Response of the Egg Parasitoids *Trissolcus basalis* and *Telenomus podisi* to Compounds from Defensive Secretions of Stink Bugs

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Abstract We tested the hypotheses that host-searching behavior of the egg parasitoids *Telenomus podisi* and *Trissolcus basalis* may be differentially influenced by the different blends of volatiles released from the metathoracic glands of adult stink bug host species. We further studied whether such a differential response is due to different individual components of these glands and whether these responses reflect host preferences. Y-tube olfactometer bio-assays were carried out with crude extracts of metathoracic glands of five different host species of neotropical stink bugs. Additionally, we tested the parasitoids' responses to synthetic standards of individual compounds identified in these stink bug glands. Results showed that females of *T. basalis* and *T. podisi* responded differentially to crude gland extracts of the different species of host stink bugs and to the compounds tested. The parasitoid *T. basalis* showed a positive taxic behavior to *Nezara viridula* methathoracxic gland extracts of a host species preferred in the field, i.e., *N. viridula*. Furthermore, *T. basalis* responded positively to 4-oxo-(*E*)-2-hexenal and (*E*)-2-decenal, two components of *N. viridula* glandular secretion. Higher residence time, reduced linear velocity, and higher tortuosity in the arm of the olfactometer supplied with 4-oxo-(*E*)-2-hexenal showed that this compound modifies the kinetics of some traits of *T. basalis* walking pattern and suggests that it might stimulate the searching behavior of this parasitoid. The parasitoid *T. podisi* was attracted to crude gland extracts of the preferred host (*Euschistus heros*) and also to 4-oxo-(*E*)-2-hexenal. Additionally, this parasitoid responded positively to (*E*)-2-hexenal and to the hydrocarbon tridecane, both of which are defensive compounds released from the metathoracic glands by several stink bugs. The results indicate some degree of specialization in the response of two generalist parasitoid species toward defensive secretions of stink bugs.

Keywords Defensive compounds · Host searching · Host–parasitoid interactions · Kairomones · Host preference · Hymenoptera · Sceolionidae · Hemiptera · Pentatomidae

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Introduction

Parasitoids are known for using semiochemicals as medium- and long-range cues when searching for hosts (Vinson 1985, 1998; Vet and Dicke 1992; Steidle and van Loon 2002; Fatouros et al. 2008). Semiochemicals that originate from the host habitat, the hosts themselves, or indirectly from stages associated to the host can be used by parasitoids during a hierarchical sequence of steps for host location and selection (Vinson 1985).

Egg parasitoids face the challenge of finding hosts that are not, or are barely apparent (eggs). Therefore, they must

rely on semiochemical cues that are more detectable than those from the eggs, such as those from stages of the host that are not suitable for parasitism (adults or immature stages) or from host plants (Vet et al. 1991, 1995; Vet and Dicke 1992; Vinson 1998; Fatouros et al. 2008).

Scelionidae that parasitize eggs from stink bugs can use several types of semiochemicals for long-range localization of habitat, microhabitat, and hosts: volatiles from plants damaged by stink bug oviposition or feeding (Colazza et al. 2004; Moraes et al. 2005a, 2008); sex pheromones (Aldrich 1985, 1995; Borges et al. 1998, 2003; Bruni et al. 2000; Silva et al. 2006); volatile defensive secretions from the metathoracic (adults), or dorsal abdominal (nymphs) glands of stink bugs (Aldrich 1985, 1995; Mattiaci et al. 1993; Borges and Aldrich 1994); or crude whole body extracts of stink bugs (Colazza et al. 1999; Salerno et al. 2006). Volatiles from nonhost stages of stink bugs, such as pheromones or defensive compounds, as well as contact chemicals (traces left by walking insects) also can be used for host location, recognition, and acceptance, thus leading to successful oviposition (Bin et al. 1993; Borges et al. 1999, 2003; Colazza et al. 1999; Conti et al. 2003). Physical stimuli such as visual and resonance cues also may be involved in successful host search (Borges et al. 1999). Laumann et al. (2007) demonstrated that foraging *Telenomus podisi* Ashmead, 1881 can orientate toward hosts by using substrate-borne vibratory signals produced during sexual communication of host stink bugs.

The Scelionidae comprise a large family with about 3,000 described species (Masner 1993) that primarily attack eggs of Heteroptera and Lepidoptera and less frequently are reported as egg parasitoids of Diptera, Orthoptera, Coleoptera, and Araneae (Arias-Penna 2002; Austin et al. 2005). In central Brazil, *Trissolcus basalis* (Wollaston 1958) and *T. podisi* are common parasitoids of stink bug eggs. In soybean agroecosystems in Brazil, *T. basalis* shows high parasitism in eggs of the pentatomid stink bug *Nezara viridula* (L., 1758) and *T. podisi* in eggs of the pentatomid *Euschistus heros* (Fabricius 1794) (Corrêa-Ferreira and Moscardi 1995; Medeiros et al. 1997, 1998; Pacheco and Correa-Ferreira 2000). These field observations were confirmed by Sujii et al. (2002) in a laboratory study, who reported that *T. podisi* showed high parasitism indices in *E. heros* eggs both in no choice and multiple-host choice experiments. In the same set of experiments, *T. basalis* showed high parasitism indices on eggs of *N. viridula* and *Piezodorus guildinii* (Westwood. 1837) in no choice experiments and showed a clear preference for eggs of *N. viridula* in multiple-host choice experiments (Sujii et al. 2002).

Aldrich (1995) pointed out that preferences observed in scelionid wasps may be based on differential long-range attraction to stink bugs allomones. There is evidence that *T.*

basalis uses (*E*)-2-decenal, a defensive compound of *N. viridula*, as a kairomone (Mattiaci et al. 1993).

In this study, we tested the following hypotheses by using *T. podisi* and *T. basalis* and five different stink bug species as hosts: (1) Host-searching behavior of the egg parasitoids is differentially influenced by blends from metathoracic glands of the different host stink bug species; (2) Individual components of these blends may be responsible for such a differential response; (3) A differential response to odor from metathoracic glands of hosts is related to the preference for these host species.

We recorded the parasitoids' behavioral responses in a Y-tube olfactometer to crude metathoracic gland extracts of five neotropical stink bugs species and to synthetic standards of individual compounds of the metathoracic glands of different species.

Methods and Materials

Insects Parasitoids used in this work were obtained from a laboratory colony that started from parasitized stink bug egg masses collected near the Embrapa Genetic Resources and Biotechnology Laboratory in Brasilia, DF, Brazil (15°47′ S and 47°55′ W). Insects were maintained in an environmental chamber in plastic cages (25-cm²-angled-neck tissue culture flasks, ICN Biomedicals) under a 14-h photophase at 26.0± 0.5°C and 65±10% relative humidity. Droplets of pure bee honey were supplied as a food source. Both parasitoid species (*T. basalis* and *T. podisi*) were reared on *E. heros* eggs. Host eggs were exposed to parasitoid females for 24 h and then removed and placed in glass tubes (7.5×1.3 cm) for incubation. Adult parasitoids obtained with these procedures were kept for 24–48 h in the plastic cages described above for mating. Later, the females were separated individually into glass tubes (4×0.5 cm) for use in bioassays. In this way, all females used in the bioassays were similarly experienced.

