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Effect of host kairomones and oviposition experience on the arrestment behavior of an egg parasitoid

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Summary

Chemical residues left by walking adults of the southern green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae) induce arrestment behavior in the egg parasitoid *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) leading to prolonged periods of walking on contaminated areas and to systematic return to the stimulus after encountering the treatment borders. In this study, we quantified how the hierarchical value of residues from host adults and oviposition experience can influence the arrestment behavior of *T. basalis* females. Our results showed that: (1) female wasps perceived host residues at different hierarchical levels depending on the host gender, with a clear preference for the chemical residues deposited by host females rather then host males; (2) wasps' arrestment response to chemical residues of host females became weaker when wasps were not rewarded by an oviposition experience, and stronger following successful oviposition; (3) repeated encounters with host male chemical residues, followed or not by oviposition experience, did not cause wasps to change their innate arrestment response; (4) in the unrewarded condition, arrestment responses of wasps varied according to the time elapsed between successive visits to areas contaminated by host females: responses were weak with a short interval (less than 24 h) and stronger with a long interval (more than 72 h), suggesting that this unrewarded experience, i.e. encounter with female traces not followed by host egg location, fade within a few hours. The potential significance of these results to the host location behavior of *T. basalis* in the field is discussed.

Key words: *Trissolcus basalis*, *Nezara viridula*, host location, infochemical detour, learning.

Introduction

Foraging behavior of insect parasitoids is generally mediated by semiochemicals associated with hosts. Because successful host location has a direct influence on a parasitoid's fitness, semiochemicals conveying reliable information on the hosts' presence can play an important role in the adaptive behavior of parasitoid wasps (Vinson, 1984; Vinson, 1991; Vinson, 1998). An extensive literature exists regarding the origin of these chemical cues, their general or specific incidence, and the innate or learned ability of female parasitoids to perceive them [(Vet and Dicke, 1992; Godfray, 1994; Vet et al., 1995; Quicke, 1997) and references therein]. According to the 'variable response model' developed by Vet et al. (Vet et al., 1995; Vet et al., 2003), wasps have innate responses to each subset of chemical cues used for locating their hosts, and such responses can be ranked according to their potential effects, so that the variability of wasp responses to each stimulus should be related to their potential benefits (Vet and Groenewold, 1990; Vet et al., 1990). Through association of innate stimuli with host-derived stimuli such as successful oviposition, female wasps can enhance their searching efficacy. Subsequently, the maintenance of such associative experience is mainly related to whether or not host eggs are successfully located (Vet et al., 1995; Vinson, 1998; Takasu and Lewis, 2003). Any chemical cue released by a host can act as a kairomone. However, when hosts are concealed or less active, their presence also can be revealed by chemicals that are not directly associated with them (Vet and Dicke, 1992; Meiners et al., 1997; Meiners and Hilker, 1997; Colazza et al., 2004a; Colazza et al., 2004b). For example, adult scales lost by *Heliothis zea* (Boddie) and *Sesamia nonagrioides* (Lefebvre) moths induce females of the egg parasitoids *Trichogramma pretiosum* Riley and *Telenomus busseolae* Gahan, respectively, to search longer on host patches where such cues are present (Beevers et al., 1981; Gardner and van Lenteren, 1986; Colazza and Rosi, 2001). In this work we investigated the effects of residues of adult hosts and oviposition experience on the host location of *Trissolcus basalis* (Hymenoptera: Scelionidae), an egg parasitoid of several pentatomid bugs, including the southern green stink bug, *Nezara viridula* (Heteroptera: Pentatomidae), a pest of a wide variety of economically important crops (Todd, 1989; Colazza and Bin, 1995). Previous studies showed that chemical residues left on the substrate by both nymphs and adults of *N. viridula* play a role as indirect host-derived cues inducing arrestment responses in the parasitoid females (Colazza et al., 1999). We developed laboratory experiments to quantify how the hierarchical value of residues from host adults and oviposition experience can influence the arrestment behavior of *T. basalis* females.

