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# Herbivore-Induced Plant Volatiles Can Serve as Host Location Cues for a Generalist and a Specialist Egg Parasitoid

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**Abstract** Herbivore-induced plant volatiles are important host finding cues for larval parasitoids, and similarly, insect oviposition might elicit the release of plant volatiles functioning as host finding cues for egg parasitoids. We hypothesized that egg parasitoids also might utilize HIPVs of emerging larvae to locate plants with host eggs. We, therefore, assessed the olfactory response of two egg parasitoids, a generalist, *Trichogramma pretiosum* (Tricogrammatidae), and a specialist, *Telenomus remus* (Scelionidae) to HIPVs. We used a Y-tube olfactometer to tests the wasps' responses to volatiles released by young maize plants that were treated with regurgitant from caterpillars of the moth *Spodoptera frugiperda* (Noctuidae) or were directly attacked by the caterpillars. The results show that the generalist egg parasitoid *Tr. pretiosum* is innately attracted by volatiles from freshly-damaged plants 0–1 and 2–3 h after regurgitant treatment. During this interval, the volatile blend consisted of green leaf volatiles (GLVs) and a blend of aromatic compounds, mono- and homoterpenes, respectively. Behavioral assays with synthetic GLVs confirmed their attractiveness to *Tr. pretiosum*. The generalist learned the more complex volatile blends released 6–7 h after induction, which consisted mainly of sesquiterpenes. The specialist *T. remus* on the other hand was attracted only to volatiles emitted from fresh and old damage after associating these volatiles with oviposition. Taken together, these results strengthen the emerging pattern that egg and larval parasitoids behave in a similar way in that generalists can respond innately to HIPVs, while specialists seems to rely more on associative learning.

**Key Words** Tritrophic interaction · Egg parasitoids · Natural enemies · Fall armyworm · Induced defense

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## Introduction

Volatiles released by plants under herbivore attack are important chemical cues for parasitoids and predators to locate their host and/or prey (Dicke et al., 1990; Turlings et al., 1990). Attraction to herbivore-induced plant volatiles (HIPVs) has been observed for many larval parasitoids and arthropod predators (Mattiacci et al., 1995; Ngi Song et al., 1996; De Moraes et al., 1998; Dicke, 1999; Kessler and Baldwin, 2001). Besides herbivory, deposition of insect eggs also can induce the production of volatiles or change leaf chemistry in a way that the plants attract and/or arrest certain egg parasitoids (Meiners and Hilker, 1997; Hilker et al., 2002; Mumm et al., 2003; Fatouros et al., 2008; Tamiru et

al., 2011). To date, *de novo* oviposition-induced volatiles have been reported for some tritrophic systems. In some systems, the combination of oviposition and herbivory trigger volatiles that attract egg parasitoids (Colazza et al., 2004; Conti et al., 2010; Michereff et al., 2011).

Apart from oviposition-induced plant volatiles, the orientation of egg parasitoids towards egg-derived odors might be an effective alternative strategy for host location. However, eggs usually emit only small amounts of volatiles and are therefore only useful as short-range cues. Moreover, eggs generally are inconspicuous and deposited on hidden sites, making their location difficult (Vinson, 1998). This is why egg parasitoids are expected to frequently exploit chemical cues from other sources for host location as well. In the case of lepidopteran hosts, this includes, for example, pheromones and compounds released from scales of the wings of ovipositing females (Shu and Jones, 1989; Colazza et al., 1997), but also HIPVs (Lou et al., 2005; Moraes et al., 2005). Although HIPVs do not directly indicate the presence of eggs, their exploitation could be of use if the host has overlapping generations, as the highly abundant and easily detectable plant odors enable them to locate host habitats and communities, and, consequently, eggs (Vinson, 1976, 1984). Furthermore, HIPVs may help phoretic egg parasitoids to locate adults on which they can "catch a ride" to new oviposition sites with eggs (Fatouros et al., 2008).

Despite the possible importance of HIPVs for host location by egg parasitoids, few studies have directly addressed this subject (Reddy et al., 2002; Lou et al., 2005; Manrique et al., 2005; Moraes et al., 2005). Also, little is known about the capacity of egg parasitoids to learn HIPVs for host location (Mumm et al., 2005; Schroder et al., 2008). This is surprising, given the fact that learning is an important strategy for parasitoids that optimizes their host searching strategy (Lewis and Takasu, 1990; Turlings et al., 1993; Vet et al., 1995). To obtain deeper insight into these topics, we tested innate and learned responses of two lepidopteran egg parasitoids towards HIPVs that are released by maize plants upon attack by larvae of the noctuid moth *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae). We assessed the behavior of the generalist *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae), which parasitizes a wide range of hosts, (Zucchi and Monteiro, 1997), and the specialist *Telenomus remus* Nixon (Hymenoptera: Scelionidae), whose host range is limited to the genus *Spodoptera*. To complement this study, we also tested whether or not *S. frugiperda* gravid females show a preference for oviposition on plants that are damaged by conspecifics. We hypothesized that if moths prefer plants that have been damaged by conspecifics, or at least do not actively avoid them, HIPVs may guide egg parasitoids not only to habitats with hosts, but potentially also to plants containing host eggs of later arriving *Spodoptera* females.

