Use of pheromones and other semiochemicals in integrated production
IOBC wprs Bulletin Vol. 25(•) 2002
pp. •-•

Volatile chemicals released by pentatomid bugs having a kairomonal effect on *Trissolcus basalis*: their role on host specificity and prospects for IPM

Gianandrea Salerno, Eric Conti, Stefano Colazza,[1] Ezio Peri[1] and Ferdinando Bin

Dept. of Arboriculture and Plant Protection, Univ. of Perugia, Borgo XX Giugno, 06121 Perugia, Italy
[1] Inst. of Agricultural Entomology, Univ. of Palermo, Viale delle Scienze 13, 90128 Palermo, Italy

Abstract: Volatile semiochemicals emitted by the host, or by different products excreted or secreted by the host itself, play a major role in enabling host microhabitat location by parasitoids. The response of the egg parasitoid *Trissolcus basalis* Wollaston (Hymenoptera: Scelionidae) to volatile compounds, from four species of pentatomid bug females (Heteroptera: Pentatomidae) in ovipositional state, was analysed in a Y-tube olfactometer. *Trissolcus basalis* was attracted by volatiles from its coevolved host, *Nezara viridula* (L.), thus confirming previous reports, whereas it did not respond to *Murgantia histrionica* (Hahn), suggesting that a previous host record from the field may be unreliable. In addition, this parasitoid did not respond to volatiles of the non coevolved host *Graphosoma semipunctatum* (F.) and of the non-host *Eurydema ventrale* (Kol.) (Heteroptera: Pentatomidae). Although the host range of Scelionidae can vary greatly, several species are reported as monophagous or oligophagous. The analysis of these results may help in explaining host specificity in *T. basalis* in order to use this parasitoid with improved efficacy and safety in biological control programmes.

Key words: Pentatomidae, *Trissolcus basalis*, kairomone, host selection, host specificity.

Introduction

Models concerning host foraging behaviour of parasitoids are usually divided into a series of hierarchical steps: host habitat location, host location, host recognition and host suitability (Vinson, 1985), which, among numerous ecological and physiological factors, are mediated by physical and chemical cues, the latter also termed semiochemicals or infochemicals (Vinson, 1985, 1998; Vet and Dicke, 1992). The ensemble of physical and chemical characters of the host egg, the substrate and the combined material and/or organisms, that elicit host location and acceptance by egg parasitoids,

has been proposed to be defined as "host unit" (Conti et al., 2000a). In the case of pentatomid bugs, a general host unit is represented by the characters of the host egg, the plant substrate, the secretion used by host female to glue the eggs onto the substrate, the contact traces left by gravid females on the substrate and volatile chemicals from adult bugs (Bin et al., 1993; Mattiacci et al., 1993; Colazza et al., 1999b; Conti et al., 2000a, 2000b; Salerno, 2000).

These information sources have, as a yet, not been used as clearly defined and quantifiable elements for the definition of host specificity, although it has been suggested (Conti et al., 2000a; Salerno, 2000). Host specificity is an important issue for researchers and practitioners in developing biological control programs, and particularly when natural enemies are introduced (i.e., classical biological control). In fact, most parasitoid species are restricted to development in relatively few hosts that either share similar life history traits or live in the same habitat. However, both in natural and laboratory conditions such parasitoids may switch (Conti et al., 2000b) or shift host (Follett et al., 2000), thus revealing an unsuspected wider host range.

This paper deals with laboratory studies carried out in order to evaluate the influences of the volatile cues (Mattiacci et al., 1993; Colazza et al., 1999b) released by four species of pentatomid bugs (Heteroptera: Pentatomidae) on host location by *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae). This cosmopolitan egg parasitoid appears to be the most polyphagous species among the pentatomid egg parasitoids, as it attacks 51 species (Salerno, 2000). In spite of that it has been used, on a worldwide scale, mainly for biological control of the southern green stinkbug, *Nezara viridula* (L.) (Jones, 1988; Jones et al., 1996) a serious cosmopolitan pest that can attack more than 30 different crops (Todd, 1989).

The pentatomid bugs tested were: 1) the coevolved host *N. viridula*; 2) a factitious (non co-evolved) host, *Graphosoma semipunctatum* (F.), which attacks Umbelliferae and, occasionally, Gramineae (Voegele, 1966); and two non-hosts, 3) the European *Eurydema ventrale* (Kol.) (Bonnemaison, 1952) and 4) the American *Murgantia histrionica* (Hahn) (McPherson, 1982), both of which attack Cruciferae and Capparidaceae. In order to better understand the role of volatiles from bug females in the determination of host specificity by the parasitoid, laboratory data are discussed in relation to those from the literature on host associations.

