



WAGENINGEN UNIVERSITY
LABORATORY OF ENTOMOLOGY

**A comparative study on the foraging behaviour of
the parasitoids *Diadegma semiclausum* and
Diadegma fenestrale: the specificity and the use of
herbivore-induced plant volatiles**

No.: 05.14
Name: Chris Veenemans
Period: 2004-2005
1^e Examiner: Dr. R.P.J. Potting
2^e Examiner: Dr. T. Bukovinszky

Contents

Abstract	
Introduction.....	2
Materials and Methods	4
Odour sources.....	4
Parasitoids	4
Leaf-damage standardizing methods	5
Y-tube bioassays.....	5
Treatments and experimental design	5
Data analysis.....	6
Results	7
Experiment 1: Influence of experience I.....	7
Experiment 2: Response to host-induced plants.....	7
Experiment 3: Specificity of induction (host vs non-host)	8
Experiment 4: Influence of experience II.....	9
Herbivore damage.....	10
Discussion.....	11
Specificity of volatile blend composition	11
Specialist versus generalist.....	12
Herbivore damage.....	14
Day-to-day variation in responsiveness	14
Behaviour of <i>Diadegma fenestrata</i>	14
Acknowledgements	15
References.....	16

Abstract

Herbivore-induced plant volatiles provide important information in the host location process of parasitoids. They can vary considerably between and within plant species but can also differ between the herbivore species causing the damage. The specificity of these volatiles together with the ability of parasitoids to distinguish different blends determines the foraging efficiency of parasitoids. In this perspective a theoretical distinction is drawn between specialist and generalist parasitoids. The specialist is thought to be superior in exploring specific volatile cues whereas the generalist is thought to be superior in adapting its foraging behaviour in a more variable environment by means of associative learning. In the study presented here, differences in foraging behaviour between two closely related parasitoid species, specialist *Diadegma semiclausum* and generalist *Diadegma fenestrata*, is investigated by means of two-choice olfactometer bioassays. The comparative approach was used to evaluate differences in the specificity and the use of plant-derived infochemicals and the influence of associative learning. The plant-host complexes used in this study consisted of *Brassica oleracea*, *Sinapis alba* and a feral population of *B. oleracea*, either uninfested or infested with *Plutella xylostella* (host) or *Pteris brassicae* (nonhost). The experiments revealed that both parasitoid species significantly preferred host-infested plants over uninfested plants of all three plant species tested, although females of specialist *D. semiclausum* discriminated more pronounced than females of the generalist *D. fenestrata*. However, when offered a choice between host-infested vs nonhost-infested plants this difference between parasitoid species disappeared. Moreover both parasitoid species could only differentiate between volatile blends of host-infested vs nonhost-infested *B. oleracea*. In comparable bioassays conducted with *Sinapis* or Feral *B. oleracea* no preferences were found. Experience increased the ability to differentiate between host-infested vs uninfested *B. oleracea*, equally for both species. Experience did not improve parasitoids ability to distinguish between host-infested and nonhost-infested Feral *B. oleracea* (only tested for *D. semiclausum*). The results of this study seem to confirm that differences in volatile blends of the plants species used, depend on both plant species and herbivore species damaging the plant. Furthermore, although diet breath of the parasitoids used in this study is expected to range considerably, the differences in their foraging behaviour were not as pronounced as expected.

Introduction

Parasitoids are of great importance for the control of pests in agricultural systems and are used in many biological control-based management programs (Talekar and Shelton, 1993). The success of these programs depends to a large extent on the available knowledge about the ecology of the parasitoids and the interaction with their hosts. An important aspect herein is the efficiency of the so called foraging behaviour. Host searching by adult female parasitoids takes place in a complex and heterogeneous environment and is done by the use of environmental cues. From these cues, volatile stimuli from the host, its microhabitat and food plant are the most important (Turlings et al., 1991; Godfray, 1994). Although direct volatile cues from the host and its by products are the most reliable ones, they are seldom detectable over long distances (Vet and Dicke, 1992). Therefore parasitoids most often have to rely on the better detectable indirect cues as indication for the presence of their host.

Indirect cues mostly refer to plant-derived infochemicals which can vary considerably between and within plant species as a result of differences in abiotic and biotic factors. One of the more important biotic factors, provoking variation in plant-derived infochemicals, is herbivore damage. Many plant species under herbivore attack respond with an increase of volatile production, providing parasitoids with information on herbivore presence (Vet and Dicke, 1992). This indirect plant defence mechanism can be very specific. For some plant species evidence is found that herbivore-induced infochemicals (synomones) diverge between herbivore species causing the damage (Takabayashi et al., 1991; Agelopoulos and Keller 1994a; 1994b; Blaakmeer et al., 1994; Geervliet, 1997) and even between different instars of the same herbivore (Takabayashi et al., 1995). From the perspective of the parasitoid the specificity of this herbivore-induced plant response is important because specific synomones can reliably predict host presence over longer distances and therefore it can be a solution for the reliability-detectability problem (Vet et al., 1991).

Different species of parasitoids, although facing similar evolutionary challenges, have evolved divergent foraging strategies and lifestyles (Godfray, 1994). Vet et al. (1990) and Lewis et al. (1990) assume naïve parasitoids to have an innate set of 'response potentials' to different stimuli at their disposal. When confronted with a set of stimuli, they have an innate preference for the stimuli with the highest host encounter expectation which is fine-tuned by natural selection. The ranking of different stimuli can be influenced by genetic differences within species due to local adaptation and more important this ranking can be subject to change over the life of the parasitoid as a result of learning from experience. Parasitoid species with foraging strategies adapted to more specific synomones are thought to have an advantage over parasitoid species with a more general response to plant-derived cues. In this perspective a theoretical distinction is drawn in the variation of host range between extreme specialists, that have only one host species, and generalists, that have a broad range of host species. Vet and Dicke (1992) formulated a broadly accepted concept in which the origin and specificity of the used infochemicals is expected to depend on dietary specialisation of both the parasitoid and its host. Furthermore they propose that the specificity and strength of innate response potentials depends on the degree of dietary specialisation as well. Specialists are thought to be superior in host-location efficiency than generalists due to their ability to use more specific long-range and short-range volatile cues (Vet and Dicke, 1992; Wang and Keller, 2002). With respect to learning from experiences, generalists are expected to be superior in this concept because they function in a more variable environment, whereas specialists more rely on innate responses to specific infochemicals.

Research and interpretation on the variability in foraging behaviour of parasitoids is studied using different approaches: the tri-trophic approach (evolution of foraging behaviour is influenced by several trophic levels), the optimally approach (successful variation in foraging decisions is rewarded with higher fitness) and the comparative approach (correlations between specific foraging behaviour of species and ecological factors can indicate adaptation) (Godfray, 1994, Vet et al., 1995). Comparative research is an interesting mean to investigate differences in foraging strategies because it gives insights in the relative importance of the underlying behavioural ecology and other tritrophic interactions that may account for variation in host location efficiency (Godfray, 1994). Therefore diet breadth has been point of departure in several comparative studies (Legaspi, 1986; Wiskerke and Vet, 1994; Geervliet, 1996, 1998; De Moraes and Lewis, 1998, 1999; Shiojiri et al., 2000; Wang and Keller, 2002; Roßenbach et al., 2005). Besides the differences in infochemical use and learning capacity, some of these studies also investigated the variability in behavioural patterns between specialists and generalists, studying how host defence behaviour and time allocation in foraging behaviour, influence foraging efficiency.

