

## RESEARCH

# Morphology and Chemical Analysis of the Metathoracic Scent Glands System in *Adelphocoris suturalis* (Hemiptera: Miridae)

Zhilin Zhang,<sup>1</sup> Jing Luo,<sup>1</sup> Yanan Wang,<sup>1</sup> Longjia Chen,<sup>1</sup> Lizhen Chen,<sup>1,2</sup> and Chaoliang Lei<sup>2,3</sup><sup>1</sup>Hubei Insect Resources Utilization and Sustainable Pest Management Key Laboratory, Huazhong Agricultural University, Wuhan 430070, Hubei, China<sup>2</sup>Corresponding authors, e-mail: lzchen@mail.hzau.edu.cn, ioir@mail.hzau.edu.cn<sup>3</sup>College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, Hubei, China

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**ABSTRACT.** The morphological structure of the metathoracic scent glands (MTGs) in *Adelphocoris suturalis* was observed by utilizing scanning electron microscope (SEM). Also, the secretions of MTGs in male and female were analyzed by using gas chromatography–mass spectrometry (GC-MS). The result showed that the MTGs comprised a reservoir and paired lateral glands, which are connected to a reservoir by duct. The MTGs belong to the diastomian type. A usually depressed channel extends from opening downward the middle of metathorax, a tongue-like structure was covered by bristles and mushroom-shaped cuticular structures, known as evaporative area. In GC-MS investigation, differences were found in quantitative or qualitative compositions of the substances between the two sexes. In our study, hexyl butyrate was the most abundant compound in the MTGs of *A. suturalis*, comprising ~85% of total secretions in both female and male, respectively. 4-oxo-(E)-2-hexenal (5.22%) was the second most abundant compound in female MTGs secretions, whereas octacosane (2.42%) followed hexyl butyrate in male MTGs secretions.

**Key Words:** secretion, scanning electron microscope, gas chromatography–mass spectrometry

The plant bug, *Adelphocoris suturalis* (Hemiptera: Miridae), has become one of the most important insect pests of cotton, *Gossypium hirsutum* L., in the Changjiang River Region of China. The damage is gradually increasing with the commercial cultivation of Bt cotton because the use of broad-spectrum insecticide applications were substantial decreased (Lu et al. 2008a, 2009, 2010; Zhang et al. 2011). The pest causes a substantial economic loss by feeding on cotton organs, causing stunting, and fruit malformation. Because of its high mobility and broad host range, it can rapidly infest a cotton fields from the surrounding vegetation. Conventional chemical control methods often led to the development of pest resistance and environmental pollution. Pheromone-baited traps are an effective tool for monitoring and detecting insect pests because of their efficiency and no harming environment as some other pest management tools (McBrien et al. 1996, 1997). However, little is yet known about it in China.

There are well-developed metathoracic scent glands (MTGs) and Brindley's glands or secretory setae in some species of the Hemiptera (Aldrich 1988). Brindley's glands were found in some families. Nevertheless, most adult hemipteran insects possess a well-developed MTGs system (Aldrich 1988, Ho and Millar 2001). The external efferent system of the MTGs is considered as an important diagnostic feature in the Miridae (Cassis and Schuh 2012). Previous studies indicated that the MTGs are composed of usually well-developed external efferent system and paired lateral glands (Cassis and Schuh 2012). For most miridae, the position of the peritreme is situated on the metepisternum of the metapleuron and opens in between mesocoxae and metecoxae. A usually depressed channel extends from the peritreme, a tongue-like structure is a smooth surface covered with mushroom-shaped cuticular structures (Cassis and Schuh 2012). However, there are suprageneric differences for the evaporative and the position of the peritreme in the Miridae (Cassis 1995).

