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Monitoring the Neotropical brown stink bug *Euschistus heros* (F.) (Hemiptera: Pentatomidae) with pheromone-baited traps in soybean fields

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Abstract

The effectiveness of the synthetic sex pheromone of the Neotropical brown stink bug, *Euschistus heros*, was evaluated both in laboratory and in field assays. Lures loaded with 1 mg of methyl 2,6,10-trimethyltridecanoate (TMTD) continuously attracted female bugs for more than 30 days to pheromone-baited traps in field trials. The pheromone-baited traps were effective in field tests even at low bug population densities, as compared with the usual monitoring technique, shake cloth sampling. Traps around borders or in the centre of soybean fields caught similar numbers of bugs. Trap captures showed a positive relationship with field populations, as monitored with the shake cloth technique, during the reproductive phase of the soybean crop, i.e. from the R1–R5 developmental stage (pod formation to pod fill). The physiological state of the trapped migrating insects was determined. The first insects arriving in the field had fewer eggs in the reproductive tract compared to later arrivals. Some cross-attraction was also observed, with *Piezodorus guildinii* and *Edessa meditabunda* also being caught in pheromone-baited traps, suggesting that these insects respond to the sex pheromone or to the defensive compounds released by *E. heros* captured in traps. In brief, the results showed that traps baited with 1 mg of the sex pheromone efficiently caught bugs, that the lures lasted for more than 1 month under field conditions and that placement of traps around the borders of the crop area was as effective as placement inside the crop area. Border-placed traps were effective at a density of one trap every 200 m.

Introduction

Several stink bug species are considered the main pests of soybean because of the damage they cause to the crop (Panizzi and Slansky 1985; Panizzi and Rossi 1991; Corrêa-Ferreira and Moscardi 1996). Bug feeding directly damages the fruit and seeds, rendering the product unsuitable for human consumption and as seed for growers (Panizzi 1991; Corrêa-Ferreira and Azevedo 2002). Species of *Euschistus*, particularly *Euschistus heros* (Fabricius) in the neotropics (Panizzi and Slansky 1985) and, to some extent *Euschistus servus* (Say) in the Nearctic (Kogan and Turnipseed 1987), cause injury to soybean.

The economic importance of stink bug damage to soybeans, combined with the necessity of developing more integrated management of stink bug populations, is motivating researchers worldwide to look for methods to reduce pesticide use for stink bug control (Corrêa-Ferreira and Moscardi 1996; Panizzi and Corrêa-Ferreira 1997; Corrêa-Ferreira and Panizzi 1999; Venzon et al. 1999; Knight and Gurr 2007). In

this aspect, the use of semiochemicals to manage and monitor these pests and their natural enemies has been proposed by several researchers (Borges et al. 1998a,b; McBrien and Millar, 1999; Cullen and Zalom 2005; Leskey and Hogmire, 2005; and references therein).

The pheromone components of *E. heros* were identified by Borges et al. (1998a,b) and consist of three components: methyl 2,6,10-trimethyldecanoate, methyl 2,6,10-trimethyltridecanoate (2,6,10 TMTD) and methyl (2*E*,4*Z*)-decadienoate. The biological activity of the three components was confirmed in a laboratory bioassay that showed 2,6,10 TMTD was the main component to attract females (Borges et al. 1998a; Costa et al. 2000). However, the presence of all stereoisomers in the racemic mixture did not have an antagonist effect in bioassays (Costa et al. 2000).

Furthermore, despite major efforts to establish biological tools to manage stink bugs populations in soybean, the main method of control of stink bugs is still with chemical pesticides (Corrêa-Ferreira and Moscardi 1996; Knight and Gurr 2007; Brier et al. 2008). The concept of integrated pest management is evolving towards a more sustainable management system in which external chemical interventions are a last resort. Sustainable agriculture requires management of the ecosystem so as to conserve the natural enemies that are instrumental in suppressing pest populations (Knight and Gurr 2007; Moraes et al. 2009; Weiss et al. 2009).

While the sustainable agriculture movement has been gaining momentum, the genetically engineered crop revolution has begun in earnest with releases of *Bt*-cotton and Bt-corn, with genetically engineered strains of soybean, sorghum, canola, alfalfa and wheat soon to follow. In *Bt*-crops, the primary pests are suppressed by the expressed *Bt* toxins, alleviating the need for insecticides for control of these insects (Naranjo 2009 and references therein). However, *Bt* toxins are not effective against sucking insects (Hemiptera) (P. Roberts, University of Georgia College of Agricultural and Environmental Sciences, Athens, GA, USA, personal communication; Sharma and Pampapathy 2006; Torres and Ruberson 2008). As a consequence, these sucking insects have surfaced as the new primary pests of these transgenic crops. The complex of stink bugs around the world has always been difficult to control, and the advent of reduced tillage practices has intensified this problem (Fidelis et al. 2003; Chocorosqui and Panizzi 2004; Seffrin et al. 2006). Future pest management strategies should accommodate semiochemical relationships with crop plants and herbivore enemies to take full advantage of stabilizing trophic webs.

The use of sex pheromone-baited traps for monitoring stink bugs catches mainly adult, sexually mature females (Borges et al. 1998a; Cullen and Zalom 2005) but damage is caused by both adult and immature stink bugs (Cottrell 2001; Millar et al. 2002). Therefore, for efficient population monitoring which might count also the immature insects, pheromone-baited trap technology could be used to establish an accurate and directly proportional relationship between insects trapped with and population density in the field (Suckling 2000). By indicating precisely the critical changes in population dynamics and behaviour of stink bugs in the field, this technology could be used to time control measures for the key pest, *Euschistus heros* (F).

