Plant Resistance Affects the Olfactory Response and Parasitism Success of *Cotesia vestalis*

Javad Karimzadeh · Jim Hardie · Denis J. Wright

Abstract Understanding the factors influencing host-selection behavior of parasitoids is essential in studies on host-parasitoid ecology and evolution, and in combining sustainable strategies of pest management, such as host-plant resistance and biological control. The effects of host-plant resistance on the olfactory response and parasitism success by *Cotesia vestalis*, a parasitoid of diamondback moth (*Plutella xylostella*) larvae were examined. Here, it was demonstrated that host-plant resistance can strongly influence foraging behavior and parasitism success of the parasitoid. In olfactometer experiments, *C. vestalis* did not differentiate between crucifer plant types with similar levels of susceptibility or resistance to *P. xylostella* but showed a strong preference for susceptible compared with partially-resistant host plants. The influence of previous oviposition activity varied with the host-plant type experienced by the parasitoid. In cage experiments, *C. vestalis* preferred to parasitize *P. xylostella* larvae on a susceptible plant compared with larvae on a partially resistant host plant when exposed to hosts for 24 h. However, this preference appeared to be transitory, and was not found after 96 h exposure. The present study suggests that combining partial host-plant resistance with biological control by *C. vestalis* for the control of *P. xylostella* may in some circumstances be antagonistic and negatively affect parasitism success.

Keywords *Plutella xylostella* · *Brassica* · *Cotesis vestalis* · olfactory behaviour · tritrophic interactions · biocontrol

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Introduction

Plants provide the primary interface between insect herbivores and their natural enemies (Howe and Schaller 2008; Dicke et al. 2009). In addition to the direct effects of plant characteristics such as plant morphology, semiochemicals and vegetation texture on natural enemies, plants can influence natural enemies indirectly through multitrophic interactions, including semiochemically-mediated processes, herbivore sequestration of plant allelochemicals, plant nutrition and resistance effects on herbivore quality, and microbial symbionts (Bottrell et al. 1998; Stiling and Moon 2005).

Host selection by parasitoids (Vinson 1984) consists of a series of behaviors, being influenced by information from the surroundings. Although physical factors such as shape, texture, size, age, movement, sound and color are involved, parasitoid host selection depends mainly on semiochemicals, which influence the searching efficiency of foraging parasitoids (Lewis and Martin 1990; Vet and Dicke 1992; Vet 1999), with plant volatiles playing a major role (Chadwick and Goode 1999; van Tol et al. 2001; Hilker and Meiners 2002; Hunter 2002; Ahuja et al. 2010). Semiochemical-mediated interactions can thus provide an extrinsic defence for plants by enhancing the effectiveness of herbivore natural enemies (Paré and Tumlinson 1999; Dicke et al. 2003a,b; Turlings and Wäckers 2004). Studies on tritrophic interactions have shown that the quality and quantity of plant secondary compounds may affect the success of natural enemies by influencing the nutritional and chemical suitability of their host (Price 1997; Raymond et al. 2002; Hunter 2003; Kopelke 2003; Campan and Benrey 2004; Helms et al. 2004).

The compatibility of host-plant resistance and biological control is of prime importance in pest management programs but this can vary between different agro-ecosystems (Price 1986; Thomas and Waage 1996; Bottrell et al. 1998; Verkerk et al. 1998; van Emden 1999; Tscharntke and Hawkins 2002). In many instances, the evidence suggests that insect-resistant plants and natural enemies are compatible, and can provide greater pest suppression when combined (e.g. van Emden 1986; McAuslane et al. 2000; Kalule and Wright 2002; Schmale et al. 2003; Velten et al. 2007; Cai et al. 2009). Other studies have shown antagonistic relationships between plant resistance and natural enemies, with plant resistance negatively affecting development and success of parasitoids (e.g. Campbell and Duffey 1979; Orr and Boethel 1986; Schuler et al. 1999; Gassmann and Hare 2005; Simmons and Gurr 2005).

The parasitoid *Cotesia vestalis* (Haliday) (*C. plutellae*) (Hymenoptera: Braconidae) is a key larval endoparasitoid of the cosmopolitan crucifer pest, the diamondback moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (Verkerk and Wright 1997; Verkerk et al. 1998). Previous studies have shown that *C. vestalis* responds to volatiles derived from the host, the host plant and from plant-host complexes (Bogahawatte and van Emden 1996; Potting et al. 1999; Shiojiri et al. 2000a,b; Reddy et al. 2002; Wang and Keller 2002; Liu and Jiang 2003; Schuler et al. 2003). The present study examined the effect of partial host-plant resistance and prior oviposition experience on the olfactory response and parasitism by *C. vestalis*. Partial host-plant resistance was shown to strongly influence the olfactory response and parasitism success of *C. vestalis*.

Materials and Methods

Plants and Insects

Chinese cabbage (*Brassica pekinensis*) cvs Tip Top (Chiltern Seeds, Ulverston, UK) and F_1 One Kilo SB, common cabbage (*B. oleracea* var. *capitata*) cvs Wheelers Imperial and Red Drumhead, turnip (*B. rapa*) cv Snowball (all Suttons Seeds, Devon, UK), and rapeseed (*B. rapa* Plant Introduction 469895 and *B. napus* Plant Introduction 470055; North Central Regional Plant Introduction Station, Ames, Iowa, USA) were grown organically under glass (25±5°C; L:D 16:8 h; Verkerk and Wright 1994). These host plants range from susceptible to partially resistant to attack by *P. xylostella* (Verkerk and Wright 1994; Ramachandran et al. 1998; Table 1). An insecticide-susceptible strain of *P. xylostella* (Roth population) and a population of *C. vestalis* were both obtained from Rothamsted Research (Harpenden, UK). Roth population had been collected originally from the Philippines, and cultured in laboratory for over 200 generations. Insects were cultured on susceptible plants (S_1, S_2, S_3 and S_6; Table 1) as described previously by Karimzadeh et al. (2004); the cultures of *P. xylostella* were maintained on the mentioned plants in ventilated Perspex oviposition cages ($35\times35\times35$ cm). Similarly, the cultures of *C. vestalis*, in turn, were maintained on *P. xylostella* larvae in ventilated Perspex oviposition cages ($45\times45\times45$ cm). Five mated female wasps, fed on honey solution (50 %), were offered individual plants infested with about 200 second instar *P. xylostella* larvae for 24 h. Plants with the parasitized larvae were then transferred to the similar cages and fed with fresh plants (from the same type and age) until cocoon formation. In order to prevent any oviposition before performing experiments, the harvested parasitoid

Table 1 Host-plant types used in experiments

Plant type	Age (weeks)	Susceptibility to attack by *P. xylostella*	Abbreviation
Chinese cabbage, *B. pekinensis* cv. F_1 One Kilo SB	4	susceptible	S_1
Chinese cabbage, *B. pekinensis* cv. Tip Top	4-5	susceptible	S_2
Common cabbage, *B. oleracea* var. *capitata* cv. Wheelers Imperial	4	susceptible	S_3
Common cabbage, *B. oleracea* var. *capitata* cv. Red Drumhead	4	susceptible	S_4
Rapeseed, *B. rapa* Plant Introduction 469895	6	susceptible	S_5
Turnip, *B. rapa* cv. Snowball	4	susceptible	S_6
Common cabbage, *B. oleracea* var. *capitata* cv. Wheelers Imperial	15	partially resistant	R_1
Common cabbage, *B. oleracea* var. *capitata* cv. Red Drumhead	15	partially resistant	R_2
Common cabbage, *B. oleracea* var. *capitata* cv. Wheelers Imperial	8	partially resistant	R_3
Rapeseed, *B. napus* Plant Introduction 470055	8	partially resistant	R_4

cocoons were allowed to emerge individually in microtubes. Both cultures were reared in a standard constant environment ($25\pm2°C$; 70 ± 5 % RH: L:D 16:8 h).

