P. brassicae* as a host. The parasitoid colony was kept at 23 ± 2 °C, L16:D8, and 60–70% r.h..

Experiments

First instar *P. brassicae* were collected from batches that had not yet begun to feed. Parasitized caterpillars were obtained in two different ways for two separate experiments. For experiment 1, parasitized caterpillars were obtained by placing individual caterpillars in a glass vial with a female *C. glomerata*. Caterpillars were assumed to be parasitized when they were observed to be stung by a parasitoid female until it finished its oviposition and had turned away from the caterpillar. Ten plants having just visible flower buds were inoculated on the youngest leaves below the inflorescence with either no (control), six unparasitized, or six parasitized caterpillars (day 1). Due to a higher mortality of the parasitized caterpillars, the number of parasitized as well as healthy caterpillars per plant was reduced to 4 on day 8 when they had reached the third instar, to have an equal number on each plant for each treatment.

Experiment 2: according to Ikawa & Suzuki (1982) and Ikawa & Okabe (1984), the clutch size of *C. glomerata* is strongly correlated with the duration of oviposition. To obtain caterpillars with different numbers of *C. glomerata* eggs, oviposition by parasitoid females was performed in a room with a temperature of approximately 18 °C, and was interrupted after 5, 10, 15 and 25 s. To obtain an oviposition time of 25 s, it was often necessary that the caterpillar be stung twice (superparasitized) by the same female wasp. A fifth group of caterpillars was left unparasitized and served as the control. A group of three caterpillars that had been exposed to the same parasitism treatment was placed on the same plant, on a mature leaf next to the lowest flowering branch. These plants had just started to flower (developmental stage 4.2 according to Harper & Berkenkamp, 1975) prior to the inoculation. All five treatments were replicated 10 times.

Each day during experiments 1 and 2 caterpillar survival, instar (L1–L5), position on the plant (leaf, stem, or flower), were recorded. For experiment 2, the number of flower buds and flowers consumed by the caterpillars were recorded and the area of leaf tissue removed from leaves attached to the plant (mm^2) was estimated using a ruler and when necessary drawn on mm-paper. These measurements were made until the unparasitized caterpillars started to pupate or until the parasitoid larvae egressed from the parasitized caterpillars. To ensure that larvae did not escape, they were reared on plants in open-mesh fabric cages that enclosed the flowers and leaves.

For experiments 1 and 2, both fresh and dry weights were obtained for *P. brassicae* pupae, clusters of *C. glomerata* and caterpillars from which wasps had egressed. Dry weights were obtained by drying in an oven at 40 °C for several days and weights measured on a daily basis until constant dry weight was obtained (approximately 72 and 120 h for caterpillars and pupae, respectively). The number of parasitoid cocoons in each cluster was counted.

Plant parameters measured in experiment 1 were: height and number of leaves present before inoculation (day 1). After pupae and caterpillars were taken off the plants at day 16, the following parameters were recorded: plant height, number of leaves remaining, number of caterpillar-damaged leaves, number of flower branches, number of flower branches still flowering, and number of flower branches with chewing damage. One week later, plant height was measured once more, and the number of new flower branches and number of siliques on original and re-grown branches were counted. The siliques were harvested (around day 30), dried and cleaned, after which the seeds were counted and weighed. Seed production on re-grown branches was likely to be underestimated due to an aphid infestation that occurred during the last phase of the experiment.

Plant parameters quantified in experiment 2 were taken from plants that were left in the greenhouse compartment until the siliques had ripened. Subsequently, the siliques of each plant were harvested, dried, and then collected seeds were separated from the chaff using a South Dakota Seed Blower (Franken BV, Goes, The Netherlands; turning speed 500 turns per minute, sieve width 0.9 mm). The total amount of seed was weighed for each individual plant to an accuracy of 1 μg (Mettler PM200 balance; Mettler-Toledo BV, Tiel, The Netherlands) and counted with a Numigral 1 seed counter (Tripette and Renaud Chopin, Villeneuve-la-Garenne, France).

Statistical analysis

For experiment 1, generalized linear models (GLM; GenStat, Edition 8.11, VSN International, Oxford, UK; Anonymous, 2005) were used to examine the effect of treatment and their interaction with the various parameters measured. For experiment 2, to be able to compare caterpillar damage across treatments, parasitoid load was pooled per plant. Hence, 'parasitoid load' represents the total number of *C. glomerata* cocoons that emerged from the caterpillars that were present on a plant. Generalized linear models were used to examine the effect of the parasitism treatment on the mean number of *C. glomerata* cocoons emerging from the caterpillars (normal distribution), the percentage of caterpillars surviving on a plant (binomial distribution, link in logit), caterpillar mortality (binomial distribution, link in logit), and the mean duration of each instar (normal distribution). Effects were considered to be significant at $P<0.05$. Two-sided t-probabilities were calculated to test differences between means. The significance threshold of these means was corrected for multiple comparisons and reduced to $P<0.0085$ when four treatments were compared and to $P<0.005$ when five treatments were compared. Linear regression analyses (GenStat) were used to examine possible relationships between (pooled) parasitoid load, dry weight, leaf and flower consumption, and number of seeds. The proportion of time spent on leaves and flowers was analysed with a GLM (binomial distribution, link in logit) and the Kolmogorov-Smirnov two-sample test (GenStat). Effects were considered to be significant at $P<0.05$.

In total, four plants were removed from the data set. Two plants did not have any *C. glomerata* larvae emerge from one of the caterpillars. Two more plants were removed from the data set due to heavy aphid infestation or because flowering was significantly delayed, compared to the other plants.