Material and methods

Insects. All pentatomid bugs were reared in a controlled condition chamber (14-h photophase, at a temperature of 25 ± 1°C; and RH 70 ± 10%), inside clear plastic food containers (300mm x 195mm x 125mm-high) with 5-cm diameter mesh-covered holes. Separate containers were used for nymphs and adults. All stages were fed with seeds, fruits and vegetative parts of their preferred food plants. Sunflower seeds (*Helianthus annus* L.) and French beans (*Phaseolus vulgaris* L.) were used to feed

N. viridula, fresh celery (*Apium graveolens* L.) and parsley seeds (*Apium petroselium* L.), fennel (*Phoeniculum vulgare* Mill.) and *Ferula communis* L. for *G. semipunctatum*, cabbage and broccoli (*Brassica oleracea* L.) for *E. ventrale* and *M. histrionica*. Food was changed every 2-3 d and water was provided weekly through cotton wool soaked in small containers. Only mated females in ovipositional state, i.e., with enlarged and slightly bloated abdomens, were used for the experiments.

Parasitoids were reared in 16-ml glass tubes and kept in an incubator (15-h photophase, 24 ± 2°C; and RH 80 ± 5%). Adult wasps were fed with a solution of sugar (10%), honey (10%), benzoic acid (10%), yeast and water (Safavi, 1968). Host eggs were exposed to parasitoids for 48h, and were removed and stored in another tube for incubation. After emergence, male and female parasitoids were kept together to allow mating. Mated females used in the experiments were isolated almost 24 h before the assays, provided with a drop of the Safavi (1968) diet, and were used when they were 2-5 d old.

Volatile chemicals bioassays. 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 humidified the air before it passed into the olfactometer. The Y-olfactometer was surrounded by a black fabric curtain to minimise possible cues from the room, and was illuminated by two 22-W cool white fluorescent tubes located above the device and by infra-red illumination (homogenous emission of wavelengths at 950 nm provided by 108 LEDs) from below. The temperature in the bioassay room was continuously maintained at ≈ 25°C. For each replicate, one adult bug, caged in a small brass mesh box (2.5 x 1.5-cm), was randomly assigned to one arm and placed near the orifice. Each bug was bioassayed separately, employing a new host adult after each set of two replicates per parasitoid, with a total of 6. Female wasps were tested singly and tests were repeated from ≈ 9:00 to 19:00. After each experimental trial, the whole system was cleaned with hexane, acetone and distilled water. A single wasp was introduced into the Y-tube at the entrance of the stem, and its walking pattern was recorded for 10 min. using a monochrome CCD videocamera (Sony SSC M370 CE) fitted with a 12.5-75 mm /F 1.8 zoom lens. The camera lens was covered with an infrared pass filter (Kodak Wratten filter 87Å) to remove visible wavelengths. Analog video signals from the camera were digitalized by a video frame grabber. Digitalized data were processed by XBug, a video tracking and motion analysis system developed for Linux (Colazza et al., 1999a).

The following parameters, that better describe differences between arms, were calculated: - residence time, computed as the percentage of time spent in each of the olfactometer arm (%), and - average linear speed in each arm (mm/sec).

Statistical analysis. Data on parasitoid response to volatiles in the test *vs*. control arms of the olfactometer were analysed with *t* tests for paired comparisons. The

possible presence of bias was evaluated in the blank tests by also comparing the two arms of the olfactometer with *t* tests for paired comparisons. Data from the test arms with the different bugs and from the blank were analysed with 1-way analysis of variance (ANOVA) and the Tukey's Honestly Significant Difference test for multiple comparisons between the means. Because both arms in the blank were obviously bug-free, after having verified the absence of bias as described, data for analysis were randomly chosen from one of the two arms. Eteroschedastic data were subjected to angular or square-root transformation (Statsoft, 1997; Sokal and Rohlf, 1998).

Results

Trissolcus basalis walking behaviour in the blank trials showed absence of bias between the olfactometer's arms, both in terms of residence time (t=0.96; df=15; p=0.35) and linear speed (t=0.45; df=15; p=0.66) (Fig. 1). Instead, as expected, female *T. basalis* exhibited a clear preference for its coevolved host, *N. viridula*, in ovipositional state (Fig. 1).

