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Field responses of stink bugs to the natural and synthetic pheromone of the Neotropical brown stink bug, *Euschistus heros* (Heteroptera: Pentatomidae)

M. BORGES,[1] F. G. V. SCHMIDT,[1] E. R. SUJII,[1] M. A. MEDEIROS,[2] K. MORI,[3] P. H. G. ZARBIN,[4] and J. T. B. FERREIRA[4]

[1]EMBRAPA/CENARGEN/ACB, Cx. Postal 02372, 70849–970, Brasília, [2]EMBRAPA/CNPH, BR 060 Km 09. 70359–970, Brasília, DF, Brazil, [3]Department of Chemistry, Science University of Tokyo, Kagurazaka I-3, Shinjuku-Ku, Tokyo 162, Japan, and [4]UFSCar/Dep. Química/Lab. Sintese Produtos Naturais, 13565–905, São Carlos, SP, Brazil

Abstract. The synthetic racemic mixture of methyl 2,6,10-trimethyltridecanoate, a component of the male produced pheromone of *Euschistus heros* (F.) (Heteroptera, Pentatomidae), was attractive to pentatomid species in a field test, using homemade pheromone trap designs. The pentatomid *Piezodorus guildinii* was caught in high numbers in field traps, during two field experiments, indicating a consistent response of this species to the *E. heros* pheromone. A correlation was found between the range of insects caught in the pheromone-baited traps and a random sampling method. The synthetic stereoisomeric mixture of methyl 2,6,10-trimethyldodecanoate, a minor component of *E. heros* pheromone, was also field tested and caught no pentatomids. Egg parasitoids were caught in traps baited with *E. heros* pheromone, indicating that this pheromone can be exploited as a kairomone. A synchrony in the periodicity of trap catch, between the egg parasitoids and their host, was also recorded.

Key words. Egg parasitoids, *Euschistus heros*, methyl-2,6,10-trimethyldodecanoate, methyl-2,6,10-trimethyltridecanoate, pheromone traps, *Piezodorus guildinii*, Scelionidae, *Telenomus podisi*, *T. teretis*, *T. urichi*.

Introduction

Soybean, *Glycine max* (L.) Merryl, is damaged by complexes of stink bugs around the world. In Brazil, the species of major importance in practically all soybean growing regions are *Nezara viridula* (L.), *Piezodorus guildinii* (Westwood) and *Euschistus heros* (F.). The Neotropical brown stink bug, *E. heros*, is a major pest of soybean (Panizzi & Rossi, 1991), mainly in the regions where the temperature is high (Cividanes & Parra, 1994). In Brazil, over 4 million litres of chemical insecticides are used annually to control stink bugs (Corrêa-Ferreira & Moscardi, 1996).

Forming part of a system of integrated pest management, the use of semiochemicals can lead to opportunities in biological control. A chemical attractant for *Euschistus* species would be valuable for monitoring these pests (Aldrich *et al*., 1991). Besides which, attractants can usefully concentrate stink bugs in early-maturing trap crops where they may be economically controlled by limited insecticide application (McPherson & Newson, 1984).

The chemical communication system of *E. heros* has been elucidated (Borges & Aldrich, 1994; Aldrich *et al*., 1994). Recently, evidence was presented demonstrating that methyl 2,6,10-trimethyltridecanoate is a component of the male-produced pheromone of *E. heros* (Borges *et al*., 1998). The racemic mixture of male-produced pheromone (methyl 2,6,10-trimethyltridecanoate) of *E. heros* and *E. obscurus* has been synthesized (Mori & Murata, 1994). To date, no field tests have been conducted to monitor the biological activity of the synthetic methyl 2,6,10-trimethyltridecanoate. Thus, this work is aimed at providing new information about the biological activity of both the natural pheromone and the synthetic stereoisomeric mixture of the methyl 2,6,10-trimethyltridecanoate in the field. Because the novel pheromone components of *E. heros* were characterized through its relative, the Nearctic *E. obscurus* (Aldrich *et al*., 1994), this work also provides the opportunity to test the synthetic stereoisomeric mixture of methyl 2,6,10-trimethyldodecanoate (Ferreira &

Correspondence: Miguel Borges, EMBRAPA/CENARGEN, SAIN – Parque Rural, Cx. Postal 02372, 70849–970, Brasília DF, Brazil.