Most of adults release a large of strong-smelling and irritating defensive chemicals when they are molested or attacked (Ho and Millar 2001, Durak and Kalender 2007a, Durak 2008). Adult of miridae possess well-developed MTGs, which play an important role in defensive and

intraspecific communication, such as defense pheromone, aggregation, and sexual behavior (Zhang and Aldrich 2003a). The volatile communication chemicals secreted from MTGs affect insect behavior depending on their concentrations (Farine et al. 1992). Those compounds are mainly composed of short-chain unsaturated aldehydes, straight-chain esters, and acids, such as (E)-2-hexenal, 4-oxo-(E)-2-hexenal, and hexyl butyrate, which have been also found in other families of Hemiptera (Leal et al. 1994; Durak and Millar 2007a,b). Zhang et al. (2011) proved that males of *A. suturalis* are attracted to the sexually mature virgin females. Moreover, recent studies suggested that some mirid species secrete aggregation or sex pheromones from MTGs (Millar et al. 1997; Staples et al. 2002; Zhang and Aldrich 2003b, 2008).

In this study, we examined the morphological structure of the MTGs in *A. suturalis* by using scanning electron microscopy (SEM). In particular, we compared the chemical composition of the MTGs secretions in female and male by using gas chromatography-mass spectrometry (GC-MS). To the best of our knowledge, this was the first report on the morphological structure and secretions of MTGs of *A. suturalis*, and we also suggested the biological functions of MTGs secretions in *A. suturalis* by compared other related species. This study established the groundwork for further research on the use of sex pheromone for managing *A. suturalis*, as well as a basis for further understanding of biosynthesis and storage of communication chemicals in this important pest species.

## Materials and Methods

**Insect Material.** Nymphs of *A. suturalis* were collected from alfalfa fields in Zhengzhou (Henan Province, China) in June 2010. Insects were maintained at 26°C and 75 ± 5% RH, with a photoperiod of 16:8 (L:D) h in plastic cages (10 by 6 by 4 cm; 20 nymphs per cage) fed on green beans, *Phaseolus vulgaris* L. and a 5% sugar solution (Lu et al. 2008b, Zhang et al. 2011). The emerged adults were separated by gender every day, and same sex were kept in one plastic cage (10 by 6 by 4 cm) with the density of 20 adults per cage before used for further experiments.

**Scanning Electron Microscopy.** The separated, orange-colored MTGs were fixed in 3% glutaraldehyde (0.1 M sodium phosphate buffer, pH = 7.2) for 3 h and then were rinsed with fresh buffer, postfixed with 1% osmium tetroxide (0.1 M sodium phosphate buffer, pH = 7.2), dehydrated in a gradient ethanol series, dried using 1,1,1,3,3,3-hexamethyldisilazane, and coated with gold. The observations were made under a JSM-6390/LV SEM.

**Chemical Analysis.** The MTGs were removed and immersed in 100  $\mu$ l analytical grade n-hexane distilled from calcium hydride and stored at  $-20^{\circ}\text{C}$ . Two independent series of extracts were made, each sex with 20 glands with 8–10-d-old virgin adults. Extracts were analyzed ( $\sim 1$   $\mu$ l of the extract) by splitless injection into a GC-MS on an Agilent 7890A GC series equipped with a polar column HP-5 (30 m by 0.32 mm by 0.25  $\mu$ m) and interfaced to an Agilent 5975C Mass Selective Detector. The GC was programmed at  $50^{\circ}\text{C}$  for 1 min, then rising to  $250^{\circ}\text{C}$  at  $10^{\circ}\text{C}/\text{min}$ , and then held for 5 min and post-run  $280^{\circ}\text{C}$  held for 2 min. The carrier gas was helium with a flow rate of 1 ml/min, and the injector and transfer line temperature was 220 and  $280^{\circ}\text{C}$ , respectively. Compounds were tentatively identified by GC-MS and identifications were comparison of the retention times and mass spectra with those of authentic samples. The molecular structure of compounds was determined by a comparison of the recorded mass spectra with the reference spectra of the NIST library and coinjections of the extracts and synthetic compounds on the two columns. The relative proportions of the compounds in the extracts were obtained by integration of the GC-peak areas.