Gland Extracts To test the influence of the defensive compounds from different host species on the response of the parasitoids, bioassays were performed with the natural blends of metathoracic glands obtained by dissecting adults of different species. Adults of *E. heros*, *N. viridula*, *P. guildinii*, *Chinavia impicticornis* (Stål 1872), and *Edessa meditabunda* (Fabricius 1794) were dissected under a stereoscopic microscope, and the metathoracic glands were extracted with microdissecting forceps. The contents of the dissected glands were extracted in *n*-hexane for 2 h. Then, the tissues were removed, and the volume was adjusted to four glands per milliliter. With this procedure, we obtained metathoracic gland extracts having the defensive compounds in similar concentrations to the range of the solutions of individual compounds used in the bioassays

(next section; M.C.B. Moraes, unpublished data). Before the bioassays, the qualitative compositions of the extracts were checked by gas chromatography (data not shown). Four to five extracts of each species were obtained with this procedure and used in the bioassays.

Chemicals For the bioassays that used individual compounds, solutions of the synthetic standards of (*E*)-2-hexenal, 4-oxo-(*E*)-2-hexenal, tridecane, (*E*)-2-decenal, and undecane were prepared at two concentrations (0.01 and 0.1 mg/ml hexane). These compounds were selected based on the following criteria: (1) compounds commonly found in defensive secretions of neotropical stink bugs and the cosmopolitan, *N. viridula*, i.e., (*E*)-2-hexenal, 4-oxo-(*E*)-2-hexenal, and tridecane (Borges and Aldrich 1992; Aldrich 1995; Zarbin et al. 2000; Moraes et al. 2005b; Borges et al. 2007; Pareja et al. 2007); (2) a compound found as a major component in glands of *N. viridula* and *Chinavia* spp. and as a minor component in *E. heros* and *Thyanta perditor* (Fabricius 1794), i.e., (*E*)-2-decenal (Borges and Aldrich 1992; Moraes et al. 2005b; Pareja et al. 2007); and (3) undecane, a compound found as a major component exclusively in *Edessa meditabunda* (Borges and Aldrich 1992) and *E. rufomarginata* (Howard and Wiemer 1983) and in low amounts (near trace quantities) in *Chinavia ubica* (Rolston 1983), *T. perditor*, *E. heros*, and *P. guildinii* (Borges and Aldrich 1992; Moraes et al. 2005b; Pareja et al. 2007).

All authentic standards were purchased from Sigma-Aldrich (St Louis, MO, USA), Fluka (Buchs., Switzerland), Bedoukian (Danbury, CT, USA), or TCI (Tokyo, Japan). Synthetic 4-oxo-(*E*)-2-hexenal was provided by J.R. Aldrich (USDA, Beltsville, MD, USA).

General Procedures for Olfactometer Bioassays A Y-tube olfactometer constructed with an acrylic block with a Y-shaped cavity (27.5×21.0 cm), placed on top of a translucent glass plate and covered with transparent glass, was used to test the influence of the chemical compounds on the behavior of the parasitoids. The trunk of the apparatus measured 8 cm, plus a 1-cm circular area at the base of the trunk for insect liberation, and each arm measured 7 cm (at an angle of 130°, id 1.5 cm). Charcoal-filtered, humidified air was passed through each arm at 800 ml/min in a push–pull system. The air flow was maintained with two aquarium pumps. The olfactometer was illuminated from above by two fluorescent lamps (40 W) and from below by two infrared lamps (homogenous emission of wavelengths at 950 nm provided by 108 LEDs). The behavior of the insects was recorded by a CCD Sony SPT M324CE camera (fitted with a 4- to 50-mm/ F1:1.6 zoom lens with an infrared filter) coupled to the Xbug software (Colazza et al., unpublished). Filter papers

(1.5-cm long, 0.5-cm wide) treated with 5 μl of the test solution [gland extract or synthetic compound (25 ml), see above] were introduced into a glass chamber located close to the air entrance in one of the arms. In the other arm, a glass chamber with a filter paper treated with 5 μl *n*-hexane was used as control. To avoid any bias in the parasitoid responses, the arms through which control and treatment odors were presented were inverted every two to three bioassays. The apparatus was cleaned after two to three bioassays with fragrance-free liquid soap, rinsed thoroughly with water, and dried in convector ovens (at 160°C for the glass material and 60°C for the acrylic box).

For each bioassay, a single naïve *T. podisi* or *T. basalis* female (24–48 h adult stage) was introduced at the base of the Y-tube, and its behavior was monitored for 10 min. Insects that had not made a choice after 5 min were considered as nonresponders, and they were not included in the statistical analyses. To test for any bias between the two olfactometer arms, blank tests were carried out, in each set of bioassays, presenting *n*-hexane in both arms (*N*=30 for each parasitoid species). All bioassays were performed between 1000 to 1600 hours in a room at 26.0±1.0°C. During each bioassay, two parameters were recorded: first choice, measured as the arm of the olfactometer into which the insect entered first for at least 1 cm and remained for at least 20 s; and residence time, measured as the percentage of total bioassay time spent in each arm of the olfactometer.

Bioassays with Defensive Gland Extracts These bioassays were performed to test the response of the parasitoids toward the natural blend of defensive glands of different stink bugs species. Gland extracts that were obtained as described above were used. As a first step, extracts of each species were contrasted with *n*-hexane to test whether the parasitoid showed a significant response to the natural blend of each species. In a second step, the gland extracts of the host species for which a parasitoid showed a positive response were contrasted to investigate in a dual choice test whether the parasitoid showed a preference for one or another host species. For each parasitoid species, 40–50 insects were tested toward each defensive blend extract against *n*-hexane and toward each relevant host species— species pair recording the same parameters as described above.

Bioassays with Synthetic Compounds To test the effect of individual components present in the defensive gland blends, bioassays were performed with authentic standards of the compounds. Compounds tested in bioassays were used in solutions at two concentrations (0.1 and 0.01 mg/ml *n*-hexane). These concentrations were chosen based on previous bioassays described by Mattiaci et al. (1993) and Pires et al. (2001). Each compound was tested using *n*-

hexane as control; 30–55 replicates were performed for each compound at each concentration. The parameters computed during the monitoring were first choice and residence time. Additionally, we investigated the effect of individual compounds on specific parameters of the parasitoids' searching behavior, i.e., linear velocity (mm/s), turning rate (°/cm), and tortuosity. Turning rate is the number of times that the insect changes its route in the olfactometer, and the tortuosity index quantifies insect kinetic movement by the calculation $T=1-mp/tl$, in which mp represents the projection of the track in general straight line of the plain and tl the total length of the track (Borges et al. 2003). The index varies between 0 (zero) for minimal and 1 (one) for maximal tortuosity. Slow linear velocity, high turning rate, and tortuosity may be indicative of searching behavior stimulated by a cue.