Zarbin, 1996), a component of the male-produced pheromone of *E. obscurus*, against the Brazilian soybean stink bug complex, because this compound may be a minor component of the *E. heros* pheromone (Aldrich *et al*., 1994).

Materials and Methods

Pheromones

Synthetic racemic E. heros *pheromone.* The synthetic stereoisomeric mixture of methyl 2,6,10-trimethyltridecanoate (Mori & Murata, 1994), was kept at –20°C. Solutions at a concentration of 4 μg μl^{-1} were prepared by diluting the synthetic compound in *n*-hexane.

Synthetic stereoisomeric E. obscurus *pheromone.* The synthetic stereoisomeric mixture of the (2R, 6S, 10S) and (2S, 6S, 10S) isomers of methyl 2,6,10-trimethyl-dodecanoate in the proportion of 7:3 (Ferreira & Zarbin, 1996), was kept at –20°C. Solutions at a concentration of 4 μg μl^{-1} were prepared by diluting the synthetic compound in *n*-hexane.

Crude male pheromone extract of E. heros. A crude pheromone extract was obtained by caging twenty sexually mature virgin males of *E. heros* in a glass Petri dish (15 cm diameter). The apparatus was allowed to stand for 24 h at room temperature (max. = 28, min = 19°C), after which the insects were removed and the Petri dish washed with acetone. Excess solvent was evaporated under pure nitrogen to 200 μl, and 1/10 of this extract was applied to each trap. The release rate of active compound calculated for *E. heros* was 0.04 μg/day/male (Aldrich *et al*., 1994).

Pheromone traps

Funnel-ended trap. The top 8 cm and the bottom 3 cm from a clear plastic 2 litre bottle were cut off, forming a cylinder. Two top sections from similar bottles were placed, inverted, into the cylinder, providing it with two inverted funnel ends. Air holes (1 mm diameter) were made in the funnel sections, in the lower floor of the trap, and a central hole made in the cylinder section for attachment/suspension of the rubber septum pheromone holder (Fig. 1a).

Mesh perforated trap. A second type of trap was made by removing the bottom 3 cm of a 2 litre clear plastic bottle, and attaching it in an inverted position. A 2-cm diameter disk was removed from the inverted bottle base and from four areas from the centre of the bottle sides. The bottom hole was covered with 0.5 mm wire mesh, secured with silicone adhesive. The holes in the sides were filled with open cones of wire mesh, leaving 0.8 cm holes for insect entry. The pheromone holder was suspended from the bottle cap (Fig. 1b).

Field testing. Experiment 1. The first field test was conducted from 29 February to 18 April 1996, in a 1-ha soybean field at Cenargen Research Center. This field test was designed for the bioassay of four treatments [synthetic racemic mixture of *E. heros*, synthetic stereoisomeric mixture of *E. obscurus*, and the crude male *E. heros* pheromone extract, and control (*n*-hexane)].