## Methods and Materials

*Plants and Insects* Maize seeds (*Zea mays* L., var. Delprim) were planted individually in plastic pots containing about 200 g potting soil ("Hortaliças Basiplant" substrate). Plants were grown in a greenhouse (12/12 h day/night cycle). Maize plants used in the bioassays had been growing for 8–9 d after emergence and had three fully expanded leaves. *Spodoptera frugiperda* caterpillars were collected on maize fields at Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba-SP, Brazil, and reared under laboratory conditions (25±3°C, 60±10% rh, 12/12 h). The caterpillars were reared individually in sterilized glass vials (8.5 cm high, 2.2 cm diam, plugged with cotton) on artificial diet (Greene et al., 1976). Emerging pupae were separated by sex and placed into PVC cages (10.0 cm diam and 22.0 cm high) that were closed at both ends with Petri dishes (14.2 cm diam). The Petri dish in the bottom of the cage was lined with filter paper. In addition, cages were lined with office paper to serve as a surface for oviposition. Adults were fed a 10% honey solution that was renewed every 2 d.

Both *Telenomus remus* Nixon (Hymenoptera: Scelionidae) and *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) were reared on 24 h-old *S. frugiperda* egg masses, which were placed in sterilized glass vials (9.0 cm high, 3.0 cm diam, sealed with PVC film) together with the parasitoids. One paper card containing eggs was offered per day in each glass vial. Each card was removed after a 5 h period and placed in a new glass vial. Upon emergence of the parasitoids, small droplets of honey were placed on the internal wall of the vials as food source. All vials were maintained in incubators (25±1°C, 60±10% rh, 12/12 h).

*Moth Oviposition Preference* In an oviposition preference test, we offered *S. frugiperda* females an undamaged maize plant (treatment UP) next to a caterpillar damaged plant (CD). To obtain CD, 10 sec-instar larvae were placed inside the whorl of the plants at 10:00am until the beginning of preference tests at 6:00pm. Plants of each treatment were placed on the opposite ends of a *voil* fabric cage (55.0 cm high, 60.0 cm long, 10.0 cm wide). At 6:00 pm, five 3-d-old gravid females were released in the cage and left for one night. The following morning, egg masses were removed from the plants, and the eggs were counted. The bioassay was replicated 20 times, and was conducted in a room under controlled conditions (25±1°C, 60±10 rh, 12/12 h).

*Olfactometer Bioassays* Olfactory responses of the egg parasitoids (*T. remus* and *Tr. pretiosum*) were tested for 3-to-4-d-old individuals that were either naïve or experienced. A Y-tube olfactometer was used to determine their preference, either between two different odor sources or one source and a blank (clean air). Experience was given to parasitoids by allowing

them to oviposit on 100 viable *S. frugiperda* while exposing them to HIPVs released by regurgitant treated (0–1, 2–3, 6–7, and 12–13 h) or to caterpillar damaged plants for 10 min. After this period, we collected only wasps that were parasitizing the egg masses. Wasps that were walking around the vial were not used as experienced wasps. Experienced parasitoids were tested on the subsequent day to the odors of the same treatment they had experienced against the undamaged plant. All bioassays were conducted in the laboratory (conditions as described above) during day time, between 10:00 and 17:00. The Y-tube olfactometer consisted of a bifurcated glass tube (2.5 cm diam), and two arms (9.0 cm long). The odor sources were placed inside glass bottles (11.5 cm i.d., 51 cm high), which were connected to the extremities of the olfactometer. From the main arm of the olfactometer, a tube from a vacuum pump was connected, and the air from the environment was purified with the use of granular activated carbon filter (40 mesh, 11 cm i.d., 35 cm high) before pulling it through the system. The air flow was adjusted to 300 ml/min using calibrated flowmeters connected to each arm. Insects then were positioned individually at the beginning of the central arm of the Y-tube and observed for 5 min. When the wasps crossed the threshold line (located in the middle of each arm) and stayed in the arm end for 20 sec, this was considered as "choice". Only insects that made a choice for one arm within the 5 min were considered for statistical analysis. Each parasitoid was used only a single time to prevent associative learning. After each trial, the olfactometer was disassembled and all glassware was washed with neutral dishwashing soap (v/v 5%), distilled water, acetone, and alcohol (v/v 90%).

To evaluate egg parasitoid responses to HIPVs, bioassays with the following combinations were carried out: (i) undamaged plant (UP) vs. blank (B); (ii) UP vs. regurgitant-treated plant (RT) at intervals 0–1, 2–3, 6–7, and 12–13 h after treatment; (iii) UP vs. caterpillar-damaged plant (CD), which was obtained by having 20 2nd instars feed overnight. The larvae were left feeding on the plant during the entire experiment. The treatment CD thus corresponds to a "host-plant-complex". The regurgitant was manually collected from 3rd-4th instar fall armyworm caterpillars fed on maize for 2 d and stored at −76°C. To simulate herbivory on plants, we scratched the 2nd and 3rd leaf (about 1 $cm^2$ of each) and applied 10 μl of regurgitant to each damaged site. Regurgitant-treated plants 0–1, 2–3, and 6–7 h were induced during the morning (8:00–10:00), and 12–13 h was induced at 22:00 and tested at 10:00 on the following day. For each bioassay, we conducted at least 30 replicates on 4–5 experimental days using different odor sources.

