

## The Response Specificity of *Trichogramma* Egg Parasitoids towards Infochemicals during Host Location

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*Parasitoids are confronted with many different infochemicals of their hosts and food plants during host selection. Here, we investigated the effect of kairomones from the adult host *Pieris brassicae* and of cues present on Brussels sprout plants infested by *P. brassicae* eggs on the behavioral response of the egg parasitoid *Trichogramma evanescens*. Additionally, we tested whether the parasitoid's acceptance of *P. brassicae* eggs changes with different host ages. The wasps did not discriminate between olfactory cues from mated and virgin females or between mated females and males of *P. brassicae*. *T. evanescens* randomly climbed on the butterflies, showing a phoretic behavior without any preference for a certain sex. The parasitoid was arrested on leaf parts next to 1-day-old host egg masses. This arrestment might be due to cues deposited during oviposition. The wasps parasitized host eggs up to 3 days old equally well. Our results were compared with former studies on responses by *T. brassicae* showing that *T. evanescens* makes less use of infochemicals from *P. brassicae* than *T. brassicae*.*

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**KEY WORDS:** host finding; oviposition-induced plant synomone; phoresy; Brussels sprout plants; *Pieris brassicae*.

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## INTRODUCTION

Chemical cues play a major role in the process of host selection by parasitoids (Vinson, 1991), which has been divided into several steps such as habitat location, host location, and host acceptance. Variation in host location ability could be a major constraint for the performance of parasitoids used as pest-control agents (Lewis *et al.*, 2003).

Egg parasitoids of herbivorous insects are known to use a wide range of chemicals for host selection, i.e., cues emitted by plants with and without host eggs, cues released by host adults, specifically the egg-laying female, and infochemicals of host eggs (Romeis *et al.*, 2005; Rutledge, 1996). *Trichogramma* egg parasitoids are considered efficient biological control agents and are used worldwide for control of lepidopteran pests in many crops (Smith, 1996; Wajnberg and Hassan, 1994). They are regarded as relatively polyphagous and therefore less host specific than specialist egg parasitoids such as some *Telenomus* spp. (Pinto and Stouthamer, 1994). However, their suitability as biological control agents may vary due to considerable inter- and intraspecific variations in tolerance to environmental conditions, preference for hosts, recognition and acceptance of crops and host searching behavior (Pak, 1988; van Dijken *et al.*, 1986; Wajnberg and Hassan, 1994). The ability to select and accept different host species may differ between *Trichogramma* strains and species (Pak *et al.*, 1986; Pak and De Jong, 1987; Pak *et al.*, 1990). Evidence is accumulating that *Trichogramma* spp. show high habitat loyalty, thus resulting in development of preferences for specific hosts and specific plants in the respective habitat (see Romeis *et al.*, 2005 and references therein).

Here, we investigated a tritrophic system consisting of Brussels sprout plants (*Brassica oleracea* var. *gemmifera* cv. Cyrus), the large cabbage white butterfly *Pieris brassicae* L. (Lepidoptera: Pieridae), and the egg parasitoid *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae).

Egg deposition by *P. brassicae* has shown to induce changes in the plant surface chemistry of Brussels sprout plants after 3 days that result in the arrestment of females of *T. brassicae* Bezdenko (Fatouros *et al.*, 2005a). Three-day-old eggs of *P. brassicae* were most successfully parasitized by *T. brassicae* (Fatouros *et al.*, 2005a). While egg deposition by *P. brassicae* induced synomones perceived by contact, no evidence was found that *T. brassicae* is attracted to oviposition-induced plant volatiles (Fatouros *et al.*, 2005a), as is known for several other egg parasitoids of herbivorous insects (Hilker and Meiners, 2002; Hilker and Meiners, 2006). However, volatiles from the adult hosts were shown to arrest *T. brassicae*. After locating mated *P. brassicae* females by the anti-aphrodisiac, benzyl cyanide, the

wasps explicitly mount them and hitchhike with them to the host plant (i.e. phoresy) (Fatouros *et al.*, 2005b).