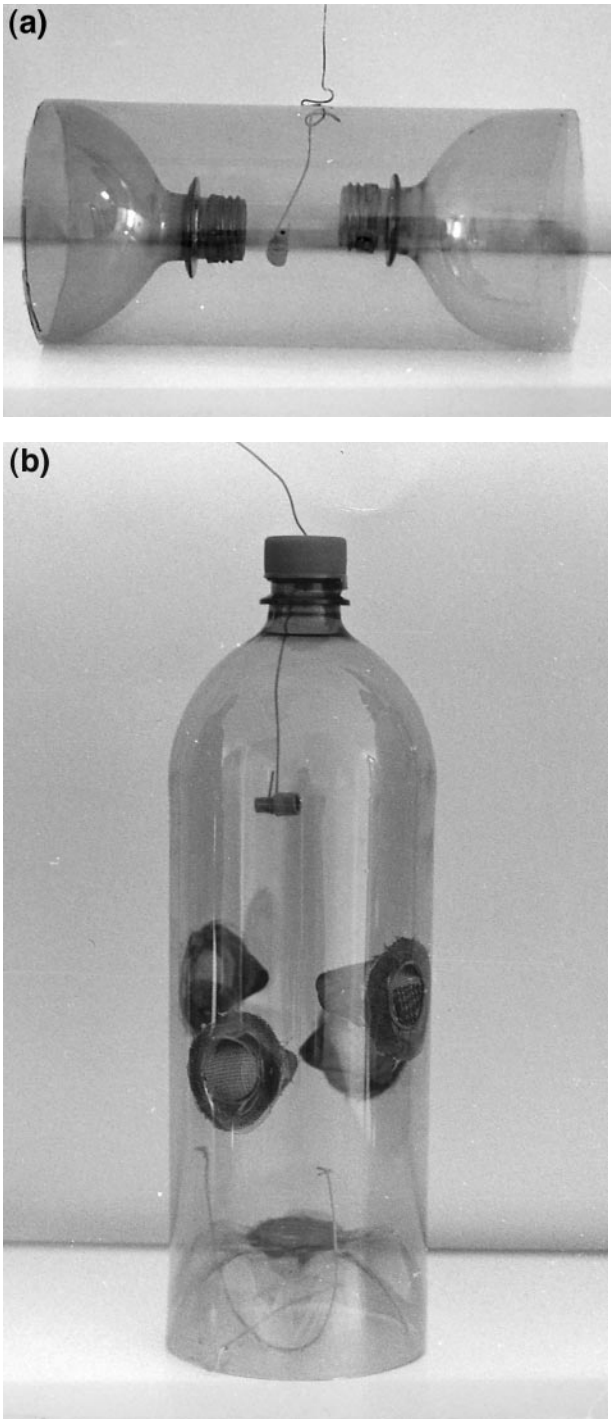

Fig. 1. Traps made from 2 litre transparent plastic bottles used in field experiments. (a) funnel-ended trap, (b) mesh perforated trap.

At the start of the experiment, the soybean crop was at the vegetative stage (V6–V7) and ended at physiological maturity (R7, Fehr *et al*., 1971). The funnel-ended traps were suspended in contact with the crop foliage from stakes (≈ 1 m above the ground), with 10 m distance between treatments. Four traps for each treatment were deployed in a 4 × 4 Latin Square

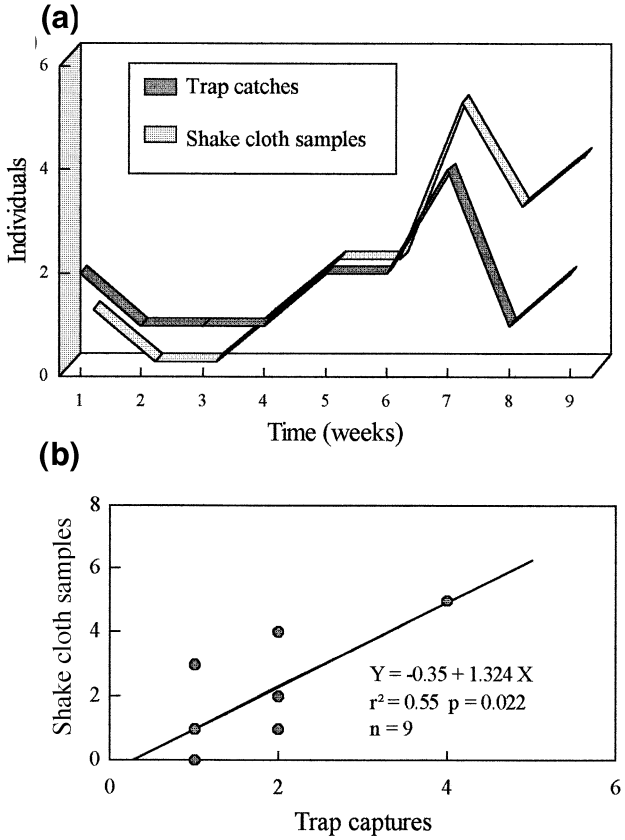

Fig. 2. Number of *Piezodorus guildinii* captured in the treatments traps in a field test during Experiment 1, plotted against their number in shake cloth samples. (a) comparison between trap capture and random samples during the experimental period, (b) regression analyses between methods.