Olfactometer Experiments

A 4-way Perspex olfactometer, similar to one described by Douloumpaka and van Emden (2003), was used (Fig. 1). A central arena (45 mm dia. × 30 mm), into which parasitoids were released, was linked to four odor-source chambers (25 mm i.d. × 95 mm) by Perspex tubing (5 mm i.d. × 40 mm). The odor source chambers were loosely sealed at each end with cotton wool. The far end of each source chamber was connected to a pot (25 mm dia. × 30 mm) filled with activated charcoal and covered with a perforated lid to enable air to pass through the odor source chamber. The central chamber was linked to a pump (CAPEX 8 C; Charles Austen Pumps Ltd., Surrey, UK) set to suck air through the apparatus at 1700 ml min^{-1}. Smoke tests showed that this rate is optimum to produce four even streams from the source chambers into the central arena. The smoke was created from the mixture of diethylamine [$(C_2H_5)_2NH$] and acetic acid [CH_3COOH] in a glass vial (Pham-Delegue et al. 1991). The olfactometer was placed inside a $60\times60\times60$ cm wooden frame whose inner sides were covered with white paint to reflect illumination from a top fluorescent light. No directional bias was observed in control tests with parasitoids using empty odor-source chambers ($\chi^2=1.25$, $P=0.870$; Fig. 2).

Ten, mated 3-day-old female *C. vestalis* were released together into the central arena of the olfactometer and were given a choice between two different plants or plant-host complexes. The choice after 30 min (the number of the wasps in each arm) was used as the preference index. The number of the wasps remaining in the central arena was recorded as being non-responsive. Each treatment was replicated eight times. One half of the replications were run with opposite arms containing the same treatment, and other half of the replications were run with adjacent arms containing the same treatment. The olfactometer was then rotated by 90° compared with its previous position before the next replication test was conducted. All tests were undertaken between 1000 h and 1600 h, the period of day in which wasps showed
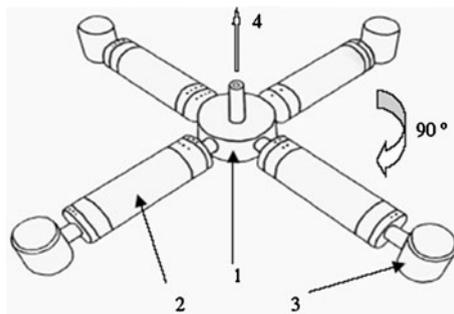
Fig. 1 Schematic picture of the 4-way olfactometer used in experiments. The parts 1, 2 and 3 are the central arena, odour source chamber and plastic pot, respectively. The number 4 shows the direction of flow suction. The curved arrow shows 90° rotation after each replication

Fig. 2 Olfactometer control test with *Cotesia vestalis* females using empty, odour-free arms
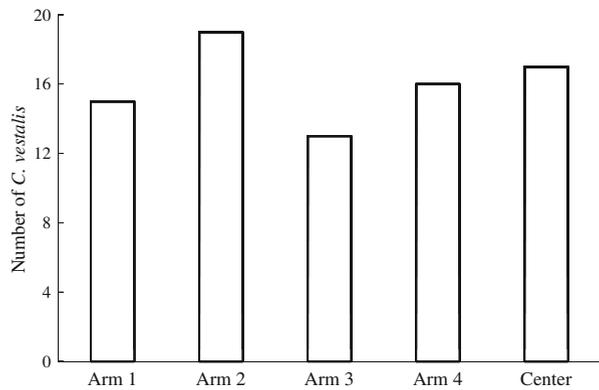
maximum activity (preliminary observations of searching behavior). After each set of replicates, the central chamber, tubing and odor-source chambers were washed with aqueous ethyl alcohol (70 %v/v), soaked in an odorless detergent (Lipsol 5 %v/v; Bibby Sterilin Ltd., Staffordshire, UK) overnight, and rinsed in distilled water before drying and reuse.

For the experiments with uninfested plants, a rolled leaf from each host plant (S_1, S_3, S_4, R_1 and R_2; Table 1) was placed in an odor-source chamber immediately before performing the test. For experiments with infested plants, 20 early-third-instar larvae of *P. xylostella* (cultured on the same plant type; S_1, R_1 and R_2; Table 1) were added to the leaf in an odor-source chamber 2 h before the parasitoids were introduced into the olfactometer. The following factors were examined for uninfested and infested plants: (1) source of the parasitoid: the host plant on which the parasitoid was reared from the host (S_1, S_3 and S_6; Table 1); (2) oviposition experience by the parasitoid: for oviposition experience, ten, mated 2-day-old female *C. vestalis* (from the same plant type used as source of the parasitoid) were released into a Perspex ventilated cage (30×30×30 cm) containing a host plant with 200 early-third-instar *P. xylostella* larvae for 24 h; these experienced parasitoids were then used immediately for olfactometer experiments.

Parasitism by *Cotesia vestalis* on Different Host Plants: Choice Experiments

To explore the role of host-plant resistance on overall parasitism success by *C. vestalis* a series of choice experiments using caged plants were conducted, where parasitoids were exposed to two types of host-infested plants for either 24 or 96 h. For each experiment, two susceptible (S_2 or S_5) and two partially resistant (R_1, R_3 or R_4) host plants were placed together in a ventilated cage (90×75×60 cm); plants from the same type were placed in opposite corners of the cage. Each plant was infested with 50 late-second-instar larvae of *P. xylostella*, which were from the same host-plant type used in the experiments. Plants were placed in trays filled with water and were prevented from touching the sides of the cage to prevent movement of *P. xylostella* larvae between plants. Aluminum foil was used as a horizontal barrier around each pot to prevent larvae from falling into the water. After 24 h, which was sufficient time

for larvae to establish on the plants, two or four, mated 2-day-old female *C. vestalis* (reared from S_2, S_3 or S_6) were released into each cage. Depending on the experiment, parasitoids were removed after 24 h or 96 h (Table 2). For each cage, replacement plants were provided ad lib from the same type as the original plants, until *P. xylostella* pupae or *C. vestalis* cocoons formed. Each treatment was replicated four times in a randomised block design and maintained under constant environmental conditions ($25\pm2°C$, 70 ± 10 % RH and L:D 16:8 h photoperiod).