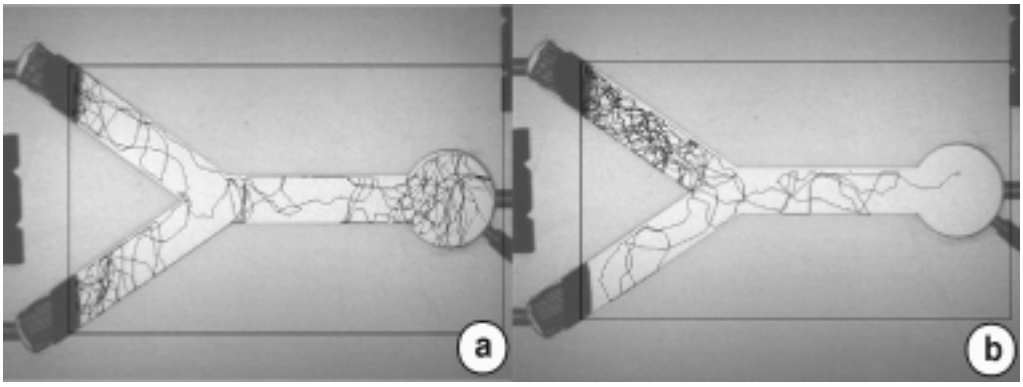

Figure 1. Tracks of *Trissolcus basalis* in Y-tube olfactometer without bugs (blank) (a) or with a gravid female of *Nezara viridula* in the right arm (b).

In fact, residence time was significantly higher (t=4.38; df=16; p<0.001) and linear speed significantly lower (t=5.09; df=14; p<0.001) in the arm containing the host compared with the control (Fig. 2). When the non coevolved host *G. semipunctatum* was tested, *T. basalis* females did not show any preference for the test arm compared with the control. Seemingly, this parasitoid spent less time in the arm containing the bug, although only at the 0.08 probability level (t=1.91; df=15; p=0.075), whereas, the linear speed was similar in both arms (t=0.41; df=13; p=0.69). Finally, *T. basalis* did not respond to volatiles from the other bugs tested (Residence time. *E.v.*: t=0.08; df=13; p=0.93. *M.h.*: t=0.98; df=14; p=0.34. Linear speed. *E.v.*: t=0.63; df=13; p=0.54. *M.h.*: t=0.70.; df=14; p=0.50) (Fig. 2).