This work is aimed at providing additional information about: (i) the efficiency of the synthetic *Euschistus heros* sex pheromone at trapping insects in the field, (ii) the efficiency of trap captures to predict population densities of the stink bug, and (iii) the relationship between the insects captured in traps and their physiological state that could be used to characterize the immigrating, emigrating, and resident female bugs.

Materials and Methods

Pheromone

Synthetic pheromone was formulated by the Fuji Flavor Co., Ltd (Tokyo, Japan). Lures were loaded with 1 mg of a stereoisomeric mixture of 2,6,10 TMTD, the main component of the male-produced pheromone of *Euschistus heros* (Borges and Aldrich 1994b; Aldrich et al., 1994; Borges et al. 1998b). The synthesis of the 2,6,10 TMTD was carried out following the methodology described by Mori and Murata (1994).

Efficiency of the lure device

To monitor the release rate of lures under field conditions, five traps with one lure each (1 mg of 2,6,10 TMTD in each lure) were maintained in the experimental area of EMBRAPA Genetic Resources and Biotechnology (Brasília, DF, Brazil) for 63 days. Weekly, all five lures were taken from the traps and submitted to two procedures. First, three randomly chosen lures out of the five being tested were subject to volatiles collection for 24 h under laboratory conditions. The procedure consisted of introducing

the lures individually into glass chambers and trapping the volatiles with 50 mg of Super Q adsorbent in a pulled filtered (activated charcoal) air system (n = 3), at 0.8 l/min, in an acclimatized room at 26.0 ± 1.0°C, 60% RH and 14-h photophase. After 24 h, the volatiles trapped on the adsorbent were eluted with 1 ml of n-hexane and concentrated to 200 μl under a gentle current of nitrogen. The release rate (ng/24 h) was calculated through quantification after lure aeration and GC analyses in relation to the area of an internal standard (IS, dodecane, 0.25 mg/ml). The lures were returned to the field for another week and the same procedure was carried out up to 9 weeks. Mean release rates in each week were used to analyse the shape of the release rate curve using nonlinear regression analyses. Mean maximum and minimum temperatures and RH% during each week of the experiment were obtained from Embrapa Cerrados climatic data bank (http://www.cpac.embrapa.br/tempoeagri/tempoeagri.html).

Second, the two remaining lures of the five being tested had their efficiency to attract *E. heros* females monitored, simultaneously with the above procedure, in olfactometer bioassays after being exposed under field conditions. Fifteen to 20 replicates were carried out each week during the 9 weeks. After field exposure, individual lures were assayed against females in an olfactometer modified from Borges and Aldrich (1994b). In brief, the olfactometer consisted of a 500-ml three-neck, round-bottom flask (24/40 joints; Kontes, Vineland, NJ). Two 250-ml rotary evaporator trap adapters (24/40 joints) were attached to the two outside arms to provide treatment and control chambers. A charcoal filter (20/40 mesh, 130 mm × 10 mm ID) connected in series to a water bubbler to humidify the incoming air was attached to the inlets of the two chambers with silicon rubber tubing (Silastic, 4.8 mm ID; VWR Scientific, Darmstadt, Germany). Air was pulled through the system (400 ml/min) by connecting the middle neck of the flask to a regulated vacuum source, with the flow measured with a Clear Flow Rotameter (Accura Flow Products, Warminster, PA). The olfactometer was positioned horizontally on a countertop in a room with bright fluorescent lights (4 × 40 W). Temperature in the bioassay room was maintained at 26.0 ± 1.0°C. Positions of control and treatment arms were alternated between replicates to avoid any positional bias. The apparatus was cleaned after each five replicates with fragrance-free liquid soap, rinsed thoroughly with water, and dried in a convection oven at 80°C.

To begin an assay, a single *E. heros* female was gently introduced into the release chamber with an artist's paint brush (Camel Hair, number 1), and allowed to acclimate for 3 min while the remainder of the apparatus was assembled. After attaching the treatment (lures with pheromone) and control (clean air) chambers and starting the air flow, the test bug's behaviours were recorded by an observer for 15 min/replicate. We recorded the first choice of the insect, determined as the first arm of the olfactometer that each insect chose and subsequently remained in for at least 100 s. Insects that did not leave the release chamber of the olfactometer during the first 10 min were considered 'non-responders', and were not included in the data analyses. Data on the responses of females to treatment or control were analysed using chi-squared tests (P = 0.05). The lures were taken to the field again for another week and the same procedure was carried out up to 9 weeks.

Field tests

Lures dosed with 1 mg synthetic 2,6,10 TMTD were used in traps assembled from 2-l transparent plastic soft drink bottles with four holes at the top of the bottle, each with an iron funnel to permit the entrance of the insects and the dispersion of the pheromone plume. The pheromone lures were hung at the level of the entrance openings, in the interior of the trap, with an iron rod. A plastic funnel was incorporated in the lower third of the bottle to retain the insects in the trap. In this way, the bottom of the bottle functioned as a collector of the insects that entered in the trap (Borges et al. 1998a; Pires et al. 2006). Field experiments were carried out in the Central Region of Brazil during three consecutive soybean seasons, 2004/2005, 2005/2006 and 2006/2007, referred to as experiment 1, experiment 2 and experiment 3, respectively.