Table 1 Plant parameters for *Brassica nigra* recorded in experiment 1 (means ± SE) by treatment (n = 9–10)

	Treatment		
Plant parameter	Undamaged	Parasitized	Unparasitized
Height at start	29.0 ± 1.7a	33.1 ± 2.5a	28.9 ± 2.7a
Height after attack	139.5 ± 4.0a	120.3 ± 6.4b	119.0 ± 4.2b
Height 1 week after attack	161.6 ± 4.1a	136.4 ± 6.9b	129.1 ± 3.8b
Number of leaves at start	12.6 ± 0.5a	11.6 ± 0.5a	12.3 ± 0.5a
Number of leaves after attack	12.8 ± 0.5a	7.6 ± 0.6c	9.3 ± 0.5b
Number of leaves damaged	0	2.4 ± 0.4a	4.1 ± 0.4b
Number of branches damaged	0	5.1 ± 0.4a	6.2 ± 0.6a
Number of branches after attack	13.0 ± 0.7a	7.8 ± 1.0b	7.3 ± 0.7b
Number of branches flowering after attack	13.0 ± 0.7a	2.7 ± 1.1b	1.1 ± 0.5b
Number of siliques	158.7 ± 18.1a	99.3 ± 20.6a	140.0 ± 44.0a
Number of seeds	461 ± 103a	357 ± 86a	79 ± 38b
Seed weight (mg)	0.62 ± 0.04a	0.68 ± 0.06a	0.74 ± 0.13a

GLM, generalized linear models.
Numbers followed by the same letter within a row are not significantly different (GLM: $P \geq 0.05$).

Results

Experiment 1: unrestrained parasitization

Caterpillar and parasitoid parameters. Mortality on day 8 was zero for healthy, unparasitized caterpillars, whereas 32% of the parasitized ones had died. Survival after day 8 until the end of the experiment was 100% for both parasitized and healthy caterpillars. No differences in developmental rate were observed between unparasitized (12.23 days) and parasitized (12.45 days) caterpillars. The mean (± SEM) number of parasitoid larvae inside a caterpillar was 18.6 ± 1.9 (n = 36). The dry weight of the moribund caterpillar carcass (28 ± 5 mg; n = 36) combined with the dry weight of the batch of *Cotesia* cocoons (22 ± 3 mg; n = 36) was 50 ± 3 mg (n = 36), which was significantly lower than the dry weight of a healthy pupa (80 ± 6 mg, $P<0.001$). The dry weight of one *C. glomerata* cocoon was 1.18 ± 0.06 mg.

As had been observed before (Smallegange et al., 2007), late second and early third instars, both healthy and parasitized, moved to and fed on the flowers of *B. nigra* plants. The first caterpillars observed on the flowers were parasitized caterpillars on day 3 with the maximum proportion of parasitized caterpillars feeding on flowers occurring on day 10 (82%). There were no significant differences in distribution over leaves, stem, and flowers between healthy and parasitized caterpillars (GLM: $P>0.05$; data not shown).

Effect of caterpillar feeding on plant parameters. Before the caterpillars were put on the plants, no differences in plant height were found between the undamaged control plants, plants damaged by healthy caterpillars, and plants damaged by parasitized caterpillars ($P = 0.37$; Table 1). No differences in height were found between plants directly after feeding by parasitized or unparasitized caterpillars had ceased and this was the same 1 week later ($P = 0.85$ and 0.31, respectively). Control plants were taller than the plants in the two treatment groups at both these time points (control vs. non-parasitized caterpillars: $P = 0.01$ and $P = 0.006$; control vs. parasitized caterpillars: $P = 0.002$ and $P<0.001$).

Control plants had on average a similar number of leaves as plants exposed to parasitized and unparasitized caterpillars at the start of the experiment ($P = 0.35$). Plants that had been damaged by either parasitized or healthy caterpillars had significantly fewer leaves left than control plants ($P<0.001$ and $P<0.001$, respectively). Plants exposed to unparasitized caterpillars had more leaves than plants exposed to parasitized caterpillars ($P = 0.04$), although the number of damaged leaves was higher for plants attacked by unparasitized caterpillars ($P = 0.014$), indicating that parasitized caterpillars had been consuming some leaves completely.

Number of flowering branches damaged by caterpillars was similar for both caterpillar treatments ($P = 0.14$) and caterpillar feeding significantly reduced the numbers of flowers present ($P<0.001$). Parasitized and unparasitized plants had similar numbers of flowers ($P = 0.16$). Control plants had more branches flowering than the caterpillar-attacked plants following the caterpillar attack ($P<0.001$). Control plants showed no re-growth of flower branches, whereas some attacked plants did. An aphid infestation developed rapidly hereafter, preventing further measurements.

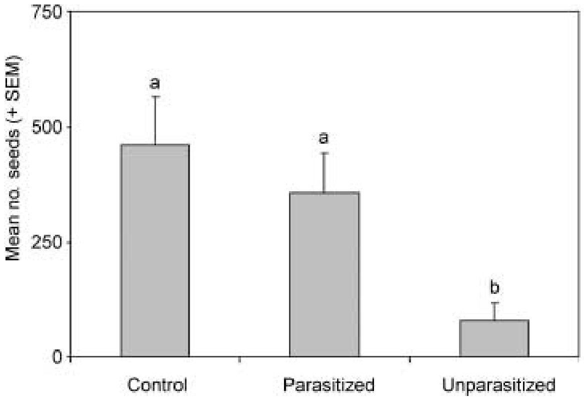

Figure 1 Seed production (mean + SE) of undamaged *Brassica nigra* control plants (n = 10), plants damaged by *Cotesia glomerata*-parasitized caterpillars (n = 9), and plants damaged by unparasitized caterpillars (n = 9).

Five of the plants fed on by unparasitized caterpillars produced no seed at all, whereas all plants fed on by parasitized caterpillars developed seed. On average control plants and plants fed on by parasitized caterpillars produced more seeds than plants attacked by healthy caterpillars (P = 0.006; Figure 1). There were no significant effects of treatment on the individual seed weight (Table 1).

Experiment 2: experimentally manipulated parasitoid load

Caterpillar survival and instar duration. The method we employed to produce variation in clutch size, by manipulating oviposition time, resulted in a wide range of parasitoid load per caterpillar (see Figure 2A). A significantly positive relationship was disclosed between oviposition duration and resulting parasitoid load, established as number of parasitoid larvae egressing and producing cocoons (GLM: P<0.001). Data from two parasitized caterpillars were excluded from the analysis as no parasitoid larvae emerged and they pupated normally.

The highest mortality of the caterpillars occurred in the first 24 h after they were introduced onto the plants (16.7%; GLM: P<0.001). The caterpillars with the highest parasitoid loads experienced the lowest survival (50.0%), whereas only a few unparasitized caterpillars died (3.3%). However, the effect of the parasitization on survival was nearly significant (GLM: P = 0.06; Table 2). On five plants all caterpillars died within the first 24 h. As these plants had undergone negligible feeding damage, they were considered as controls with respect to their seed production (see below).