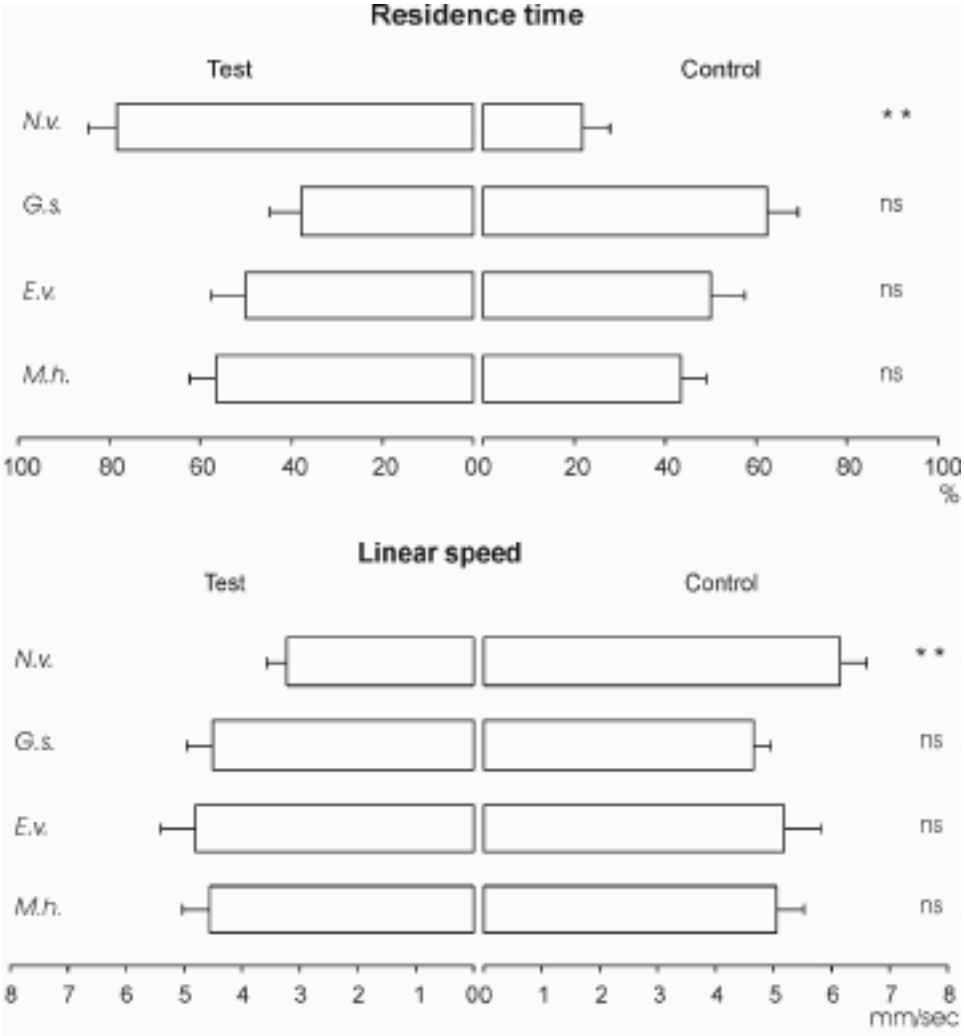

Figure 2. Response of *Trissolcus basalis* to volatiles of *Nezara viridula* (*N.v.*), *Graphosoma semipunctatum* (*G.s.*), *Eurydema ventrale* (*E.v.*) and *Murgantia histrionica* (*M.h.*). Comparisons of residence time and linear speed (Mean ± SE) between test and control arms (** $p<0.01$; ns not significant; t-tests for paired comparisons).

Differences in parasitoid residence time and linear speed were also found when blanks and test arms containing the different bugs were compared (Fig. 3). Residence time (F=6.51; df=4, 73; $p<0.001$) was significantly higher in the presence of cues from *N. viridula* compared with *G. semipunctatum*, *E. ventrale* and the blank, whereas it was intermediate on *M. histrionica*. Linear speed (F=3.11; df=4, 71.; p=0.02) partially confirms these results, being significantly lower on *N. viridula* compared with *E. ventrale* and the blank (Fig. 3).

Discussion

Adults of the coevolved host *N. viridula* release both volatile and contact cues that have a kairomonal effect on *T. basalis* females. Volatile cues are used by *T. basalis* to