Bioassays with Defensive Blends vs. Individual Compounds To test whether individual compounds that elicit positive response in parasitoids have the same effect as complete natural blends from defensive glands, bioassays were performed that contrasted the complete defensive gland blends against individual compounds for which each parasitoid showed a positive response; 40–50 bioassays were performed, and first choice and residence time were recorded.

Statistical Analyses The choices made by the parasitoids in the bioassays were analyzed by logistic regression and estimation of the probability of choosing the test odor. The model fitted contained a factor for the side (left or right) on which the test odor was presented to control for this variability. The hypothesis of no preference (50% first choice to each odor) was tested by means of a χ^2 Wald test. The percentages of the total bioassay time spent in each odor field and in blank tests (residence time) were analyzed by Wilcoxon's matched-pairs test after arcsine transformation of the data. Mean average linear velocity, turning rate, and tortuosity in treatment and control arms for each compound were compared with Student's *t* test or Mann–Whitney test when the data were not normally distributed. Insects that made a choice but remained immobile for more than 300 s were excluded from the residence time, linear velocity, turning rate, and tortuosity analyses.

Results

Y-tube olfactometer bioassays with metathoracic gland extracts of the five adult stink bug species showed that each parasitoid species responded selectively to the gland extract of its preferred stink bug host species. The egg parasitoid *T. basalis* preferred odor from extracts of *N. viridula* when compared to the control ($\chi^2_1=5.33$, $P=0.021$, $N=46$; Fig. 1a). Residence time in this treatment was higher than in the control ($W=395.0$, $P=0.014$, $N=43$; Fig. 1b). Odors of metathoracic glands of the other stink bug species did not elicit significant preference nor did they affect residence time (Fig. 1a and b). Since *T. basalis* showed a response only to extracts of *N. viridula*, no comparisons were necessary with extracts of other species.

The egg parasitoid *T. podisi* significantly preferred the odor from gland extracts of *E. heros* ($\chi^2_1=13.88$, $P<0.001$, $N=40$; Fig. 2a). When extracts of *N. viridula* were tested,
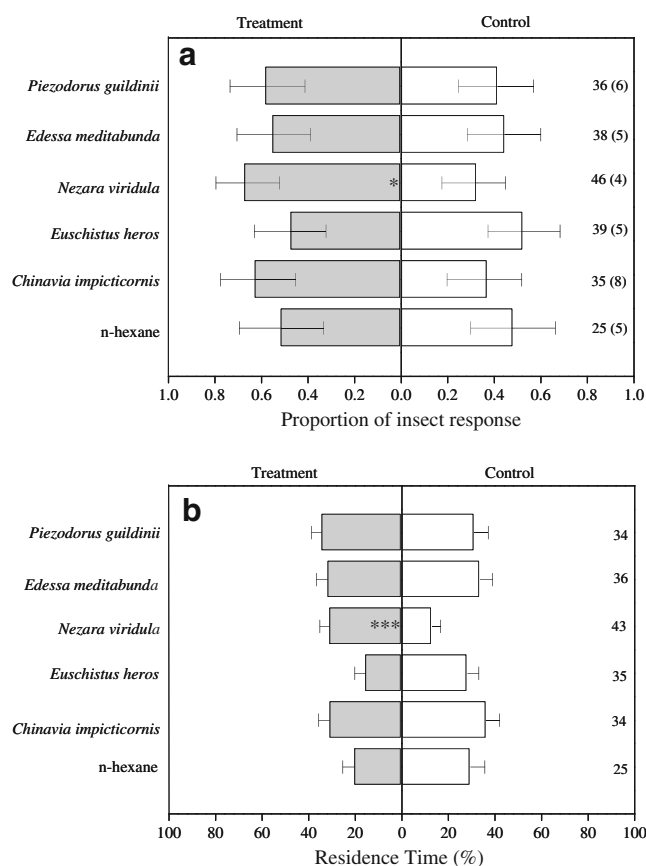

Fig. 1 First choice (**a**) and residence time (**b**) of the parasitoid *T. basalis* in Y-tube olfactometer bioassays with blends of metathoracic glands of different species (four glands per milliliter *n*-hexane) and *n*-hexane as control. Analyses of first choices were carried out by logistic regression and a Wald χ^2 statistic to assess significance. Mean residence time in treatment and control arms was analyzed by Wilcoxon's matched-pairs test. *0.05>*P*>0.01, ***P*<0.001. *Bars* indicate the mean values of the parameters, and *lines* are the 95% confidence interval for first choice and SE for residence time. *Numbers on the right side of the figures* are the total number of insects tested. *Numbers in brackets in figure* (**a**) represent the number of insects that did not respond to the treatment tested