On average, the first stage of caterpillars that had been parasitized for 25 s lasted longer than for the caterpillars that had not been parasitized or had been parasitized during a shorter period (GLM: P<0.001; Table 3). The duration of L3 was longer in caterpillars that had been parasitized for 15 s than for unparasitized caterpillars (GLM: P = 0.01). No significant differences in the duration of L2 and L4 stages were detected between the treatments (GLM: P = 0.13 and 0.25, respectively). The fifth instar was shorter for parasitized than for unparasitized caterpillars (GLM: P = 0.01). The total time between the onset of an experiment and the egression of *C. glomerata* larvae from their host tended to increase with the parasitoid load (GLM: P = 0.03; Table 3).

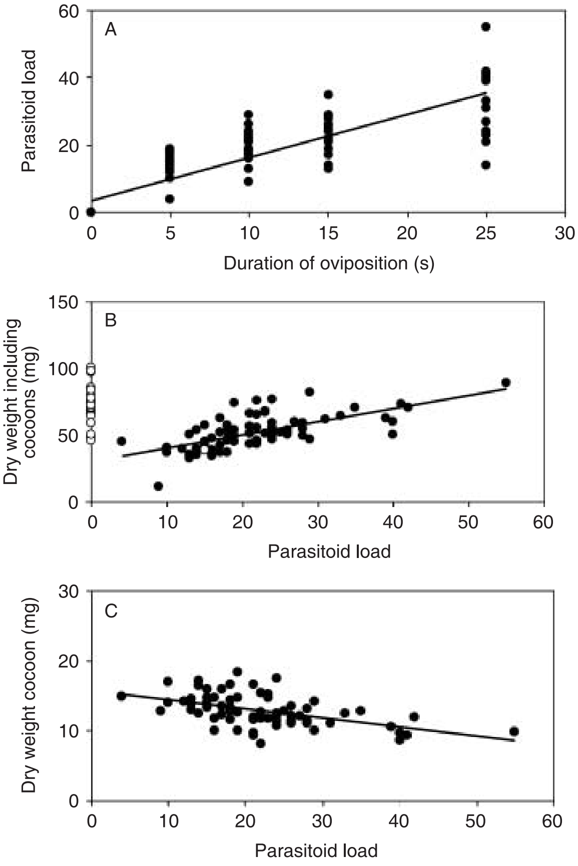

Figure 2 (A) Relationship between manipulated duration of oviposition by *Cotesia glomerata* into *Pieris brassicae* and parasitoid load; regression equation: $y = 0.8615x + 10.599$; $r^2 = 0.472$ (GLM: $P<0.001$). (B) Relationship between pooled parasitoid load and the combined dry mass of the parasitoid-host complex. Regression equation: $y = 0.001x + 0.0306$; $r^2 = 0.437$ (GLM: $P<0.001$). Open circles on the *y*-axis indicate pupal weights reached by unparasitized caterpillars. (C) Relationship between pooled parasitoid load and the dry weight of an individual *Cotesia glomerata* cocoon. Regression equation: $y = 1.6 - 0.01x$; $r^2 = 0.244$ (GLM: $P<0.001$).

Table 2 Survival (mean ± SE) of *Pieris brassicae* caterpillars and number of caterpillars feeding for each treatment in experiment 2. Numbers of *Brassica nigra* plants are given between brackets

	Treatment					
	Unparasitized	Parasitized 5 s	Parasitized 10 s	Parasitized 15 s	Parasitized 25 s	GLM[1]
Survival (%)[2]	96.7 ± 3.3 (10)a	71.4 ± 18.4 (7)a	76.7 ± 11.2 (10)a	77.8 ± 5.6 (9)a	50.0 ± 13.4 (10)a	P = 0.06
Number of caterpillars on plant[3]	3.0 ± 0.1 (10)a	3.0 ± 0.0 (5)a	2.6 ± 0.2 (9)a	2.3 ± 0.2 (9)a	2.1 ± 0.3 (7)a	P = 0.05

GLM, generalized linear models.

[1] Numbers followed by the same letter within a row are not significantly different (GLM, binomial distribution, link in logit, respectively, Poisson distribution, link in logarithm; the significance threshold was corrected for multiple comparisons).

[2] Mean survival including plants on which all caterpillars died.

[3] Mean number of caterpillars excluding plants on which all caterpillars died.

Effect of parasitoid load on host and cocoon weight. The dry weight of healthy pupae and combinations of caterpillar carcass after egression and batch of egressed cocoons, plotted against parasitoid load (the number of cocoons in a batch) is shown in Figure 2B. The combined dry mass of the parasitoid-host complex was positively correlated with parasitoid load ($P<0.001$). The weight of an individual cocoon correlated negatively with parasitoid load ($P<0.001$; Figure 2C).

Effect of parasitoid load on caterpillar position. First and second instars consumed mainly leaf tissue. A few parasitized caterpillars (three that had been parasitized for 5 s and three for 25 s) moved from the leaves to flowers during their first or second instar. However, irrespective to whether they were parasitized or not, most *P. brassicae* larvae started moving upwards towards the flowers as soon as they had molted to L3 (from day 5). Before arriving at flowers, they often consumed the small leaves at the base of a flowering branch. On day 9, more than 90% of the caterpillars were observed eating flower petals.

Although parasitized caterpillars appeared to stay proportionately longer on the leaves before moving to the flowers (50.3 vs. 43.9%), no significant differences were found between unparasitized and parasitized caterpillars using the interval-observation method (Table 4; Kolmogorov-Smirnov two-sample test: $P>0.25$). Furthermore, no significant effect of parasitism on the distribution of the caterpillars on the plants was found when caterpillars were categorized in three classes based on parasitoid load pooled for the caterpillars on the same plant (0–20, 20–50, and 50–110 *C. glomerata* larvae per three host caterpillars; Kolmogorov-Smirnov two-sample test: $P>0.05$; data not shown), although the 5-, 10-, and 15-s treatment groups on average spent less time in the flowers.