*Volatile Collection and Analysis* To analyze the volatiles released by regurgitant-treated (RT) and caterpillar-damaged plants (CD), maize seedlings were grown as described in Erb et al. (2010). Maize seedlings were subjected to *S. frugiperda* feeding and regurgitant treatment as described above and following the same timing as the bioassays. Regurgitant-treated plants (RT) then were placed into glass bottles (9.5 cm i.d., 54 cm high), and volatiles were sampled at intervals 0–1, 2–3, 6–7, and 12–13 h after induction using super-Q® filters. Caterpillar damaged plants (CD) were sampled on the following morning (10:00 am) after one-night of feeding, as wasps were tested at this time for attraction to CD odors. The super-Q® traps were extracted with 150 μl dichloromethane (Suprasolv; Merck, Dietikon, Switzerland), and 200 ng of n-octane and n-nonyl acetate (Sigma, Buchs, Switzerland) in 10 μl dichloromethane were added to the samples as internal standards. All extracts were stored at −76°C until analyses. Traps were washed with 3 ml dichloromethane before they were reused for a next collection. HIPVs of the experiments were identified with a gas chromatograph (Agilent 6890 Series GC system G1530A) coupled to a mass spectrometer that operated in electron impact mode (Agilent 5973 Network Mass Selective Detector; transfer line 230°C, source 230°C, ionization potential 70 eV, scan range 33–280 amu). A 2-μl aliquot of each sample was injected in the pulsed splitless mode onto an apolar capillary column (HP-1, 30 m, 0.25 mm ID, 0.25 μm film thickness; Alltech Associates). Helium at constant flow (0.9 ml/min) was used as carrier gas. After injection, the column temperature was maintained at 40°C for 3 min and then increased to 100°C at 8°C/min and subsequently to 200°C at 5°C/min followed by a postrun of 5 min at 250°C. Detected volatiles were identified by comparison of their mass spectra with those of the NIST 05 library and with the retention times of analyses from previous studies (Turlings et al., 1998; D'Alessandro and Turlings, 2006).

*Response of Wasps to Synthetic Volatiles* We measured the response of inexperienced *Tr. pretiosum* wasps towards different HIPVs in a Y-tube olfactometer following the procedure described above. First a synthetic GLV mixture comprised of 80% (*Z*)-3-hexanal, 10% (*Z*)-3-hexenol, 8% (*E*)-2-hexenal, and 2% (*Z*)-3-hexenyl-acetate (Sigma Aldrich, Switzerland) (Von Mérey et al., 2011), which corresponds to a typical GLV mix released by freshly wounded plants, was tested. Second, we evaluated the attractiveness of the sesquiterpene (*E*)-*β*-farnesene (Sigma Aldrich, Switzerland), a major sesquiterpene that is released from herbivore-induced maize plants. Hexane solutions of synthetic plant volatiles were prepared at concentrations of 1,000, 500, 50, and 5 μl/ml. For each replicate, 2 μl of synthetic plant volatile solution and hexane (control) were incorporated into filter paper squares of 2 $cm^2$, which were placed on the ends of olfactometer arms. Maize plants can release 2–3 μg of the tested volatiles following the first 12 h of induction (Erb et al., 2011), which means that the lowest concentration we tested can be reached by a stand of five or more infested plants in a dense canopy with little air circulation.

*Statistical Analysis* Levene's and a Kolmorogov-Smirnov tests were carried out to determine heteroscedasticity of error variance and normality of the data. Number of eggs from preference tests performed with fall armyworm moths were analyzed by paired *t-tests* ($\alpha$=0.05). Differences between behavioral responses of parasitoids under pairs of treatments were analyzed by *chi-square* tests ($\alpha$=0.05 and $\alpha$=0.01). Individuals that did not make a choice were excluded from the statistical analysis. Statistical analysis was performed using the software Statistica 6.0.

## Results

*Herbivore Oviposition Choice. Spodoptera frugiperda* females laid, on average, 283 eggs on undamaged plants (UP) and 293 eggs on caterpillar-damaged plants (CD). There was no significant difference between the treatments in number of eggs (paired *t* test, $N$=20, $t$=−0.0951, $P$=0.925) or number of egg masses laid by *S. frugiperda* (data not shown).