The arrestment response of wasps to traces of host adults is a host location strategy commonly observed in scelionid (Hymenoptera: Scelionidae) egg parasitoids of pentatomid bugs (Heteroptera: Pentatomidae). To date, the presence of arresting kairomones has been observed in the following host–egg parasitoid associations: *Nezara viridula* (L.) – *Trissolcus basalis* (Wollaston) (Colazza et al., 1999; Salerno et al., 2006), *Murgantia histrionica* Hahn – *Trissolcus brochymenae* (Ashmead) (Conti et al., 2003), *Eurydema ventrale* Klt. – *Trissolcus simoni* (Mayr) (Conti et al., 2004) and *Euschistus heros* (F.) – *Telenomus podisi* (Ashmead) (Borges et al., 2003). Female wasps that fail to locate host egg masses while exploring a contaminated area should be less likely to respond to the same stimulus in the near future, whereas female wasps that successfully located host egg masses should have their responses reinforced. The objectives of the present study were thus to determine how wasps' arrestment responses were influenced by (1) chemical residues left by *N. viridula* adults, (2) successful oviposition experience, and (3) repeated encounters with areas contaminated by host chemical residues.

Materials and methods

Insects

Nezara viridula and *T. basalis* cultures were started from materials collected during summer 2003 from fields located around Palermo, Italy. The *N. viridula* colony was held in plastic cages (30×19.5×12.5 cm), ventilated with mesh-covered holes (5 cm in diameter), in a environmental room (25±1°C, 70±10% relative humidity and 16 h:8 h L:D), and fed with fresh broad beans and French beans.

Adults of *T. basalis* emerged from parasitized egg masses were kept in 16-ml glass tubes and fed with a honey-water solution. Egg masses of *N. viridula* were collected daily and used to maintain cultures of both *N. viridula* and *T. basalis*. Single *N. viridula* egg masses were exposed to three to four female wasps, and then held in the same environmental conditions until adult emergence. Before being exposed to parasitoid females, egg masses of *N. viridula* used to maintain *T. basalis* colonies were removed from the original oviposition substrate and glued on a new strip of paper to avoid wasp contact with any possible chemicals residues from adults. For bioassays, female wasps 2–3-days old and naive with respect to both oviposition experience and contact with cues released by *N. viridula* were individually isolated in a small vial with a drop of honey-water solution and acclimatized in the bioassay room for about 24 h.

Bioassay procedure

Bioassays were conducted in open arenas consisting of a rectangular sheet of filter paper (40×20 cm; insect:arena surface ratio of about 1:400). In the middle of each arena, a circular area (6 cm diameter) was defined and exposed for 30 min to a single adult of *N. viridula*, leaving the surrounding area untreated. This was achieved by constraining the *N. viridula* adults under a steel mesh cover (6 cm diameter, 1 cm high, 0.01 cm mesh) to ensure constant contact of the bug legs with the filter paper and, at the same time, to avoid the atmosphere from becoming saturated by any possible bug volatiles that might have been retained on the filter paper surface. Filter papers contaminated by bug's faeces were not used for bioassays. After removing the bug, one female wasp was gently released in the middle of the circular area and its arrestment behavior was followed by a video tracking system (see below) until it flew away from or walked off the whole arena. The arrestment response behavior of scelionid egg parasitoids to host-contaminated areas is characterized by an initial prolonged motionless period with the antennae kept in contact with the surface. Then the wasps, while drumming the surface with the antennae, start to walk slowly with a walk characterized by a klinotactic response, i.e. variation in orthokinetic and klinotaxic locomotion activity, so that the resulting tortuous path keeps the wasps in the contaminated areas. If no host eggs are found, the response decreases and the normal walking behavior is gradually resumed (Colazza et al., 1999). Tested female wasps that did not show the arrestment behavior, and either flew away or walked off the arena immediately as a consequence of lack of contact of their antennae with the treated area, were excluded from the analysis. Adults of *N. viridula* used to treat the inner circular area were either males or females taken from the colony and kept isolated about 3 days before the bioassays. Experiments were carried out in an isolated room (2.8×2.4×2.0 m) illuminated by two 18 cm long fluorescent tubes. All experiments were carried out from 09:00 h to 12:00 h and at 26±1°C. Wasps' walking patterns were scanned with a CCD camera (Sony M370) equipped with a zoom lens and mounted above the center of the arena. The video camera was connected to a video monitor (Sony PVM 2130QM) and a desktop PC equipped with a video frame grabber (Miro Pc-TV – Pinnacle Systems, Mountain View, CA, USA). A composite video signal from the camera was fed into computerized video tracking and motion analysis software, developed for the Linux operating system (Colazza et al., 1999). A threshold value of 1 mm was adopted during the acquisition process to avoid detection of artifact slight movements caused by any noise in the system. The arrestment responses of the female wasps were quantified over the entire arena (pooling both outside and inside the circular contaminated area) by means of the following parameters: (1)