(E)-2-hexenal (J&K Chemical Co. Ltd., Beijing, China), hexyl acetate (Sigma/Aldrich Co., Ltd., Shanghai, China), 1-hexanol, hexyl butyrate, pentyl butyrate, hexyl hexanoate, hexyl isobutyrate, pentadecane, hexadecane, and Eicosane (TCI Chemicals Co. Ltd, Shanghai, China) were used. (E)-4-oxo-2-hexenal was synthesized as previously described by [Moreira and Millar \(2005\)](#).

## Results

**Studies on MTGs by SEM.** Micrographs showed that the MTGs of *A. suturalis* are composed of well-developed reservoir and paired lateral glands (Fig. 1A). The reservoir is long and bag shaped, and the wall of reservoir comprises some sac-like secretory cells and collecting canals (Fig. 1B). The secretions of sac-like secretory are collected by the collecting duct and transferred to the reservoir. The reservoir is connected to the lateral glands by duct. The lateral glands are multitubular (Fig. 1C). The MTGs have paired openings located on the metathoracic basisternum in between mesocoxae and metacoxae (Fig. 1D). A usually depressed channel is long and extends from opening downward the middle of metathorax. The content of MTGs is probably released through the paired openings and spreads over the cuticle that surrounds each of the openings, known as evaporative areas, while the structure is a tongue-like structure. Bristle and mushroom-shaped cuticular structures are found on the surface of the evaporative areas. However, the bristle only locates the lateral area and points laterad (Fig. 1E). The mushroom-shaped cuticular structure is long and narrow, irregular polygonal surrounding around the peritreme of MTGs (Fig. 1F). Generally, mushroom-shaped cuticular structure extends out four to six ridges, and they are linked to other mushroom-shaped cuticular structure. Furthermore, mushroom-shaped cuticular structures are tightly connected to each other by numerous trabeculae.

**Analysis of MTGs by GC-MS.** The contents of MTGs from *A. suturalis* are analyzed separately by gender. Differences in quantitative or qualitative compositions of the substances are found between the two sexes. Nineteen different chemical substances are determined in the female, 21 different chemical substances are determined in the male, among which only 15 are common (Table 1). The results indicate that hexyl butyrate (85.44%) is most abundant, and heneicosane (0.07%) is least abundant in the females (Table 1; Fig. 2). Hexyl butyrate (84.12%) is the most abundant, whereas pentadecane (0.06%) is the least abundant in the male (Table 1; Fig. 3).

