

# Comparison of associative learning of host-related plant volatiles in two parasitoids with different degrees of host specificity, *Cotesia marginiventris* and *Microplitis croceipes*

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**Abstract** The ability of parasitoids to learn a wide range of volatiles, including ecologically relevant and novel odors, and respond favorably to the learned stimuli has been documented for various species. Comparison of odor learning in closely related species can elucidate adaptive differences in species responses to infochemicals. The present study used a Y-tube olfactometer to compare odor learning and priming of behavioral responses of two parasitoids, *Cotesia marginiventris* (Cresson) and *Microplitis croceipes* (Cresson), to four selected host-related volatile compounds which have been previously shown to elicit behavioral response in both species; *trans*-2-hexanal,  $\alpha$ -pinene, *cis*-3-hexenyl butyrate, and (*E,E*)- $\alpha$ -farnesene. The two parasitoid species differ in their degree of specialization at the host level with *M. croceipes* having a more specialized host range, but otherwise represent infochemical generalist parasitoid species at the plant level. Naïve females as well as females that had been trained to associate sugar water with the test compounds were tested. Compared to naïve females, trained females of *C. marginiventris* showed a significant increase in behavioral

response to all four tested compounds. In contrast, trained females of *M. croceipes* showed a significant increase in behavioral response only to  $\alpha$ -pinene, and (*E,E*)- $\alpha$ -farnesene. Overall, the species with a wider host range, *C. marginiventris* showed a greater learning-induced increase in response than the species with a relatively specialized host range, *M. croceipes*. The results imply that animals with different ecological constraints may show variations in learning. The adaptive significance of behavioral responses as related to dietary specializations of the parasitoids is discussed.

**Keywords** *Cotesia marginiventris* · *Microplitis croceipes* · Associative learning · Host-related plant volatiles · Y-tube olfactometer

## Introduction

The ability to learn and respond favorably to the learned stimuli has been demonstrated in a wide range of vertebrate species including numerous fish, birds and mammals (Woody and Mathis 1995; Mahometa and Domjan 2005). Learning has also been demonstrated for many invertebrate species, including, snails (Dalesman et al. 2006), isopods (Baker 2005), phytophagous insects (Dukas and Bernays 2000; Xue et al. 2007), various parasitic wasp species (Lewis and Tumlinson 1988; Vet and Groenewold 1990; Turlings et al. 1993; Vet et al. 1995; Takasu and Lewis 2003; Bleeker et al. 2006; Müller et al. 2006; Segura et al. 2007), and social insects (Farina et al. 2005). The role of learning in the foraging behavior of parasitic wasps (parasitoids) is well studied not only because they are used in biological control (Lewis et al. 1990) but also because they are ideal laboratory animals to test theoretical questions in behavioral ecology (Potting et al. 1997). Several studies

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have shown that parasitoids can learn olfactory or visual cues associated with successful host location and use these odors for subsequent foraging decisions (Turlings et al. 1993; Vet et al. 1995). Learning could be in a form of experience or associative learning. For some species, prior experience with a host or host-related products could lead to a general increase in responsiveness (i.e. priming) or an increase in responsiveness to those specific cues that had been experienced (i.e. preference induction) (Turlings et al. 1993; Vet et al. 1995; Potting et al. 1997).

Associative learning, which is the establishment through experience of an association between two stimuli or between a stimuli and a response, has also been demonstrated for various parasitoid species (Lewis and Tumlinson 1988). Parasitoids can learn to respond to new compounds by associating them with either a food reward (Wäckers et al. 2002), or the host (Lewis and Tumlinson 1988). Associative learning of host-associated information by parasitoids may occur at the immature stage (Hérard et al. 1988) or at the adult stage (Lewis and Takasu 1990; Vet and Dicke 1992; Turlings et al. 1993; Vet et al. 1995). Various authors have emphasized the importance of considering an animal's ecology when studying and interpreting its learning abilities (Johnston 1982; Kamil 1994). Therefore, if learning is of adaptive significance in the foraging success of a species we might expect correlations between the ecology of an organism and its ability to learn. Because of the tight linkage between successful host location and fitness (Potting et al. 1997), parasitoids are expected to have evolved the ability to use or learn only those stimuli that enhance their probability of finding suitable hosts (Vet et al. 1995; Potting et al. 1997).

The adaptive value of variations in the learning ability of different parasitoid species can be elucidated through a comparative approach (Vet and Dicke 1992; Poolman Simons et al. 1992; Geervliet et al. 1998; Röse et al. 1998; Fukushima et al. 2002; Meiners et al. 2003; Tamò et al. 2006; Bleeker et al. 2006). In this paper, we compare associative learning of odor in two parasitoid species (Hymenoptera: Braconidae) of cotton caterpillars that differ in the degree of specialization at the host level; but otherwise represent infochemical generalist parasitoid species at the plant level. *Cotesia marginiventris* (Cresson) is considered to be a generalist at both the host level and the plant level (Steidle and van Loon 2003). It attacks a wide range of highly polyphagous lepidopteran caterpillar species, including *Spodoptera exigua* (Hübner), *Helicoverpa zea* (Boddie), and *Heliothis virescens* (Fab) (Lepidoptera: Noctuidae) (Jalali et al. 1987). *Microplitis croceipes* (Cresson), on the other hand, is more specialized at the host level and attacks *Heliothis* and *Helicoverpa* caterpillars (Eller 1990); however its hosts are highly polyphagous feeding on >100 species of plants from

several families (Li et al. 1992; Steidle and van Loon 2003). Thus, at the host level *C. marginiventris* is a generalist while *M. croceipes* can be classified as a specialist (but a generalist at the plant level).