In the study presented here, differences in foraging behaviour between two closely related parasitoid species were investigated in a tritrophic system by means of two-choice olfactometer bioassays. At the third trophic level the parasitoid species *Diadegma semiclausum* and *Diadegma fenestrata* were used. Both species are solitary feeding larval endoparasitoids with the mutual preferred host, *Plutella xylostella* (Legaspi, 1986). They differ however in their degree of specialization: *D. semiclausum* is considered to be a specialist (Abbas, 1988; Talekar & Yang, 1991) whereas *D. fenestrata* is described as ‘very polyphagous’ (Hardy, 1938, in Fitton and Walker, 1992). The herbivores at the second trophic level consisted of *Plutella xylostella* and *Pieris brassicae* (respectively host and non-host for both parasitoids). At the first trophic level Brussels sprouts, White mustard and a feral population of *B. oleracea* were used. The comparative approach was used to evaluate differences in the specificity and the use of plant-derived infochemicals and the influence of associative learning. More specific questions were formulated as follows:

1. Do parasitoids differ in their ability to discriminate between host-infested and uninfested plants?
2. Do parasitoids differ in their ability to discriminate between host- and nonhost-infested plants?
3. Is plants species influencing the parasitoids ability to discriminate?
4. Does experience influence the ability to discriminate between uninfested and host-infested plants to different extent for both parasitoid species?
5. Does experience influence the ability to discriminate between host and non-host infested plants?

Materials and Methods

Odour sources

The plants used in the experiments, Brussels sprouts (*Brassica oleracea* L. gemmifera cv Cyrus) (Capparales, Brassicaceae), White mustard (*Sinapis alba* L. cv Carnaval) (Capparales, Brassicaceae), and a naturalized population of *Brassica oleracea* L.^{*}, were grown in the University greenhouses under 16L; 8D photoperiod, 20-28°C and 40-80% R.H. For details on rearing conditions and biomass standardisation (to obtain comparable leaf surface resulting in comparable emission of volatiles when damaged by the caterpillars) see Bukovinszky et al. (2005).

Specific volatile emission was provoked by infesting the plants 18-24 hours in advance with \pm twenty-five 2nd to 3rd instar caterpillars, evenly distributed over the leaves. Caterpillars used in the experiments were *Plutella xylostella* (host for both parasitoids) and *Pieris brassicae* (non-host for both parasitoids), obtained from colonies maintained on Brussels sprouts in a climate room at 20-22°C, 50-70% RH under a L16:D8 photoregime. Thirty minutes preceding the experiments caterpillars and by-products were removed, the plants were removed from their pots, the below-ground parts wrapped in aluminium foil and placed in the experimental setup. Plants served during a maximum of one hour for experiments and a minimum of eight plant sets was used per bioassay.

Parasitoids

The parasitoids used in the experiments, *Diadegma semiclausum* (Hellén) (Hymenoptera, Ichneumonidae) and *Diadegma fenestrata* (Holmgren) (Hymenoptera, Ichneumonidae), originated from wild populations collected in the vicinity of Wageningen in Brussels sprouts fields. Both species were reared at the Laboratory of Entomology, on the plant/host combination of Brussels sprouts/*P. xylostella* (8D: 16L photoperiod, 20 \pm 2 °C and 70% R.H.). Cardboard strips containing the pupating parasitized host larvae were transferred from the rearing into clean wooden cages (50x25x25) to prevent emerging parasitoids to get in contact with plant material or hosts. From emergence parasitoids were provided *ad libitum* with water and honey. At least 24 hour prior to the experiments parasitoids were stored at 21.9 °C (male:female ratio 1:4). On experimental days parasitoids were transferred individually in glass vials from the wooden cage into the experimental setup. For the experiments only 5 to 10 days old mated females were used. Parasitoids were tested individually and each female was used only once.

During the experiments parasitoids were used with different levels of experience. Naïve parasitoids (N) had no contact with either plant or host material; Sensitized parasitoids (S) were placed on host-damaged plant material until they showed probing behaviour; Experienced parasitoids (E) had contact with damaged plant material and 3 oviposition experiences on their host (*P. xylostella*); Negative experienced parasitoids (Neg-E) were confronted with non-host *P. brassicae* until oviposition attempt or being attacked until bedaubed with non-host saliva; Positive/Negative-experienced parasitoids (Pos-Neg-E) had both experiences in the mentioned order. When experience was required, this was given 18-24 hours in advance of the experiment, with the exception for the Sensitization, which was done within 5 minutes prior to performing in the experimental setup. Parasitoids were

* This feral population was found in a roadside hollow in 2001, it probably “escaped” from a local farm, and it is unknown how long it has been growing in the wild (Harvey et al., 2003)

experienced individually in a Petri-dish on plant material of the plant species used in the experiment.

Leaf-damage standardizing methods

To avoid differences in volatile emission due to differences in damage by the different caterpillar species on the different plant species, the number of larvae of the both herbivore species was chosen in such a way that eaten leaf surface was approximately equal. This was achieved by conducting several preliminary tests preceding the experiments, with different quantities of caterpillars of both species to obtain data on eaten leaf tissue. For each test two sets of three plants were infested with different quantities and instars of *P. xylostella* and *P. brassicae*. After 24 hours, all caterpillars were removed and feeding damage was measured (in cm² removed leaf tissue) from the scanned leaf images, with *Scion Image Beta 4.0.2*. (Scion Corporation © 2000). Based on these results from each caterpillar species fifteen 2nd instar and ten early 3rd instar per plant were used in all experiments. Standardizing for leaf-damage was not conducted on *S. alba* because of its morphological unsuitability for this method in combination with the feeding behaviour of *P. brassicae*.

During experiments 3 and 4 (see below) a second method for standardising feeding damage was used, alongside the above described. Quintessence of this method was that the length of the affected leaf area (sum of the perimeters) was measured instead of eaten surface. Editing and measurements of the scanned images was done with *Adobe® Photoshop® version 6.0* and *Scion Image Beta 4.0.2*.

Y-tube bioassay

All bioassays were conducted in a glass Y-tube olfactometer (ø 3.5 cm; length of stem section 22 cm; length of both arms 12 cm) as described by Takabayashi and Dicke (1992) and Bukovinszky et al. (2005). Both arms of the Y-tube were attached to 5 litre glass vessels by means of two sieves and a glass tube (ø 3.5 cm, length 7 cm) per arm. The airflow through each vessel (4 l/m) was filtered first over active charcoal and extracted at the base of the Y-tube at 8 l/m. The experimental setup was placed in a blinded room at 22 ± 2 °C and illuminated one meter from above with high frequency fluorescent lights at an intensity of 30–35 μmol photons/m²/sec.

At 30 cm from the base of the stem a line was drawn on both arms of the Y-tube (2 cm from the first sieve) which served as ‘finish’. A choice was defined as a parasitoid crossing this line and not returning to the junction for at least 15 seconds. From the point of release at the base of the Y-tube, parasitoids were given max. 10 minutes to make a choice, if not, a non-choice was recorded. However, when the junction was not reached within 5 minutes after release, this was also considered as a non-choice and the individual was removed. To prevent asymmetries in the experimental setup to influence the results, the Y-tube was turned frequently as well as the odour sources were exchanged.

To exclude day-to-day variation a block design was used as described by Steinberg et al (1993) and for each experiment data were collected on 4 to 7 different days. The experiments were carried out from January 2005 through April 2005.