Field experiment 1 was carried out during the 2004/2005 season, to evaluate the attractiveness of the synthetic 1 mg 2,6,10 TMTD lure vs. controls (blank lures), and to determine the best field distribution of traps (along borders or in the centre of the soybean field). Thus, treatment and control traps were distributed in pairs in a randomized way in the centre (inside) and borders of a 16-ha soybean field, with four repetitions of the pairs along the borders and four repetitions in the centre, with a minimum of 100 m between repetitions and 50 m between traps of each pair.

The experiment was set out at the end of the vegetative stage (V7) and the beginning of the reproductive stage of the soybean crop (R1) (Fehr et al. 1971). This stage is when stink bugs start to colonize the crop, and it is the beginning of the most critical stage of the soybean crop, that is from pod formation (R3) to pod fill (R5–R6), when the pod is still tender (Panizzi 1991; Panizzi et al. 2000; Corrêa-Ferreira and Azevedo 2002). The field test was allowed to run until physiological maturation of the crop (R7–R8) (Fehr et al. 1971). Following previous experience (Borges et al. 1998a; Pires et al. 2006), the traps in this and in field experiments 2 and 3 were hung from iron rods (2 m long × 0.005 m d.) with the openings of the trap just above the canopy of the soybean plant. Traps were checked weekly throughout the experiment and the insects collected were recorded as to species and sex.

The shake cloth sampling technique (Kogan and Pitre 1980; Herbert and Harper 1983) was used once a week to provide an independent estimate of population densities (number of insects/m^2) of stink bug species present during the period of the field experiment. Ten randomized complete sample units per week were taken in the experimental area. The sample unit consisted of a 1-m long cloth placed under the plants. The plants were shaken and the number of insects falling on the cloth was recorded and converted to number of insects/m^2.

Influence of the treatments (pheromone-baited traps vs. control traps) and position in the field (border vs. inside) in relation to total number of insects collected/trap during the 12 weeks of the experiment were tested with generalized linear models (GLM) and analyses of deviance (ANODEV) using Poisson errors and log as a link function. The statistical analyses were carried out using the R programming language (R Development Core Team 2008).

Field experiment 2 was carried out during the 2005/2006 soybean season, with the goal of predicting the number of traps per hectare required to give useful data for the assessment of stink bug populations. Thus, nine plots of 1 ha each were demarcated in three blocks (each with three plots) in a soybean field of 35 ha, with 150 m between each plot. Inside each block the experimental plots received one of three treatments in a randomized distribution: T1, one trap baited with 1 mg synthetic 2,6,10 TMTD; T2, two traps baited with 1 mg synthetic 2,6,10 TMTD; and T3 = four traps baited with 1 mg synthetic 2,6,10 TMTD.

The pheromone lure was replaced each 30 days. Inside each experimental plot the traps were distributed with a minimal distance of 50 m between each trap. The experiment was set at the V7 soybean stage and allowed to run until the stage of physiological maturation (R7–R8) (Fehr et al. 1971). Traps were checked once a week, recording the number of stink bugs trapped.

Insect sampling was performed following the same methodology described for Experiment 1, above. The recorded data (mean number of insects/trap/week and the mean density from samples with the shake cloth technique, n = 10 per plot) were used to plot curves of the population fluctuation and the relationship between the capture in traps vs. the shake cloth sampling. GLM and ANODEV (using Poisson errors and log as a link function) were conducted to test the significance of the mean captures in traps using week and treatment as factors. The original model including block effects was simplified because no significant block effect was found. The statistical analyses were carried out using the R programming language (R Development Core Team 2008).

Field experiment 3 was carried out during the 2006/2007 soybean season, to relate the number of insects sampled in the pheromone-baited traps to the population density in the experimental area. The pheromone-baited traps were placed along the borders (n = 7) around a soybean sampling area of 13 ha. The distance between traps was at least 200 m. The pheromone lure was replaced each 30 days. Traps were checked weekly, recording the number of stink bugs trapped. The experiment was set at the V7 soybean stage (just before flowering) and allowed to run until the stage of physiological maturation (R7–R8) (Fehr et al. 1971).

Insect sampling was performed following the same methodology described for Experiment 1. The recorded data (mean number of insects trapped and the mean number sampled in the shake cloth technique, n = 10) were used to plot curves of the population fluctuation and the relationship among the capture in the traps vs. the shake cloth sampling. The data were analysed using GLM to test the relationship between capture in the traps (independent variable) with the population density estimated from the surveys performed with the shake cloth sampling technique (dependent variable). Total number of stink bugs (adults and/or nymphs) captured in the pheromone traps and in the cloth sampling were used to analyse the relative abundance of each species in the field. Statistical analyses were performed using R programming language (R Development Core Team 2008).

Physiological state of trapped insects

Previous field tests (Borges et al. 1998a) showed that at the end of the soybean season (physiological maturation, R7/R8) (Fehr et al. 1971), pheromone traps would loose effectiveness in trapping stink bugs as compared with the shake cloth sampling technique. Therefore, field experiment 3 was also designed to monitor the difference in the physiological state of females migrating into and out of the crop from those females remaining in the crop during the development of the soybean cycle. For this purpose, a sample (n = 15/date) from all the females collected in the traps during field experiment was taken into the laboratory for dissection and recording the number of developing eggs in the reproductive tract.