Given the polyphagous nature of the hosts attacked by both species, they would have to cope with a huge variety of stimuli as infochemical generalists. Learning is, therefore, a factor that is involved in the success of both parasitoids. Previous studies have reported that both *C. marginiventris* and *M. croceipes* females learn to associate different odors with hosts, host byproducts, host plant-related chemicals and food, and search for hosts and food based on factors such as age, ovipositional status, physiological state and experience, colonization and environmental variable such as temperature, relative humidity and even barometric pressure (Lewis and Takasu 1990; Turlings et al. 1993; De Moraes et al. 1998; Geervliet et al. 1998; Lewis and Tumlinson 1988; Meiners et al. 2002; Olson et al. 2003). Recent studies also show that when conditioned with sucrose water, *M. croceipes* females can learn and detect a wide spectrum of novel chemicals (Olson et al. 2003; Tomberlin et al. 2005). Even though both species have demonstrated the ability to learn, we hypothesized that learning of host-related cues should be greatly adaptive for the *C. marginiventris* as compared to *M. croceipes*, since *C. marginiventris* attacks a wide variety of host species with a broader food plant range.

We used Y-tube olfactometer bioassays to compare the responses of naïve and trained females of *C. marginiventris* and *M. croceipes* to a shortlist of select compounds representing two categories of volatiles produced by cotton, a principal plant host of both species: green leaf volatiles (GLVs) which are released constitutively by plants and herbivore-induced plant volatiles (HIPVs). Based on the results of previous studies by our group on the response of both parasitoid species to host-related volatiles (Chen and Fadamiro 2007; Ngumbi et al. 2009, 2010), and a preliminary Y-tube olfactometer study that tested their response to a select group of synthetic GLVs and HIPVs, the following four synthetic compounds were selected for further testing in the present study: *trans*-2-hexenal,  $\alpha$ -pinene, *cis*-3-hexenyl butyrate and (*E,E*)- $\alpha$ -farnesene. The two GLVs tested are among the most common volatiles released constitutively by plants or at the beginning of herbivore feeding damage, whereas the two HIPVs tested are released in large amounts after several hours of herbivore damage. Two main hypotheses were tested: (1) trained females of both species would show increased behavioral response than naïve females to host-related volatiles, and (2) trained females of the generalist, *C. marginiventris* would show greater learning-induced increase in response to host-related volatiles than trained females of the host specialist, *M. croceipes*. In consonant with the second

hypothesis, we also investigated possible differences in the latency of response of both parasitoid species to host-related volatiles, to test whether the generalist would be slower at responding to host-related volatiles in the olfactometer.

## Materials and methods

### Insects

The parent cultures of *C. marginiventris* and *M. croceipes* were provided by the University of Georgia (Tifton campus, contact: John Ruberson) and the USDA-ARS, Insect Biology and Population Management Research Laboratory (Tifton, Georgia), respectively. *Cotesia marginiventris* was reared on caterpillars of *S. exigua*, whereas *M. croceipes* was reared on *H. virescens*. The rearing procedures of both parasitoids were similar to those of Lewis and Burton (1970). Eggs purchased from Benzene Research (Carlisle, PA, USA) were used to start laboratory colonies of the two lepidopteran host species, *S. exigua* and *H. virescens*. Caterpillars of both species were reared on a laboratory-prepared pinto bean diet (Shorey and Hale 1965) at  $25 \pm 1^\circ\text{C}$ , LD 14:10 h, and  $75 \pm 5\%$  RH. For each parasitoid species, newly emerged adults were collected prior to mating, sexed, and placed in groups of two individuals of opposite sex (mated individuals) in a 6-cm diameter plastic Petri dish supplied with water and sugar solution. Water was provided by filling a 0.5 ml microcentrifuge tube with distilled water and threading a cotton string through a hole in the cap of the tube. About 5 drops (2  $\mu\text{L}$  per drop) of 10% sugar solution were smeared on the inside of the Petri dish cover with a cotton-tipped applicator. Mated naïve and trained female parasitoids (aged 3–5 days) were used for the bioassays.

### Test compounds

Four synthetic compounds were tested: *trans*-2-hexenal,  $\alpha$ -pinene, *cis*-3-hexenyl butyrate and (*E,E*)- $\alpha$ -farnesene. The first two compounds (*trans*-2-hexenal, and  $\alpha$ -pinene) are components of GLVs, while *cis*-3-hexenyl butyrate and (*E,E*)- $\alpha$ -farnesene are HIPVs in cotton and several other plants (Loughrin et al. 1994; McCall et al. 1994; Röse et al. 1998; Ngumbi et al. 2009). Furthermore, recent and ongoing studies by our group have revealed key differences in electrophysiological and behavioral responses of *C. marginiventris* and *M. croceipes* to the select test compounds (Chen and Fadamiro 2007; Ngumbi et al. 2010; Ngumbi and Fadamiro unpublished). Compounds were purchased from Sigma<sup>®</sup> Chemical Co. (St. Louis, MO, USA) with purity >97%. Solutions of synthetic volatile compounds were formulated in hexane. Each compound was tested at a dose of 100  $\mu\text{g}$ , which was shown in

preliminary tests to elicit significant olfactometer response in both parasitoid species (unpublished data).

### Odor training procedure

Wasps were trained following the procedures described by Rains et al. (2004) with some modifications. Mated wasps (3- to 5-day old) were first starved for 6 h prior to training. Sugar solution (10%) served as the unconditional stimulus for both parasitoids in associative learning of volatiles. Both *M. croceipes* and *C. marginiventris* were fed with artificial sugar solutions during rearing. In addition, recent studies showed that *M. croceipes* (Nafziger and Fadamiro 2011) and *C. marginiventris* (H. Fadamiro, unpublished data) can both feed on nectar, hence sugar solution is deemed as a good reward (unconditional stimulus) for both species. To train female parasitoids to the test chemical compounds, 10  $\mu\text{L}$  of the compound to be tested was applied on a filter paper square (2  $\times$  2 cm), which was subsequently placed on the floor of a 250-ml glass jar that was covered with an 8  $\times$  8 cm sheet of aluminum foil. Several holes (each  $\sim$ 1 mm diameter separated by  $\sim$ 2 mm) were made on the aluminum foil covering the glass jar. The filter paper containing 10  $\mu\text{L}$  of 10% sugar solution (unconditional stimulus) was placed in the center of the ringlet of holes on the aluminum foil. While the female parasitoids were contacting the sugar solution, they were concurrently exposed to the volatiles emitted from the test compound. Wasps were exposed to the volatiles for 30 s three times, with 1-min interval between trainings (Rains et al. 2004). The control group (naïve wasps) was trained using similar procedures but only trained to clean air (i.e. exposed to blank filter papers only) while feeding. Clean air (and not the solvent) was used as control odor during training since associative learning of the solvent by the parasitoids could not be ruled out. Trainings were done at the laboratory 3–5 h into the photophase, between 1000 (10 AM) and 0230 at  $25 \pm 1^\circ\text{C}$  and  $75 \pm 5\%$  RH. Y-tube olfactometer bioassays followed immediately after training.