total arena residence time (s), (2) average walking speed (mm s^{-1}) and (3) tortuousity index. This last parameter is a spatial index computed from the coordinates of the wasps (sample rate=15 points s^{-1}). It was calculated as $1-mp/tl$ where mp=maximum projection of the track in a general straight line of the plane, and tl=total length of the track. The value can range from 0.0 to 1.0, with higher values corresponding to more tortuous walking paths (Colazza et al., 1999).

Experimental protocols

Hierarchical value of host chemical residues

A first experiment was done to evaluate a possible hierarchical value of areas contaminated by chemical residues left by *N. viridula* females or males on the arrestment response of *T. basalis* females. Naive wasps were tested on host-contaminated areas. They were then recaptured, isolated in small vials and tested 1 h later on newly treated arenas according to the following four cross-combinations: (1) experienced and tested on male traces (MM; N=31), (2) experienced on male traces and tested on female traces (MF; N=36), (3) experienced on female traces and tested on male traces (FM; N=35), or (4) experienced and tested on female traces (FF; N=34).

Oviposition experience

In a second experiment, we investigated the influence of successful oviposition experience (rewarding experience) on the wasps' arrestment responses. This second experiment also aimed to quantify the wasps' ability to associate an oviposition experience with host chemical residues during a successive visit to a host-contaminated area. Single naive *T. basalis* females were released onto a circular area contaminated with residues of host males or females and with a host egg-mass (five to six eggs) in the middle. During this phase, the walking pattern of the wasp was not recorded. Then, experienced wasps (i.e. those that had located the egg-mass and parasitized at least one egg) were recaptured and kept isolated in a small vial for 1 h. They were then tested in open arenas treated with chemical residues from host females or males as previously described, and their walking pattern was recorded. These tests were run using two combinations: (1) oviposition on female traces and tested on female traces (female_kairomone_female, f_ko_f, N=34) or (2) oviposition on male traces and tested on male traces (male_kairomone_male, m_ko_m, N=30). For *T. basalis* females, successful oviposition is known to increase klinokinesis and decrease orthokinesis in a manner analogous to contact with host chemical residues (Bin et al., 1993). Thus, to separate the effect of oviposition experience and host chemical residues, experienced female wasps tested on uncontaminated areas were used as controls, leading to two additional treatments: (3) oviposition on female residues and tested on uncontaminated arena (female_kairomone_blank, f_ko_b, N=15), or (4) oviposition on male residues and tested on uncontaminated arena (male_kairomone_blank, m_ko_b, N=15).

Time intervals between successive encounters with contaminated areas

A third experiment was conducted to examine the arrestment behavior of female wasps encountering successive host-contaminated areas at different time intervals without successful oviposition (unrewarding experience). Thus, the same *T. basalis* females were exposed to four consecutive open arenas treated with resides of *N. viridula* females at intervals of 1, 23 and 72 h. After each exposure, wasps were recaptured and kept isolated in a small vial until the successive test. It has been demonstrated in several parasitoid species that extended periods without host contact can affect their host-searching behavior (for a review, see Jervis and Ferns, 2004). Therefore, to disentangle the effect of unrewarded experience and increased duration without successful oviposition on wasps' arrestment behavior, as a control we used naive female wasps tested at the same time as experienced females were re-tested. Each treatment was replicated 24 times.

Statistical analysis

Data were first checked for assumptions of normality with a Shapiro–Wilk test with P=0.05 considered statistically significant. The assumption of normality was met for walking speed data, whereas data of arena residence time and tortuousity index were normalized by logarithmic transformation and arcsine transformation, respectively. Data from the first experiment were statistically analyzed using Student's t-tests for paired comparisons. Data from the second experiment and data from the third experiment concerning naive females tested at defined periods were analyzed by one-way ANOVA followed by Tukey's HSD *post hoc* test for multiple comparisons between the means. Data for the third experiment regarding experienced female wasps retested on consecutive open arenas were analyzed by repeated-measures ANOVA followed by Tukey's HSD *post hoc* test for multiple comparisons between the means. Contrast analyses between naive and experienced female wasps from the third experiment were done using t-tests. All statistical analyses were done using Statistica 6.0 for Windows (StatSoft Inc., Tulsa, OK, USA).

