行政院國家科學委員會補助專題研究計畫 ☑ 成 果 報 告
□期中進度報告

應用固相微萃取法在果實蛀蟲性費洛蒙之研究

Use of solid phase microextraction in the investigation of sex pheromone of fruit pests

計畫類別：☑ 個別型計畫　　□ 整合型計畫
計畫編號：NSC 91－2626－M－164－001－
執行期間：　91 年 08 月 01 日 至　92 年 07 月 31 日

計畫主持人：朱紫雲
共同主持人：
計畫參與人員：

成果報告類型(依經費核定清單規定繳交)：□精簡報告　☑完整報告

本成果報告包括以下應繳交之附件：
□赴國外出差或研習心得報告一份
□赴大陸地區出差或研習心得報告一份
□出席國際學術會議心得報告及發表之論文各一份
□國際合作研究計畫國外研究報告書一份

處理方式：除產學合作研究計畫、提升產業技術及人才培育研究計畫、列管計畫及下列情形者外，得立即公開查詢
□涉及專利或其他智慧財產權，□一年□二年後可公開查詢

執行單位：修平技術學院化工系
中　華　民　國　92 年 8 月 24 日

Use of solid phase microextraction in the investigation of sex pheromone of fruit pests

Tzu-Yun Chu [1]*, Chau-Chin Hung [2], Chung-yuan Hsu [1], Weih-Ling Chen [1], Mu-Pai Huang [1], Pei-Ying Lin [1]

[1] Department of Chemical Engineering, Hsiuping Institute of Technology, Dali, Taichung, Taiwan 412, R.O.C.

[2] Taiwan Agricultural Chemicals and Toxic Substances Research Institute (TACTRI), Wu-Feng, Taichung, Taiwan 413, R.O.C.

Abstract

A simple and efficient technique that does not require solvent and uses less operating time for the investigation of the sex pheromones by utilizing headspace solid-phase microextraction (SPME) followed by GC-MS analysis has been developed. Variables such as the types of SPME fiber, number of pests, temperature and extraction time have been studied. Whole sex glands of *Eucosma notanthes* Meyrick were dissected from 5 virgin insects, placed in a 2 mL vial, equilibrated at 170°C for 10 min, and then head spaced at room temperature for 5 min. The results of the GC-MS analyses of headspace SPME of these sex glandular solid samples were much better than those obtained with hexane extraction of sex glandular from 117 insects followed by either with headspace SPME or direct injection due to higher absorption efficiency. The simplicity of this technique renders it a very suitable method for research on the biological control of pests.

Keywords: headspace solid-phase microextraction; *Eucosma notanthes* Meyrick; sex pheromone; carambola fruit

1. Introduction

A number of sex pheromone components of various insect species have been investigated [1-6]. The discovery of sex pheromone in related species allowed the evolution of insect lures and trap designs using synthetic sex pheromone and improved pest control, thus minimizing the harm of fruits and shoots of orchard.

Eucosma notanthes Meyrick is the major pest on carambola fruits in Taiwan. The investigation of the major components from pheromone gland of carambola fruit borer has been reported [7]. *Z*-8-dodecenyl acetate and *Z*-8-dodecenol were isolated by solvent extraction and analyzed by gas chromatography-mass spectrometry. The bioassay of the components from the pheromone gland has also been studied [8-10].

Classical methods of analyzing insect pheromones involve extraction by solvents. These methods often require tedious and solvent consumptive procedures plus hundreds to thousands of insects are needed for the extraction of the pheromone before analytical studies can be carried out [7,11,12]. Furthermore, unwanted components originated from the insects or the glands will also be extracted by this process. Recently, the volume of extracting

solvent has been cut down considerably to microliters of solvent for extracting only a few insects [13-15]. However, this procedure still could not avoid contamination from the living tissues. Absorption method has also been used by first trapping volatile pheromones onto an absorbent tube and then eluting the trapped organic compounds with a solvent system [16,17]. The solid phase microextraction (SPME) is a viable alternative to solvent extraction and offers a convenient, solvent-free and time-saving method. Numerous SPME sampling studies have been published [18-28]. The first report of SPME being used to analyze the air-borne volatile pheromones released from the sugar cane weevil *Metamasius hemipterus* was sampled by a polydimethylsiloxane fiber appeared in 1995 [29], which initiated the application of SPME for insect studies [30]. The headspace SPME sampling results are comparable with those obtained with solvent extractions [31]. This method significantly reduces the time and the organic solvent required for sample examination. Other reports revealed that SPME allows for experiments on just a few insects [32-36]. Yet, the use of SPME in analyzing insect pheromones is at a very early stage in Taiwan. To the best of our knowledge, there has no report from Taiwan using SPME for this purpose. This report has studied the parameters for the use of headspace SPME technique for the isolation and analysis of the sex pheromones of the carambola fruit borer, which infested the tropical fruits in Taiwan as well as in South East Asia.

2. Experimental

2.1. Materials

Eucosma notanthes Meyrick were acquired from TACTRI (Wu-Feng, Taichung, Taiwan, R.O.C.). Synthetic *Z*-8-dodecenyl acetate (*Z*8-12:Ac) and *Z*-8-dodecenol (*Z*8-12:OH) were purchases from Chemtech (Nertherlands). A manual SPME fiber holder and three types of SPME fibers, 100 μm Polydimethylsiloxane (PDMS), 85 μm Polyacrylate (PA) and 65 μm Polydimethylsiloxane-divinylbenzene (PDMS-DVB) were purchase from Supelco (Bellefonte, PA, USA).

