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Influence of semiochemicals present in the scales of *Spodoptera frugiperda* on chemotactic behavior of *Trichogramma pretiosum*

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Abstract

Trichogramma species (Hymenoptera: Trichogrammatidae) are known to use the host's sex pheromone as a kairomone in foraging strategies. *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) deposit scales on eggs during oviposition, which may be a mechanical barrier or facilitate the action of parasitoids due to the presence of semiochemicals. This study identified the compounds in *S. frugiperda* scales and evaluated the chemotactic responses of *Trichogramma pretiosum* Riley to volatiles in host eggs and scales, as well as to synthetic equivalents of substances identified therein. Bioassays were performed with scales extracted from the wings and abdomen of females under three conditions: virgin, mated, or mated and oviposited. The parasitoids were tested in a two-choice olfactometer comparing scales, eggs, and synthetic compounds. The extracts were analyzed by gas chromatography and mass spectrometry (GC-MS) and 20 compounds were identified, among them two acetate components of the sex pheromone of *S. frugiperda* (*Z*9-14:OAC and *Z*11-16:OAC). *Trichogramma pretiosum* females did not differentiate between scales from wings and abdomen. They were more attracted to scales from virgin and mated *S. frugiperda* females, and these treatments did not trigger different chemotactic responses compared with eggs. The parasitoid was significantly attracted to *Z*9-14:OAC and *Z*11-16:OAC. We conclude that *T. pretiosum* use chemical clues in the scales to search for *S. frugiperda* eggs.

Introduction

Trichogramma species (Hymenoptera: Trichogrammatidae) are important biological control agents of agricultural pests (Woelke et al., 2017) and have more than 400 host species, especially Lepidoptera (Harba & Idris, 2018). The success of egg parasitoids in the field is closely associated with their search capacity (Siqueira et al., 2011), which is mainly mediated by chemical stimuli from hosts (Strand & Vinson, 1982; Vinson, 1998). Substances on host eggs have been recorded as kairomones for *Trichogramma brassicae* Bezdenko, which responded to volatiles from the Lepidoptera *Ostrinia nubilalis* Hübner, *Ostrinia furcanalis* (Guenée), and *Mamestra brassicae* (L.) (Renou et al., 1992). These parasitoids can also use pheromones as kairomones (Vet & Dicke, 1992; Arakaki et al., 1997; Collaza et al., 1997; Boo & Yang, 2000). Females of *Trichogramma evanescens* Westwood were attracted by (*Z*,*E*)-9,12-tetradecadienyl acetate, a substance present in the sex pheromones of *Anagasta kuehniella* (Zeller) and *Plodia interpunctella* (Hübner) (Schöller & Prozell, 2002). Similar results were found for *Trichogramma oleae* (Voegele & Poitale), *Trichogramma cacoeciae* Marchal, and *Trichogramma bourarachae* Pintureau & Babault when exposed to (Z)-7-tetradecenal, a major molecule of the sex pheromone of *Prays oleae* (Bern) (Milonas et al., 2009).

Scales and other host substances deposited near or on the eggs during oviposition can repel (Beserra & Parra, 2013) or attract (Ferreira et al., 1979) the parasitoid during

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the search for hosts. Lewis et al. (1972) verified that the extract from *Cadra cautella* Walker on the deposited eggs increased their attractiveness to *T. evanescens*. The increase in *T. brassicae* parasitism on *Pieris brassicae* (L.) eggs was also shown by Fatouros et al. (2005), who found that egg clusters with scales were more parasitized by species of *Trichogramma* than egg clusters without scales. Chemical analysis of *Heliothis zea* (Boddie) scales indicated the presence of the linear hydrocarbon tricosane, which attracted *T. evanescens* (Jones et al., 1973). Shu et al. (1990) isolated 13,17-dimethylnonatriacontane hydrocarbons from *O. nubilalis* scales and observed that both were bioactive for *Trichogramma nubilale* Ertle & Davis.

Spodoptera frugiperda Smith (Lepidoptera: Noctuidae) is a key pest of maize, soybean, rice, and others crops (Besserra et al., 2002; Ko et al., 2014). Female *S. frugiperda* leave scales from wings and abdomen on the eggs (Urretabizkaya et al., 2010). The egg parasitoid *Trichogramma pretiosum* Riley, a tool for pest management (Fig ueiredo et al., 2015), might use scales from *S. frugiperda* to find eggs. Information about cues employed by *T. pretiosum* is important to increase its efficiency in the field. However, the influence of volatile compounds in *S. frugiperda* scales on the parasitism of *T. pretiosum* has not been studied. Thus, the objectives of this work were (1) to evaluate whether *T. pretiosum* uses volatiles from *S. frugiperda* scales and eggs to locate the host, and (2) to identify possible kairomones in these volatiles.