Data Analysis

Data from the olfactometer experiments (the number of parasitoids responding to different plants or plant-host complexes) were pooled between replications and compared against a null hypothesis of random choice using a Chi-square test (Crawley 2002). The Chi-square values were also calculated for heterogeneity between replications, and a statistically signifcant comparison only accepted if accompanied by non-significant heterogeneity (Gomez and Gomez 1984). Non-responsive wasps were not included in the analyses. Data from cage experiments were analyzed for percentage parasitism (with exclusion of host mortality from unknown factors) and wasp cocoon numbers. Differences in the levels of percentage parasitism between host-plant types were analyzed using logistic analysis of deviance (binomial error; Crawley 2002). In case of overdispersion, the model was refitted using quasibinomial rather than binomial errors. To achieve the minimal adequate model, non-significant terms were removed through model simplification; models were compared by *F*-test (Crawley 2005). The differences in the number of parasitoid cocoons between the host-plant types were analyzed using Student's *t*-test (Crawley 2002). All statistical analyses were completed in S-Plus 6.1 (Insightful Corp., Seattle).

Table 2 Parasitism success of *Cotesia vestalis* on different host plants

Experiment	Parasitoid			Host plant choice [b]	Cocoons per plant (mean±s.e.)	*P*	Proportion parasitism	*P*
	Source [a]	Number	Exposure (h)					
1	S_2	4	24	S_2	38.4±1.6	<0.005	0.84	<0.001
				R_3	19.0±2.5		0.46	
2	S_2	4	24	S_2	35.4±1.8	<0.001	0.82	<0.001
				R_1	9.6±1.3		0.31	
3	S_3	4	24	S_2	29.3±1.9	<0.001	0.69	<0.001
				R_1	6.5±0.7		0.19	
4	S_2	2	96	S_5	34.9±2.0	< 0.05	0.97	0.748
				R_4	25.6±2.1		0.95	
5	S_6	2	96	S_2	31.8±1.8	< 0.05	0.72	0.385
				R_1	25.3±1.8		0.76	

[a] The host plant on which parasitoids were reared from *P. xylostella* larvae

[b] Host-plant type: S = susceptible, R = partially resistant (as are shown in Table 1)

Results

Preference of Naive *C. vestalis* Reared on Hosts from Chinese Cabbage (S$_1$)

Cotesia vestalis reared from hosts on Chinese cabbage (S$_1$) preferred the odor from uninfested young Chinese cabbage (S$_1$) compared with uninfested old common cabbage (R$_1$=Wheelers Imperial or R$_2$=Red Drumhead) (Fig. 3; S$_1$ vs R$_1$: χ^2= 18.5, *P*<0.001; S$_1$ vs R$_2$: χ^2=17.8, *P*<0.001). The odor from infested young Chinese cabbage was also preferred compared with infested old common cabbage (Fig. 3; S$_1$-host vs R$_1$-host: χ^2=20.0, *P*<0.001; S$_1$-host vs R$_2$-host: χ^2=10.6, *P*< 0.005). *Cotesia vestalis* was more responsive to infested old common cabbage (Wheelers Imperial) compared with uninfested young Chinese cabbage (Fig. 3; R$_1$-host vs. S$_1$: χ^2=21.8, *P*<0.001).

    Cotesia vestalis did not show a significant preference between the odors from uninfested young Chinese cabbage (S$_1$=F$_1$ One Kilo SB) and uninfested young common cabbage (S$_3$=Wheelers Imperial or S$_4$=Red Drumhead) (Fig. 3; S$_1$ vs. S$_3$: χ^2=0.46, *P*=0.497; S$_1$ vs S$_4$: χ^2=1.61, *P*=0.204) or between uninfested old Wheelers Imperial and uninfested old Red Drumhead (R$_1$ vs. R$_2$: χ^2=0.33, *P*= 0.564).

Preference of Experienced *C. vestalis* Reared on Hosts from Chinese Cabbage (S$_1$)

Irrespective of the host plant (S$_1$ or R$_1$) on which the parasitoids had previously experienced oviposition in host larvae, parasitoids preferred uninfested young Chinese cabbage compared with uninfested old common cabbage (Wheelers Imperial) (Fig. 4; S$_1$ vs R$_1$, experienced on S$_1$: χ^2=37.6, *P*<0.001; S$_1$ vs R$_1$, experienced on R$_1$: χ^2=12.8, *P*<0.001). The same result was found when infested host plants were compared (Fig. 4; S$_1$-host vs R$_1$-host, experienced on S$_1$: χ^2=50.2, *P*<0.001; S$_1$-host
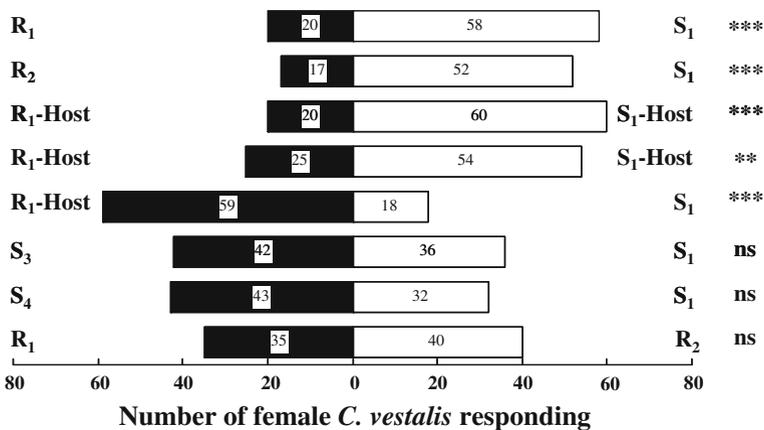
Fig. 3 Olfactory response of naive *Cotesia vestalis* females (reared from hosts feeding on young Chinese cabbage, S$_1$) towards uninfested and infested plants. The numbers within bars indicate the number of wasps that made a choice for each of the two odor sources offered; R and S refer to partially-resistant and susceptible host plant, respectively (Table 1); asterisks indicate significant differences between choice tests: ***, ** and ns, no significant difference (*P*<0.001, < 0.005 and >0.05, respectively)
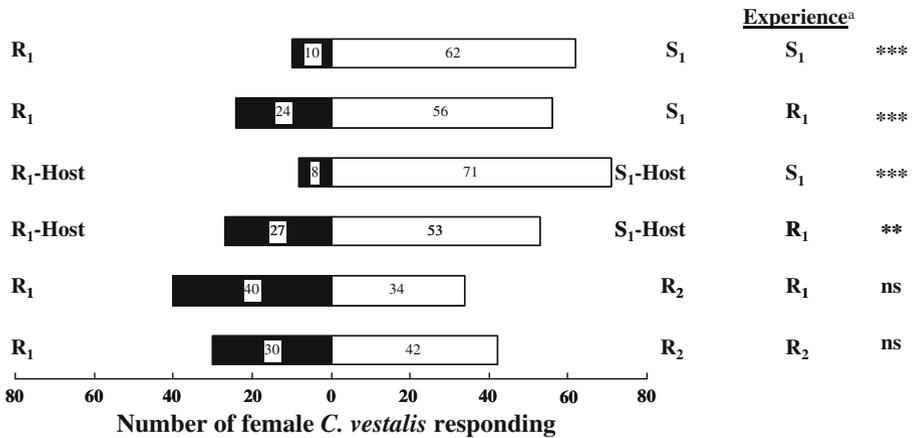
Fig. 4 Olfactory response of experienced *Cotesia vestalis* females (reared from hosts feeding on young Chinese cabbage, S_1) towards uninfested and infested plants. The numbers within bars indicate the number of wasps that made a choice for each of the two odor sources offered; R and S refer to partially-resistant and susceptible host plant, respectively (Table 1); [a] the host plant on which the parasitoids experienced multiple ovipositions on the host larvae before performing the test; asterisks indicate significant differences between choice tests: ***, ** and ns, no significant difference ($P<0.001$, < 0.005 and >0.05, respectively)