## Discussion

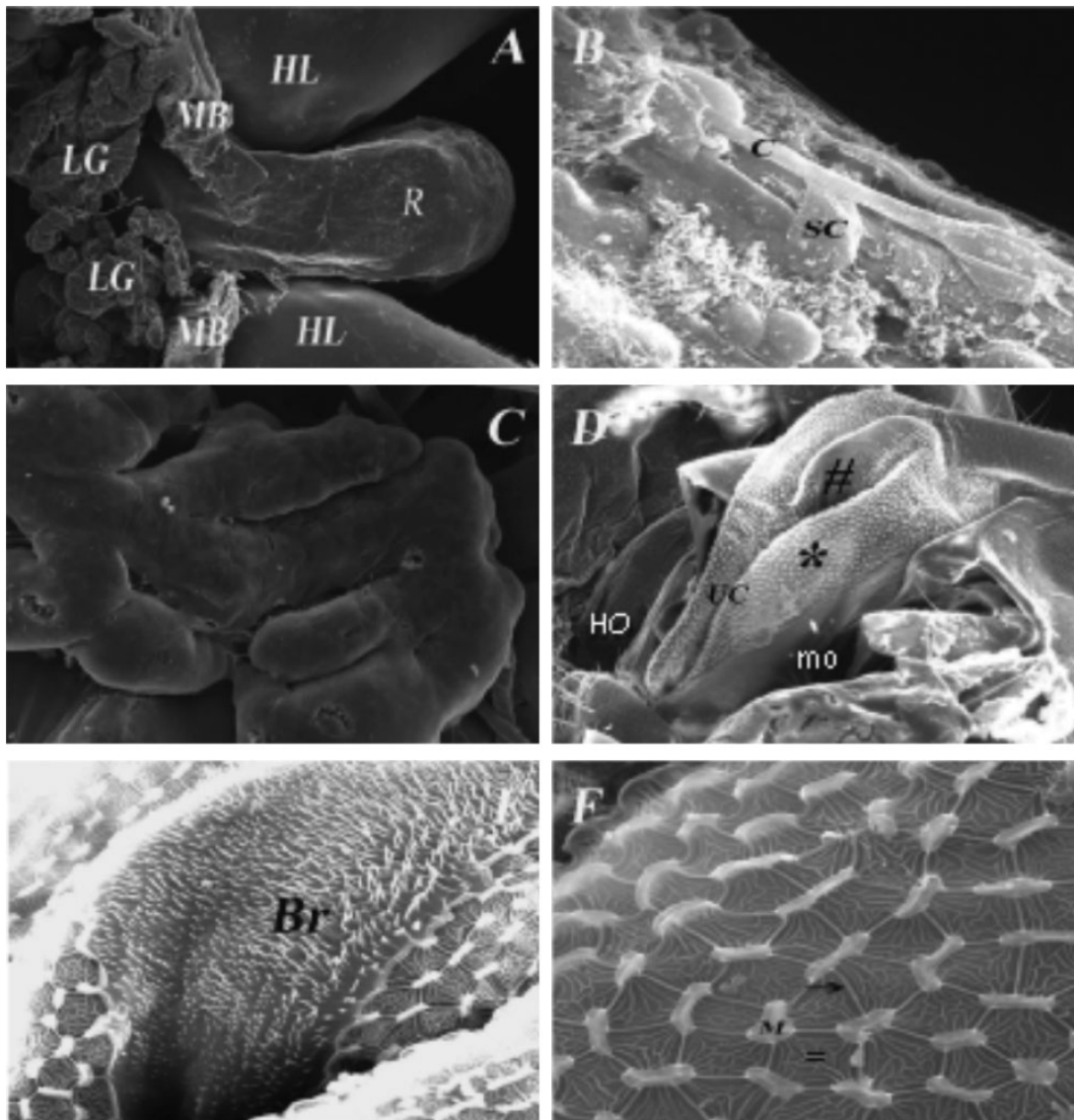
This study examined the morphological structure of *A. suturalis* MTGs in detail by SEM. Moreover, we analyzed the chemical contents of MTGs in both males and females by utilizing GC-MS. The results indicated that there are not differences in between the two sexes. However, differences in quantitative or qualitative compositions of the MTGs volatiles are found between the two sexes.

As exhibited in Fig. 1, the structure of MTGs in *A. suturalis* resembled that of *Nezara viridula* ([Gilby and Waterhouse 1967](#)), constituting of paired lateral glands and one reservoir. Moreover, there was no sexual dimorphism in both female and male of *A. suturalis*. A previous study showed that the shape, size, and development of MTGs in a Reduviid bug varied depending on the nutritional state ([Santos-Mallet and De Souza 1990](#)). Here, we observed various differences in the reservoir related to the nutrition state, whereby in nonfed *A. suturalis* the glands only reached the metathoracic segment. In Hemiptera, there are two types of MTGs on the basis of the number of the opening, the diastomian and the omphalian types ([Carayon 1971](#)). Our findings indicated that the MTGs in *A. suturalis* belong to the diastomian type with paired openings.

In the opening and the evaporation area, there are diverse structures in different families of Hemiptera ([Weirauch 2006](#)). [Cassis and Schuh \(2012\)](#) reported that the evaporative areas of MTGs could be extensive in distribution, greatly reduced or absent in miridae. Our study showed that the evaporation area of *A. suturalis* is extensive in distribution. There are polygonal mushroom-shaped cuticular structures and bristles on the evaporation surface in *A. suturalis*. Furthermore, the mushroom-shaped cuticular structure is present in the Pentatomomorpha, some Cimicomorpha, and Scutelleridae ([Weirauch 2006](#), [Durak and Kalender 2007b](#)). [Remold \(1963\)](#) reported that the mushroom-shaped cuticular structure could prevent the secretion spreading on the evaporative area because of its high viscosity and structure, which is regarded as obstacles to prevent further spreading. Moreover, [Durak and Kalender \(2009a\)](#) hypothesized that its function of the polygonal mushroom-shaped cuticular structure and ridge might have increased evaporation of the MTGs secretions in *Graphosoma lineatum*. We found that bristle only located on the outboard of the opening in *A. suturalis*. In several Reduviidae, a row of bristles located the anterior lateral surface of each hind coxa. And while walking, the bristles brush over the opening of gland to disseminate the secretions ([Schofield and Upton 1978](#), [Weirauch 2006](#)). The polygonal mushroom-shaped cuticular structure might have the functional roles as increasing evaporation of MTGs secretions. However, the function of bristles is unknown.