The studied *T. evanescens* population was collected on *Mamestra brassicae* L. eggs (Lepidoptera: Noctuidae) found on *Brassica nigra*. Several strains of *T. evanescens* have been tested on their host acceptance for the three most harmful lepidopteran pest species in cabbage, i.e. *Mamestra brassicae*, *Pieris brassicae*, and *P. rapae* (van Dijken *et al.*, 1986). The infochemicals known to be used by *T. evanescens* for host location of these cabbage pest species are: the host sex pheromone of *M. brassicae* and volatiles from virgin *P. brassicae* females (Noldus and van Lenteren, 1985a), cues of their wing scales or other contact cues like chemicals from the host accessory gland (Noldus and van Lenteren, 1985b). However, the identity of the used *Trichogramma* spp. was later revised to *T. maidis* (= *T. brassicae*) (Noldus, 1989), which makes a comparison to these results difficult. Here, we used a population that was clearly identified as *T. evanescens* by internally transcribed spacer 2 (ITS2) DNA sequences (Stouthamer *et al.*, 1999).

In the present study the response of the field-collected *T. evanescens* to specific host cues of the adult large cabbage white butterfly and to cues associated with an egg deposition on the plant, as well as their acceptance to host eggs of different ages were tested. These responses were compared to our earlier study on *T. brassicae* (Table I). Since *T. evanescens* was found in *M. brassicae* eggs, we hypothesize that *T. evanescens* is less host specific

**Table I.** Response of Females of *T. brassicae* and *T. evanescens* to Infochemicals of Their Host *P. brassicae* and Brussels Sprout Plants Infested with *P. brassicae* Eggs

Infochemical	Source		Response	
			<i>T. brassicae</i>	<i>T. evanescens</i>
Volatile	Plant	Egg-laden (24–72 h)	No response <sup>a</sup>	<i>Not tested</i>
		Host	Virgin female	
	Mated female		Arrestment <sup>b</sup>	
	Males		Arrestment <sup>b</sup>	
Contact /close range	Plant	Egg-laden (up to 24 h)	Arrestment <sup>a</sup>	Arrestment
		Egg-laden (72–96 h)	Arrestment <sup>a</sup>	
	Host	Virgin female	Not attractive <sup>b</sup>	No discrimination
		Mated female	Mounting <sup>b</sup>	
		Males	Not attractive <sup>b</sup>	
		Scales/deposits (24-h-old)	Arrestment <sup>a</sup>	
Scales/deposits (72-h-old)	No response <sup>a</sup>	<i>Not tested</i>		

<sup>a</sup> Fatouros *et al.* 2005a.

<sup>b</sup> Fatouros *et al.* 2005b.

in its response to infochemicals of *P. brassicae* than *T. brassicae* that was tested previously (Fatouros *et al.*, 2005a).

## MATERIAL AND METHODS

### Plants and Herbivores

Brussels sprout plants were reared in a greenhouse ( $18 \pm 2^\circ\text{C}$ , 70% rh, L16:D8). Plants (8–12 weeks old) with ca. 14–16 leaves were used for rearing of *Pieris brassicae* and for the experiments.

*Pieris brassicae* was reared on Brussels sprout plants in a climate room ( $21 \pm 1^\circ\text{C}$ , 50–70% r.h., L16:D8). Each day a plant was placed into a large cage ( $80 \times 100 \times 80$  cm) with more than 100 adults for approximately 24 hours to allow egg deposition.

### Parasitoids

*Trichogramma evanescens* Westwood were collected from the field in *Mamestra brassicae* eggs in 2003 and reared since then in eggs of *P. brassicae* ( $25^\circ\text{C}$ , 50–70% rh, L16:D8). For the rearing, 1–3-day-old *P. brassicae* eggs on leaves were used. Naïve wasps (no oviposition experience) were used for the host location tests with the adult hosts and for the host-age suitability tests. All two-choice contact bioassays with leaf disks were conducted with oviposition-experienced female wasps, because naïve *Trichogramma* wasps were shown to have a low response level toward plant cues (Fatouros *et al.*, 2005a). An oviposition experience was given for a period of 18 h prior to the experiment with 2–3-day-old *P. brassicae* eggs deposited on Brussels sprout leaves. All wasps were mated and about 2–5 days old when tested. They were always provided with honey solution prior to the experiment.

