



Study on Volatile Components of Butterfly Nectar Plants and Host Plants

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Volatile components of butterfly nectar plants, *Lantana camara* L., *Vitex trifolia* L. and *Hibiscus rosa-sinensis* L. and host plants, *Cleome spinosa* L., *Dregea volubilis* (Linn. f.) Benth. ex Hook. f. and *Parsonsia laevigata* (Moon) Alston, were analyzed by automatic thermal desorption-gas chromatography-mass spectrometry process (ATD-GC-MS). Of the three nectar plants, the most dominant components were terpenes, which total content exceeded 60 %, together with eucalyptol with higher content. Also there were some hydrocarbons, alcohols and aromatic compounds detected in the three nectar plants. Host plant volatiles presented a different composition. *C. Spinosa* volatiles were mainly composed of terpenoids (36.35 %), aldehydes (29.07 %), ethers (10.48 %) and acids (9.15 %). *D. volubilis* volatile compounds were mainly esters (21.03 %), next were terpenoids (19.53 %). *P. laevigata* volatiles were composed mainly of aldehydes (32.2 %) and esters (19.41 %).

Key Words: Volatile components, Butterfly, Nectar plants, Host plants, ATD-GC-MS.

INTRODUCTION

Butterflies are insects belonged to the *Rhopalocera lepidoptera*. Butterflies are also one kind of pollinators, most of which not only have high ornamental value but also play an important role in maintaining the ecological balance of the nature¹.

Plant volatiles are produced and emitted by plants during growth and metabolism processes. Plant volatile components are short-chain hydrocarbons and their derivatives such as terpenes, alcohols, esters, acids, aldehydes, ketones and aromatic compounds *etc.*²⁻⁴. Some of those compounds can transmit chemical information in nature, which can regulate behaviours of insects, such as food and host recognition⁵⁻⁷. So it is necessary to study volatiles of butterfly host plants and nectar plants for its population reproduction.

The isolation of volatile components can be carried out by several methods *i.e.*, liquid solvent extraction⁸, steam distillation⁹, supercritical fluid extraction (SFE)⁹, solid phase microextraction (SPME)¹⁰ and thermal desorption¹¹. The thermal desorption method can be carried out automatically (automatic thermal desorption, ATD) on-line with the gas chromatography-mass spectrometry process (GC-MS). Comparing with the first four methods, the last method is simple, accurate, and can determine plants volatiles *in vivo*. *L. camara*, *V. trifolia* and *H. rosa-sinensis* are nectar plants of some

common ornamental butterflies such as *Tirumala limniace*, *Danaus chrysippus* and *Euploea core*¹² and *C. Spinosa*, *D. volubilis* and *P. laevigata* are their host plants¹³. In this paper, plants volatiles *in vivo* were isolated using the thermal desorption method and then analyzed by ATD-GC-MS.

EXPERIMENTAL

Nectar plants, flowering *L. camara*, *V. trifolia* and *H. rosa-sinensis*, were planted in Yuanjiang Experimental Station, Chinese Academy of Forestry (Yunnan, China) in November 2010. Host plants, living *C. Spinosa*, *D. volubilis* and *P. laevigata*, were also planted in Yuanjiang experimental station in November 2010.

Isolation of volatile components: The flowers or parts of the plant in natural state were putted in one closed, clean sampling bag. Volatile compounds was pumped by an air pump and collected by the sampling tube with adsorbents Tenax TA (0.2 g, 60-80 mesh, Perkin-Elmer). The collection time was 20 min. Sampling tubes were sealed and stored at low temperature (4 °C), ready for automatic thermal desorption and GC-MS analysis.

Automatic thermal desorption (ATD) conditions: Samples were desorbed using a commercial automatic thermal desorption system (Turbomatrix 650, Perkin-Elmer). The desorption temperature of sampling tube was at 260 °C for 10 min. A flow of

helium (25 mL min⁻¹) transferred the desorbed substances into a cold trap (at -30 °C, packed with Tenax TA), with a 15 mL min⁻¹ inlet split. After the desorption period, the cold trap was rapidly heated (40 °C s⁻¹) to 300 °C (5 min) in order to inject the sample as a narrow band into the chromatographic column, with a 20 mL min⁻¹ outlet split. Total split ratio was about 22:1.

GC-MS conditions: GC-MS analysis was performed on a Perkin-Elmer Clarus 600 GC-MS instrument equipped with a DB-5 fused silica column (30 m × 0.25 mm, 0.25 μm film thickness). Column temperature was initially kept at 40 °C for 2 min, then increased to 130 °C at a rate of 6 °C min⁻¹, held for 5 min and finally raised to 280 °C at 15 °C min⁻¹ for 5 min. Helium was used as the carrier gas at the constant flow of 1.5 mL min⁻¹. Mass spectra were recorded in EI mode with a 29-500 amu scan range, scan time 0.2 s. Interface temperature 250 °C; ion source temperature 220 °C; ionization voltage 70 eV.