Effect of parasitoid load on caterpillar leaf and flower consumption. Linear regression showed that number of flowers consumed from a plant significantly increased with the pooled parasitoid load ($P<0.001$; Figure 3A). The relationship between leaf consumption and parasitoid load was close to significance ($P = 0.05$; Figure 3B). In comparison, a group

Table 3 Host measurements: instar duration of *Pieris brassicae* caterpillars in days (mean ± SE; replicate number between brackets) for each treatment

	Treatment					
Instar	Unparasitized	Parasitized 5 s	Parasitized 10 s	Parasitized 15 s	Parasitized 25 s	GLM[1]
L1	3.7 ± 0.1 (30)a	3.4 ± 0.1 (15)a	3.5 ± 0.1 (23)a	3.4 ± 0.1 (21)a	4.3 ± 0.2 (16)b	P<0.001
L2	2.5 ± 0.1 (31)a	2.0 ± 0.0 (15)a	2.4 ± 0.2 (23)a	2.7 ± 0.3 (21)a	2.7 ± 0.2 (15)a	P = 0.13
L3	3.0 ± 0.2 (31)a	3.9 ± 0.4 (15)ab	3.6 ± 0.2 (23)ab	4.2 ± 0.3 (20)b	3.7 ± 0.3 (15)ab	P = 0.01
L4	3.0 ± 0.2 (30)a	3.3. ± 0.3 (15)a	3.2 ± 0.2 (23)a	2.6 ± 0.2 (20)a	2.6 ± 0.3 (14)a	P = 0.25
L5	3.0 ± 0.1 (30)b	2.1 ± 0.1 (15)a	2.3 ± 0.2 (23)a	2.5 ± 0.2 (20)ab	2.4 ± 0.4 (14)ab	P = 0.01
Total L1–L5	15.2 ± 0.1 (29)ab	14.7 ± 0.2 (15)a	14.9 ± 0.2 (23)ab	15.2 ± 0.1 (20)ab	15.6 ± 0.3 (14)b	P = 0.03

GLM, generalized linear models.

[1] Numbers followed by the same letter within a row are not significantly different (GLM, normal distribution; the significance threshold was corrected for multiple comparisons).

Table 4 Position of *Pieris brassicae* caterpillars on *Brassica nigra* plants in experiment 2 given as percentage of time (mean ± SE) spent on leaves (true and small), stem, and flowers

	Treatment					
Position	Unparasitized	Parasitized 5 s	Parasitized 10 s	Parasitized 15 s	Parasitized 25 s	GLM[1]
Number of plants	10	5	9	9	7	
Leaf	43.9 ± 2.6 a	50.3 ± 7.7a	52.8 ± 3.4a	53.2 ± 3.5a	50.1 ± 6.2a	P = 0.48
Stem	1.5 ± 1.0a	3.2 ± 2.0a	3.4 ± 1.1a	1.1 ± 0.8a	0 ± 0a	P = 0.05
Flower	54.5 ± 2.3a	46.5 ± 8.3a	43.8 ± 3.4a	45.7 ± 3.3a	49.9 ± 6.2a	P = 0.37

GLM, generalized linear models.

[1] Numbers followed by the same letter within a row are not significantly different (GLM, binomial distribution, link in logit).

of three caterpillars that had not been parasitized consumed (mean ± SE) 407 ± 63 buds and flowers and 4180 ± 1019 mm^2 leaf tissue.

Effect of parasitoid load and consumption by Pieris brassicae *on seed production in* Brassica nigra. Seed production of *B. nigra* plants was negatively related to both number of flowers ingested and to total parasitoid load (P = 0.003; Figure 4A; and P = 0.01; Figure 4B, respectively). The total amount of leaf tissue consumed by parasitized caterpillars showed no significant relationship with the number of seeds produced (P = 0.24, data not shown). However, the weight of a single seed was lower when more leaf tissue had been eaten (P = 0.001; Figure 4B). Pooled parasitoid load had a significantly negative effect on the average weight of a single seed (P = 0.02; Figure 4D). Plants exposed to unparasitized caterpillars produced (mean ± SE) 362.0 ± 107.0 seeds. From the five plants on which all caterpillars died within 24 h (mean ± SE) 805.0 ± 164.0 seeds per plant were harvested.

To allow for direct comparison between experiments 1 and 2, seed numbers produced by plants in the five treatment groups in experiment 2 (as represented in Figure 4B) were plotted in Figure 5 as they were in Figure 1, that is, splitting up the range of pooled parasitoid loads over three classes (15–45, 46–60, and 60–80 parasitoid larvae per host). An analysis of the data, thus categorized, disclosed significant differences between treatment groups (one-way ANOVA: F = 2.67, P<0.05). When pooled parasitoid load ranged between 15 and 45, plants damaged by parasitized caterpillars produced a similar number of seeds as undamaged control plants (least significant difference post-hoc test: P<0.05). At both higher ranges of parasitoid load (above a value of 45), plants fed on by parasitized caterpillars produced significantly lower amounts of seeds, similar to the seed production achieved by plants fed on by unparasitized caterpillars.

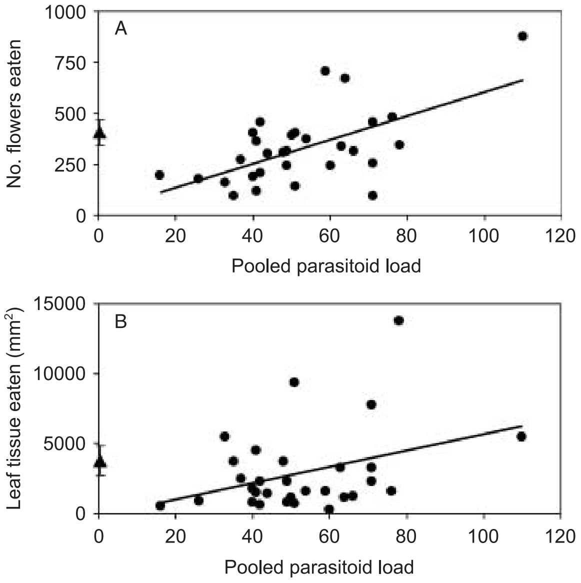

Figure 3 (A) Relationship between pooled *Cotesia glomerata* parasitoid load and number of *Brassica nigra* flowers consumed. Regression equation: $y = 21.0 + 5.9x$; $r^2 = 0.337$. (B) Relationship between pooled parasitoid load and leaf tissue consumption. Regression equation: $y = -183.0 + 58.8x$; $r^2 = 0.132$. The triangles on the *y*-axes indicates mean ± SE for plants fed on by unparasitized caterpillars.