*Parasitoid Response to Maize Volatiles* Neither wasps *T. remus* nor *Tr. Pretiosum* distinguished the odor of undamaged plants (UP) and air from blank arms (data not shown). Therefore, for the following bioassays, UP was used as a control. Results in detail were as follows: Naïve *T. remus* females did not show any preference for odors emitted by regurgitant-treated plants (RT) 0–1 h (Fig. 1; Table 1). Experienced females preferred odors from RT 0–1 h to UP (Table 1). The generalist *Tr. pretiosum* showed a significant preference for RT 0–1 h odors independently of previous experience (Fig. 1; Table 1). For the following time point, again, naïve *T. remus* showed no preference for odors emitted by RT 2–3 h (Fig. 1; Table 1), but a significant number of experienced females preferred RT 2–3 h to UP (Table 1). Both naïve and experienced *Tr. pretiosum* females showed a significant preference for RT 2–3 h odors compared to UP (Fig. 1; Table 1). Both *T. remus* and *Tr. pretiosum* showed a similar response towards volatiles released from RT 6–7 h. Naïve wasps showed no preference to RT 6–7 h or UP (Fig. 1; Table 1), while, after oviposition experience, they significantly preferred the RT 6–7 h odors to UP (Table 1). Neither naïve nor experienced *T. remus* and *Tr. pretiosum* showed a preference to RT 12–13 h compared to UP odors (Fig. 1; Table 1). *Telenomus remus* and *Tr. pretiosum* females did not distinguish between the odors of caterpillar-damaged plants (CD) or UP (Fig. 2; Table 1). However, significantly more experienced *T. remus* wasps preferred CD odors to UP (Table 1). *Trichogramma pretiosum* experienced wasps did not distinguish between CD and UP odors (Table 1).

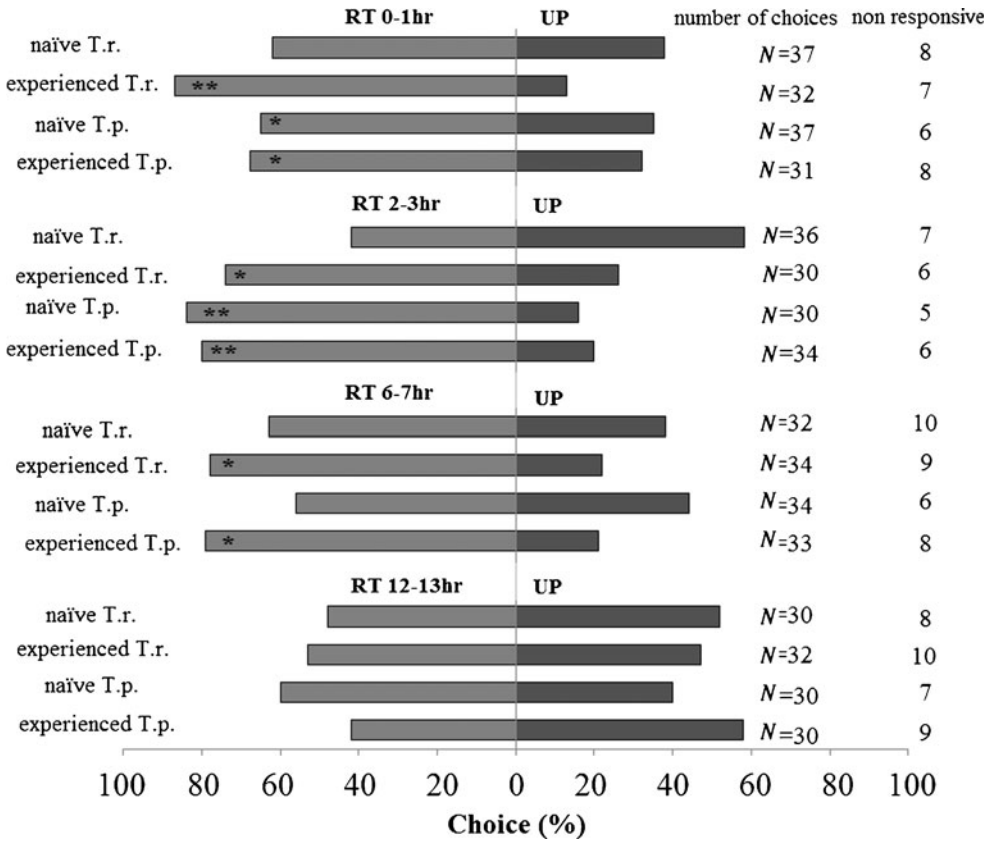


**Fig. 1** Response of naïve and experienced *Telenomus remus* and *Trichogramma pretiosum* to volatiles emitted by undamaged plant (UP) and *Spodoptera frugiperda*-regurgitant treated plant (RT) 0–1, 2–3, 6–7, and 12–13 h. T.r. = *T. remus*; T.p. = *Tr. pretiosum*. * significant at 5% according to chi-square; ** significant at 1% according to *chi-square*

**Table 1** Results of *chi-square* tests on the wasp choices in the Y-tube olfactometer between the undamaged plant and *Spodoptera frugiperda* regurgitant-treated (RT) or caterpillar-damaged plant (CD)

| Wasp | | Treatment | *N* | χ2 | *P* |
|---|---|---|---|---|---|
| *T. remus* | Naïve | RT 0–1 h | 37 | 2.189 | 0.139 |
| | | RT 2–3 h | 36 | 1.000 | 0.317 |
| | | RT 6–7 h | 32 | 2.000 | 0.157 |
| | | RT 12–13 h | 30 | 0.133 | 0.715 |
| | | CD | 39 | 0.100 | 0.753 |
| | Experienced | RT 0–1 h | 32 | 15.125 | <0.001 |
| | | RT 2–3 h | 30 | 4.800 | 0.028 |
| | | RT 6–7 h | 34 | 5.452 | 0.020 |
| | | RT 12–13 h | 32 | 0.125 | 0.724 |
| | | CD | 35 | 4.829 | 0.028 |
| *Tr. pretiosum* | Naïve | RT 0–1 h | 37 | 4.568 | 0.033 |
| | | RT 2–3 h | 30 | 13.333 | <0.001 |
| | | RT 6–7 h | 34 | 0.471 | 0.493 |
| | | RT 12–13 h | 30 | 1.200 | 0.273 |
| | | CD | 41 | 0.095 | 0.758 |
| | Experienced | RT 0–1 h | 31 | 3.903 | 0.048 |
| | | RT 2–3 h | 34 | 11.765 | 0.001 |
| | | RT 6–7 h | 33 | 6.125 | 0.013 |
| | | RT 12–13 h | 30 | 0.533 | 0.465 |
| | | CD | 42 | 0.024 | 0.876 |