### Host Suitability: Oviposition Tests with Different Host Ages

*P. brassicae* eggs of 5 different ages (<12, 24, 48, 72 and 96 h) were offered on a leaf piece (ca.  $1 \text{ cm}^2$ ) excised from egg-carrying plants (see procedure of egg infestation as described for the contact bioassays) to 1-day-old mated females of *T. evanescens*, which had had no previous contact to host eggs. An egg clutch consisting of 15 eggs of the same age was offered for a period of 24 h to a female confined in a glass vial ( $7 \times 1$  cm). After that period the wasp was removed from the vial. Eight females were

tested per host age and parasitoid species. When the eggs turned black (approximately 4 days after exposure to the parasitoids), the number of parasitized eggs was counted. Each parasitized egg was isolated and individually kept in a gelatin capsule to determine the number of emerging wasps.

### **Host Location: Effect of Contact Plant Cues From Egg-Carrying Plants**

For the experiments, test plants were placed into the cage with more than 100 *P. brassicae* adults to allow deposition of eggs, wing scales, and host odors onto the plants for a period of 8 h. After this exposure to the butterflies the treated plants were either tested immediately or were kept in a climate chamber ( $21 \pm 2^\circ\text{C}$ , 70% rh, L16:D8) for 1–3 days longer. Thus, the period during which eggs or butterfly deposits were on the cabbage plant ranged from less than 12 h, to 24, 48, 72 or 96 h (compare Fatouros *et al.*, 2005a). Control plants had never been in contact with *P. brassicae* or any other insect. They were grown under the same abiotic conditions as treated plants.

A test leaf square (2 cm<sup>2</sup>) was cut from an egg-laden treated plant right next to an egg mass (3–5 cm away from the eggs). Such a test leaf square could elicit arrestment of wasps by either local oviposition-induced plant surface cues or by butterfly deposits such as scales that are always present close to eggs (Fatouros *et al.*, 2005a). A control square of the same size was cut from a leaf of corresponding position relative to the topmost leaves. A wasp was released in the center of a small glass Petri dish (5.5 cm diameter) lined with filter paper and simultaneously offered a test and a control leaf square. The total duration of time spent in the arena was observed for a period of 300 sec using The Observer software 3.0 (Noldus Information Technology 1993<sup>®</sup>). The time spent searching in the area outside the leaf squares was scored as “no response.” Ten wasps were tested per day and plant. Test and control squares were changed after having tested 3 wasps. Each wasp was used only once and was then discarded.

### **Host Location: Olfactometer Bioassays with Butterfly Odors**

The experiments were carried out in a two-chamber olfactometer described in detail by Fatouros *et al.* (2005a). This olfactometer was a modified version of the four-chamber olfactometer of Steidle and Schöller (1997). The time spent by the wasps in one of the two odor fields was observed for 300 sec. Two butterflies were introduced per chamber as odor source. A number of 10–15 naïve wasps was tested per day. To avoid biased results

due to positional preferences of the parasitoids, the olfactometer was rotated 180° after every third insect. The response of *T. evanescens* to odor of the following *P. brassicae* combinations was tested: a) virgin females vs. mated females and b) mated females vs. males. A total number of 40 wasps per combination was tested.