### Y-tube olfactometer bioassays

A Y-tube olfactometer (Analytical Research Systems, Inc, Gainesville, FL, USA) was used to test the attraction of 3- to 5-day-old naïve and trained female *C. marginiventris* and *M. croceipes* to the four selected synthetic plant volatiles. The system consists of a central tube (13.5 cm long, 24 mm diameter) and two lateral arms (5.75 cm long, 24 mm diameter). A sieve inlay in the lateral arms and extending glass tube 5.25 cm away from the connection prevents escape of insects and serves as an end point of each lateral arm. Humidified and purified air was passed into the extending glass tube through a Teflon connection

at 150 mL/min. The Y-tube olfactometer was inverted following preliminary experiments which showed that the parasitoids preferred to walk vertically up the glass tube than horizontally (unpublished data). Illumination was provided by vertically hanging an office lamp (20 W, 250 Lux) above (~50 cm high) the olfactometer tube. Parasitoids were introduced individually into the central arm of the Y-tube. The initial choice of a parasitoid that responded by walking into one of the two arms and remaining there at least 15 s was recorded. If a parasitoid did not make a choice within 5 min of being released, it was removed and discarded. Parasitoids that did not walk into any of the arms were not counted. After four individual parasitoids had been tested, the olfactometer arms were flipped around (180°) to minimize positional effect. After eight individuals had been bioassayed to the same test compound, the olfactometer set up was rinsed with soap water and acetone, and then air-dried. Each compound (100 µg of *trans*-2-hexenal,  $\alpha$ -pinene, *cis*-3-hexenyl butyrate, or (*E,E*)- $\alpha$ -farnesene) was delivered as 10-µL sample placed on No. 1 filter paper strips (7 × 40 mm, Whatman® no. 1). After allowing for solvent evaporation (~15 s, the filter paper strip was inserted into a designated arm of the olfactometer. A similar filter paper strip without compound or solvent was inserted into the second arm and served as clean air control. The two species were tested daily using a random order. On a given day and for each compound, a total of 4 parasitoids of each species (2 naïve and 2 trained) were tested. Experiments were carried for over a period of 3 months.

#### Influence of associative learning on response of parasitoids to host-related odor

To determine whether associative learning increased the responsiveness of the parasitoids (*C. marginiventris* and *M. croceipes*) to host-related plant volatiles, both naïve females as well as females that had been trained to associate sugar water with the test compounds were tested. Each parasitoid species was tested separately. In each test, parasitoids were offered a choice between odors that they had experienced during training and clean air. Responsiveness was measured as the percentage of the females tested that had walked upwind and chose one of the arms of the Y-tube olfactometer. To determine the net benefit from associative learning, the net gain in response (defined as percent increase in response after training) was calculated. To determine the effect of training on the latency of response of the parasitoids (naïve or trained) to the compounds, the amount of time taken by each individual parasitoid to respond to each compound was recorded. For each species, 25 naïve or trained individuals were bioassayed to each test compound. Data were analyzed by the

use of a Chi-square test ( $P < 0.05$ ; JMP® 7.0.1, SAS Institute 2007). Significant differences in the latency of response of naïve versus trained parasitoids of the same species or between the two species (naïve and trained individuals compared separately) were established by using Student's *t* test ( $P < 0.05$ ; JMP® 7.0.1, SAS Institute 2007).