vs R_1-host, experienced on R_1: $\chi^2=8.45$, $P<0.005$). When old uninfested common cabbage types (Wheelers Imperial and Red Drumhead) were compared, *C. vestalis* did not show a significant preference (Fig. 4; R_1 vs R_2, experienced on R_1: $\chi^2=0.49$, $P=0.486$; R_1 vs R_2, experienced on R_2: $\chi^2=2.0$, $P=0.157$).

Preference of *C. vestalis* Reared on Hosts from Common Cabbage (S_3)

When uninfested plants were compared, both naive parasitoids and ones that had experienced parasitization of hosts feeding on old common cabbage showed no preference between young Chinese cabbage and old common cabbage (Fig. 5; S_1 vs R_1: $\chi^2=0.24$, $P=0.628$ and S_1 vs R_1, experienced on R_1: $\chi^2=0.89$, $P=0.346$, respectively). Parasitoids that had experienced parasitization of hosts feeding on young Chinese cabbage preferred uninfested young Chinese cabbage compared with uninfested old common cabbage (Fig. 5; S_1 vs R_1, experienced on S_1: $\chi^2=5.41$, $P<0.05$).

When infested plants were compared, both naive parasitoids and ones that had experienced parasitization of hosts feeding on young Chinese cabbage preferred young Chinese cabbage compared with old common cabbage (Fig. 5; S_1-host vs R_1-host: $\chi^2=5.88$, $P<0.05$ and S_1-host vs R_1-host, experienced on S_1: $\chi^2=22.2$, $P<0.001$, respectively). Parasitoids that had experienced parasitization of hosts feeding on old common cabbage showed no preference between young Chinese cabbage and old common cabbage (S_1-host vs R_1-host, experienced on R_1: $\chi^2=3.20$, $P=0.074$).

Preference of *C. vestalis* Reared on Hosts from Turnip (S_6)

When uninfested plants were compared, both naive parasitoids and ones that had experienced parasitization of hosts feeding on old common cabbage showed no
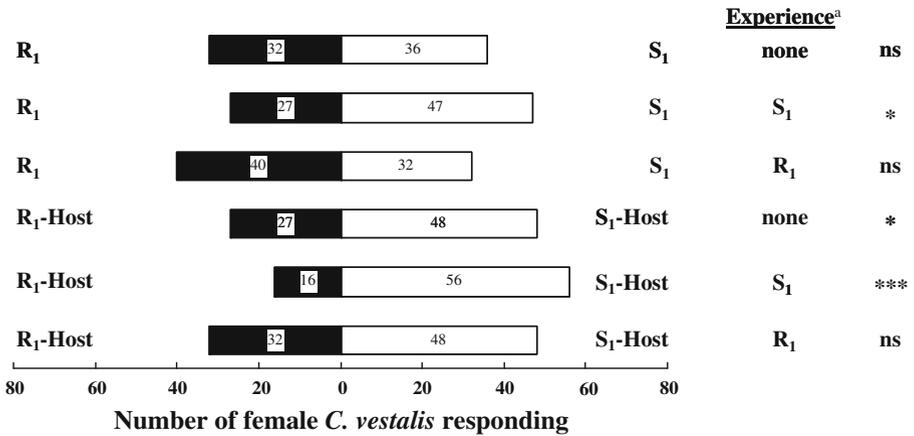
Fig. 5 Olfactory response of naive and experienced *Cotesia vestalis* females (reared from hosts feeding on young common cabbage cv Wheelers Imperial, S_3) towards uninfested and infested plants. The numbers within bars indicate the number of wasps that made a choice for each of the two odor sources offered; R and S refer to partially-resistant and susceptible host plant, respectively (Table 1); [a] the host plant on which the parasitoids experienced multiple ovipositions on the host larvae before performing the test; asterisks indicate significant differences between choice tests: ***, * and ns, no significant difference ($P<0.001$, < 0.05 and >0.05, respectively)

preference for young Chinese cabbage or old common cabbage (Fig. 6; S_1 vs R_1: $\chi^2 = 0.64$, $P=0.425$ and S_1 vs R_1, experienced on R_1: $\chi^2=2.25$, $P=0.133$, respectively). Parasitoids that had experienced parasitization of hosts feeding on young Chinese cabbage preferred young Chinese cabbage compared with old common cabbage (S_1 vs R_1, experienced on S_1: $\chi^2=43.1$, $P<0.001$).

When infested plants were compared, naive *C. vestalis* preferred young Chinese cabbage compared with old common cabbage (S_1-host vs R_1-host.: $\chi^2=8.67$, $P<$
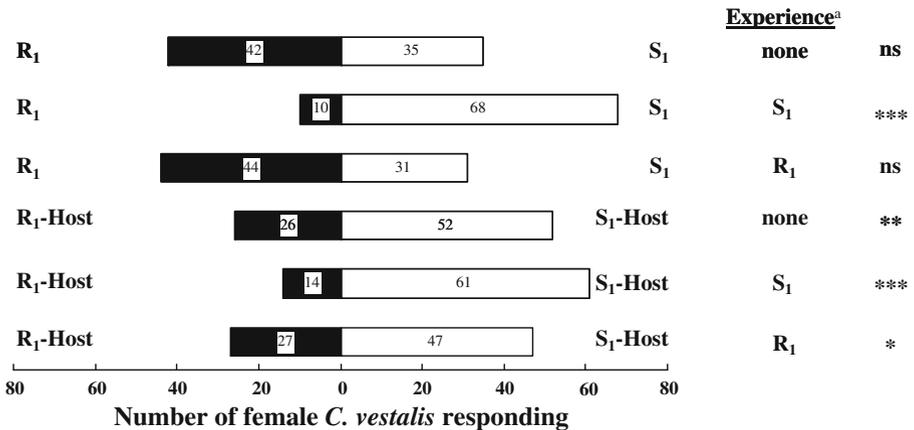
Fig. 6 Olfactory response of naive and experienced *Cotesia vestalis* females (reared from hosts feeding on turnip, S_6) towards uninfested and infested plants. The numbers within bars indicate the number of wasps that made a choice for each of the two odor sources offered; R and S refer to partially-resistant and susceptible host plant, respectively (Table 1); [a] the host plant on which the parasitoids experienced multiple ovipositions on the host larvae before performing the test; asterisks indicate significant differences between choice tests: ***, **,* and ns, no significant difference ($P<0.001$, < 0.005, < 0.05 and >0.05, respectively)