Results

Efficiency of the lure device

The (2,6,10 TMTD) pheromone release rate from lures exposed to field conditions was fit to an inverse sigmoid curve ($F_{2,9} = 67.81$, $P < 0.001$, $R^2 = 0.937$) (fig. 1a). Mean maximal temperature during the period of the experiment was 29.1 ± 1.5°C, mean minimal temperature was 17.6 ± 2.2°C and mean relative humidity was 34.3 ± 7.2 (fig. 1b). The lures remained significantly attractive to females of *E. heros* until Day 49 (fig. 2).

Field tests

From field experiment 1 during the 2004/2005 soybean crop season, pheromone-baited traps captured significantly more insects than control traps (ANODEV for treatment effect $P < 0.001$, d.f. = 1,14) (fig. 3a). The number of insects captured in different field locations by pheromone-baited traps, i.e. traps placed on borders or in the centre of the soybean field, was not statistically different (ANODEV for position $P = 0.21$, d.f. = 1,6) (fig. 3b). Pheromone-baited traps were able to catch stink bugs in conditions of very low population levels throughout the experimental period (fig. 3c).

In field experiment 2 during the 2005/2006 soybean crop season, the number of traps per hectare (1–4 traps/ha) was evaluated. No statistical difference was found amongst the treatments (ANODEV $P = 0.86$, d.f. = 2,86), suggesting that 1 trap/ha could be used to monitor stink bugs. Traps were more efficient than shake cloth sampling until the medium to late reproductive stage of soybean. After this period, the traps became less effective than shake cloth sampling, through the final physiological stages of the soybean culture (fig. 4).

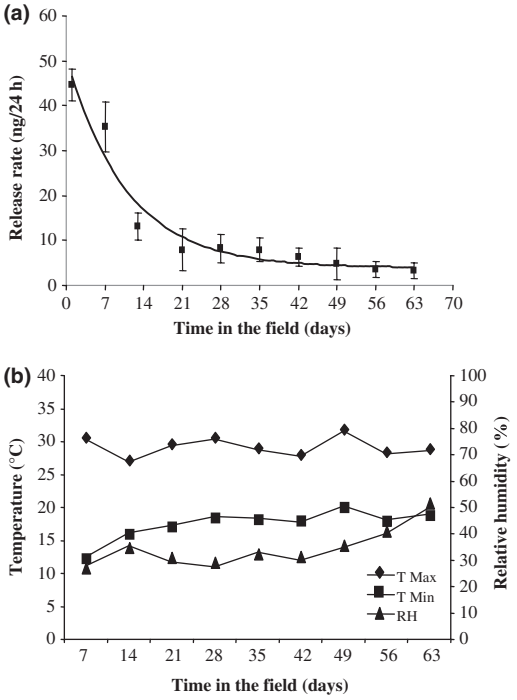

Fig. 1 (a) Release rate (ng/24 h) from field-aged lures with 1 mg of methyl 2,6,10-trimethyltridecanoate (2,6,10 TMTD). Data are mean values from three replicates. Quantification after lure aeration (24 h) and GC analyses with IS (dodecane 0.25 mg/ml). (b) Climatic conditions during the period when lures were deployed in the field. Data are mean values of 1-week periods.

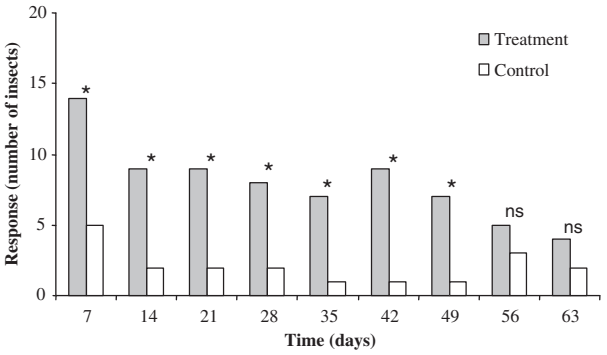

Fig. 2 Efficiency of lures for attracting *Euschistus heros* females in olfactometer bioassays after exposure of lures under field conditions for periods of 1 week. *Significant differences between number of insects that choose treatment or control (filtered air) chi-squared test ($P < 0.05$). N = 15–20 for each period of exposition.

