



## Research Article

## Recognition of native host volatiles by laboratory reared *Trichogramma chilonis* Ishii

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**ABSTRACT:** It is well known that many trichogrammatids use volatiles from hosts or host plants during host location. When such parasitoids are reared continuously on factitious hosts in the laboratory it is believed that they may lose their ability to recognize the volatiles from their native hosts or change their host preference. Hence, this study was carried out to investigate whether *Trichogramma chilonis* Ishii reared on *Corcyra cephalonica* (Stainton) was able to recognize the volatiles of two of its native hosts in sugarcane, viz., *Chilo infuscatellus* Snellen and *Chilo sacchariphagus indicus* (Kapur). The per cent parasitoids responding to the olfactory cues, namely, the hexane-washings of eggs, scales, adult body and pheromone lures of target hosts, was assessed in no-choice and dual-choice bioassays in a “Y”-tube olfactometer. For comparison, *C. cephalonica* washings were used. The results indicated attraction of *T. chilonis* to volatiles from washings of all host sources at different levels with highest per cent parasitoids attracted to *C. sacchariphagus indicus* egg washings. Overlapping significant levels of attraction was found with the scale and adult body washings of fresh mated moths of all three species in no-choice tests, while *C. sacchariphagus indicus* was the most preferred in dual-choice tests. Volatiles from male moths were more attractive to *T. chilonis* than those from female moths. The parasitoids failed to respond to the body washings of >72h old moths of all the three species. Attraction of *T. chilonis* to the synthetic sex pheromone lures of two native hosts was observed but the parasitoid did not discriminate between them. These findings suggest that though reared on a factitious host, *T. chilonis* is able to recognize and respond to the native host cues and there is no change in the innate preference of the parasitoid which may have to be further confirmed with parasitism studies.

**KEY WORDS:** *Trichogramma chilonis*, *Chilo sacchariphagus indicus*, *Chilo infuscatellus*, *Corcyra cephalonica*, host volatiles, olfactometer

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### INTRODUCTION

World wide *Trichogramma* egg parasitoids are used for management of many lepidopterous pests. Among the factors that decide the field efficacy of an egg parasitoid, its competence to locate the host eggs is the most important. To facilitate host location, various so-called infochemical detour strategies have been developed by egg parasitoids (Fatouros *et al.*, 2008) of which chemical signals associated with hosts and host plants are vital. Though these cues are not directly associated with the attacked host stage, they help the parasitoids in restricting the search area (Colazza *et al.*, 1997). While many species of egg parasitoids have been observed to be attracted to aggregation pheromones, sex pheromones or virgin males of their hosts (Colazza *et al.*, 1997; Hardie *et al.*, 1994; Kennedy, 1979; McClain *et al.*, 1990; Rice and Jones, 1982; Rutledge, 1996), differential response of trichogrammatids being either repelled by or attracted or unresponsive to a wide range of host and host plant-derived volatiles has been noted

by several workers (Ananthakrishnan *et al.*, 1991; Bakthavatsalam *et al.*, 2006 ; Fatouros *et al.*, 2005b; Fatouros *et al.*, 2007; Gardner *et al.*, 2007; Geetha, 2009; Milonas *et al.*, 2009; Noldus and van Lenteren, 1985; Romeis *et al.*, 2005; Usharani *et al.*, 2007) .

The host volatiles proven to be attractive to one species of *Trichogramma* need not necessarily be so for another though both species parasitize the same host. For instance, *Trichogramma evanescens* had been indifferent to the volatiles from *Pieris brassicae* adults, while *Trichogramma brassicae* was found to be arrested though both accepted *P. brassicae* as host. Longer period of rearing of the latter on *P. brassicae* was cited as the reason for its adaptation to the cues from such host (Fatouros *et al.*, 2007) and for resultant variations in response. Similarly a species of parasitoid might have preference among its hosts which may depend on host attraction or suitability or learning effect. Rearing *Trichogramma* on a non-preferred host can often improve

the attraction of the parasitoid to such a host (Kaiser *et al.*, 1989). Further, previous exposure either in pre-imaginal stage or during adult life can influence the preference by *Trichogramma* (Bjorksten and Hoffman, 1995, 1998; Kaiser *et al.*, 1989). It is also felt that when reared on factitious hosts in the laboratory, the parasitoids tend to adapt to such hosts and ambience (Takada *et al.*, 2001) and during inundative releases this may result in a certain loss of ability to spot cues from original hosts and host plants leading to less-than-expected performance in the field.