*Parasitoid Response to Synthetic Volatiles* Inexperienced *Tr. pretiosum* wasps showed a significant preference to GLV synthetic mixture at all tested concentrations (Fig. 3; 1,000 µl/ml$N$=35, χ2=6.429, $P$=0.011; 500 µl/ml$N$=35, χ2=4.829, $P$=0.028; 50 µl/ml$N$=35, χ2=10.314, $P$=0.001; 5 µl/ml$N$=35, χ2=4.829, $P$=0.028). In contrast, wasps did not show any attraction to (*E*)-*β*- farnesene at 5 or 50 µl/ml (Fig. 4; 5 µl/ml$N$=35, χ2=0.257, $P$=0.612; 50 µl/ml$N$=35, χ2=0.029, $P$=0.866), and preferred control (hexane) over (*E*)-*β*- farnesene at 500 and 1,000 µl/ml (500 µl/ml $N$=35, χ2=4.829, $P$=0.028; 1,000 µl/ml$N$=35, χ2=8.257, $P$=0.004).

*Plant Volatile Collection* The composition of the volatile mixture released by the different treatments is shown in Table 2. The proportions of compounds in the mixture are depicted in Fig. 5. It should be noted that, as the absolute quantities of the different volatiles cannot be directly compared with the given method, the approach used here is valid for relative differences between compounds at different time

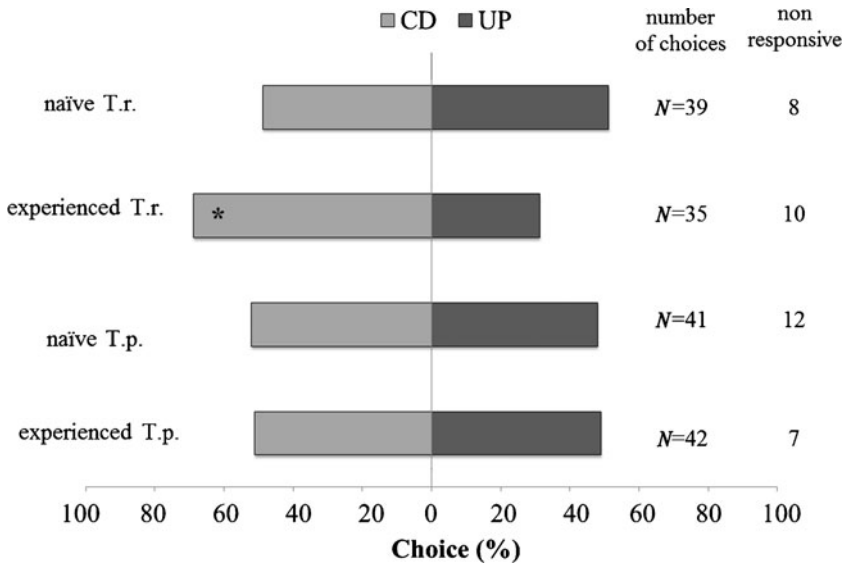


**Fig. 2** Response of naïve and experienced *Telenomus remus* and *Trichogramma pretiosum* to volatiles emitted by undamaged plant (UP) and *Spodoptera frugiperda*-caterpillar damaged plant overnight (CD). T.r. = *T. remus*; T.p. = *Tr. pretiosum*. * significant at 5% according to *chi-square*