Our results indicated that the MTGs secretions of both *A. suturalis* sexes existed some differences in quantity and quality. The results we obtained agreed with the results of other authors. For example, [Zhang and Aldrich \(2008\)](#) reported that both sexes of *Phytocoris call* have some differences in quantity and quality by analyzing the MTGs content. Furthermore, principal component is hexyl butyrate in male *P. call* as we observed in *A. suturalis*. In our study, we found that hexyl isobutyrate, hexyl hexanoate, fumaric acid dec-4-enyl propyl ester, and tetradecane are only found in the female, whereas pentadecane, eicosane, tetracosane, nonadecane, hexadecane, and octacosane are only found in the male. Moreover, chemical analyses of the scent glands have been extensively studied in Hemiptera, e.g. Reduviidae, Scutelleridae, Pentatomidae, and Coreidae ([Durak and Kalender 2007a, 2009a; Marques et al. 2007; Moraes et al. 2008](#)), which also show that the common compounds between male and female are different in the quantity. However, concerning on the qualitative variations of composition of MTGs secretions of *A. suturalis*, the biological function for these secretions is questionable and needs further study.

Hexyl butyrate is a major compound of MTGs secretions with diverse function in many mirids ([Zhang and Aldrich 2003b](#)). Some studies propose that the esters and aldehydes secreted by the MTGs of Miridae have a dual function depending on their concentration.



**Fig. 1.** (A) Scanning electron micrograph of MTGs of *A. suturalis*: LG, lateral gland; R, reservoir; MB, metathoracic basisternum; HL, hind leg. (B) Micrograph of reservoir: C, canal; SC, secretory cell. (C) Multitubular lateral glands of MTGs. (D) Opening: UC, usually depressed channel; \*, evaporation areas; #, Opening; HO, hind leg coxal cavity; MO, middle leg coxal cavity; (E) Br, bristle. (F) Evaporation areas: M, mushroom-shaped cuticular structure; →, ridge; =, trabecule.

In *Stenotus rubrovittatus* and *Lygus rugulipennis*, this compound acts as one of sex attractant pheromones (Innocenzi et al. 2001, Yasuda et al. 2008). However, the male secretes to prevent other males from copulating with a female in *Phytocoris difficilis*, *L. lineolaris*, and *Lygocoris pabulinus* (Groot et al. 2001, Zhang et al. 2007, Zhang and Aldrich 2008). Furthermore, Ho and Millar (2002) demonstrated that this compound could attract scavenging chloropid and milichiid flies in *Lygus* spp. (Zhang and Aldrich 2003c). Hexyl butyrate was identified in both sex of *A. suturalis*, suggesting that this compound may have a dual function, and we are planning to investigate the functions of hexyl butyrate in *A. suturalis* both in the lab and the field.