Treatments and experimental design

Experiment 1: Individuals of both *D. semiclausum* and *D. fenestrata* with different levels of experience (N/S-E/E) were offered a choice between *P. xylostella*-infested and

- uninfested Brussels sprouts to investigate differences in response between the mentioned experience levels. Furthermore this experiment would indicate the suitability of the olfactometer for testing searching behaviour of *D. fenestrata*.
- Experiment 2: Naïve individuals of both *D. semiclausum* and *D. fenestrata* were offered a choice between *P. xylostella*-infested and uninfested Brussels sprouts, White mustard and Feral *B. oleracea* to investigate differences in preference between parasitoid species.
- Experiment 3: Naïve individuals of both *D. semiclausum* and *D. fenestrata* were offered a choice between *P. xylostella*-infested and *P. brassicae*-infested Brussels sprouts, White mustard and Feral *B. oleracea* to investigate differences between parasitoid species with regard to host / non-host preferences.
- Experiment 4: Individuals of *D. semiclausum* with different levels of experience (N/E/Neg-E/Pos-Neg-E) were offered a choice between *P. xylostella*-infested and *P. brassicae*-infested Feral *B. oleracea* to investigate the influence of experience. The choice for Feral *B. oleracea* was based on the findings in *experiment 3*. The choice to use only *D. semiclausum* for this experiment was based on practical difficulties with *D. fenestrata* (the rearing could not provide enough females standardised for age on experimental days).

Table 1 outlines the used plant/caterpillar/parasitoid combinations used for the different experiments.

	Plants	Caterpillars	Parasitoids
Experiment 1	<i>Brassica oleracea</i>	<i>Plutella xylostella</i>	<i>Diadegma semiclausum</i> (N/S/E) <i>Diadegma fenestrata</i> (N/S/E)
Experiment 2	<i>Brassica oleracea</i> <i>Brassica oleracea</i> (feral) <i>Sinapis alba</i>	<i>Plutella xylostella</i>	<i>Diadegma semiclausum</i> (N) <i>Diadegma fenestrata</i> (N)
Experiment 3	<i>Brassica oleracea</i> <i>Brassica oleracea</i> (feral) <i>Sinapis alba</i>	<i>Plutella xylostella</i> <i>Pieris brassicae</i>	<i>Diadegma semiclausum</i> (N) <i>Diadegma fenestrata</i> (N)
Experiment 4	<i>Brassica oleracea</i> (feral)	<i>Plutella xylostella</i> <i>Pieris brassicae</i>	<i>Diadegma semiclausum</i> (N/E/Neg-E/Pos-Neg-E)

Table 1

Data analysis

Experiments consisted of 39 to 62 individuals that made a choice (parasitoids that did not make a choice - 8.3 % of 1168 - were excluded from the statistical analyses). Preferences in dual choice tests were analyzed with a two-tailed binomial test, assuming an even distribution of choices over the odour sources. Significance (when $p < 0.05$) indicates preference for one of the two odour sources tested. To investigate differences between experience levels, parasitoid species and plant species a generalized linear model (GLM) was used, assuming no differences in performance on the different plant species, nor between experience levels or parasitoid species. Data on herbivore damage in experiment 3 and 4 was pooled for plant and for herbivore species separately and analyzed for both removed leaf tissue and perimeter of the damaged area using GLM, testing the assumption that neither plant species nor herbivore species influences the surface amount of leaf tissue or perimeter eaten.

Results

Experiment 1: Influence of experience I

To investigate the role of experience on parasitoids ability to recognize a host-infested plant, Brussels sprouts plants were infested with *P. xylostella* and tested against uninfested Brussels sprouts plants. Volatiles from the infested plants were preferred by all categories of both *D. semiclausum* ($p < 0.0001$, binomial test) and *D. fenestratale* ($p \leq 0.01$, binomial test) (Figure 1). Comparison between parasitoid species showed a higher preference for infested plants of *D. semiclausum* ($p < 0.001$, $\text{Chi}^2 = 11.33$, $\text{DF} = 1$, GLM). Increasing experience with the plant-host complex resulted for both species in a higher preference for the infested plants ($p = 0.008$, $\text{Chi}^2 = 9.68$, $\text{DF} = 2$, GLM). However, the relationship between level of experience and preference for infested plants did not depend on the parasitoid species ($p = 0.1$, $\text{Chi}^2 = 4.38$, $\text{DF} = 2$, GLM).

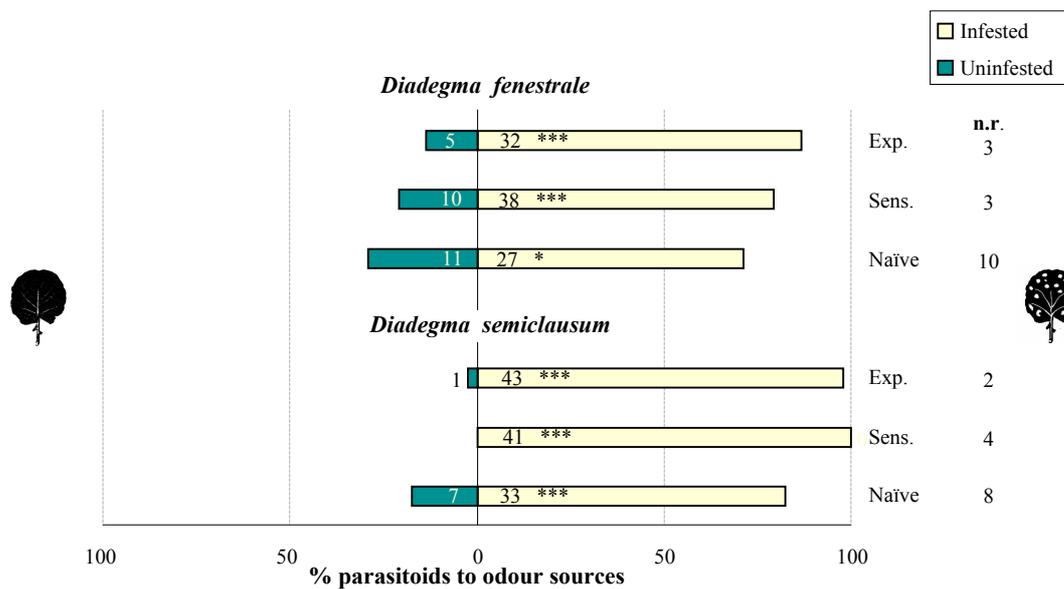


Figure 1. The percentage of *D. fenestratale* and *D. semiclausum* (per level of experience) that made a choice for either *P. xylostella*-infested Brussels sprouts or uninfested plants. The numbers in the bars reflect the number of parasitoids, the asterisks indicate statistical significance (* $0.01 < p < 0.05$; ** $0.001 < p < 0.01$; *** $p < 0.001$). Numbers next to the graph reflect the number of non-responding individuals (n.r.).

Non-response levels in this experiment varied between 4% (experienced *D. semiclausum*) and 21% (naïve *D. fenestratale*), though differences between experience levels were not significant ($p = 0.3$, $\text{Chi}^2 = 2.72$, $\text{DF} = 2$, GLM), neither were the differences between parasitoid species ($p = 0.4$, $\text{Chi}^2 = 0.85$, $\text{DF} = 1$, GLM). Based on these results, naïve females were used for experiments 2 and 3.