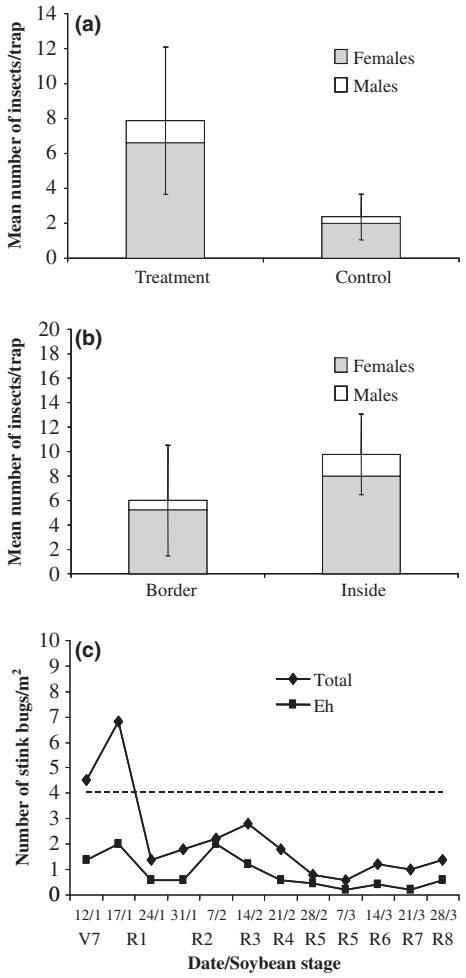

Fig. 3 Mean number of *Euschistus heros* adults/trap during the experimental period (12 weeks). (a) Comparisons between 1 mg of the methyl 2,6,10-trimethyltridecanoate (2,6,10 TMTD) baited traps (Treatment n = 8) with traps without pheromone (Control, n = 8). (b) Comparisons between pheromone-baited traps located at borders (Border, n = 4) or inside soybean fields (Interior, n = 4). Differences between means were significant for a (treatment vs. control) and non-significant for (b) (Border vs. Inside), GLM analyses $z = 4.896$ $P < 0.001$ for (a) and $z = 1.871$ $P = 0.06$ for (b). (c) Mean number of stink bugs (Total stink bugs) and *Euschistus heros* (Eh) per m^2 in the experimental field survey with the shake cloth sampling technique (n = 10), during the period of the 2004/2005experiment. The dotted line indicates the control threshold (in number of insects/m^2) estimated from production recommendations for Brazil by Embrapa (2008) (two stink bugs bigger than 0.5 cm/linear m) and a typical interline space of 0.5 m.

Finally, comparison of trap captures in field experiment 3 with the population density as determined by shake sampling showed that the number of insects in the pheromone-baited traps was significantly correlated with the mean population density only during the initial to medium reproductive stages of the soybean (R1–R5) (table 1). When compared over the total period of the bioassays the relationships were not significant (table 1). Thus, the traps lose their efficacy in catching *E. heros* at the end of the soybean reproductive stages (R6 to R7–R8) (fig. 5a). During the soybean reproductive stages, *E. heros* female captures in traps were correlated with the females' reproductive status (measured as mean number of eggs in the reproductive system). During the initial to medium soybean reproductive period, there was a rise in the captures and also an increase in the mean number of eggs in the reproductive tract of *E. heros* females, paralleling the curves of population density (fig. 5b). At the final stages of the soybean reproductive period, both number of insects in traps and eggs in the reproductive system decreased (fig. 5a). The results presented in fig. 5a also show that the density of stink bug nymphs followed the typical fluctuation as recorded for eggs and adults during the period. The guild of Pentatomidae in the experimental field was dominated by *E. heros* nymphs and adults (fig. 6) with *Piezodorus guildinii* (Westwood) and *Edessa meditabunda* (F.) being less abundant. Others species occasionally found in the experimental field included *Thyanta perditor* (F.) and *Chinavia* spp. (formerly *Acrosternum*). The patterns of insects captured in traps were representative of the stink bug guild, but in this case only adults were sampled (fig. 6). That is, captures in the traps were dominated by *E. heros* adults (72.4% of the total insects captured in traps during the experiment), but others stink bugs species also were caught in the baited traps, including *Edessa meditabunda* (14% of the total captures) and *Piezodorus guildinii* (11.6% of the total captures), along with a few of the other stink bugs present in the field (1.6% of the total insects captured).

Discussion

Pheromone lures remained attractive for more than 30 days in both laboratory and field assays, attracting females of the Neotropical brown stink bug, *Euschistus heros*, indicating that the 1 mg synthetic pheromone lure was suitable for field application.

The field experiments demonstrated that the pheromone-baited traps were more efficient than the shake cloth sampling technique even at population densities lower than the economic threshold level (4 individuals/m^2) (Embrapa, 2008), mainly during the colonization of the culture. These results are in concordance with those obtained for *Euschistus conspersus* by Cullen and Zalom (2000, 2005) in tomatoes fields in California, USA. Furthermore, the field

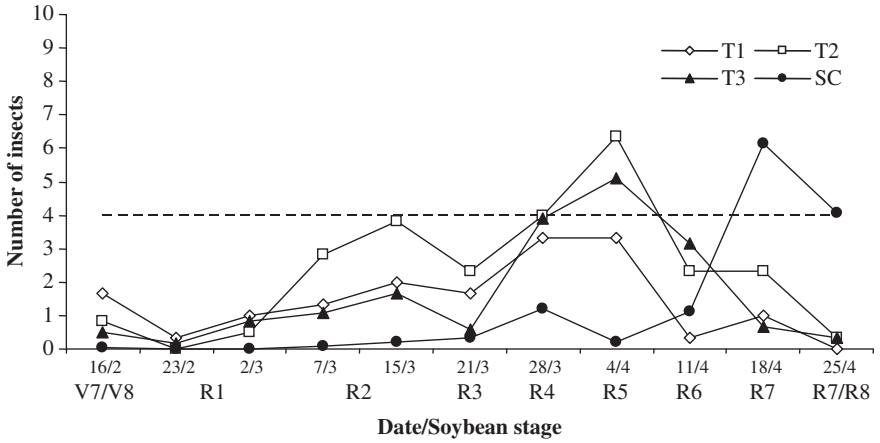

Fig. 4 Trap catches in soybean in PAD-DF (DF). T1 = 1 trap/ha baited with 1 mg of methyl 2,6,10-trimethytridecanoate, T2 = 2 trap/ha baited with 1 mg of methyl 2,6,10-trimethytridecanoate, T3 = 4 trap/ha baited with 1 mg of methyl 2,6,10-trimethyltridecanoate. Points indicate mean number of stink bugs/trap/week (N = 3 experimental plot/treatment, n = 3 for T1, n = 6 for T2, n = 12 for T3) or mean density (number of stink bugs/m^2, n = 90) for shake cloth sampling. Means were obtained from insects captured in traps from three replicates of the experimental design. Treatment 1: 1 trap/ha – Treatment 2: 2 traps/ha – Treatment 3 :4 traps/ha; SC, shake cloth sampling. The dotted line indicates the control threshold (in number of insects/m^2) estimated from production recommendations for Brazil by Embrapa (Embrapa, 2008) (two stink bugs bigger than 0.5 cm/linear m) and a typical interline space of 0.5 m.

