COMPOUNDS IN METATHORACIC GLANDS OF ADULTS OF THE SUNN PEST, *EURYGASTER INTEGRICEPS* (PUTON)(HETEROPTERA: SCUTELLERIDAE)

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[Hassani, S., Pour Abad, R. F., Djozan, D. & Fazel, M. M. 2010. Compounds in metathoracic glands of adults of the Sunn Pest, *Eurygaster integriceps* (Puton) (Heteroptera: Scutelleridae). Munis Entomology & Zoology, 5 (1): 232-239**]**

ABSTRACT: Sunn Pest, *Eurygaster integriceps* (Puton) (Heteroptera: Scutelleridae) is a serious pest of wheat and barley in countries of west and central Asia. The contents of metathoracic glands (MTG) of adults of the sunn pest *E. integriceps* were tested by two methods *in vitro* and *in vivo*. Volatiles were collected by solid-phase microextraction (SPME) fibers. Chemical composition of the glandular secretion was identified by gas chromatography (GC), coupled GC-mass spectrometry, matching retention times and mass spectra with those of authentic samples. No sexual dimorphism exists in the glandular composition in this species. A total of 7 compounds (E)-2-Hexenal, 2(5H)-Furanone, 5-Ethyle, 2-Hexen-1-ol, acetate, Limonene, 5-Decyne, Tridecane and Nonadecane were identified. Among the identified compounds (E)-2-Hexenal and Tridecane comprised nearly 95% of the total secretion in both females and males. In the MTG of both females and males of *E. integriceps*, (E)-2-Hexenal was determined in maximum amount and 2(5H)-Furanone,5-Ethyle was determined in minimum amount. Comparing results of *in vitro* and *in vivo* SPME sampling methods indicates that *in-vivo* method had high quality for detecting compounds than *in vitro* method. Quantity and area under GC peaks increased in *in vivo* method.

KEY WORDS: GC-MS, SPME, Scent glands, (E)-2-Hexenal, Tridecane.

Stink bugs produce large quantities of strong-smelling and irritating defensive chemicals, which are released when the bugs are disturbed or molested (Aldrich, 1988).

Odorous compounds are produced by the scent gland of adult and immatures. The scent glands are epidermal glands formed from epidermis by invaginations. The name is originated from the parts of the body (abdomen, metathorax). Production, storage and finally release of odoriferous substances are the main physiological function of the scent glands in Heteroptera (Staddon, 1979). Different roles such as defense against predation, alarm, mating, aggregation have been reported by researchers for the scent gland compounds (Ho & Millar, 2001).

Sunn Pest, *Eurygaster integriceps* (Puton), is a serious pest of wheat and barley in countries of west and central Asia. Nymphs and adults cause damage via feeding on leaves, stems and grains. During feeding they inject chemicals that reduce the baking quality of flour made from damaged grains (Parker et al., 2002).

The main objective of this study was to characterize the exuded compounds produced in the metathoracic glands of the Sunn pest, *E. integriceps* adults. This

information could be used in developing a new management strategy in the control of insects and as a tool to anticipate and predict potential damage to cereals. Rudimentary identification of compounds was made by using gas chromatography (GC) and final identification of extracted compounds carried out by GC coupled mass spectrometry (GC-MS).

MATERIAL AND METHODS

Insects

Adults *E. integriceps* were collected from wheat plants in the Hamedan region, from May through July, 2007. Insects were reared and maintained at 22-24°C and 60±10% RH With a 16: 8 (L:D) photoperiodic regime in plastic jars in the laboratory. Bugs were maintained on fresh host-plants until dissection.

Extraction

To prevent the premature discharge of gland contents, mature bugs were killed by freezing (Marques et al., 2007). Then an adult of *E. integriceps* was pinned in a Petri dish with the dorsal side up. The dissection process consisted of cutting the dorsal abdominal edges of the insect cuticle up to the metathoracic region and under the scutelum. The dorsal abdominal cuticle was pulled back and the viscera were removed (fig1-A). The scent gland complex, located at the ventral abdominal metathoracic region, could be reached and removed with the aid of small surgical scissors (Zarbin et al., 2000). The 20 glands reservoir of male and female were removed and immersed in 1ml dichloromethane and stored at -20°C until analysis.

In other method (*in vivo*), 3 alive insects were transferred into 4 ml vials, excited by shaking vial, up and down, 12 times. Released volatile compounds were adsorbed on modified HB pencil lead fiber in a diameter of 0.35mm, length 60mm from Rotring Co.(Germany), prepared as described below, was mounted in the homemade SPME device and the exposed fiber was trimmed to 2cm (Djozan et al., 2005). Fiber was located on the head space of vials (fig1-B), for 30 minutes, then injected to GC and GC-MS for identification. Adsorbed volatile compounds, were desorbed in the GC-MS injection port at 260°C for 1 min.