Fig. 2 First choice (**a**) and residence time (**b**) of the parasitoid *T. podisi* in Y-tube olfactometer bioassays with blends of metathoracic glands of different species (four glands per milliliter *n*-hexane) and *n*-hexane as control. Analyses of first choices were carried out by logistic regression and a Wald χ^2 statistic to assess significance. Mean residence time in treatment and control arms was analyzed by Wilcoxon's matched-pairs test. *0.05>P>0.01, **0.01>P>0.001, and ***P<0.001. *Bars* indicate the mean values of the parameters, and *lines* are the 95% confidence intervals for first choice and SE for residence time. *Numbers on the right side of the figures* are the total number of insects tested. *Numbers in brackets in figure* (**a**) represent the number of insects that did not respond to the treatment tested
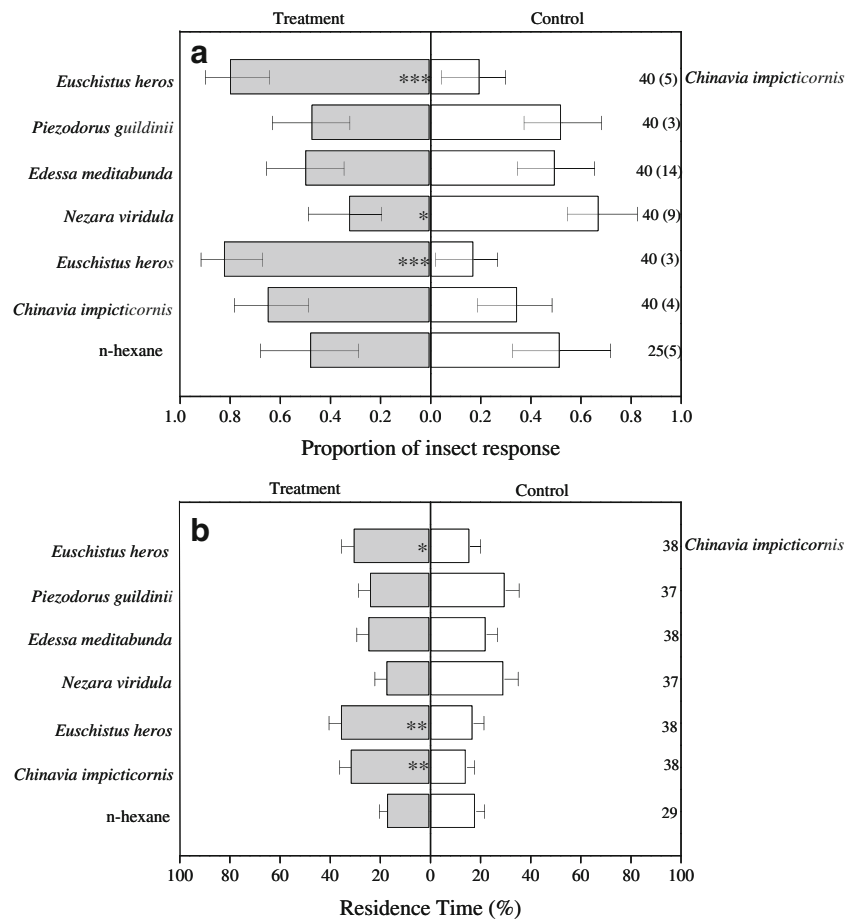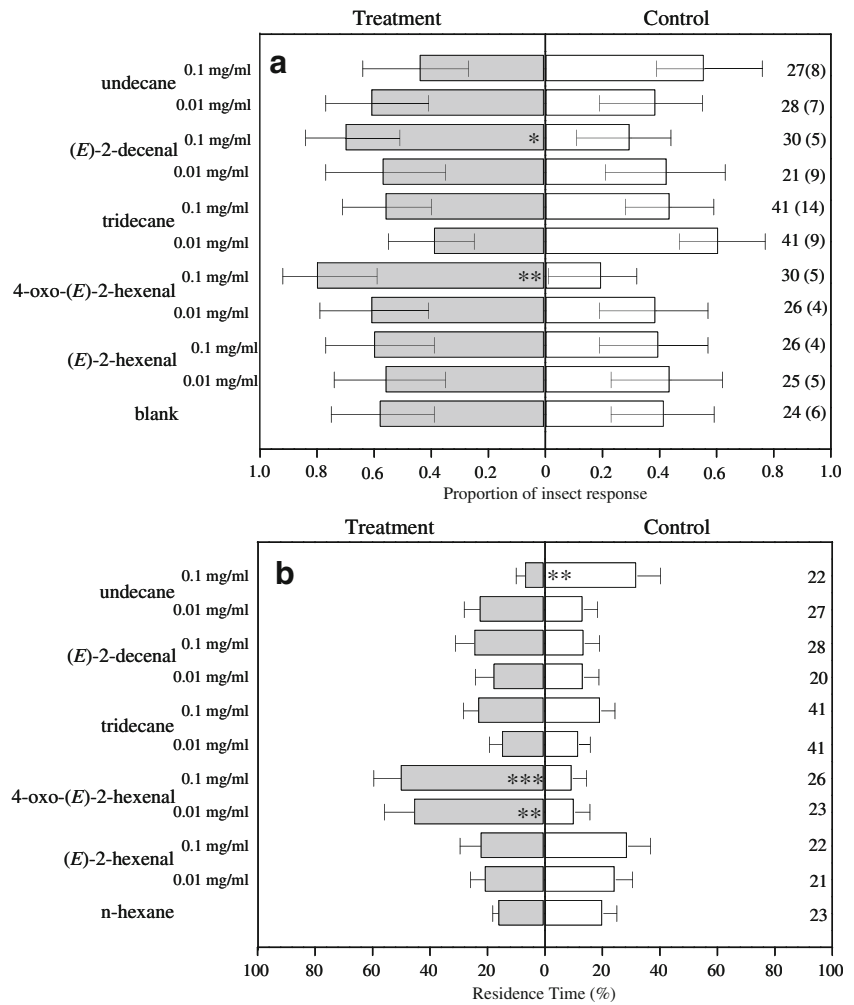

the parasitoid preferred the control arm of the olfactometer (χ^2_1=4.69, *P*=0.030, *N*=40; Fig. 2a). Parasitoids spent more time in the olfactometer arm supplied with extract of *E. heros* glands than in the control arm (*W*=352.0, *P*=0.011, *N*=38). Odor from extracts of *C. impicticornis* glands (Fig. 2b) also elicited a longer residence time (*W*=349.0, *P*=0.012, *N*=38), whereas the first choice for this odor was not significant (χ^2_1=3.49, *P*=0.062, *N*=40). When the metathoracic gland extracts of *E. heros* were tested against those of *C. impicticornis*, female *T. podisi* showed a clear preference for *E. heros* extracts (χ^2_1=12.30, *P*<0.001, *N*=40 for initial choice and *W*=307.0, *P*=0.026, *N*=38 for residence time; Fig. 2a and b).

The parasitoids showed clear differential responses to the different synthetic compounds tested. *T. basalis* preferred 4-oxo-(*E*)-2-hexenal (χ^2_1=7.68, *P*=0.005, *N*=30) and (*E*)-2-decenal (χ^2=4.53, *P*=0.033, *N*=30) at the higher concentration tested (0.1 mg/ml) against the control (80 and 70% choosing the arm with treatments, respectively; Fig. 3a). This positive response to 4-oxo-(*E*)-2-hexenal was confirmed when the percentage residence time was analyzed. Residence time in the treatment arm supplied with

4-oxo-(*E*)-2-hexenal was higher when compared to the residence time spent in the control arm for the two concentrations tested (*W*=178.0, *P*=0.007, *N*=23 and *W*=271.0, *P*<0.001, *N*=26 for 0.01 and 0.1 mg/ml, respectively; Fig. 3b). (*E*)-2-Hexenal, tridecane, and (*E*)-2-decenal did not show significant effects on *T. basalis* behavior, whereas undecane at the higher concentration tested (0.1 mg/l) caused the parasitoid to spend more time in the control arm (*W*=151.0, *P*=0.015, *N*=22; Fig. 3).