(E)-2-hexenal is identified in female of *A. suturalis*. It has been attested as a defensive compound in ants, beetles, cockroaches, and true bugs (Staples et al. 2002). However, this compound also acts as an alarm pheromone in *Cimex lectularius* (Harraca et al. 2010). Moreover, it is found to be an aggregation or repellent pheromone in *N. viridula* according to the amount (Farine et al. 1992). 4-Oxo-(E)-2-hexenal is identified in both male and female of *A. suturalis*. It is a defensive compound to protect the nymphs against predation in *C. hemipterus* and

*C. lectularius* (Harraca et al. 2010, Liedtke et al. 2011). Besides, Elyahu et al. (2012) reported that the 4-oxo-(E)-2-hexenal is most abundant compound as the defensive secretions of fifth-instar nymphs of *Chinavia aseada*, *Ch. impicticornis*, *Dichelops melacanthus*, and *Piezodorus guildinii*. However, 4-oxo-(E)-2-hexenal is also identified to be one of sex-attractant pheromones in *S. rubrovittatus* and *L. rugulipennis* (Innocenzi et al. 2001, Yasuda et al. 2008). The authors suggest that this compound may have a dual function.

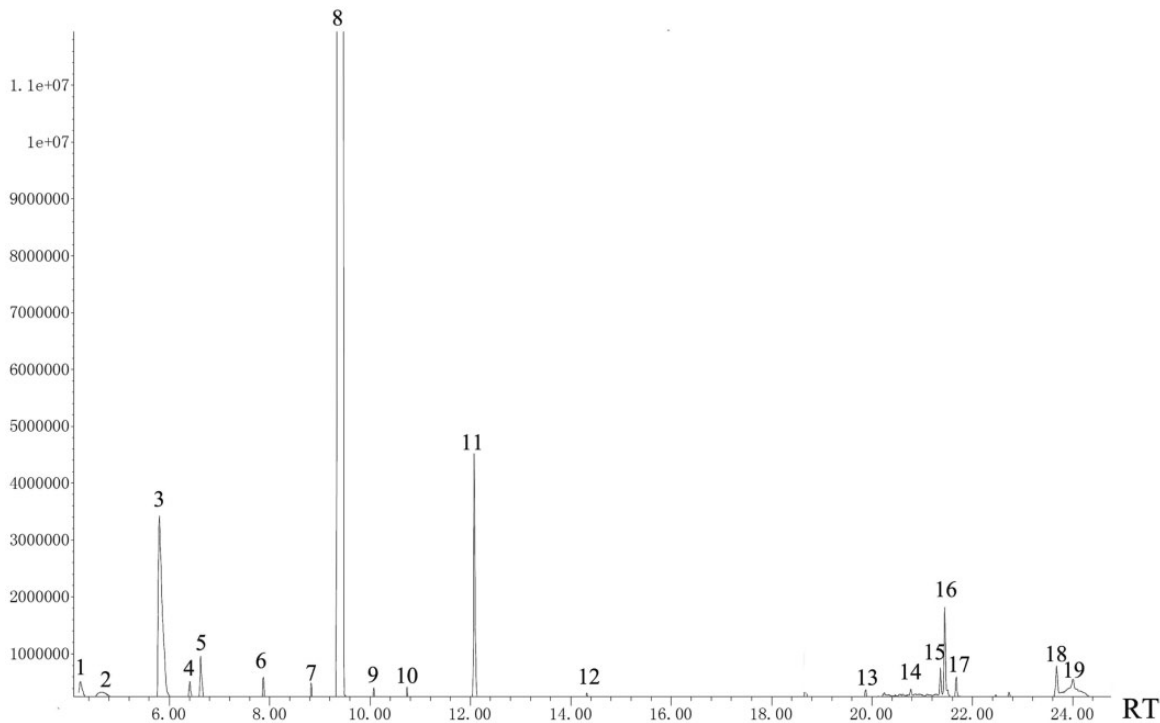
Some studies revealed that the 1-hexanol is found in male and female volatiles of *Campylomma verbasci* and *P. calli* Knight as our result in the GC-MS investigation (Smith et al. 1991, Zhang and Aldrich 2008). Chinta et al. (1994) found that 1-hexanol possesses EAD active for *L. lineolaris*. Moreover, Wardle et al. (2003) found that it may serve as allelochemicals rather than pheromones in the study of inactivity of 1-hexanol against adult *L. lineolaris*. However, the biological function is still unknown in this insect.

