

# Chemical Ecology Studies in Soybean Crop in Brazil and Their Application to Pest Management

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## 1. Introduction

Considering the current state of soybean production and markets around the world, it is readily apparent that it is possible to divide the countries in the world in two halves: producer and consumers'. Consumers' countries are mainly those belonging to the European Union that have their need for proteins used for animal feeding supplied in their majority by soybean seed or meal imports (Dros, 2004). The majority of soybean production is shared (80%) between four countries: the United States, Brazil, Argentina and China (Dros, 2004). Therefore, if we consider only those countries that may supply their internal needs and exporting either seeds, meals or oils, only the USA, Brazil and Argentina remain as exporting countries (Daydé et al., 2009). In this context, Brazil is currently the world second largest soybean producer (18%) and exporter (19%), with a cultivated area for soybean around 23 million ha and production around 3 ton/ha, reaching yearly a total production of approximately 68 million ton (CONAB, 2010).

Worldwide, scientists in different countries are trying to increase both the productivity and profitability of the agricultural sector of their economies, to feed growing populations and to increase the quality of life for millions of people. In recent years there has been a growing concern about environmental changes, and about how we are using the resources available in natural habitats. These concerns have triggered a search for natural products as a source of medicines, cosmetics, fuel, nutrients for humans and animals, and, especially, as alternatives to synthetic pesticides. These have led to an awareness of the importance of studying natural products, and of conserving the natural habitats that remain largely unexplored in the tropics and subtropics. Within this context, and bearing in mind that research with semiochemicals has been producing more efficient tools for insect pest management, this review opens opportunities for discussions of new technologies to reduce the use of toxicant chemical insecticides against insect pest species. The development of new monitoring methods based on semiochemical interactions, sexual pheromone baits, species-specific attractants (including natural enemies), molecular biology studies and vibrational communication offers great prospects for its application in Pest Management and Area Wide Pest Management Programs of insect populations.

Despite Brazil being one of the largest producers and exporters of soybean in the world (CONAB 2010), millions of tonnes of grains and products are lost every year due to damage by pests, mainly stink bugs (Hemiptera: Pentatomidae) (Panizzi, 1997, 2007).

Phytophagous stink bugs feed on a wide variety of plants, including numerous important crop plants such as grains, forage and fiber crops, vegetables, and perennial crops such as tree fruits and nuts (Panizzi, 2000). Damage is caused by both immatures and adults, but only adults are winged and capable of long-distance movement. Injury to young seeds, fruits, or nuts produces necrotic lesions and often results in premature abortion, while attacked leaves may wilt and die. Stink bugs are also known or implicated as vectors of plant pathogens such as yeast, fungi, and bacteria (Pannizzi 1997).

Stink bugs are reported attacking soybean crops during reproductive stages (Panizzi, 1997), and throughout this period they suck pods and grains causing direct damage and indirect injury. In Brazil, several species of stink bugs have been associated with soybean, and the more frequently observed are: *Euschistus heros* (Fabricius), *Nezara viridula* (Linnaeus), *Piezodorus guildinii* (Westwood), *Chinavia impicticornis* (Stål), *Chinavia ubica* (Rolston), *Dichelops melacanthus* (Dallas), *Dichelops furcatus* (Fabricius), *Thyanta perditor* (Fabricius) and *Edessa meditabunda* (Fabricius) (Panizzi and Slansky, 1985; Panizzi and Corrêa Ferreira, 1997; Medeiros et al., 1997; Laumann et al., 2008b; Vieira et al., 2008) (Figure 1). In this species complex the brown stink bug, *Euschistus heros* is the key pest, principally in central Brazil, due to its high abundance and heavy damage to the crop.

The stink bugs produce a wide variety of chemical compounds that show potential to be used as a toll for managing these insects. Among these compounds are the pheromones, which can be classified as either sexual, alarm or aggregation pheromones (Aldrich, 1988). Stink bug management by means of the use of semiochemicals is an alternative that has shown potential for application in soybean and other crops (Aldrich, 1988; Borges et al., 1998a; b; 2010; Cullen & Zallom, 2000; 2005; 2006). Effective bug management hinges on the rapid detection of these invasions so that appropriate control measures can be implemented before serious crop damage occurs.

Associated with this more ecologically based approach, egg parasitoids and soybean resistant varieties also can be manipulated to manage stink bugs. Egg parasitoids (Hymenoptera: Scelionidae) are the principal natural enemies of stink bugs. These egg parasitoids have biological attributes and behavioral responses to host density that could lead to density-dependent parasitism and efficient local regulation of stink bug populations (Laumann et al., 2008; 2010). Some species of this parasitoid family are being considered and used in many countries for biological control of stink bugs, by mass or inoculative liberations, especially the cosmopolitan *Trissolcus basalis* (Wollaston) (Caltagirone, 1981; Clarke, 1990) and the neotropical *Telenomus podisi* Ashmead (Côrrea-Ferreira, 2002) (Figure 1).

The egg parasitoids might be used through mass release and their behavior might be managed using semiochemicals from its host or plants. The use of natural enemies as biological control tool of pests has been confirmed as very positive to the economy and environment. Studies have reported that around \$ 20.92 billions of dollars are lost to native pests in the USA, and when the natural control is acting these losses decrease to \$ 7.32 billion (Losey & Vaughan, 2006). Within natural control are included parasitoids, predators, pathogens, climatic conditions and host-plant resistance. Soybean resistant varieties have been recommended for pest control because the pest populations can be reduced to levels below the economic threshold reducing the disturbance and pesticide inputs to the agroecosystem without extra costs for the growers. Additionally, the resistant cultivars

could be compatible with chemical, microbial, and cultural control methods, including synergistic effects with natural enemies. Therefore, it is important that host-plant resistance combine positively with the action of natural enemies.

In this review we will give an overview of the main semiochemicals related to the Brazilian soybean stink bug complex, illustrating the diversity of the compounds and the possibilities of using these compounds in pest control. Other topics related to chemical ecology and behavioral ecology of stink bugs, their host plants and their natural enemies will be considered.

## 2. Pheromones and other stink bugs semiochemicals

Semiochemicals mediate interactions of insects with other organisms and they can be used in different ways for population monitoring, mass trapping, sexual confusion and in indirect applications, such as behavioral manipulation of natural enemies with parasitic wasps, that use both sex pheromones and defensive compounds during their foraging behavior (Vinson, 1985; Borges et al., 1998a; Bruni et al., 2000; Fiaboe et al., 2003). Defensive compounds also are used as defence against predators, since many are repellent, irritant or toxic (Eisner, 2003).

### 2.1 Defensive compounds

The stink bugs from the soybean complex, *Chinavia impicticornis*, *Chinavia ubica*, *Dichelops melacanthus*, *Euschistus heros* and *Piezodorus guildinii* have had their blends of defensive compounds evaluated both qualitatively and quantitatively. All these studies showed that the blends of defensive compounds are species-specific. Thus, different species may have exactly the same compounds in the blend, but these compounds are present in different ratios.

This specificity can potentially play an important role in intra-specific communication and may help to understand the phylogeny of some pentatomid groups, through chemotaxonomy, and in understanding the evolution of insect defence. For example the sister species, *C. impicticornis* and *C. ubica* are more similar to each other than the species *E. heros*, *P. guildinii* and *D. melacanthus* (Fig. 2A) (Pareja et al., 2007).

The main compounds identified from the glands of Brazilian stink bugs are: 2-alkenals (aldehydes with an insaturation on the second carbon), mainly the *trans* isomer with very small quantities of the *cis* isomer; saturated aliphatic hydrocarbons; and 4 oxo-(*trans*)-2-alkenals (C<sub>6</sub>, C<sub>8</sub> and C<sub>10</sub>). Esters, alcohols and unsaturated hydrocarbons are present as well, but in lower quantities and in some species, such as for the males of *P. guildinii*, many sesquiterpenes were identified (Borges et al., 2007). The 4-oxo-alkenals are present in higher quantities in nymphs of the pentatomids when compared with MTG of adult insects (Aldrich, 1988). These compounds were responsible for separating nymphal blends from adult blends of Neotropical pentatomids (Fig. 2B) (Pareja et al., 2007).

The biological role of most of these defensive compounds still needs to be studied. Three of the compounds that were identified in higher concentrations in the Brazilian species, namely (*E*)-2-octenal, (*E*)-2-decenal and (*E*)-2-hexenal, were reported initially as defensive compounds (Gilby & Waterhouse, 1967) and later as alarm pheromones (Pavis et al., 1994, Lockwood, 1987). The esters, although in lower concentrations when compared to the aldehydes have also been shown to have biological importance, mainly as an alarm pheromone in *N. viridula* (Lockwood & Story, 1987).