Design. Treatment traps, with synthetic samples, were rebaited every other day with 80 µg of the active compound, on a rubber septum (5 × 9 mm, Thomas Scientific, Philadelphia, U.S.A.). Traps baited with natural pheromone were rebaited in the same way using an equivalent concentration of two individuals per treatment (0.8 µg). Control traps were unbaited. Surveys recording captured individual insects were made every second day.

Experiment 2. The two treatments that yielded the highest trap catches in the previous experiment were tested once more, with captured individuals recorded twice a day (morning and afternoon). The aim of this was to understand better the insects' response to the pheromone in the field.

The second field experiment was conducted from 20 November to 11 December 1996, in a 1-ha soybean field at Cenargen Research Center. This field test was designed to bioassay three treatments, i.e. the synthetic racemic mixture of *E. heros* pheromone, the crude male pheromone extract of *E. heros*, and the control. The soybean crop had a temporary irrigation set-up owing to the fact that the crop was planted out of the Brazilian soybean calendar (three months early). Traps were deployed in a randomized block design, with four replicates, and 25 m distance maintained between treatments in a block. Traps used in this test were modified from the previous experiment (Fig. 1b) in an attempt to improve their efficiency in the retention of captured insects. The same trap rebaiting procedure used previously was applied for this experimental design. At the start of the experiment, the soybean crop was at the R2 stage, and ended at R7.

Insect sampling. Insect pests. The shake cloth sampling technique (Herbert & Harper, 1983) was used once a week in order to characterize the population densities of stink bugs species present during the period of the field experiment. Ten randomized complete sample units per week were taken into the experimental area for this purpose.

Insect egg parasitoids. For sampling egg parasitoids (Hymenoptera: Scelionidae), host egg batches were collected throughout the crop once a week throughout the experimental period. The survey unit consisted of two people, collecting as many egg batches as they could in one hour. The egg parasitoids emerging from egg batches were kept in 70% alcohol, and sent for taxonomic identification (Dr M. S. Loiácono, Faculdad de Ciencias Naturales and Museo de La Plata, Argentina).

Statistical analysis. The mean numbers of individuals captured in the experiments were compared by Analysis of Variance followed by the Tukey Test for comparisons between means (Sokal & Rohlf, 1981).

Results

Experiment 1

The sampling cloth method demonstrated that the pentatomid complex in the experimental area consisted of *P. guildinni* (51%), *Acrosternum aseadum* (21%), *N. viridula* (13%), *Thyanta perditor* (6%) and *E. heros* (6%), and *Edessa meditabunda* (3%) ($n = 72$).

Egg parasitoids were not caught either in the treatment traps or in the control traps. However, they were present in the sampled egg batches, with individuals present belonging to *Telenomus podisi* (67%), *Trissolcus teretis* (17%), *Trissolcus urichi* (8%), and *Trissolcus brachymenae* (8%) ($n = 30$ egg masses collected).

Piezodorus guildinii was caught in the baited traps in significantly greater numbers than other insects ($F = 5.79$, $P < 0.05$). Some dipterans (possible Tachinidae) and some predators (mainly spiders and field wild cockroach), were also observed in the traps, but the low number of captures did not allow any significant conclusions to be made. The crude pheromone extract was less effective in attracting *P. guildinii*, but did attract the pentatomid *T. perditor*, although numbers caught were not significant (Table 1). *Euschistus heros* was not captured during Experiment 1, probably due to their low population density, as indicated by the shake cloth data.

Experiment 2

The shake cloth method demonstrated that *P. guildinii* was, once again, the main species in the experimental area,

Table 1. Individuals (Pentatomidae) captured in the *E. heros* pheromone-baited traps during Experiment 1.