In our study, the quantity of alkane secreted by the MTGs in *A. suturalis* is less than that of ester. Moreover, the result indicated that alkane in male is more in quantity or quality than that of female. In the

**Table 1. Relative abundance of compounds in metathoracic gland scents secretion of female and male *A. suturalis***

Groups	Chemical compounds	<i>A. suturalis</i>		
		Female %	Male %	
Alcohol	1-Hexanol	1.42	2.23	
Aldehyde	(E)-2-Hexenal	0.79	ND	
	4-oxo-(E)-2-Hexenal	5.22	1.00	
Ester	Pentyl butyrate	0.31	0.34	
	Hexyl isobutyrate	0.25	0.25	
	Hexyl butyrate	85.44	84.12	
	Isovalericacid hexylester	0.15	0.18	
	Hexyl n-valerate	0.16	0.129	
	Hexyl hexanoate	2.22	0.07	
	Fumaric acid dec-4-enyl propyl ester	0.10	ND	
	Hexyl acetate	0.71	0.32	
	Tributyl acetylcitrate	0.23	ND	
Alkanes	Pentadecane	ND	0.06	
	Hexadecane	ND	0.09	
	Pentadecane,2,6,10,14-tetramethyl	ND	0.19	
	Eicosane	ND	0.11	
	Heneicosane	0.07	1.58	
	Docosane	0.21	0.51	
	9-Tricosene	0.81	1.12	
	Tricosane	0.18	1.33	
	Tetracosane	ND	1.78	
	1-Heptacosane	0.32	0.27	
	Octacosane	0.44	2.42	
	Benzene	Benzene,1,2,3-trimethyl	0.69	ND
	Alkenes	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-	ND	0.112

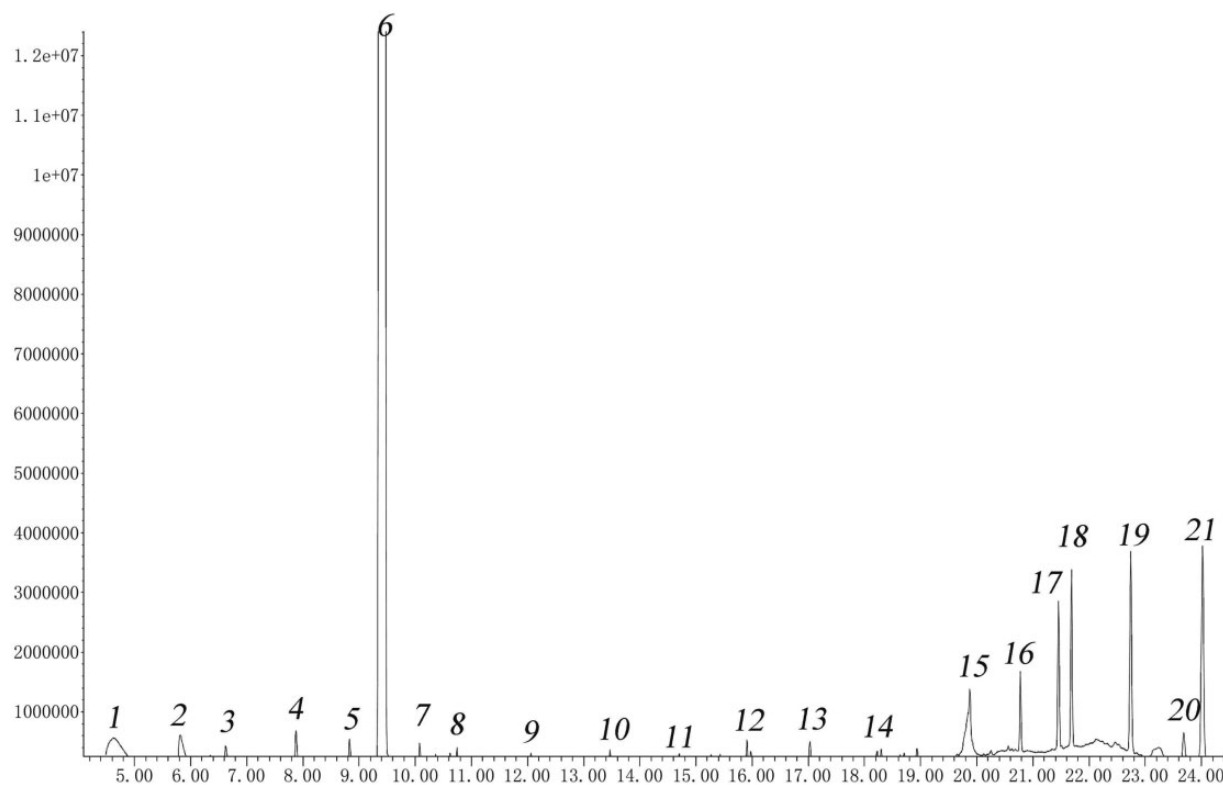
ND, not detected.