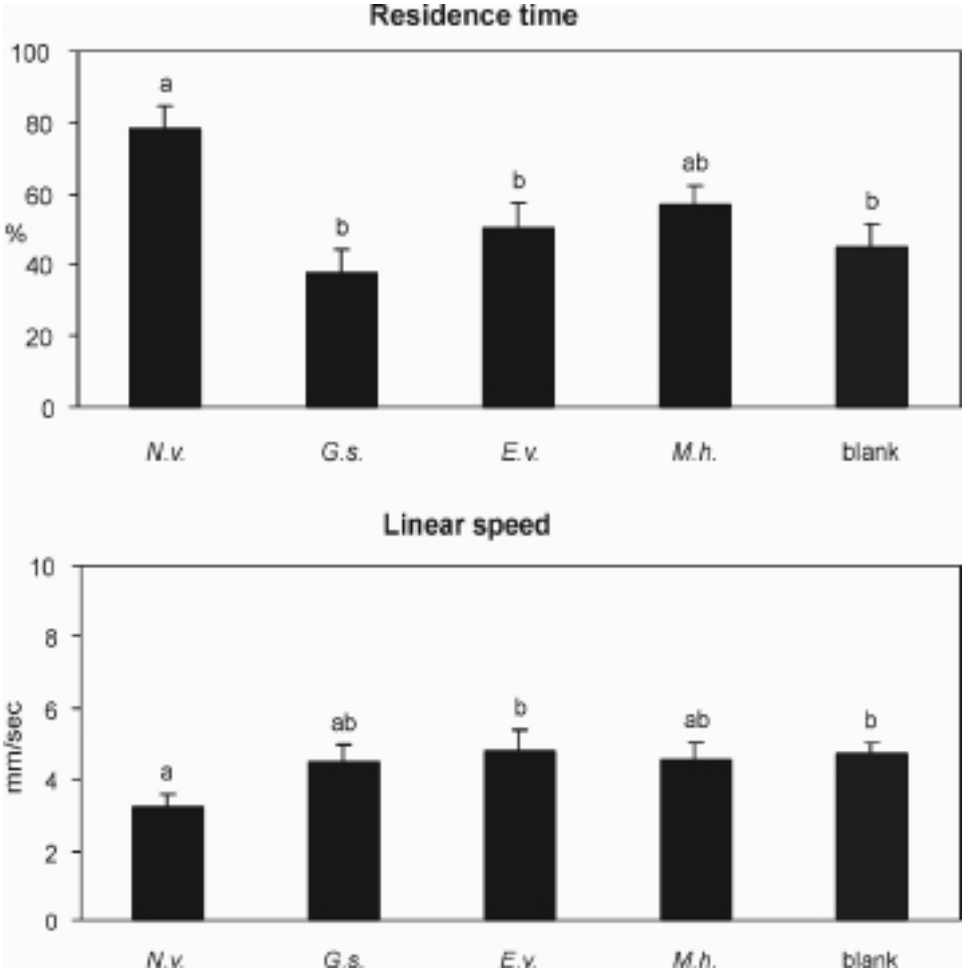

Figure 3. Comparisons of residence time and linear speed (Mean ± SE) of *Trissolcus basalis* as response to volatiles of *Nezara viridula* (*N.v.*), *Graphosoma semipunctatum* (*G.s.*), *Eurydema ventrale* (*E.v.*) and *Murgantia histrionica* (*M.h.*). Columns with the same letter are not significantly different ($p<0.05$; ANOVA, Tukey HSD).

locate the potential host community (Mattiacci et al., 1993, Colazza et al., 1999b), whereas adult traces left on the substrate provide additional information to the parasitoid about the presence of host egg masses (Colazza et al., 1999b). Once the host eggs are encountered, the wasp's acceptance is affected by a contact kairomone present on the egg surface (Bin et al., 1993). Parasitoid response to volatile cues is characterised by an increase of residence time and a reduction in linear speed. Both behaviours suggest an increased searching in the area contaminated by host volatiles (Bell, 1990).

Trissolcus basalis also recognises and accepts the eggs of *G. semipunctatum* (Voegele, 1966; Kartavtsev et al., 1977), and such response may be chemically mediated by the adhesive secretion present on eggs, which most likely have properties similar to those of *N. viridula* (Bin et al., 1993). However, because this host selection

process lacks cues necessary for host location, it seems improbable that *T. basalis* could also attack the eggs of *G. semipunctatum* in field conditions. In fact, this parasitoid does not respond to volatiles from adult females of *G. semipunctatum* and shows a behaviour that may even suggest some kind of repellence. However, this hypothesis needs to be confirmed with more tests.

In addition, *T. basalis* does not respond to volatiles from adult females of *E. ventrale*. It also rejects the eggs of this non-host pentatomid bug, both in field and laboratory conditions (unpublished data), indicating that different cues are involved in such steps of the host selection sequence.

Finally, one field association reported in the literature, was not confirmed by laboratory tests. This regards a record of *T. basalis* on *M. histrionica* in Brazil (Correa-Ferreira and Moscardi, 1995). In the laboratory, this parasitoid did not respond to volatile cues, although partially responded to contact chemicals left on the substrate (Salerno et al., 2000) and partially recognised *M. histrionica* eggs, but did not develop in the eggs (unpublished data). Therefore, parasitoid adaptation to such a potential new host appears unlikely because at least part of the cues used are not shared with its coevolved host.

Host-parasitoid associations are characterised by complex interactions that are often difficult to understand. Comparing such interactions with those implying possible novel hosts and non-hosts may help to explain the mechanisms involved in the host selection steps. This may help defining host preference in *T. basalis* in order to use this parasitoid with improved efficacy and safety (Nechols et al., 1992) in IPM programmes. Finally, this system may be used as a model for host range studies that can also be applied in other host-parasitoid systems.

Acknowledgments

We are grateful to Jeff Aldrich and Dave Truesdale for their helpful collaboration. This work was financially supported by MURST-PRIN 1998-2000 "Reproductive strategies of parasitoid Hymenoptera for biological control of crop insect pests".