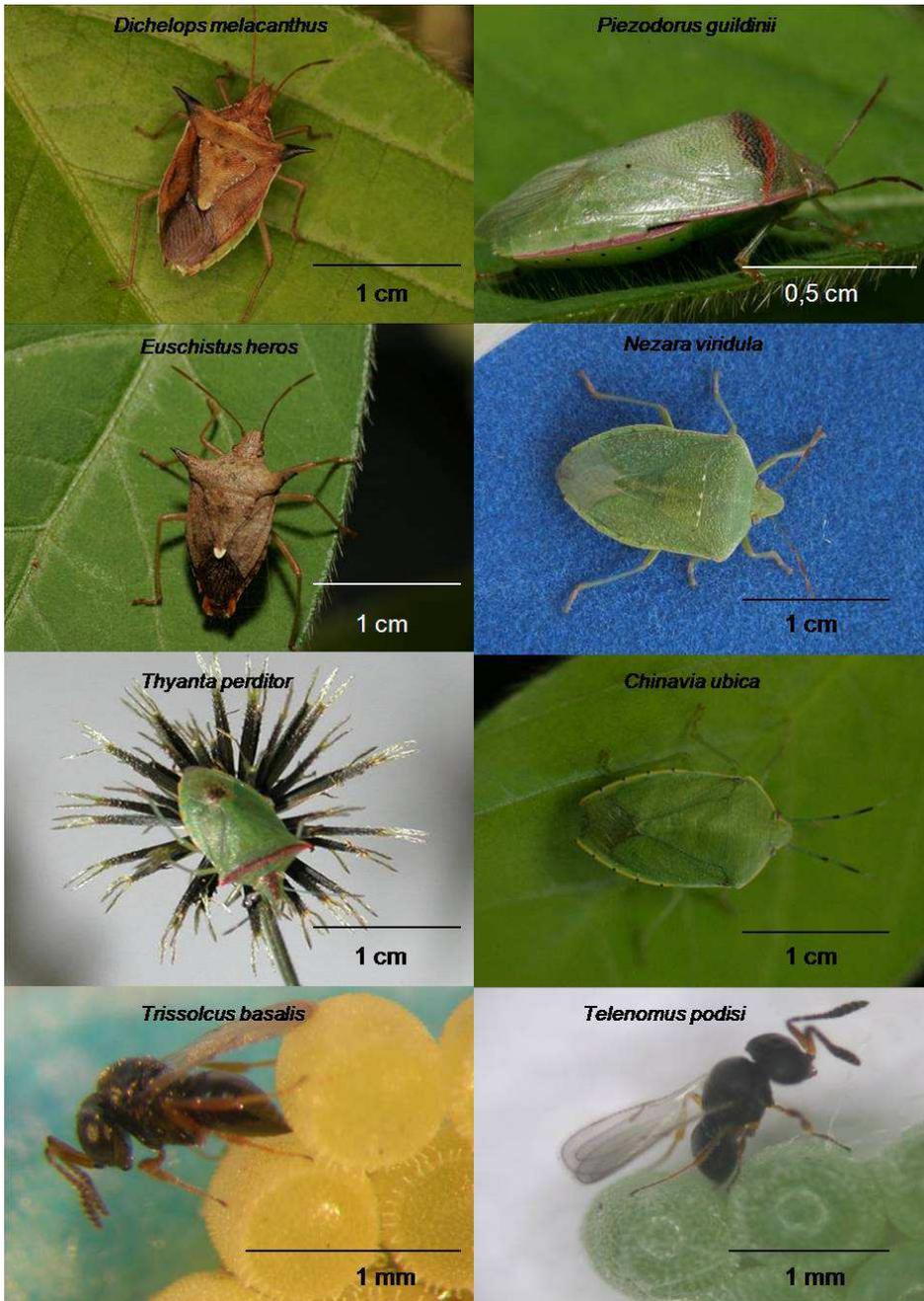


Fig. 1. The main stink bugs and their natural enemies commonly find on soybean crops in Brazil. Stink-bugs pictures by Cecília Vieira, parasitoids photographs by Raúl Laumann.

These aldehydes have also been shown to have important anti-microbial action, in particular against the entomopathogenic fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin (Borges et al., 1993). These blends could therefore be important in determining susceptibility of these insects to pathogens, a consideration of likely importance in biological control efforts.

The aliphatic hydrocarbons are present in large quantities, and in most species tridecane is one of the principal compounds found in gland or aeration extracts (Moraes et al. 2005a, Aldrich et al., 1989, 1994, Borges et al., 2007, Pareja et al., 2007). In *Edessa rufomarginata* (De Geer) (Howard & Wiemer, 1983) and *Edessa mediatubunda* (Moraes et al., unpublished data) undecane is the most abundant aliphatic hydrocarbon. Lockwood & Story (1985) found that tridecane has an action as both an aggregation and as an alarm pheromone for *N. viridula*, in a dose-dependent manner, though recently, Fucarino et al. (2004) did not find any biological activity for tridecane in *N. viridula*. Lockwood & Story (1985) only found effects at very high (alarm) and very low (aggregation) concentrations. It is difficult to determine whether these concentrations are biologically relevant, or those used by Fucarino et al. (2004) are more realistic, since very high amounts (up to 1 mg) of tridecane can be present in the MTG at any given time. However the aggregation effect at very low concentrations showed that these insects can detect the compound in small amounts and this is likely to be a true aggregation effect. Aldrich (1988) proposed that one of the functions of the aliphatic hydrocarbons is to serve as solvents that modulate the evaporation of the other compounds, and this can explain the high quantities of these compounds found in extracts of the stink bugs.

The 4-oxo-(*E*)-2-alkenals are compounds that have only been reported in the Hemiptera. Fucarino et al. (2004) showed that 4-oxo-(*E*)-2-decenal elicits aggregation behavior in first instar nymphs of *N. viridula*, as had been proposed by Borges & Aldrich (1992). However there is no further information on the biological activity of these compounds, since they are unique to these species, and therefore difficult to obtain. Compounds with very similar mass spectra to, and eluting just after 4-oxo-(*E*)-2-hexenal have been detected in Pentatomidae (Pareja et al., 2007) and other Hemiptera (Drijfhout et al., 2002). However, it is not yet clear whether these are produced by the insects or are breakdown or rearrangements of 4-oxo-(*E*)-2-hexenal.

## 2.2 Sex pheromones

The Hemiptera show a fantastic variety in the chemical structure of the few sex pheromones identified so far. In contrast to the Lepidoptera, the Hemiptera do not show a structural pattern of the compounds associated to families or subfamilies (Moraes et al., 2008a). However, for some species of Pentatomidae it is possible to observe a structural pattern inside the same genus.

So far all the studies with stink bugs have detected the male as the producer of the sex pheromone (McBrien & Millar, 1999, Moraes et al., 2005a, Borges et al. 2006, Borges et al., 2007). In addition males are responsible for emitting sounds that attract females during courtship and mating (Čokl et al., 2000, Moraes et al., 2005b).

### Species of *Nezara* group

*Nezara* group include more than 100 species with 8 genera and they are cosmopolitan with higher diversity in Afro- and Neotropical regions. Schwertner & Grazia (2006) conducted a cladistic study of this group considering *Chinavia* a valid genus that include Nearctic and Neotropical species formerly classified as *Acrosternum*. Following this new classification all *Acrosternum* from the regions cited above will be referred as *Chinavia* in this text.

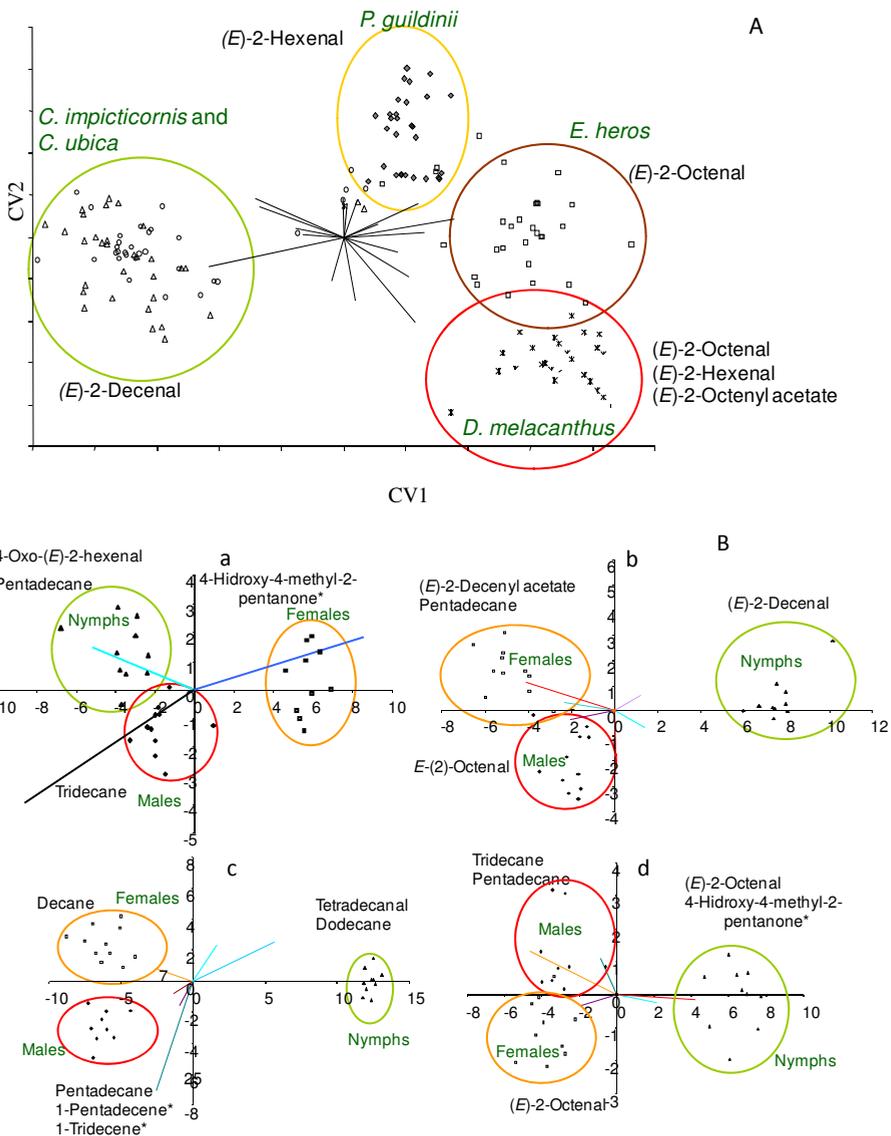


Fig. 2. Canonical variates analysis (CVA) biplot for (A) the analysis differentiating between species, and (B) the stage analysis independent of different species a) *Chinavia impicticornis*, b) *Chinavia ubica*, c) *Euschistus heros* and d) *Dichelops melacanthus*. The points are the individual scores for each replicate, calculated from the CVA equation that maximises differences between treatments along the two dimensions defined by the two canonical variates (CV1 and CV2). The lines are the loadings for each of the variates (compounds), only the main compounds that separate the species and different stages insects are shown. Asterisks indicate tentative identification.