2.2. Sample preparation

2.2.1. Headspace SPME analysis of solid sample

Eucosma notanthes female moths were placed in plastic bags with a L:D = 12:12 photoperiod regime. The calling behavior began from 1 to 4 hours after light on. Whole sex pheromone glands were dissected from five virgin insects during the calling period and placed in a 2 mL screw-top vial furnished with PTFE silicone septa. The vial was inserted into a temperature controlled sand bath and allowed to equilibrate at 170°C for 5 min. After the vial was removed from the sand bath, a SPME syringe was then immediately inserted into the vial. The fiber was exposed to the headspace over the sample and extracted for 5 min at room temperature.

2.2.2. Direct injection of hexane extraction

Whole pheromone glands of *Eucosma notanthes* female (117, during calling period as previously mentioned) were dissected and immersed into 300 μL hexane in a 2 mL graduated vial. Additional hexane had to be added to bring the total solvent volume to 300 μL due to

the absorption of hexane by the glands. After two days, the glands were carefully removed by tweezers and the remaining solution was stored at –20°C until sample analysis. A 1 μL volume of hexane extract was injected into the GC-MS inlet for analysis.

2.2.3. Headspace SPME analysis of hexane extract

A 6 μL volume of hexane extract was placed in a 2 mL vial and then extracted at headspace under the same conditions as for SPME analysis of solid samples.

2.3. Standard solution

A solution of 20.2 ng μL^{-1} of synthetic *Z*8-12:Ac and *Z*8-12:OH (in H_2O:MeOH = 3:2 solvent) was used as reference. A 6 μL volume of standard solution was placed in a 2 mL vial and then extracted at headspace under the same conditions as for SPME analysis of solid samples.

2.4. GC/MS parameters

Gas chromatography-mass spectrometry was performed on a Hewlett-Packard 6890 gas chromatograph, interfaced to a HP 5973 MSD. Gas chromatographic separation was conducted using a DB-5MS capillary column (30 m x 0.25 mm i.d., 0.25 μm film thickness) in splitless injection mode. Carrier gas was He (purity 99.995%) at 1.0 mL/min flow rate. The initial oven temperature was 90 °C, held for 2 min, the temperature was raised to 180°C at a rate of 20°C/min, held for 1 min, then the temperature was raised to 240°C at a rate of 10°C/min, and finally, held for 3 min, the total elution time was 16.50 min. The injection-port was set to 260°C. For SPME analysis a Supleco 0.75 mm i.d. GC inlet liner was used. SPME samples were injected by exposing the fiber in the hot injector of GC for 5 min and the chromatogram was then acquired.

3. Results and Discussion

3.1. Development of SPME method

In order to find the optimum conditions for the analysis of sex pheromone of *Eucosma notanthes*, several parameters have been examined. Three types of SPME fiber coatings were evaluated to select the appropriate fiber for the method. Duplicate authentic standard solutions were analyzed and the results were shown in figure 1. The extraction efficiency of PDMS-DVB was lower than those of PDMS and PA, while PDMS and PA gave comparable responses for both *Z*-8-12:OH and *Z*-8-12:Ac. Since the reproducibility of PDMS fiber was better than that of PA and the PDMS is a more resistant coating than PA, PDMS was chosen as the fiber for the rest of experiments.

The effects of temperature and extraction time were also evaluated. Five virgin moths were extracted by headspace SPME at different temperatures and extraction times. The retention times and the mass spectra of the components identified in the sex gland were compared with those of synthetic standards. Table 1 showed that much lower extraction responses were observed for *Z*8-12:OH than that of Z8-12:Ac when the fiber was inserted into

the vial to extract the sample without prior equilibration at 140°C. This large differences in absorption might be a result of incomplete vaporizing of the more polar *Z*8-12:OH. The peak area ratio for the two analytes decreased at higher equilibration temperature and resulted in closer extraction efficiency when the extraction was carried out after equilibration at 120°C and 140°C respectively. For a more convenient SPME operating procedure, sample vial was removed from the sand bath after equilibration and the SPME syringe was inserted immediately to perform the extraction. The results summarized in Table 2 demonstrated that much closer peak area ratios were achieved under all of the extraction conditions. For the same equilibration and extraction time, the amount of the analytes extracted increased as the equilibrating temperature increased. The highest extraction performance was achieved by equilibrating at 170°C for 5 min and extracting for 5 min at room temperature and therefore it was chosen as the optimum HSSPME conditions for the rest of experiments.

Absorption time profiles were examined by plotting the area counts versus the extraction time (Figure 2). The amount of *Z*8-12:OAc absorbed reached while the absorbed amount of *Z*8-12:OH almost approached to a constant after 5 min absorption. Therefore, 5 min exposure time was taken as the adequate extraction time for the study of sex pheromone of the carambola fruit borer.

To study carryover effect, blank tests were run after desorption of SPME samples. No signal of pheromone components was detected for all the cases examined. To ensure a complete desorption of other high boiling point or high molecular weight compounds from the sex glands, the SPME fiber was exposed for another 5 min in hot injector after the vent system was open. The GC inlet was set to open the vent system 2 min after start run.