In India *Trichogramma chilonis* is mass produced mainly on a factitious host, *Corcyra cephalonica* (Stainton) and used extensively in south India for management of internode borer, *Chilo sacchariphagus indicus* (Kapur) in sugarcane. It is possible that these parasitoids reared for generations continuously on the factitious host may be so adapted to that host that the actual hosts may no longer be attractive to or recognized by the parasitoid. Hence, in the present study, the host recognition ability and shift if any, in host preference of the laboratory population of *T. chilonis* reared on *C. cephalonica* were examined through their response to the volatiles from two of its native hosts, *viz.*, sugarcane shoot borer, *Chilo infuscatellus* Snellen and internode borer in a “Y” tube olfactometer and compared with its response to the factitious host, *C. cephalonica*. This information is crucial not only for understanding of learning behaviour but also the quality of natural enemies when reared on a factitious host (van Lenteren, 2003) for use in field releases against native hosts.

## MATERIALS AND METHODS

A laboratory population of *T. chilonis* maintained on UV-sterilized, 1–2 days old eggs of factitious host, *C. cephalonica* for many years at Sugarcane Breeding Institute, Coimbatore, was used in this study. Naïve mated wasps less than 24h old, which did not have oviposition experience were used for all the bioassays with olfactometer. *C. cephalonica* eggs, scales and adults were derived from the moths obtained from laboratory culture maintained on broken pearl millet diet ( $28 \pm 2^\circ\text{C}$ , 50–60% RH). The original populations of the two native hosts, *viz.*, *Chilo infuscatellus* (SB) and *C. sacchariphagus indicus* (INB) were collected from sugarcane fields as larvae and reared to adults on shoot bits and were mated to harvest the host eggs. One set of such eggs was used to raise larvae which were reared to adults on a semi-synthetic diet, for collection of scales and whole body washings. On emergence the adult moths of the test species were segregated further based on sex for olfactometer studies.

The response of *T. chilonis* to volatiles from i) egg washings, ii) scales, iii) whole body of males and females,

iv) fresh mated moths, and v) more than 72 hrs old moths of the three test species was observed in no-choice and dual-choice tests in a “Y”-tube olfactometer in the combinations shown in Table 1. A separate set of tests involving only the native host sex pheromones also was conducted using commercial pheromone lures both in no-choice as well as dual-choice conditions.

**Table 1. Combinations of host volatiles (washings of egg / scales / whole body / fresh mated moths / >72h old moths) tested in Y-tube olfactometer for response of *T. chilonis***

Arm 1	Arm 2
No-choice test	
<i>C. infuscatellus</i> (SB)	Hexane
<i>C. sacchariphagus indicus</i> (INB)	Hexane
<i>C. cephalonica</i>	Hexane
Dual-choice tests	
<i>C. infuscatellus</i> (SB)	INB
<i>C. infuscatellus</i> (SB)	<i>C. cephalonica</i>
<i>C. sacchariphagus indicus</i> (INB)	<i>C. cephalonica</i>

The host washings of each category tested were prepared by soaking the i) eggs (approximately 2500 eggs), ii) scales of 25 adult moths, and iii) whole body of 25 adult moths of respective gender/age of the target species, separately in glass vials in 10ml of hexane at  $4^\circ\text{C}$  for 24 hrs. The volatiles from scales were obtained by brushing the body and wings into hexane in the glass vial. Adult moths were used either in fresh mated condition (less than 48 h old) or aged condition (>72hrs old) for assessment of their effect on *T. chilonis*. Hexane was used as it is a non-polar solvent which can dissolve the short range volatiles involved in host location. Pheromone lures of shoot borer and INB were obtained from M/s Rajshree Sugars, Theni, and stored in a freezer till use for the studies. As known, in the pheromone lures, sex pheromones are embedded in rubber septa which were used as a source of attraction for tests.