Of the species within the genus *Nezara* and *Chinavia* studied from different geographic areas in the world the major components identified were: *trans*-(Z)-bisabolene epoxide (*trans*-Z-EBA) ((Z)-(1'S,3'R,4'S)(-)-2-(3',4'-epoxy-4'-methylcyclohexyl)-6-methylhepta-2,5-diene) and the corresponding *cis*-isomer (*cis*-Z-EBA) (Baker et al., 1987; Aldrich et al., 1987; 1993; McBrien et al., 2001) (Table 1). These studies showed that the specificity is guaranteed by the different ratios of the two components produced by the different species (Aldrich et al., 1993; McBrien et al., 2001; MCB Moraes, RA Laumann & M Borges, unpublished data). Miklas et al. (2000) using solid phase micro-extraction (SPME) methodology with *N. viridula* from different parts of the world reported that there is a great variability in the quantities produced of the sex pheromones between males of the same species, but the ratio of *cis* to *trans*-Z-EBA is constant.

Only two species of genus *Nezara*, *N. antennata* and *N. viridula*, have had their sex pheromone identified, but nine *N. viridula* populations were studied from different geographical areas. All of these populations showed the *trans* isomer in higher quantities than the *cis* isomer. In contrast to the *Nezara* species, the *Chinavia* species presented higher quantities of the isomer *cis*-Z-EBA. The exceptions are *C. pennsylvanica* (Say), which has a ratio close to one between the isomers, and *C. impicticornis*, which only produces the *trans* isomer (Moraes et al., 2008a). It is worthwhile to notice that *C. impicticornis* has the ratio between the components more similar to the blend identified on *Nezara* spp. than the others *Chinavia* species.

The function of the ratio of *trans* to *cis*-Z-EBA is still unclear. Aldrich et al. (1989) reported that in laboratory experiments males of *N. viridula* from Japan mated with females of *N. viridula* from the USA (Mississippi), and the offspring were fertile and produced a ratio of *trans* to *cis*-Z-EBA of 1.53, which is intermediate between the two parental blends. Electroantennography experiments with female antenna of *Chinavia ubica* and *C. impicticornis* showed that the females responded to both isomers *cis* and *trans* (Z)-EBA, but in bioassays they did not respond for heterospecific males, what indicate that they have receptors for different isomers, and the ratio between the compounds is essential to locate the partners of the same species (M.C.B. Moraes; R.A. Laumann & M. Borges data not published).

Bioassays in the laboratory carried out with *N. viridula* from Brasilia, Brazil, showed that females were attracted only to synthetic EBA when both isomers are present in the correct ratio (Borges, 1995). The absolute configuration was identified as being (1'S,3'R,4'S) (1) (Baker et al., 1987), and later Borges (1995) reported that females did not show the same level of response (attraction) to the racemic mixture than to the (1'S,3'R,4'S) enantiomer. A similar result was obtained by McBrien et al. (2001) working with *Chinavia hilaris* (Say). Females of *C. hilaris* responded only to the pheromonal mixture containing both *cis* and *trans*-Z-EBA in the same ratio released by the males, additionally the absolute configuration identified for this species was the same as found by Baker et al. (1987).

### ***Euschistus heros***

The pheromone components of *E. heros* were identified by (Borges et al., 1994, and Aldrich et al., 1994) and consists of three components: methyl 2,6,10-trimethyldecanoate, methyl 2,6,10 trimethyldodecanoate, and methyl 2E,4Z decadienoate (Table 1). Zhang et al. (2003) confirmed the ratio among the three components by previously proposed (Borges & Aldrich, 1994; Aldrich et al., 1994; Borges et al., 1998a) as being: 53 % methyl 2E,4Z-decadienoate, 3% methyl -2,6,10- trimethyldodecanoate and 44 % methyl- 2,6, 10-trimethyltridecanoate. Recently, Moraes et al. (2008b) carried out a study with *E. heros* that

revealed the importance of understanding the physiology and the behaviour of stink bugs in order to identify the correct sex pheromone blend. When the insects were aerated with food (*Phaseolus vulgaris* (L.) pods) males of *E. heros* released all three components in the ratio reported by Zhang et al. (2003) during seven consecutive days. On the other hand, when the insects were kept in aeration without food, after 48 h they stopped releasing the main component of the sex pheromone (methyl-2*S*,6*R*,10*S*-trimethyltridecanoate), and they released in higher quantities the first component of the blend, methyl-(2*E*,4*Z*)-decadienoate. The biological activity of the three components of *E. heros* species was confirmed in a laboratory bioassay and showed that methyl 2,6,10-trimethyltridecanoate was the main component in attracting females (Borges et al., 1998a). Costa et al. (2000) carried out a set of experiments that showed that the racemic mixture of methyl 2,6,10 trimethyltridecanoate was efficiently attractive to females in laboratory bioassays. Methyl-2,6,10-trimethyltridecanoate has 8 possible stereoisomers. The absolute configuration of methyl 2,6,10 trimethyltridecanoate was determined by means of bioassays using the 8 stereoisomers separately, which were synthesized by Mori & Murata (1994). Costa et al. (2000) reported that females respond better to isomer 2*S*,6*R*,10*S* than the other isomers when compared with a solvent. Borges et al. (1998b) using the racemic mixture of methyl 2,6,10 trimethyltridecanoate captured *E. heros* in field traps. The experiments were carried out placing rubber septum with 10 µg of racemic mixture of methyl 2,6,10 trimethyltridecanoate placed in a soybean crop (Borges et al., 1998b). Traps containing the racemic mixture caught a higher number of *E. heros*, along with another pentatomid, *P. guildinii*, when compared with a trap containing only the septum with solvent (Borges et al., 1998b). Additionally, the racemic mixture in a lure formulation showed great power to attract *E. heros* in field conditions and a potential to be used for population monitoring of this species (Borges et al., 2010).

### *Thyanta perditor*

Males of *T. perditor* produce the ester, methyl-2*E*,4*Z*,6*Z*-decatrienoate ((*E*2,*Z*4,*Z*6)-10:COOMe) as the main sex pheromone component (Moraes et al. 2005a) (Table 1). The same component was also identified in the pheromone blend of the two nearctic species *Thyanta pallidovirens* and *Thyanta custator acerra* (Millar et al., 1997; McBrien et al., 2002). As in *Nezara* and *Chinavia* species, *Thyanta* spp have a very similar blend, and one of the compounds is the same, which could suggest that closely related species of stink bugs share the same or similar blends of compounds as sex pheromone and specificity is due to different ratios of these compounds. Bioassays in the laboratory showed that 4 µg of the (2*E*,4*Z*,6*Z*) methyl decatrienoate was attractive to females of *T. perditor* (Moraes et al., 2005a). In addition to bioassays in the laboratory, the sex pheromone of *Thyanta perditor* was the subject of field trials. Traps baited with 1 mg (*E*2,*Z*4,*Z*6)-10:COOMe, were more efficient in capturing *T. perditor* than control traps. Additionally, traps baited with the sex pheromone captured a significantly higher number of insects than the sampling cloth technique, as well as, some tachinid parasitoids of stink bugs (R.A., Laumann; M.C.B., Moraes; A., Krimian & M., Borges not published). Results from field tests showed that the (*E*2,*Z*4,*Z*6)-10:COOMe has potential for monitoring populations of *T. perditor*, and this technique is more easily performed, than the traditional cloth sampling.

### *Piezodorus guildinii*

The Neotropical redbanded stink bug, *P. guildinii* is one species of the complex of stink bugs that are serious pests of soybean in Central and South America, especially in Brazil (Panizzi & Rossi, 1991). Borges et al. (1999a) reported the identification of two components of *E. heros*

sex pheromone in the sex pheromone blend of *P. guildini*, methyl-2*S*,6*R*,10*S*-trimethyltridecanoate and methyl-2,4,6-trimethyldodecanoate. After this, Borges et al. (2007) identified a new compound specific to males when gas chromatograms of extracts of volatiles collected from sexually mature virgin males and females were compared. This compound was not found in aerations of sexually immature females or males, and its retention time and mass spectra matched the sesquiterpene  $\beta$ -sesquiphellandrene (Borges et al., 2007) (Table 1). The quantitative analysis of 10 extracts obtained from volatile collection of 20 males resulted in a mean liberation of sexual pheromone of approximately 40 ng/24 h. For *Piezodorus guildinii* the bioassays showed that females of the species responded preferentially to (7*R*) -  $\beta$ -sesquiphellandrene and the females also showed response to the isomer (7*S*) -  $\beta$ -sesquiphellandrene (Borges et al., 2007). Furthermore, the role and biological activity of *P. guildinii* compounds as a mediator of reproductive behavior under natural conditions still remains to be determined.

It is likely that the pheromone will act in concert with the substrate-borne vibrational signals that this insect uses for communication over shorter ranges (Moraes et al., 2005b). Thus, developing pheromone-based monitoring systems for this insect may be, in the near future, considered an integration of both the pheromonal (chemical) and vibrational (physical) signals to be incorporated into trap design.

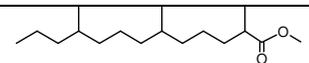
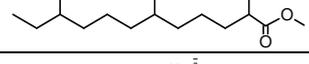
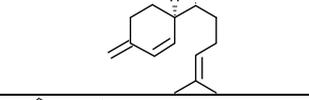
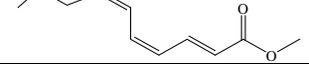
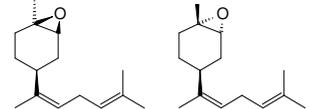
Compounds	Structure	Species
Methyl 2,6,10-trimethyltridecanoate		<i>Euschistus heros</i>
Methyl <i>E</i> 2, <i>Z</i> 4- decadienoate		<i>Euschistus heros</i>
Methyl 2,6,10-trimethyldodecanoate		<i>Euschistus heros</i>
(7 <i>R</i> )-(+)- $\beta$ -Sesquiphellandrene		<i>Piezodorus guildinii</i>
Methyl <i>E</i> 2, <i>Z</i> 4, <i>Z</i> 6-decatrienoate		<i>Thyanta perditor</i>
<i>cis</i> - <i>Z</i> -Epoxybisabolene <i>trans</i> - <i>Z</i> -Epoxy-bisabolene		<i>Chinavia</i> sp. <i>Nezara</i> sp.