Results

Hierarchical value of host chemical residues

Naive *T. basalis* females discriminated between areas contaminated by chemical residues left by a host female or host male, with a clear preference for the former. Arena residence time of female wasps was almost twice as high when wasps were placed on areas contaminated by host female *versus* host male residues (mean ± s.e.m.; 216±12.84 s *vs* 93.03±7.11 s; t=8.53, P<0.001; t-test). There were also significant differences between the parameters describing *T. basalis* walking paths. On areas contaminated by host female residues, the mean velocity of naive wasps was lower (6.58±0.25 *vs* 10.89±0.40 mm s^{-1}; t=–9.10, P<0.001, t-test), and the tortuosity indexes described a more convoluted locomotion pattern (0.80±0.009 *vs* 0.73±0.01; t=3.36, P<0.01, t-test) than on host male traces.

Female wasps experienced with host female chemical residues and re-encountering the same type of chemical cue showed a significantly reduced arrestment response (group FF, arena residence time: $t=-4.93$, $P<0.001$; mean velocity: $t=-7.24$, $P<0.001$; tortuosity index: $t=3.33$, $P<0.001$, d.f.=33, paired t-tests) (Fig. 1). By contrast, wasps with experience on *N. viridula* male residues did not change their arrestment response upon re-encountering host male residues, despite a significant increase in their mean velocity (group MM, arena residence time: $t=-0.67$, $P=0.5$; mean velocity: $t=-4.46$, $P<0.001$; tortuosity index: $t=-0.27$, $P=0.78$; d.f.=30, paired t-tests) (Fig. 1). Wasps experienced on female residues showed a decreased interest upon encountering areas contaminated by host male residues, as their arena residence time was reduced, their mean velocity increased, and their walking path was less convoluted (group FM; arena residence time: $t=10.21$, $P<0.001$; mean velocity: $t=-8.02$, $P<0.001$; tortuosity index: $t=4.20$, $P<0.001$, d.f.=34, paired t-tests) (Fig. 1). Finally, wasps experienced on male residues showed more interest in female residues (group MF; arena residence time: $t=-4.93$, $P<0.001$; mean velocity: $t=5.59$, $P<0.001$, d.f.=35; tortuosity index: $t=-0.85$, n.s., paired t-tests) similar to the results obtained for