0.005). Parasitoids that had experienced parasitization of hosts feeding on young Chinese cabbage (S_1) or old common cabbage (R1) both preferred young Chinese cabbage (S_1-host vs R_1-host, experienced on S_1: $\chi^2 = 29.5$, $P < 0.001$; and S_1-host vs R_1-host, experienced on R_1: $\chi^2 = 5.41$, $P < 0.05$).

Parasitism by *Cotesia vestalis* on Different Host Plants: Choice Experiments

Parasitoids Exposed to Infested Plants for 24 h

Cotesia vestalis reared on hosts on young Chinese cabbage (S_2, Tip Top) parasitized a significantly greater proportion of hosts on Chinese cabbage (S_2) compared with hosts on an intermediate aged common cabbage (R_3, Wheelers Imperial) (Table 2; $F_{1,6} = 44.26$, $P < 0.001$), and the mean number of parasitoid cocoons per plant was significantly greater on Chinese cabbage ($t_6 = 6.4828$, $P < 0.005$).

The proportion of hosts parasitized on Chinese cabbage (S_2) was also significantly greater compared with old common cabbage (R_1, Wheelers Imperial) (Table 2; $F_{1,6} = 141.78$, $P < 0.001$), and the mean number of parasitoid cocoons per plant was significantly greater on Chinese cabbage ($t_6 = 11.4799$, $P < 0.001$).

Cotesia vestalis reared on hosts from young common cabbage (S_3, Wheelers Imperial) parasitized a significantly greater proportion of hosts on Chinese cabbage (S_2) compared with old common cabbage (R_1) (Table 2; $F_{1,6} = 75.30$, $P < 0.001$), and the mean number of parasitoid cocoons per plant was significantly greater on Chinese cabbage ($t_6 = 10.9551$, $P < 0.001$).

Parasitoids Exposed to Infested Plants for 96 h

The proportion of hosts parasitized by *C. vestalis* reared on hosts from young Chinese cabbage (S_2, Tip Top) was not significantly different for hosts on a susceptible rapeseed (S_5, *B. rapa* Plant Introduction 469895) compared with hosts on a partially-resistant rapeseed (R_4, *B. napus* Plant Introduction 470055) (Table 2: $F_{1,6} = 0.11$, $P = 0.748$) but the mean number of parasitoid cocoons per plant was significantly greater on the susceptible rapeseed ($t_6 = 3.1516$, $P < 0.05$).

The proportion of hosts parasitized by *C. vestalis* reared on hosts from turnip (S_6, *B. rapa*) was not significantly different for hosts on Chinese cabbage (S_2) compared with hosts on old common cabbage (R_1) (Table 2; $F_{1,6} = 0.88$, $P = 0.385$) but the mean number of parasitoid cocoons per plant was significantly greater on Chinese cabbage ($t_6 = 2.5828$, $P < 0.05$).

Discussion

This work suggests that plant resistance may substantially influence searching efficiency and parasitism success of a major parasitoid of diamondback moth. From a pest management viewpoint, the movement of *C. vestalis* to different plant-host complexes is fundamental to the success of this parasitoid in combination with host-plant resistance (Cortesero et al. 2000). In an olfactometry study, Bogahawatte and van Emden (1996) found that *C. vestalis* preferred the odor of a partially-resistant

cabbage on which they had developed compared with the odor of a susceptible cauliflower; the parasitoid rearing history was so influential that the odor of intact partially-resistant plant was preferred to the odor of infested susceptible host plant.

The present study, using a wider range of host plants, gives somewhat different results. It was found that irrespective of the plant on which the parasitoid was reared, *C. vestalis* preferred a susceptible host plant to a partially-resistant plant, taking into consideration that both plants were in the same condition (either both were uninfested or both were infested with host larvae). Indeed, this preference was likely to be either due to parasitoid rearing history on Chinese cabbage but not on common cabbage, or due to oviposition experience on Chinese cabbage but not on common cabbage. The present study also showed that such a preference can change towards the partially-resistant plant under different conditions (when the partially-resistant plant is under attack by the host while the susceptible plant is intact). To clarify these dissimilarities, chemical studies (e.g., Vuorinen et al. 2004) on intact and *P. xylostella*-damaged crucifers are required to determine the quality and quantity of the semiochemicals that affect *C. vestalis* behavior.

Olfactometry experiments showed that *C. vestalis* did not differentiate between odors from the different plant types tested with similar levels of susceptibility or resistance to *P. xylostella* but responded preferentially to odors from susceptible compared with partially-resistant host plants. This suggests that host resistance of crucifers may significantly reduce host location by female *C. vestalis*. The observed plant preference was either because *C. vestalis* had developed on hosts feeding on Chinese cabbage, or because *C. vestalis* (which had developed on hosts feeding on common cabbage or turnip) had experienced multiple ovipositions of the hosts feeding on Chinese cabbage. The former may be due to experience by the parasitoid larvae of plant chemicals present in the *P. xylostella* larval body (Bogahawatte and van Emden 1996); whereas the latter is experience by the parasitoid adults of plant or plant-host allelochemicals (Potting et al. 1999; Liu and Jiang 2003).

Experience by the parasitoid larvae (i.e. parasitoid rearing history) and adults (i.e., multiple oviposition experience) on common cabbage did not affect *C. vestalis* choice towards old common cabbage. Secondary plant products, such as glucosinolates and their relatives, play an important role in producing allelochemicals that attract parasitoids (Bradburne and Mithen 2000; Hadacek 2002; Dicke and van Poecke 2002; Ahuja et al. 2010). It is known that the glucosinolate contents vary markedly among host plants of *P. xylostella* (van Etten et al. 1976; Hopkins et al. 1998; Moyes et al. 2000; Ulmer and Dosdall 2006), in particular by plant age (Karowe et al. 1997; Hopkins et al. 1998; Ciska et al. 2000). The lower responsiveness of *C. vestalis* to old common cabbage in present study might be due to reduced secondary plant products, such as glucosinolates, and reduced myrosinase activity in older crucifers (Porter et al. 1991; Wallace and Eigenbrode 2002; Lambdon et al. 2003). The fact that increased glucosinolate production in young crucifers favors both herbivores and parasitoids (Benrey et al. 1998; Harvey et al. 2003) may explain the preference of *C. vestalis* for a susceptible host plant, young Chinese cabbage.