Table 1. Sex pheromone structures from the complex of the Brazilian stink bugs (Hemiptera: Pentatomidae) soybean pests.

### 2.3 Stink bug semiochemicals and behavior of natural enemies'

The potential for utilising semiochemicals to manage parasitic Hymenoptera to improve biocontrol programs has been discussed by several authors (Smiths 1982; Lewis & Nordlund, 1985, Lewis & Martin 1990; Powell & Pickett, 2003). The identification of factors leading to host recognition, acceptance and oviposition, and *in vitro* rearing and mass

production may offer possibilities for making previously artificial and unacceptable hosts, acceptable after treatment with kairomones, for example (Vinson, 1985).

The selection process of a host by a parasitoid involves both location and recognition behavior that precedes oviposition (Vinson, 1984, 1985). In each phase of the selection process, physical and chemical stimuli acting as cues, play an important role and may alter parasitoid behavior (Weseloh, 1981; Nelson & Roitberg, 1995). Therefore, the cues may have different origins. These can be derived from the host plant, the host itself or from a host product. In this case, they are called kairomones (Noldus & van Lenteren, 1983; Blum, 1996). Some of these cues are concerned with host location and involve volatile semiochemicals that act at long-distances (Sales et al., 1979; Aldrich et al., 1984; Bin et al., 1987; Borges et al., 1998b; Dicke, 1994). Other cues act at short range and are involved in host recognition (Buleza & Mikheev, 1978; Leonard et al., 1987; Nordlund et al., 1987; Borges et al., 1998b). Various stimuli have a certain range over which they can provide information to the parasitoid. Once the parasitoid is within a potential host community, shape and semi-volatile factors increase in importance and when the host is contacted, contact chemicals, shape, size and texture then become important (Vinson, 1984).

Scelionidae that parasitize eggs from stink bugs can use several types of semiochemicals for long-range localization of habitat, microhabitat, and hosts: volatiles from plants damaged by stink bug oviposition or feeding (Colazza et al., 2004; Moraes et al., 2005c, 2008c); sex pheromones (Aldrich, 1995; Borges et al., 1998a,b; 2003; Bruni et al., 2000; Silva et al., 2006); volatile defensive secretions from the metathoracic (adults) or dorsal abdominal (nymphs) glands of stink bugs (Aldrich 1995, Mattiacci et al., 1993; Borges & Aldrich 1992); or crude whole body extracts of stink bugs (Colazza et al. 1999; Salerno et al. 2006). Volatiles from non-host stages of stink bugs, such as pheromones or defensive compounds, as well as contact chemicals (traces left by walking insects) can also be used for host location, recognition, and acceptance leading to successful oviposition (Bin et al. 1993; Borges et al., 1999b, 2003; Colazza et al., 1999; Conti et al. 2003). Physical stimuli such as visual and resonance cues may also be involved in successful host search (Borges et al., 1999b).

The foraging behavior of *Telenomus podisi* in the presence of selected stimuli from its host, *Euschistus heros* were recorded in a closed arena bioassay. The parasitoids were given the choice between single and combined stimuli presented to them simultaneously (egg mass; virgin males and females volatile extracts; volatile extracts of sexually mature males and females; synthetic standards of male sex pheromone; (*E*)-2-hexenal, a component of the alarm pheromone, hexane and air). To find the host egg, *T. podisi* primarily uses the sensory cues released from the male insects, and the results suggest that the eggs did not release any volatile that could enhance or decrease the attraction of the egg parasitoid. *T. podisi* responded to the male extract, sex pheromone components and to (*E*)-2-hexenal (Silva et al., 2006). In brief, in a multisensory condition, *T. podisi* use olfactory signals to find its host at long distance. This confirms previous studies that host location is oriented primarily by olfactory cues released directly out from the non target host egg, which is more detectable and reliable (Borges et al., 1998a).

The long range host searching behavior of the egg parasitoids *Telenomus podisi* and *Trissolcus basal* is differentially influenced by the blends of volatiles released from the metathoracic glands of different stink bug species. We have studied whether such variable response is due to different individual components of these glands and whether these responses reflect host preferences. Y-tube olfactometer bioassays were carried out with crude extracts of metathoracic glands of five different host species of neotropical stink bugs. Furthermore, we

tested the parasitoids' response to synthetic standards of individual compounds identified in these stink bug glands. Results showed that females of *T. basalis* and *T. podisi* responded differentially to crude gland extracts of the different species of host stink bugs and to the compounds tested. The parasitoid *T. basalis* showed a positive taxic behavior to *Nezara viridula* methathoracic gland extracts (Laumann et al., 2009) its preferred host species (Sujii et al., 2002). Furthermore, *T. basalis* responded positively to 4-oxo-(*E*)-2-hexenal and (*E*)-2-decenal, two components that are in higher quantities present in *N. viridula* glandular secretion. The compound 4-oxo-(*E*)-2-hexenal modified the kinetics traits of *T. basalis* walking pattern and suggests that this compound might stimulates the searching behavior of this parasitoid (Laumann et al., 2009).

The parasitoid *T. podisi* was attracted to crude gland extracts of the preferred host (*Euschistus heros*) and had its walking pattern altered when stimulated with 4-oxo-(*E*)-2-hexenal. In addition, *T. podisi* also responded positively to (*E*)-2-hexenal and to the hydrocarbon tridecane, both of which are defensive compounds released from the metathoracic glands by several stink bugs. These results indicate some degree of specialization in the response of two generalist parasitoid species towards defensive secretions of stink bugs (Laumann et al., 2009). The use of semiochemicals for behavioral manipulation of parasitoids have been proposed and discussed extensively. In recent years, semiochemicals have also been discussed as a tool to improve biological control (Vet & Dicke, 1992; Lewis & Martin, 1990; Powell & Pickett, 2003). Specific knowledge about host - parasitoid relationships mediated by semiochemicals are important for improving the effectiveness of applications of semiochemicals in integrated pest control.

#### 2.4 Vibrational communication in stink bugs

Aside from chemical communication stink bugs use vibratory signals during mating behavior. Vibratory signals provide information about species, sex, position on a plant and receptivity to mating (Čokl et al., 2000; Čokl & Virant-Doberlet 2003; Miklas et al., 2003a; b; Gogala, 2006). Vibratory signals emitted by pentatomids are efficiently transmitted through the plant (Čokl et al, 2005). Species- and sex-specific songs have been described in several species, and different vibratory songs have been described and correlated with reproductive behaviour in the Neotropical species *Chinavia impicticornis*, *Chinavia ubica*, *Euschistus heros*, *Piezodorus guildinii* and *Thyanta perditor* (Fig. 3) (Moraes et al., 2005b; Lopes et al., 2006).

Neotropical stink bug species feed and mate on the same host plants and in Brazil constitute major components of the soybean pest complex. During mating they communicate with species and sex specific vibratory signals. Songs differ between species in the time structure and amplitude modulation of their units. The repertoire of *C. impicticornis*, *E. heros* and *T. perditor* fits into the scheme described for most investigated stink bugs: females call with a sequence of pulses which differ among species in their duration and repetition rate, and males respond with courtship songs of species specific temporal structure and amplitude modulation of complex pulse trains (Čokl & Virant-Doberlet, 2003; Gogala, 2006). Female calling and male courtship songs are the main constituents of vibratory communication between sexes in the mating period. The other vibratory emissions represent either transitional song, support recognition during close-range courtship or are involved in male rivalry. First recorded vibratory emissions of *P. guildinii* confirm that genus *Piezodorus* represents an exception between Pentatominae (Moraes et al., 2005b). Irregularly repeated female vibratory signals do not trigger typical male courtship responses like in small stink

bugs *Holcostethus strictus* (Fabricius) and *Murgantia histrionica* (Hahn). On the other hand, complex rivalry with extensive frequency modulation of pulses as described also in *P. lituratus* opens a new insight into the role of vibratory communication in stink bugs (Moraes et al., 2005b).

Except for *P. guildinii*, songs of investigated species from Brazil fit well into the general pattern of vibrational communication in Pentatominae, though several questions remain unanswered. Studies on the functional value of statistically determined differences between songs are lacking and there are no data about the role of different host plants on the quality of vibratory signals exchanged during communication at different distances. Bimodal communication with olfactory and vibratory signals needs detailed studies on the nature of chemical signals involved and finally signals of other modalities like touch, vision and even low frequency near-field sound emitted by mates immediately before copulation (Moraes et al., 2005b). This knowledge could be applied in stink bug management if, in the future, the technology development can design micro-devices emitting vibrational signals that could be a powerful tool to be applied in the field to enhance the efficiency of the capture of stink bugs, and also to attract and keep the natural enemies in the field.