### Host Location: Mounting Bioassays

The selective mounting behavior of *T. evanescens* females was tested in a two-choice bioassay conducted at  $23 \pm 2^\circ\text{C}$  in a plastic container (9 cm high, 13.5 cm diameter). Two adults of *P. brassicae* were placed in the arena after cooling them down in a refrigerator ( $6^\circ\text{C}$ ) for ca. 10 min to decrease their mobility. A naïve *T. evanescens* female was introduced and then continuously observed till it made a first mount on one of the two butterflies. When a wasp did not choose for one of the two butterflies by mounting it within 5 min, this was recorded as “no response.” The following butterfly combinations were tested: (a) mated females vs. males and (b) mated females vs. virgin females of *P. brassicae*. After each 10th wasp the butterflies were replaced by new ones. For each combination, 40 wasps were tested. Virgin female butterflies were obtained by separating pupae. Mated females had been observed to mate before they were used for the bioassay.

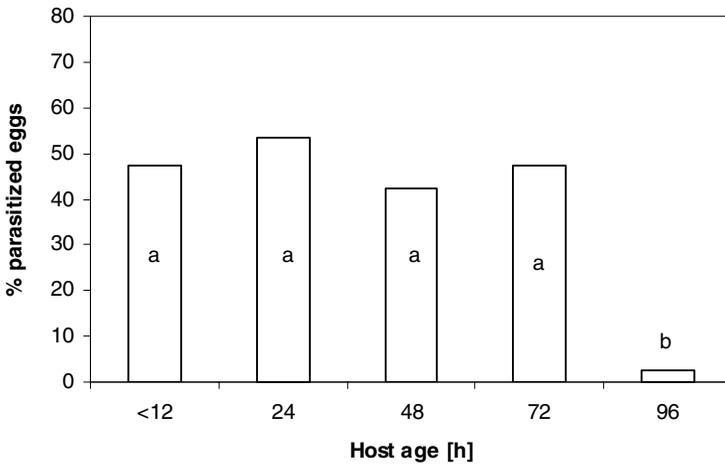
### Statistics

Both the contact and the olfactometer bioassay were analyzed using Wilcoxon’s matched pairs signed rank test. A  $\chi^2$ -test was used to analyze the choices in the mounting bioassay. Parasitism rates for different host ages were analyzed with  $5 \times 2$  contingency tables, and individual  $\chi^2$ -tests were carried out, corrected by the sequential Bonferroni procedure for table-wide  $\alpha$  levels.

## RESULTS

### Host-Age Suitability

Wasps of *T. evanescens* showed a preference for host eggs 12–72 h old. About 50% of the offered eggs were parasitized. However, eggs that were 96 h old were unsuitable for the wasps showing a very low parasitization rate (Fig. 1,  $5 \times 2$  contingency test,  $df = 4$ ,  $P < 0.001$ ).



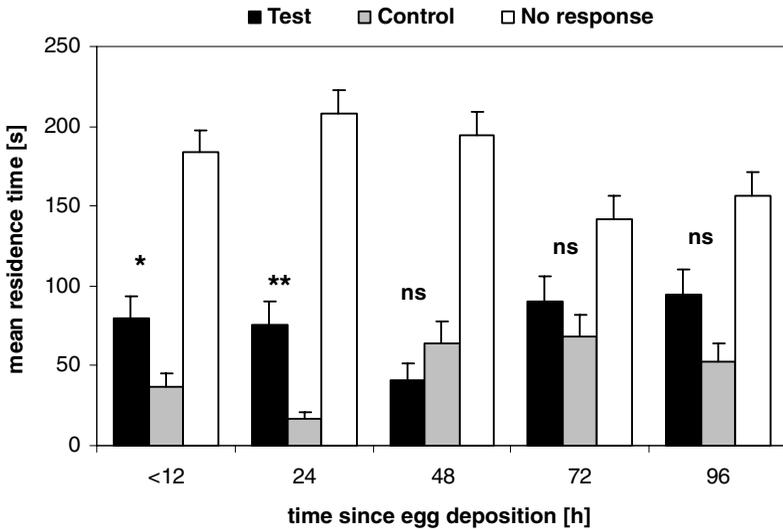
**Fig. 1.** Host-age suitability of eggs from *Pieris brassicae* for *Trichogramma evanescens*. 15 eggs of different ages (<12, 24, 48, 72, 96 h) were offered on leaf pieces to 1-day old parasitoid females for 24 h. Per egg age a number of 8 females was tested. Different letters in the columns indicate significant differences ( $P < 0.05$ ) ( $5 \times 2$  contingency tables using  $\chi^2$ ).