Experiment 2: Response to host-induced plants

In this experiment naïve females of both parasitoid species were offered a choice between host-infested and uninfested Brussels sprouts, host-infested vs uninfested mustard and between host-infested vs uninfested Feral *B. oleracea*. With 87% to 98% for *D. semiclausum* and 75% to 80% for *D. fenestratale*, both parasitoid species significantly preferred the volatile blends from all three infested plant sources over the uninfested conspecifics ($p < 0.0001$, binomial) (Figure 2). The ability of *D. semiclausum* to recognize a host-infested plant was

significantly higher than of *D. fenestrata* ($p=0.008$, $\text{Chi}^2=6.95$, $\text{DF}=1$, GLM), irrespective of the tested plant species ($p=0.51$, $\text{Chi}^2=1.36$, $\text{DF}=2$, GLM). Plant species did not influence the preferences of either parasitoid species ($p=0.3$, $\text{Chi}^2=2.54$, $\text{DF}=2$, GLM).

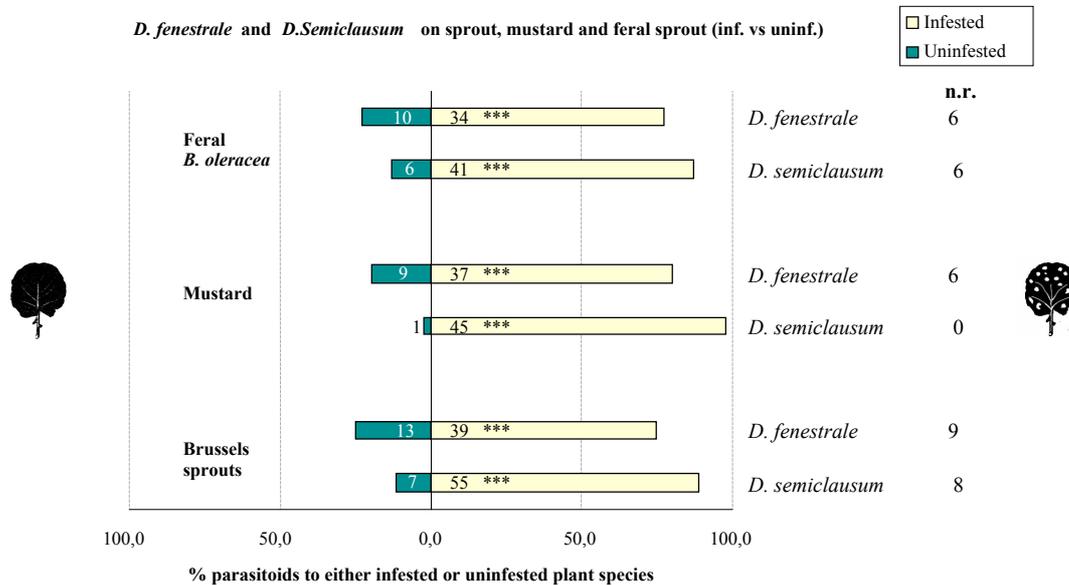


Figure 2. The percentage of *D. fenestrata* and *D. semiclausum* (naïve females) that made a choice for either *P. xylostella*-infested plants or uninfested plants. The bars in the graph present the results for both parasitoid species on Feral *B. oleracea*, Mustard and Brussels sprouts in that order from top to bottom. The numbers in the bars reflect the number of parasitoids, the asterisks indicate statistical significance (***) $p<0.001$). Numbers at the right-hand side of the graph reflect the number of non-responding individuals (n.r.).

When looked at percentages of non-responding wasps, which varied between 0% (*D. semiclausum* on Mustard) and 15% (*D. fenestrata* on Brussels sprouts), like in the previous experiment no significant differences were found between parasitoid species ($p=0.1$, $\text{Chi}^2=2.58$, $\text{DF}=1$, GLM) or for plant species ($p=0.07$, $\text{Chi}^2=5.31$, $\text{DF}=2$, GLM).

Experiment 3: Specificity of induction (host vs non-host)

When offered a choice between the volatiles of host-infested vs nonhost-infested plants, naïve females of both parasitoid species could discriminate on Brussels sprouts. Seventy-six percent of *D. semiclausum* went for the *P. xylostella*-damaged plants ($p<0,001$, binomial test) whereas 78% of *D. fenestrata* did ($p<0.001$, binomial test). However when offered host- vs nonhost-infested Mustard or Feral *B. oleracea*, none of the two parasitoid species could discriminate ($p\geq 0.28$, binomial test). Here 46% (*D. fenestrata* on Feral *B. oleracea*) to 57% (*D. semiclausum* on Mustard) of the females went for the volatile cues of the *P. brassicae*-induced plants (Figure 3). The blends of different plant species clearly provoke different reaction (preferences) of the parasitoids ($p<0.001$, $\text{Chi}^2=20.01$, $\text{DF}=2$, GLM). Further data-analysis showed no significant difference in behaviour between parasitoid species ($p=0.19$, $\text{Chi}^2=1.73$, $\text{DF}=2$, GLM).

Response did not vary between females of *D. semiclausum* and *D. fenestrata* ($p=0.28$, $\text{Chi}^2=1.15$, $\text{DF}=1$, GLM), but in contrast to previous experiments the percentages of responding individuals did differ between plant species ($p=0,04$, $\text{Chi}^2=6.41$, $\text{DF}=2$, GLM). Response percentages on Mustard were higher when compared to Brussels sprouts and Feral *B. oleracea*. No significant interaction was found between plant species and parasitoid species

indicating that both parasitoid species showed the same responses to odours from the different plant species ($p=0.68$, $\chi^2=0.75$, $DF=2$, GLM).

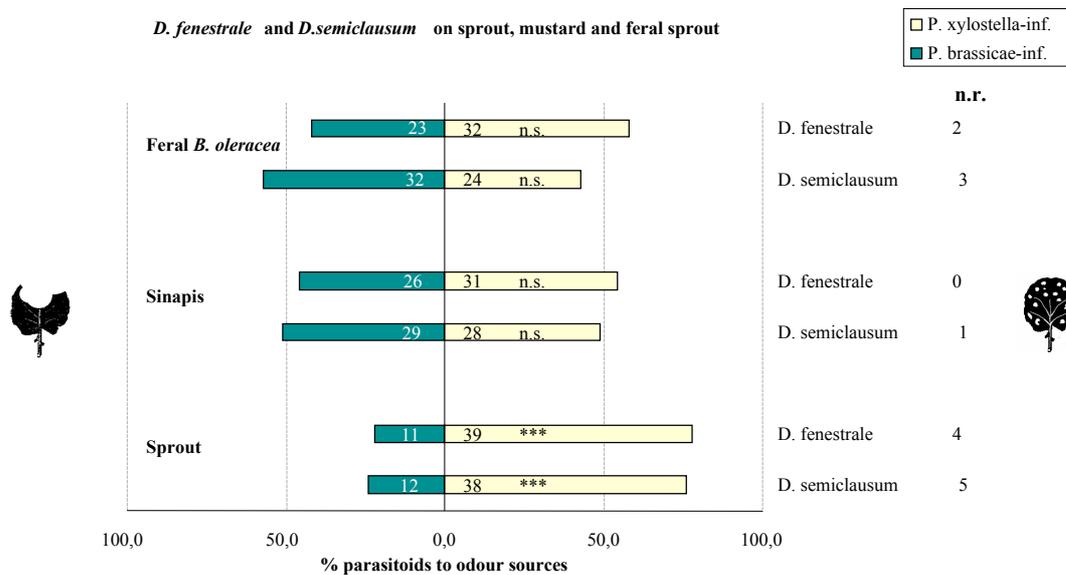


Figure 3. The percentage of *D. fenestrata* and *D. semiclausum* (naïve females) that made a choice for either *P. xylostella*-infested Brussels sprouts plants or *P. brassicae*-infested plants. The numbers in the bars reflect the number of parasitoids, asterisks indicates statistical significance (***) $p < 0.001$, n.s. means no statistical significance was found. Numbers next to the graph indicate the number of non-responding individuals (n.r.).