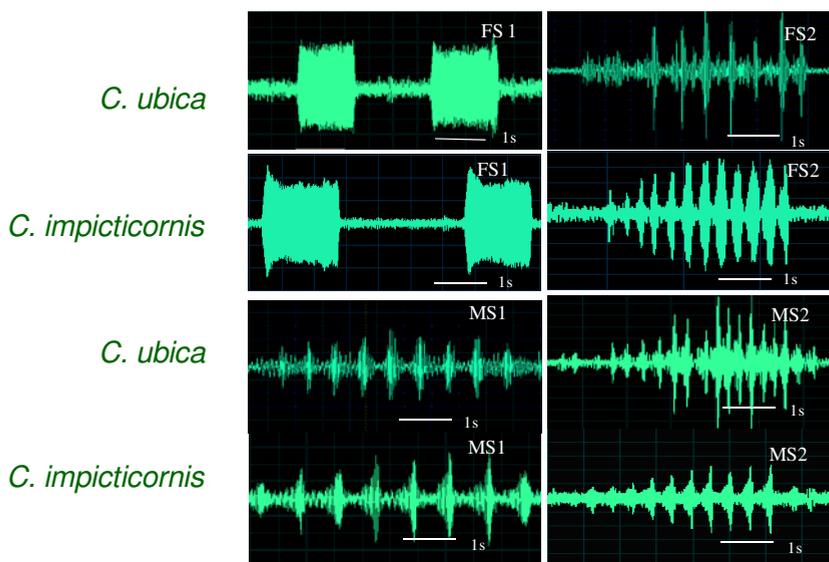


Fig. 3. Example of songs emitted by different *Chinavia* species stink bugs. FS1 = Female song 1; FS2 = Female song 2; MS1 = Male song 1; MS2 = Male song2.

Vibratory signals emitted by pentatomids are efficiently transmitted through the plant (Čokl et al., 2005) and can be used by natural enemies to obtain information about host presence and position.

Males and females of the Neotropical brown stink bug, *Euschistus heros*, communicate with vibratory songs transmitted through plants. The egg parasitoid *Telenomus podisi*, a natural enemy of these bugs, shows taxis to the vibratory signals of female songs on a plant and on an artificial substrate. This reaction is specific to the signal type since directionality is elicited by the songs of *E. heros* females, but not males or duet songs or by continuous pure tone vibratory signals. Vibratory signals do not influence other components of *T. podisi*

foraging behavior, such as linear velocity, turning rate and tortuosity. These results are the first register of a parasitoid eavesdropping sexual vibratory signal of insects. When *T. podisi* females were stimulated with songs of *E. heros* females they showed an oriented response (taxis) toward the emission points, with significant differences in the first choice and residence time in areas (leaves of plants or arms of the arena) vibrated with the songs. Additionally, a higher turning rate of *T. podisi* females when stimulated with this song indicates some effect on their kinetic locomotion behavior (Laumann et al. 2007). In addition to selectively responses to females' songs *T. podisi* also can discriminate between songs of different stink bugs species showing selective responses toward song for their preferred host *E. heros* (R. Laumann; A. P. S. Lopes; M.C.B. Moraes; A. Čokl & M. Borges, not published). The use of vibratory signals of *E. heros* females over intermediate distances (on the same plant) can be favoured because these cues can be more reliable than chemical or visual cues from eggs. *Telenomus podisi* probably uses the vibratory signals emitted by females to locate sites where there is a high probability of finding eggs. This hypothesis can be valid in the case that stink bug females copulate and oviposit on the same plant; field experiments are needed to confirm or reject this hypothesis (Laumann et al., 2007).

## 2.5 Odor chemoreception

Insects communicate through visual, vibrational, chemical and tactile signals, the last two being perceived by sensory receptors known as sensilla located mainly in the antennae, legs and bodies labial (Silva et al., 2010). There are several studies reporting the importance of morphology and ultrastructure of the antennal sensilla (chemosensory hairs) to understand the mechanisms of olfaction (Hallberg et al., 1994; Isidoro et al., 2001; Gómez & Carasco, 2008); however, for pentatomids, few studies have been conducted in this area. In this group of insects the representative studies have focused on the morphology and ultrastructure of antennal sensilla of *Lygaeus kalmii* (Slifer & Sekhon, 1963), *Lygus lineolaris* (Dickens et al., 1995; Chinta et al., 1997), *Nezara viridula* (Brézot et al., 1997) *Odontopus nigricornis*, *Cyclopelta siccifolia* and *Chrysocoris purpurea* (Rani & Madhavendra, 2005).

To characterize chemical communication in stink bugs, it is necessary to know the structure of the olfactory system of these insects. Therefore, the morphology, abundance, and distribution of antennal sensilla in males, females and 5<sup>th</sup> instar nymphs of *E. heros*, *P. guildinii* and *E. meditabunda* were studied.

A transitional difference in sensilla density between nymphs and adults was recorded and this factor seems to be a common phenomenon reported for the stink bug *N. viridula* by Brézot et al. (1997). There is a general tendency for insects to increase the number of receptors at the adult stage, particularly chemoreceptors, such as basiconic and trichoid sensilla (Catalá, 1997). As reported by Chapman (1982), this may be related to different chemical communication. The increase in the number of chemoreceptors at the adult stage would reflect additional sensorial requirements for reproduction and dispersal in the host community.

Studies of ultrastructure and function of sensilla and labial parts were initiated through scanning electron microscopy (SEM) with the aim of identifying the olfactory sensilla on the antennae of *E. heros* and other stink bugs species. These studies may help in the interpretation of how the signal processing occurs in the insect and, indirectly, to develop more efficient tools to control these pests (Silva et al., 2010).

In addition to the main chemosensory receptors, the trichoid and basiconic sensilla, two additional types were identified: the knob-shaped and the slit-tipped basiconic sensilla. The sexual dimorphism in numbers of sensilla trichodea (ST1), sensilla basiconica (SB1) and

(SB2) constitutes an important feature in the chemical communication of these stink bugs. The results presented are important for ongoing studies on mating and host plant seeking behaviors in these species, and more detailed studies regarding the odor perception in the pentatomid group (Silva et al., 2010) (Fig. 4).

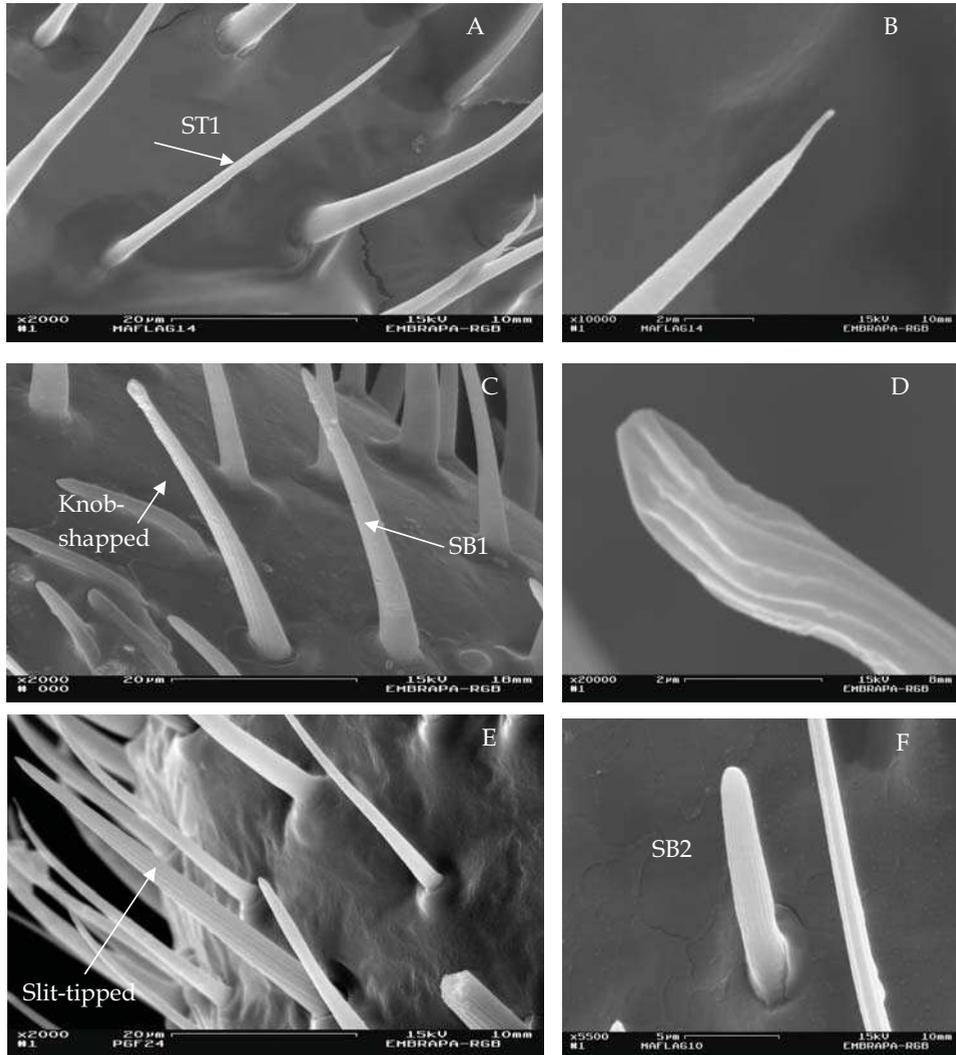


Fig. 4. SEM micrographs of the olfactory sensilla on the antennae of *Euschistus heros* and others stink bugs. (A) The long straight, sharp-tipped sensillum trichoid 1 (ST1) with smooth wall on the flagellum of *E. heros*. (B) Tip of the sensillum at higher magnification showing its hooked point. (C) knob-shaped and basiconic sensillum type 1 (SB1). (D) Tip of the sensillum at higher magnification showing deep grooves. (E). Long slit-tipped sensillum with longitudinal grooves. (F) Basiconic sensillum type 2 (SB2).