## Results

### Influence of associative learning on response of parasitoids to host-related odor

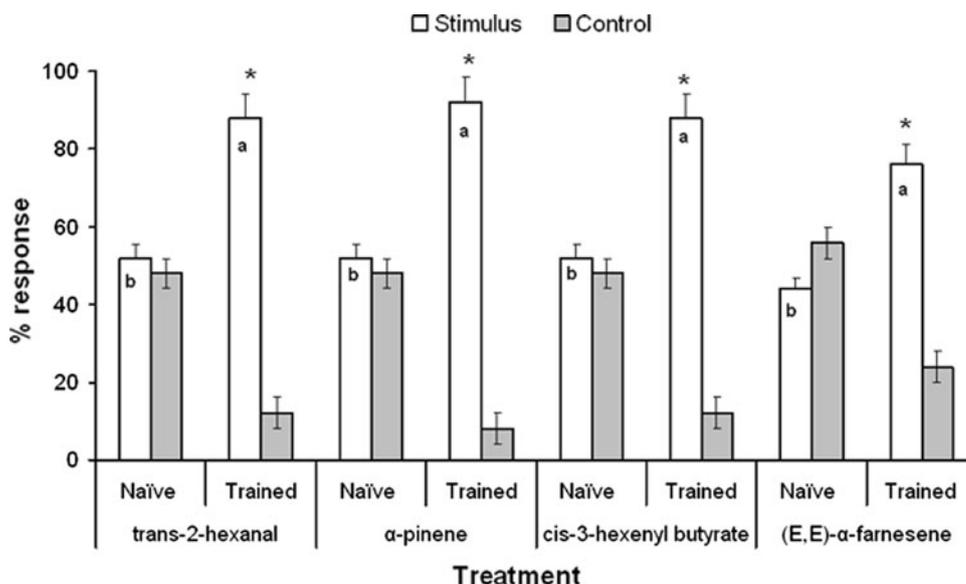
#### *Cotesia marginiventris*

Naïve *C. marginiventris* showed no significant attraction to any of the tested compounds in the Y-tube olfactometer (Chi-square test: *trans*-2-hexenal:  $\chi^2 = 0.08$ ,  $df = 1$ ,  $P = 0.7773$ ;  $\alpha$ -pinene:  $\chi^2 = 0.722$ ,  $df = 1$ ,  $P = 0.3956$ ; *cis*-3-hexenyl butyrate:  $\chi^2 = 0.08$ ,  $df = 1$ ,  $P = 0.7773$ ; (*E,E*)- $\alpha$ -farnesene  $\chi^2 = 0.08$ ,  $df = 1$ ,  $P = 0.7773$ ; Fig. 1). In other words, naïve individuals did not differentiate between the (clean air) control and any of the tested compounds. However, odor training resulted in significant increase in the response of *C. marginiventris* to all compounds (Fig. 1). Trained individuals showed significant attraction to *trans*-2-hexenal (Chi-square test:  $\chi^2 = 32.6$ ,  $df = 1$ ,  $P < 0.0001$ ),  $\alpha$ -pinene (Chi-square test:  $\chi^2 = 14.2$ ,  $df = 1$ ,  $P = 0.0002$ ), *cis*-3-hexenyl butyrate (Chi-square test:  $\chi^2 = 32.6$ ,  $df = 1$ ,  $P < 0.0001$ ), and (*E,E*)- $\alpha$ -farnesene (Chi-square test:  $\chi^2 = 41.4$ ,  $df = 1$ ,  $P < 0.0001$ ). In other words, odor training significantly enhanced the response of *C. marginiventris* to host-related odor. No significant differences were recorded in the latency of response (time taken to respond to each odor compound in the olfactometer) of naïve and trained *C. marginiventris* to any of the tested compounds (Student's *t* test:  $t = 0.4390$ ,  $df = 1$ ,  $P = 0.3313$  for *trans*-2-hexenal;  $t = 1.7297$ ,  $df = 1$ ,  $P = 0.9548$  for  $\alpha$ -pinene;  $t = 0.5196$ ,  $df = 1$ ,  $P = 0.6971$  for *cis*-3-hexenyl butyrate;  $t = 0.2971$ ,  $df = 1$ ,  $P = 0.6162$  for (*E,E*)- $\alpha$ -farnesene; Fig. 4). However, naïve parasitoids appeared to have numerically shorter latencies of response than trained parasitoids to the tested compounds.

#### *Microplitis croceipes*

Naïve *M. croceipes* showed significant attraction to *trans*-2-hexenal (Chi-square test:  $\chi^2 = 3.9$ ,  $df = 1$ ,  $P = 0.0462$ ) and *cis*-3-hexenyl butyrate (Chi-square test:  $\chi^2 = 19.2$ ,  $df = 1$ ,  $P < 0.0001$ ), but not to  $\alpha$ -pinene (Chi-square test:  $\chi^2 = 0.722$ ,  $df = 1$ ,  $P = 0.3956$ ) and (*E,E*)- $\alpha$ -farnesene

**Fig. 1** Influence of associative learning on response of *Cotesia marginiventris* to host-related odor. Asterisk indicates significant differences between stimulus and control (Chi-square test,  $P < 0.05$ ). Letters (a and b) indicate significant differences in the response of naïve versus trained individuals to each compound ( $\chi^2$  test,  $P < 0.05$ )



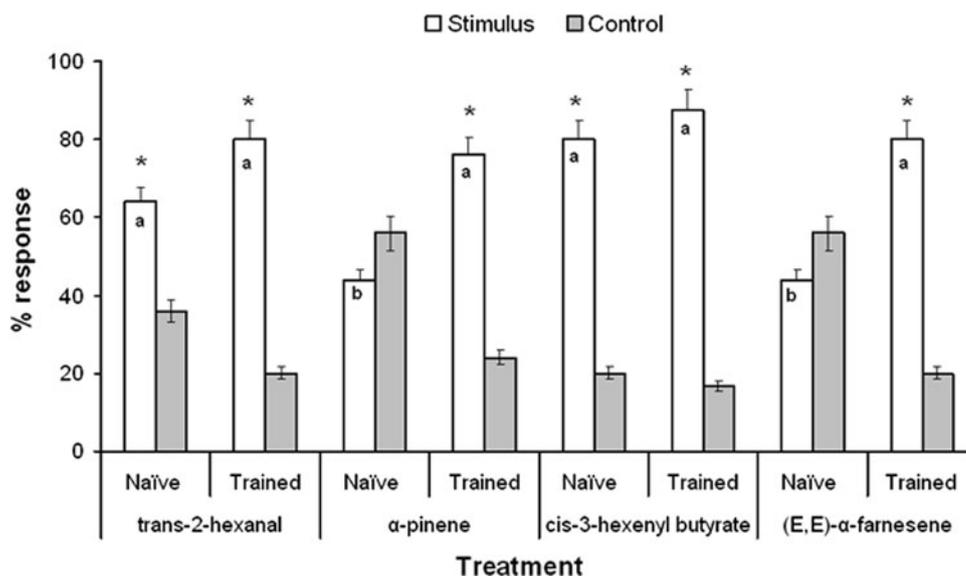
(Chi-square test:  $\chi^2 = 0.722$ ,  $df = 1$ ,  $P = 0.3956$ ) (Fig. 2). Odor training increased the response of this parasitoid to two of the compounds. Trained individuals showed significant attraction to  $\alpha$ -pinene (Chi-square test:  $\chi^2 = 19.2$ ,  $df = 1$ ,  $P < 0.0001$ ) and (E,E)- $\alpha$ -farnesene (Chi-square test:  $\chi^2 = 14.2$ ,  $df = 1$ ,  $P = 0.0002$ ) (Fig. 2). Although trained individuals showed significant attraction to trans-2-hexanal (Chi-square test:  $\chi^2 = 19.2$ ,  $df = 1$ ,  $P < 0.0001$ ) and cis-3-hexenyl butyrate (Chi-square test:  $\chi^2 = 25.3$ ,  $df = 1$ ,  $P < 0.0001$ ), this was not significantly greater than the proportion of naïve individuals which were attracted to the same compound. In other words, training enhanced the response of *M. croceipes* to  $\alpha$ -pinene, and (E,E)- $\alpha$ -farnesene, but not to trans-2-hexanal and cis-3-hexenyl butyrate. Although, trained *M. croceipes* tended to have numerically shorter latencies of response than naïve individuals, no

significant differences were recorded in the latencies of response of naïve versus trained individuals to the tested compounds (Student's *t* test:  $t = 0.8475$ ,  $df = 1$ ,  $P = 0.2005$  for trans-2-hexanal;  $t = 1.1507$ ,  $df = 1$ ,  $P = 0.1285$  for  $\alpha$ -pinene;  $t = 0.1203$ ,  $df = 1$ ,  $P = 0.4524$  for cis-3-hexenyl butyrate;  $t = 0.8583$ ,  $df = 1$ ,  $P = 0.8023$  for (E,E)- $\alpha$ -farnesene; Fig. 4).