The experiments were carried out in a “Y”-tube olfactometer made of glass. The olfactometer had two arms of equal length (10 cm with a diameter of 5.5 cm) separated from a single basal arm of 18.5cm length. The hexane washings of host(s) were introduced in the arms of the olfactometer to be odour cue(s) as 10ml solution spotted on 1 cm<sup>2</sup> Whatman’s No. 42 paper strips. One of the two arms of the “Y”-tube olfactometer contained the host volatile while the other arm contained only hexane in no-choice tests whereas both the arms of the olfactometer contained volatiles from two different hosts in dual-choice

tests. Both the arms were closed at the end with rubber corks. Free wind flow in the arms through a carbon filter was maintained with an air pump powered by a motor from an air compressor (pressure cut off at 4 kg cm<sup>-1</sup>).

The wasps were observed for 5 min. for their decision to move towards either of the odour fields in the two arms or to remain in the basal arm. Parasitoids moving to either of the arms were accounted to be attracted correspondingly while the wasps remaining in the basal arm were considered to be indecisive. At least 50 wasps (for tests with egg volatiles) and 75 wasps (for tests with other volatiles) were tested for each observation and 20 such observations formed 20 replications for each volatile tested.

From the data obtained from these experiments, two separate comparisons were made statistically: i) to test whether there is acceptance / attraction to a specific volatile source, and ii) to compare the performance of each source when offered in no-choice and dual-choice conditions, and to identify the most attractive kairomone source of the three hosts (pooled analysis).

The per cent parasitoids that moved towards an odour source / hexane or remained unresponsive was calculated by the following formula:

$$\text{Percentage of parasitoids moved} = \frac{\text{Actual number of parasitoids moved towards "X" source}}{\text{Total number of parasitoids tested}} \times 100$$

Pooled analysis and comparison of volatiles of the three hosts was done by comparing per cent parasitoids moved to each host volatile in no-choice (pooled analysis of no-choice tests) and the per cent parasitoids moved towards each of the two volatiles offered in dual-choice in all of the combinations tested (pooled analysis of dual-choice tests).

The data on the per cent of parasitoids moved towards each arm were subjected to angular transformation. Transformed data was analysed through one-way ANOVA (SPSS version 11.5) and the treatment means were compared by LSD (Least Significant Difference;  $P = 0.05$ ). Comparison of means in case of per cent parasitoids attracted to pheromone lures was done by "t"(independent samples) test.

## RESULTS AND DISCUSSION

The laboratory reared *T. chilonis* adults responded to all sources of the host volatiles, albeit in varying degrees. In general, INB volatiles were the most attractive (as shown by the % parasitoids moved) often overlapping with SB

volatiles followed by *C. cephalonica* as indicated by the results of pooled analysis of no-choice and dual-choice tests. Presence of two volatiles (dual-choice tests) resulted in distraction of the parasitoid movement thus reducing the per cent parasitoids attracted to each host volatile. Yet dual-choice increased the % total respondents to odour cues reducing the % parasitoids remaining indecisive. Parasitoids responded more to male moths than female moths. While fresh mated moths were attractive source of volatiles, >72-hr old moths did not elicit response from the parasitoids. The host sex pheromones too proved to be attractive to the parasitoids. In most of the tests, though INB was the preferred kairomone source, SB and the factitious host too elicited response showing the true generalist adaptive nature of *T. chilonis* responding to a broad range of volatiles from many host species. Based on the results, it could be said that the parasitoid has an inherent preference to INB as proven by higher attraction to its volatiles.