Material and methods

Insects were reared in the laboratory under controlled conditions (25 ± 1 °C, 65 ± 10% r.h., L14:D10 photophase). The *T. pretiosum* colony was obtained from eggs of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) collected in a maize field, located in the municipality of Santa Maria, RS, Brazil (29°41'24"S, 53°48'42"W). The strain of *T. pretiosum* was kept on *A. kuehniella* eggs, maintained on a diet based on wheat flour (97%) and brewer's yeast (3%), at 25 ± 1 °C, 65 ± 10% r.h., and L14:D10 photophase (Parra, 1997). The methodology explained in Parra (2001) was used for culturing *S. frugiperda* under the same climatic conditions.

Obtaining the scales

Scales from *S. frugiperda* were obtained from females that were virgin (V), mated (M), or mated and oviposited (O). To obtain virgin female scales, the pupae were separated by sex and the females were placed in plastic pots (35 ml). After emergence, the females (up to 24 h old) were killed by freezing (–20 °C) and the scales were separated with a brush nr. 0 (Tigre, Joinville, Brazil). To extract the scales of mated females, males and females of up to 24 h old were paired in cylindrical cages (10 cm high, 15 cm diameter) of polychloroethene (PVC), and after visual recording of the first copula, females were separated and the scales were removed as from the virgins. For O scales, females and males were paired under the same conditions as 'M'; immediately after the first copulation, they were separated from the males, then the scales were removed after the first oviposition. Females in all three conditions were killed by freezing. The scales of up to three females were extracted from the ventral part of the wings and abdomen (between segments VII and IX) (1:1). A standardized amount of 2.5 ± 0.1 mg of scales (i.e., the equivalent of scales of one female) was used in the olfactory bioassays within 1 h of extraction. Six extracts of the scales were produced and used for the bioassays.

Extraction and identification of scale compounds

The scales were placed in a 4-ml glass vial (Agilent, Santa Clara, CA, USA), in which 3 ml of hexane solvent (P.A., HPLC grade, or equivalent; Sigma Aldrich, San Luis, MO, EUA) was added covering all scales. The flasks with the scales soaking in the solvent were kept in an ultrasonic bath for 10 min to extract the compounds. Then, the extracts were filtered through glass wool packed in a Pasteur pipette (230 mm long). Six extractions were conducted, each with 25 females (62.5 ± 0.5 mg of scales). Each *S. frugiperda* scale extract was concentrated on N_2 flow to 20 µl. For quantitative analysis, 2 µl of each of the samples was injected into a gas chromatograph (GC) coupled to a 7895 B flame ionization detector (Agilent), equipped with a DB-5MS apolar column (30 m long × 0.25 mm internal diameter and 0.25 µm film thickness) (J&W Scientific, Folsom, CA, USA). Helium was used as carrier gas. The initial slope temperature was 50 °C for 2 min with a gradual increase from 5 °C per min to 180 °C, followed by a second step increase of 10 °C per min to 250 °C, and held at this temperature for 20 min. The detector and injector were maintained at 270 and 250 °C, respectively. The compounds were quantified using the internal standard method, and 1 µl of a 0.25 µg ml^{-1} solution of 16-hexadecanolactone was added to each scale sample. The response factor of all compounds was considered one. Data were collected using GC-MS ChemStation 2.1 software (Agilent) and analyzed using an Excel spreadsheet.

For qualitative analysis, selected samples were analyzed on a GC (Agilent 7890) coupled to a mass spectrometer (MS) (Agilent 5975-MSD) equipped with a quad-polar analyzer with a non-polar DB-5MS column (30 m long × 0.25 mm ID and 0.25 µm film thickness; J&W Scientific) and a split-splitless injector, with helium as the

drag gas. Ionization was by electron impact (70 eV and ionization source temperature of 230 °C). The oven temperature was maintained at 50 °C for 2 min and programmed to increase at 5 °C per min to 180 °C, then at 10 °C per min to 250 °C, and held for 20 min. Data were collected and analyzed with GC-MS ChemStation 2.1 software. Compounds were identified by comparing GC retention times and fragmentation patterns of commercially available chemical (Sigma Aldrich/Bedoukian) mass spectra as well as by calculation of Kováts indices.