Parasitoid rearing history was not influential when infested plants were compared; *C. vestalis* had a strong preference for an infested susceptible plant (young Chinese cabbage) compared with an infested partially-resistant plant (old common cabbage). It has been found that *C. vestalis* is most responsive to plant-host derived synomones

(Bogahawatte and Van Emden 1996; Potting et al. 1999; Shiojiri et al. 2000b). The distinct observed response of *C. vestalis* to the susceptible plant-host complex may be because of high release of herbivore-induced synomones (Mattiacci et al. 1994; Geervliet et al. 1997; Souissi et al. 1998) by infested young Chinese cabbage; more extensive feeding damage by *P. xylostella* resulted in stronger response of *C. vestalis* to this host plant (Schuler et al. 2003). However, the significant movement of *C. vestalis* towards infested old common cabbage compared with uninfested young Chinese cabbage observed in this study points to the importance of the host as a paramount factor in the host location behavior by this parasitoid (Bogahawatte and Van Emden 1996; Potting et al. 1999; Shiojiri et al. 2000a,b).

The cage experiments indicated that *C. vestalis* preferred initially (24 h exposure) to parasitize host larvae kept on a susceptible plant (young Chinese cabbage) compared with the host larvae on a partially resistant plant (intermediate or old common cabbage). Such a preference is likely to be linked to a greater 'attraction' of *C. vestalis* towards infested Chinese cabbage, as shown in the olfactometry experiments. *Cotesia vestalis* has also been reported to have a relatively narrow-area search around feeding damage sites (Wang and Keller 2002), which implies that the parasitoid would remain in a preferred microhabitat, such as infested Chinese cabbage, rather than searching other microhabitats. However, this preference disappeared when the exposure time to the parasitoid was increased to 96 h; *C. vestalis* did not differentiate between the host larvae kept on a susceptible plant (*B. rapa* or young Chinese cabbage) or the host larvae on a partially resistant plant (*B. napus* or old common cabbage). This has implications for patch use and redistribution of parasitoids based on the frequency of unparasitized hosts encountered by parasitoids (Godfray 1994). The greater number of *C. vestalis* cocoons (as a measure of successful parasitism) on a susceptible host plant (*B. rapa* or young Chinese cabbage) compared with a partially-resistant host plant (*B. napus*, intermediate or old common cabbage) may be either due to the preference of *C. vestalis* for susceptible host plants (as was indicated by olfactometry tests) or due to a lower rate of survival of parasitized larvae on partially-resistant host plants (Verkerk and Wright 1994).

The lower parasitism on partially-resistant host plants could be a result of differential immune response of *P. xylostella* larvae to the parasitoid attack (Karimzadeh and Wright 2008; Vogelweith et al. 2011), resulting in a reduced ability to encapsulate the parasitoid eggs or larvae. However, immunity experiments using measures of cellular and humoral effectors in our laboratory have failed to support such a hypothesis (Karimzadeh and Wright 2008). Plants can influence the performance and preference of parasitoids directly, by reducing or enhancing their ability to locate hosts, or indirectly by affecting the fitness of herbivores and thus of parasitoids. The reduced fitness of *P. xylostella* larvae feeding on partially-resistant host plants may cascade up to higher trophic levels by affecting the parasitoid fitness measures, such as developmental time, fecundity, longevity and size (Caron et al. 2008; Sarfraz et al. 2009).

Despite potential for integrating technologies such as host-plant resistance and biological control in sustainable pest management programs (Price 1986; Thomas and Waage 1996; Verkerk and Wright 1996; Lewis et al. 1997; Verkerk et al. 1998; Thomas 1999), specific studies testing the effects of host-plant resistance on the preference and performance of major parasitoids of diamondback moth are rare

(Bogahawatte and van Emden 1996; Schuler et al. 2003, 2004; Karimzadeh et al. 2004; Karimzadeh and Wright 2008; Sarfraz et al. 2008). In present study, the negative effects of partially-resistant host plants on *C. vestalis*, in terms of both reduced preference for and less parasitism success on such plants, suggests that integration of such plants and *C. vestalis* for control of *P. xylostella* is inadvisable. Field studies are necessary to consider the consequences of combining such partially resistant host plants and *C. vestalis* for *P. xylostella* management during the crop cycle (Puente et al. 2008).

References

Ahuja I, Rohloff J, Bones AM (2010) Defence mechanisms of Brassicaceae: implications for plant-insect interactions and potential for integrated pest management- a review. Agron Sustain Dev 30:311–348

Benrey B, Callejas A, Rios L, Oyama K, Denno RF (1998) The effects of domestication of *Brassica* and *Phaseolus* on the interaction between phytophagous insects and parasitoids. Biol Control 11:130–140

Bogahawatte CNL, van Emden HF (1996) The influence of the host plant of diamond-back moth (*Plutella xylostella*) on the plant preferences of its parasitoid *Cotesia vestalis* in Sri Lanka. Physiol Entomol 21:93–96

Bottrell DG, Barbosa P, Gould F (1998) Manipulating natural enemies by plant variety selection and modification: a realistic strategy? Annu Rev Entomol 43:347–367

Bradburne RP, Mithen R (2000) Glucosinolate genetics and the attraction of the aphid parasitoid *Diaeretiella rapae* to *Brassica*. Proc R Soc B 267:89–95

Cai Q, Ma X, Zhao X, Cao Y, Yang X (2009) Effects of host plant resistance on insect pests and its parasitoid: a case study of wheat-aphid–parasitoid system. Biol Control 49:134–138

Campan E, Benrey B (2004) Behavior and performance of a specialist and a generalist parasitoid of bruchids on wild and cultivated beans. Biol Control 30:220–228

Campbell B, Duffey S (1979) Tomatine and parasitic wasps: potential incompatibility of plant antibiosis with biological control. Science 205:700–702

Caron V, Myers JH, Gillespie DR (2008) Fitness-related traits in a parasitoid fly are mediated by effects of plants on its host. J Appl Entomol 132:663–667

Chadwick D, Goode J (1999) Insect-plant interactions and induced plant defence. Wiley, Chichester

Ciska E, Martyniak-Przybyszewska B, Kozlowska H (2000) Content of glucosinolates in cruciferous vegetables grown at the same site for 2 years under different climatic conditions. J Agr Food Chem 48:2862–2867

Cortesero AM, Stapel JO, Lewis WJ (2000) Understanding and manipulating plant attributes to enhance biological control. Biol Control 17:35–49

Crawley MJ (2002) Statistical computing: an antroduction to data analysis using S-plus. Wiley, Chichester

Crawley MJ (2005) Statistics: an introduction using R. Wiley, Chichester

Dicke M, van Poecke RP (2002) Signaling in plant-insect interactions: signal transduction in direct and indirect plant defence. In: Scheel D, Wasternack C (eds) Plant signal transduction. Oxford University Press, Oxford, pp 289–316