The findings indicate that the laboratory reared *T. chilonis* was able to recognize and was attracted to all types of volatiles from all the three hosts, be it egg, scales or body washings. Arrestment of other species of *Trichogramma* by volatiles from eggs, adults and pheromones (Fatouros *et al.*, 2005a, b, 2007, 2008;

Gardner *et al.*, 2007; Milonas *et al.*, 2009; Noldus *et al.*, 1991) has been reported whether they were reared on the same host or not.

### *Attraction to the egg surface volatiles*

Egg surface volatiles from *C. cephalonica*, SB and INB were attractive to *T. chilonis* as shown by the significant per cent of parasitoids moved towards volatile source than those moved to hexane or that remained indecisive (Table 2) in no-choice volatile tests. However, comparison among the volatiles (pooled analysis of no-choice tests) indicated that INB volatiles were the most attractive (75.26%) while the SB and *C. cephalonica* egg volatiles attracted significantly lesser per cent (64.91 and 67.62%) of parasitoids. In dual-choice tests too, significantly higher per cent parasitoids moved to SB and INB egg washings (Table 2) compared to the washings of *C. cephalonica* eggs. This is in contrast with the results of Gardner *et al.* (2007), who did not find differences in the responses of *T. evanescens* to contact kairomones of two of its hosts.

**Table 2. Per cent parasitoids moved towards to volatiles from host eggs in no-choice and dual-choice bioassays**

% parasitoids moved towards volatiles in Y-tube olfactometer (Mean $\pm$ S.E)		
Arm 1	Arm 2	% parasitoids that remained indecisive
No-choice tests with volatile from		
<i>C. cephalonica</i> eggs	Hexane	
67.62 $\pm$ 2.02b <b>A</b>	15.43 $\pm$ 1.28a	16.96 $\pm$ 1.47a
SB eggs	Hexane	
64.91 $\pm$ 2.36b <b>A</b>	18.38 $\pm$ 1.26 a	16.71 $\pm$ 1.73a
INB eggs	Hexane	
75.26 $\pm$ 1.98b <b>B</b>	12.91 $\pm$ 1.11a	11.83 $\pm$ 1.20a
Dual-choices tests with volatiles from		
SB eggs	INB eggs	
45.55 $\pm$ 1.10b	45.18 $\pm$ 1.45b	9.27 $\pm$ 0.86a
<i>C. cephalonica</i> eggs	INB eggs	
33.06 $\pm$ 1.65b	56.39 $\pm$ 2.06c	10.55 $\pm$ 0.78a
<i>C. cephalonica</i> eggs	SB eggs	
30.77 $\pm$ 1.36b	57.31 $\pm$ 1.68c	11.92 $\pm$ 2.55a

Analysis by ANOVA and separation of means by LSD ( $P = 0.05$ ); Means followed by similar letters in a row are not significantly different; pooled analysis of no-choice test showed significant differences among the means of percent parasitoids attracted to egg volatiles as indicated by different letters (upper case) in a single column; pooled analysis of dual-choice data showed that when two choices were offered, attraction to volatiles of SB eggs and INB eggs were not different statistically but significantly higher than the per cent parasitoids attracted to *C. cephalonica* eggs.

#### Attraction of the parasitoids to moth scales

In no-choice tests, more than 70% of total number of parasitoids moved to the volatiles from SB scales and INB scales which were significantly different from those attracted to either hexane or those remained indecisive (Table 3). Pooled analysis of no-choice tests showed that attraction by SB scales was on par with INB scales. The latter was similar to the attraction of *T. chilonis* by *C. cephalonica* scales.

In dual-choice tests, washings from INB scales was better than SB scales in attracting the parasitoids and *C. cephalonica* was significantly lower than these two in eliciting parasitoid movement. Pooled analysis of dual-choice tests showed a similar trend with INB scales being the best source of kairomones followed by SB scales. Volatiles from scales of *C. cephalonica* were the least attractive among all.