Table 1 Relationship between mean numbers of insects/week sampled in pheromone-baited traps and shake cloth samples, for stink bugs and *Euschistus heros*, during the total experimental period and the critical phase of the soybean crop (R1–R5 stages) in experiments carried out in Rio Verde, Goiás. Data were analysed with GLM regression procedures using a Poisson distribution as error family wise and the values of P are related to the test for the significance of the parameter *b* in the regression line

Shake cloth technique	Stink bugs Total (including nymphs)	Stink bugs Adults	*Euschistus heros* Total	*Euschistus heros* Adults
Pheromone-baited traps				
Total experimental period (V7 to physiological maturation)				
Total adults (all species)	t = 1.15	t = 0.94	–	–
	P = 0.276	P = 0.347		
Euschistus heros (adults)	t = 1.45	t = 1.22	t = 1.450	t = 1.209
	P = 0.178	P = 0.221	P = 0.178	P = 0.227
R1–R5 soybean stages				
Total adults (all species)	t = 3.74	t = 5.11	–	–
	P = 0.033	P = 0.014		
Euschistus heros (adults)	t = 4.46	t = 4.32	t = 1.92	t = 3.17
	P = 0.021	P = 0.023	P = 0.150	P = 0.034

ns, not significant.

Relationship between captures in traps as a function of captures in shake cloth samples.

tests showed that in areas of approximately 15–20 ha, trap catches were similar in traps deployed around the field borders or in the centre of blocks. This result is relevant for growers, suggesting that surveys of insect densities with traps may be carried out only on the edges of the crop, minimizing the effort required to monitor large areas. This finding may be especially appealing when the costs and benefits of other monitoring techniques are taken into account. In fields with larger areas and higher area/perimeter ratios, a different disposition of the traps may be necessary to ensure that the trap data are representative of the populations in these large blocks. This different configuration will need to be established in specific field tests.

There was some concern that using pheromone-baited traps may not be suitable for predicting the real size of the damaging population in a field, due

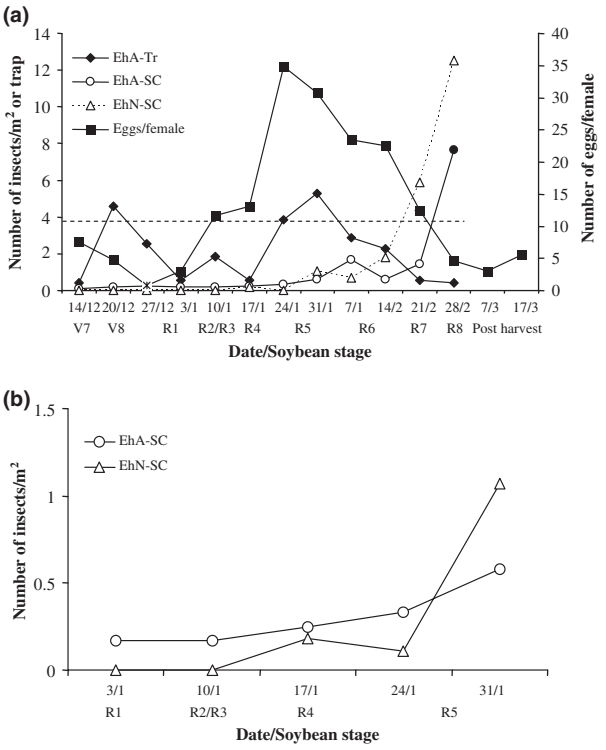

Fig. 5 (a) Relationship between the pheromone-baited traps and the shake cloth sampling showing the fluctuation of adult *Euschistus heros* populations in field experiments. EhA-T, mean *Euschistus heros* adults in traps; EhA-SC, mean *Euschistus heros* in shake cloth samples. Eggs female = mean eggs/female reproductive system. Soybean stage: V, vegetative; R, reproductive. Arrow indicates the harvest time of soybean in the experimental area. (b) Adult and nymphs fluctuation by the sampling cloth technique during the critical stage of the soybean crop.