Olfactometry

The bioassays were conducted in a Y-tube olfactometer with a diameter of 1 cm, initial arena of 12 cm long, bifurcated into two 5-cm-long arms each under 60-W fluorescent light (luminance 290 lux) under controlled conditions (25 ± 1 °C and 65 ± 10% r.h.). An air stream, previously filtered with activated charcoal (4-12 mesh; Supelco, Bellefonte, PA, USA), was conducted into the system with the aid of an air pump connected to a flow meter and a humidifier at a rate of 0.3 l per min. The olfactometer was inverted horizontally (180° rotation) after every three repetitions. After every sixth test, it was washed with neutral soap, ethyl alcohol, and distilled water, and then dried in a sterilization autoclave at 150 °C. At each wash of the olfactometer, the filter papers were changed and treated with new aliquots of the treatments. Mated females, kept without feeding, were inserted separately in the test arena with a fine brush (000). The parasitoids that reached one of the odor sources or crossed at least 3 cm of the arms and remained in this area for at least 1 min (first choice) were considered responsive. Insects that did not move within the first 5 min or had not reached one of the two arms of the olfactometer after 10 min were considered nonresponsive and were excluded from statistical analysis. All *T. pretiosum* females used in the bioassays had *A. kuehniella* as original host and were at most 24 h old. Before the beginning of the tests, the females were isolated and acclimatized for 1 h in the bioassay room. Between 40 and 45 repetitions were performed for each treatment. Repetitions and contrasts between treatments were performed on different days.

Chemotactic responses of *Trichogramma pretiosum*

Initially, wing and abdomen scales from *S. frugiperda* were obtained in three physiological conditions (V, M, and O) and each one was placed in a plastic tube (4 cm high, 0.5 cm diameter) open at the ends, which was inserted into one of the arms of the olfactometer. The chemotaxis of *T. pretiosum* to the egg volatiles (maximum 24 h old) was also evaluated in the presence and absence of scales (2.5 ± 0.1 mg) from V, M, or O females. Eggs were removed using a brush (000) and distilled water to separate the eggs, and placed on sulfite paper cards (75 g m^{-2}) containing 100 eggs (<24 h old). In the treatments with eggs and scales, the scales were placed on the eggs with the same brush. The cards were inserted into the plastic tube, as already described. The two-choice assays evaluated: (1) wing scales vs. abdomen scales; (2) eggs vs. eggs + V scales (wings + abdomen); (3) eggs vs. eggs + M scales (wings + abdomen); (4) eggs vs. eggs + O scales (wings + abdomen); (5) eggs vs. V scales (wings + abdomen); (6) eggs vs. M scales (wings + abdomen); and (7) eggs vs. O scales (wings + abdomen).

Chemotactic responses to synthetic scale compounds

The response of *T. pretiosum* was verified by mixing the following compounds obtained from Sigma-Aldrich (Gillingham, UK): linalool (97%), hexan-2-ol (99%), 4-hydroxy-4-methylpentanone (99%), 6-methyl-5-hepten-2-one (99%), nonanal (95%), decanal (98%), octadecanal (99%), nonanoic acid (99%), benzoic acid (99.5%), eicosane (99%), heneicosane (98%), dodecosane (99%), tetracosane (99%), pentacosane (99%), hexacosane (99%), heptacosane (99%), octadecane (99%), nonacosane (99%), (*Z*)-9-tetradecenyl acetate (*Z*9-14 OAC) (97%), and (*Z*)-11-hexadecenyl acetate (*Z*11-16 OAC) (95%). The total mixture (TM) was made using all compounds except the acetates at the same concentration (0.01 μg μl^{-1}) in equal amounts. The TM volume tested was 10 μl contrasted with the same solvent hexane. The responses of *T. pretiosum* to hexane solutions of *Z*9-14:OAC or *Z*11-16 OAC, at concentrations of 1, 0.1, and 0.01 μg μl^{-1}, in the volume of 10 μl were compared with hexane (10 μl).

Statistical analysis

The chemotactic responses were compared with χ^2 and binominal testes alternatives analyzed by the program BioEstat v.5.0 (Ayres et al., 2007), at a significance level of 5%.