Experiment 4: Influence of experience II

The last experiment was conducted with *D. semiclausum* only and tested whether associative learning would influence the ability to discriminate between host-infested and nonhost-infested Feral *B. oleracea* plants. As in the previous experiment naïve females could not discriminate (56% chose for host-infested plants, $p > 0.05$, binomial test). Neither could the female parasitoids which were exposed to different learning experiences (Exp. 59%, $p > 0.05$; Neg-E 51%, $p > 0.05$; Pos-Neg-E 60%, $p > 0.05$, binomial test) (figure 4).

GLM analyses, conducted to compare variety between experience levels, did not show differences ($p=0.16$, $\chi^2=2.01$, $DF=3$). Response levels varied between 91% (N) and 100% (Neg-E). Although differences in response levels may be significant analyses were not made because of the relatively small absolute numbers.

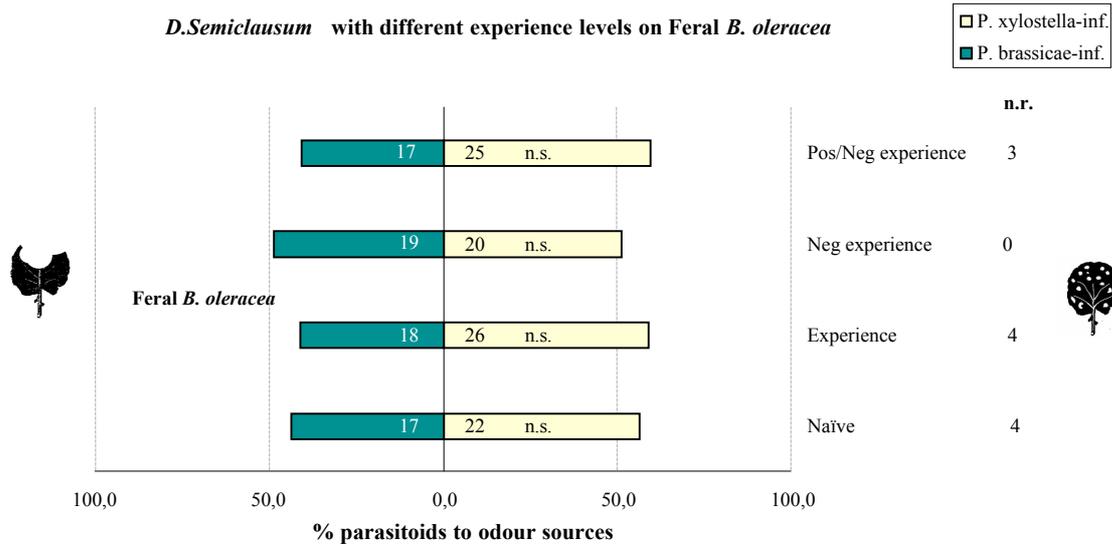


Figure 4. The percentage of *D. semiothisa* (per level of experience) that made a choice for either *P. xylostella*-infested Feral *B. oleracea* plants or *P. brassicae*-infested plants. The numbers in the bars reflect the number of parasitoids, n.s. means no statistical significance was found. Numbers next to the graph reflect the number of non-responding individuals (n.r.).

Herbivore damage

For the last two experiments herbivore damage was measured on all used plant sets used to investigate whether possible differences in amount of feeding may have influenced (non)preferences. Data was pooled for plant species and for caterpillar species and analyzed for both removed leaf tissue and perimeter of the damaged area. On removed leaf tissue no significant differences were found between plant species ($p=0.99$, $F\text{-stat}<0.001$, $DF=1$, GLM) or herbivores ($p=0.07$, $F\text{-stat}=3.3$, $DF=1$, GLM) indicating that *P. xylostella* and *P. brassicae* ate comparable quantities of leaf material on both Brussels sprouts and Feral *B. oleracea*. The perimeters of the affected leaf area were comparable for both plant species ($p=0.48$, $F\text{-stat}=0.5$, $DF=1$, GLM), however, differed considerably for herbivore species ($p<0.001$, $F\text{-stat}=70$, $DF=1$, GLM). So although caterpillars had eaten similar amounts of leaf tissue, the diverging feeding patterns between herbivore species resulted in higher amounts of affected leaf edges (damaged plant cells) for *P. xylostella* than *P. brassicae* (figure 5).

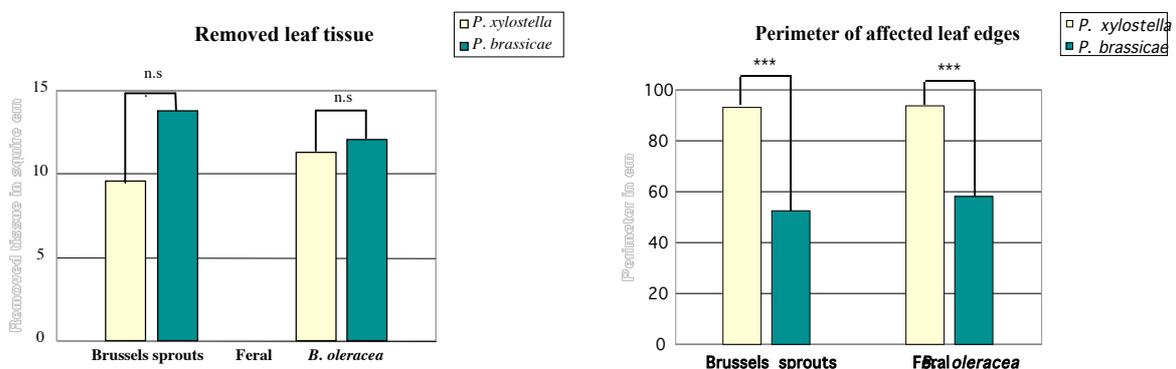


Figure 5. A. Herbivore damage measured in cm^2 removed leaf tissue for *P. xylostella* and *P. brassicae* on Brussels sprouts and Feral *B. oleracea*. **B.** Herbivore damage measured in cm of affected area (sum of perimeters) for *P. xylostella* and *P. brassicae* on Brussels sprouts and Feral *B. oleracea*. Asterisks indicates statistical significance ($*** p < 0.001$), n.s. means no statistical significance was found.

Discussion

In this study differences in foraging behaviour between two closely related parasitoid species, specialist *Diadegma semiclausum* and generalist *Diadegma fenestrata*, was investigated by means of two-choice olfactometer bioassays. The comparative approach was used to evaluate differences in the specificity and the use of plant-derived infochemicals and the influence of associative learning. The plant-host complexes used in this study consisted of *Brassica oleracea*, *Sinapis alba* and a feral population of *B. oleracea*, either uninfested or infested with *Plutella xylostella* (host) or *Pieris brassicae* (nonhost). The experiments revealed that both parasitoid species significantly preferred host-infested plants over uninfested plants of all three plant species tested, although females of specialist *D. semiclausum* discriminated more pronounced than females of the generalist *D. fenestrata*. However, when offered a choice between host-infested vs nonhost-infested plants this difference between parasitoid species disappeared. Moreover both parasitoid species could only differentiate between volatile blends of host-infested vs nonhost-infested *B. oleracea*. In comparable bioassays conducted with *Sinapis* or Feral *B. oleracea* no preferences were found. Experience increased the ability to differentiate between host-infested vs uninfested *B. oleracea*, equally for both species. Experience did not improve parasitoids ability to distinguish between host-infested and nonhost-infested Feral *B. oleracea* (only tested for *D. semiclausum*). The results can be interpreted from the perspective of (1) the specificity of plant induction at the first trophic level and (2) the differences in foraging behaviour of parasitoids at the third trophic level.

