Volatile and Contact Chemicals Released by *Nezara viridula* (Heteroptera: Pentatomidae) Have a Kairomonal Effect on the Egg Parasitoid *Trissolcus basalis* (Hymenoptera: Scelionidae)

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The responses of females of the egg parasitoid *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) to volatile and contact chemicals from its host *Nezara viridula* (L.) (Heteroptera: Pentatomidae) were investigated in a Y-tube olfactometer and under open arena conditions. In the Y-tube tests, volatiles from virgin males and from females in a preovipositional state attracted *T. basalis* females, while volatiles from host virgin females did not. In an open arena, traces left by *N. viridula* adults in different physiological conditions function as contact cues inducing the wasps to remain longer in the arena and to change the pattern of their walking behavior. However, only contact kairomones from *N. viridula* mated females in a preovipositional condition induced an arrestment response characterized by an increase in patch searching time and turning rates and a reduction in linear speed. The chemical ecological implications of these results on this host–parasitoid association are discussed.

**Key Words:** *Trissolcus basalis; Nezara viridula; egg parasitoid; pheromone; kairomone; host location; arrestment; foraging behavior.*

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**INTRODUCTION**

Models concerning host foraging behavior of parasitoids are usually divided into a series of hierarchical steps: host habitat location, potential host community location, host location, host acceptance, host suitability, and host regulation (Vinson, 1985). Among numerous ecological and physiological factors, chemical cues, also termed infochemicals (Vet and Dicke, 1992), play an important role in all of these steps (Vinson, 1985, 1998). Many studies have been carried out to document the role of chemical cues used by insect parasitoids to locate their hosts (for recent reviews see Vet and Dicke, 1992; Godfray, 1994; Vinson, 1998), and those involved in host location have been categorized in different ways by many authors. According to functional criteria, Vinson (1991) classified the cues involved in the host location into five groups. More recently, Godfray (1994) proposed dividing them into three main categories: (1) stimuli coming from the host microhabitat or foodplant, (2) stimuli indirectly associated with the presence of the host, and (3) stimuli coming from the host itself. The chemical cues included in the second group are, among others, used by egg parasitoids. Generally, egg parasitoids use cues that do not directly originate from the attacked host stage. Instead, their origin is closely related in time with the egg stage and the cues may arise from the activity of the adult hosts, as in the case of sex pheromones, or can be produced by the plant in response to herbivory, frass, or other waste products (Vinson, 1991, 1998; Godfray, 1994). These habits allow egg parasitoids to reach the areas where host mating is in progress or where the eggs have just been laid, in either case before the hosts are too old to be physiologically acceptable (Godfray 1994; Vet et al., 1995). Adult host products, such as the attractant pheromone of the spined soldier bug, *Podisus maculiventris* (Say) (Aldrich, 1996), or sex pheromones of the moth *Heliothis zea* (Boddie) (Lewis et al., 1982; Noldus, 1989), contain volatile chemicals used by their egg parasitoids, *Telenomus calvus* Johnson and *Trichogramma pretiosum* Riley, respectively, as host location cues. Another such example is lepidopteran scales shed during oviposition, which are used by egg parasitoids as host-finding cues (Beever et al., 1981; Shu and Jones, 1985).

The possibility of manipulating parasitoids’ behavior before or after their release through the use of infochemicals that improve the ability of mass-reared parasitoids to locate the host has been suggested by many authors (Lewis et al., 1982; Vet et al., 1995). Due to the importance of infochemicals as tools to improve the efficiency of parasitoids in biological control programs (Vinson, 1985), we carried out a series of investigations to better understand the chemical ecology of
the host–parasitoid association, *Nezara viridula* (L.)–*Trissolcus basalis* (Wollaston). The southern green stink bug, *N. viridula*, is a serious pest of several cultivated plants in most areas of the world (Todd, 1989). In Italy, its economic importance is related mainly to soybeans and, occasionally, vegetable crops (Colazza and Bin, 1995; Colazza et al., 1996). One of the main biological control agents acting on *N. viridula* populations in most affected regions of the world is the egg parasitoid *T. basalis*, which has been used in biological control programs in several countries (Jones, 1988; Colazza and Bin, 1995; Jones et al., 1996). For example, in Brazil, inundative releases of this parasitoid on trap crops with high concentrations of southern green stink bugs have yielded good results in the control of this pest (Correa-Ferreira and Moscardi, 1996).