Going deeply into the sensilla structure, chemical signals cross the pores and are transported through the sensillum lymph to interact with the chemoreceptors, which in turn activate the cascade of events leading to spike activity in sensory neurons (Pelosi, 1996; Stengl et al., 1999). The most important proteins involved in the recognition of chemical cues comprise odorant-binding proteins (OBPs), and odor degrading enzymes (ODEs), which are involved in peripheral olfactory processing, and the chemoreceptor proteins family formed by the olfactory receptors (ORs). Insect OBPs are small globular proteins (about 135–220 amino acids long) that bind and solubilize hydrophobic odorants such as pheromones or allelochemicals, and transport and deliver them to chemoreceptors located on the sensory neurons (Matsuo et al., 2007; Laughlin et al., 2008). In the first case they are called pheromone binding proteins (PBP) and in the second general odorant binding proteins (GOBPs) (Leal et al., 1999; Scaloni et al., 1999). In the antennae the OBPs are compartmentalized in specific sensilla, the PBP are related to the sensilla trichodea (Steinbrecht et al., 1992) and GOBPs in sensilla basiconica (Laue et al., 1994). The CSPs (also known as olfactory specific-D, OS-D, or sensory appendage protein, SAP) comprise another class of small binding proteins (about 130 amino acids long), which are expressed in antennal and non-antennal tissues and are capable of binding odorants, however showing less binding specificity than the OBPs (Jacquin-Joly et al., 2001). The ODEs are selectively evolved to degrade pheromones and allelochemicals (Rybczynski et al., 1989). Such enzymes attack specific functional groups, such as acetate esters, aldehydes, alcohols, ketones and epoxides. A few semiochemical degrading enzymes have been identified and characterized in detail in contrast with current efforts on OBPs and ORs. The ORs are expressed by olfactory receptor neurons (ORNs) and localized in the membranes of the ciliated dendrites as seven-transmembrane domain receptors of about 400 amino acids that bind environmental compounds, thereby transforming the chemical signal into the activation of neurons in the higher processing centres in the brain, which in turn mediate the appropriate behavior (Clyne et al., 1999; Engsontia et al., 2008).

All these protein components (OBP, ODE, and OR, sometimes CSP) of the olfactory system are extensively prospected as candidates for the development of biotechnological tools for pest control by different research groups around the world. Since the identification of the first PBP in the silk moth *Antheraea polyphemus* (Cramer) (Vogt & Riddiford, 1981), many other labs became interested in molecular components of the olfactory system mainly for insects of the Lepidoptera order. These became faster in insects from different orders with the advance of the genome assessment, greatly expanding knowledge of the range of species possessing these proteins and knowledge of the size and diversity of the olfactory gene families. Until now the genomes of nine insect species from different orders have been sequenced, allowing us to compare the organization richness of the olfactory system and helping to clarify the evolution of the olfaction machinery (Table 2). Nevertheless, the majority of the olfactory proteins identified are putative, based on their sequence similarity with sequences stored in a database, therefore the functional validation has not been provided in most of the cases.

In the Hemiptera order 21 OBPs have been identified and only for two hemipterans the genome sequencing information was provided, *Acyrtosiphon pisum* (Harris) (Aphidoidea) and *Rhodnius prolixus* Stål (Reduviidae). The group of scientists at Embrapa Genetic Resources and Biotechnology in Brazil is putting effort into sequencing the genome of at least one stink bug species, *Euschistus heros*, from the soybean complex. Using cDNA library construction from antennae and sequencing of 1000 clones two putative OBPs (called

Insect	OBP/CSP	OR
<i>Acyrtosiphon pisum</i> (Hemiptera: Aphidoidea)	49.6/5.4	86.9
<i>Anopheles gambiae</i> (Diptera: Culicidae)	60	-
<i>Apis mellifera</i> (Hymenoptera: Apidae)	58	16
<i>Nasonia</i> sp (Hymenoptera: Pteromalidae)	1	15
<i>Tribolium castaneum</i> (Coleoptera: Tenebrionidae)	3	13

Table 2. Genes representation of the olfactory system in insect genomes available in the NCBI site (National Center for Biotechnology Information).

*Eher*OBP1 and *Eher*OBP2, Genbank HM347779) and three putative CSPs (called *Eher*CSP1, *Eher*CSP2 and *Eher*CSP3) (Genbank in submission) have been identified. The *Eher*OBP1 presented the highest similarity to *Lygus lineolaris* (E-value  $2e-20$ ) and the *Eher*OBP2 to *Cimex lectularius* L. (E-value  $7e-07$ ) (Fig 5a). By BLAST similarity search, both *Eher*OBPs are hypothetically related to PBP family, and they are expressed in antennae in the gender producer of the sexual pheromone (males) and in the gender attracted (females), as evidenced by quantitative PCR analyses. The *Eher*OBP1 and *Eher*OBP2 are also expressed in legs, wings, abdomen and heads (without antennae), although in smaller amounts than in the antennae. The *Eher*CSPs identified are also expressed in the antennae of males and females and are more similar to each other than to the others CSP of other hemipterans (Fig. 5b) (L.R. Farias, M. Borges, M.C.B. Moraes, R.A. Laumann, D.P. Paula, unpublished data).



Fig. 5. Partial alignments of some putative OBPs identified in Hemiptera order with: a. Deduced amino acid sequences of the putative *Eher*OBP1 and *Eher*OBP2; b. Nucleotide sequences of the putative *Eher*CSP1, *Eher*CSP2 and *Eher*CSP3 (ClustalX 1.81 program and edition using BoxShade 3.21). The gray color indicates the similarity and the black color the identity. The sequences are indicated by the GenBank code followed by the abbreviation of the genus and species. The species are: *Sitobion avenae* (Sav); *Acyrtosiphon pisum* (Apis); *Myzus persicae* (Mper); *Nasonovia ribis-nigri* (Nrib); *Lygus lineolaris* (LAP); *Aphis fabae* (Afa); *Aphis gossypii* (Agos); *Nilaparvata lugens* (Nlug); *Rhodnius prolixus* (Rpr); *Apolygus lucorum* (Aluc); *Cimex lectularius* (Clec); *Adelphocoris lineolatus* (Ali).

Considering the number of OBPs identified in other insect species and also by the proteomic results of the antennae (Fig. 6), it can be inferred that there is the potential to identify more olfactory proteins from *E. heros*. The differences in the polypeptide composition in the proteomic profile of the antennae of nymphs, males and females *E. heros* confirm the sexual dimorphism in numbers of sensilla trichodea and basiconica showed by the antenna morphology studies.

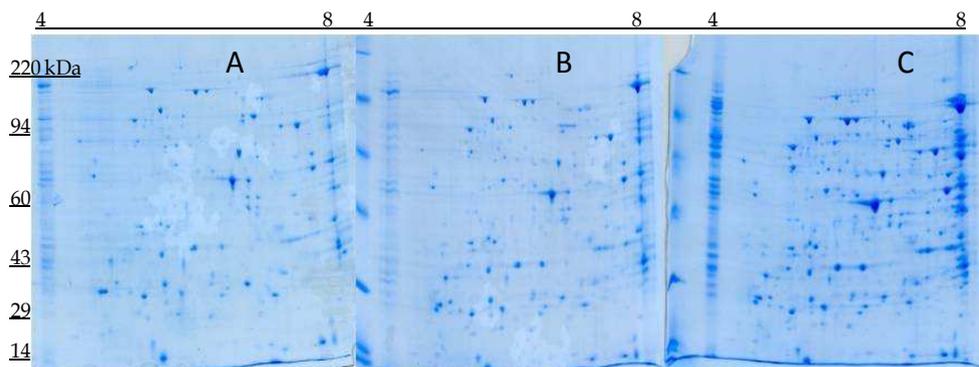


Fig. 6. Proteomic profile of total *E. heros* antennae proteins by two-dimensional SDS-PAGE. The pH linear range of the first dimension gel (isoelectric focusing = IEF, 13 cm) is indicated above the gels. The positions of protein standards (kDa) used in the second dimension gel (SDS-PAGE 15%) are indicated at the left of first gel. A. Fifth instar nymphs, B. Males and C. Females (600 antennae each, ~500  $\mu$ g of total protein), both virgins 12 days-old.

The efforts concerning the identification of more chemoreception proteins are being continually conducted in order to know the complete genetic charge of the pheromone olfaction and in this way systemically knowing how the recognition of pheromones works, as well as the potential to recognize different categories of pheromones at the same time, and the developing of the olfaction according to the stink bug life cycle and the biotic and abiotic factors that can influence chemoreception. Furthermore the studies will be expanded to the other stink bugs species in order to richly characterize the olfaction mechanisms in stink bugs and by this way contribute to the development of biotechnological tools to aid in pest management schemes.

### 3. Tri-trophic interactions soybean - stink bugs - natural enemies

Plants commonly suffer continuous damage by insects in their natural habitat. There are two main strategies of defence against herbivore feeding damage. In one strategy, the plants directly affect the insect; for example, the plant reduces the supply of essential metabolites to the herbivore or synthesizes compounds that minimize herbivore feeding, such as antinutritive or antidigestive compounds, which is known as direct defence (Dicke, 1994a; b; Kessler & Baldwin, 2002). In the other strategy, the plant affects the herbivore indirectly by attracting their natural enemies, such as parasitoids, which is known as indirect defence (Dicke, 1994a; b; Kessler & Baldwin, 2002).

Several studies have demonstrated that plants injured by herbivore feeding produce specific blends of odours, which can be attractive to parasitoids and predators. The profile of the volatiles emitted is markedly different from those of undamaged or mechanically damaged plants (Dicke & van Loon, 2000; De Moraes et al., 1998; Kessler & Baldwin, 2001; Turlings et al., 1990; 1998; Hoballah & Turlings, 2001). The volatile organic compounds (VOCs) emitted by plants are of fundamental importance in the association involving plants, phytophagous insects and their natural enemies.

The possibility of plant volatiles to play important role in the attraction and retention of the natural enemies is researched by scientists the world over. Chemical ecology studies using soybean in Brazil are concerned with the possibility of utilizing plant volatiles to manipulate the behavior of the egg parasitoids of the stink bugs, for their application in pest management programs.

### **3.1 Stink bugs in soybean (interaction with plants, bioecology and damage)**

Most phytophagous pentatomids are polyphagous, feeding on cultivated and uncultivated (wild) plants. They are major pests of economically important crops throughout the world, including legumes such as soybean and beans; cereals such as rice and wheat; and tree crops such as citrus, oil palms and coconut, cocoa, and coffee (Panizzi, 1997 and references therein).