Discussion

Our experiments show that parasitoid load is a crucial factor determining whether parasitism by the gregarious koinobiont braconid *C. glomerata* will increase plant fitness compared to plants attacked by unparasitized caterpillars. Compared to the published findings for solitary koinobiont parasitoids (van Loon et al., 2000; Fritzsche-Hoballah & Turlings, 2001), measurement of the effect of parasitism by gregarious koinobiont parasitoids on plant fitness is complicated due to variability in parasitoid load. Elzinga et al. (2003) argued that the host plant *Silene latifolia* will probably benefit from parasitism

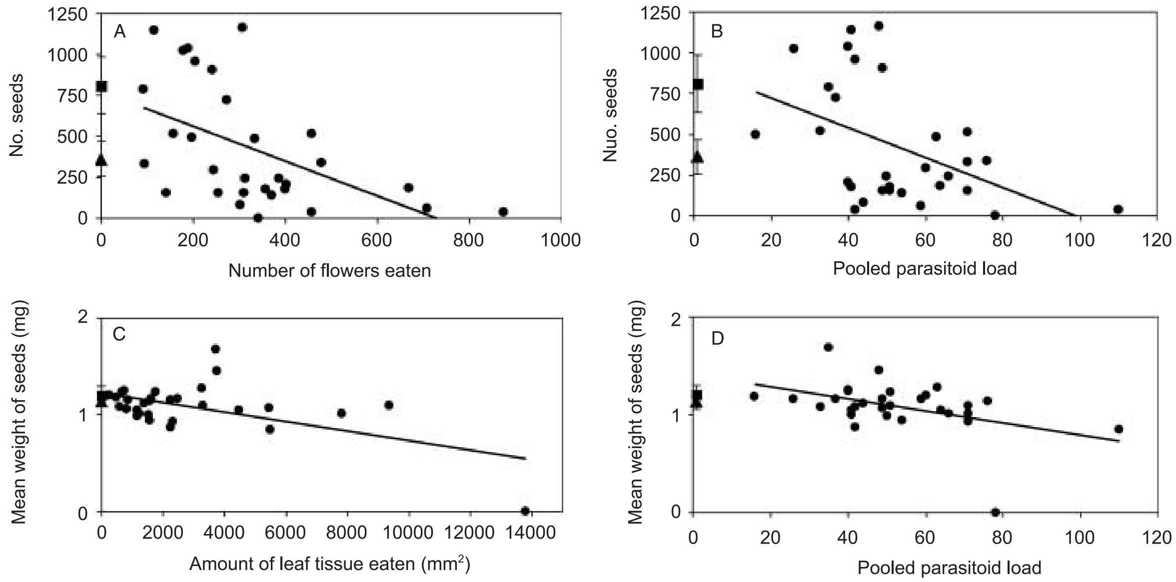

Figure 4 (A) Relationship between number of flowers ingested by *Cotesia glomerata*-parasitized caterpillars and number of seeds produced by *Brassica nigra*. Regression equation: $y = 776.0 - 1.1x$; $r^2 = 0.249$. (B) Relationship between pooled parasitoid load and number of seeds produced by *B. nigra*. Regression equation: $y = 909.0 - 9.2x$; $r^2 = 0.187$. (C) Relationship between amount of leaf tissue consumed and the weight of a single seed. Regression equation: $y = 1.2282 - 0.05x$, $r^2 = 0.293$. (D) Relationship between pooled parasitoid load and weight of a single seed. Regression equation: $y = 1.4100 - 6.2x$; $r^2 = 0.162$. For all four figures, the square on the y-axis indicates the mean value (± SE) for five plants that had a little initial feeding damage; the triangle indicates the mean (± SE) value for plants that were fed on by unparasitized caterpillars.

of the caterpillar *Hadena bicuris* by its gregarious koinobiont, *Microplitis tristis*, because parasitized caterpillars consumed significantly less plant material than unparasitized caterpillars, although they did not study the effect on plant reproduction. Coleman et al. (1999) observed that *P. brassicae* caterpillars that were parasitized by *C. glomerata* consumed more excised leaf material of *B. oleracea* than unparasitized caterpillars and concluded that the host plant could not benefit from parasitism by this parasitoid. However, the authors did not examine parasitoid load nor did they explicitly measure reproductive traits in damaged plants, such as seed number and plant size. Furthermore, they also did not consider the possibility that *P. brassicae* caterpillars may preferentially feed on flower buds and flowers (and even siliques), which clearly has a higher impact on plant fitness than folivory (Smallegange et al., 2007).

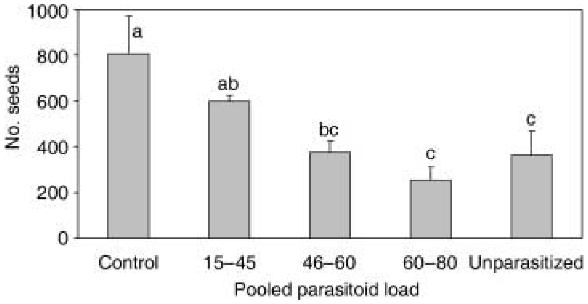

Figure 5 Seed numbers produced by *Brassica nigra* plants in the five treatment groups in experiment 2 (as represented in Figure 4B) were plotted as in Figure 1, the range of pooled parasitoid load split up over three classes: 15–45 (n = 12), 46–60 (n = 10), and 60–80 (n = 7). Control plants: n = 5; plants fed on by healthy caterpillars: n = 10. Bars capped with the same letters are not significantly different (least significant difference post-hoc test: $P \geq 0.05$).

Rahman (1970), Parker & Pinnell (1973), Smith & Smilowitz (1976), and Slansky (1978) showed that *P. rapae* caterpillars parasitized by *C. glomerata* consumed a larger amount of leaf tissue of cabbage plants than unparasitized caterpillars. This was explained either by a prolonged larval period or by exploitation of the host by the endoparasitic larvae, but none of these studies considered the influence of brood size. We did not observe a prolonged larval period for parasitized compared to unparasitized *P. brassicae*.