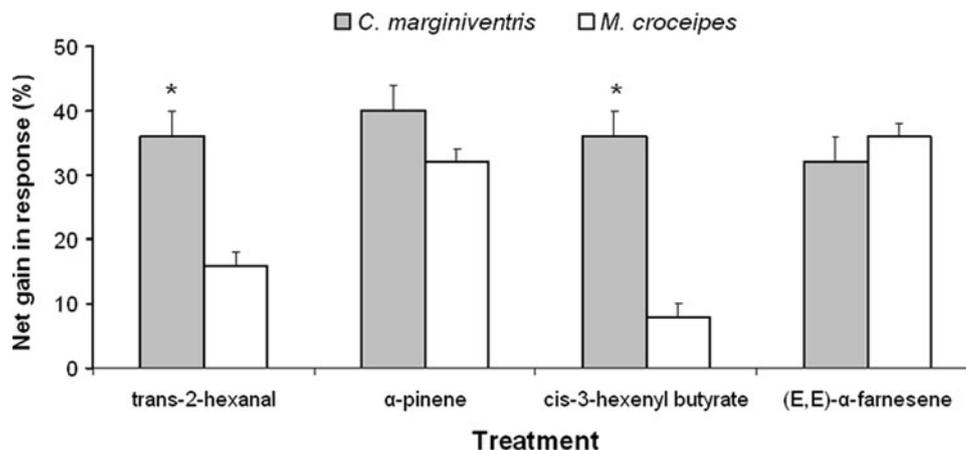
Comparing the two species

*Cotesia marginiventris* (generalist) showed a greater learning-induced increase in response than *M. croceipes* (specialist) to three of the tested odor compounds (Fig. 3). The net gain in response (defined as percent increase in response after training) after odor training was significantly higher for *C. marginiventris* compared to *M. croceipes* for

**Fig. 2** Influence of associative learning on response of *Microplitis croceipes* to host-related odor. Asterisk indicates significant differences between stimulus and control (Chi-square test,  $P < 0.05$ ). Letters (a and b) indicate significant differences in the response of naïve versus trained individuals to each compound (Chi-square test,  $P < 0.05$ )



**Fig. 3** Comparing effect of training on increased response of the two species; *Cotesia marginiventris* and *Microplitis croceipes*. Asterisk indicates significant differences between species (Chi-square test,  $P < 0.05$ )



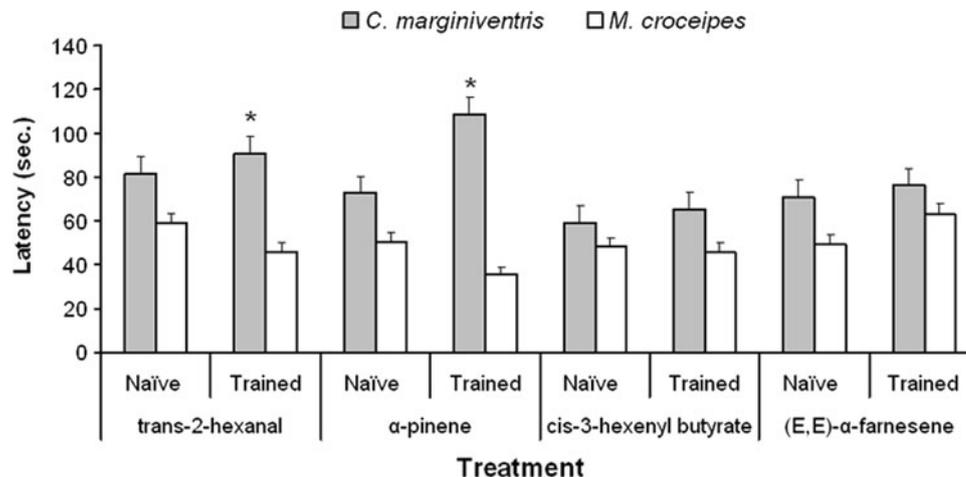
*trans*-2-hexanal (Chi-square test:  $\chi^2 = 7.692$ ,  $df = 1$ ,  $P = 0.0055$ ; Fig. 3), and *cis*-3-hexenyl butyrate (Chi-square test:  $\chi^2 = 17.818$ ,  $df = 1$ ,  $P = < 0.0001$ ; Fig. 3). However, no significant difference was recorded between the two species in their net gain in response to  $\alpha$ -pinene (Chi-square test:  $\chi^2 = 0.8888$ ,  $df = 1$ ,  $P = 0.3458$ ), and (*E,E*)- $\alpha$ -farnesene (Chi-square test:  $\chi^2 = 0.5882$ ,  $P = 0.4431$ ) after training (Fig. 3). In general, *M. croceipes* had shorter latencies of response than *C. marginiventris* to all the tested compounds, and this trend was true for both naïve and trained individuals. For example, trained *M. croceipes* had significantly shorter latencies of response than trained *C. marginiventris* to *trans*-2-hexanal (Student's *t* test:  $t = 2.5006$ ,  $df = 1$ ,  $P = 0.0081$ ; Fig. 4), and  $\alpha$ -pinene (Student's *t* test:  $t = 4.1565$ ,  $df = 1$ ,  $P = 0.0001$ ; Fig. 4).