T. podisi showed a positive response to (*E*)-2-hexenal (χ^2_1=4.50, *P*=0.034, *N*=25) and tridecane (χ^2_1=4.61, *P*=0.032, *N*=47) at 0.01 mg/ml and to 4-oxo-(*E*)-2-hexenal at 0.1 mg/ml (χ^2_1=9.22, *N*=0.002, *N*=25). On the other hand, (*E*)-2-decenal and undecane did not elicit a significant response by this species (Fig. 4a). Accordingly, *T. podisi* females showed a higher proportion of residence time in arms of the olfactometer with (*E*)-2-hexenal (0.01 mg/ml) (*W*=211.0, *P*<0.001, *N*=22) and 4-oxo-(*E*)-2-hexenal at the two concentrations (*W*=114.0, *P*=0.03, *N*=20 and *W*=116.0, *P*=0.035, *N*=22 for 0.01 and 0.1 mg/ml, respectively; Fig. 4b). *T. podisi* females did not show higher residence time in the olfactometer arms supplied with

Fig. 3 First choice (a) and residence time (b) of the parasitoid T. basalis in Y-tube olfactometer bioassays with different stink bug defensive compounds as treatments in two dosages (5 μl of 0.01 and 0.1 mg/ml n-hexane) and n-hexane as control. Analyses of first choices were carried out by logistic regression and a Wald χ^2 statistic to assess significance. Mean residence time in treatment and control arms was analyzed by Wilcoxon's matched-pairs test. *0.05>P> 0.01, **0.01>P>0.001, and *** indicate P<0.001. Bars indicate the mean values of the parameters, and lines are the 95% confidence intervals for first choice and SE for residence time. Numbers on the right side of the figures are the total number of insects tested. Numbers in brackets in figure (a) represent the number of insects that did not respond to the treatment tested

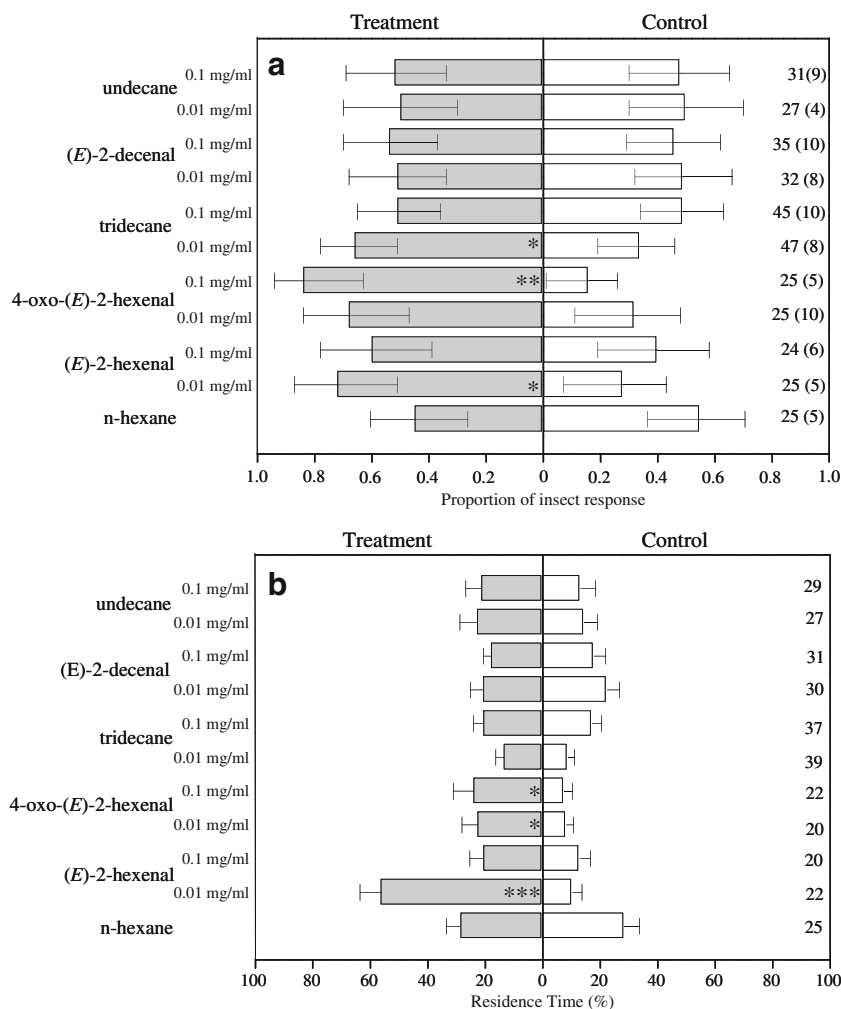(E)-2-decenal, tridecane, or undecane compared to controls (Fig. 4b).

T. basalis showed slower linear velocity (t=2.81, df= 22, P=0.01) and a higher tortuosity index (t=2.16 df=23 P=0.04) in olfactometer arms with 4-oxo-(E)-2-hexenal (0.1 mg/ml) when compared to hexane (control; Fig. 5). Linear velocity was slower when exposed to tridecane at 0.01 mg/ml compared to the control (Mann–Whitney test U=819.0, N=32, 326, P<0.001), and (E)-2-decenal reduced the turning rate of the parasitoid in the control arm (Mann–Whitney test U=318.0, N=15, 17, P=0.008). The other compounds did not show significant effects on T. basalis walking patterns parameters (Fig. 5).

T. podisi showed slower linear velocity when exposed to (E)-2-hexenal at both 0.1 and 0.01 mg/ml (Mann–Whitney test U=149, N=10, 12, P=0.027, and t test t=2.41, df=20, P=0.021 for 0.01 and 0.1 mg/ml, respectively) and to 4-oxo-(E)-2-hexenal at 0.1 mg/ml (Student t test t=2.71, df= 20, P=0.013; Fig. 6). Tortuosity was higher when the

parasitoid was exposed to (E)-2-hexenal (0.01 mg/ml; Student's t test t=2.20, df=24, P=0.037) and 4-oxo-(E)-2-hexenal (0.1 mg/ml; Student's t test t=2.97, df=24, P= 0.007). Exposure to 4-oxo-(E)-2-hexenal led to an increase of the turning rate of T. podisi females (Student's t test t=2.14, df=28, P=0.04). Tridecane, (E)-2-decenal, and undecane did not show significant effects on T. podisi walking pattern parameters (Fig. 6).

When the compounds to which the parasitoids showed significant positive responses were tested against the crude metathoracic gland extract preferred when tested singly, T. basalis did not show a preference for the N. viridula gland extracts when tested against 4-oxo-(E)-2-hexenal at 0.01 mg/ml, but preferred the arm with gland extracts when it was contrasted against 4-oxo-(E)-2-hexenal at 0.1 mg/ml (χ^2_1=4.59, P=0.032, N=36). In contrast, when N. viridula gland extracts were tested against (E)-2-decenal (at 0.1 mg/ml), the parasitoid preferred the arms treated with the aldehyde (χ^2_1=4.59, P=0.032, N=35; Fig. 7 a). In

Fig. 4 First choice (**a**) and residence time (**b**) of the parasitoid *T. podisi* in Y-tube olfactometer bioassays with different stink bug defensive compounds as treatments in two dosages (5 µl of 0.01 and 0.1 mg/ml *n*-hexane) and *n*-hexane as control. Analyses of first choices were carried out by logistic regression and a Wald χ^2 statistic to assess significance. Mean residence time in treatment and control arms was analyzed by Wilcoxon's matched-pairs test. *0.05>*P*>0.01, **0.01>*P*> 0.001, and *** indicate *P*< 0.001. *Bars* indicate the mean values of the parameters, and *lines* are the 95% confidence intervals for first choice and SE for residence time. *Numbers on the right side of the figures* are the total number of insects tested. *Numbers in brackets in figure* (**a**) represent the number of insects that did not respond to the treatment tested

contrast, the residence time in arms of the olfactometer with the stink bug gland extract or with individual defensive compounds was the same for all combinations tested (Fig. 7b).