### Effect of Contact Plant Cues From Egg-Carrying Plants

In order to examine whether plant surface chemicals in the vicinity of an egg mass serve as cues that indicate the close-by host eggs, leaf squares were excised right next to an egg mass and offered together with a leaf square from an egg-free "clean" leaf from a control plant. The leaf square from a plant, on which eggs had been deposited <12 h prior to the assay significantly arrested *T. evanescens* (Fig. 2,  $P = 0.03$ , Wilcoxon's matched pairs test). The same behavior was observed when leaf squares were offered from plants, on which eggs had been deposited 24 h prior to the assay. The wasps were significantly arrested on the test square (Fig. 2,  $P = 0.01$ , Wilcoxon's matched pairs test). Females of *T. evanescens* were not arrested on leaf squares cut from an egg-carrying leaf, on which eggs had been deposited 48 h or longer prior to the assay. In all experiments, the wasps showed a relatively low response and spent about 50% or more of the total time in the "no response" area in all tested treatments (Fig. 2).

### Response to Butterfly Odors

In two-choice olfactory bioassays the *T. evanescens* wasps showed no preferences for odors of *P. brassicae* females or males (Fig. 3). When odor



**Fig. 2.** Contact 2-choice bioassay. Response of *Trichogramma evanescens* females to test leaf parts right excised from a leaf next to an egg mass deposited <12, 24, 48, 72, 96 h prior to the bioassay (black bars) and to egg-free control leaf parts (grey bars). White bars: Time spent by wasps on bioassay surface other than leaves (i.e. “no response”). Number of tested females per treatment  $N = 50$ . Mean residence time and standard error are shown. Asterisks indicate significant differences between test and control within the same treatment \* $P < 0.05$ , \*\* $P < 0.01$ , ns, not significant (Wilcoxon’s matched pairs signed rank test).

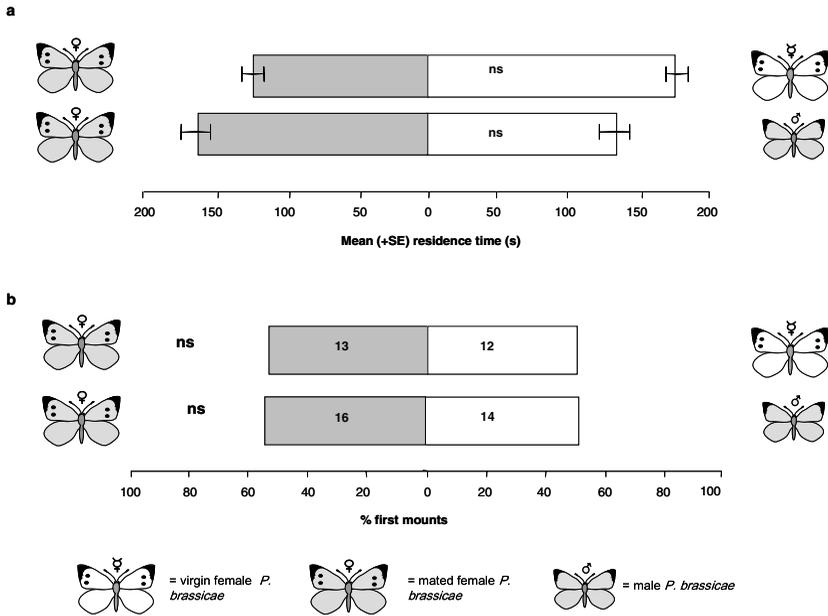
from virgin female butterflies was offered against odor of mated female butterflies, the wasps did not show any preference either.