Dicke M, de Boer JG, Hofte M, Rocha-Granados MC (2003a) Mixed blends of herbivore-induced plant volatiles and foraging success of carnivorous arthropods. Oikos 101:38–48

Dicke M, van Poecke RP, de Boer JG (2003b) Inducible indirect defence of plants: from mechanisms to ecological functions. Basic Appl Ecol 4:27–42

Dicke M, van Loon JJA, Soler R (2009) Chemical complexity of volatiles from plants induced by multiple attack. Nat Chem Biol 5:317–324

Douloumpaka S, van Emden HF (2003) A maternal influence on the conditioning to plant cues of *Aphidius colemani* Viereck, parasitizing the aphid *Myzus persicae* Sulzer. Physiol Entomol 28:108–113

Gassmann AJ, Hare JD (2005) Indirect cost of a defensive trait: variation in trichome type affects the natural enemies of herbivorous insects on *Datura wrightii*. Oecologia 144:62–71

Geervliet JBF, Posthumus MA, Vet LEM, Dicke M (1997) Comparative analysis of headspace volatiles from different caterpillar-infested or uninfested food plants of *Pieris species*. J Chem Ecol 23:2935–2954

Godfray HCJ (1994) Parasitoids: behavioural and evolutionary ecology. Princeton University Press, Princeton

Gomez KA, Gomez AA (1984) Statistical procedures for agricultural research. Wiley, New York

Hadacek F (2002) Secondary metabolites as plant traits: current assessment and future perspectives. Cr Rev Plant Sci 21:273–322

Harvey JA, van Dam NM, Gols R (2003) Interactions over four trophic levels: foodplant quality affects development of a hyperparasitoid as mediated through a herbivore and its primary parasitoid. J Anim Ecol 72:520–531

Helms SE, Connelly SJ, Hunter MD (2004) Effects of variation among plant species on the interaction between a herbivore and its parasitoid. Ecol Entomol 29:44–51

Hilker M, Meiners T (2002) Induction of plant responses to oviposition and feeding by herbivorous arthropods: a comparison. Entomol Exp Appl 104:181–192

Hopkins RJ, Ekbom B, Henkow L (1998) Glucosinolate content and susceptibility for insect attack of three populations of *Sinapis alba*. J Chem Ecol 24:1203–1216

Howe GA, Schaller A (2008) Direct defenses in plants and their induction by wounding and insect herbivores. In: Schaller A (ed) Induced plant resistance to herbivory. Springer, Berlin, pp 7–29

Hunter MD (2002) A breath of fresh air: beyond laboratory studies of plant volatile-natural enemy interactions. Agr Forest Entomol 4:81–86

Hunter MD (2003) Effects of plant quality on the population ecology of parasitoids. Agr Forest Entomol 5:1–8

Kalule T, Wright DJ (2002) Effect of cabbage cultivars with varying levels of resistance to aphids on the performance of the parasitoid, *Aphidius colemani* (Hymenoptera: Braconidae). B Entomol Res 92:53–59

Karimzadeh J, Wright DJ (2008) Bottom-up cascading effects in a tritrophic system: interactions between plant quality and host-parasitoid immune responses. Ecol Entomol 33:45–52

Karimzadeh J, Bonsall MB, Wright DJ (2004) Bottom-up and top-down effects in a tritrophic system: the population dynamics of *Plutella xylostella* (L.)-*Cotesia plutellae* (Kurdjumov) on different host plants. Ecol Entomol 29:285–293

Karowe DN, Seimens DH, MitchellOlds T (1997) Species-specific response of glucosinolate content to elevated atmospheric CO2. J Chem Ecol 23:2569–2582

Kopelke JP (2003) Natural enemies of gall-forming sawflies on willows (*Salix spp*) (Hymenoptera: Tenthredinidae: *Euura*, *Phyllocolpa*, *Pontania*). Entomol Gen 26:277–312

Lambdon PW, Hassall M, Boar RR, Mithen R (2003) Asynchrony in the nitrogen and glucosinolate leaf-age profiles of *Brassica*: is this a defensive strategy against generalist herbivores? Agr Ecosyst Environ 97:205–214

Lewis WJ, Martin WRJ (1990) Semiochemical for use with parasitoids: status and future. J Chem Ecol 16:3067–3089

Lewis WJ, van Lenteren JC, Phatak SC, Tumlinson JH (1997) A total system approach to sustainable pest management. P Natl Acad Sci USA 94:12243–12248

Liu SS, Jiang LH (2003) Differential parasitism of *Plutella xylostella* (Lepidoptera: Plutellidae) larvae by the parasitoid *Cotesia plutellae* (Hymenoptera: Braconidae) on two host plant species. B Entomol Res 93:65–72

Mattiacci L, Dicke M, Posthumus MA (1994) Induction of parasitoid attracting synomone in brussels sprouts plants by feeding of *Pieris brassicae* larvae: role of mechanical damage and herbivore elicitor. J Chem Ecol 20:2229–2247

McAuslane HJ, Simmons AM, Jackson DM (2000) Parasitism of *Bemisia argentifolii* on collard with reduced or normal leaf wax. Fla Entomol 83:428–437

Moyes CL, Collin HA, Britton G, Raybould AF (2000) Glucosinolates and differential herbivory in wild populations of *Brassica oleracea*. J Chem Ecol 26:2625–2641

Orr DB, Boethel DJ (1986) Influence of plant antibiosis through four trophic levels. Oecologia 70:242–249

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

Pham-Delegue MH, Trouiller J, Bakchine E, Roger B, Masson C (1991) Age dependency of worker bee response to queen pheromone in a four-armed olfactometer. Insect Soc 38:283–292

Porter AJR, Morton AM, Kiddle G, Doughty KJ, Wallsgrove RM (1991) Variation in the glucosinolate content of oilseed rape (*Brassica napus* L) leaves. I. Effect of leaf age and position. Ann Appl Biol 118:461–467

Potting RPJ, Poppy GM, Schuler TH (1999) The role of volatiles from cruciferous plants and pre-flight experience in the foraging behaviour of the specialist parasitoid *Cotesia plutellae*. Entomol Exp Appl 93:87–95

Price PW (1986) Ecological aspects of host plant resistance and biological control: interactions among three trophic levels. In: Boethel DJ, Eikenbary RD (eds) Interactions of plant resistance and parasitoids and predators of insects. Wiley, Chichester, pp 11–30

Price PW (1997) Insect ecology. Wiley, New York

Puente ME, Kennedy GG, Gould F (2008) The Impact of herbivore-induced plant volatiles on parasitoid foraging success: a general deterministic model. J Chem Ecol 34:945–958

Ramachandran S, Buntin GD, All JN, Raymer PL (1998) Diamondback moth (Lepidoptera: Plutellidae) resistance of *Brassica napus* and *B. oleracea* lines with differing leaf characteristics. J Econ Entomol 91:987–992