3.2. Chemical identification

The chromatogram from five calling *E. notanthes* females was compared with that from five noncalling females. Figure 3 demonstrates that the total ion chromatograms of calling and noncalling females both have the same two major peaks except the peak areas of the calling females were significantly larger than those of the noncalling females. It suggests that these are the components of the sex pheromone because they are released in much greater amount during calling period. The confirmation of peaks was achieved by comparison of the authentic synthetic standard mixture which gave identical retention times and mass spectral fragmentations as those of the pheromones obtained from gland extract (Figures 4 and 5). The characteristic major fragment ions and their relative abundance of the pheromone and the authentic sample in this assay are listed in Table 2. The molecular ion, $[M]^+$, in all of the mass spectra is vanishingly small. The EI mass spectrum of the earlier eluting compound gave a base peak at m/z 41, and the peak at m/z 166, resulting from a loss of water from the parent ion $[M–H_2O]^+$, suggested that the compound might be an alcohol. The EI mass spectrum of the second eluting compound gave an acyliun ion base peak at m/z 43, $[CH_3CO]^+$, and a peak at m/z 166, resulted from the loss of an acetic acid from the parent ion, $[M–AcOH]^+$, suggested that the compound might be an acetate. Therefore, the identities of the major constituents of gland extracts could be established by the GC-MS data.

3.3. Evaluation of the proposed method

Linearity, detection limit and reproducibility were evaluated to ensure the viability of this HSSPME method. The results were shown in Table 3. Six different concentrations of the

authentic standard mixture were analyzed in triplicate using the optimum conditions developed above. Calibration graphs were linear for the concentration range from 1.26 to 40.3 ng ml^{-1}. The precision of the proposed procedure was estimated by determining of five replicates at two different concentration levels. The RSD values were between 6.2% and 13.6 % revealed that HSSPME/GC-MS analysis yielded good reproducibility. Real samples from five female moths were also investigated to verify the reproducibility of this method. Higher RSD values were obtained which might be caused by the unequal amount of pheromone among individual insects. Detection limits were calculated with the formula LOD = 3 x SD on account of seven replicate analysis results for the 10 ng ml^{-1} concentration of *Z*8-12:OH and *Z*8-12:Ac. These were 2.3 and 1.1 ng ml^{-1} respectively.

3.4. Comparison of SPME with solvent extraction method

Comparison of the optimized HSSPME/GC-MS solid sample method with the hexane extraction method was launched. Hexane extract was analyzed by two different ways: (1) 1 μL of extract was injected directly into the GC-MS inlet, (2) 6 μL of extract was headspace extracted by PDMS fiber, followed by GC-MS analysis. The total ion chromatograms were shown in figure 6. When five sex pheromone glands were cut apart carefully without any portion of abdomen, the chromatogram was very clean, only two significant major signals were found by HSSPME solid sample method, and the identification was confirmed by comparison of retentions times and mass spectra with the standard references. The headspace SPME chromatogram of the hexane extract could detect only one of the pheromone responses in low intensity, which was identified as *Z*8-12:Ac by mass spectrum. Furthermore, some earlier eluting peaks, not found in the HSSPME of solid sample, were present in hexane extraction method, presumably is the result of other unwanted biological compounds that originate from the insect glands being extracted into the hexane solution. In the direct injection of hexane extract method, no pheromone peaks were observed. Consequently, HSSPME of solid sample method was a more convenient with higher extraction efficiency than the hexane extraction method, both in the direct injection or the headspace SPME procedure.

The relative amount of the identified compounds was estimated. Table 4 showed the ratio of total amount of Z8-12:OH and Z8-12:Ac, obtained from the HSSPME method of 5 females, were 2.2 : 1. This result was in good agreement with that reported by Hung, which was extracted from 52,820 females by hexane.[7]

3.5. Field Test[7]

Field tests were conducted in carambola orchards at Changhua, Taiwan. The number of males captured in trap baited with different blend ratios of two authentic compounds were calculated and evaluated. The results were shown in Table 5. Synthetic mixtures (1 mg) in ratios of Z8-12:OH and Z8-12:Ac ranging from 100:50 to 100:150 were found more attractive to *E. notanthes* male moths in orchards.

4. Conclusions

An optimized HSSPME method coupled with GC-MS has been developed for the

determination of the sex pheromone of *Eucosma notanthes* Meyrick. Headspace extraction of solid sample by 100 μm PDMS fiber gave the highest absorption effect when the glands were equilibrated at 170°C for 5 min, and then extracted for 5 min at room temperature. Compare to classical solvent extraction method, the optimized HSSPME method was easier to perform, faster and more efficient, consumed no solvent, and suffered much less contamination from the living tissues. HSSPME is a practical method in research on the sex pheromone of fruit pests. There is specification of insect species attacking certain kind of fruit, the unique sex pheromone components of different species of pests are not identical. Thus, the development of a practical identification method for the sex pheromone of diverse fruit borers is a conscious work. Consequently, we plan to further pursue the determination of the pheromones of important fruits pests in Taiwan to assist the control pests harmful to the agriculture of Taiwan.

Acknowledgment

Financial support for this research by the National Science Council of the Republic of China is gratefully acknowledged (NSC 91-2626-M-164-001). The authors wish to thank our colleagues at TACTRI, who contributed to the study by providing of live moths.