#### Attraction of the parasitoids to pheromones

The per cent parasitoids moved towards SB or INB sex pheromone lures was high (Table 4). Pooled analysis

of no-choice tests did not show differences between attraction by the two pheromone lures. But when both the lures were offered simultaneously in dual-choice test, per cent parasitoids moved to INB pheromone lures was significantly higher (39.56%) than that moved to SB pheromones (28.7%).

**Table 3. Per cent parasitoids moved towards to volatiles from host scales in no-choice and dual-choice bioassays**

% parasitoids moved towards volatiles in Y-tube olfactometer (Mean $\pm$ S.E)		
Arm 1	Arm 2	% parasitoids that remained indecisive
No-choice tests with volatiles from		
<i>C. cephalonica</i> scales	Hexane	
64.35 $\pm$ 0.85c <b>A</b>	19.75 $\pm$ 0.84b	16.20 $\pm$ 0.63a
SB scales	Hexane	
72.99 $\pm$ 3.57b <b>B</b>	13.67 $\pm$ 2.16a	13.52 $\pm$ 1.69a
INB scales	Hexane	
70.88 $\pm$ 3.08b <b>A</b>	15.66 $\pm$ 1.53a	13.46 $\pm$ 1.83a
Dual-choice tests with volatiles from		
SB scales	INB scales	
35.15 $\pm$ 1.33b	47.52 $\pm$ 1.29c	17.33 $\pm$ 1.62a
<i>C. cephalonica</i> scales	SB scales	
31.95 $\pm$ 0.70b	54.92 $\pm$ 0.81c	13.12 $\pm$ 0.68a
<i>C. cephalonica</i> scales	INB scales	
31.15 $\pm$ 1.15c	58.95 $\pm$ 1.02b	9.90 $\pm$ 0.55a

Analysis by ANOVA and separation of means by LSD ( $P = 0.05$ ); Means followed by similar letters in a row are not significantly different. Pooled analysis of means of % parasitoids attracted to different volatiles in no-choice test showed significant differences (means followed by different upper case letters in a column); in the dual-choice tests attraction to all the three species was significantly different (INB > SB > *C. cephalonica*)

Different kinds of pheromones of target hosts have been proved to be a strong kairomonal source for trichogrammatids. Anti-aphrodisiacs from males transferred onto females during mating that discourage conspecifics from mating and pheromones deposited on the egg surface by females that deter oviposition by conspecifics have been proven to be an indicator and arrestant for *Trichogramma* spp. (Fatouros *et al.*, 2005b; Noldus and van Lenteren, 1985). Such a detection of moth sex pheromones may result in an enhanced searching effort by *Trichogramma* wasps in an area with host eggs and eventually lead to increased parasitism of these eggs (Milonas *et al.*, 2009).

### Attraction to kairomones from adult washings

The washings of the adults of both sexes were attractive to the parasitoid though higher per cent of parasitoids was attracted to male SB washings (79.4%) than female SB (67.9%) washings. A similar trend was observed with both male and female INB and *C. cephalonica* washings also (Table 5). This is in contrast to the report by Fatouros *et al.* (2007) who found that two species of *Trichogramma* wasps did not discriminate between olfactory cues from mated and virgin females or between mated females and males of the host.

Dual-choice tests of fresh mated moths showed significantly higher per cent parasitoids moved towards INB body washings (50.49%) than to SB body washings (39.07%). When tested with washings of *C. cephalonica*, both SB washings and INB washings were significantly more attractive to the parasitoid than the factitious host. There was no attraction by volatiles of old moths of any of the species to the parasitoids as indicated by random movement of parasitoids in all arms (Table 5). Pooled analysis showed a similar trend.

Similar finding with host scales serving as attractants to *T. chilonis* has been observed by many workers. Kairomonal compounds from target host as well as factitious host scales with or without additive compounds increase parasitism of *T. chilonis* in the laboratory conditions (Ananthakrishnan *et al.*, 1991; Bakthavatsalam *et al.*, 2006). It is also reported that hexane extracts of the adult host body stimulate ovipositor probing of *Trichogramma japonicum* (Usharani *et al.*, 2007).