### Mounting of Butterflies

We exposed adult butterflies to female wasps in two-choice bioassays. Indeed, the wasps mounted adult butterflies. However, the *T. evanescens* did not discriminate between mated females, virgin female or male butterflies (Fig. 3).

## DISCUSSION

*Trichogramma evanescens* did not show any preference for volatile cues emitted by adult *P. brassicae*. They showed phoretic behavior by climbing onto the butterflies but did not discriminate between males and females, or mated and virgin females. Since such a mounting behavior



**Fig. 3.** Response of *T. evanescens* wasps to cues from adult *P. brassicae* butterflies. **(a).** Mean residence time ( $\pm$  SE) spent by wasps in test and control fields of a two-chamber olfactometer;  $N = 40$  tested wasps per experiment; ns, not significant (Wilcoxon's matched pairs signed ranks test). **(b).** Proportion of first mounts (%) of wasps on adult *P. brassicae* butterflies. The number of responding wasps is shown inside each bar;  $N = 40$  wasps tested per combination; ns, not significant ( $\chi^2$ -Test).

involves some risk from the butterfly defending itself (N. Fatouros, personal observations), it is highly unlikely that this behavior just coincidentally happened. Some phoretic egg parasitoids are known to mount also male hosts and then often transfer during mating to the host female (Bruni *et al.*, 2000; Fatouros, 2006). Our findings suggest that *T. evanescens* may be phoretic on males as well and then shifts to females during mating, which would be less efficient but not maladaptive. Leaf disks of egg-laden plants arrested the wasps until 24 h after egg deposition. Here, a leaf contamination with host cues like wing scales, always deposited in the vicinity of eggs, could have caused this increased searching behavior. An arrestment to such deposits on a host plant was shown for *T. brassicae* (Fatouros *et al.*, 2005a). Jones *et al.* (1973) showed that *T. evanescens* is able to detect tricosane from adult host scales (*Heliothis zea*), which stimulates parasitization. In our study, leaf disks from plants with eggs and deposits older than 24 h did not arrest the wasps. Host eggs that had been offered to the wasps were

equally parasitized until the age of 72 h, indicating that other cues than the tested contact cues on the plant surface play a role as well.

There are interesting parallels and differences between the responses of *T. evanescens* and *T. brassicae* to egg-laden leaves and host cues (compare Table 1):

- (a) Like *T. evanescens*, *T. brassicae* is arrested on leaf areas right next to *P. brassicae* eggs. The arrestment response vanishes 24 h after egg deposition in both *T. brassicae* and *T. evanescens* (Fatouros *et al.*, 2005a).
- (b) However, 72 h after egg deposition, *T. brassicae* was again arrested on leaf area next to an egg mass. A series of different experiments indicated that the eggs of *P. brassicae* induce a change in the leaf surface of Brussels sprout plants arresting *T. brassicae* wasps at a time when eggs are most suitable for parasitization, i.e. 72 h after egg deposition (Fatouros *et al.*, 2005a). Such a response to oviposition-induced plant surface changes has not been observed in *T. evanescens*.
- (c) *T. evanescens* did not show a preference for mated females. In contrast, *T. brassicae* preferred mated over virgin females, which was mediated by benzyl cyanide, the anti-aphrodisiac of *P. brassicae* that is transferred from males to females during mating to render the females less attractive to conspecific males (Andersson *et al.*, 2003).

Even though both *T. evanescens* and *T. brassicae* are known as host generalists, at least from laboratory studies, that readily accept *P. brassicae* as host, we found such species specific differences in their responsiveness to host cues relevant for host location. How to explain these differences?