References

[1] T. Takanashi, S. Ohno, Y.P. Huang, S. Tatsuki, H. Honda, Y. Ishikawa, Chemoecology 10 (2000) 143.
[2] T.G. Gray, R.F. Shepherd, G. Gries, R. Gries, The Canadian Entomologist 128 (1996) 1139.
[3] J.G. Millar, A.E. Knudson, J.S. McElfresh, R. Gries, G. Gries, Bioorganic & Medicinal Chemistry 4 (1996) 331.
[4] A. Svatos, B. Kalinova, M. Hoskovec, J. Kindl, O. Hovorka, I. Hrdy, Tetrahedron Letters 40 (1999) 7011.
[5] F.C. Griepink, T.A.v. Beek, M.A. Posthumus, A.d. Groot, Tetrahedron Letters 37 (1996) 411.
[6] C.R. Unelius, A. Eiras, P. Witzgall, M. Bengtsson, A. Kovaleski, E.F. Vilela, A.K.B. Karlson, Tetrahedron Lett. 37 (1996) 1505.
[7] C.C. Hung, J.S. Hwang, M.D. Hung, Y.P. Yen, R.F. Hou, J. Chem. Ecology 27 (2001) 1855.
[8] C.C. Hung, J.S. Hwang, R.F. Hou, Plant Protection Bulletin 41 (1999) 165.
[9] J.S. Hwang, C.C. Hung, C.Y. Liu, Chinese Journal of Entomology 20 (2000) 97.
[10] C.C. Hung, J.S. Hwang, R.F. Hou, Plant Protection Bulletin 38 (1996) 119.
[11] T.C. Baker, W. Francke, J.G. Millar, C. Lofstedt, B. Hansson, J.W. Du, P.L. Phelan, R.S. Vetter, R. Youngman, J.L. Todd, J. Chem. Ecology 17 (1991) 1973.
[12] A.B. Attygalle, G.N. Jham, A. svatos, R.T.S. Frighetto, F.A. Ferrara, E.F. Vilela, M.A. Uchoa-fernandes, J. Meinwald, Bioorganic & Medicinal Chemistry 4 (1996) 305.
[13] E.A. Malo, M. Renou, A. Guerrero, Talanta 52 (2000) 525.
[14] W. Wu, J.W. Zhu, J. Millar, C. Lofstedt, Insect Biochem. Molec. Biol 28 (1998) 895.
[15] C. QUERO, J. BAU, A. GUERRERO, M. BREUER, A.D. LOOF, H.-G. KONTZOG, F. CAMPS, J. Agric. Food Chem. 51 (2003) 2987.
[16] J.G. Millar, D.M. Hinkens, J.S. McElfresh, Tetrahedron Letters 42 (2001) 1619.
[17] M.M. Rai, A. Hassanali, R.K. Saini, H. Odongo, H. Kahoro, J. Insect Physiol. 43 (1997) 83.

[18] J.V. Wooten, D.L. Ashley, A.M. Calafat, Journal of Chromatography B 772 (2002) 147.
[19] S.S. Yang, C.B. Huang, I. Smetena, Journal of Chromatography A 942 (2002) 33.
[20] M. Llompart, K. Li, M. Fingas, Talanta 48 (1999) 451.
[21] F. Guan, K. Watanabe, a. Ishii, H. Seno, T. Kumazawa, H. Hattori, O. Suzuki, J. Chromatogr. A 714 (1998) 205.
[22] S. Vichi, A.I. Castellote, L. Pizzale, L.S. Conte, S. Buxaderas, E. Lopez-Tamames, Journal of Chromatography A, 983 (2003) 19.
[23] G. Prokupkova, K. Holadova, J. Poustka, J. Hajlova, Analytica Chimica Acta 457 (2002) 211.
[24] C. Garcya-Jares, M. Llompart, M. Polo, C. Salgado, S. Macyas, R. Cela, Journal of Chromatography A, 963 (2002) 277.
[25] J.C.R. Demyttenaere, C. Dagher, P. Sandra, S. Kallithraka, R. Verhe, N.D. Kimpe, Journal of Chromatography A 985 (2003) 233.
[26] A. Navalon, A. Prieto, L. Araujo, J.L. Vylchez, Journal of Chromatography A 975 (2002) 355.
[27] L. Jirovetz, D. Smith, G. Buchbauer, J. Agric. Food Chem. 50 (2002) 4643.
[28] N. Raikos, K. Christopoulou, G. Theodoridis, H. Tsoukali, D. Psaroulis, Journal of Chromatography B 789 (2003) 59.
[29]C. Malosse, P. Ramirez-Lucas, D. Rochat, J. Morin, J. High Res. Chromatogr. 18 (1995) 669.
[30] G.R. Jones, N.J. Oldham, J. Chromatogr. A 843 (1999) 199.
[31] M.F. Sledge, G. Moneti, G. Pieraccini, S. Turillazzi, Journal of Chromatography A 873 (2000) 73.
[32] S.R. Clarke, F.R. Dani, G.R. Jones, E.D. Morgan, S. Turillazzi, Journal of Insect Physiology 45 (1999) 877.
[33] J. Auger, S. Rousset, E. Thibout, B. Jaillais, J. chromatogr. A 819 (1998) 45.
[34] D. Rochat, P. Ramirez-Lucas, C. Malosse, R. Aldana, T. Kakul, J.P. Morin, J. Chromatogr. A 885 (2000) 433.
[35] J.M. Bland, W.L.A. Osbrink, M.L. Cornelius, A.R. Lax, C.B. Vigo, Journal of Chromatography A, 932 (2001) 119.
[36] A. Peppuy, A. Robert, E. Semon, C. Ginies, M. Lettere, O. Bonnard, C. Bordereau, Journal of Insect Physiology 47 (2001) 445.

