Study of the Alarming Volatile Characteristics of *Tessaratoma papillosa* Using SPME–GC–MS

Zhuo-Min Zhang[1,2], Wen-Wei Wu[1], and **Gong-Ke Li[1,*]**

[1]School of Chemistry and Chemical Engineering, Sun Yat-Sen University, Guangzhou 510275, P.R. China and [2]Key Laboratory of Analysis & Detection for Food Safety (Fuzhou University) Ministry of Education, College of Chemistry and Chemical Engineering, Fuzhou University, Fuzhou, Fujian 350002, P.R. China

Abstract

The stinkbug's volatile compositions would alter very much before and after stinkbugs were disturbed or irritated, which caused the alarming effect. An efficient headspace solid-phase microextraction sampling method was established to study the alarming volatile characteristics and potential alarming volatiles of stinkbugs (*Tessaratoma papillosa*) followed by gas chromatography–mass spectrometry detection. The number of volatiles identified was 16 and 22 before and after stinkbug irritation, respectively. Long-chain alkanes, alkenes, and alcohols consisted of the main volatile compositions of *Tessaratoma papillosa*. When stinkbugs were disturbed, the typical unsaturated volatiles were released, especially including a series of tridecane derivatives. In comparison with the volatile compounds of lichi leaf and flower (plants the stinkbug eats), it could be seen that most stinkbug alarming volatiles were synthesized by the insects themselves, and that they do not originate from their food. The different statistical alarming volatile characteristics of *Tessaratoma papillosa* before and after irritation were interpreted by principal component analysis in the original Chromatography Data Processing System. However, temperature and light did not affect the alarming volatile characteristics. The variety of the stinkbug alarming volatile characteristics before and after irritation was specified by common model strategy. Tridecane, [E]-2-hexenal, dodecane, [E]-2-hexen-1-ol acetate, and 2,3-dimethyl-1-pentene contributed most to the various alarming volatile characteristics before and after irritation, which might be the potential alarming volatiles. It is hoped that this work will provide useful information for insect control.

Introduction

Insects produce all kinds of chemical substances, including ester, aldehyde, alcohol, and other compounds, which are related to insect communication, defense, and alarm. Many compounds have been identified as useful stinkbug pheromones, such as hexyle butyrate, [E]-2-hexenyl butyrate, [E]-2-hexenal, hexanol, hexyle butanoate, and so on (1–4). In particular, the stinkbug alarming pheromones have aroused great interest from analysts. Most stinkbug alarming pheromones are volatile emanations from their scent glands. Volatile compositions change, obviously, when stinkbugs feel an approaching menace. The volatiles released by the irritated stinkbugs repel the other creatures or give warning information to their congeners, which is called alarming effect (5).

Tessaratoma papillosa is a kind of common stinkbug (5). *Tessaratoma papillosa* usually lives on the lichi trees in South China and sucks the plant juice, which make the leaf and flower sear. Thus, *Tessaratoma papillosa* has been considered as a pest of lichi plants. It is very important to study the alarming volatile compositions of *Tessaratoma papillosa* and distill potential alarming volatiles, which would provide helpful clues for the interdiction of insect communication and facilitate insect control. Although several previous works have focused on the qualification or semi-quantification of stinkbug secretion components, there are still no systematic and/or statistical reports studying the variety of all alarming volatile characteristics of *Tessaratoma papillosa* before and after irritation. The modern chromatographic methodologies, such as gas chromatography–mass spectrometry (GC–MS), and the corresponding chemometric strategies could effectively interpret the statistical alarming volatile characteristics of *Tessaratoma papillosa* and distill potential alarming volatiles.

Conventional sampling methods for the stinkbug volatiles were mainly in-vitro modes. Stinkbugs were anesthetized with CO_2 or ethyl acetate and then killed by freezing. Stinkbug scent glands were removed from the insect bodies and immersed in small volumes of organic solvent for the volatile extraction (1-4). These methods required long time, large amounts of solvents, and multiple steps. Moreover, the volatile compounds obtained by the conventional sampling method cannot represent the original volatile composition of stinkbugs. However, due to their relative simplicity and straightforwardness, the conventional sampling methods were still applied to collect the insect emanations. Solid-phase microextraction (SPME), developed by Pawliszyn and his co-workers (6,7), is a simple and solvent-free sampling method. SPME has been widely used in the environmental (8), biological (9), pharmaceutical (10), field analyses (11), and fragrance and aroma studies (12). Headspace (HS) SPME has been considered a suitable sampling method for volatile organic compounds from living biological samples (12,13). HS-SPME has especially been used to collect the

* Author to whom correspondence should be addressed: email cesgkl@mail.sysu.edu.cn

volatiles from animal samples, such as human skin, cow breath, and insects, achieving better sampling efficiency than the conventional methods (14–16).