## Discussion

The results of this study revealed differences on the effect of associative learning on the response of our parasitoid

models, *C. marginiventris* (generalist) and *M. croceipes* (specialist at host level) to host-related odor. As expected, trained individuals of both parasitoid species showed greater response than naïve individuals to most of the tested compounds, suggesting that both parasitoid species can improve their foraging efficiency through associative learning. However, *C. marginiventris* showed greater learning-induced increase in response to host-related plant volatiles than *M. croceipes*, in support of our hypothesis. Whereas, the generalist showed significant increase in response to all four tested compounds (*trans*-2-hexanal,  $\alpha$ -pinene, *cis*-3-hexenyl butyrate and (*E,E*)- $\alpha$ -farnesene) after training, the specialist (at host level) showed significant increase in response only to  $\alpha$ -pinene and (*E,E*)- $\alpha$ -farnesene. Thus, the net benefits from associative learning appeared to be higher for the generalist than for the specialist. Our findings are in agreement with previous reports on odor learning in parasitoids in general (Jaenike 1988), and support the prediction that odor learning is of greater adaptive value to generalists than specialist parasitoids (Vet and Groenewold 1990; Vet and Dicke 1992; Steidle and van Loon 2003).

**Fig. 4** Influence of training on time taken to make a choice on the trained odor (latency). Asterisk indicates significant differences between species (Student's *t* test,  $P < 0.05$ )



Associative learning has been documented before in *C. marginiventris* and *M. croceipes* (Turlings et al. 1993; Lewis and Tumlinson 1988). Furthermore, several studies have used a comparative approach to determine differences in the net effect of learning between generalist and specialist parasitoids (Poolman Simons et al. 1992; Vet et al. 1993; Geervliet et al. 1998; Röse et al. 1998). Geervliet et al. (1998) compared effect of experience on host finding behavior of two *Cotesia* (Hymenoptera: Braconidae) species that differ in their degree of specialization. The data showed that after multiple experiences with host larvae the generalist, *Cotesia glomerata* L. showed a clear preference for *Pieris brassicae* infested Brussels sprouts leaves over *P. rapae* infested Brussels sprouts (i.e. preference learning at the herbivore level). In contrast, the specialist *Cotesia rubecula* Marshall did not show preference learning at the herbivore level after multiple experiences with host larvae. These results support the hypothesis that learning plays a more important role in generalists than in specialist parasitoids.

Whereas naïve *C. marginiventris* did not show significant response to any of the tested compounds in the olfactometer, trained individuals were highly attracted to the compounds. This result is in line with the ‘varying response model’ proposed by Vet et al. (1990), and implies that *C. marginiventris* benefited from the brief training experience with the tested compounds, as has been observed in previous studies (Turlings et al. 1993). Recent studies showed that though *C. marginiventris* can perceive most of the volatiles associated with fresh leaf damage and caterpillar-induced volatiles (Hoballah and Turlings 2005; Ngumbi et al. 2009), it shows higher electrophysiological (Chen and Fadamiro 2007; Ngumbi et al. 2009) and behavioral (Cortesero et al. 1997; Hoballah and Turlings 2005) responses to green leaf volatiles than to induced volatiles. The parasitoid showed significant response to induced volatiles after female parasitoids associate such volatiles with the presence of hosts (Hoballah and Turlings 2005). Other generalist parasitoid species that have been shown to increase their behavioral response to host-related plant volatiles after experience include *Cotesia kairayi* Watanabe (Fukushima et al. 2002) and *Microplitis rufiventris* Kok (Tamò et al. 2006). The results which showed that training enhanced the response of *M. croceipes* to  $\alpha$ -pinene and (*E,E*)- $\alpha$ -farnesene, but not to *trans*-2-hexanal, and *cis*-3-hexenyl butyrate, are partly in agreement with previous studies which demonstrated that *M. croceipes* can learn to respond to odors associated with their hosts (Lewis and Tumlinson 1988; Meiners et al. 2002). The lack of significant increase in the response of *M. croceipes* to *trans*-2-hexanal and *cis*-3-hexenyl butyrate by trained individuals compared to naïve individuals is likely due to an already high inherent response of naïve *M. croceipes* to

these compounds. Furthermore, the data which showed that both naïve and trained *M. croceipes* responded equally to *cis*-3-hexenyl butyrate may suggest an important role for *cis*-3-hexenyl butyrate in the host location behavior of this specialist parasitoid, since naïve animals are expected to show the highest responses to those stimuli that, in evolutionary time, are predictably correlated with host location (Vet et al. 1990). *cis*-3-hexenyl butyrate is a major HIPV component emitted by cotton plants damaged by both *H. virescens* and *S. exigua* caterpillars (Ngumbi et al. 2009), and has been shown to elicit behavioral response in *M. croceipes* (Whitman and Eller 1992). More recently, we reported that *cis*-3-hexenyl butyrate is emitted in greater amounts by plants damaged by *H. virescens* compared to plants damaged by *S. exigua* (Ngumbi et al. 2009) and that it elicits higher EAG responses in *M. croceipes* compared to *C. marginiventris* (Ngumbi et al. 2010).

It is noteworthy that in our associative learning experiments, we used sugar solution and not host-related odor (e.g., frass) as our unconditional stimulus. Thus, the results showed that parasitoids can learn these odors in association with sugar water, in agreement with previous studies that demonstrate that females of *M. croceipes* can learn to associate host and non-host related odors with sugar water or sucrose (Takasu and Lewis 1996; Wäckers et al. 2002; Olson et al. 2003). In both studies, females that had experienced an odor while feeding on sucrose water showed a strong preference for the odor which they had experienced in conjunction with the food. In addition, if parasitoids are to be eventually used for biological control, their ability to be trained with sugar as the unconditional stimuli instead of real host is beneficial for the parasitoid because *H. virescens* larvae will very aggressively defend themselves and may occasionally bite and injure the wasps (Röse et al. 1997).

Interestingly, the results also revealed differences in the latencies of response (time taken to respond) of both species to the tested compounds. In general, *M. croceipes* had shorter latencies of response than *C. marginiventris* to virtually all the tested compounds, and this trend was true for both naïve and trained individuals. In other words, *M. croceipes* was faster to respond to host-related odor than *C. marginiventris*.

In conclusion, our results showed that both generalist and specialist parasitoid species potentially could improve their foraging efficiency through associative learning. This finding may have practical significance in the use of parasitoids as biological control agents. Parasitoids could be mass conditioned (trained) before field release to prime their response to host-related odor. When comparing the net benefits from associative learning in the two species, it was apparent that for the compounds tested, the generalist benefited the most. This finding provides evidence for

species-specific learning, whereby variation in learning correlates with a natural difference in the animal's behavioral ecology (Poolman Simons et al. 1992; Smid et al. 2007; Hoedjes et al. 2011), and implies that animals with different ecological constraints may show variations in learning success. While it is likely that our results reflect real differences in the learning success of generalist and specialist parasitoids, it is possible that the results may simply reflect differences in the phylogeny of both species. Future studies are necessary to understand the neurophysiological and behavioral mechanisms of learning in both parasitoid species.