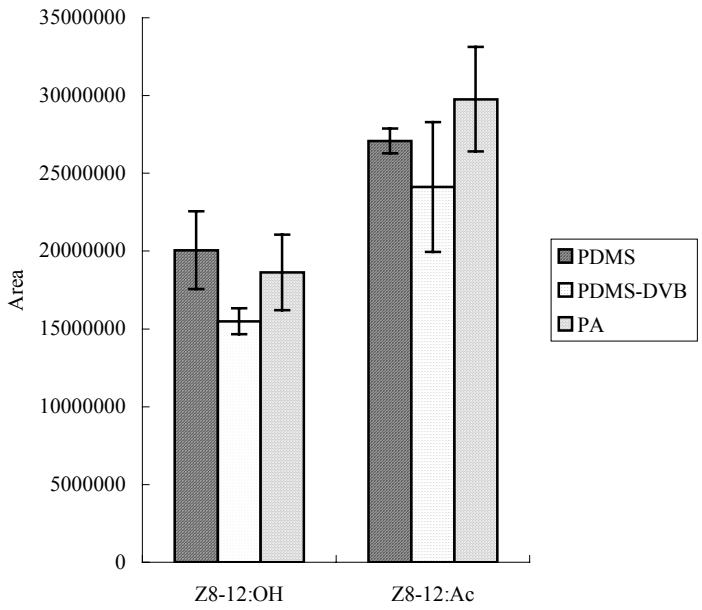

Figure 1. The results of GC-MS analysis of headspace SPME injections of standard solution using different fiber types.

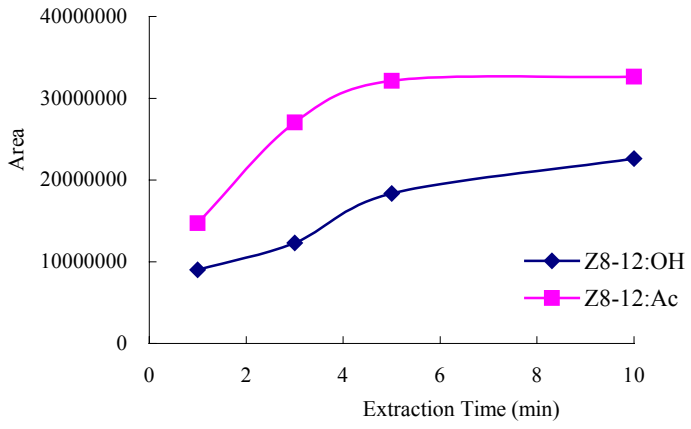

Figure 2. Absorption time profile of *Z*-8-12:OH and *Z*-8-12:Ac by HSSPME with PDMS fiber under the optimum conditions

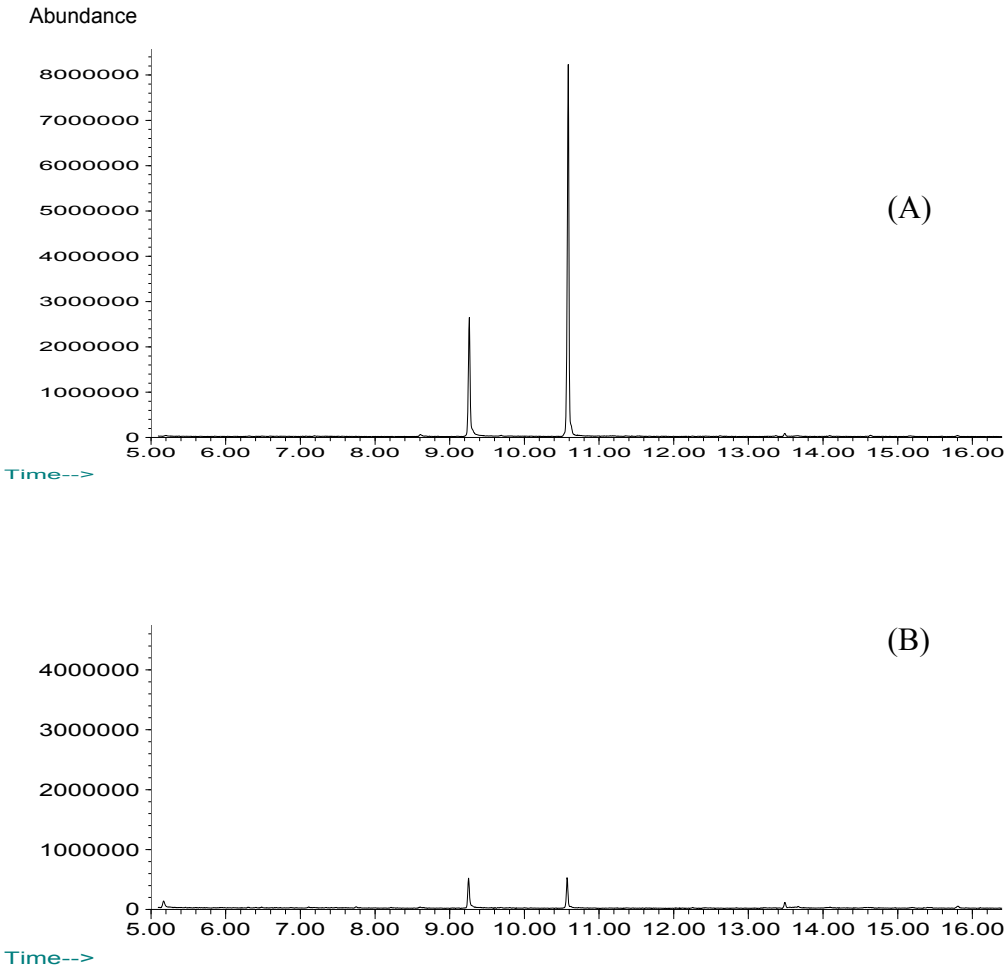

Figure 3. Chromatograms of HS-SPME of sex gland from 5 calling females (A) and 5 non-calling females (B) evidencing the two components of the female sex pheromone.