Results

Chemotactic responses of *Trichogramma pretiosum*

Trichogramma pretiosum did not differentiate between scales collected from wings vs. abdominal scales of either of the three types of females moths (V: $\chi^2 = 0.20$, P = 0.82; M: $\chi^2 = 0.80$, P = 0.50; O: $\chi^2 = 0.20$, P = 0.82; all d.f. = 1) (Figure 1). More V and M *T. pretiosum* females were attracted to the volatiles of scales (wings + abdomen) than to the control (air) (V: 61%, $\chi^2 = 7$, P = 0.014; M: 65.9%, $\chi^2 = 9.8$, P = 0.004; both d.f. = 1). However, when exposed to volatiles of scales removed after the first oviposition, the parasitoids preferred the control (air) (62.5%,

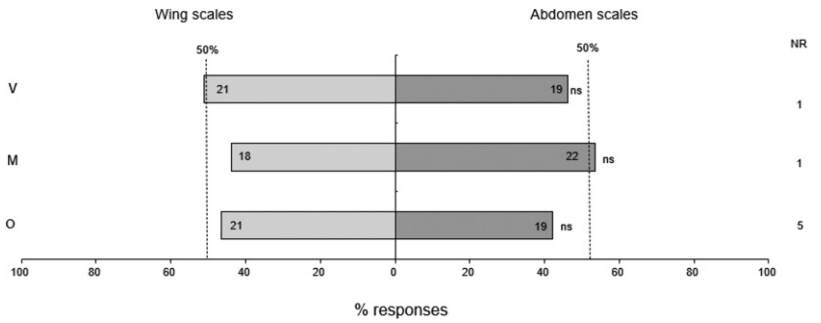

Figure 1 Chemotactic responses (%) of *Trichogramma pretiosum* females (<24 h old) in a two-choice olfactometer when offered volatiles from wing vs. abdomen scales of *Spodoptera frugiperda* females that were virgins (V), mated (M), or mated and oviposited (O). The numbers inside the bars indicate the responding insects. NR, nonresponsive insects; ns, non-significant difference (χ^2 test: P>0.05).

$\chi^2 = 5$, d.f. = 1, P = 0.044) (Figure 2). More parasitoids headed to volatiles of eggs combined with scales from V females (57.8%, $\chi^2 = 7.2$, d.f. = 1, P = 0.014) or with scales from M females (61%, $\chi^2 = 5.0$, d.f. = 1, P = 0.044) than to volatiles of eggs without scales (Figure 3). No difference was observed when parasitoids was offered the choice between volatiles from eggs with scales of O females (53.7%) vs. without scales (43.9%; $\chi^2 = 0.80$, d.f. = 1, P = 0.50) (Figure 3). There was no difference in response to scales of V (46.7%; $\chi^2 = 0.20$, d.f. = 1, P = 0.82) and M (54.8%; $\chi^2 = 1.80$, d.f. = 1, P = 0.26) females compared to eggs (42.2 and 54.8%, respectively). However, when parasitoids were exposed to the scales of O females vs. eggs, they preferred eggs (62.8%; $\chi^2 = 6.86$, d.f. = 1, P = 0.016) (Figure 4).

Chemical analysis of scales

Twenty compounds were identified in the extracts of *S. frugiperda* scales, including linalool, hexan-2-ol, 4-hydroxy-4 methylpentanone, 6-methyl-5-hepten-2-one, nonanal, decanal, octadecanal, nonanoic acid, benzoic acid, straight chain hydrocarbons (nC_{20} to nC_{29}), and the two acetates (*Z*)-9-tetradecenyl acetate (*Z*9-14:OAC) and (*Z*)-11-hexadecenyl acetate (*Z*11-16:OAC), which are known to be part of the sex pheromone of *S. frugiperda* (Figure 5; Table 1).

Chemotactic responses to synthetic scale compounds

Trichogramma pretiosum did not exhibit a chemotactic response to the mixture of the compounds (TM) identified in the scales in absence of the acetates (46.7%) when tested against hexane (51.3%; $\chi^2 = 0.20$, d.f. = 1, P = 0.82). Female parasitoids were attracted to *Z*9-14:OAC at the three concentrations evaluated when tested against hexane (1 μg μl^{-1}: 60.5%, $\chi^2 = 8.91$, P = 0.005; 0.1 μg μl^{-1}: 63.4%, $\chi^2 = 7.20$, P = 0.013; 0.01 μg μl^{-1}: 62.8%, $\chi^2 = 9.80$, P = 0.003; all d.f. = 1) (Figure 6). For the compound *Z*11-16:OAC compared to hexane, female parasitoids preferred the compounds at the lowest concentrations (0.1 μg μl^{-1}: 61.9%, $\chi^2 = 7.20$, P = 0.014; 0.01 μg μl^{-1}: 60.5%, $\chi^2 = 5.90$, P = 0.027; both d.f. = 1) (Figure 6).