Two important aspects would improve the understanding of the chemical ecology of this host–parasitoid relationship: the identification of the chemical composition of the kairomones used in host location and recognition and the parasitoid behavior elicited by these kairomones. Previous reports on the chemical cues used by *T. basalis* to locate their hosts have observed that *N. viridula* adults play an important role in both the host finding (Bin et al., 1987; Mattiacci et al., 1993) and host acceptance (Bin et al., 1993; Mattiacci et al., 1993; Aldrich et al., 1995). Although these reports give interesting information about the chemical relationships between *T. basalis* and one of its hosts, *N. viridula*, the functions of the chemical compounds acting at each step of the foraging behavior still remain fragmented and in need of study. Investigations to clarify these aspects have been initiated (Clemente and Colazza, 1997).

In this study, we have focused our attention on the analysis of the behavioral responses of *T. basalis* to chemical cues released by *N. viridula* adults rather than on the identification of the chemical compound involved. Therefore, observations of *T. basalis* responses to volatile and contact chemicals from both host males and females under different physiological conditions were carried out. Here we report that a complex of short-range stimuli originating from adult hosts either retains the parasitoid in a contaminated area or stimulates host searching.

**MATERIALS AND METHODS**

**Insect Cultures**

Pairs of adult *N. viridula* were caged in ~0.02-liter plastic containers, ventilated with 5-cm-diameter mesh-covered holes, and were fed with sunflower seeds (*Helianthus annus* L.) and French beans (*Phaseolus vulgaris* L.). Food was changed daily and separate containers were used for nymphs and adults. Paper towels were hung from the inside edge of each adult cage as an oviposition substrate. Egg masses were collected daily to prevent cannibalism. Adult hosts were tested under the following conditions: virgin males (8–10 days old); virgin females (8–12 days old); and mated females in a previposition state (7–10 days after mating), defined as those whose abdomens appeared enlarged and slightly bloated.

A *T. basalis* colony was established from wasps emerging from *N. viridula* egg masses collected in central Italy and was cultured for three to five generations on laboratory-reared *N. viridula* egg masses. Host eggs were exposed to parasitoids for 24 h and were removed and stored in another tube for incubation. Adult wasps were fed with a honey–water solution after emerging. Male and female parasitoids were caged together in 16-ml glass tubes to allow mating and were kept in the laboratory at 18-h photophase, at a temperature of 24 ± 2°C, and RH of 70 ± 5%. Mated females used in the experiments were isolated almost 24 h before the assays, provided with a drop of honey–water solution, and used when they were ~2–3 days old.

**Y-tube Olfactometer Behavioral Assays**

The Y-tube olfactometer (stem 9 cm; arms 8 cm at 130° angle; ID 1.5 cm) used and the device adopted for the observations were similar to the ones described by Colazza et al. (1997). Medical-grade compressed air flowed through both arms, creating an air stream of 144 ml/min per arm. The flow was regulated by flowmeters, and bubbling through a water jar before the air passed into the olfactometer provided humidity. The Y-olfactometer was surrounded by a paper wall to minimize possible cues from the room and was illuminated by two 22-W cool white fluorescent tubes located above the device. The temperature in the bioassay room was maintained at ~26°C at all times. For each replicate, one adult bug, caged in a small box (2.5 × 1.5 cm), was randomly assigned to one arm and placed near the orifice. Each bug was bioassayed separately or under choice conditions, employing a new adult after each set of three replicates. Female wasps were tested singly and tests were repeated from ~9:00 to 18:00. After each experimental trial, the whole system was cleaned with hexane, chloroform, and acetone. A single wasp was introduced into the Y-tube at the entrance of the stem and observed with the aid of a behavior recording program, “The Observer 3.0” Software Package (Noldus Information Technology, Wageningen, The Netherlands). Each wasp was allowed 10 min to choose one of the arms of the olfactometer; then it was discarded whether or not it had made a choice. A choice was considered to have been made when a wasp passed a line 3 cm into any arm and remained there for 20 s.
Open Arena Behavioral Assays

Arena experiments were conducted on filter paper (Whatman, No. 1, 18.5 cm diameter) which served as an open area (ratio of wasp/arena of about 0.003) where wasps could move in an unconstrained field. In the center of the filter paper, a circular area 6 cm in diameter was either left untreated or exposed for 1 h to a single *N. viridula* adult constrained under a plastic cover (6 cm in diameter, 1 cm high). Filter papers exposed to frass or other host waste products were not used for the bioassays. Following application of the treatments, wasps were gently placed into the middle of the circular area. Continuous observation started immediately and was stopped when the wasp flew from or walked out of the arena.