References

Bell WJ (1990) Searching Behaviour. The Behavioural Ecology of Finding Resources. Chapman and Hall, London

Bin F, Vinson SB, Strand MR, Colazza S, Jones WA (1993) Source of an egg kairomone for *Trissolcus basalis*, a parasitoid of *Nezara viridula*. Physiol Entomol 18: 7-15

Bonnemaison L (1952) Morphologie et biologie de la punaise ornée du chou (*Eurydema ventralis* Kol.). Annal de l'I.N.R.A 2: 257-252

Colazza S, Peri D, Salerno G, Peri E, Lo Pinto M, Liotta G (1999a) Xbug, a video tracking and motion analysis system for Linux. XII International Entomophagous insects workshop. Asilomar. USA. September 26-30, 1999

Colazza S, Salerno G, Wajnberg E (1999b) Volatile and contact chemicals released by *Nezara*

viridula (Hemiptera: Pentatomidae) have a kairomonal effect on the egg parasitoid *Trissolcus basalis* (Hymenoptera: Scelionidae). Biol Control 16: 310-317

Colazza S, Rosi MC, Clemente A (1997) Response of the egg parasitoid *Telenomus busseolae* to sex pheromone of *Sesamia nonagrioides*. J Chem Ecol 23: 2437-2444

Conti E, Bin F, Vinson SB (2000a) Host range in egg parasitoids: a tentative approach through the analysis of the host unit. Antonie van Leeuwenhoek Symposium 7th European Workshop on Insect Parasitoids, Haarlem, 1-6 October 2000: 32

Conti E, Bin F, Vinson SB, Williams HJ (2000b) Egg parasitoids attacking homologouis hosts: equivalences and differences between two allopatric cabbage bugs. XXI International Congress of Entomology, Brazil, August 20-26, 2000: 368

Correa-Ferreira BS, Moscardi F (1995) Seasonal occurrence and host spectrum of egg parasitoids associated with soybean stink bugs. Biol Control 5:196-202

Follett PA, Duan J, Messing RH, Jones VP (2000) Parasitoid drift after biological control introduction: re-examining Pandora's box. American Entomologist 46: 82-94

Kartavtsev NI, Voronin KE, Sumaroka AF, Dzyuba ZA, Pukinskaya GA (1975) Investigations over many years on the seasonal colonisation of telenomines in the control of the noxious pentatomid in the Krasnodar region. Trudy Vsesoyuznogo Nauchno issledovatel'skogo Instituta Zashchity Rastenii, recd. 1977, 44

Mattiacci L, Vinson SB, Williams HJ, Aldrich JR, Bin F (1993) A long-range attractant kairomone for egg parasitoid *Trissolcus basalis*, isolated from defensive secretion of its host, *Nezara viridula*. J Chem Ecol 19: 1167-1181

McPherson RM, Pitts JR, Newsom LD, Chapin JB, Herzog DC (1982) Incidence of tachinid parasitism of several stink bug (Heteroptera: Pentatomidae) species associated with soybean. J Econ Entomol 75: 783-786

Nechols JR, Kauffman WC, Schaefer PW (1992) Significance of Host Specificity in Classical Biological control. In: Proceedings Thomas Say Publications in Entomology. Rockstein M (ed), Entomological Society of America, Lanham, Maryland, pp. 41-52

Safavi M (1968) Etude biologique et ecologique des hymenopteres parasites des oeuefs des punaises des cereales. Entomophaga 13: 381-495

Salerno G (2000). Evaluation of host specificity in pentatomid parasitoids through their response to the host unit. Ph.D. thesis, University of Perugia, 169 pp

Salerno G, Conti E, Peri E, Colazza S, Bin F (2000) Arrestment response in egg parasitoids of pentatomid bugs: its role in host specificity. Antonie van Leeuwenhoek Symposium 7th European Workshop on Insect Parasitoids, Haarlem, 1-6 October 2000: 44

Sokal R, Rohlf FJ (1998) Biometry. W.H. Freeman and Company, New York, 887 pp

StatSoft (1997) STATISTICA per Windows [user’s manual]. StatSoft Italia S.r.l., via Parenzo, 3 - 35010 Vigonza (Padova)

Todd JW (1989) Ecology and Behavior of *Nezara viridula*. Annu Rev Entomol 34: 273-292

Vet LEM, Dicke M (1992) Ecology of infochemicals use by natural enemies in tritrophic context Annu Rev Entomol 37: 141-172

Vinson SB (1985) The behaviour of parasitoids. In:. Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol. 9. G.A. Kerkut and L.I. Gilbert (Eds.) Pergamon Press, Elmsford, New York, pp. 417-469

Vinson SB (1998) The general host selection behavior of parasitoid Hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species. Biol Control 11: 79-96

Voegele J (1966) Biolopgie et morphologie de *Graphosoma semipunctata* Fabricius (Heteroptera, Pentatomidae). Al Awamia 20: 43-102