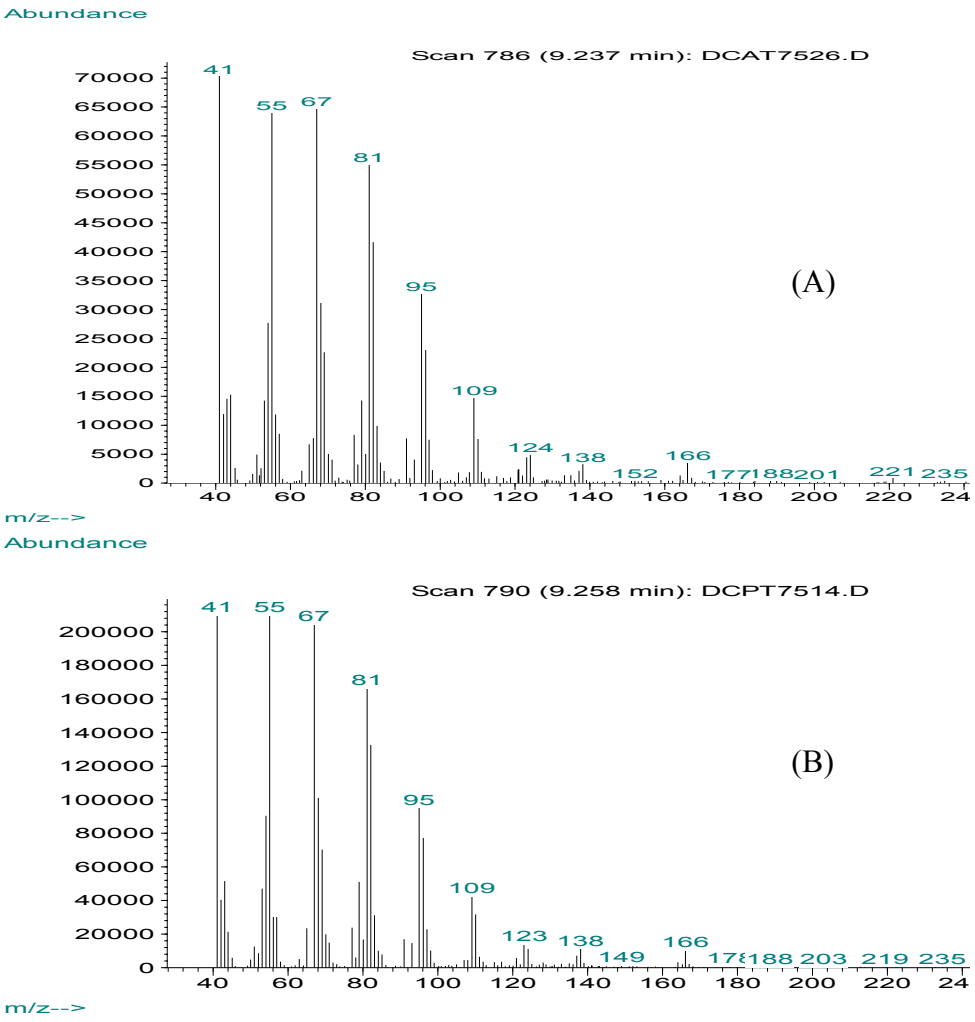

Figure 4. Mass spectra (in scan mode) of *Z*-8-dodecenol obtained by (A) standard solution and (B) headspace SPME of sex glands from 5 *E. notanthes* Meyrick.