The arena was lit from above by infrared illumination (homogenous emission of wavelengths at 950 nm provided by 108 LEDs) and was observed using a video monitor (Sony PVM 1371QM) connected with a monochrome CCD camera (Sony SSC M370CE) fitted with a 12.5–75 mm/F1.8 zoom lens. The camera’s lens was covered with an infrared pass filter (Kodak Wratten filter 87Å) to remove visible wavelengths. Two 22-W cool white fluorescent tubes that simulated daylight located above the device illuminated the arena. Analog video signals from the camera were digitalized by a video frame grabber (PC-VISION plus Imaging Technology), and data was processed by the “Etho Vision 1.90” Software Package (Noldus Information Technology, Wageningen, The Netherlands). We computed the arena residence time(s), i.e., the time from when a wasp first entered the arena until it flew from or walked out of the arena, and the patch retention time(s), the time from when a wasp was in contact with the treated area to when it left the area for more than 2 s. From the coordinates of the insect (sample rate = 5 images/s), the following parameters were determined to characterize the individual kinetic reaction to the treatments: linear speed (cm/s), angular speed (°/s), and turning rate (°/cm) (Bell, 1990). Test wasps had no previous host contact and were used only once. All experiments were carried out from ∼9:00 to 18:00. The temperature in the bioassay room was ∼26°C at all times.

### Statistical Analysis

In the Y-tube olfactometer experiments, wasp choices were analyzed with a χ² test. In open arena experiments, variable values were tested for normality (Kolmogorov–Smirnov test). Because there was no significant deviation from normal distribution, the variable values were then analyzed with parametric tests. All

<table>
<thead>
<tr>
<th>Response (n)</th>
<th>Pa</th>
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<tr>
<td>air</td>
<td>male</td>
</tr>
<tr>
<td>air</td>
<td>female</td>
</tr>
<tr>
<td>air</td>
<td>pre-ovi. female</td>
</tr>
<tr>
<td>male</td>
<td>pre-ovi. female</td>
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**FIG. 1.** The number of *T. basalis* females that made a response and the percentage of their choices in each arm of the Y-test olfactometer to *N. viridula* adults in different physiological states. There were 60 replicates of each experiment. *, χ² test; ns, P > 0.05.
the data were analyzed using the “Statistica 5.1” Statistical Package (StatSoft, Inc., 1997).

RESULTS

**Wasp Responses to Adult Host Volatile Kairomones**

When only clean air was passing through the Y-tube olfactometer, *T. basalis* females did not show any preference for either arm of the olfactometer (*P* > 0.05) and explored randomly over the olfactometer’s entire surface. Wasp females exhibited a significant preference for volatiles from an adult host male and, in separate experiments, for volatiles from a host female in a preovipositional state (*P* = 0.03 and *P* = 0.0002, respectively) (Fig. 1). In contrast, in the presence of a virgin female of *N. viridula*, wasp females showed no significant preference for one of the two olfactometer arms (Fig. 1). Testing these volatiles against clean air, wasps seem to respond more strongly to a host female in a preovipositional state than to a host male. New, comparative experiments were carried out to investigate this preference. Given a choice, the percentage of the wasps that actually made a choice rose to 75%. These wasps selected significantly more often the arm with the cues left by preovipositional female (*P* = 0.02) (Fig. 1).

**Wasp Responses to Adult Host Contact Kairomones**

Under the control conditions, *T. basalis* residence time in the arena was short. Before flying away, wasp females spent little time in the arena (8.23 ± 3.21 s), walking fast (1.46 ± 0.37 cm/s) with few turns (62.66 ± 15.80°/s) and, consequently, exploring only small sections of the arena’s surface (Fig. 2A). Under the test conditions, contact with the treated surface caused the wasps to stay longer in the arena. Once having touched the treated area, wasps remained stationary and drummed the surface with their antennae. After a few seconds, wasps began to move, searching intensively around the starting point. Females continued to explore the surrounding area, returning to the treated area a few times and increasing progressively the distance from the starting point until they flew from or walked out of the arena (Fig. 2B). These flight delays were differently affected by the treatments (Fig. 3). The residence time in the arenas treated with one preovipositional host female was significantly longer, about twice as long, than that recorded in the arenas treated with males or virgin females (*F* = 27.25; *df* = 3; *P* < 0.00001) (Fig. 3).