Raymond B, Vanbergen A, Pearce I, Hartley SE, Cory JS, Hails RS (2002) Host plant species can influence the fitness of herbivore pathogens: the winter moth and its nucleopolyhedrovirus. Oecologia 131:533–541

Reddy GVP, Holopainen JK, Guerrero A (2002) Olfactory responses of *Plutella xylostella* natural enemies to host pheromone, larval frass, and green leaf cabbage volatiles. J Chem Ecol 28:131–143

Sarfraz M, Dosdall LM, Keddie BA (2008) Host plant genotype of the herbivore *Plutella xylostella* (Lepidoptera: Plutellidae) affects the performance of its parasitoid *Diadegma insulare* (Hymenoptera: Ichneumonidae). Biol Control 44:42–51

Sarfraz M, Dosdall LM, Keddie BA (2009) Fitness of the parasitoid *Diadegma insulare* is affected by its host's food plants. Basic Appl Ecol 10:563–572

Schmale I, Wackers FL, Cardona C, Dorn S (2003) Combining parasitoids and plant resistance for the control of the bruchid *Acanthoscelides obtectus* in stored beans. J Stored Prod Res 39:401–411

Schuler TH, Poppy GM, Kerry BR, Denholm I (1999) Potential side effects of insect-resistant transgenic plants on arthropod natural enemies. Trends Biotechnol 17:210–216

Schuler TH, Potting RPJ, Denholm I, Clark SJ, Clark AJ, Stewart CN, Poppy GM (2003) Tritrophic choice experiments with *Bt* plants, the diamondback moth (*Plutella xylostella*) and the parasitoid *Cotesia plutellae*. Transgenic Res 12:351–361

Schuler TH, Denholm I, Clark SJ, Stewart CN, Poppy GM (2004) Effects of *Bt* plants on the development and survival of the parasitoid *Cotesia vestalis* (Hymenoptera: Braconidae) in susceptible and *Bt*-resistant larvae of the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). J Insect Physiol 50:435–443

Shiojiri K, Takabayashi J, Yano S, Takafuji A (2000a) Flight response of parasitoids toward plant-herbivore complexes: a comparative study of two parasitoid-herbivore systems on cabbage plants. Appl Entomol Zool 35:87–92

Shiojiri K, Takabayashi J, Yano S, Takafuji A (2000b) Herbivore-species-specific interactions between crucifer plants and parasitic wasps (Hymenoptera: Braconidae) that are mediated by infochemicals present in areas damaged by herbivores. Appl Entomol Zool 35:519–524

Simmons AT, Gurr GM (2005) Trichomes of *Lycopersicon* species and their hybrids: effects on pests and natural enemies. Agr Forest Entomol 7:265–276

Souissi R, Nenon JP, Le Ru B (1998) Olfactory responses of parasitoid *Apoanagyrus lopezi* to odor of plants, mealybugs, and plant-mealybug complexes. J Chem Ecol 24:37–48

Stiling P, Moon DC (2005) Quality or quantity: the direct and indirect effects of host plants on herbivores and their natural enemies. Oecologia 142:413–420

Thomas MB (1999) Ecological approaches and the development of "truly integrated" pest management. P Natl Acad Sci USA 96:5944–5951

Thomas MB, Waage JK (1996) Integration of biological control and host plant resistance breeding: a scientific and literature review. TCARCEU, Wageningen

Tscharntke T, Hawkins BA (2002) Multitrophic level interactions. Cambridge University Press, Cambridge

Turlings TCJ, Wäckers F (2004) Recruitment of predators and parasitoids by herbivore-injured plants. In: Cardé RT, Millar JG (eds) Advances in insect chemical ecology. Cambridge University Press, Cambridge, pp 21–75

Ulmer BJ, Dosdall LM (2006) Glucosinolate profile and oviposition behavior in relation to the susceptibilities of Brassicaceae to the cabbage seedpod weevil. Entomol Exp Appl 121:203–213

van Emden HF (1986) The interaction of plant resistance and natural enemies: effects on populations of sucking insects. In: Boethel DJ, Eikenbary RD (eds) Interactions of plant resistance and parasitoids and predators of insects. Ellis Horwood, Chichester, pp 138–150

van Emden HF (1999) Are two methods better than one? Int J Pest Manag 45:1–2

van Etten CH, Daxenbichler ME, Williams PH, Kwolek WF (1976) Glucosinolates and derived products in cruciferous vegetables: analysis of the edible part from twenty-two varieties of cabbage. J Agr Food Chem 24:452–455

van Tol RWHM, van der Sommen ATC, Boff MIC, van Bezooijen J, Sabelis MW, Smits PH (2001) Plants protect their roots by alerting the enemies of grubs. Ecol Lett 4:292–294

Velten G, Rott AS, Cardona C, Dorn S (2007) Effects of a plant resistance protein on parasitism of the common bean bruchid *Acanthoscelides obtectus* (Coleoptera: Bruchidae) by its natural enemy *Dinarmus basalis* (Hymenoptera: Pteromalidae). Biol Control 43:78–84

Verkerk RHJ, Wright DJ (1994) Interactions between the diamondback moth, *Plutella xylostella* L and glasshouse and outdoor-grown cabbage cultivars. Ann Appl Biol 125:477–488

Verkerk RHJ, Wright DJ (1996) Multitrophic interactions and management of the diamondback moth: a review. B Entomol Res 86:205–216

Verkerk RHJ, Wright DJ (1997) Field-based studies with the diamondback moth tritrophic system in Cameron Highlands of Malaysia: implications for pest management. Int J Pest Manage 43:27–33

Verkerk RHJ, Leather SR, Wright DJ (1998) The potential for manipulating crop-pest-natural enemy interactions for improved insect pest management. B Entomol Res 88:493–501

Vet LEM (1999) From chemical to population ecology: infochemical use in an evolutionary context. J Chem Ecol 25:31–49

Vet LEM, Dicke M (1992) Ecology of infochemical use by natural enemies in a tritrophic context. Annu Rev Entomol 37:141–172

Vinson SB (1984) How parasitoid locate their hosts: a case of insect espionage. In: Lewis T (ed) Insect communications. 12th Symp. Roy. Entomol. Soc. London. Academic, London, pp 325–348

Vogelweith F, Thiéry D, Quaglietti B, Moret Y, Moreau J (2011) Host plant variation plastically impacts different traits of the immune system of a phytophagous insect. Funct Ecol 25:1241–1247

Vuorinen T, Nerg A, Ibrahim MA, Reddy GVP, Holopainen JK (2004) Emission of *Plutella xylostella*-induced compounds from cabbages grown at elevated CO_2 and orientation behavior of the natural enemies. Plant Physiol 135:1984–1992

Wallace SK, Eigenbrode SD (2002) Changes in the glucosinolate-myrosinase defense system in *Brassica juncea* cotyledons during seedling development. J Chem Ecol 28:243–256

Wang XG, Keller MA (2002) A comparison of the host-searching efficiency of two larval parasitoids of *Plutella xylostella*. Ecol Entomol 27:105–114