**Fig. 2.** Gas chromatogram of extract of the MTG secretion of *A. suturalis* female. 1, (E)-2-hexenal; 2, 1-hexanol; 3, 4-oxo-(E)-2-hexenal; 4, benzene,1,2,3-trimethyl; 5, hexyl acetate; 6, pentyl butyrate; 7, hexyl isobutyrate; 8, hexyl butyrate; 9, isovalericacid hexylester; 10, hexyl n-valerate; 11, hexyl hexanoate; 12, fumaric acid dec-4-enyl propyl ester; 13, heneicosane; 14, docosane; 15, tributyl acetylcitrate; 16, 9-tricosene; 17, tricosane; 18, 1-heptacosane; 19, octacosane; RT, retention time in minutes.

study of *Rhaphigaster nebulosa* and *G. lineatum*, n-tetracosane was detected only in male (Durak and Kalender 2009a,b), which is similar to our study. Pentadecane is detected only in male in our study. However, Durak (2008) reported that pentadecane was detected in both sex in *Dolycoris baccarum*. The alkanes are reported that they function

as short range attractants and defense pheromones (Lockwood and Story 1985, Borges and Aldrich 1992, Krall et al. 1999), while which are diverse in different insect species. Some articles reported that the pentadecane and n-tetracosane have functions of defensive pheromone in Reduviidae, Scutelleridae, Pentatomidae, and Coreidae (Zarbin et al.



**Fig. 3.** Gas chromatogram of extract of the MTG secretion of *A. suturalis* male. 1, 1-hexanol; 2, 4-oxo-(E)-2-Hexenal; 3, hexyl acetate; 4, pentyl butyrate; 5, hexyl isobutyrate; 6, hexyl butyrate; 7, isovaleric acid hexylester; 8, hexyl n-valerate; 9, hexyl hexanoate; 10, pentadecane; 11, hexadecane; 12, pentadecane, 2,6,10,14-tetramethyl-; 13, eicosane; 14, 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-; 15, heneicosane; 16, docosane; 17, 9-tricosene; 18, tricosane; 19, tetracosane; 20, 1-heptacosane; 21, octacosane; RT, retention time in minutes.

2000; Durak and Kalender 2007a, 2009a). In addition, their function is thought to be solvent and released substrates of other volatile compounds in other families of Hemiptera (Gunawardna and Herath 1991, Ho and Millar 2001). And octacosane is found to possess the function in penetrating the cuticle of enemies and delaying evaporation as other alkane (Blum and Brand 1972, Farine et al. 1992, Durak 2008). Krall et al. (1999) indicate that alkane may have some other functions in *Cosmopepla bimaculata*. Nevertheless, their biological function roles of alkanes in *A. suturalis* are unknown.

In conclusion, this study is the morphological description and chemical composition analysis of MTGs in *A. suturalis*. Ester, aldehyde, and alkane are the major compounds in MTGs contents. In mirid, ester, and aldehyde of MTGs, contents are primarily for defense and have role as sex or aggregation pheromones. Moreover, males of *A. suturalis* could be attractive to females (Zhang et al. 2011). Further studies are necessary to investigate the biological function of ester-aldehyde combination and each compound. In summary, this research has provided useful information on MTGs compounds of *A. suturalis*, and the results will be essential parts for further studies on functional roles and application in pest control of *A. suturalis*.

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