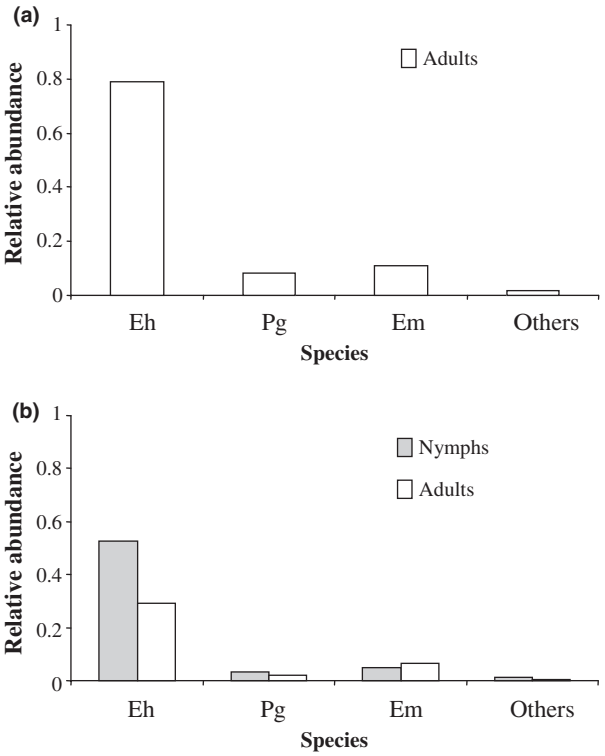

Fig. 6 Relative abundance of species of stink bugs in the fields where the experiments were conduced, calculated from the total insects sampled. (a) Stink bugs captured by pheromone-baited traps and (b) stink bugs sampled in shake cloth technique. Eh, *Euschistus heros*; Pg, *Piezodorus guildinii*; Em, *Edessa meditabunda*. Relative abundance was calculated from the total of stink bugs sampled in the shake cloth surveys during the experiment.

to the fact that the traps sample mainly the sexually mature females' population. However, the results of field experiment 3 showed that this should not be a concern; during the most critical period of the soybean crop (from R1 to R5) there was a significant positive relationship when comparing adults of *E. heros* (or the total adult stink bug population including other species) captured in traps with the total of all stink bugs, including nymphs, sampled with the shake cloth technique. On the other hand, considering the entire soybean season, from V7 to physiological maturation (R7/R8), the results did not show a significant positive relationship between the insects captured in pheromone traps and the shake cloth samples in accordance to the results reported by Cullen and Zalom (2005) for *E. conspersus* in tomatoes fields. However, it is important to note that, the majority of the insects sampled in the shake cloth technique during the physiological maturation of the soybean were nymphs turning into migrating adults (fig. 5a) (Panizzi and Corrêa-Ferreira 1997). Thus, those insects would not be considered as damaging insects to the soybean season. Additionally, nymphs turning into adults are not sexually mature until the 11th to 12th days (Costa et al. 1998) and do not respond to the pheromone. This phenomenon should also explain the reason of the collection of insects in the shake cloth technique but not into the traps.

To get a better understanding of these phenomena, some bioecologial characteristics of *E. heros* need to be taken into account. In Brazil, this insect feeds on leguminous plants, mainly soybean, during the rainy season (corresponding to spring/summer in the temperate regions) and uses alternative hosts, such as *Euphorbia hetherophylla* (L.) (Euphorbiacea) and *Acanthospermum hispidum* DC. (Asteracea) at the end of the rainy season and start of the dry season (corresponding to the end of summer and start of fall in temperate regions) (Panizzi 1997). During the dry

season (winter to beginning of spring for temperate regions), the insect may develop reproductive diapauses under culture debris and leaf litter (Panizzi 1997). However, in central Brazil the diapauses phenomenon is not observed since the insects can develop in irrigated cultures as dry beans, sorghum and maize or in alternative host plants in wet habitats such as riparian or stream margins (M. Borges, M.C.B. Moraes and R. Laumann, field observations).

Euschistus heros has been reported to be multivoltine with number of generations ranging from two (in the southernmost part of Brazil – cold regions) to six (in the central Region of Brazil between the Tropic of Capricorn and the Equator) (Cividanes and Parra, 1994). In the laboratory, under constant temperatures and diverse food supply this species showed a mean development time of 38 days from eggs to adults, with adult longevity in mated stink bugs of approximately 50 days (Costa et al. 1998). Thus, in central Brazil we can expect a dynamic of *E. heros* in soybean as follows: colonizing insects from dry season refuges/host plants, first generation from migrating (inside) insects and a second generation before the physiological maturation of the crop by migrant (outside) adults.

Probably, the relationship observed between trap catches and population density in the period R1–R5 of the soybean is influenced by the development of the first generation from the migrating insects, in this period we may expect an *E. heros* population with higher number of adults than nymphs, because the nymphs of the first generation became adults and the second nymphs population/generation is at the beginning. Further in the crop cycle the nymph population became higher than the adult population and this could be responsible for the absence of a relationship between trap catches and population densities.

Furthermore, the traps seemed to be effective during the colonization of the crop (V7–R1 soybean stages) and this could be influenced by the effect of attractant pheromone on the migration of females, as pointed out by Aldrich (1988) as an important component of the field colonization process. The absence of relationship with the population density estimated by the sampling cloth technique may be explained by the weakness of this technique at very low population density during the colonization process.

The efficiency of the traps in the early colonization process of the culture could be an important factor to develop a monitoring programme for stink bugs and to understand the stink bug population evolution, with a degree-day phenology model based on development time as was observed by Cullen and Zalom (2000, 2005) for *E. conspersus* in tomatoes.

This model may help to predict the necessity for the application of chemical pesticides or use of other control techniques to maintain the population under economic injury levels. Additionally, the application of a premature control treatment when the first insects are trapped in the borders of the fields may help to reduce the use of insecticides by the application in a reduced area and reduce the further application by reducing the initial population in the crop.