**Table 4. Percent parasitoids moved towards host pheromone lures in no-choice and dual-choice bioassays**

% parasitoids moved towards (Mean $\pm$ S.E)		
Arm 1	Arm 2	% parasitoids that remained indecisive
No-choice tests with volatiles from		
SB pheromone	No lure	
77.14 $\pm$ 2.24b	12.72 $\pm$ 1.30a	10.14 $\pm$ 1.38a
INB pheromone	No lure	
76.93 $\pm$ 1.71b	11.11 $\pm$ 1.30a	11.96 $\pm$ 0.92a
Dual-choice tests with volatiles from		
SB pheromone	INB pheromone	
28.71 $\pm$ 3.24b	39.56 $\pm$ 4.06c	11.94 $\pm$ 1.40a

Analysis by ANOVA and separation of means by LSD ( $P = 0.05$ ); Means followed by similar letters in a row are not significantly different. Pooled data analysis of the no-choice test showed no significant differences between per cent of parasitoids attracted to SB pheromone lure and INB pheromone lure (independent "t" test, NS; t (sig. two tailed) = 0.851)

### Has rearing resulted in adaptation of *T. chilonis* to factitious host?

The present results mainly suggest that i) the native host volatiles were attractants to *T. chilonis*, and ii) there was no change in the preference status despite the fact that tested *T. chilonis* population was reared for several years on *C. cephalonica*. Loss of acceptance of native host is reported in parasitoids after being reared on factitious host (Rojas *et al.*, 1999), but no such consequence has been reported in some strains/species of *Trichogramma* spp. (Fatouros *et al.*, 2007; Hassan, 1989; Lui *et al.*, 1998; Pavlik, 1993; Takada *et al.*, 2001) which is true of the present finding too.

The reasons for the varied levels of attraction to the three hosts could be many. Variations in the natural ability of different strains and species of *Trichogramma* in selection and acceptance of different host species have been reported (Fatouros *et al.*, 2007) in which the preliminary step of attraction to the hosts and their habitat may have been fundamental. In the present study there were subtle and in some cases, stark differences in the response of *T. chilonis* to the volatiles from three of its hosts. However, in most cases INB and SB volatiles were highly preferred by the parasitoids to the volatiles of *C. cephalonica*. While two polyphagous *Trichogramma* species with similar host ranges responded in a contrasting manner to a single host infochemicals (Fatouros *et al.*, 2007), equal attraction to kairomones of two different hosts have been obtained with *T. evanescens* regardless of previous exposure (Gardner *et al.*, 2007). Though differential reactions of *T. chilonis* responding differently to volatiles of different hosts are not reported, its contradicting reactions of attraction and repulsion by diverse plant volatiles affecting parasitism have been well documented (Romeis *et al.*, 2005). More than adaptation to rearing host, it is the innate response that might have played a major role in the present study since the parasitoids were not exposed to either of the native hosts prior to the experiments and still responded highly to them.

Trichogrammatids are known to respond to chemical stimuli produced by its lepidopteran hosts using them as indicators of host presence (Gardner *et al.*, 2007). They learn about host and host plant cues constantly to sustain themselves but that does not result in rigid addiction to a particular host. Host acceptance is enhanced with learning in *Trichogramma* females through contact with rearing host and oviposition, linking the hosts with host volatiles and plant volatiles (Bjorksten and Hoffmann, 1998; Kaiser *et al.*, 1989; Romeis *et al.*, 2005). Persistence of such learned effects has been observed to last during that generation (Bjorksten and Hoffmann, 1998) though it is not understood whether that knowledge is transferred to the next generation of the parasitoids. However, learning helps in accepting a non-preferred host and does not override the inherent preference (Bjorksten and Hoffmann, 1998). In this context, it can be said that *C. cephalonica*

on which *T. chilonis* had been reared might probably have been a non-preferred host but due to continuous rearing had adapted to it and hence the resultant attraction to its volatiles. However, it could not be more than the attraction to the original host volatiles. Host preference based on conservative genetic determination has been noted in *T. brassicae* which had a greater spontaneous affinity for its host *Ostrinia nubilalis* than for *Ephestia kuehniella* with respect to host acceptance even though it was reared on the latter for more than 100 generations (Fatouros *et al.*, 2007) which might be true for *T. chilonis* also.