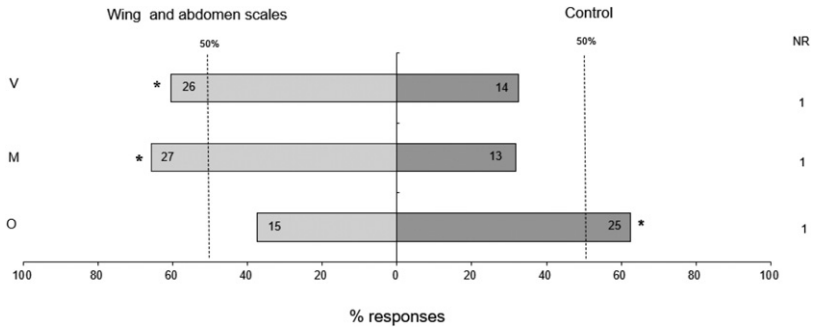

Figure 2 Chemotactic responses (%) of *Trichogramma pretiosum* females (<24 h old) in a two-choice olfactometer when offered clean air (control) vs. volatiles from wing and abdomen scales of *Spodoptera frugiperda* females that were virgins (V), mated (M), or mated and oviposited (O). The numbers inside the bars indicate the responding insects. NR, nonresponsive insects. Asterisks indicate significant preference for one of the options (χ^2 test: P<0.05).

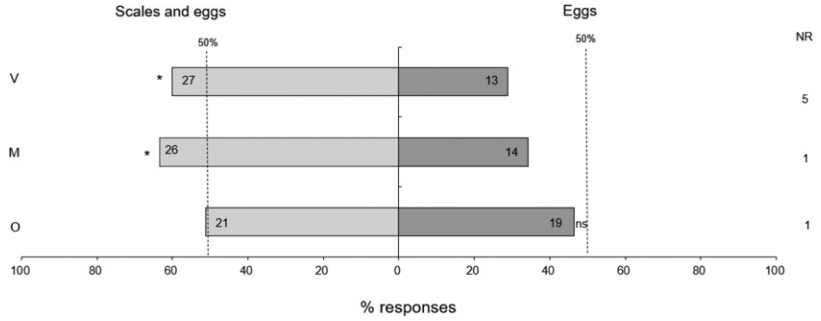

Figure 3 Chemotactic responses (%) of *Trichogramma pretiosum* females (<24 h old) in a two-choice olfactometer when offered volatiles from eggs vs. eggs combined with scales (wing + abdomen) from *Spodoptera frugiperda* females that were virgin (V), mated (M), or mated and oviposited (O). The numbers inside the bars indicate the responding insects. NR, nonresponsive insects. Asterisks indicate significant preference for one of the options (χ^2 test: P<0.05; ns, P>0.05).

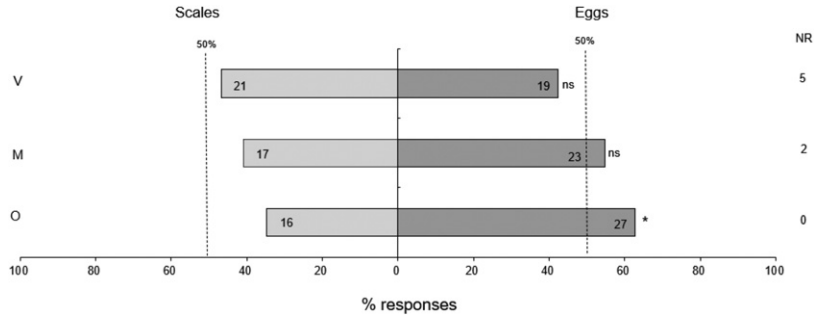

Figure 4 Chemotactic responses (%) of *Trichogramma pretiosum* females (<24 h old) in a two-choice olfactometer when offered volatiles from eggs vs. scales (wing + abdomen) from *Spodoptera frugiperda* females that were virgin (V), mated (M), or mated and oviposited (O). The numbers inside the bars indicate the number of responding insects. NR, nonresponsive insects. The asterisk indicates a significant preference for one of the options (χ^2 test: P<0.05; ns, P>0.05).

Discussion

Trichogramma pretiosum did not respond differently to scales from wings or abdomen of females in the three physiological conditions (V, M, or O). In *S. frugiperda*, the pheromonal gland is located between the abdominal segments VII and IX, and its eversion begins the liberation of pheromonal molecules that attract the male to copulate (Leiderman & Sauer, 1953). In the laboratory, *S. frugiperda* females initiate this process on average 24 h after their emergence (Blomquist & Vogt, 2003), and the behavior is repeated once each scotophase, until the female mates (Beserra & Parra, 2013). Thus, the proximity of this gland to the scales on both wings and abdomen, causes them to become soaked with the pheromone.