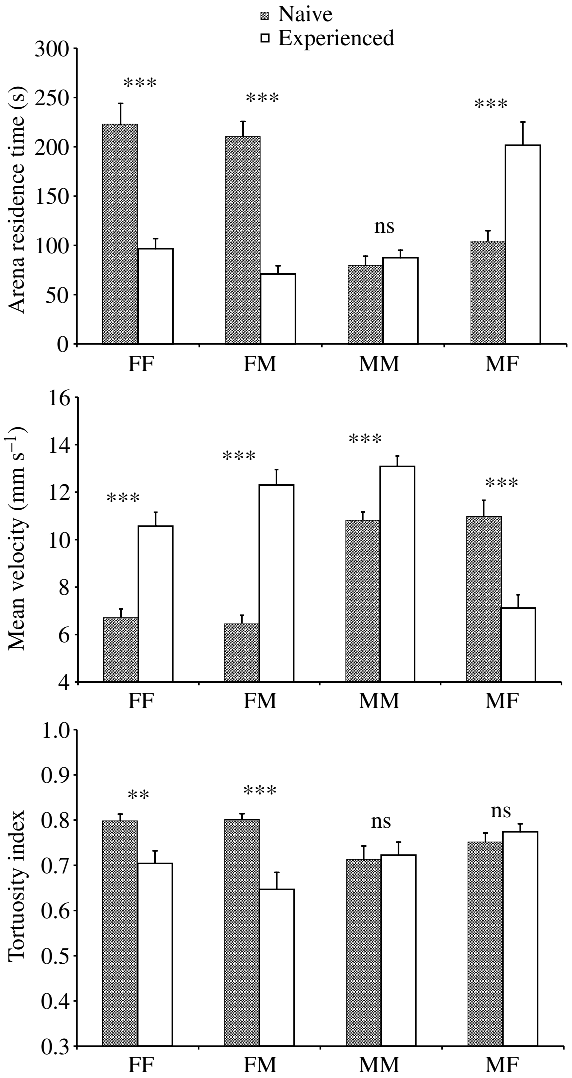

Fig. 1. Mean (± s.e.m.) response of *T. basalis* females encountering for the first time (naive, in grey) or re-encountering (experienced, in white) *N. viridula* adult resides. MM, experienced and tested on male traces; MF, experienced on male traces and tested on female traces; FM, experienced on female traces and tested on male traces; FF, experienced and tested on female traces. Asterisks indicate values that differed significantly within each condition (ns $P>0.05$, $^{**}P<0.01$, $^{***}P<0.001$).

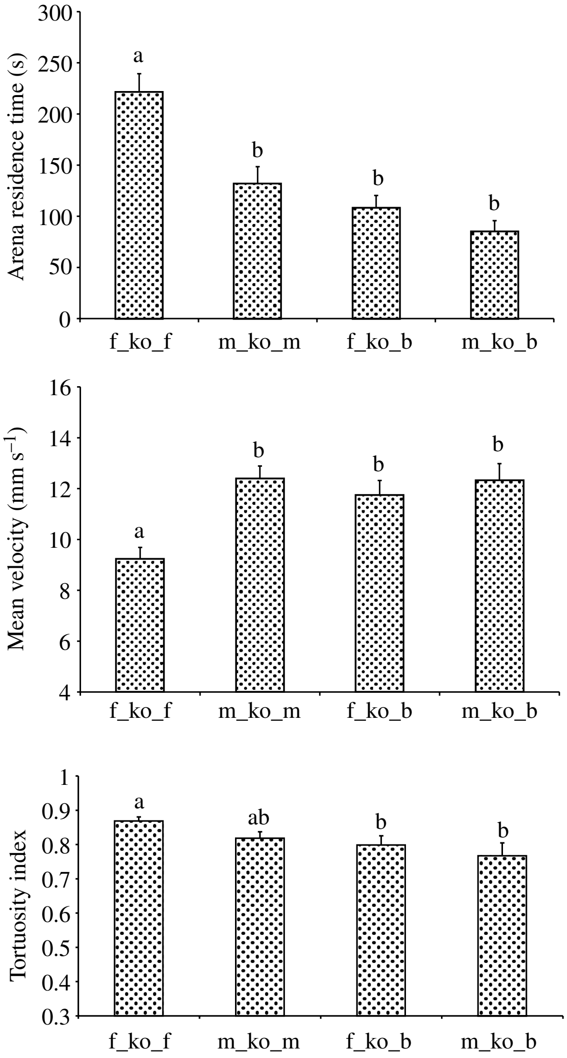

Fig. 2. Mean (± s.e.m.) arrestment response of *T. basalis* females allowed to oviposit on a host egg-mass in the presence of either host male (m_ko; male kairomone) or female residues (f_ko), and then tested according to different conditions (e.g. oviposition on male traces and tested on male traces: m_ko_m; see text for a detailed explanation. b, blank – uncontaminated area). Different letters above the bars indicate significantly different means (ANOVA, Tukey's HSD).

naive female wasps encountering host female traces for the first time (Fig. 1).

Oviposition experience

The arrestment behavior of *T. basalis* females when successively tested in arenas contaminated by host chemical residues was influenced by previous successful oviposition experience (one-way ANOVA; arena residence time: $F_{3,90}=12.76$, $P<0.001$; mean velocity: $F_{3,90}=9.79$, $P<0.001$; tortuosity index: $F_{3,90}=4.15$, $P=0.008$) (Fig. 2). Rewarded females re-encountering host female residues (group f_ko_f) showed longer arena residence time and increased arrestment responses compared to rewarded females encountering host male residues (group m_ko_m). The influence of oviposition experience *per se* on the wasps' arrestment response could be excluded because females with successful oviposition experience did not show a strong arrestment response when re-tested on untreated arenas (group f_ko_b). On the other hand, arrestment responses of rewarded wasps in the presence of host male residues remained unchanged when they re-encountered host male residues (group m_ko_m), and the response was similar to that recorded from control females (group m_ko_b) (Fig. 2). Therefore, positive oviposition experience elicited host-seeking behavior by *T. basalis* females only in the presence of host female residues, but not in the presence of host male residues.