In this study, alarming volatile characteristics and alarming volatiles of *Tessaratoma papillosa* were studied by HS-SPME coupled with GC–MS. The statistical alarming volatile characteristics before and after irritation of stinkbugs were interpreted by principal component analysis (PCA) in the original Chromatographic Data Processing System. Potential stinkbug alarming volatiles related to the variety of alarming volatile characteristics both before and after irritation and were specified by a common model strategy.

Methods and Materials

Tessaratoma papillosa

More than 200 stinkbugs (*Tessaratoma papillosa*) were obtained from a lichi orchard in Zengchen (Guangdong, China) during lichi florescence in 2006. Sample collection was conducted once a day in the morning. All stinkbugs were selected for uniformity in size and color. The body length of stinkbug imagoes was selected as > 2.0 cm, while the body length of stinkbug larvae was selected as < 0.5 cm. After collection, stinkbugs were transferred to the lab within one day and fed with lichi leaves. Stinkbugs were randomly distributed into groups of 6 samples and stored in glass bottles. Each batch of stinkbugs was analyzed within 3 days.

Sample preparation

The experimental conditions of HS-SPME potentially influencing the extraction process in the study included the type of SPME fiber coating and extraction time. Replicate measurements were performed to optimize HS-SPME conditions in the study. The number of the volatiles identified and the normalized amount of tridecane (the volatile with highest amount) were used as the symbols to decide the efficiency of HS-SPME and the desorption efficiency of the volatiles. The type of SPME fiber coating preferred in the study was 65 µm polydimethylsiloxane-divinylbenzene (PDMS–DVB) (Supelco, Inc., Bellefonte, PA) due to their similar polarities to the non- or mid-polar volatiles.

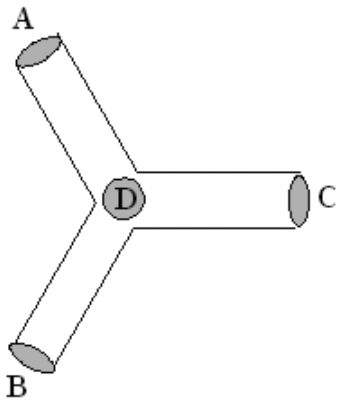

Figure 1. The original Y-shaped apparatus for studying the alarming effect.

The extraction time of 30 min was chosen as the optimal extraction time according to the extraction efficiency in the work in comparison with other extraction times (5, 15, 30, and 45 min). Stinkbugs could also obtain enough air in the 40-mL glass vial when the extraction was conducted for 30 min.

For each measurement, a stinkbug from one group was placed in a 40-mL glass vial, followed by HS-SPME exposure for 30 min. Then, the stinkbugs were irritated by perforating their hypogastriums with a needle. After irritation, the stinkbug volatiles were sampled by HS-SPME again. Stinkbugs were kept alive during the sampling procedure. Finally, HS-SPME fiber coating containing volatiles was thermally desorbed by inserting the fiber into GC injector set at 250°C in splitless mode for 5 min. For studying the effect of temperature change on the alarming volatile characteristics, a comparison study was conducted by putting the glass vial in a 50°C water bath for 10 min, followed by HS-SPME sampling. For studying the effect of light change to the alarming volatile characteristics, a comparison study was based on the data between diurnal and night sampling projects.

In order to preliminarily discuss the resource of the stinkbug alarming volatiles, the volatile compounds of lichi leaves and flowers, respectively, were also studied by HS-SPME–GC–MS. Both lichi leaves and flowers were washed with tap water, followed by rinsing with deionized water to remove the dirt on the surface, and dried naturally. Every 10.0 g lichi leaf or flower was randomly distributed to one group. Then 2.0 g leaf or flower from one group was rubbed with 5 mL deionized water in a commercial glass mortar (Guangdong Huabopi, Guangzhou). After that, 2.0 g sample tissue homogenate was put in a 15-mL glass vial followed by HS-SPME (65 µm PDMS–DVB) exposure for 30 min. Finally, the volatile compounds were thermally desorbed by inserting the fiber into the GC injector set at 250°C in splitless mode for 3 min.