When the scales were contrasted with air, *T. pretiosum* females were attracted to virgin (V) and mated (M) females, indicating that they contained kairomone compounds. Nordlund et al. (1983) reported the attraction of *T. remus* by extracts from scales of virgin *S. frugiperda* females. Similarly, Arakaki et al. (1996) demonstrated that volatiles emitted by virgin females of *Euproctis taiwana* (Shiraki) attracted *Telenomus euproctidis* Wilcox. However, none of these studies indicated which compounds may be involved in the attraction of trichogrammatids. De Lury et al. (1999) found that the parasitoid *Ascogaster quadridentata* Wesmael uses kairomones in the scales of *Cydia pomonella* (L.) eggs, and that compounds – such as heptanal, octanal, nonanal, decanal, undecan-2-one, dodecanal, pentadecan-3-one, (*Z*)-6-pentadecen-2-one, (*Z*)-9-hexadecenal, (*Z*)-6-heptadecene-2-one, and 3,7,11-trimethyl-2*E*,6*E*,10-dodecatrien-1-ol acetate – could be involved in this attraction. These compounds are not part of the sex pheromone mixture released by *C. pomonella*.

The lack of chemotaxis by the parasitoids to scales of females that had already performed the first oviposition may be because the females do not evert the pheromonal gland after the first copulation, despite accepting other

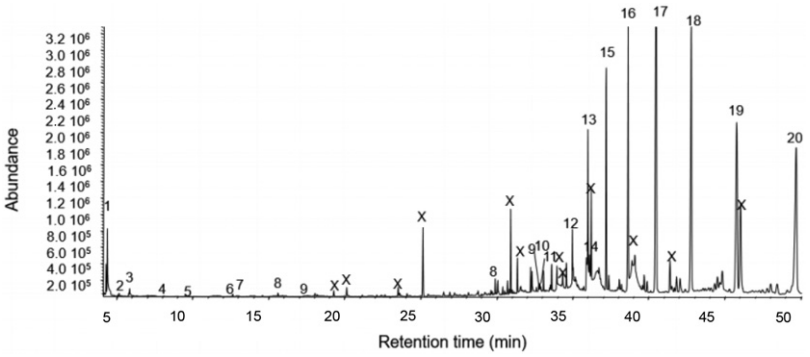

Figure 5 Profile of the gas chromatographic-mass spectrometric analysis, indicating the compounds identified in wing and abdomen scales from *Spodoptera frugiperda* virgin females. (1) hexan-2-ol; (2) 4-hydroxy-4-methyl-pentanone; (3) 6-methyl-5-hepten-2-one; (4) linalool; (5) nonanal; (6) decanal; (7) nonanoic acid; (8) (*Z*)-9-tetradecenyl acetate (Z9-14:OAC); (9) (*Z*)-11-hexadecenyl acetate (*Z*11-16:OAC); (10) eicosane; (11) octadecanol; (12) heneicosane; (13) dodecosane; (14) benzoic acid; (15) tetracosane; (16) pentacosane; (17) hexacosane; (18) heptacosane; (19) octacosane; and (20) nonacosane.

Table 1 Mean (± SE) quantity (ng mg^{-1} of scale) and retention index (RI) of the compounds, present on wing and abdominal scales of virgin female *Spodoptera frugiperda*

Compounds	Quantity (ng mg^{-1})	IR
Hexan-2-ol	0.26 ± 0.124	803
4-Hydroxy-4-metylpentanone	0.02 ± 0.009	818
6-Metyl-5-hepten-2-one	0.09 ± 0.014	985
Linalool	0.01 ± 0.008	1100
Nonanal	0.10 ± 0.032	1103
Decanal	0.10 ± 0.027	1205
Nonanoic acid	0.01 ± 0.008	1275
(*Z*)-9-tetradecenyl acetate	0.13 ± 0.082	1795
(*Z*)-11-hexadecenyl acetate	0.06 ± 0.040	1993
Eicosane (C20)	0.43 ± 0.171	2000
Octadecanol	0.10 ± 0.028	2079
Heneicosane (C21)	0.75 ± 0.200	2100
Dodecosane (C12)	1.71 ± 0.554	2200
Benzoic acid	0.01 ± 0.072	2380
Tetracosane (C24)	3.17 ± 1.740	2400
Pentacosane (C25)	5.26 ± 3.206	2500
Hexacosane (C26)	3.76 ± 3.243	2600
Heptacosane (C27)	16.04 ± 5.974	2700
Octacosane (C28)	12.61 ± 4.84	2800
Nonacosane (C29)	12.04 ± 4.32	2900

males for mating in the laboratory (Besserra & Parra, 2013). As a consequence, the scales would not re-saturate; the molecules adsorbed by them before copulation would have already volatilized. Thus, the scales left with the first ovipositions would probably have the greatest amount of pheromone. *Trichogramma pretiosum* was more attracted to the hexane, probably due to changes in the chemical profile of the O scales, which could have resulted in repellency. A chemical analysis of these scales was not yet done.