Specificity of volatile blend composition

In literature plant response to herbivore damage is categorised based on changes in volatile blend (Dicke 1999, Dicke and Vet, 1999). The two main categories distinguished are: plant response that is predominantly qualitatively different upon infestation and plant response that is predominantly quantitative different. In the first category herbivore damage results in the production of a large set of novel compounds dominating the blend (which are not present in the blends of undamaged or mechanically damaged plants). In the second category the herbivore-induced volatiles are qualitatively similar to volatiles of uninfested conspecifics but quantities and ratios of the compounds may differ. On cabbage systems various studies have found evidence for differences in blend composition as a result of herbivory, either by means of behavioural response experiments with parasitoids or by means of gas-chromatographic mass spectrometric analyses, or both (amongst others Agelopoulos and Keller 1994; Mattiacci et al., 1994; Geervliet et al., 1997; De Moares et al., 1998; Bukovinszky et al., 2005). The results of the first two experiments show that both *Diadegma* species could differentiate between volatile blends of uninfested and *P. xylostella*-infested plants for all plant species used and therefore provide evidence that (the composition) of volatile blends differ. Earlier findings of Ohara et al (2003) and Bukovinszky et al. (2005) already showed preference for host-infested cabbage (different cultivars) and mustard of *D. semiclausum*. The preference of *D. fenestrata* for host-infested plants was up till now unknown, although it was expected based on results of studies with other generalist parasitoid species (Steinberg et al, 1993; Geervliet et al., 1996).

Interpretation of the results found in the third experiment however is slightly more complicated. Hypotheses for this experiment were that specificity of herbivore induced volatiles depends on both plant species and herbivore species. The results reveal that both parasitoid species could discriminate between *P. xylostella* and *P. brassicae* on Brussels sprouts but not on the other two plant species tested. Hence, this seems to confirm that volatile blends differ between plant species and herbivore species. However, the fact that

neither parasitoid species could discriminate between host-infested and nonhost-infested Feral *B. oleracea* or Mustard, raises questions on the specificity of volatile blends induced by different herbivores for these plants. Various authors found differences in volatile blends induced by different herbivores on different plant species (see review Dicke and Vet, 1999). For several cabbage cultivars variation in blends was predominantly quantitative (Agelopoulos and Keller, 1994; Blaakmeer et al., 1994; Geervliet et al., 1997). When looked at parasitoids ability to differentiate between host-induced and nonhost-induced blends, literature is kaleidoscopic and appears to vary for all tritrophic systems studied. For instance, the specialist parasitoid *Cardiochiles nigriceps* can distinguish infestation by its host on corn, maize and tobacco (De Moraes et al., 1998) whereas the specialist parasitoid *Cotesia rubecula* was not able to discriminate between blends of host and non-host on various cabbage cultivars (Agelopoulos and Keller, 1994; Geervliet et al., 1998). Interpreting the reviews and studies of the authors mentioned above it seems unlikely that volatile blends of Feral *B. oleracea* and Mustard induced by *P. xylostella* or *P. brassicae* are qualitatively and quantitatively identical. Therefore it is expected that both parasitoids either could not detect the differences or that their expectancy to find a suitable host was equal for both odour sources offered.

Another explanation for the results of this experiment might be found in early adult learning. Findings of Hérard et al. (1988) and Van Emden et al. (2002) indicate that parasitoids may acquire odour information from the host cocoon directly upon emergence which can influence subsequent foraging decisions. Kalule and Wright (2004) found that parasitoids develop a preference for the specific cabbage cultivar on which they were reared. However, this preference disappeared when parasitoids were offered a choice between the infested cabbage cultivar of rearing and another infested cabbage cultivar. Douloumpaka and Van Emden (2003) showed that parasitoids of which the host was reared on an artificial diet did not have preference for any of the odour sources offered. Since both *D. semiclausum* and *D. fenestrata* are reared on *P. xylostella*-Brussels sprouts[†] and could only distinguish host- from nonhost-infestation on this plant-host complex, this might be caused by early adult learning. To investigate whether the results of this experiment are due to rearing history, the experiment should be repeated with parasitoids emerged from hosts reared on Feral *B. oleracea*, Mustard or an artificial diet, for several generations.

Specialist versus generalist

In the introduction the concept of Vet and Dicke (1992) is described in which the degree of dietary specialization is used to explain differences in foraging behaviour of parasitoids with reference to the use of specific infochemicals and the ability to improve searching efficiency by associative learning. Before interpreting the results according to this concept it is relevant to briefly elaborate the degree of specialization of the parasitoids used in this study. According to Vet and Dicke (1992) dietary specialization of parasitoids can occur both at plant and herbivore levels. Therefore in a tritrophic system four extreme groups can be distinguished: (A) Specialists at herbivore and plant level; (B) Generalists at herbivore level and specialists at plant level; (C) Specialists at herbivore level and generalists at plant level, and (D) Generalists at both levels. In reality there will be a continuum from one group to the next. In several studies *D. semiclausum* is considered to be a specialist in group A, successfully developing on 9 host species of which *P. xylostella* is the most important (Abbas, 1988; Talekar & Yang, 1991). On *D. fenestrata* only scarce literature can be found. Hardy (1938) indicated *D. fenestrata* to attack 24 species of Lepidoptera and one coleopteran,

[†] Although paper strips with the cocoons were put in a clean case before emergence of the parasitoids, it is possible that the cocoons have absorbed volatiles from Brussels sprouts previously.

describing it as ‘very polyphagous’ (in Fitton and Walker, 1992). Azidah (2000) however considers that *D. fenestrata* often has been misidentified taxonomically. Hence, existing literature is notoriously unreliable. Although diet breadth of the parasitoids used in this study is expected to range considerable the above mentioned may illustrate that the distinction is rather relative.

Expectations based on the specialist-generalist concept were: (1) that naïve females of *D. semiclausum* would show a more pronounced preference for host-infested plants over undamaged plants than females of *D. fenestrata*; (2) that experience would have less influence on foraging efficiency of *D. semiclausum* than of *D. fenestrata*; (3) that naïve females of *D. semiclausum* would be able to discriminate between host- and nonhost-infestation whereas naïve females of *D. fenestrata* would not. The results reveal for all three comparative experiments more pronounced preferences of *D. semiclausum*, although the difference in the third bioassay was not statistically significant. However the findings did not match expectations regarding the influence of experience nor the ability to discriminate between host- and nonhost-infested plants. No differences between parasitoid species were demonstrated indicating that parasitoid species do not differ in their innate responses to *P. xylostella*-induced volatile blends. A study of De Moraes and Lewis (1999) did find differences between a generalist and a specialist on host vs non-host discrimination. Here specialist *Cardiochiles nigriceps* proved capable to distinguish between host infested and non-host infested plants whereas generalist *M. croceipes* could not. Based on their findings these authors suggest that specialist *C. nigriceps* exhibits superior host-location proficiency. However, this conclusion cannot be justified because the non-host species used in the experiments (*Heliothis zea*) actually appears to be a host of *M. croceipes*. Other comparative studies on generalist versus specialist parasitoids have been conducted by several authors (see introduction) often using *Cotesia* species. Out of these, the study of Geervliet (1996) is most similar as for experimental setup and design. Like in the study at hand, authors found that both *Cotesia* species had a clear preference for host-infested plants over uninfested plants. An interesting difference though, is that naïve females of both parasitoid species could not discriminate between host-infested and nonhost-infested Brussels sprouts (plant species of rearing) whereas they could on White cabbage. This may invalidate the above described ideas on rearing history and point in the direction of preferences for specific plant-host complexes of *Diadegma* species (in a study of Roßenbach et al. (2005), investigating foraging behaviour of *D. semiclausum* and generalist *D. mollipa*, this is confirmed for the former but contradicted for the latter).