Time intervals between successive encounters of contaminated areas

Repeated contacts with areas contaminated by host female residues without successful oviposition experience significantly influenced wasps' arrestment response (ANOVA repeated measures; arena residence time: $F_{3,69}=32.58$, $P<0.001$; mean velocity: $F_{3,69}=16.46$, $P<0.001$; tortuosity index: $F_{3,69}=6.95$, $P<0.001$) (Fig. 3). Experienced female wasps re-encountering host female residues at intervals of 1 h showed a decreased interest in the chemical cue, in line with the results recorded in the first experiment. Then, arrestment behavior gradually increased as the time elapsed between successive visits increased. After 72 h, arrestment behavior reached a level similar to that displayed by naive females (arena residence time: $t=-1.92$, $P=0.06$; mean velocity: $t=-1.06$, $P<0.29$; tortuosity index: $t=-1.83$, $P=0.07$; d.f.=46, t-tests). Possible influences of extended periods without host contact on wasps' arrestment responses can be excluded as naive female wasps tested at the same times did not show differences in the arrestment response (one-way ANOVA: arena residence time: $F_{3,92}=2.42$, $P=0.07$, mean velocity: $F_{3,92}=0.17$ $P=0.91$; tortuosity index: $F_{3,92}=0.92$, $P=0.44$) (Fig. 3).

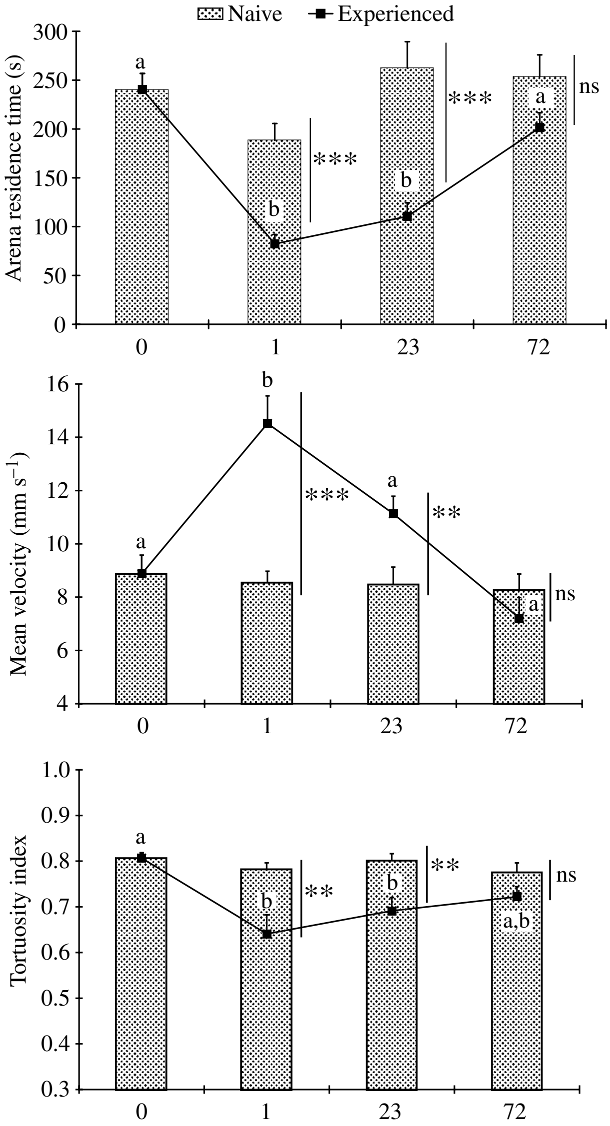

Fig. 3. Mean (± s.e.m.) arrestment response of *T. basalis* females exposed to four consecutive arenas at intervals of 1, 23, and 72 h treated with residues of *N. viridula* females (solid lines; experienced females) and *T. basalis* females tested at the same times (stippled bars; naive females). Means represented by lines and indicated by the same letters were not significantly different by ANOVA repeated measures followed by Tukey's test for multiple comparisons. Means represented by bars were not statistically different by one-way ANOVA. Vertical dotted lines indicate means that differ significantly based on t-test (ns $P>0.05$, $^{**}P<0.01$, $^{***}P<0.001$).