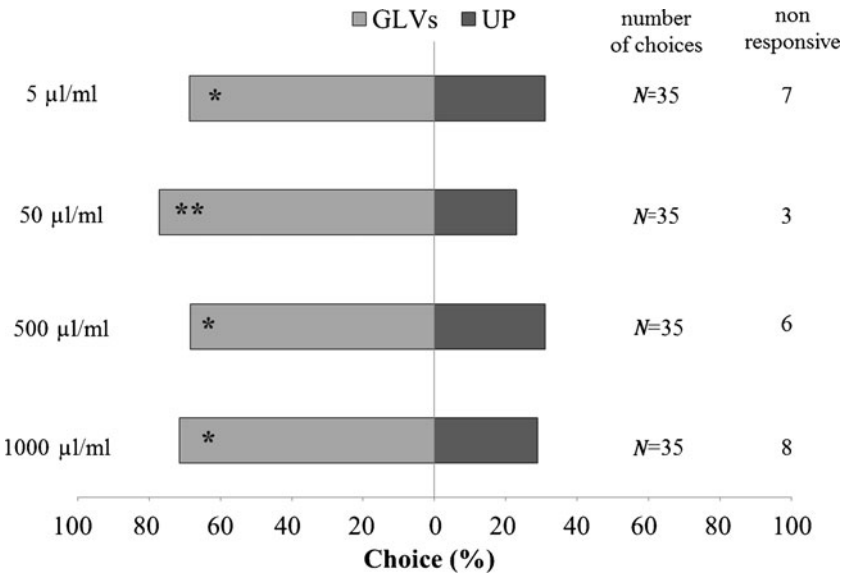


**Fig. 3** Response of naïve *Trichogramma pretiosum* to different concentrations of synthetic GLV mixture (GLVs) at 5, 50, 500, and 1,000 µl/ml and hexane as control. * significant at 5% according to *chi-square*; ** significant at 1% according to *chi-square*

points. We observed that GLVs (*Z*)-3-hexenal, (*E*)-2-hexenal, (*E*)-3-hexen-1-ol, and (*Z*)-3 hexenyl acetate are predominantly released by RT 0–1 h, and monoterpenes, such as linalool and $\beta$-myrcene are present in small amounts (Fig. 5). On the following time interval, RT 2–3 h, the plants started to emit monoterpenes and aromatic compounds (phenethyl acetate, benzyl acetate, geranyl acetate e indole) in greater amounts, and the homoterpene (3*E*)-4,8-dimetil-1,3,7-nonatriene (DMNT) was abundant in the mixture (Fig. 5). Moreover, at this time interval, sesquiterpene emission commenced. By contrast, emission of GLVs almost ceased, and (*Z*)-3 hexenyl acetate was the only GLV that could still be detected.

At 6–7 h after induction, sesquiterpenes [(*E*)-$\beta$-caryophyllene, (*E*)-$\alpha$-bergamotene, and (*E*)-$\beta$-farnesene] were emitted in highest amounts (Fig. 5). All aromatic compounds (except benzyl acetate), DMNT, and monoterpenes also were present in the blend. At this time interval, no GLVs were detected.

The composition of volatiles emitted 12–13 h after induction (induction at night time) was largely similar to the blend at 6–7 h, with exception of geranyl acetate, which was absent. Sesquiterpenes still dominated the mixture, while the proportion of homoterpenes (DMNT) was reduced. Monoterpenes were still present and in higher amounts than at 6–7 h (Fig. 5). In general, overall amounts of volatiles were reduced at 12–13 h compared to emission of RT 6–7 h, with exceptions of (*E*)-$\beta$-caryophyllene, which was the major compound among

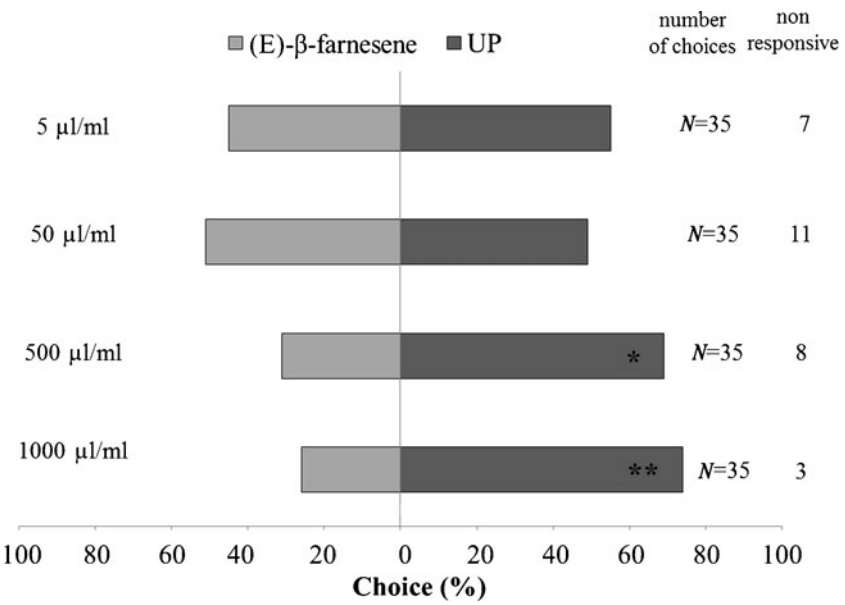


**Fig. 4** Response of naïve *Trichogramma pretiosum* to different concentrations of synthetic (E)-β-farnesene at 5, 50, 500, and 1,000 µl/ml and hexane as control. * significant at 5% according to chi-square; ** significant at 1% according to chi-square