The parasitoid *T. podisi* did not prefer odor of *E. heros* glands when this was tested against individual compounds (Fig. 8a, b).

Discussion

Scelionid parasitoids show clear host preferences (Sujii et al. 2002) for stink bug eggs that maximize their biological performance (Pacheco and Corrêa-Ferreira 1998; Kivan and Kilic 2002, 2004; Laumann et al. 2008). Egg parasitoids that search for nonapparent hosts may rely especially on easily detectable cues such as host pheromones or host allomones (Vet and Dicke 1992). Aldrich (1995) postulated that the differential use of adult stink bug host allomones by egg parasitoids should reflect

the host preference observed in different species of Scelionidae.

Our results confirm the hypothesis that females of *T. basalis* and *T. podisi* respond differentially to metathoracic gland extracts of different species of stink bugs. Furthermore, the data support the hypothesis that this differential response is related to the preference of hosts reported by Corrêa-Ferreira and Moscardi (1995), Medeiros et al. (1997, 1998), and Pacheco and Correa-Ferreira (2000) from field data, and Sujii et al. (2002) from laboratory experiments. Even when considering that the response of *T. podisi* to *E. heros* gland extracts may be influenced by preimaginal or emergence experience of the parasitoids that were reared on *E. heros* eggs, the response of *T. basalis*, reared on the same host, to gland extracts of *N. viridula* clearly indicates an innate response of this parasitoid to blends of their favorite host. The relevance of individual components of the attractive odor of host metathoracic glands for the parasitoids' host-searching behavior is discussed below.
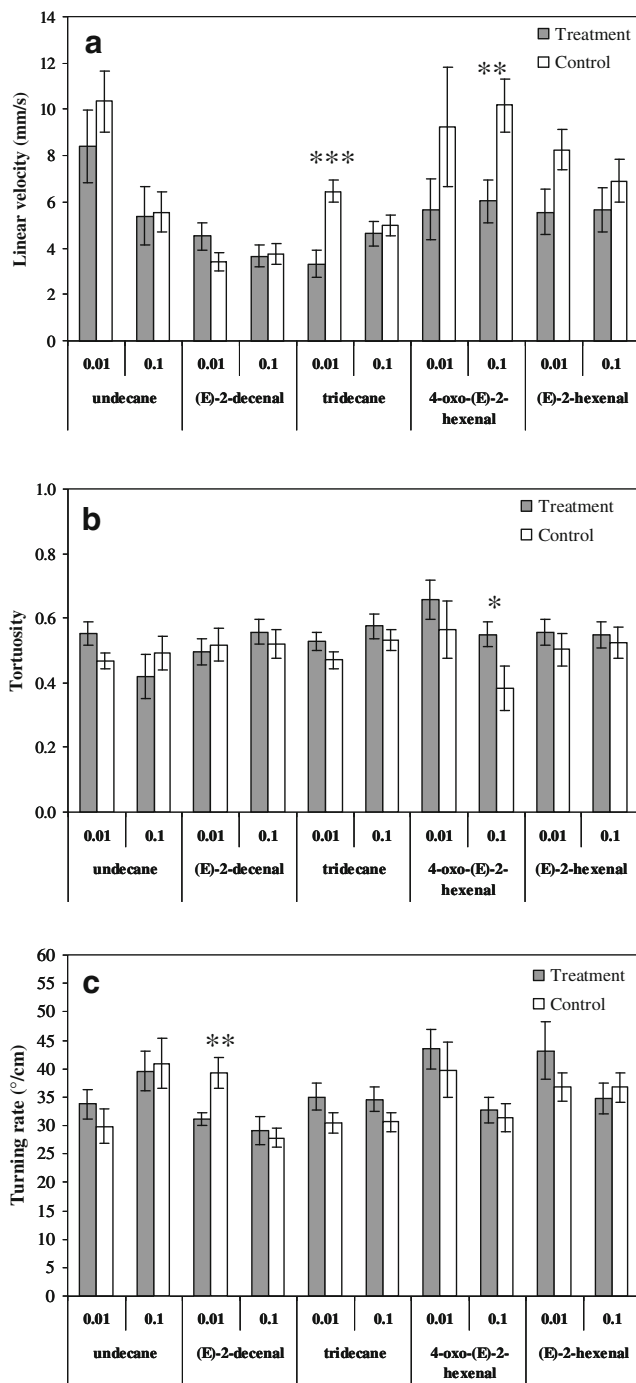
long-range kairomone by *T. basalis*. When testing *T. basalis,* the higher values of residence time, tortuosity, and reduced linear velocity in olfactometer arms supplied with 4-oxo-(*E*)-2-hexenal suggest that this compound can
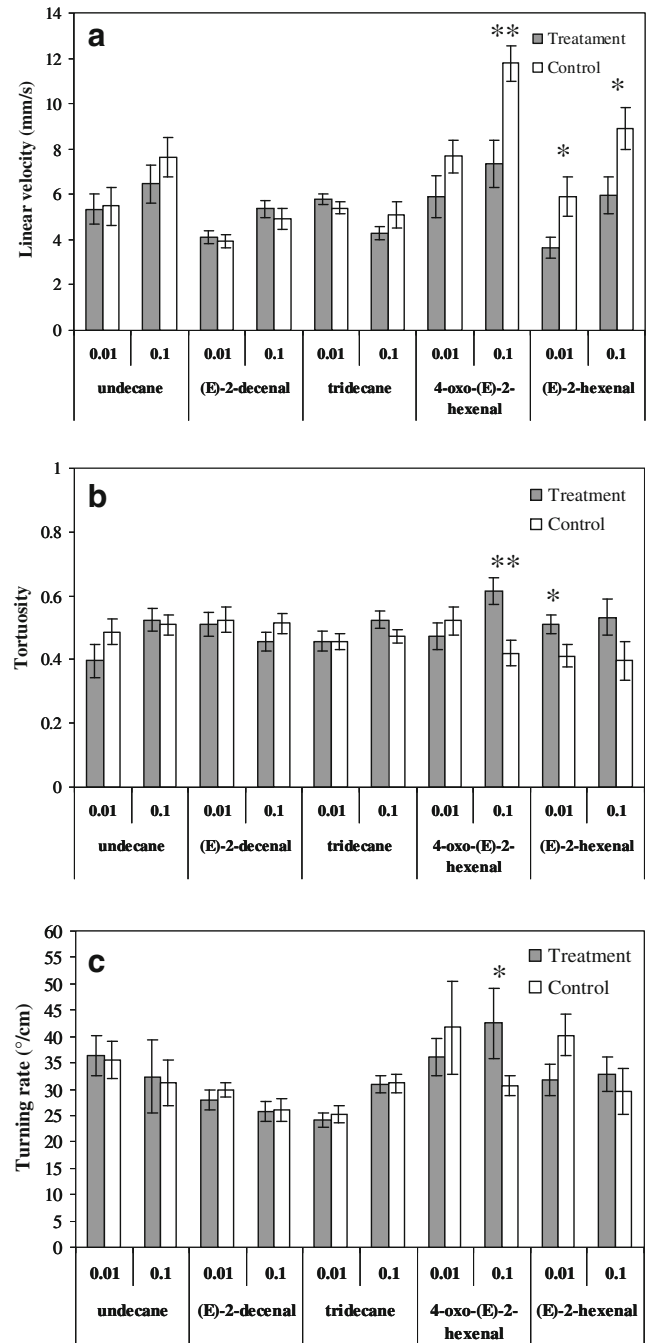