GC–MS analysis

A Hewlett-Packard (HP) 6890 GC HP 5973 mass detector system was used in the study. Chromatographic separation was

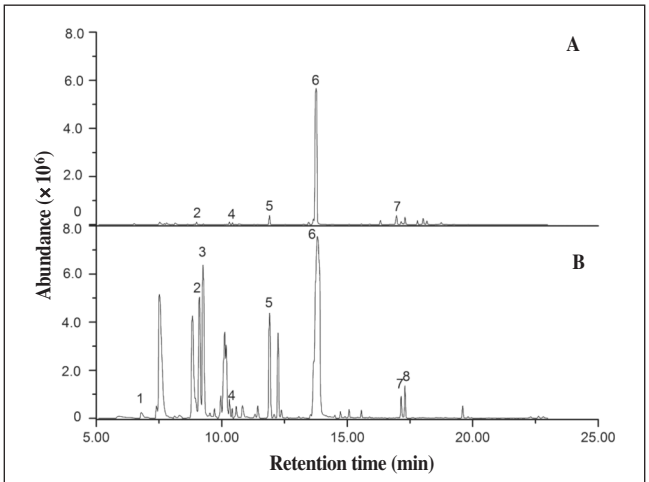

Figure 2. The volatile chromatograms of *Tessaratoma papillosa* before (A) and after (B) irritation by HS-SPME. The peak numbers correspond to the main volatiles of irritated *Tessaratoma papillosa*. 1, 2,3-Methyl-1-pentene; 2, [E]-2-hexen-1-ol acetate; 3, [E]-2-hexenal; 4, undecane; 5, dodecane; 6, tridecane; 7, 1-hexadecanol; 8, pentadecane.

performed with an HP-VOC (Agilent Scientific, Palo Alto, CA) capillary column (60 m × 0.32 mm i.d. × 1.8 µm film thickness) using the following instrumental conditions: Ultra-pure helium flow, 1 mL/min; injector temperature, 250°C; transfer line temperature, 280°C; energy of electron, 70 eV; oven temperature ranged from 60°C to 200°C at ramp rate of 20°C/min, held at 200°C for 5 min, and then from 200°C to 260°C at a ramp rate of 5°C/min, 260°C for 5 min. The parameters of HP 5973 mass detector were: ion mass/charge ratio, 30–550 *m/z*; scan mode.

Study of alarming effect by original Y-shape apparatus

Once stinkbugs were disturbed or irritated, they would strongly release typical volatiles alarming their congeners to evade the coming menaces or dangers. In the paper, the original aluminous Y-shape apparatus (Figure 1) was designed to preliminarily study the alarming effect of *Tessaratoma papillosa*. A, B, and C ports were in three vertexes of an equilateral triangle. The distance from A, B, and C ports to the entrance, D, was the same. One, 2, or 3 irritated stinkbugs were placed in the A port of the Y-shaped apparatus, and 15 normal stinkbugs were put into the apparatus through the D entrance. The total stinkbug numbers leaving the apparatus from B and C ports were calculated to evaluate the alarming effect. Duplicated experiments were done in the study.

Chromatographic data processing system

In this study, the chromatographic data were analyzed by an original Chromatographic Data Processing System (17) based on the Matlab 6.5. The original data of the chromatograms acquired from the GC–MS were exported and transformed to an "$m \times 2$" matrix ("m" represents frequencies of MS data-collecting). The chromatograms were smoothed by wavelet transform and polynomial smoothing strategies in this system. The first and second columns in this "$m \times 2$" matrix represented the time of MS data-collecting and the corresponding MS responses, respectively. After normalization, the data of the total chromatograms of all the investigated samples were merged into an "$m \times n$" matrix ("n" represents the numbers of the aroma chromatograms). Finally, PCA and common model were performed based on this "$m \times n$" matrix.