Superparasitism by *C. glomerata* was observed to lengthen parasitoid development, reduce survivorship and increase the feeding period and consumption of excised cabbage leaves and weight growth in *P. brassicae* larvae compared to a single oviposition (Gu et al., 2003). Several caterpillars in our study were self-superparasitized by the same parasitoid female when we allowed them a total oviposition time of 25 s. Survival of the caterpillars that were parasitized over 25 s, either during one or two ovipositions, was lower than in other groups and development of the parasitoids was slightly prolonged (Table 3).

In contrast to studies with gregarious koinobiont parasitoids, many studies have reported that the growth of hosts parasitized by solitary koinobionts is greatly reduced (reviewed by Mackauer & Sequeira, 1993; Harvey, 2005). The larvae of solitary endoparasitoids, such as *C. rubecula* and *Microplitis demolitor*, usually emerge during the fourth instar of the host, in contrast with gregarious species such as *C. glomerata* and a closely related species, *C. congregata*, whose larvae usually emerge late during the host's final instar (Beckage & Riddiford, 1983; Harvey et al., 1999, 2004). As *Pieris* caterpillars typically consume more than 90% of their total food intake during their final (L5) instar (Parker & Pinnell, 1973; Smith & Smilowitz, 1976), it is not surprising that caterpillars parasitized by solitary koinobionts consume less than healthy caterpillars (Rahman, 1970; Parker & Pinnell, 1973). This can be understood in a nutritional context, as a single parasitoid larva, even if having, say, twice the maximum body mass, has lower nutritional requirements than a small brood size of a gregarious parasitoid species. Hymenopteran parasitoids are known to regulate the feeding behaviour and nutritional physiology of their hosts by virtue of a suite of biochemical mechanisms, including the injection of polyDNA virus and venom during oviposition (Harvey, 2005; Pennacchio & Strand, 2006).

Our results show that unparasitized and superparasitized caterpillars consumed more flowers than caterpillars with single parasitoid broods and that this resulted in a reduction in seed production. Therefore, we conclude that in the association under investigation, the effect of parasitism of the herbivore on plant reproduction depends on parasitoid load. An obvious benefit in terms of seed production and thus instantaneous plant fitness was found at small parasitoid load (brood size 15–20 parasitoid larvae per caterpillar), whereas at higher values, seed production was similar to that of plants damaged by unparasitized caterpillars. Thus, the relative costs and benefits are critically correlated with parasitoid load. To assess the relevance of our greenhouse results for field conditions, information on brood sizes occurring in the field is required. Harvey (2000) presented evidence that typical primary broods of *C. glomerata* in the same host species are between 10 and 40, with a mean of approximately 25. In a field study conducted by Gu et al. (2003), brood size of *C. glomerata* in *P. brassicae* feeding on *B. oleracea* was 45, but varied over a wide range (3–158), and the rate of superparasitization was estimated to range from 20 to 29%. A more narrow range of average brood sizes (22–39) was found in another field study on three plant species conducted by our group (Geervliet et al., 2000). The highest average brood sizes (30–39) were recorded for *B. oleracea* (Brussels sprouts). Naturally occurring brood sizes are expected to vary due to several factors. A major factor is the availability of unparasitized hosts. When this availability is low (high parasitoid to host ratios), superparasitization will occur and has been proposed to be adaptive for the parasitoid (Gu et al., 2003). A second factor is the quality of the host caterpillars, which is largely determined by the nutritional quality of their host plant (Karowe & Schoonhoven, 1992; Geervliet et al., 2000). We conclude that brood sizes found on the cultivated, non-flowering *B. oleracea* reported above are not representative for those occurring on feral, flowering *B. nigra*. In field experiments on *B. nigra* we found an average brood size value of 20, a value that was stable over several years of field studies (RC Smallegange, unpubl.), suggesting that superparasitization was rare.

We observed that a higher parasitoid load leads to scramble competition via sibling competition resulting in a decrease in cocoon weight (Figure 2C; Tagawa et al., 1982). This reduction may lead to a lower survival of parasitoid pupae and a decrease in adult wasp body weight and fitness (Ikawa & Okabe, 1985; Harvey, 2000; Elzinga et al., 2003). If there is a trade-off for the parasitoid, one may expect that, under natural conditions, females of gregarious koinobiont parasitoids will adjust the primary clutch in such a way as to optimize the fitness of the brood (the 'Lack clutch size' sensu Elzinga et al., 2005). Brood size is a phenotypically plastic trait and is dependent on physiological status of the parasitoid female, her experience, and her perception of the environment in terms of competition, experienced as encounters with already parasitized hosts, and risks of instantaneous mortality, for example, due to predation (Ikawa & Okabe, 1985; Le Masurier, 1991; Gu et al., 2003).

Although several circumstances in a greenhouse are optimized for plant growth, 1.5-l pots limit plant growth considerably. Therefore, under natural conditions plants will often grow larger, especially in nutritional, riverine soils where *B. nigra* is most common. Consequently, they will develop more flowers and will produce more seed when under attack by the same number of caterpillars as used in this study (RC Smallegange, unpubl.). Soil fertility appeared to influence the ability for *B. nigra* plants to compensate for herbivory (Meyer, 2000). Therefore, caution should be taken to extrapolate our data directly to natural circumstances.

Another unknown factor is the average number of *P. brassicae* caterpillars that attack *B. nigra* plants in nature.

Pieris brassicae females oviposit batches of ca. 50 eggs on suitable host plants, but mortality is often very high; 95% cumulative mortality during development from neonate to pupa is no exception (Kristensen, 1994). These two values were used to arrive at the caterpillar densities we studied.

Previous studies have reported that leaf defoliation affects the seed weight of *B. nigra* (Ramana & Ghildiyal, 1997). Although leaf consumption had no effect on seed production in our experiments, its negative effect on seed weight could have a significant impact on seed germination and seedling growth (Hendrix, 1984; Hendrix et al., 1996; Smart & Moser, 1999), which may be even more important for plant reproduction than seed numbers in case plant recruitment to the next generation is microsite-limited rather than seed-limited, which seems to be valid for *B. nigra*.