Treatments	*Piezodorus guildinii*	*Thyanta perditor*
Methyl 2,6,10-trimethyltridecanoate	11	0
Methyl 2,6,10-trimethyldodecanoate	0	0
Crude male-produced pheromone	3	5
Control (unbaited)	1	0

Table 2. Percentage of individuals of different species captured in the treatment traps baited with the synthetic racemic mixture of male-produced pheromone (methyl 2,6,10-trimethyltridecanoate) of *E. heros*, crude pheromone extract of *E. heros*, and the control, during Experiment 2, listed in order of appearance in the experimental area. Numbers in brackets are number of specimens captured.

	Individuals captured			
	Treatments			Soybean stage
Species	Synthetic	Crude	Control	
Pentatomidae (*n* = 38)				
Piezodorus guildinii	(11) 29	(13) 34	(3) 8	R6
Euschistus heros	(2) 5	(1) 3	– –	R6
Nezara viridula	(3) 8	– –	– –	R6
Thyanta perditor	(1) 3	– –	(1) 3	R6
Acrosternum aseadum	(1) 3	– –	– –	R6/R7
Edessa meditabunda	(2) 5	– –	– –	R7
Scelionidae (*n* = 23)				
Trissolcus teretis	(3) 13	(6) 26	(3) 13	R6
Telenomus podisi	(3) 13	(5) 22	– –	R6/R7
Trissolcus urichi	(2) 9	(1) 4	– –	R7

comprising 75% of the individuals of the pentatomid fauna, followed by *E. heros* (9%), *E. meditabunda* (8%), *Acrosternum aseadum* (5%), *N. viridula* (2%), and *T. perditor* (1%) (N = 196).

Trap captures followed the same pattern as in Experiment 1, with captured fauna comprising pentatomids, dipterans and predators. *Piezodorus guildinii* was, again, the main species caught (55%), but this time followed by *N. viridula* (15%), *E. heros* (10%), *E. meditabunda* (10%), *A. aseadum* (5%), and *T. perditor* (5%). A total of twenty pentatomids from the stink bug complex were recorded from the traps baited with the synthetic sample only (Table 2). For the crude male extract, the catch was *P. guildinii* (93%) followed by *E. heros* (7%). A total of fourteen pentatomids from the stink bug complex were caught in traps baited with the crude pheromone sample only. However, a significant number of Hymenoptera egg parasitoids were also caught. The parasitoid sampling method showed that the main species present in the area consisted of *Telenomus podisi* (74%), *T. teretis* (15%), *T. urichi* (6%), and *T. basalis* (5%) (n = 287 egg masses collected).

The number of egg masses parasitized at the start (R2 stage) of the experiment was 16%, reaching 71% at the end (R7 stage). The egg parasitoid fauna initially comprised 86% *T. teretis* and 14% *T. basalis*. At the end of the trial, *Telenomus podisi* comprised 85% of the egg parasitoid fauna, followed by *T. urichi*, comprising 15%.

Table 3. Fluctuation in the number of individuals captured in the pheromone baited traps in the field test during Experiment 2, during different period of the day.

Period of day	Pentatomidae	Scelionidae
Morning	12	4
Afternoon	22	16

There was no significant difference in the number of pentatomids captured by the two treatment traps, but these numbers were significantly higher than in the control traps ($F = 33.51$, $P < 0.001$). A similar result was observed for the hymenopteran egg parasitoids ($F = 17.77$, $P < 0.001$; Table 2). The presence of dipterans and some predators was observed in the treatment traps, but again, the low number of captures do not allow any significant conclusions to be made.

The numbers of individual stink bugs and egg parasitoids caught fluctuated according to the time of day. For instance, the percentage of stink bugs caught during the morning was 38%, compared to 62% in the afternoon. However, a bigger fluctuation was observed for the Scelionidae egg parasitoids, where 16% were caught in the morning, compared with 84% in the afternoon (Table 3).