Table I. Volatiles of *Tessaratoma papillosa* Before/After Irritation by HS-SPME

Volatiles	Retention time (min)	Fit*	Normalized amounts of volatiles (%)† (n = 6)	
			Before irritation	After irritation
Alkenes				
2,3-Dimethyl-1-pentene	6.79	87	–	2.71 ± 0.42
[E]-1-Phenyl-1-butene	10.72	91	< 0.50	–
[Z]-3-Hexadecene	14.51	90	–	< 0.50
[E]-3-Tetradecene	15.42	97	–	< 0.50
α-Farnesene	17.80	95	< 0.50	< 0.50
β-Bisabolene	18.17	98	< 0.50	< 0.50
2,6-Dimethyl-2,4,6-octatriene‡	18.74	86	< 0.50	–
Alkanes				
Undecane	10.30	92	1.07 ± 0.15	0.55 ± 0.07
Dodecane‡	11.90	96	6.84 ± 0.71	6.67 ± 0.55
Tridecane‡	13.76	97	62.16 ± 5.27	26.75 ± 0.76
7-Methyl tridecane	14.65	94	–	< 0.50
5-Methyl tridecane	14.73	90	–	< 0.50
2-Methyl tridecane	14.91	90	–	< 0.50
3-Methyl tridecane	15.07	91	< 0.50	< 0.50
Tetradecane	15.56	96	< 0.50	< 0.50
Pentadecane	17.30	95	–	0.97 ± 0.08
Alcohols				
1-Tridecanol	13.65	91	0.74 ± 0.11	–
1-Hexadecanol§	17.14	87	< 0.50	0.81 ± 0.14
Aldehydes				
[E]-2-Hexenal‡,§	7.53	95	–	17.21 ± 0.91
[E,E]-2,4-Hexadienal‡	8.30	93	< 0.50	< 0.50
[E]-2-Octenal	10.11	87	–	< 0.50
Nonanal§	10.67	86	< 0.50	–
Octadecanal	19.60	95	–	< 0.50
Ketones				
5-Ethyl-2[5H]-furanone	8.99	86	0.68 ± 0.17	–
1,4-Cyclohexanedione	9.96	91	–	< 0.50
3-Methyl-2-cyclohexene-1-one	10.07	87	–	< 0.50
Esters				
[E]-2-Hexen-1-ol acetate	9.25	91	< 0.50	6.25 ± 0.66
Nonanoic acid methyl ester	12.52	86	< 0.50	–

* Fit value indicates to what degree the target spectrum matches the standard spectrum in the NIST library (100 relates to a perfect fit).
† Normalized amounts of volatiles (%) = Peak area of a volatile / Total peak area of all volatiles ± SD. The SDs of stable main volatiles (> 0.5%) are calculated in the table.
‡ The compound is also found in the volatile compounds of lichi leaves and flowers.
§ The volatile is further identified using the corresponding standard compound.

Results and Discussion

The results of the alarming effect experiments

When stinkbugs feel approaching menaces, they strongly release typical alarming volatiles to inform their congeners to evade the coming menaces or dangers. In the study, an original aluminous Y-shaped apparatus was designed to preliminarily study the alarming effect. When 1, 2, and 3 irritated bugs were put into port A of the Y-shaped apparatus, respectively, most normal stinkbugs left the apparatus from ports B and C. The number of stinkbugs that left the apparatus from ports B and C was 11, 12, and 13, corresponding to the 1, 2, and 3 irritated bugs in port A, respectively. The results tentatively suggested that the typical volatiles released by the irritated *Tessaratoma papillosa* might give warning information to their congeners and cause the alarming effect. However, due to the limitation of the stinkbug samples, further confirmation of the alarming effect should be done in the future when more stinkbugs are available.

The volatile compositions of *Tessaratoma papillosa* before and after irritation

The volatiles thermally desorbed from the SPME fiber coating were identified according to the standard mass spectra of the National Institute of Standards and Technology (NIST) MS library. When the mass spectral fit values were at a default value of 85 or above, volatiles were considered "identified". Some crucial volatiles were further confirmed by comparing their retention times with corresponding standards when available. The variability of the retention times between the volatiles and corresponding standards was < 0.05 min. The chromatograms of volatile compounds of *Tessaratoma papillosa* before and after irritation by HS-SPME are illustrated in Figure 2. According to different degrees of certainty, the identified number of volatiles was 16 and 22 before and after irritation, respectively. The qualification and semi-quantification results are listed in Table I. The percentage of the area counts of the identified peaks in the aroma chromatograms was more than 61.11% of the total area counts of the peaks in the total ionization chromatograms (TICs). Except the identified peaks, there are many other discernable peaks in the chromatograms with the fit value < 85, which need further confirmation when the authentic compounds are available. The percentage of the area counts of the unidentified peaks in the aroma chromatograms was more than 38.89%. The juice of lichi leaves and flowers are the food for *Tessaratoma papillosa*. In order to preliminarily discuss the resource of stinkbug volatiles, the volatiles of lichi leaves and flowers were also sampled and identified by HS-SPME-GC–MS (Table II). The reproducibility of volatile compositions of stinkbugs before and after irritation can be evaluated from the standard deviation (SD, n = 6) of the main volatiles present (> 0.5% of total composition). The corresponding relative standard deviations (RSDs) ranged from 2.8% to 25.0%. The fluctuation of the retention times of all the identified peaks was < 0.05 min.