Phytophagous pentatomids feed sucking up nutrients from host plants using their stylets. They can feed in different structures of the plants but seed and immature fruits are the preferred feeding sites (Panizzi et al., 2000). In soybean and other legumes stink bugs feed mainly on pods and grains causing direct damage such as seed abortion, reduction of oil content and mechanical damage or indirect injury like fungal transmission and physiological alterations (Villas-Bôas et al., 1990; Sosa-Gómez & Moscardi, 1995; Boethel et al., 2000).

The preferred feeding sites are ephemeral resources, therefore a succession of host plants are necessary to develop successive generations along crop seasons or continuous breeding (Panizzi, 1997). Specific host plant relationships may be found in different crops and geographical sites, additionally some species show feeding preferences by certain plant taxa (Panizzi, 1997).

The host plant/stink bug interaction is also related to the bio-ecology of the insect. In temperate climates adult stink bugs enter a reproductive diapause after the crop season (fall) and spend the winter protected in different places (under the bark of trees, litter or culture debris) (Ehler, 2000, Mourao & Panizzi, 2000). In spring they develop a first generation in wild hosts and then migrate to cultivated crops where the numbers of generations that develop depend on climatic conditions (Ehler, 2000).

In tropical or sub-tropical conditions the phenology can be different. For example, in southern Brazil (Parana State) Panizzi (1997) describe continuous generations for *N. viridula* and *P. guildinii* and a diapause period in the winter for *E. heros*. On the other hand, in central Brazil (Federal District) *E. heros* and others pentatomids, due to high temperatures throughout the year, could be found during dry season (winter for temperate and sub-tropical regions of the southern hemisphere) on alternative hosts, wild or cover crops plants that grow in more humid situations, with reproductive activity and developing successive generations. Additionally the host plant spectra used by pentatomids in central Brazil is different than that observed in southern region of the country (Laumann et al., 2008b; Vieira et al., 2008).

The specific knowledge of cultivated and wild hosts may be relevant to stink bug management (Panizzi, 1997). Information about invasion time in the crop fields could help in monitoring and control, knowledge of alternative host plants could help to develop trap crops and the dynamic of the use of crop/wild hosts and their influence in natural enemy guilds could be useful to biological control strategies. Additionally, the semiochemical complex related to crop/wild host plants could be useful to identify chemicals to repel or attract stink bugs or attract and retain natural enemies in specific areas.

### 3.2 Interaction soybean stink bugs - Direct and Indirect defenses

Working on signalling through volatiles induced by *E. heros* on two legumes has demonstrated that these volatiles influence the attraction and retention of the egg parasitoid, *T. podisi*. Air-borne extracts obtained from two host plants of *E. heros*, soybean, *Glycine max* L., and pigeon pea, *Cajanus cajan* (L.) (Leguminosae), produce a different blend of emitted volatiles when attacked by adult males or females and nymphs of the pest species compared with the undamaged plants. The same results were obtained when the plants were treated with extracted saliva of *E. heros*, mechanically introduced into the plants. This indicates that some substance in the saliva contributed to the release of the volatiles (Moraes et al., 2005c). Olfactometer studies with female *T. podisi* and treated plants confirmed the significant preference of the egg parasitoid for herbivory damaged plants by *E. heros* when compared to undamaged plants. On the other hand, volatile extracts obtained from soybean subjected to the velvetbean caterpillar, *Anticarsia gemmatilis* Hübner, a non-host species for *T. podisi*, showed a different blend of volatiles compared to those obtained from plants damaged by *E. heros* feeding, and the extract obtained from *A. gemmatilis* herbivory damaged plants did not attract the egg parasitoid. These results indicate that *E. heros* causes the induction of indirect defense of soybean and it is possibly caused by an elicitor present in the pest saliva. This work has demonstrated the potential of these volatiles to play an important role in the attraction and retention of the egg parasitoid, *T. podisi* (Moraes et al., 2005c).

Phytohormones have been used as inductors of defense of plants to improve the resistance of the plants to pests (Birkett et al., 2000; Moraes et al., 2008d; Pickett et al., 2007). *cis*-Jasmone is a volatile compound derived from fatty acid pathway, which has been used to induce the chemical defense of plants (Birkett et al., 2000; Moraes et al., 2008d). Soybean plants treated with *cis*-jasmone also had their chemical defense induced provoking changes in the volatile organic compounds (VOCs) profile when compared to untreated soybean (L.) (var. BR16) and the volatiles released by the *cis*-jasmone treated soybean attracted the egg parasitoid *T. podisi*. The main components induced by *cis*-jasmone were camphene, myrcene, (*E*)-ocimene, methyl salicylate and (*E,E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT). Thus, *cis*-jasmone appears to induce defence pathways in soybean similar to those induced by stink bug damage, and this phenomenon appears to be a promising tool for the manipulation of beneficial natural enemy in future sustainable stink bug control strategies (Moraes et al., 2009).

These results suggest that *cis*-jasmone has great potential for the development of new strategies for stink bug control using the natural defense of plants to attract natural enemies in soybean crops. Further laboratory and field studies are underway to evaluate how *cis*-jasmone can be used in a productive agricultural context, not only with soybean but with other economically important Brazilian crops (Moraes et al., 2009).

### 3.3 Soybean varieties and response to stink bugs

Several studies have shown that herbivore induced plant volatiles act directly on herbivores and indirectly on their natural enemies (Moraes et al., 2005c and references therein). However, little has been reported about the effect of herbivore damage on resistant and susceptible plant cultivars and its effect on natural enemies. Thus, to evaluate the attraction of *Telenomus podisi* and *Euschistus heros* to different types of damage (herbivory, herbivory+oviposition and oviposition) in two stink bug resistant soybean cultivars, Dowling and IAC100, and one susceptible cultivar, Silvânia were carried out. In olfactometer studies, the parasitoids were attracted to herbivory and herbivory+oviposition damaged soybean plants when compared to undamaged soybean plants for the resistant cultivars, but did not show preference for the susceptible cultivar Silvânia in any of the damage treatments. In olfactometer studies, *E. heros* females did not show preference for odors of damaged or undamaged soybean plants of the three cultivars studied. The susceptible cultivar Silvânia produced a very low level of herbivory induced plant volatiles (HIPVs) whereas resistant cultivars (IAC100 and Dowling) produce a higher quantity of HIPVs (Michereff et al., 2009). The compounds that most contributed to the divergence between damaged soybean plants compared to undamaged plants were (*E,E*)- $\alpha$ -farnesene, methyl salicylate, (*Z*)-3-hexenyl acetate and (*E*)-2-octen-1-ol. In addition, it is apparent that each cultivar has a different response to herbivore damage, eliciting different responses by the egg parasitoid. Inasmuch, the level of indirect defense in response to herbivory or oviposition damage in resistant cultivars is greater than that in susceptible cultivars and that there is a synergistic effect between the different types of damage in soybean in attraction of the egg parasitoid *T. podisi* (Michereff et al., 2009; Moraes et al., 2008c).

In brief, *E. heros* induces the indirect defense of soybean resistant cultivars Dowling and IAC100 and *T. podisi* is attracted to the blend of herbivory and herbivory+oviposition damaged plants. *E. heros* did not distinguish in an olfactometer bioassays between damaged and undamaged soybean nor between resistant and susceptible cultivars. Further laboratory studies are necessary to investigate the importance of these compounds in the attraction of *T. podisi*. Once understood, these volatiles could help to develop new strategies for control of *E. heros* using natural enemies in soybean crops (Michereff et al., 2009).

In addition, the effects of direct defences of different soybean genotypes on nymphal development of the southern green stink bug *Nezara viridula*, showed that in resistant genotypes the mortality of nymphs was around 66.2% and in susceptible cultivar it was around 27.5% (Piubelli et al., 2003). The quantities of flavonoids in soybean appear to be involved in the direct defence and resistance against herbivorous (Piubelli et al., 2005).

These studies may be used to develop new tools for pest management such as attracting naturally the natural enemies and influencing, in this way, the level of parasitism that may occur or to control herbivorous insects increasing natural pest mortality. Selection of semiochemicals, from plants, with the power to increase the beneficial action of parasitoids will enable the use of an efficient methodology for management of stink-bugs with minimal impact on the environment.

### 3.4 Metabolic pathways induced

The composition of herbivore induced volatiles can change among plant and herbivore species, even when comparing different plant varieties, different developmental stages of the plant and under abiotic stress (Viswanathan et al., 2007; Moraes et al., 2005c; 2008c; Turlings et al., 1998).

The herbivore induced volatiles come from several different metabolic pathways, the most important are the isoprene-derived terpenoids and fatty acid derived the green leaf volatiles, mainly when the damage is provoked by chewing insects (Arimura et al., 2009). Pathogens and sucking insects, like aphids and white flies, has a unique aspects that they induce a third and important pathway, the salicylic acid pathway, in a higher expression compared to when plants are attacked by chewing insects (Walling, 2000; Kaloshian & Walling, 2005).

Terpenoids are produced from the assembly of the C<sub>5</sub> building block called isoprene units (2-methyl-1,3-butadiene), which can originate from the mevalonic acid pathway or the pyruvate pathway (Gerhenzon and Kreis, 1999, Arimura et al., 2009). The monoterpenes (C<sub>10</sub>) and diterpenes (C<sub>20</sub>) are synthesized in plastids and the sesquiterpenes in the cytosol. Other important class of terpenes, the homoterpenes, such as TMTT, can be produced from sesquiterpenes and diterpenes, therefore they come from cytosol or plastid route. The fatty acid derived compounds come from the octacanoic signaling pathway, which breaks down fatty acids (linolenic and linoleic acid) to provide the green leaf volatiles (GLV), which includes, C<sub>6</sub> aldehydes, alcohols, and their esters, and also jasmonic acid and its derivatives, such as *cis*-jasmonone and methyl jasmonate (Hatanaka, 1993; Dudareva et al., 2006; Pickett et al., 2007). Salicylic acid dependent responses are very important upon attack by plant pathogens (Walling, 2000; Kaloshian & Walling, 2005), the compounds derived from this pathway are aromatic compounds, for example, methyl salicylate and indole, that have important functions in insect-plant communication (De Boer & Dicke, 2005).