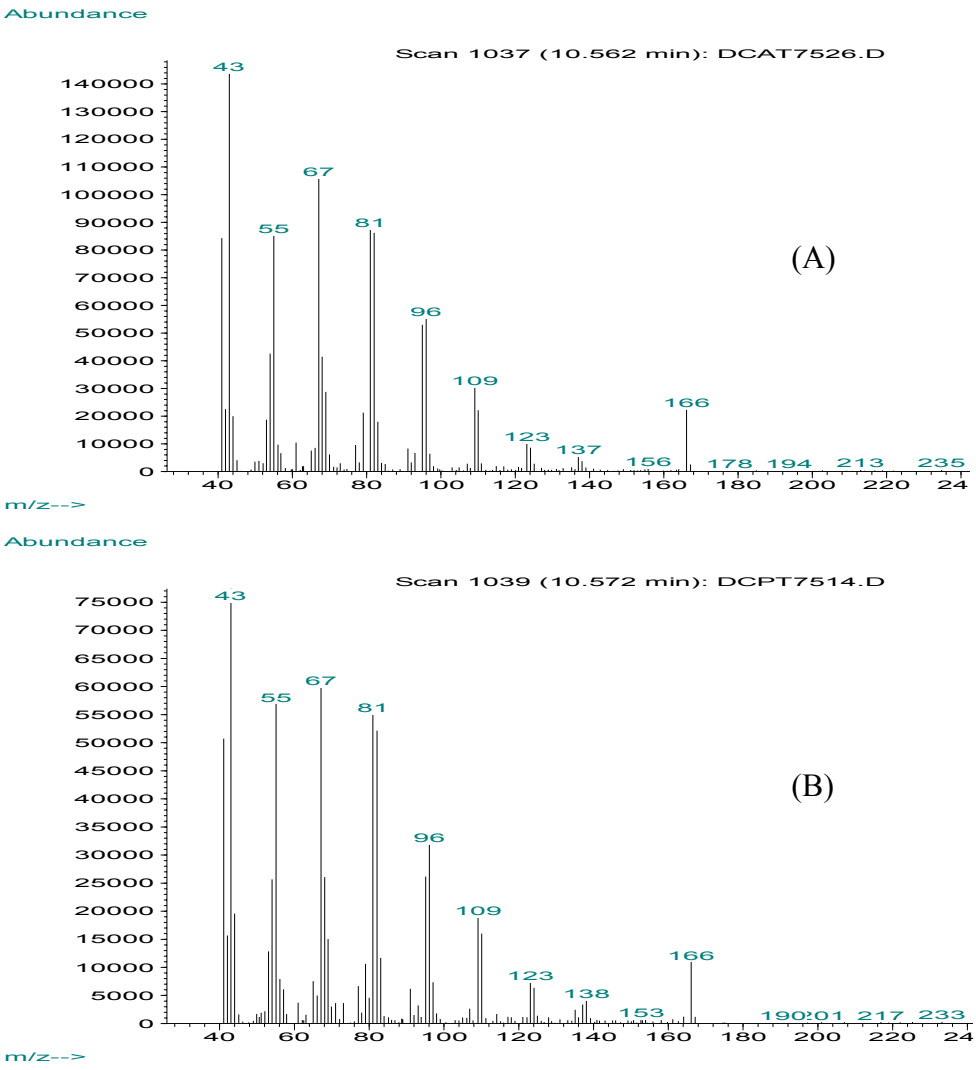

Figure 5. Mass spectra (in scan mode) of *Z*-8-dodecenyl acetate obtained by (A) standard solution and (B) headspace SPME of sex glands from 5 *E. notanthes*

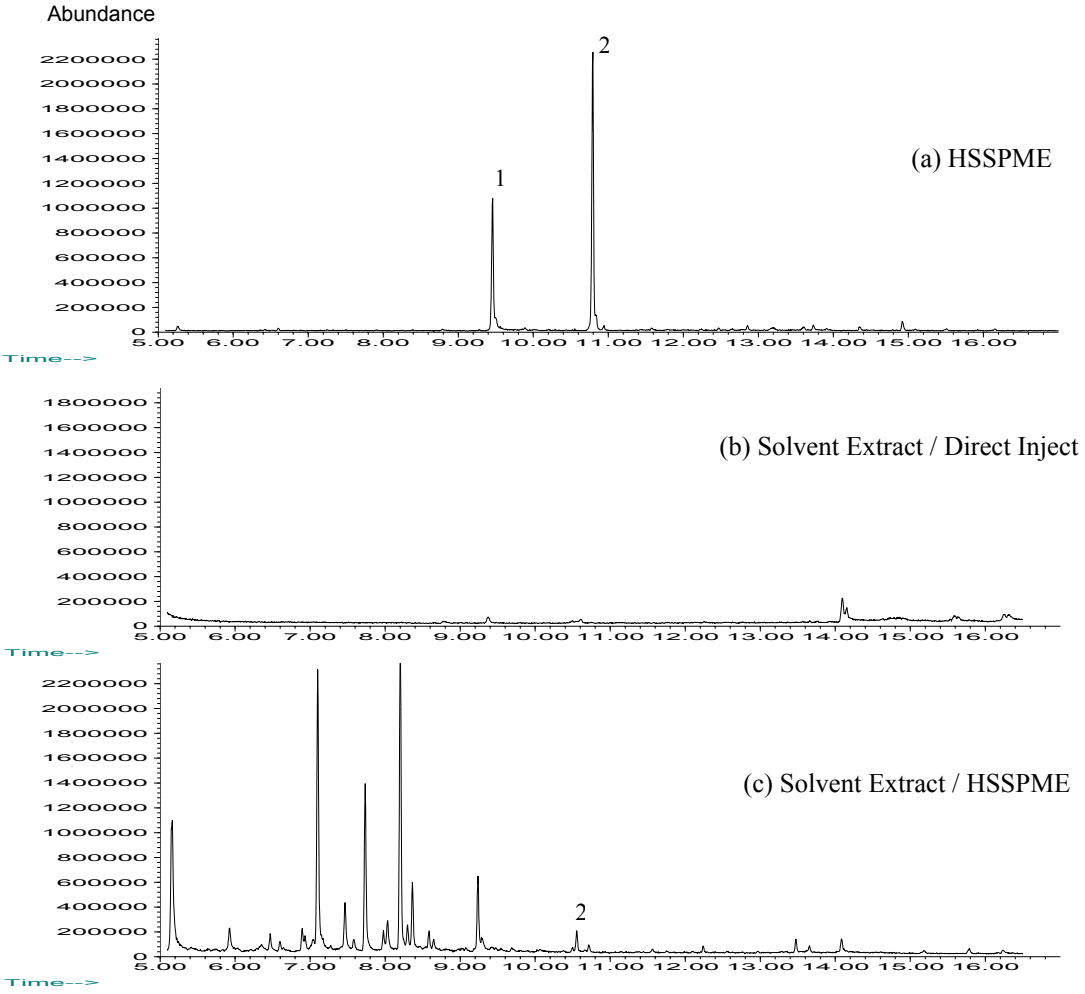

Figure 6. Comparison of methods. Total ion chromatogram of GC-MS analysis of sex glands from 5 *E. notanthes* by headspace SPME analysis of solid sample (a), direct injection of 1 µL of 117 *E. notanthes* hexane extract (b), and headspace SPME analysis of 6 µL of 117 *E. notanthes* hexane extract (c). Peaks are identified as (1) *Z*-8-dodecenol and (2) *Z*-8-dodecenyl acetate.