**Table 2** Amount of volatiles (ng/h) released by *Spodoptera frugiperda* regurgitant- treated (RT) and caterpillar-damaged plant (CD)

| Volatile compound | UP | Regurgitant-treated plant | | | | CD |
|---|---|---|---|---|---|---|
| | | 0–1 h | 2–3 h | 6–7 h | 12–13 h | |
| Green leaf volatiles (GLVs) | | | | | | |
| (*Z*)-3-hexanal | | 26.3±6.1 | | | | 122.5±25.1 |
| (*E*)-2-hexanal | | 103.9±14.9 | | | | 18.4±2.6 |
| (*E*)-3-hexen-1-ol | | 10.00±2.3 | | | | 22.1±2.5 |
| (*Z*)-3-hexen-1-ol acetate | | 93.9±12.3 | 5.5±1.5 | | | 64.1±10.9 |
| Aromatics | | | | | | |
| benzyl acetate | | | 2.00±0.8 | | | 5.3±2.0 |
| phenethyl acetate | | | 4.1±1.3 | 7.0±5.2 | 2.3±1.1 | 5.3±2.2 |
| indole | | | 77.7±36.5 | 69.4±29.0 | 4.2±2.0 | 193. 2±78.7 |
| Monoterpenes | | | | | | |
| geranyl acetate | 6.5±1.2 | | 6.8±2.4 | 13.9±8.3 | | 8.8±2.5 |
| $\beta$-myrcene | 8.7±0.3 | 4.4±0.40 | 4.0±1.2 | 2.0±1.3 | 3.3±0.8 | 6.7±0.7 |
| linalool | 94.0±13.4 | 18.4±5.0 | 33.3±9.5 | 59.2±34.1 | 41.6±10.8 | 93.9±25.2 |
| Homoterpenes | | | | | | |
| (3*E*)-4,8-dimethyl-1,3,7-nonatriene | | | 180.6±51.1 | 161.0±78.8 | 1.3±1.3 | 30.4±6.0 |
| Sesquiterpenes | | | | | | |
| (*E*)-$\beta$-caryophyllene | | | 2.6±1.0 | 13.5±2.8 | 75.5±5.5 | 62.3±6.2 |
| (*E*)-$\alpha$-bergamotene | | | 14.2±3.9 | 132.0±55.8 | 38.4±10.7 | 24.7±5.5 |
| (*E*)-$\beta$-farnesene | | | 27.3±9.3 | 282.2±119.7 | 53.8±19.5 | 39.6±12.5 |
| Total | 109.2±43.5 | 261.7±31.2 | 360.1±99.7 | 741.2±205.0 | 224.6±43.4 | 701.1±146.9 |

the sesquiterpenes, and $\beta$-myrcene. Continuous attack of *S. frugiperda* caterpillars overnight (treatment CD) triggered the emission of a complex volatile blend on the next day, which comprised all chemical groups simultaneously: GLVs, aromatics, homoterpenes, monoterpenes, and sesquiterpenes (Fig. 5). The relative quantities gave rise to a unique pattern of VOCs (Fig. 5).

## Discussion

HIPVs can be detected over long distance, making them important signals in the host finding behavior of parasitoids (Vet and Dicke, 1992). While HIPVs can provide specific information about the attacking herbivore, such as species and herbivore stage (Takabayashi et al., 1995; De Moraes et

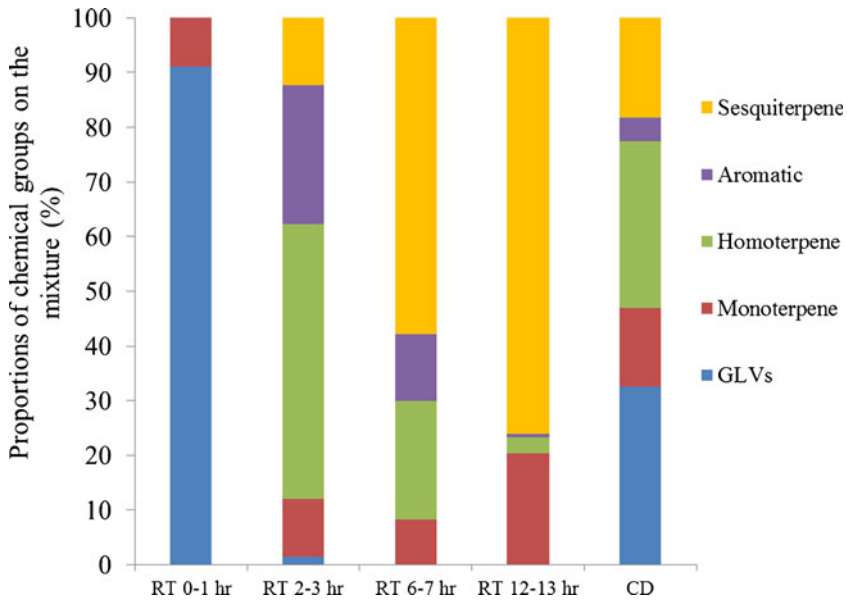


**Fig. 5** Proportions of each chemical group present on the mixture released by *Spodoptera frugiperda*-regurgitant induced plant at 0–1 h (RT 0–1 h), 2–3 h (RT 2–3 h), 6–7 h (RT 2–3 h), 12–13 h (RT 12–13 h) and induced by larva feeding overnight (CD)

al., 1998; Mumm et al., 2005), our work shows that they also may serve as indirect cues, possibly giving away the presence of more inconspicuous host stages like eggs. Our results reveal that both generalist and specialist egg parasitoids may use plant volatiles to locate plants with potential hosts. Until now, few other studies have compared the strategies of egg parasitoids with different host ranges, and little has been done regarding their capacity to learn to respond to plant volatiles (Meiners et al., 2003).