Stink bugs in general feed preferentially on soybean seeds, but some species, such as *Edessa meditabunda* prefer the vegetative parts of the plants (Panizzi, 2007). The stink bugs use a stylet to pierce the tissue, destroying few cells, resulting in a minor mechanical damage, and consuming great quantities of fluid during long periods (Panizzi, 2007).

Soybean plants damaged by *E. heros* feeding induced the three main pathways described above, both those involved with chewing herbivore damage (terpenoids and fatty-acids) and also the salicylic acid pathway. The main herbivore induced volatiles in soybean were methyl salicylate, the GLV, (Z)-3-hexenyl acetate and the sesquiterpene, (E,E)- $\alpha$ -farnesene. The soybean response to stink bug feeding resembles that to pathogens in many ways as described for aphids and white flies by Kaloshian and Walling (2005). Another interesting difference in the plant's response when damaged by stink bug feeding when compared to chewing insects is that for chewing insects the green leaf volatiles are produced and released immediately after the attack and the terpenoids only few hours later or when the plants reach the next photosynthetic phase, (next day). In soybean plants damaged by stink bugs the green leaf volatiles and terpenoids were induced only after 72-96 hours of damage (Moraes et al., 2005c; 2008c; 2009). The interactions between pathways and the timing of the compounds released are raising many interesting questions that will need further of work to be responded.

### 3.5 Herbivory induced volatiles and natural enemy behavior

Egg parasitoids have a short time frame in which their host eggs are suitable for parasitism, and in several systems these parasitoids respond to plant volatiles induced by feeding damage of the herbivore as a means of finding suitable hosts. By reviewing all the work carried out regarding indirect defence induced by pentatomids, it has been demonstrated that these volatiles play an important role in the attraction and retention of the egg

parasitoids, *T. basalis* and *T. podisi* (Colazza et al., 2004; Moraes et al., 2005c; 2008c; 2009). As discussed above, the volatiles derived from the host plants appear to be a powerful tool for the manipulation of egg parasitoid behaviour and their exploitation for pest control. The potential use for that should be attracting beneficial insects in selected environments and influencing the levels of parasitism that may occur by the use of indirect defence of plants may enhance integrated pest management.

We conclude that indirect defences are produced by these plants, when attacked by *E. heros*, as an induced response indirectly favouring the egg parasitoid as a biological control agent of this harmful soybean pest species (Moraes et al., 2005c; 2008c; 2009).

The results obtained and recorded from all studies regarding the tritrophic interactions in the Brazilian soybean crop; indicate that plant volatiles maybe one of the main strategy used by this parasitoid for location of habitats where stink bugs eggs are more likely to be found (Moraes et al., 2005c; 2008c; 2009).

#### 4. Application to stink bugs management

The economic importance of stink bug damage to soybeans, combined with the necessity of developing more integrated management of stink bug populations, are motivating researchers worldwide looking for methods to reduce pesticide use for stink bug control (Corrêa-Ferreira & Moscardi, 1996; Panizzi & Corrêa-Ferreira, 1997; Venzon et al., 1999; Corrêa-Ferreira & Panizzi, 1999; Knight & Gurr, 2007). The concept of integrated pest management (IPM) is evolving toward a more sustainable management system in which external chemical interventions are a last therapeutic resort. Sustainable agriculture requires management of the ecosystem so as to conserve the natural enemies that are instrumental in suppressing pest populations (Knight & Gurr, 2007; Weiss et al., 2009; Moraes 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, -corn and -potato, 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 & Pampapathy, 2006; Torres & Ruberson, 2008) and, as a consequence, have surfaced as the new primary pests in 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 & Panizzi, 2004; Seffrin et al., 2006).

The use of sex pheromone baited traps for monitoring stink bugs catches mainly the adult sexually mature females individuals (Borges et al., 1998a) but damage is caused by both adult and immature stink bugs (Millar et al., 2002). Therefore, for efficient population monitoring, which might counts also the immatures insects, the pheromone baited traps technology could be able to establish an accurate relation between insects trapped with the population density in the field, to indicate precisely the critical changes in population dynamics and behaviour of stink bugs in the field, that could be used for timing control measures for key pest as *E. heros*.

The effectiveness of the synthetic sex pheromone of the Neotropical brown stink bug, *E. heros*, was evaluated both in laboratory and field assays. 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 relation with field populations, as monitored with the shake cloth technique, during the reproductive phase of the soybean crop from the R1 to R5 developmental stage, i.e., from pod formation to pod fill. 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 and that the lures lasted one month under field conditions, and that traps could be used only in the borders of the crop area at a density of one trap every 200 meters (Borges et al., 2010).

In conclusion, the synthetic *E. heros* sex pheromone can be used to monitor the seasonal fluctuations in stink bug populations infesting soybean. The results gave a more precise indication of the critical changes in population dynamics and behavior of these bugs in the field, that can be used for timing control measures for the key pest, *E. heros*, with more precision in soybean in the Central Region of Brazil. Because environmental conditions vary in different regions of Brazil, ranging to 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 (Borges et al., 2010). Additionally, in recent year stink bugs have adapted to new crops such as cotton (Willrich et al., 2004 a; b; c; 2005), maize (Townsend & Sedlacek 1986, Avila & Panizzi, 1995) and sunflower (Panizzi & Machado-Neto, 1992; Malaguido & Panizzi 1999), so that the monitoring technology of pheromone baited traps for stink bugs could lead to establish effective biocontrol strategies that contribute to solving current and future stink bug pest problems.

Another case of study in Brazil was concerned with the species *Thyanta perditor* that is one of the stink bugs of the guild attacking soybean and may be found on others crops such as wheat, sunflower and sorghum. The field attractiveness of synthetic *T. perditor* pheromone baited traps were tested using the two-liter transparent plastic soft drink bottles traps baited with rubber septa impregnated with the treatments: 1mg methyl-(E2,Z4,Z6)-decatrienoate ((E2,Z4,Z6)-10:COOMe), the male sex pheromone of *T. perditor*, and traps with rubber septa impregnated with hexane (control). The experiment was performed in a soybean field during the soybean reproductive stages. Traps were monitored every week and the captures were compared to the population density estimated by the sampling cloth and visual inspection monitoring techniques. Traps baited with (2E,4Z,6Z)-10:COOMe were more effective in capturing *T. perditor* than control traps. The *T. perditor* pheromone traps also showed cross-attraction to other species of stink bugs such as *E. heros*, *E. meditabunda*, *P. guildinii* and *N. viridula*. In addition, results of this case of study showed that, the synthetic sex pheromone of the Neotropical stink bug *T. perditor* is a powerful female attractant and is highly efficient for population monitoring (Laumann, Moraes, Krimian & Borges not published).

The sampling cloth technique is a reference method for stink bug monitoring and is considered to be a non biased method to estimate the stink bug species population (Kogan & Pitre, 1980). The similar performance of the pheromone traps and the sampling cloth to estimate the species occurrence and their relative abundance could indicate that the pheromone baited traps may be a tool not only for population density estimations but also

to identify the relative composition of the stink bug guilds, at least in guilds where the pheromone baited traps used are that of the dominant species.

Future pest management strategies should accommodate the semiochemical relationships including crop plants and herbivore enemies to take full advantage of stabilizing trophic webs. In the past decades, scientists have worked on the chemical ecology of different stink bug species in an attempt to identify semiochemicals that might be useful for monitoring pest populations (Borges et al., 1998a; Millar et al., 2002) or for manipulation of the behaviour of beneficial insects (Borges & Aldrich, 1994a; Borges et al., 1998a; 1999).

Furthermore, it is likely that the pheromone will act in concert with the substrate-borne vibrational signals that this insect uses for communication over shorter ranges (Moraes et al., 2005c). It may be possible to develop pheromone-based monitoring systems for this insect, particularly if vibrational signals also could be incorporated into the trap design. Such monitoring systems, when used in conjunction with strategies such as trap cropping, may provide an integrated pest management solution for control of these pest bug species.

Regarding the application of semiochemicals for the manipulation of the behavior of the egg parasitoids to stink bugs management, Peres (2004) showed that application of (*E*)-2-hexenal in field conditions, in southern Brazil, may result in increased parasitism of eggs naturally deposited by caged females, in a week-long experiment. On the other hand, in a full crop season time experiment applications of (*E*)-2-hexenal in soybean plots (400 m<sup>2</sup>) showed that the treated areas with 4 mg of (*E*)-2-hexenal applied in rubber septa increased the number of scelionid wasps in relation to non treated areas, however the increase in parasitoid abundance was not associated with the occurrence and intensity of parasitism (Vieira 2010). In another study where *cis*-jasmone was sprayed in a soybean field, the structure of the community of Scelionidae in terms of species richness, was similar in general between treatment and control plots and equity was higher in treated plots. The total number of Scelionidae, mainly *Telenomus* spp. and *Trissolcus* spp. was significantly higher in treated plots. However, no significant difference in rates of parasitism and number of stink bugs were found between plots (Vieira, 2010).

These results suggest that semiochemicals have the potential to be used as a behavior manipulator of egg parasitoids. Notwithstanding, prior to an efficient field use in pest management specific concentrations, dosages, formulations and application times need to be established to obtain a total comprehension of their influence in scelionid wasp field behavior.

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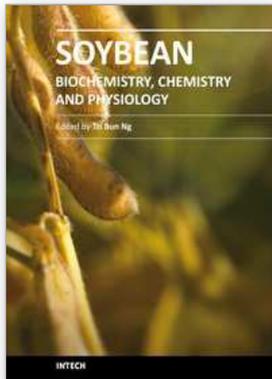
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