Trichogramma pretiosum was more attracted to scales on egg masses from V and M females, than to egg masses that did not contain these scales. However, when parasitoids were only exposed to V or M scales, in contrast to eggs, there was no significant difference. These results indicate that scales from both V and M, as well as eggs, contain compounds that were attractive to *T. pretiosum*. Jones et al. (1973) reported that volatiles present on Lepidoptera scales attract parasitoids at long distances, suggesting that the encounter with the host is also mediated by scales left in the environment or on eggs. In addition, volatiles emitted by eggs act on the chemotaxis of parasitoids at short distances, aiding in host recognition and/or localization (Renou et al., 1992; Colazza et al., 2010; Tognon et al., 2017). The responses of *T. pretiosum* to O scales combined with eggs were similar to only eggs, which is evidence of the absence of kairomones on this type of scale. However, when the parasitoid was exposed to only O scales vs. eggs, females were directed more towards the eggs indicating that the eggs contain bioactive compounds.

The chemical analyses of the extracts from the scales found the presence of several volatile compounds, such as monoterpenes, linear hydrocarbons with up to 19 carbons, and the two major components of the sex pheromone of *S. frugiperda*, i.e., *Z*9-14:OAC and *Z*11-16:OAC (Batista-Pereira et al., 2000). The parasitoids were not responsive to the mixture containing the 18 compounds identified, without the presence of the two acetates. The alcohols, aldehydes, and monoterpenes identified in the scales are common plant compounds (Férnandez-Martínez et al., 2018), and this is the first study to identify them in insect scales. However, 6-methyl-5-hepten-2-one and linalool, released by plants after herbivory, have been shown to influence parasitoid seeking behavior (Du et al., 1998;

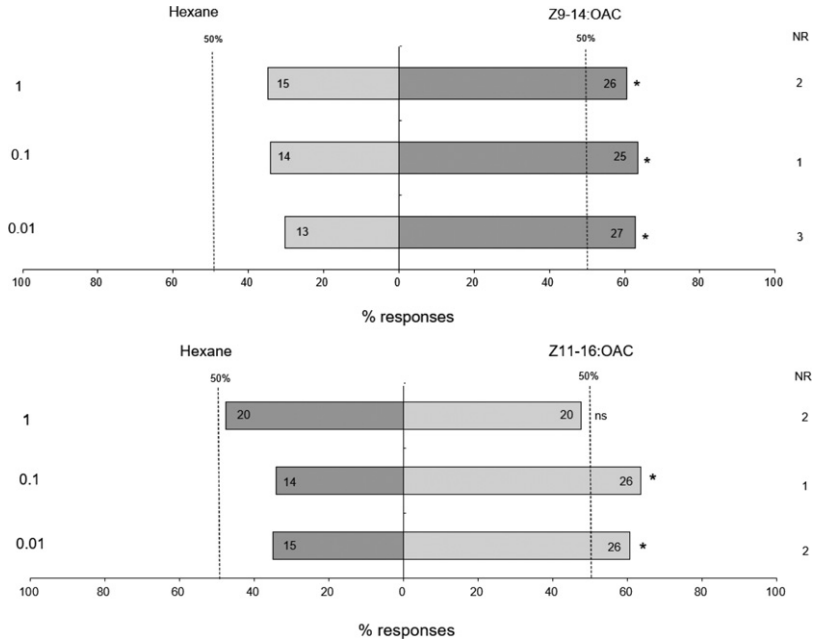

Figure 6 Chemotactic responses (%) of *Trichogramma pretiosum* females (<24 h old) in a two-choice olfactometer to hexane (control) vs. 1, 0.1, or 0.01 μg μl^{-1} (*Z*)-9-tetradecenyl acetate (*Z*9-14:OAC) or (*Z*)-11-hexadecenyl acetate (*Z*11-16:OAC). The numbers inside the bars indicate the responding insects. NR, nonresponsive insects. Asterisks indicate a significant preference for one of the options (χ^2 test: P<0.05; ns, P>0.05).