Discussion

Many insects leave chemical residues when moving around on substrates and these residues can play a role as contact kairomones for insect parasitoids (Quicke, 1997). Insect parasitoids eavesdropping on host chemical residues can receive either directional information about host location (systematic search), or information inducing them to intensify their host searching behavior (random search) (Godfray, 1994). Systematic search seems more commonly adopted by wasps attacking active hosts where cues are a reliable indication of their presence, as is the case for *Poecilostictus cothurnatus* Gravenhorst, a larval parasitoid of pine looper moth, *Bupalus* *pinarius* L, which is able to find hosts by following the chemical trails left by the moth larvae (Klomp, 1981). Random search is expected to be adopted mainly by parasitoids that respond to contact chemical cues indirectly associated with the hosts (Vinson, 1991; Godfray, 1994). Congruent with this view, *T. basalis* females seem to adopt a random search for locating host eggs as a consequence of an arrestment response induced by chemical residues left by adults of *N. viridula*. In this way, once on infested plants, the possibility of finding host eggs are improved as a consequence of lowered flight propensity, prolonged stay on the plant, reduced movement, and increased klinokinesis (Colazza et al., 1999). However, the decision of *T. basalis* females to remain in the plant canopy to search for hosts could be influenced by the reproductive success accumulated while foraging on plant surfaces contaminated by host residues. Foraging insect parasitoids are known to learn which environmental stimuli are associated with rewarding or aversive outcomes to improve their chance of future host location and hence their reproductive success (Lewis and Tumlinson, 1988; Lewis and Martin, 1990; Vet and Groenewold, 1990; Petitt et al., 1992; Turlings et al., 1993; Dutton et al., 2000). The results presented in this paper show that *T. basalis* has an innate response to host chemical residues, with a strong preference for female residues. Oviposition experience enhanced the arrestment responses of the wasps when they were associated with host female residues, and this appears to be congruent with the form of experience defined as 'α-conditioning' (*sensu* Vinson, 1998) where host or host's products can provide a reward to foraging that increases the innate response to a stimulus. The magnitude of variability observed for *T. basalis* females responding to host female chemical residues as a consequence of oviposition experience seems fairly constant and predictable in accordance with the 'variable response model' developed by Vet et al. (Vet et al., 1995). *Trissolcus basalis* females that were not rewarded by successful oviposition within a certain amount of time gradually lost their arrestment response and progressively moved back to a more general host search behavior. The adaptive meaning of these results may be that chemical residues of female *N. viridula* cannot guarantee the presence of host eggs or provide directional information. Hence, even when searching in areas contaminated by 'promising host cues', it could be adaptive for females to give up and leave the area if host eggs are not found after a certain amount of time. Generally, experience effects on 'α-conditioned' wasps are not permanent (McAuslane et al., 1991). Our results showed that the arrestment response of *T. basalis* females was influenced by the time elapsed between two successive unrewarding encounters, leading to the conclusion that about 72 h is the time needed by wasps to 'forget' negative experiences.

Trissolcus basalis females also respond to residues left by males and nymphs of *N. viridula* (Colazza et al., 1999). Moreover, we found that the innate wasp response to host male residues was not modified by experience gained during the wasp's foraging activity. We thus can speculate that host residues might convey to foraging wasp females not only indirect information about host egg presence, but also direct information on the presence of their host species. The actual role of host residues on host specificity of *T. basalis* was recently addressed by Salerno et al. (Salerno et al., 2006), who demonstrated that *T. basalis* females were able to discriminate between coevolved and non-coevolved host species which may be present on the same infested plants. An analogous situation could be predicted for another egg parasitoid, *T. brochymenae*, the females of which showed arrestment responses to residues of third and fifth instars, and adults of *M. histrionica* (Conti et al., 2003).

Our study provides a starting point in the understanding of the importance of chemical residues left by adults of *N. viridula* on the host location behavior of *T. basalis* females. Further studies are needed to elucidate the intricate processes involved in the host searching behavior of this parasitoid, and to determine the specific compounds used by *T. basalis* females in locating their hosts and host habitats.

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