In the study, the identified volatiles could be mainly divided into six groups according to the diverse functional groups, which are alkene, alkane, alcohol, aldehyde, ketone, and ester. Most volatiles of *Tessaratoma papillosa* were even-numbered compounds, especially C6 compounds. However, the volatile with the highest relative amounts was the impar-carbon tridecane, which has been identified as one of the most important compounds released by stinkbugs (1,3,18). The comparison between the tridecane spetrum in a real sampling project and NIST spectrum is demonstrated in Figure 3. Many volatiles possessed the stable *trans*-structure, such as [E]-1-phenyl-1-butene, [E]-3-tetradecene, [E]-2-hexenal, etc. Only one *cis*-structure compound, [Z]-3-hexadecene, was detected in the study. The previous reports also suggested that the stinkbug volatiles were usually stable compounds with *trans*-structure (19). The comparison of stinkbug volatiles with those of lichi leaves and flowers suggested that only five volatiles, namely 2,6-dimethyl-2,4,6-octatriene, dodecane, tridecane, [E]-2-hexenal, and [E,E]-2,4-hexadienal, were found in the volatile compositions of both stinkbugs and their food. Moreover, these compounds were not the main volatile components of lichi leaf and flower. The results suggested that most volatiles released by *Tessaratoma papillosa* are synthesized by insects themselves and not from their food, which has also been potentially proved by the previous works (1,20). The volatile compositions of

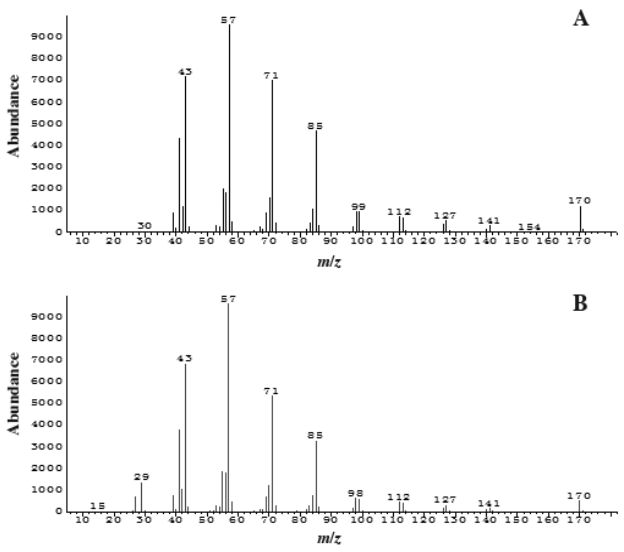

Figure 3. The comparison between the tridecane spetrum in a real sampling project (A) and NIST spectrum (B).

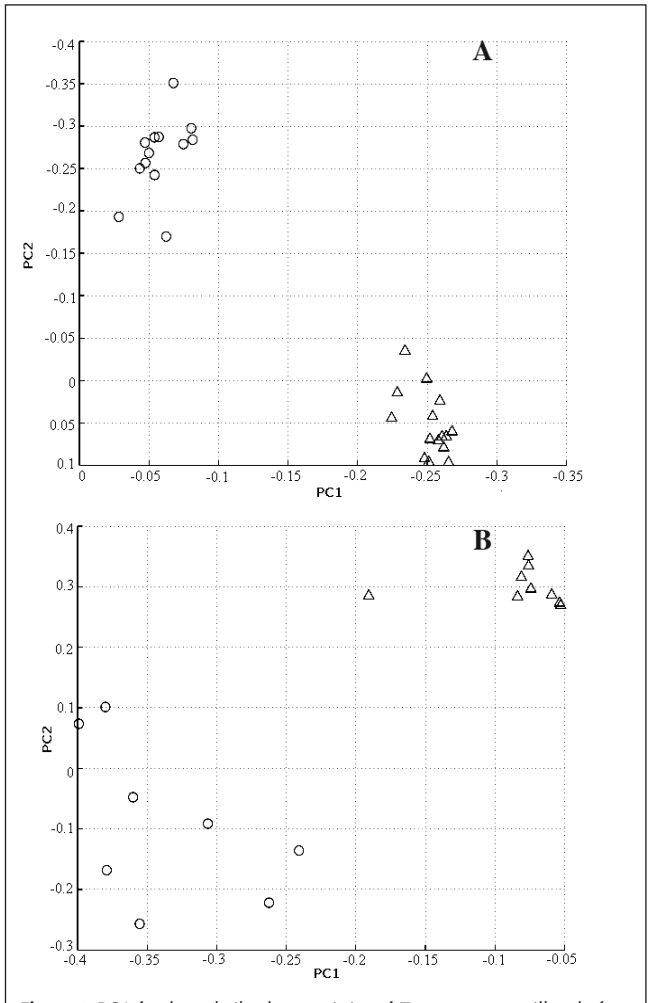

Figure 4. PCA for the volatile characteristics of *Tessaratoma papillosa* before (°) and after (Δ) irritation by HS-SPME: Imagoes (A); Larvae (B).