**Table 5. Percent parasitoids moved towards host body-washings in no-choice and dual-choice bioassays**

% parasitoids moved towards body washings of (Mean $\pm$ S.E)		
Arm 1	Arm 2	Indecisive
No-choice tests with volatiles from		
Male <i>C. cephalonica</i>	Hexane	
57.71 $\pm$ 1.95b	20.21 $\pm$ 1.16a	21.09 $\pm$ 1.23a
Female <i>C. cephalonica</i>	Hexane	
61.31 $\pm$ 1.50b	20.17 $\pm$ 0.78a	18.52 $\pm$ 0.99a
Male SB	Hexane	
79.36 $\pm$ 2.22b	9.63 $\pm$ 1.31a	11.01 $\pm$ 1.20a
Female SB	Hexane	
67.90 $\pm$ 1.71b	15.62 $\pm$ 1.61a	16.48 $\pm$ 1.58a
Male INB	Hexane	
72.56 $\pm$ 2.36b	12.96 $\pm$ 1.30a	14.48 $\pm$ 1.69a
Female INB	Hexane	
69.85 $\pm$ 3.03b	12.71 $\pm$ 1.12a	11.08 $\pm$ 1.15a
Dual-choice tests with volatiles from fresh moths of		
SB	INB	
39.07 $\pm$ 1.50b	50.49 $\pm$ 1.19c	10.44 $\pm$ 1.38a
<i>C. cephalonica</i>	SB	
34.19 $\pm$ 0.99b	43.06 $\pm$ 0.82c	22.75 $\pm$ 1.05a
<i>C. cephalonica</i>	INB	
32.26 $\pm$ 0.71b	55.05 $\pm$ 1.66c	10.98 $\pm$ 0.85a
volatiles from >72h old moths of		
SB	INB	
33.58 $\pm$ 1.86	30.97 $\pm$ 1.63	35.45 $\pm$ 2.51 NS
<i>C. cephalonica</i>	SB	
29.48 $\pm$ 1.02c	33.15 $\pm$ 1.04b	37.37 $\pm$ 1.08a
<i>C. cephalonica</i>	INB	
31.75 $\pm$ 1.48a	36.43 $\pm$ 1.49b	31.92 $\pm$ 1.36a

Analysis by ANOVA and separation of means by LSD ( $P = 0.05$ ); Means followed by similar letters in a row are not significantly different; pooled analysis of no-choice tests indicated volatiles from *C. cephalonica* were significantly lower in attraction than SB and INB volatiles which were on par with each other. Pooled analysis of dual-choice tests of fresh moths showed movement of percent of parasitoids to volatiles in the order of INB > SB > *C. cephalonica* volatiles which differed significantly. Pooled analysis of dual-choice tests with 72h old moths showed that the volatiles were not attractive as indicated by random movement of parasitoids in the arms of olfactometer.

In the present experiments, the target hosts though native to the parasitoids were not associated with the parasitoids at all during the rearing, yet were readily attractive to the parasitoid showing innate preference or elasticity in host selection (when exposed for the first time) which increases their survival. The attraction to both the native hosts as seen in the present study will be of good use for the parasitoids in the field in responding to host population fluctuations as the two tested pests do not occur simultaneously but chronologically, in the field based on crop age enabling the parasitoid to sustain itself throughout the crop season. The results of the present study also show that continuous rearing on the factitious host has made *T. chilonis* to adapt to *C. cephalonica*, but has not led to loss of ability in recognizing its native hosts thus upholding the host-location quality of the parasitoid. Nevertheless, these results are confined to attraction of *T. chilonis* to hosts and should be confirmed with host acceptance studies with assessment of parasitism rates on target hosts.

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