The specialist parasitoid, *T. remus*, did not show an innate response towards volatiles emitted by regurgitant-induced maize or by fall armyworm attacked plants (CD). Nevertheless, after experience, *T. remus* was able to learn to associate odors emitted by the artificially induced and attacked plants. This suggests that in a situation where the parasitoid encounters eggs on plants that are attacked by emerging *S. frugiperda* larvae, it can adjust to this situation and use HIPVs as host-finding cues. The plasticity in behavior may be advantageous to the egg parasitoid, as HIPVs may be associated only with eggs under certain environmental situations: In times of high herbivore density, for example, it may be fairly common to find egg masses and larvae occurring simultaneously on the same plants, while in times of low occurrence, this would be less likely. Alternatively, HIPVs might play a role as long-range cues to egg parasitoids. Once wasps find the host community, host odors and subtle oviposition induced cues (Fatouros et al., 2008; Tamiru et al., 2011) from neighboring plants may precisely indicate the presence of the host.

Our results indicate that the multivoltine *S. frugiperda* does not avoid plants infested with conspecifics for oviposition, lending support to the concept that multiple host stages are likely to be present on maize plants under heavy pest pressure.

In contrast to *T. remus*, the generalist *Tr. pretiosum* was attracted innately to volatiles emitted by regurgitant-induced plants at 0–1 and 2–3 h (Fig. 1). These mixtures contained major amounts of GLVs compared to the later time points (Table 2 and Fig. 5). GLVs are thus main candidates for the innate attraction of *Tr. pretiosum*, and our bioassays with synthetic GLVs show that the parasitoid is indeed responding positively to these cues (Fig. 3). Maize larval parasitoids are known to be strongly attracted to fresh damage (Hoballah and Turlings, 2005; Shiojiri et al., 2006; Ngumbi et al., 2009), and other *Trichogramma* species have been shown be reactive to GLVs (Reddy et al., 2002; Romeis et al., 2005; Sen et al., 2005). Interestingly, *Tr. pretiosum* was not attracted to the complete blend elicited by larval feeding, which did contain substantial amounts of GLVs. This suggests that the composition of the HIPVs plays an important role in the attraction of the parasitoid, and some later induced volatiles like aromatic compounds and sesquiterpenes may distort and/or mask the attractiveness of the active volatiles. Additional evidence for the negative impact of these compounds comes from the fact that *Tr. pretiosum* was not attracted to caterpillar induced plants, even after having had a positive oviposition experience (Fig. 2), and from our finding that (*E*)-$\beta$-farnesene repelled the parasitoid, at least at high doses. It remains to be determined why *Tr. pretiosum* did not learn the CD blend, although it contained compounds that it was clearly able to perceive and learn. One obvious constraint of the data present here is that volatile collections and olfactometer assays took place in two different locations, and small differences in plant growth conditions may have altered the volatile blends (Gouinguené and Turlings, 2002).

According to the theory proposed by Vet and Dicke (1992), specialist parasitoids should respond innately to volatiles released by host-damaged plants, while generalists should rely more strongly on learning. Our results, however, show the exact opposite: the specialist seemed to rely more strongly on learning, while the generalist showed innate attraction to HIPVs (Fig. 1). This is in accordance with a series of studies showing that innate responses are important in generalists, and specialists retain a high capacity for learning (Dickens, 1999; Shiojiri et al., 2000; Smid et al., 2002; Steidle and Van Loon, 2003; Gouinguené et al., 2005; Hoballah and Turlings, 2005).

Both *T. remus* and *Tr. pretiosum* show innate responses to host odors, such as pheromones and body scales (Beevers et al., 1981; Nordlund et al., 1983; Noldus et al., 1990; Gazit et al., 1996). Similar to HIPVs, these are indirect cues for the egg parasitoids. The innate attraction to such signals may be advantageous because of their relative stability (i.e., pheromones vary very little) and their potential to guide the parasitoids to habitats that are likely to have eggs present within days.

In general, our results are in agreement with findings of Manrique et al. (2005) that studied the egg parasitoid *Anaphes iole* Girault (Hymenoptera: Mymaridae). This parasitoid develops exclusively in hosts of *Lygus* spp. (Hemiptera: Miridae), a group of polyphagous herbivores. In this system, only experienced parasitoids were attracted by volatiles induced by feeding of their hosts. Equally, Moraes et al. (2005, 2009) showed that *Telenomus podisi* (Ashmead) (Hymenoptera: Scelionidae), which is considered a generalist egg parasitoid of pentatomids, is innately attracted by HIPVs released by soybean and chickpea. Taken together, the emerging pattern is that generalist egg parasitoids seem to respond innately to HIPVs, while specialists rely on learning at least when their host is polyphagous. Learning HIPVs could allow specialist parasitoids to exploit odors from different plants and plant genotypes that carry their host (Gouinguené et al., 2001; Degen et al., 2004). This study is, to our best knowledge, the first to compare directly a generalist and specialist egg parasitoid within the same system, and further research comparing several generalist and specialist species is

required to confirm the pattern and explain the observed phenomenon in detail.

Differently from larval parasitoids, egg parasitoids can prevent injuries to crops by killing herbivores before hatching. Our results provide a better understanding on egg parasitoid host searching behavior, and may help to develop techniques that optimize the biological control efficiency of egg parasitoids in the field.

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