Tessaratoma papillosa before and after irritation by SPME were different. The volatile species and amounts of the irritated stinkbugs dramatically increased and resulted in a strong aroma. Unsaturated 2,3-methyl-1-penten, [E]-2-hexenal, and [E]-2-hexen-1-ol acetate firstly appeared in the volatile compositions of irritated stinkbugs in high amounts. Another interesting point was the appearance of a series of tridecane derivatives, such as 2-methyl tridecane, 5-methyl tridecane, and 7-methyl tridecane. Tridecane predominated over the main volatile compositions of irritated stinkbugs. Therefore, it was expected that these tridecane derivatives originated from tridecane after irritation of stinkbugs. On the other hand, the relative amounts of [E]-1-phenyl-1-butene, 2,6-dimethyl-2,4,6-octatriene, 1-tridecanol, [E]-2-octenal, 5-ethyl-2[5H]-furanone, and nonanoic acid methyl ester decreased after irritation.

Table III. Top 5 Compounds Contributing to the Difference of the Stinkbug Volatile Characteristics Before and After Irritation

Volatile compounds	Contribution (%)*
Tridecane	34.57
[E]-2-Hexenal	28.83
Dodecane	10.21
[E]-2-Hexen-1-ol acetate	9.31
2,3-Dimethyl-1-pentene	4.23

* Contribution (%) = Peak area of a volatile in common model chromatogram / Total peak area of all volatiles in common model chromatogram × 100.

Table II. The Volatiles of Lichi Leaves and Flowers by HS-SPME

Volatiles	Retention time (min)	Fit*	Normalized amounts of volatiles (%)† (n = 6)	
			Lichi leaves	Lichi flowers
Alkenes				
α-Pinene	8.87	95	0.13 ± 0.02	–
β- Myrcene	9.40	90	–	0.12 ± 0.01
β-Pinene	9.72	96	< 0.10	–
[E]-3,7-Dimethyl-1,3,6-octatriene	10.15	94	–	< 0.10
[Z]-3,7-Dimethyl-1,3,6-octatriene	10.40	93	–	< 0.10
3-Carene	10.99	87	–	< 0.10
3,7-Dimethyl-2,4,6-octatriene	11.58	94	–	< 0.10
2,6- Dimethyl-2,4,6-octatriene	12.16	94	–	< 0.10
1,3,8-p-Menthatriene	12.44	93	–	< 0.10
3-Isopropenyl-1-isopropyl-4-methyl-4-vinyl-1-cyclohexane	18.00	97	–	0.23 ± 0.03
α-Cubebene	18.35	98	0.25 ± 0.02	1.00 ± 0.03
Ylangene	19.22	99	–	0.28 ± 0.01
Copaene	19.34	99	1.58 ± 0.08	5.42 ± 0.13
Caryophyllene	20.86	99	12.23 ± 0.41	10.15 ± 0.60
α-Caryophyllene	21.87	97	3.51 ± 0.35	6.87 ± 0.21
Alkanes				
Sabinene	9.48	97	–	< 0.10
Dodecane	13.38	92	0.12 ± 0.01	–
Tridecane	15.86	93	< 0.10	–
β-Elemene	19.59	91	–	0.28 ± 0.01
Alcohols				
[Z]-3-Hexen-1-ol	7.49	96	0.24 ± 0.03	< 0.10
1-Octen-3-ol	9.22	90	–	0.16 ± 0.01
Aldehydes				
[E]-2-Hexenal	7.55	91	3.00 ± 0.30	0.27 ± 0.04
[E,E]-2,4-Hexadienal	8.46	91	0.24 ± 0.02	–

* Fit value indicates to what degree the target spectrum matches the standard spectrum in the NIST library (100 relates to a perfect fit).
† Normalized amounts of volatiles (%) = Peak area of a volatile / Total peak area of all volatiles ± SD.