The wasp’s kinetic reactions in response to the treated area are summarized in Table 1. The kinetic reaction demonstrated a significant difference in the path shape when the wasp was in or out of the kairomone-treated patches. In fact, treated areas elicited both a reversed orthokinesis and an increased klinokinesis, indicating that adult host chemical cues cause arrestment in wasp females. Female wasps walked slower and turned more frequently per unit path length, resulting in a more convoluted walking pattern. These arrestment behaviors of *T. basalis* occurred in a manner dependent on the host’s physiological condition. In the area treated with a preovipositional female, wasps increased patch retention time (*F* = 30.61, *df* = 2; *P* < 0.00001) and reduced speed (*F* = 22.70; *df* = 2; *P* < 0.00001), and the search path became more tortuous (*F* = 9.51; *df* = 2; *P* < 0.0002; for angular speed; *F* = 10.59; *df* = 2; *P* < 0.0001; for turning rate) compared to values in the area treated with a host female or a host male (Fig. 4). Significant differences between cues left by the host
male and the host female were observed only in the patch retention time and the linear speed, while wasps’
kinetic reactions were not affected differently.

These results suggest that a variety of host cues,
acting as volatile and contact kairomones, play a role in
host location by *T. basalis* and that these cues seem
related to different physiological host conditions. The
hierarchy of the responses, deduced from the Y-
olfactometer tests, was confirmed in the open arena
test.

**DISCUSSION**

Females of *T. basalis* react to cues emitted by *N.
viridula* adults. The responses observed were (1) more
frequent preference for the Y-tube olfactometer arms
containing an adult host and (2) both flight delay and
ortho- and klino-kinesis variations in an open area. The
strength of the responses was varied. *T. basalis* reacted
differently according to the sex and the physiological
condition of the host. Wasps were attracted by volatile
cues released by *N. viridula* virgin males and mated
females in preovipositional state but were not attracted
by those released by *N. viridula* virgin females. A
previous study (Bin *et al.*, 1987), reporting that both
male and female adult host attracted *T. basalis* fe-
male, might be explained by the undefined physiologi-
cal states of the females utilized and/or by the fact that
males were not kept isolated from females before being
tested, possibly causing cross-contamination. Cues from
host males and preovipositional females seem to play a
role in the parasitoid’s host location process through a
basalis* was drawn to host males, but was even more

**TABLE 1**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Parameters</th>
<th>Central area</th>
<th>Central area</th>
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<tr>
<td></td>
<td>In Means ± SD</td>
<td>Out Means ± SD</td>
<td><em>P</em></td>
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<tr>
<td>Virgin female</td>
<td>Linear speed (cm/s)</td>
<td>0.44 ± 0.10</td>
<td>1.40 ± 0.25</td>
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<tr>
<td></td>
<td>Angular speed (%/s)</td>
<td>147.79 ± 31.95</td>
<td>83.56 ± 20.57</td>
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<td></td>
<td>Turning rate (%/cm)</td>
<td>40.93 ± 10.18</td>
<td>17.78 ± 4.51</td>
</tr>
<tr>
<td>Virgin male</td>
<td>Linear speed (cm/s)</td>
<td>0.57 ± 0.24</td>
<td>1.49 ± 0.41</td>
</tr>
<tr>
<td></td>
<td>Angular speed (%/s)</td>
<td>139.06 ± 40.72</td>
<td>77.55 ± 21.36</td>
</tr>
<tr>
<td></td>
<td>Turning rate (%/cm)</td>
<td>37.63 ± 14.57</td>
<td>16.94 ± 5.64</td>
</tr>
<tr>
<td>Female in pre-ovipositional state</td>
<td>Linear speed (cm/s)</td>
<td>0.28 ± 0.16</td>
<td>1.29 ± 0.44</td>
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<tr>
<td></td>
<td>Angular speed (%/s)</td>
<td>186.33 ± 51.31</td>
<td>85.44 ± 17.81</td>
</tr>
<tr>
<td></td>
<td>Turning rate (%/cm)</td>
<td>54.82 ± 17.25</td>
<td>19.63 ± 6.41</td>
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*Note.* Each mean represents 26 observations.