*T. evanescens* has been collected from *Mamestra brassicae* eggs on *Brassica nigra* and was reared for a shorter period on *P. brassicae* than *T. brassicae*. The latter was collected from *E. kuehniella* eggs placed on cardboard cards in a cabbage garden. Even though the original host of *T. brassicae* is unknown, the longer rearing period on *P. brassicae* might have resulted in a stronger adaptation to cues of *P. brassicae* or plant cues induced by this species. The longer rearing period might have caused a stronger selection of those *T. brassicae* individuals that are most sensitive to *P. brassicae* cues. Kaiser *et al.* (1989) showed that the affinity for an initially non-preferred host was enhanced when *Trichogramma* was reared on this species. Such an enhancement might also be due to pre-imaginal conditioning (Bjorksten and Hoffmann, 1995; Kaiser *et al.*, 1989) or adult-experience (Bjorksten and Hoffmann, 1998; Kaiser *et al.*, 1989; van Dijken *et al.*, 1986), if rearing periods are too short to allow selective effects. However, this is not the case when comparing *T. evanescens* and *T. brassicae*. The latter

species was reared at least for 30 generations longer on *P. brassicae*. When studying the selective power of rearing conditions, already Pimentel *et al.* (1967) could show that oviposition preferences may shift more and more to a new host with an increasing number of generations reared on this new host.

However, host preferences of generalist parasitoids may also be stable at varying rearing conditions, indicating very conservative and robust sensitivities to host cues. *T. maidis* (= *T. brassicae*) had a greater spontaneous affinity for *Ostrinia nubilalis* than for *Ephestia kuehniella* with respect to their host acceptance (Kaiser *et al.*, 1989). This preference was based on conservative genetic determination, because it survived the rearing of the parasitoid for more than 100 generations on the non-preferred host. Similar results were observed for other *Trichogramma* spp. as well (Pak, 1988). Chemical parameters, like surface kairomones, play a role in the specific recognition of host eggs. Thus, *Trichogramma* spp. may have an innate ability to use specific kairomones of certain hosts during the host selection procedure.

There is growing evidence for *Trichogramma* spp. “being more prevalent in certain habitats or on specific plants” (Romeis *et al.* 2005). Such a habitat/plant loyalty might be due to their limited moving abilities. Like the effects of laboratory rearing, habitat/plant loyalty might change the wasps responsiveness to certain host and plant cues. Egg parasitoids living in monocultures or habitats of little biodiversity would encounter only a small range of host species when they do not leave these sites. Therefore, some *Trichogramma* spp. with high habitat/plant loyalty might show a higher specificity with respect to the infochemicals necessary for host location than generally assumed. They could still keep the ability to parasitize most host egg species as soon as encountered, so that they remain generalists with respect to the host acceptance.

The response of closely related parasitoid species to infochemicals of the same host has been compared only in a few studies. Bruni *et al.* (2000) tested the kairomonal activity of the attractant pheromone of the spined soldier bug, *Podisus maculiventris* (Hemiptera: Pentatomidae) on two *Telenomus* species, one a generalist egg parasitoid of pentatomids (*T. podisi*) and the other a phoretic specialist on *Podisus* eggs (*T. calvus*). There was no evidence that *T. podisi* uses the pheromone as a kairomone. In contrast, *T. calvus* females oriented to the volatile chemicals of their host, to find areas likely to contain hosts and to use the pheromone to guide their phoretic behavior. Here, the dietary breadths of the two *Telenomus* wasps are different and were likely to explain their different response toward the specific host cue.

In conclusion, our results do not provide evidence that *T. evanescens* responds to volatile infochemicals released from adult *P. brassicae* or from leaves with eggs of *P. brassicae*. However, *T. evanescens* does accept *P. brassicae* eggs for parasitization. This non-response to volatile infochemicals of *P. brassicae* and its host plant Brussels sprouts contrasts to the response of the closely related *T. brassicae*, which was shown to innately use the anti-aphrodisiac benzyl cyanide of mated *P. brassicae* females and to learn oviposition-induced plant cues after landing on the host plant (Fatouros *et al.*, 2005a; 2005b). Thus, the two polyphagous egg parasitoids with very similar host ranges respond very differently to host and plant infochemicals. Further studies are needed to elucidate whether these differences are specific for a *Trichogramma* species or whether such differences can be detected even between *Trichogramma* populations with high, but different habitat/plant loyalty.

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