Chemical analysis

Gas chromatography – MS analysis of volatiles collected on SPME fibers was carried out by GC-MS with a Agilent 6890 series fitted with a HP-5MS column (30m×0.25mm I.,D. ×0.25µm film) and interfaced to an Agilent 5973 mass selective detector (electron impact ionization, 106 eV). The GC was programmed at 60°C/2 min, then 5° C/min to 140 °C, then 20° C/min to 220 °C. Helium carrier gas was programmed for constant flow (2 ml/min). the injection at 260 °C was splitless for 1 min. Compounds were tentatively identified by GC-MS, and identifications were confirmed by comparison of the retention times and mass spectra with those of authentic samples. The relative amount of each compound was determined from the area under GC peaks (Durak & Kalender, 2007a).

RESULTS

Analysis of MTG of *E. integriceps* was carried out separately for both sexes, by two methods *in vitro* and *in vivo* SPME sampling.

(E)-2-Hexenal, 2(5H)-Furanone,5-Ethyle, 2-Hexen-1-ol,acetate, Limonene, 5-Decyne, Tridecane and Nonadecane were determined in the male and female. In the analysis of MTG of both sexes of *E. integriceps*, (E)-2-Hexenal was

determined in the largest amount and 2(5H)-furanone,5-ethyle was determined in the smallest quantity. All of the chemical compounds are qualitatively similar on each male and female but they have slight differences in their quantity (fig. 2 and table 1).

Results of *in vitro* and *in vivo* methods indicates that *in vivo* method had a higher quality for detecting compounds than *in vitro* method, for example, 2-Hexen-1-ol,acetate only detected by *in vivo* method (fig 3). Also area under GC peaks increase in *in vivo* method (table 2).The advantages of the *in vivo* methods are: no need for solvent and dissection. In addition, this method needs fewer samples. Also compounds can be identified without changes in their type and relative ratio.

DISCUSSION

Compounds of Heteroptera scent glands have 2-15 carbon chain lengths and are most commonly acidics, aldehydes, ketones, acetone, alcohols and esters (Staddon, 1979; Aldrich, 1988). The main function of these compounds is defense, alarm, mating and aggregation (Regnier & Low, 1968).

The contents of metathoracic glands of adults of the sunn pest *E. integriceps* were analyzed by two methods *in vitro* and *in vivo* and 7 different chemical substances were determined for both sexes. In the male and females of *E. integriceps* the following substances were found**:** Aldehyde, lactone, acetate, cycloalcen, alcene and two types of alcanes. The components of MTG of adults in both sexes of the Sunn pest were similar and typical of what has been reported for several other Heteroptera species (Aldrich, 1988). In addition to biosynthetic parsimony, the similarity in the defensive chemical blends shared by numerous species may provide another benefit of serving as a generic warning signal and strong deterrent to attack. These blends of hydrocarbons with aldehydes and esters appear to be highly conserved, being shared both within and across genera and even between bug families (Aldrich, 1995). The aldehydes and esters are strongly scented and are strong irritants, providing both an easily detected warning signal and a strong defense. The function of the hydrocarbons is less clear, but they may serve as solvent and as controlled-release substrates for the more volatile aldehydes (Remold, 1962; Gunawardena & Herath, 1991).

One of the main compounds detected in MTG of *E. integriceps*, is (E)-2-Hexenal as reported in other species of Heteroptera. This compound has been identified in many species of Pentatomide (Aldrich, 1988; Ho & Millar, 2001; Zarbin et al., 2000), Rhopalidae (Aldrich, 1988), Lygaeida (Staddon & Olagbemiro, 1984), Coreidae (Steinbauer & Davier, 1995), Alydidae (Yasuda et al., 2007), Miridae (Drijfhout et al., 2007), *Hotea gambae* (Aldrich, 1988) and *E. maura* (Durak & Kalender, 2007a) (scutelleridae). This component has also been found in aphid sexual pheromone (Kye & Hardie, 2002). It becomes attractant at low concentrations and as a repellent at high concentrations (Durak & Kalender, 2007a). (E)-2-Hexenal may possess two functions: 1) as a defense against predators and 2) as an alerting pheromone warning and dispersing other individuals in an aggregation (Calam & Youdeowei, 1968). Levinson and Barllan (1971) bioassayed the major components of the bed bug scent glands and found that (E)-2-Hexenal has been categorized as a bed bug alarm pheromone (Levinson & Barllan, 1971 and Levinson et al., 1974). This compound is toxic to dipteran eggs and perhaps provides protection against tachinid eggs (Aldrich, 1978).