*Paired t test.*
strongly attracted to females nearly ready to lay an egg mass. In addition, the presence of both cues caused a general increase in the wasps' responsiveness, leading parasitoids to prefer females in a preovipositional state. One of the possible cues affecting \textit{T. basalis} females is probably the host sex attractant pheromone, which is emitted by mature males (Aldrich et al., 1993; Brezet et al., 1994; Sturaro et al., 1994). This pheromone is also a kairomone for host location by the adult parasitoid, the tachinid \textit{Trichopoda pennipes} F. (Mitchell and Mau, 1971; Aldrich et al., 1987). However, in contrast to this tachinid parasitoid, which seeks adult hosts, polyphagous egg parasitoids respond weakly to such host-derived cues (Aldrich, 1996). In fact, several conditions, e.g., the tendency of \textit{N. viridula} females to lay eggs far from aggregation sites (personal observations), should induce nonphoretic wasps, such as \textit{T. basalis}, to orient themselves mainly to more reliable host-derived cues (Aldrich, 1996). In all likelihood, male-derived cues direct \textit{T. basalis} toward host population aggregations, while cues from preovipositional females may not only attract the wasps but also affect the transition from flying to walking behavior.

When a \textit{T. basalis} female encounters a patch contaminated by chemicals deposited by adults of \textit{N. viridula}, the female varies its locomotory path in such a way as to show an arrestment response. The pattern of this behavior is characterized by a flight delay and an intensive antennal drumming of the substrate and, therefore, a reduction in linear speed and an increase in turning rate (Kennedy, 1978; Waage, 1979; Vinson, 1985). The arrestment response of an egg parasitoid induced by cues from host stages other than eggs is very common in \textit{Trichogramma} species (Beevers et al., 1981; Gardner and van Lenteren, 1986; Noldus, 1989; Shu and Jones, 1985) and in \textit{Telenomus} species (Gazit et al., 1996). Parasitic Hymenoptera may discover the host by responding to the host scales and/or the host sex pheromone. Here, \textit{T. basalis} seems arrested on the patches that are defined by chemical traces left by adults of \textit{N. viridula}. To observe the wasps under virtually unconstrained conditions (without any cover on the arena), the parasitoid must be released in the middle of the patch to force it to delay its flight, which happens only following physical contact by a wasp with the kairomone. Therefore, from the present results we cannot exclude the involvement of host volatile cues in

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure4}
\caption{Retention time and analysis of walking paths of \textit{T. basalis} females in the patch treated with \textit{N. viridula} adults in different physiological conditions. Bars indicate mean ± SE. Each mean represents 26 observations. Different letters above bars indicate significantly different means (Tukey test, $\alpha = 0.05$).}
\end{figure}
the arrestment response of *T. basalis*. However, in order to be absorbed and retained in the natural substrates, these substances must have low volatility. Possible candidates as contact kairomones might be the substances secreted by both the metathoracic glands and the dorsal abdominal glands common to both bug sexes, which induce wasps to antennate and attempt to oviposit (Mattiacci et al., 1993; Aldrich et al., 1995). Host acceptance behavior in *T. basalis* was observed by Mattiacci et al. (1993) and Aldrich et al. (1995), who applied gland extracts on glass beads to emulate natural eggs. However, these substances have never been detected on actual eggs of *N. viridula* (Borges and Aldrich, 1992; Mattiacci et al., 1993), and, therefore, this evidence may be due to the association of chemical stimuli with physical ones (the glass beads). Our results suggest that products from the metathoracic glands and the dorsal abdominal glands are more likely to act as contact arrestment kairomones, keeping *T. basalis* in areas with higher expectancy of egg masses. In addition, our results from open arena bioassays help better define the searching strategy of *T. basalis*. Again, as was observed for volatile kairomones, contact kairomones act hierarchically in *T. basalis*. Because the host’s trace itself is not an indication of the precise location of the host eggs, wasps benefit by prolonging their searching only in those kairomone-treated patches where the cues perceived are strongly correlated with the target. Wasp females spend more time in patches that contain traces left by females in a preovipositional state, and wasps may perceive different kairomones and thus increase their foraging activity in an area where host egg masses are more likely to be present. Consequently, the switchover from faster to slower searching induced by host females ready to oviposit would be advantageous to *T. basalis*.

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