Fig. 5 Walking patterns (mean±SE) of *T. basalis*. **a** Linear velocity, **b** tortuosity, and **c** turning rate. Bioassays were performed in a Y-tube olfactometer with different stink bug defensive compounds as treatments in two dosages (5 μl of 0.01 and 0.1 mg/ml *n*-hexane) and *n*-hexane as control. Means of each treatment and control were compared with Student's *t* test or Mann–Whitney test. *0.05>*P*>0.01, **0.01>*P*>0.001, and ***P<0.001

Fig. 6 Walking patterns (mean±SE) of *T. podisi*. **a** Linear velocity, **b** tortuosity, and **c** turning rate. Bioassays were performed in a Y-tube olfactometer with different stink bug defensive compounds as treatments in two dosages (5 μl of 0.01 and 0.1 mg/ml *n*-hexane) and *n*-hexane as control. Means of each treatment and control were compared with Student *t* test or Mann–Whitney test. *0.05>*P*>0.01 and **0.01>*P*>0.001

T. basalis showed taxic behavior to two individual compounds tested: 4-oxo-(*E*)-2-hexenal and (*E*)-2-decenal. These findings corroborate the results reported by Mattiaci et al. (1993) who showed that (*E*)-2-decenal is used as a

Fig. 7 First choice (**a**) and residence time (**b**) of the parasitoid *T. basalis* in Y-tube olfactometer bioassays contrasting the blends of metathoracic glands of *N. viridula* with different stink bug defensive compounds. Analyses of first choices were carried out by logistic regression and a Wald χ^2 statistic to assess significance. Mean residence time in treatment and control arms was analyzed by Wilcoxon's matched-pairs test. *$0.05 > P > 0.01$. *Bars* indicate the mean values of the parameters, and *lines* are the 95% confidence intervals for first choice and SE for residence time. *Numbers on the right side of the figures* are the total number of insects tested. *Numbers in brackets in figure* (**a**) represent the number of insects that did not respond to the treatment tested
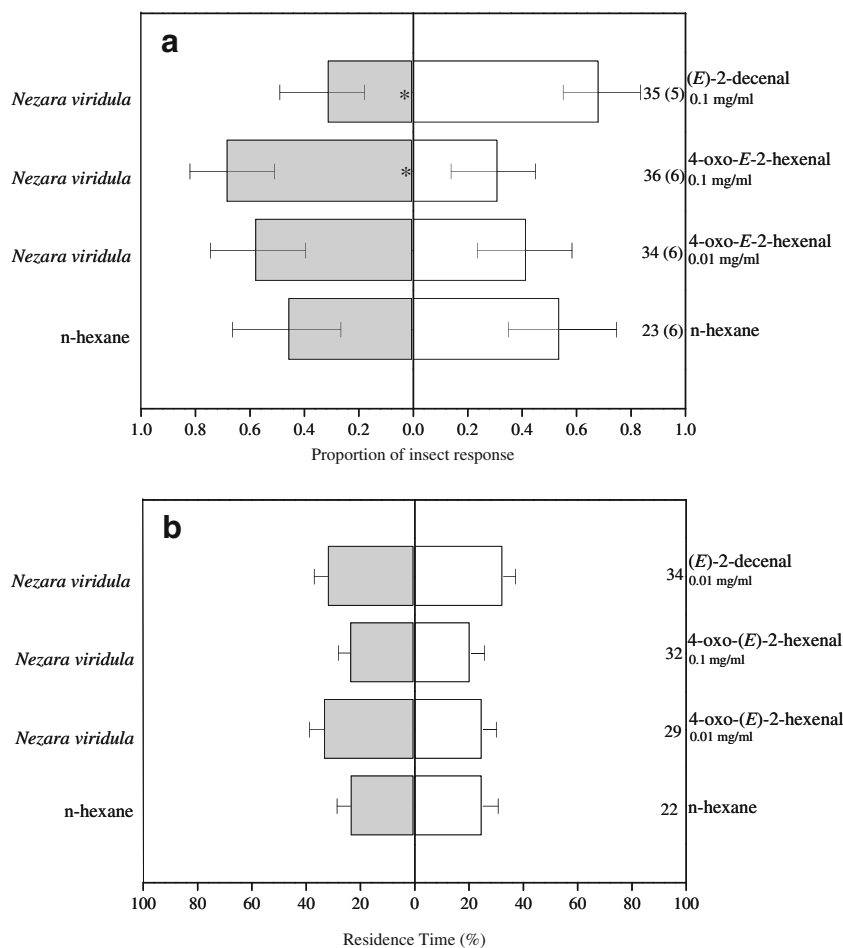
stimulate searching behavior of the parasitoid and modify kinetic components of the walking pattern.

T. podisi showed significantly positive response to (*E*)-2-hexenal and 4-oxo-(*E*)-2-hexenal, and these compounds also modified the kinetic search pattern. In contrast, tridecane affected only the initial choice of the parasitoid. The individual responses of *T. podisi* to these compounds are in concordance with those reported by Pires et al. (2001) by using olfactometer bioassays with groups of ten *T. podisi* females.

When both individual compounds and crude blends of metathoracic gland extracts that elicited positive taxic responses by the parasitoids were contrasted, the individual compounds had similar attraction power, suggesting that they can be used by the parasitoid during foraging behavior with the same efficiency as the whole blend released from glands. The only exceptions were 4-oxo-(*E*)-2-hexenal and (*E*)-2-decenal for *T. basalis*. The first compound showed lower attraction than extracts of *N. viridula* glands, and the second was more attractive than the extracts, confirming the strong kairomonal effect previously reported by Mattiaci et al. (1992) for this compound.