The results of the final bioassay showed for *D. semiclausum* that associative learning did not improve the ability to discriminate between *P. xylostella*-infested and *P. brassicae*-infested Feral *B. oleracea*. This was surprising because observations of escaped females in the experimental room had given indication that experienced females preferred host-infested plants (especially after a confrontation with *P. brassicae*, a negative experience). On the other hand it was in accordance with the hypothesis that a specialist displays more fixed behaviour according to a set of innate responses. Other authors found similar behaviour for specialist parasitoids although in different tritrophic settings. For instance, Geervliet et al. (1998) found no preference learning at herbivore level for females of the specialist *C. rubecula*. In the same study however she did find adapted behaviour of generalist *C. glomerata* after multiple experiences. The latter could indicate that when this experiment would have been conducted with *D. fenestrata*, outcomes may have been different for that parasitoid species. The choice to investigate the foraging behaviour of only *D. semiclausum* in this experiment was based on unfortunate practical difficulties with *D. fenestrata* (the rearing could not provide enough

females standardized for age on experimental days). Although this last experiment did not provide evidence that *D. semiclausum* improved its ability to discriminate at herbivore level as a result of associative learning, a preliminary experiment indicated that *D. semiclausum* could alter its behaviour at plant level. When either experience was given on *P. xylostella*-Feral *B. oleracea* or *P. xylostella*-Brussels sprouts, *D. semiclausum* showed a preference for the infested plant on which experience was given. It may be interesting to confirm these findings in further research (preferably conducted with both parasitoid species enabling to investigate inter- and intraspecific differences).

Herbivore damage

During preliminary tests on feeding damage a difference in feeding behaviour between the caterpillar species was observed. Gregariously feeding *P. brassicae* predominantly removed leaf tissue from the edges whereas solitary feeding *P. xylostella* punched holes leaving a buckshot pattern. Standardizing herbivore damage was initially done based on removed leaf tissue because the induced release of volatiles is not limited to the site of damage but can occur systemically (Dicke, 1999). However, because parasitoids were especially interested in the affected leaf edges when given experience, the impression occurred that the length of affected leaf area may influence the quantity of volatiles emitted by the plant. Therefore herbivore damage was measured both for the amounts of removed leaf tissue and the length of affected leaf area on the plants used in the last two experiments. Measurements showed that for comparable amounts of removed leaf tissue, the length of affected leaf area was significantly higher on *P. xylostella*-infested plants. However, when the results of the choice experiments per plant set were investigated in detail, no indication was found that parasitoid preferences were influenced by the differences measured.

Day-to-day variation in responsiveness

In a study, Steinberg et al. (1993) found that the direction of shifts in atmospheric pressure affects the responsiveness of *C. glomerata*, rather than pressure per se. Therefore in the study at hand a block design was used as recommended by Steinberg et al. (1993). Nevertheless on several experimental days the bioassays were ended prematurely because parasitoids showed no interest in the odour sources offered. Although not thoroughly investigated, changes in barometric pressure seem also to influence the responsiveness of both parasitoid species used in this study. However, no differences were observed between parasitoid species.

Behaviour of D. fenestrata

As mentioned only scarce literature can be found on *D. fenestrata* and therefore it is interesting to briefly pay attention to some observations on its behaviour, done during this research. Prior to this study it was unclear whether the chosen experimental setup would be appropriate to investigate the foraging behaviour of this parasitoid. Fortunately, in the olfactometer bioassays females behaved similar to females of *D. semiclausum* (in general walking towards odour sources, occasionally -restlessly- flying). On the other hand, handling *D. fenestrata* appeared to be more difficult (females seemed more tempestuous). For the bioassays were experience was required, *D. fenestrata* females took more time to parasitize the required amount of hosts and showed considerable less interest in the host-damaged leaves and the host itself than *D. semiclausum*. After an oviposition experience, females were inclined to leave the patch instantaneously and to remain on the ceiling of the Petri-dish. The latter may find its origin in the degree of specialization resulting in less adapted foraging behaviour towards *P. xylostella* (Vos et al, 1998; Wajnberg, 2003) and might also explain the difficulties with rearing females in ample amounts.

Acknowledgements

This thesis was the last part of my MSc study at the Wageningen University and Research Centre. For six months and the dust I worked at the Department of Entomology where I enjoyed the very pleasant atmosphere.

I would like to thank my supervisors, Roel Potting and Tibor Bukovinszky, for providing me excellent guidance and support throughout the whole research period. Their ‘quick and dirty’ comments as well as the profound discussions greatly improved my research skills and knowledge. Furthermore I would like to thank André Gidding and Leo Koopman for the maintenance of the plant/caterpillar/parasitoid rearings and their time spend explaining me many little practical matters concerning the materials I worked with.

Els, thanks for your patience and support. Koen, thanks for keeping me awake!

References

- Abbas, M. S. T. (1988). "Biological and Ecological-Studies on *Diadegma-Semiclausum* Hellen (Hym, Ichneumonidae), a Larval Parasite of the Diamond-Back Moth, *Plutella-Xylostella* (L) (Lep, Plutellidae) in Egypt." *Anzeiger Fur Schadlingskunde Pflanzenschutz Umweltschutz* 61(1): 1-2.
- Agelopoulos, N. G. and M. A. Keller (1994). "Plant Natural Enemy Association in the Tritrophic System *Cotesia-Rubecula-Pieris-Rapae-Brassicaceae* (Cruciferae) .2. Preference of *C-Rubecula* for Landing and Searching." *Journal of Chemical Ecology* 20(7): 1735-1748.
- Agelopoulos, N. G. and M. A. Keller (1994). "Plant Natural Enemy Association in Tritrophic System, *Cotesia-Rubecula-Pieris-Rapae-Brassicaceae* (Cruciferae) .3. Collection and Identification of Plant and Frass Volatiles." *Journal of Chemical Ecology* 20(8): 1955-1967.
- Azidah, A. A., M. G. Fitton, et al. (2000). "Identification of the *Diadegma* species (Hymenoptera : Ichneumonidae, Campopleginae) attacking the diamondback moth, *Plutella xylostella* (Lepidoptera : Plutellidae)." *Bulletin of Entomological Research* 90(5): 375-389.
- Blaakmeer, A., J. B. F. Geervliet, et al. (1994). "Comparative Headspace Analysis of Cabbage Plants Damaged by 2 Species of *Pieris* Caterpillars - Consequences for in-Flight Host Location by *Cotesia* Parasitoids." *Entomologia Experimentalis Et Applicata* 73(2): 175-182.
- Bukovinszky, T., R. Gols, et al. (2005). "Variation in plant volatiles and attraction of the parasitoid *Diadegma semiclausum* (Hellen)." *Journal of Chemical Ecology* 31(3): 461-480.
- De Moraes, C. M. and W. J. Lewis (1999). "Analyses of two parasitoids with convergent foraging strategies." *Journal of Insect Behavior* 12(5): 571-583.
- De Moraes, C. M., W. J. Lewis, et al. (1998). "Herbivore-infested plants selectively attract parasitoids." *Nature* 393(6685): 570-573.
- Dicke, M. (1999). "Are herbivore-induced plant volatiles reliable indicators of herbivore identity to foraging carnivorous arthropods?" *Entomologia Experimentalis Et Applicata* 91(1): 131-142.
- Dicke, M. and L. E. M. Vet (1999). Plant-carnivore interactions and ecological consequences for plant, herbivore and carnivore. *Herbivores: between plants and predators*. H. Olf, V. K. Brown and R. H. Drent. Oxford, UK, Blackwell Science Ltd.: 483-520.
- Douloupaka, S. and H. F. van Emden (2003). "A maternal influence on the conditioning to plant cues of *Aphidius colemani* Viereck, parasitizing the aphid *Myzus persicae* Sulzer." *Physiological Entomology* 28(2): 108-113.
- Van Emden, H. F., I. Eletherianos, et al. (2002). "Aphid parasitoids detect that an alien plant was present nearby during their development." *Physiological Entomology* 27(3): 199-205.
- Fitton, M. and A. Walker (1992). Hymenopterous parasitoids associated with diamondback moth: the taxonomic dilemma. *Diamondback Moth and other crucifer pests*, Tainan, Taiwan.
- Geervliet, J. B. F., M. A. Posthumus, et al. (1997). "Comparative analysis of headspace volatiles from different caterpillar-infested or uninfested food plants of *Pieris* species." *Journal of Chemical Ecology* 23(12): 2935-2954.
- Geervliet, J. B. F., L. E. M. Vet, et al. (1996). "Innate responses of the parasitoids *Cotesia glomerata* and *C-rubecula* (Hymenoptera: Braconidae) to volatiles from different plant-herbivore complexes." *Journal of Insect Behavior* 9(4): 525-538.