Table 1 Comparison of peak area from headspace SPME in relation to sample equilibration and extraction conditions

Equilibration		Adsorb		Peak Area*		
Temp.(°C)	Time(min.)	Temp.(°C)	Time(min.)	Z8-12:OH	Z8-12:Ac	ratio
140	0	140	5	5434724	314405415	1 : 58
170	0	170	5	14741497	99367508	1 : 6.7
120	5	120	5	--**	2397414	--
140	5	140	5	16855512	36853882	1 : 2.2
120	10	ambient	5	5916490	9415987	1 : 1.6
140	10	ambient	5	12245368	14136752	1 : 1.2
170	10	ambient	5	23909991	32562323	1 : 1.4
170	15	ambient	3	10333627	19629233	1 : 1.9
170	15	ambient	5	17917021	21893523	1 : 1.2
170	15	ambient	10	19724285	32860743	1 : 1.7

*Analysis of 5 *Eucosma notanthes* Meyrick.
**Not detected.

Table 2. Fragmentation patterns of the sex pheromone obtained by (a) synthetic compound and (b) gland extract[a]

Pheromone Components			
Z-8-12:OH		Z-8-12:Ac	
(a)	(b)	(a)	(b)
41(100)	41(100)	43(100)	43(100)
55(88)	55(96)	55(60)	55(72)
67(89)	67(92)	67(63)	67(76)
81(76)	81(75)	81(54)	81(70)
82(57)	82(60)	82(56)	82(66)
95(45)	95(43)	95(34)	95(34)
96(32)	96(35)	96(36)	96(40)
109(21)	109(19)	109(17)	109(23)
166(5)	166(5)	166(12)	166(14)

[a]: m/z, relative abundance ratio in parenthesis.

Table 3 Linearity, limit of detection and reproducibility for the HSSPME method

Compound	Linearity			LOD	RSD (%)		
					Synthetic		Gland extract[b]
	Slope	Intercept	r	(ng ml^{-1})	(ng ml^{-1})[a]		females
					10	40	5
Z8-12:OH	2001932	–67945	0.9988	2.3	7.8	13.6	25.0
Z8-12:Ac	1576472	–1900886	0.9835	1.1	6.2	12.7	35.9

[a]Relative standard deviation of five determination.
[b]Relative standard deviation of seven determination.

Table 4 The amount ratio of two component in pheromone of *Eucosma notanthes*

	Z8-12:OH : Z8-12:Ac	
	Peak Area Ratio	Amount Ratio
Synthetic	0.36 : 1	1 : 1
SPME (5 females)	0.78 : 1	2.2 : 1
Solvent Extract* (52,820 females)		2.7 : 1

*C.C. Hung [7].

Table 5 The mean number of male *Eucosma notanthes* moths captured in traps baited with different blend ratios of Z8-12:Ac mixed with Z8-12:Ohin carambola orchards*

	Amount Ratio	**Numbers of**
	Z8-12:OH : Z8-12:OAc	Male Moths Captured**
Experiment 1	100 : 50	19.4
	100 : 100	23
	100 : 150	22.3
Experiment 2	100 : 100	67.5
	100 : 150	55.2
	100 : 270	39.4

*C.C. Hung [7].
**Males per trap per week

應用固相微萃取法在果實蛀蟲性費洛蒙之研究

朱紫雲[1]*, 洪巧珍[2], 許仲元[1], 陳韋伶[1], 黃慕白[1], 林佩瑩[1]

[1] 修平技術學院 化工系
台中縣大理市 412 工業路 11 號.
[2] 行政院農業委員會農業藥物毒物試驗所
台中縣霧峰鄉舊正村光明路 11 號

摘要

本研究以頂空固相微萃取法針對楊桃花姬捲葉蛾作性費洛蒙之萃取分離，發展出一種簡短有效且不需用任何有機溶劑的萃取技術。以 PDMS 纖維成功的由五隻娥性腺体萃取出性費洛蒙，再以氣相層析質譜儀作成分鑑定分析。並探討各種分析條件之確效，以及與傳統的溶劑萃取方法作比較。

楊桃、荔枝及蕃石榴等是台灣地區高經濟果樹，其重要果樹害蟲為果實蛀蟲，直接影響品質及產量。然而特定種類之害蟲對不同果樹獨具危害性，且不同種類果實蛀蟲釋放之性費洛蒙成分均不相同，故極須對此本土性害蟲研究一有效的偵測方法，並研究開發一種安全、無副作用、且具專一性的昆蟲性費洛蒙誘引劑作為替代殺蟲劑之生物防治方法，提供果農參考，以達經濟有效的害蟲防治。

關鍵詞：性費洛蒙、固相微萃取、氣相層析/質譜儀、楊桃花姬捲葉蛾