Long-range host location and selectivity mediated by semiochemicals has been previously reported for *Trissolcus* spp. responding to volatiles from stink bug nymphs and/or adults in bioassays with living insects (Colazza et al. 1999; Conti et al. 2003, 2004; Salerno et al. 2006; Silva et al. 2006). These previous studies showed that the parasitoids have stronger preference to cues from host stink bug females in a preovipositional state (Colazza et al. 1999). In concordance with the observations here, the parasitoids' response to volatiles from living host insects during host location behavior are related to cues derived from their preferred host or from hosts with old association history (Conti et al. 2004; Salerno et al. 2006).

The response of *T. podisi* and *T. basalis* to 4-oxo-(*E*)-2-hexenal may indicate a general response toward a characteristic compound of Heteroptera (Borges and Aldrich 1992; Aldrich 1995; Pareja et al. 2007). The response to (*E*)-2-decenal (*T. basalis*) and tridecane (*T. podisi*) indicates that even parasitoids with broad host spectra (Orr 1988; Austin et al. 2005) show a certain degree of specialization in the use of cues during searching behavior. The preference of *N. viridula* by *T. basalis* is reflected by its

Fig. 8 First choice (a) and residence time (b) of the parasitoid *T. podisi* in Y-tube olfactometer bioassays contrasting the blends of metathoracic glands of *E. heros* with different stink bug defensive compounds. Analyses of first choices were carried out by logistic regression, and a Wald χ^2 statistic to assess significance. Mean residence time in treatment and control arms was analyzed by Wilcoxon's matched-pairs test. *Bars* indicate the mean values of the parameters, and *lines* are the 95% confidence intervals for first choice and SE for residence time. *Numbers on the right side of the figures* are the total number of insects tested. *Numbers in brackets in figure* (a) represent the number of insects that did not respond to the treatment tested
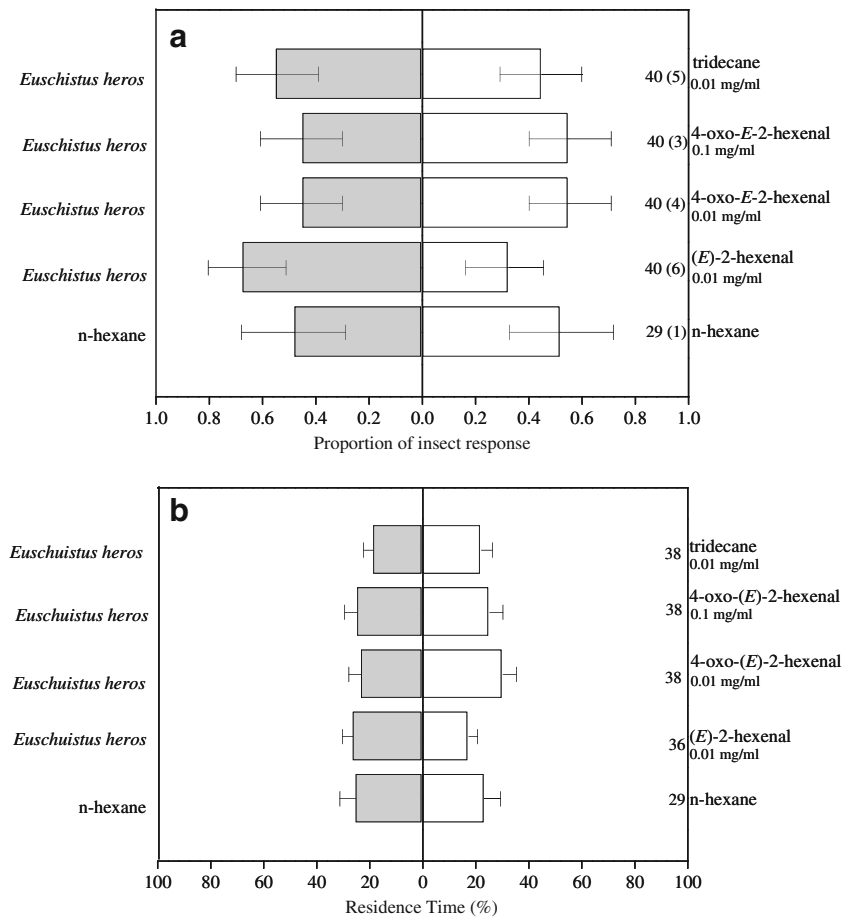
preference to (*E*)-2-decenal, a major component of defensive glands of the host *N. viridula* (Borges and Aldrich 1992). In addition, *Edessa* spp. release undecane as a major compound from their metathoracic glands (Howard and Wiemer 1983; Borges and Aldrich 1992), and they are not known as hosts of *T. basalis* and *T. podisi* in Brazil (Côrrea-Ferreira and Moscardi 1995; Medeiros et al. 1998). Neither parasitoid species showed a significant response toward undecane, suggesting that these parasitoids may use defensive compounds to discriminate unsuitable hosts.

The response of *T. podisi* to (*E*)-2-hexenal may characterize a general response to cues from the habitat where hosts can be found because this compound is usually found in glands of stink bugs: (*E*)-2-hexenal is also a common green leaf volatile present in volatile blends from plants (Hatanaka 1993) and has been reported as a component of soybean headspace (Moraes et al. 2008), which is the major host plant of stink bugs in Central Brazil. This general cue may be replaced, in more advanced steps of host-searching behavior, by specific cues from the host, such as 4-oxo-(*E*)-2-hexenal or tridecane.

A paradigm of host search in parasitic insects predicts that generalist parasitoids use nonspecific cues, while specialists use specific ones (Vet and Dicke 1992; Meiners

et al. 2000, Steidle and van Loon 2003). Phoretic scelionids and many other egg parasitoid species with reduced host spectra are known to use species-specific host sex pheromones (Nordlund et al. 1983; Aldrich 1985; Arakaki et al. 1996, 1997; Colazza et al. 1997; Bruni et al. 2000). *T. podisi* uses the sex pheromone of its preferred host, *E. heros* (Sujii et al. 2002; Borges et al. 1998, 1999; Silva et al. 2006). The results presented here indicate that, in addition to this specific response to host sex pheromones, a specific response to host allomones may help the parasitoid find hosts where the performance of their progeny will be high. Thus, even though the scelionid species studied here have quite a broad host spectrum, host species-specific cues might help parasitoids orientate to the most preferred host species.

The use of semiochemicals for behavioral manipulation of parasitoids has been proposed and discussed extensively. In recent years, semiochemicals have also been discussed as a tool to improve biological control (Vet and Dicke 1992; Lewis and Martin 2000; Powell and Pickett 2003). Specific knowledge about host–parasitoid relationships mediated by semiochemicals is important for improving the effectiveness of applications of semiochemicals in integrated pest control.

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