Tridecane, another main compound detected in this study, was found in MTG of other bugs especially several species of pentatomidae (Borges et al., 2001; Ho & Millar, 2001; Zarbin et al., 2000), Pyrrhocoridae, lygaeidae (Aldrich, 1988), *E. maura* (Durak & Kalender, 2007a) and *Pachycoris stallii* (Williams et al., 2001) (Scutelleridae). Promotion of penetration of the toxic scent carbonyls through cuticle in arthropode predators and acting as fixative, to delay the evaporation of the scent carbonyls from the body surface of the scent emitter are two main function of Tridecane in insects (Staddon, 1979). This component also was identified in compounds of alarm pheromone in ants (Regnier and Law, 1968).

It was reported for pentatomid that (E)-2-Hexenal and n-Tridecane were more effective as repellents to insects when combined than when individually tested. Furthermore, other n-alkanes when combined with (E)-2-Hexenal were not as effective deterrents towards other insects as n-Tridecane. Hence, n-Tridecane appears to be the optimal n-alkane to work synergistically with the other scent compounds (Zarbin et al., 2000).

2(5H)-Furanone,5-Ethyle is antifungal and antibacterial compound (Paulitz et al., 2000; Johne et al., 2006) and acts against *Fusarium*, *Pythium*, *Rhizoctonia*, *Thielaviopsis* and *Trichoderma* (Paulitz et al., 2000). This compound was identified in sexual pheromones of *Eurycolis florionda* (Slaughter, 1999) and *Popillia japonica* (Nation, 2002) and MTG of *Graphosoma semipunctatum* (Durak & Kalender, 2007b).

Limonene is a plant monoterpenoide with antibacterial function (Dormsn & Deans, 2000). this compound has been reported in defense pheromone of *Hotea gambiae* (Scutelleridae) (Aldrich, 1988) and *Sehrious cinctus cinctus* (Cydnidae), also as aggregation pheromone in *Cimex lectularius* (Cimicidae) (Siljander et al., 2008) and Scolytidae beetles (Hick et al., 1999).

5-Decyne, it is the first report of this compound in Heteroptera MTG although 1-Decyne has been reported in the anterior glands of Dysdercus *cingulatus* that may be involved in maintaining aggregation (Farine et al., 1992). Biological function of this compound in MTG still needs further study.

Nonadecane compound were identified as toxic, irritant or repellant in different insects (Zarbin et al., 2000). It is released by stink-bugs in response to disturbance, showing that they are responsible for chemical defenses (Durak & Kalender, 2007a) and may also have the same function in *E. integriceps*. Also it is found in *Nezara viridula* (Aldrich et al., 2005), *E. maura* (Durak & Kalender, 2007a) MTG and *Oecophylla smaragdina* (Keegans et al., 1991).

2-Hexen-1-ol,acetate, had been reported from MTG of *Dolycoris baccarum* (Durak, 2008) and in released compounds from damaged leaf of *Macaranga myrmecophytes* (Inui & Itioka, 2007).

As the *E. integriceps* has two behavioral phases: 1) At the end of feeding, adults aggregate on the nearest fields to hibernating sites and after they migrating collectively to mountain. 2) At the spring, migration again takes place collectively and most of them temporarily establish on the nearest fields. Then they disperse to adjacent fields. In attention to our obtained results and other researches it may be supposed that the MTG components, specially Tridecane and (E)-2-Hexenal act critically in two behavioral phases. So that we can use these components for repelling or aggregating in our expected locations and then controlling them. Also it has been reported that the egg parasitoid *Trissolcus basalis* utilizes a defensive substance produced by its host bug as a long-range attractant kairomone (Zarbin et al., 2000). So the MTG components of adults suun pest could be used as kairomone to synchronize the parasitoid population at the beginning of the host

flight season and pave the way for the development of invaluable tools in integrated pest management programs for this important pest.

ACKNOWLEDGMENTS

We thank the analytical chemistry department of Tabriz University for supporting our analysis, and we thank M. Arzanlo, T. Baheri and D. Mohammadi.

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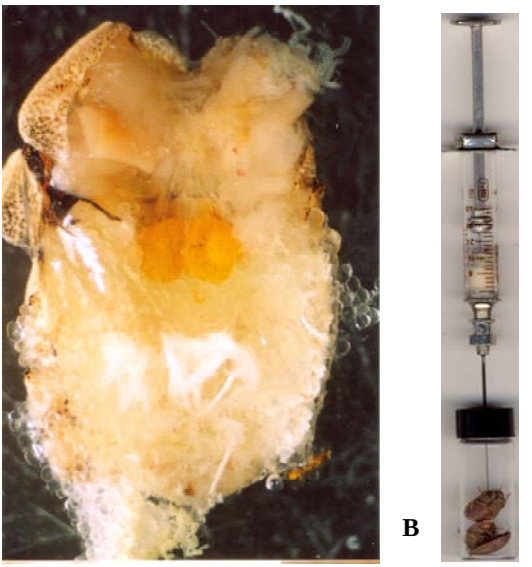