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## References

- Baker MB (2005) Experience influences settling behavior in desert isopods, *Hemilepistus reaumuri*. *Animal Behav* 69:1131–1138
- Bleeker MAK, Smid HM, Steidle JLM, Kruidhof M, van Loon JJA, Vet LEM (2006) Differences in memory dynamics between two closely related parasitoid wasp species. *Animal Behav* 71:1343–1350
- Chen L, Fadamiro HY (2007) Differential electroantennogram response of females and males of two parasitoid species to host-related green leaf volatiles and inducible compounds. *Bull Entomol Res* 97:515–522
- Cortesero AM, De Moraes CM, Stapel JO, Tumlinson JH, Lewis WJ (1997) Comparisons and contrasts in host foraging strategies of two larval parasitoids with different degrees of host specificity. *J Chem Ecol* 23:1589–1606
- Dalesman S, Rundle S, Coleman RA, Cotton PA (2006) Cue association and antipredator behavior in a pulmonate snail *Lymnaea stagnalis*. *Animal Behav* 71:789–797
- De Moraes CM, Lewis WJ, Pare PW, Alborn HT, Tumlinson JH (1998) Herbivore-infested plants selectively attract parasitoids. *Nature* 393:570–573
- Dukas R, Bernays EA (2000) Learning improves growth rate in grasshoppers. *Proc Natl Acad Sci USA* 97:2637–2640
- Eller FJ (1990) Foraging behavior of *Microplitis croceipes*, a parasitoid of *Heliothis* species. Dissertation, University of Florida, Gainesville
- Farina WM, Grutter C, Diaz PC (2005) Social learning of floral odors inside the honeybee hive. *Proc R Soc Lond B* 272:1923–1928
- Fukushima J, Kainoh Y, Honda H, Takabayashi J (2002) Learning of herbivore-induced and nonspecific plant volatiles by a parasitoid, *Cotesia kariyai*. *J Chem Ecol* 28:579–585
- Geervliet JBF, Vreugdenhil AI, Dicke M, Vet LEM (1998) Learning to discriminate between infochemicals from different plant–host complexes by the parasitoids *Cotesia glomerata* and *Cotesia rubecula* (Hymenoptera: Braconidae). *Entomol Exp Appl* 86:241–252
- Hérad F, Keller MA, Lewis WJ, Tumlinson JH (1988) Beneficial arthropod behavior mediated by airborne semiochemicals. IV. Influence of host diet on host-oriented flight chamber responses of *Microplitis demolitor* Wilkinson. *J Chem Ecol* 14:1597–1606
- Hoballah ME, Turlings TCJ (2005) The role of fresh versus old leaf damage in the attraction of parasitic wasps to herbivore-induced maize volatiles. *J Chem Ecol* 31:2003–2018
- Hoedjes KM, Marjolein Kruidhof H, Huigens ME, Dicke M, Vet LEM, Smid H (2011) Natural variation in learning rate and memory dynamics in parasitoid wasps: opportunities for converging ecology and neuroscience. *Proc R Soc Lond B* 278: 889–897
- Jaenike J (1988) Effects of early adult experience on host selection in insects: some experimental and theoretical results. *J Insect Behav* 1:3–15
- Jalali SK, Singh SK, Ballal CR (1987) Studies on host age preference and biology of exotic parasite, *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae). *Entomol* 12:59–62
- Johnston TD (1982) Selective costs and benefits in the evolution of learning. *Adv Study Behav* 12:65–106
- Kamil AC (1994) A synthetic approach to the study of animal intelligence. In: Real LA (ed) *Behavioural mechanisms in evolutionary ecology*. The University of Chicago Press, USA, pp 11–45
- Lewis WJ, Burton RL (1970) Rearing *Microplitis croceipes* in the laboratory with *Heliothis zea* as host. *J Econ Entomol* 63: 656–658
- Lewis WJ, Takasu K (1990) Use of learned odours by a parasitic wasp in accordance with host and food needs. *Nature* 348:635–636
- Lewis WJ, Tumlinson JH (1988) Host detection by chemically mediated associative learning in a parasitic wasp. *Nature* 331:257–259
- Lewis WJ, Vet LEM, Tumlinson JH, van Lenteren JC, Papaj DR (1990) Variations in parasitoid foraging behavior: essential element of a sound biological control theory. *Environ Entomol* 19:1183–1193
- Li Y, Dickens JC, Steiner WWM (1992) Antennal olfactory responsiveness of *Microplitis croceipes* (Hymenoptera: Braconidae) to cotton plant volatiles. *J Chem Ecol* 18:1761–1773
- Loughrin JH, Manukian A, Heath RR, Tumlinson JH (1994) Diurnal cycle emission of induced volatile terpenoids by herbivore-injured cotton plants. *Proc Natl Acad Sci USA* 91:11836–11840
- Mahometa MJ, Domjan M (2005) Classical conditioning increases reproductive success in Japanese quail, *Coturnix japonica*. *Anim Behav* 69:983–989
- McCall PJ, Turlings TCJ, Loughrin J, Proveaux AT, Tumlinson JH (1994) Herbivore-induced volatile emissions from cotton (*Gossypium hirsutum* L.) seedlings. *J Chem Ecol* 20:3039–3050
- Meiners T, Wäckers F, Lewis JW (2002) The effect of molecular structure on olfactory discrimination by the parasitoid *Microplitis croceipes*. *Chem Senses* 27:811–816
- Meiners T, Wäckers F, Lewis JW (2003) Associative learning of complex odors in parasitoid host location. *Chem Senses* 28: 231–236
- Müller C, Collatz J, Wieland R, Steidle JLM (2006) Associative learning and memory duration in the parasitic wasp *Lariophagus distinguendus*. *Anim Biol* 56:221–232
- Nafziger TD, Fadamiro HY (2011) Suitability of some farmscaping plants as nectar sources for the parasitoid wasp, *Microplitis croceipes* (Hymenoptera: Braconidae): effects on longevity and body nutrients. *Biol Control* 56:225–229
- Ngumbi EN, Chen L, Fadamiro HY (2009) Comparative GC-EAD responses of a specialist (*Microplitis croceipes*) and a generalist (*Cotesia marginiventris*) parasitoid to cotton volatiles induced by two caterpillar species. *J Chem Ecol* 35:1009–1020
- Ngumbi EN, Chen L, Fadamiro HY (2010) Electroantennogram (EAG) responses of *Microplitis croceipes* and *Cotesia marginiventris* and their lepidopteran hosts to a wide array of host-related and non host-related compounds: correlation between EAG response and degree of host specificity. *J Insect Physiol* 56:1260–1268
- Olson DM, Rains GC, Meiners T, Takasu K, Tertuliano M, Tumlinson JH, Wäckers FL, Lewis WJ (2003) Parasitic wasps