Michereff et al., 2011). The ratio between the components in the volatile mixture is crucial for the parasitoid to associate this information with the presence of the host (Bruce & Pickett, 2011). Thus, we cannot state that plant volatiles do not influence the attraction of *T. pretiosum*; however, in the proportions and quantities evaluated in this work, they did not influence the search behavior of the parasitoid. On the other hand, hydrocarbons, due to their low volatility, would be less perceptible to the parasitoids at short distances, but may be important as contact traces to recognize and locate the host (Wölfling & Rostás, 2009). Future studies using arena bioassays, in which the parasitoid has physical contact with the semiochemicals, could assess whether the identified hydrocarbons could influence the efficiency of parasitism by *T. pretiosum*, as already observed for *Trissolcus bassalis* (Wollaston) in eggs of Heteroptera (Peri et al., 2013).

In contrast, the two components from the sex pheromone of *S. frugiperda* were attractive for *T. pretiosum*. The attractiveness to these acetates had already been reported in other parasitoids, including *T. remus* to *Z*9-14:OAC (Norlund et al., 1983; Lewis & Norlund, 1984) and *T. evanescens* and *T. pretiosum* to *Z*11-16:OAC (Noldus et al., 1991).

Trichogramma pretiosum was able to recognize *Z*9-14:OAC at the three concentrations tested, unlike *Z*11-16:OAC, which was only attractive at concentrations of 0.1 and 0.01 μg μl^{-1}. The latter compound is found in a smaller quantity in the *S. frugiperda* pheromone (Descoins et al., 1988; Batista-Pereira et al., 2000) and was detected in smaller amounts in the extracts of the scales, which may explain the response of *T. pretiosum*. The presence of chemical compounds by parasitoids is closely linked to the specific proteins present in the antennal sensilla known as OBPs (Isidoro et al., 2001; Fan et al., 2011). These proteins are responsible for intercepting the chemical molecules and transducing this signal to the sensilla nerve endings (Fan et al., 2011). *Trichogramma pretiosum* may have a lower number of OBPs that perceive *Z*11-16:OAC; therefore, excessive amounts of this compound probably saturated these proteins, causing the lack of response to this stimulus. This result may also be associated with genetic adaptation (Milonas et al., 2009), as *Z*9-14:OAC and *Z*11-16:OAC are present in the sex pheromone of several host species of *T. pretiosum* (Lin & Guo, 1980); the generalist wasp could perceive various intensities of the molecules to increase the chance of host finding and thus reproductive output. The component *Z*11-16:OAC was quantified in extracts of *S. frugiperda* moths from Brazilian populations in a ratio 7× lower than *Z*9-14:OAC (Batista-Pereira et al., 2000). The release rates of the components are unknown. In the present study, *Z*11-16:OAC was released at 0.06 ng mg^{-1}, 2× less than *Z*9-14:OAC. This compound is also produced in several other species of moths, always as a minor component in the pheromonal mixture, e.g., in *Spodoptera cosmioides* (Walker) (Blassioli-Moraes et al., 2016), *Spodoptera eridania* (Cramer) (Teal et al., 1985), *Spodoptera desconsi* Lalanne-Cassou & Silvain (Monti et al., 1997), *Spodoptera praefica* (Grote) (Landolt et al., 2003), *Spodoptera sunia* (Guen) (Bestmann et al.,

1988), and *Spodoptera exigua* (Hübner) (Mitchell & Tumlinson, 1994). Our study found that the response of *T. pretiosum* to this pheromone is dose dependent and that the proportion between the compounds may help them distinguish different hosts in the field. The use of pheromones in chemical espionage is an important strategy for egg parasitoids, because it increases the search possibilities and the speed of encounter with new egg masses (Clausen, 1976), as observed for *T. brassicae* (Fatouros et al., 2005), *T. oleae, Trichogramma cacoeciae* Marchal, and *Trichogramma bourarachae* Westwood (Milonas et al., 2009).

Trichogramma pretiosum females live on average 10 days (Knutson, 1998) and are unable to travel long distances (Suverkropp et al., 2009), which increases the pressure to find a host (Fatouros & Huigens, 2012). The perception of pheromone molecules released by adults can lead the parasitoid to mating sites, where copulation is about to occur or in progress. In addition, parasitoids can use pheromonal molecules to find females with the intention of performing phoresis (Woelke et al., 2017). According to Fatouros et al. (2008), scales impregnated with pheromones deposited on the eggs can provide evidence of oviposition sites, and the substances present in the egg masses and the chorion can also act as short-distance kairomones (Bin et al., 1993). Our results increase knowledge about the strategies that *T. pretiosum* employ to search for *S. frugiperda* eggs. Future studies could assess whether the combination of the two components provides a synergistic effect interfering with the chemotactic response of *T. pretiosum*.

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