Chemometric study for alarming volatile characteristics and potential alarming volatiles

The volatile compositions of *Tessaratoma papillosa* before and after irritation have tentatively suggested a difference in alarming volatile characteristics. To statistically interpret the different alarming volatile characteristics, a PCA method was established to study the data of the corresponding chromatograms of volatile compounds of stinkbugs before and after irritation from the HS-SPME sampling projects in the original Chromatographic Data Processing System. The first two principal components, PC1 and PC2, were used to provide a convenient visual aid for identifying in-homogenity in the data sets. The clustering principles of stinkbug alarming volatile characteristics before and after irritation in PCA model were demonstrated in Figures 4–6. In the PCA model, for both imagoes and larvae the alarming volatile characteristics of *Tessaratoma papillosa* before and after irritation showed different clustering principles (Figure 4). However, temperature and light seemed not to obviously affect the alarming volatile characteristics. The alarming volatile characteristics did not show great different PCA segregations (Figure 5 and Figure 6) when temperature and light conditions changed. Because PCA was conducted on all chromatographic data, the clustering difference was caused by the entire difference in alarming volatile characteristics.

The statistical differences of alarming volatile characteristics were related to typical volatiles, which might possess the possibility to be the stinkbug's potential alarming volatiles. To further specify typical volatiles inducing the obvious segregations of stinkbug alarming volatile characteristics before and after irritation, a common model strategy was performed to compare the chromatograms of two respective data groups in the data processing system. The top five volatiles contributing greatly to the difference in alarming volatile characteristics are exported in Table III. The statistical results suggested that long-chain alkenes, alkanes, aldehydes, and esters formed the primary distinction of alarming volatile characteristics. Some volatiles have been considered as potential chemical bio-markers containing corresponding bio-information, such as tridecane (18,21) and [E]-2-hexenal (4), in previous works. Tridecane was the volatile with the highest relative amount released by

Tessaratoma papillosa, which could accelerate the transferring of other toxic secretions though the arthropod body (21), and slow down the volatility of other emanations from the surface of stinkbugs (18). It is remarkable that in our study, the volatiles contributing to the difference in stinkbug volatile characteristics before and after irritation contained these two compounds in previous works, and could be tentatively considered as potential alarming volatiles. The next phase of work should focus on quantifying the important volatiles and further revealing the potential alarming volatiles, when the corresponding authentic compounds are commercially available.

Acknowledgements

The authors would like to thank the National Natural Science Foundation of China for financially supporting the research under contract No. 20575081 and 20805008.

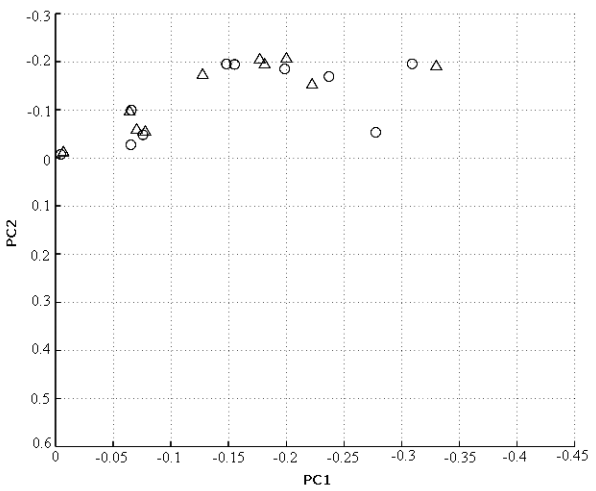

Figure 5. The effect of temperature change to the volatile characteristic of Tessaratoma papillosa by HS-SPME (○ = Room temperature; △ = 50°C water bath).

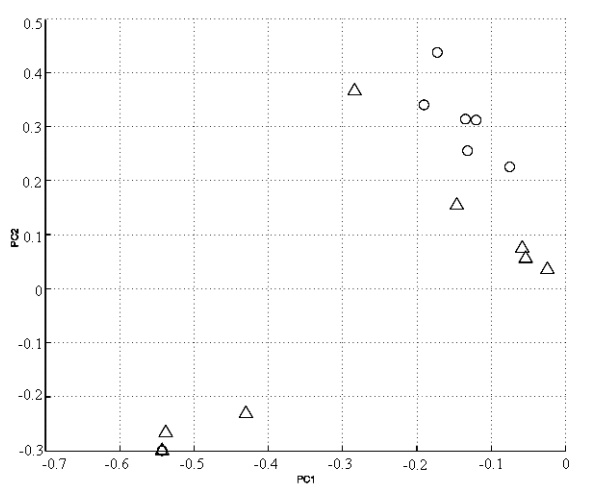

Figure 6. The effect of light change to the volatile characteristic of Tessaratoma papillosa by HS-SPME (○ = Diurnal sampling projects; △ = Night sampling projects).