Figure 1. Sampling methods of contents of metathoracic glands of adults of the *E. integriceps,* A) *In vitro* B) *In vivo*

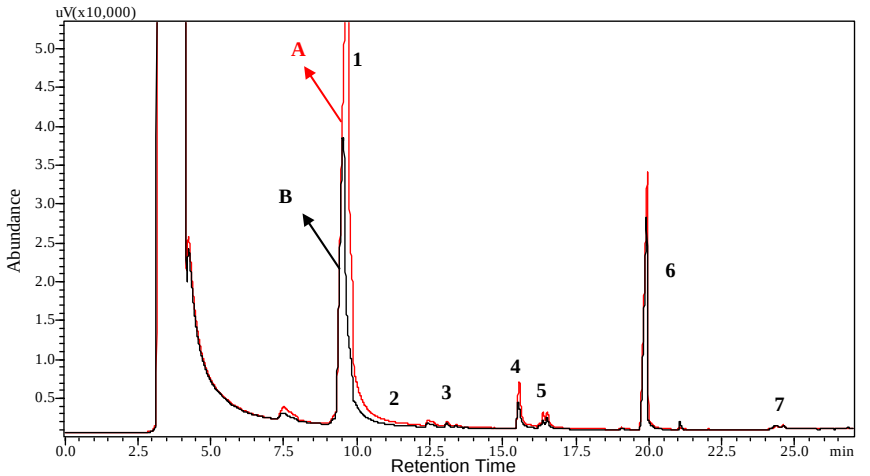

Figure 2. Gas chromatograms of metathoracic scent gland contents from *E. integriceps*, (A)Female, (B) Male. Compounds numbers 1.(E)-2-Hexenal 2.2(5H)-Furanone,5-Ethyle 3.2-Hexen-1-ol,acetate 4.Limonene 5. 5-Decyne 6.Tridecane 7.Nonadecane

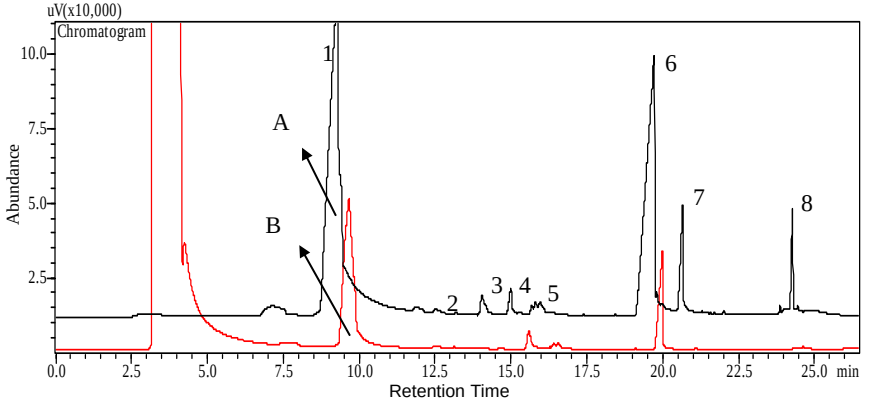

Figure 3. Gas chromatograms of metathoracic scent gland contents from *E. integriceps* in two methods *in vivo* (A) and *in vitro* (B).

Table 1. percentage of compounds in metathoracic gland contents of female and male of *E. integriceps*.

Groups	Chemical compounds	Percentage of compounds	
		Female	Male
Aldehyde	(E)-2-Hexenal	78/1	74/70
lactone	2(5H)-Furanone,5-Ethyle	0/1	0/11
acetate	2-Hexen-1-ol,acetate	2/59	3/14
cycloalcen	Limonene	0/28	0/27
alcene	5-Decyne	0/32	0/44
alcanes	Tridecane	18/11	20/98
	Nonadecane	0/5	0/36

Table 2. comparing area under peaks of detected compounds from *E. integriceps* in two methods (*in vivo* and *in vitro*).

Peak number	Groups	Compounds	Area under GC peaks	
			In vivo	*In vitro*
1	Aldehyde	(E)-2-Hexenal	2513364.7	1375086
2	lactone	2(5H)-Furanone,5-Ethyle	16619.8	4402
3	acetate	2-Hexen-1-ol,acetate	75036	-
4	cycloalcen	Limonene	61649.8	47105.5
5	alcene	5-Decyne	52129.7	1770
6	alcane	Tridecane	1632330.1	279040.2
7	-	unknown	290397.4	1541.8
8	alcane	Nonadecane	135689.1	2614.6