In experiment 1, *B. nigra* plants were exposed to feeding by *P. brassicae* caterpillars (six reduced to four in the L3 developmental stage) at an earlier flowering stage, that is, when the flower buds had just become visible. These plants, when consumed by unparasitized caterpillars, produced significantly fewer seeds than plants exposed to parasitized caterpillars and the latter produced similar numbers of seeds to undamaged plants. Considered in conjunction with the somewhat different results of experiment 2, during which flower feeding commenced at a slightly later stage of flowering, this suggests that the timing in plant development of oviposition by a female butterfly, together with the number of caterpillars on a plant, is an important factor in determining the impact of parasitism by *C. glomerata* on the seed production of that plant. Caterpillars cause a more severe reduction in seed production when they reach the reproductive structures of the mustard plants when these are still in bud stage. The benefit of parasitism for the plant will in that case be more pronounced.

In conclusion, we demonstrated that parasitism by gregarious koinobiont parasitoids, such as *C. glomerata*, can benefit plant fitness depending on parasitoid load and a number of interacting temporal, nutritional and ecological factors that can only be disentangled in an ecologically relevant manner through field studies. In our view, this largely explains existing controversies about the putative benefit of recruiting gregarious koinobiont parasitoids for plant reproduction. For parasitoid loads that occur as a result of superparasitism, the benefit of indirect plant defence through recruitment of gregarious parasitoids may be negated. In this context, it is particularly interesting to refer to a recent study performed in our laboratory in which it was shown that *C. glomerata* is capable of sensing the presence of parasitized *P. brassicae* caterpillars on a *B. oleracea* plant in flight and displayed a plant synomone-mediated preference for plants under attack by healthy caterpillars. This discrimination was ascribed to a significantly reduced emission of three terpenoid volatiles by plants damaged by parasitized caterpillars. These findings suggest that *Brassica* plants have evolved a mechanism to down-regulate synomone production that reduces the likelihood of superparasitization to occur (Fatouros et al., 2005). This trait could have evolved to preserve the fitness benefit of the plant resulting from recruiting parasitoids that attack herbivorous insects.

Acknowledgements

We thank André Gidding, Frans van Aggelen, and Leo Koopman for rearing *Pieris brassicae* and *Cotesia glomerata* and Plantkundig Proefcentrum Wageningen personnel for sowing and planting *Brassica nigra*. Johan Calis and Willem-Jan Boot were so kind as to lend us a beehive. We are grateful to John van der Lippe and Henk Vleeming for drying and cleaning the mustard seeds and Wim van der Slikke for allowing us to use the seed counter.

This research was financially supported by Earth and Life Sciences (project number 811.34.006) of The Netherlands Organisation of Scientific Research and by a fellowship of the Graduate School Experimental Plant Sciences to SEB.

References

Anonymous (2005) The Guide to GenStat® Release 8.11. VSN International, Oxford, UK.

Beckage NE & Riddiford LM (1983) Growth and development of the endo-parasitic wasp *Apanteles congregatus* – dependence on host nutritional status and parasite load. Physiological Entomology 8: 231–241.

Blatt SE, Smallegange RC, Hess L, Harvey JA, Dicke M & van Loon JJA (2008) Tolerance of Brassica nigra to Pieris brassicae herbivory. Botany (in press).

Coleman RA, Barker AM & Fenner M (1999) Parasitism of the herbivore *Pieris brassicae* L. (Lep., Pieridae) by *Cotesia glomerata* L. (Hym., Braconidae) does not benefit the host plant by reduction of herbivory. Journal of Applied Entomology 123: 171–177.

D'Alessandro M & Turlings TCJ (2006) Advances and challenges in the identification of volatiles that mediate interactions among plants and arthropods. Analyst 131: 24–32.

Dicke M (1999) Are herbivore-induced plant volatiles reliable indicators of herbivore identity to foraging carnivorous arthropods? Entomologia Experimentalis et Applicata 91: 131–142.

Dicke M & van Loon JJA (2000) Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. Entomologia Experimentalis et Applicata 97: 237–249.

Elzinga JA, Harvey JA & Biere A (2003) The effects of host weight at parasitism on fitness correlates of the gregarious koinobiont parasitoid *Microplitis tristis* and consequences for food consumption by its host, *Hadena bicruris*. Entomologia Experimentalis et Applicata 108: 95–106.

Elzinga JA, Harvey JA & Biere A (2005) Age-dependent clutch size in a koinobiont parasitoid. Ecological Entomology 30: 17–27.

Fatouros NE, van Loon JJA, Hordijk KA, Smid HM & Dicke M (2005) Herbivore-induced plant volatiles mediate in-flight host discrimination by parasitoids. Journal of Chemical Ecology 31: 2033–2047.

Fritzsche-Hoballah ME & Turlings TCJ (2001) Experimental evidence that plants under caterpillar attack may benefit from attracting parasitoids. Evolutionary Ecology Research 3: 553–565.

Führer E & Keja TD (1976) Physiologische Wechselbeziehungen zwischen *Pieris brassicae* und dem Endoparasiten *Apantales glomeratus*. Der Einfluss der Parasitierung auf Wachstum und Körpergewicht des Wirtes. Entomologia Experimentalis et Applicata 19: 287–300.

Geervliet JBF, Verdel MSW, Snellen H, Schaub J, Dicke M & Vet LEM (2000) Coexistence and niche segregation by field populations of the parasitoids *Cotesia glomerata* and *C. rubecula* in the Netherlands: predicting field performance from laboratory data. Oecologia 124: 55–63.

Gomez JM & Zamora R (1994) Top-down effects in a tritrophic system: parasitoids enhance plant fitness. Ecology 75: 1023–1030.

Gu H, Wang Q & Dorn S (2003) Superparasitism in *Cotesia glomerata*: response of hosts and consequences for parasitoids. Ecological Entomology 28: 422–431.

Harper FR & Berkenkamp B (1975) Revised growth-stage key for *Brassica campestris* and *B. napus*. Canadian Journal of Plant Science 55: 657–658.

Harvey JA (2000) Dynamic effects of parasitism by an endoparasitoid wasp on the development of two host species: implications for host quality and parasitoid fitness. Ecological Entomology 25: 267–278.

Harvey JA (2005) Factors affecting the evolution of development strategies in parasitoid wasps: the importance of functional constraints and incorporating complexity. Entomologia Experimentalis et Applicata 117: 1–13.