Discussion

Despite the almost complete absence of the target species in the field during Experiment 1, significant capture of non-target species from the soybean stink bug complex, in particular *P. guildinii*, was observed. When the data, including the numbers of *P. guildinii* caught in the traps baited with synthetic racemic mixture of the methyl 2,6,10-trimethyltridecanoate, were plotted against the corresponding shake cloth samples, the insects caught in the traps correlated with the population in the field ($r = 0.743$, $P = 0.022$), i.e. the insects appeared to have responded to the treatment according to their population density. Due to the low coefficient of determination observed, it is likely that more samples may be necessary to construct a model to estimate field densities of *P. guildinii*. The pheromone traps should probably be tested with a wide range of insect densities, to account for this effect on the capture rates. Importantly, a deviation in the sex ratio of *P. guildinii* captured in the treatments traps was observed, i.e. 85% were females. These data may be another significant piece of information for model validation in the future. Although this observation was recorded for *P. guildinii*, owing to the absence of the target species, this may be a factor that should be taken into account to adapt the model for other species.

Conversely, synthetic stereoisomeric methyl 2,6,10-trimethyldodecanoate did not attract any pentatomids from the stink bug complex, nor did it attract any parasitoids or predators. The failure to capture *E. heros* in field traps may be attributed to the low population density of this species in the field during the experiments. However, this compound was attractive to

E. heros females in a preliminary laboratory bioassay. Possibly, this compound plays a role in short-range attraction and courtship for *E. heros* (M. Borges, unpublished data).

The same pattern of trap captures occurred in both experiments, i.e. the principal pentatomid caught using the synthetic racemic mixture of the methyl 2,6,10-trimethyltridecanoate was *P. guildinii*. This high percentage of *P. guildinii* in field traps indicates a consistent response of this species to the *E. heros* pheromone.

Aldrich *et al*. (1993), working on a male-specific volatile with different populations of the pentatomid *N. viridula*, from different origins, i.e. Japan, U.S.A. and Brazil), found that at least two distinct pheromones exist for these populations. Analyses of volatiles from *N. antennata* and *Acrosternum aseadum* males were also reported, showing that the native Japanese *Nezara* sp., and species in the sister genus *Acrosternum*, produce species-specific blends based on the same compounds as *N. viridula*. Amongst *Euschistus* species, pheromone sharing seems to be more widespread (Aldrich *et al*., 1991).

To date, no studies exist on the sex pheromone of *P. guildinii* or *T. perditor*. The attractiveness of the *E. heros* pheromone to these species might be explained by their sharing a similar composition. Natural enemies might recognize and respond to the pheromone of the potential host (Aldrich, 1995). Although scelionids are not specific, they do display host preference. This may explain the complete absence in field traps of the egg parasitoid *T. basalis*, which is known to have a preference for *N. viridula* (Corrêa-Ferreira, 1993; Corrêa-Ferreira & Moscardi, 1995).

The difference in concentration, almost 100-fold, between the synthetic samples and crude pheromone extract, apparently did not significantly affect trap capture rates. This may indicate that, in the field, pheromone concentration might not be as critical for pentatomids species as for other insects species (Renwick & Vité, 1981), or that the racemic mixture is not as attractive as the natural pheromone.

The higher trap catch of egg parasitoids during the afternoon may indicate a fine synchrony between the egg parasitoids and their host. Female *E. heros* have a periodicity of response to males releasing pheromone, from afternoon to midnight (Borges *et al*., 1997). Thus, this field experiment reinforces this finding and supports other research on Pentatomidae, reporting that pheromone can be exploited as a kairomone (Aldrich, 1995).

Interestingly, it was observed in both experiments, but particularly during Experiment 2, that *T. teretis* is the first egg parasitoid to arrive, or at least the first to be detected, in the crop. However, it does not build up its population through the soybean season. This species was overtaken by later-arriving *Telenomus podisi*. This observation suggests that *Telenomus podisi* may be better adapted to compete in the field for the hosts of its spectrum of preference (Corrêa-Ferreira & Moscardi, 1995).

Trap efficiency in the retention of captured insects was not analysed. This may at least partly explain the difference in the species composition of stink bugs complex captured in the two experiments. The mesh perforated trap will be applied in further field tests owing to its ease of handling and avoidance of rain water accumulation.

To date, no other attempt has been made to test pentatomid pheromones of the stink bug complex on soybean. Thus, these findings should be of particular importance for an understanding of the interactions between the members of the soybean stink bug complex and their natural enemies, for instance, the egg parasitoids.

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