On the other hand, in the final stages of soybean physiological development (R7–R8), the traps became less effective at the end of the soybean season. That is, at the final physiological maturation stage, pheromone-baited traps may not be satisfactory for monitoring stink bug populations. However, this may have no consequences for growers because during this period insecticides generally are not applied to the crop because at this stage, the soybeans are less susceptible to stink bug attack (Panizzi et al. 2000; Corrêa-Ferreira and Azevedo 2002). Additionally, during this period the bugs migrate to alternative hosts or refuge areas outside the crop (Pinto and Panizzi 1994; Panizzi and Corrêa-Ferreira 1997). This might explain why the stink bugs no longer respond to the pheromone (Link 1979). We checked this by monitoring the physiological state of females when they were migrating in and out of the experimental area. Thus, we compared the number of eggs in the reproductive tract of females that were migrating into the crop at the beginning of the experiments, with those of females sampled during the soybean stages, and with those of females present during the final reproductive and physiological maturation of soybean. Few eggs were present in the reproductive tracts of females migrating into the crop, and similarly, few eggs were found in those females that were migrating out of the crop late in the season.

These results corroborate the observations of Panizzi and Corrêa-Ferreira (1997), suggesting that the bugs' life cycles are synchronized with the phenological stage of the soybean and also agree with findings for *E. conspersus* (Cullen and Zalom 2006). Another parallel with the last work is that some morphometric observations of *E. heros* females and males wings (data not shown) showed no evidence for the oogenesis-flight syndrome hypotheses (Johnsons 1969) similar to that observed in *E. conserus* (Cullen and Zalom 2006). The similar responses observed in *E. conspersus* and *E. heros* should indicate that stink bugs respond to pheromones traps in the same way

and may help to develop monitoring techniques for other stink bugs species.

From examination of the population fluctuation curves (fig. 5a), the different stages of development of the *E. heros* population appeared to be synchronized with those of the crop, including the number of eggs in the reproductive tract of the females of the species from the first date of collection in the field. Thus, after the arrival of the first immigrant insects, colonization of the soybean crop begins. Thus, damaging populations build up and peak during the most critical stages of the soybean crop, between late R3 and late R5, originating from the first migrant adults population that first infested the crop. With this hypothesis in mind, new field tests are being designed for the next soybean season in Brazil. Specifically, we will test whether application of control measures as soon as the first migrating bugs are detected will result in decreases in peak populations during the critical soybean stage.

The apparent response of other stink bug species such as *Edessa meditabunda, Piezodorus guildinii*, *Thyanta perditor* and *Chinavia* spp. to the synthetic 2,6,10 TMTD and the proportion of the insects of each species trapped was in concordance with the composition of the stink bug guild as determined from the shake cloth technique, an observation similar to that reported by Pires et al. (2006) in previous experiments in soybean fields. Thus, it appears that pheromone traps may be used to estimate both the population density and the relative composition of the stink bug guild, at least in guilds dominated by *E. heros*.

The capture of different species of stink bugs suggests that these species may use the sex pheromone of *E. heros*, which is the most abundant stink bug in soybean crops in the Central region of Brazil, to find food and oviposition sites. This phenomenon was first reported by Borges et al. (1998a) in field experiments using the sex pheromone of *E. heros*, and a similar guild of stink bug species was attracted. A similar phenomenon has been proposed by several authors (Tada et al. 2001a,b; Endo et al. 2006; Funayama 2008; Aldrich et al. 2009). It is known that these stink bug species produce a series of other compounds, such as aggregation pheromones (Harris and Todd 1980; Aldrich et al. 1991; Zahn et al. 2008) and defensive compounds (Pareja et al. 2007; Moraes et al. 2008a), that may be used by conspecific or heterospecific stink bugs to form clusters of immatures or adults (Borges and Aldrich 1992; Fucarino et al. 2004; Zahn et al. 2008). These compounds are also used by natural enemies as cues to find their hosts (Mattiaci et al. 1993; Borges and Aldrich 1994a; Borges et al. 1999; Laumann et al. 2009). The possibility of using the defensive compounds either alone or together with the sex pheromone to improve the efficiency of trap captures to monitor different species of stink bugs may be considered in future studies. However, it first will be necessary to study the function and concentration of each defensive compound individually and in blends, taking into account the variety of soybean being cultivated, because the economic importance of these pest species varies greatly between and within a species, depending on the variety attacked (Panizzi et al. 2000).

In conclusion, we have shown that the synthetic *Euschistus heros* sex pheromone can be used to monitor the seasonal fluctuations in stink bug populations infesting soybean, and that the responsiveness to the pheromone is correlated with the physiological state of the insects. Our results give a more precise indication of the critical changes in population dynamics and behaviour of these bugs in the field, that can be used for timing control measures for the key pest, *Euschistus heros,* with more precision in soybean in the Central Region of Brazil. Because environmental conditions vary in different regions of Brazil, ranging from wet tropical in the north to temperate in the south, a more complete evaluation of this technology in other soybean producing regions is needed to validate or adapt the sex pheromone-baited trap technology to different environments and stink bug guilds (Moraes et al. 2008b). Additionally, stink bugs in the last years adapted to new crops such as cotton (Willrich et al. 2004a,b,c, 2005), maize (Townsend and Sedlacek 1986; Ávila and Panizzi 1995) and sunflower (Panizzi and Machado-Neto 1992; Malaguido and Panizzi 1999), so that monitoring technology for stink bugs based on pheromone-baited traps could lead to effective biocontrol strategies that contribute to solving both current and future stink bugs pest problems.

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