Harvey JA, Jervis MA, Gols R, Jiang N & Vet LEM (1999) Development of the parasitoid, *Cotesia rubecula* (Hymenoptera: Braconidae) in *Pieris rapae* and *Pieris brassicae* (Lepidoptera: Pieridae): evidence for host regulation. Journal of Insect Physiology 45: 173–182.

Harvey JA & Strand MR (2002) The developmental strategies of endoparasitoid wasps vary with host feeding ecology. Ecology 83: 2439–2451.

Harvey JA, Witjes LMA & Wagenaar R (2004) Development of hyperparasitoid wasp *Lysibia nana* (Hymenoptera: Ichneumonidae) in a multitrophic framework. Environmental Entomology 33: 1488–1496.

Hendrix SD (1984) Reactions of *Heracleum lanatum* to floral herbivory by *Depressaria pastinacella*. Ecology 65: 191–197.

Hendrix SD, Nielsen E, Nielsen T & Schutt M (1996) Are seedlings from small seeds always inferior to seedlings from large seeds? Effects of seed biomass on seedling growth in *Pastinaca sativa* L. New Phytologist 119: 299–305.

Ikawa T & Okabe H (1984) Two methods for estimating the number of eggs laid in a host by the gregarious endoparasitoid, *Apanteles glomeratus* (Hymenoptera: Braconidae). Applied Entomology and Zoology 19: 389–390.

Ikawa T & Okabe H (1985) Regulation of egg number per host to maximize the reproductive success in the gregarious parasitoid, *Apanteles glomeratus* L. (Hymenoptera: Braconidae). Applied Entomology and Zoology 20: 331–339.

Ikawa T & Suzuki Y (1982) Ovipositional experience of the gregarious parasitoid, *Apanteles glomeratus* (Hymenoptera, Braconidae), influencing her discrimination of the host larvae, *Pieris rapae crucivora*. Applied Entomology and Zoology 17: 119–126.

Jones RL & Lewis WJ (1971) Physiology of the host-parasite relationship between *Heliothis zea* and *Microplitis croceipes*. Journal of Insect Physiology 17: 921–927.

Karowe DN & Schoonhoven LM (1992) Interactions among three trophic levels: the influence of host plant on performance of *Pieris brassicae* and its parasitoid *Cotesia glomerata*. Entomologia Experimentalis et Applicata 62: 241–251.

Kristensen CO (1994) Investigations on the natural mortality of eggs and larvae of the large white *Pieris brassicae* (L.) (Lep., Pieridae). Journal of Applied Entomology 117: 92–98.

Le Masurier AD (1991) Effect of host size on clutch size in *Cotesia glomerata*. Journal of Animal Ecology 60: 107–118.

van Loon JJA, de Boer JG & Dicke M (2000) Parasitoid-plant mutualism: parasitoid attack of herbivore increases plant reproduction. Entomologia Experimentalis et Applicata 97: 219–227.

Mackauer M & Sequeira R (1993) Patterns of development in insect parasites. Parasites and Pathogens of Insects (ed. by NE Beckage, SN Thompson & BA Federici), pp. 1–20. Academic Press, New York, NY, USA.

Mattiacci L & Dicke M (1995) The parasitoid *Cotesia glomerata* (Hymenoptera: Braconidae) discriminates between first and fifth larval instars of its host *Pieris brassicae*, on the basis of contact cues from frass, silk, and herbivore-damaged leaf tissue. Journal of Insect Behavior 8: 485–498.

van der Meijden E & Klinkhamer PG (2000) Conflicting interests of plants and the natural enemies of herbivores. Oikos 89: 202–208.

Meyer GA (2000) Interactive effects of soil fertility and herbivory on *Brassica nigra*. Oikos 88: 433–441.

Paré PW & Tumlinson JH (1999) Plant volatiles as a defense against insect herbivores. Plant Physiology 121: 325–331.

Parker FD & Pinnell RE (1973) Effect on food consumption of the imported cabbageworm when parasitised by two species of *Apanteles*. Environmental Entomology 2: 216–219.

Pennacchio F & Strand MR (2006) Evolution of developmental strategies in Hymenoptera. Annual Review of Entomology 51: 233–258.

Rahman M (1970) Effect of parasitism on food consumption of *Pieris rapae* larvae. Journal of Economic Entomology 63: 820–821.

Ramana S & Ghildiyal MC (1997) Contribution of leaf photosynthesis towards seed yield in *Brassica* species. Journal of Agronomy and Crop Science 178: 185–187.

Slansky F Jr (1978) Utilization of energy and nitrogen by larvae of the imported cabbage worm, *Pieris rapae*, as affected by parasitism by *Apanteles glomeratus*. Environmental Entomology 7: 179–185.

Slansky F Jr (1986) Nutritional ecology of endoparasitic insects and their hosts: an overview. Journal of Insect Physiology 32: 255–261.

Smallegange RC, van Loon JJA, Blatt SE, Harvey JA, Agerbirk N & Dicke M (2007) Flower vs. leaf feeding by *Pieris brassicae*: glucosinolate-rich flower tissues are preferred and sustain higher growth rate. Journal of Chemical Ecology 33: 1831–1844.

Smart AJ & Moser LE (1999) Switchgrass seedling development as affected by seed size. Agronomy Journal 91: 335–338.

Smith CL & Smilowitz Z (1976) Growth and development of *Pieris rapae* larvae parasitised by *Apanteles glomeratus*. Entomologia Experimentalis et Applicata 19: 189–195.

Tagawa J, Sato Y & Tanaka T (1982) Developmental interactions between the army worm *Leucania separata* (Lep., Noctuidae) and its parasite *Apanteles ruficrus* (Hym., Braconidae). Entomophaga 27: 447–454.

Trumble JT, Kolodny-Hirsch DM & Ting IP (1993) Plant compensation for arthropod herbivory. Annual Review of Entomology 39: 93–119.

Turlings TCJ & Tumlinson JH (1992) Systemic release of chemical signals by herbivore-injured corn. Proceedings of the National Academy of Sciences of the United States of America 89: 8399–8402.

Vet LEM & Dicke M (1992) Ecology of infochemical use by natural enemies in a tritrophic context. Annual Review of Entomology 37: 141–172.

Vinson SB (1972) Effect of the parasitoid *Campoletis sonorensis* on the growth of its host, *Heliothis virescens*. Journal of Insect Physiology 18: 1509–1514.