References

1. J.T. Baker and P.A. Kemball. Volatile constituents of the scent gland reservoir of the coreoid, *Pternistria bispina* stal. *Austral. J. Chem.* **20:** 395–98 (1967).
2. D.H. Calam and A. Youdeowei. Identification and functions of secretion from the posterior scent gland of fifth instar larva of the bug *Dysdercus intermedius*. *J. Insect Physiol.* **14:** 1147–58 (1968).
3. B.W. Staddon, I.J. Everton, and D.E. Games. Organ specificity and scent constitution in Corixidae (Heteroptera: Hydrocorisae). *Comp. Biochem. Physiol.* **62:** 259–62 (1968).
4. T. Ishiwatari. Studies on the scent of stink bugs (Heteroptera: Hydrocorisae). *Japan. Soc. Appl. Entonol. Zool.* **9:** 153–58 (1974).
5. Y.L. Han, W.Z. Cai, X.L. Xu, W. Chen. Scent glands of true bugs. Entomol. *Knowledge* **41:** 607–10 (2004).
6. C.L. Arthur and J. Pawliszyn. Solid phase microextraction with thermal desorption using fused silica optical fibers. *Anal. Chem.* **62:** 2145–48 (1990).
7. J. Pawliszyn. New directions in sample preparation for analysis of organic compounds. *Trends Anal. Chem.* **14:** 113–22 (1995).
8. A. Peñalve, E. Pocurull, F. Borrull, and R.M. Marcé. Trends in solid-phase microextraction for determining organic pollutants in environmental samples. *Trends Anal. Chem.* **18:** 557–68 (1999).
9. G.A. Mills and V. Walke. Headspace solid-phase microextraction procedures for gas chromatographic analysis of biological fluids and materials. *J. Chromatogr. A* **902:** 267–87 (2000).
10. S. Ulrich. Solid-phase microextraction in biomedical analysis. *J. Chromatogr. A* **902:** 167–94 (2000).
11. J. Koziel, M.Y. Jia, A. Khaled, J. Noah, and J. Pawliszyn. Field air analysis with SPME device. *Anal. Chim. Acta* **400:** 153–62 (1999).
12. F. Augusto, A.L. Lopes, and C.A. Zini. Sampling and sample preparation for analysis of aromas and fragrances. *Trends Anal. Chem.* **22:** 160-169 (2003).
13. Z.M. Zhang and G.K. Li. Advances on the analysis of biogenic volatile organic compounds. *Chem. Online* **69:** 1–8 (2006).
14. U.R. Bernier, D.L. Kline, D.R. Barnard, C.E. Schreck, and R.A. Yost. Analysis of human skin emanations by gas chromatography/mass spectrometry. 2. Identification of volatile compounds that are candidate attractants for the yellow fever mosquito (*Aedes aegypti*). *Anal. Chem.* **72:** 747–56 (2000).
15. J.P. Spinhirne, J.A. Koziel, and N.K. Chirase. Sampling and analysis of volatile organic compounds in bovine breath by solid-phase microextraction and gas chromatography–mass spectrometry. *J. Chromatogr. A* **1025:** 63–69 (2004).
16. D. Djozan, T. Baheri, R. Farshbaf, and S. Azhari. Investigation of solid-phase microextraction efficiency using pencil lead fiber for in vitro and in vivo sampling of defensive volatiles from insect's scent gland. *Anal. Chim. Acta* **554:** 197–201 (2005).
17. Z.M. Zhang, J.J. Cai, G.H. Ruan, and G.K. Li. The study of fingerprint characteristics of the emanations from human arm skin using the original sampling system by SPME-GC/MS. *J. Chromatogr. B* **822:** 244–52 (2005).
18. M.S. Blum and J.G. Traynham. The chemistry of the pentatomid scent gland. *Int. Kongr. Ent. Vien.* **3:** 48–52 (1960).
19. A.R. Gilby and D.F. Waterhouse. Secretions from the lateral scent glands of the green vegetable bug, *Nezara viridula*. *Nature.* **216:** 90–91 (1967).
20. H.T. Gordon. Incorporation of acetate-C14 into scent constituents by the green vegetable bug. *Nature* **197:** 818 (1963).
21. H.Z. Remold. Uber die biologische bedeutung der duftdrusen bei landwanzen (Geocorisae). *Zeitschr Vergl Physiol.* **45:** 636–94 (1962).

Manuscript received January 10, 2008;
revision received May 24, 2008.