- learn and report diverse chemicals with unique conditional behaviors. *Chem Senses* 28:545–549
- Poolman Simons MTT, Suverkropp BP, Vet LEM, de Moed G (1992) Comparison of learning in related generalist and specialist eucoilid parasitoids. *Entomol Exp Appl* 64:117–124
- Potting RPJ, Otten H, Vet LEM (1997) Absence of learning in the stemborer parasitoid *Cotesia flavipes*. *Anim Behav* 53:1211–1223
- Rains GC, Tomberlin JK, D'Alessandro M, Lewis WJ (2004) Limits of volatile chemical detection of a parasitoid wasp *Microplitis croceipes*, and an electronic nose: a comparative study. *Am Soc Agric Eng* 47:2145–2152
- Röse USR, Alborn HT, Makranczy G, Lewis WJ, Tumlinson JH (1997) Host recognition by the specialist endoparasitoid *Microplitis croceipes* (Hymenoptera: Braconidae): role of host- and plant-related volatiles. *J Insect Behav* 10:313–330
- Röse USR, Lewis WJ, Tumlinson JH (1998) Specificity of systemically released cotton volatiles as attractants for specialist and generalist parasitic wasps. *J Chem Ecol* 24:303–319
- SAS Institute (2007) JMP 7.0.1. Statistical discovery software. SAS Institute Statistical Analysis System, Cary
- Segura DF, Viscarret MM, Carabajal Paladino LZ, Ovruski SM, Cladera JL (2007) Role of visual information and learning in habitat selection by a generalist parasitoid foraging for concealed hosts. *Anim Behav* 74:131–142
- Shorey HH, Hale RL (1965) Mass rearing of the larvae of nine noctuid species on a simple artificial medium. *J Econ Entomol* 58:55–68
- Smid HM, Wang G, Bukovinszky T, Steidle JLM, Bleeker MAK, van Loon JA, Vet LEM (2007) Species-specific acquisition and consolidation of long-term memory in parasitic wasps. *Proc R Soc Lond B* 274:1539–1546
- Steidle JLM, van Loon JJA (2003) Dietary specialization and infochemical use in carnivorous arthropods: testing a concept. *Entomol Exp Appl* 108:133–148
- Takasu K, Lewis WJ (1996) The role of learning in adult food location by the larval parasitoid *Microplitis croceipes*. *J Insect Behav* 9:265–281
- Takasu K, Lewis WJ (2003) Learning of host searching cues by the larval parasitoid *Microplitis croceipes*. *Entomol Exp Appl* 108:77–86
- Tamò C, Ricard I, Held M, Davison AC, Turlings TCJ (2006) A comparison of naïve and conditioned responses of three generalist endoparasitoids of lepidopteran larvae to host-induced plant odors. *Anim Biol* 56:205–220
- Tomberlin JK, Tertuliano M, Rains G, Lewis WJ (2005) Conditioned *Microplitis croceipes* Cresson (Hymenoptera: Braconidae) detect and respond to 2,4-DNT: development of a biological sensor. *J Forensic Sci* 50:1187–1190
- Turlings TCJ, Wackers FL, Vet LEM, Lewis WJ, Tumlinson JH (1993) Learning of host-finding cues by hymenopterous parasitoids. In: Papaj DR, Lewis AC (eds) *Insect learning: ecological and evolutionary perspectives*. Chapman and Hall, New York, pp 51–78
- Vet LEM, Dicke M (1992) Ecology of infochemical use by natural enemies in a tritrophic context. *Ann Rev Entomol* 37:141–172
- Vet LEM, Groenewold AW (1990) Semiochemicals and learning in parasitoids. *J Chem Ecol* 16:3119–3135
- Vet LEM, Lewis WJ, Papaj DR, van Lenteren JC (1990) A variable-response model for parasitoid foraging behavior. *J Insect Behav* 3:471–490
- Vet LEM, Sokolowski MB, MacDonald DE, Snellen H (1993) Responses of a generalist and a specialist parasitoid (Hymenoptera: Eucoilidae) to drosophilid larval kairomones. *J Insect Behav* 6:615–624
- Vet LEM, Lewis WJ, Cardé RT (1995) Parasitoid foraging and learning. In: Cardé RT, Bell WJ (eds) *Chemical ecology of insects*, 2nd edn. Chapman and Hall, London, pp 65–101
- Wäckers FL, Bonifay C, Lewis JW (2002) Conditioning of appetitive behavior in the Hymenopteran parasitoid *Microplitis croceipes*. *Entomol Exp Appl* 103:135–138
- Whitman DW, Eller FJ (1992) Orientation of *Microplitis croceipes* (Hymenoptera: Braconidae) to green leaf volatiles: dose-response curves. *J Chem Ecol* 18:1743–1753
- Woody DR, Mathis A (1995) Acquired recognition of chemical stimuli from an unfamiliar predator: associative learning by adult newts, *Nolophthalmus viridescens*. *Copeia* 4:1027–1031
- Xue HJ, Egas M, Yang XK (2007) Development of a positive preference–performance relationship in an oligophagous beetle: adaptive learning? *Entomol Exp Appl* 125:119–124