- Geervliet, J. B. F., A. I. Vreugdenhil, et al. (1998). "Learning to discriminate between infochemicals from different plant-host complexes by the parasitoids *Cotesia glomerata* and *C. rubecula*." *Entomologia Experimentalis Et Applicata* 86(3): 241-252.
- Godfray, H. C. J. (1994). *Parasitoids, Behavioral and Evolutionary Ecology*. Princeton, New Jersey, Princeton University press.
- Herard, F., M. A. Keller, et al. (1988). "Beneficial Arthropod Behavior Mediated by Airborne Semiochemicals .3. Influence of Age and Experience on Flight Chamber Responses of *Microplitis-Demolitor* Wilkinson (Hymenoptera, Braconidae)." *Journal of Chemical Ecology* 14(7): 1583-1596.
- Legaspi, B. A. C. (1986). "Host Discrimination in 2 Species of Ichneumonid Wasps, *Diadegma* Spp Attacking Larvae of *Plutella-Xylostella*." *Entomologia Experimentalis Et Applicata* 41(1): 79-82.
- Lewis, W. J., L. E. M. Vet, et al. (1990). "Variations in Parasitoid Foraging Behavior - Essential Element of a Sound Biological-Control Theory." *Environmental Entomology* 19(5): 1183-1193.
- Kalule, T. and D. J. Wright (2004). "The influence of cultivar and cultivar-aphid odours on the olfactory response of the parasitoid *Aphidius colemani*." *Journal of Applied Entomology* 128(2): 120-125.
- Mattiacci, L., M. Dicke, et al. (1994). "Induction of Parasitoid Attracting Synomone in Brussels-Sprouts Plants by Feeding of *Pieris-Brassicae* Larvae - Role of Mechanical Damage and Herbivore Elicitor." *Journal of Chemical Ecology* 20(9): 2229-2247.
- Ohara, Y., A. Takafuji, et al. (2003). "Response to host-infested plants in females of *Diadegma semiclausum* Hellen (Hymenoptera : Ichneumonidae)." *Applied Entomology and Zoology* 38(1): 157-162.
- Rosbach, A., B. Lohr, et al. (2005). "Generalism versus specialism: Responses of *Diadegma mollipla* (Holmgren) and *Diadegma semiclausum* (Hellen), to the host shift of the diamondback moth (*Plutella xylostella* L.) to peas." *Journal of Insect Behavior* 18(4): 491-503.
- Shiojiri, K., J. Takabayashi, et al. (2000). "Flight response of parasitoids toward plant-herbivore complexes: A comparative study of two parasitoid-herbivore systems on cabbage plants." *Applied Entomology and Zoology* 35(1): 87-92.
- Shiojiri, K., J. Takabayashi, et al. (2001). "Infochemically mediated tritrophic interaction webs on cabbage plants." *Population Ecology* 43(1): 23-29.
- Steinberg, S., M. Dicke, et al. (1993). "Relative Importance of Infochemicals from 1st and 2nd Trophic Level in Long-Range Host Location by the Larval Parasitoid *Cotesia-Glomerata*." *Journal of Chemical Ecology* 19(1): 47-59.
- Takabayashi, e. a. (1991). "Variation in composition of predator-attracting alleochemicals emitted by herbivore-invested plant: relative influence of plant and herbivore." *Chemoecology*(2): 1-6.
- Takabayashi, J., S. Takahashi, et al. (1995). "Developmental Stage of Herbivore *Pseudaletia-Separata* Affects Production of Herbivore-Induced Synomone by Corn Plants." *Journal of Chemical Ecology* 21(3): 273-287.
- Talekar, N. S. and A. M. Shelton (1993). "Biology, Ecology, and Management of the Diamondback Moth." *Annual Review of Entomology* 38: 275-301.
- Talekar, N. S. and J. C. Yang (1991). "Characteristic of Parasitism of Diamondback Moth by 2 Larval Parasites." *Entomophaga* 36(1): 95-104.
- Turlings, T. C. J., J. H. Tumlinson, et al. (1991). "Larval-Damaged Plants - Source of Volatile Synomones That Guide the Parasitoid *Cotesia-Marginiventris* to the Microhabitat of Its Hosts." *Entomologia Experimentalis Et Applicata* 58(1): 75-82.

- Vet, L. E. M. and M. Dicke (1992). "Ecology of Infochemical Use by Natural Enemies in a Tritrophic Context." *Annual Review of Entomology* 37: 141-172.
- Vet, L. E. M., W. J. Lewis, et al. (1995). Parasitoid foraging and learning. In: *Chemical ecology of insects*, Chapman and Hall: 65-101.
- Vet, L. E. M., W. J. Lewis, et al. (1990). "A Variable-Response Model for Parasitoid Foraging Behavior." *Journal of Insect Behavior* 3(4): 471-490.
- Vet, L. E. M., F. L. Wackers, et al. (1991). "How to Hunt for Hiding Hosts - the Reliability-Detectability Problem in Foraging Parasitoids." *Netherlands Journal of Zoology* 41(2-3): 202-213.
- Vos, M., L. Hemerik, et al. (1998). "Patch exploitation by the parasitoids *Cotesia rubecula* and *Cotesia glomerata* in multi-patch environments with different host distributions." *Journal of Animal Ecology* 67(5): 774-783.
- Wajnberg, E., P. A. Gonsard, et al. (2003). "A comparative analysis of patch-leaving decision rules in a parasitoid family." *Journal of Animal Ecology* 72(4): 618-626.
- Wang, X. G. and M. A. Keller (2002). "A comparison of the host-searching efficiency of two larval parasitoids of *Plutella xylostella*." *Ecological Entomology* 27(1): 105-114.
- Wiskerke, J. S. C. and L. E. M. Vet (1994). "Foraging for Solitarily and Gregariously Feeding Caterpillars - a Comparison of 2 Related Parasitoid Species (Hymenoptera, Braconidae)." *Journal of Insect Behavior* 7(5): 585-603.