Identification of volatile components: Peak identification was accomplished by comparison of the mass spectra with those stored on the GC-MS databases (NIST 08 and Wiley 2000) and reported in the literature. Relative percent of compositions was directly calculated by peak areas from reconstructed total ion current (TIC) trace using the area normalization method.

RESULTS AND DISCUSSION

Volatile components of nectar plants, *L. camara*, *V. trifolia* and *H. rosa-sinensis*: The identified components and their percentages of nectar plants are given in Table-1 according to their elution order on a DB-5 column. Of the three nectar plants, the most dominant components were terpenes, which total contents exceeded 60 %. Terpenes of *L. camara* were diverse, including 3-carene (15.15 %), β-phellandrene (7.49 %), α-caryophyllene (6.43 %) and limonene (5.90 %) *etc.* The main terpenes of *V. trifolia* was α-pinene (23.87 %), together with sabinene (18.76 %), α-phellandrene (6.60 %) and β-pinene (6.03 %). The most abundant terpene compounds of *H. rosa-sinensis* was limonene (41.42 %) and next was β-myrcene (8.17 %). Many kinds of terpenes, such as β-phellandrene, caryophyllene, limonene, had the special odour, which could attract insects. Eucalyptol was present in the three nectar plants with higher contents, 11.39 % in *L. camara*, 22.20 % in *V. trifolia* and 12.37 % in *H. rosa-sinensis*. Also there were some hydrocarbons, alcohols and aromatic compounds detected in the three nectar plants, including *p*-cymene (17.55 %) in *L. camara* and others with a small amounts. Most of those compounds had fragrant odour and were the ingredients of spices.

Volatile components of host plants, *C. spinosa*, *D. volubilis* and *P. laevigata*: The main volatile compositions of the determined host plants were different (Table-2). *C. spinosa* volatiles were mainly composed of terpenoids (36.35 %), aldehydes (29.07 %), ethers (10.48 %) and acids (9.15 %). *D. volubilis* volatile compounds were mainly esters (21.03 %), including pentyl methoxyl acetate (7.16 %), isopentyl acetate (4.15 %), ethyl hexanoate (3.99 %), isobutyl acetate (3.53 %) and hexyl acetate (2.20 %) and next were terpenoids (19.53 %), particularly α-pinene (13.98 %). *P. laevigata* volatiles were composed mainly of aldehydes (32.2 %), particularly nonanal (17.03 %) and decanal (7.81 %) and esters (19.41 %), particularly isopentyl acetate (6.69 %) and ethyl hexanoate (6.03 %).

TABLE-1
VOLATILE COMPONENTS AND RELATIVE PER CENT
OF FLOWERS OF *L. camara*, *V. trifolia* AND *H. rosa-sinensis*
FROM NECTAR PLANTS *in vivo*

Compounds	Relative per cent of volatiles of flowers (%)			
	<i>L. camara</i>	<i>V. trifolia</i>	<i>H. rosa-sinensis</i>	
Terpenes	α-Phellandrene	2.45	6.60	2.84
	α-Pinene	4.8	23.87	1.24
	Camphene	2.64	0.18	-
	Sabinene	-	18.76	9.61
	β-Phellandrene	7.49	-	-
	β-Pinene	4.24	6.03	0.70
	β-Myrcene	2.14	-	8.17
	3-Carene	15.15	-	-
	4-Carene	2.04	-	-
	Limonene	5.90	-	41.42
	Terpinene	-	4.14	-
	Terpinolene	-	3.34	4.42
	Copaene	2.04	-	-
	β-caryophyllene	4.01	-	-
	α-caryophyllene	6.43	-	-
	1-Methyl-4-(1-methylethyl)-1,4-cyclohexadiene	2.89	-	-
Alcohols	Eucalyptol	11.39	22.20	12.37
	1,3-Butandiol	3.07	-	-
	3-Hexen-1-ol	2.70	-	1.17
	3-Methylbutanol	-	3.95	-
	1-Octen-3-ol	-	-	1.5
	3-Nonen-1-ol	-	-	0.63
	Linalool	0.23	-	-
Aromatic compounds	<i>p</i> -Cymene	17.55	3.22	-
	<i>o</i> -Cymene	-	-	1.60
	Benzaldehyde	-	-	0.23
Hydrocarbons	2,3-Epoxy-2-methylbutane	-	4.04	-
	Cyclopentane	-	-	3.59
	Octane	-	-	0.31
Esters	<i>cis</i> -3-Hexenyl acetate	-	-	1.72

“-” meant no detected

TABLE-2
VOLATILE COMPONENTS AND RELATIVE PER CENT
OF *C. spinosa*, *D. volubilis* AND *P. laevigata*
FROM HOST PLANTS *in vivo*

Compounds	Relative per cent of volatiles of hostplants (%)			
	<i>C. spinosa</i>	<i>D. volubilis</i>	<i>P. laevigata</i>	
Terpenes	1-Octene	-	1.71	0.87
	α-Pinene	21.87	13.98	7.01
	Limonene	0.70	1.66	1.42
	Ocimene	-	2.18	-
	Copaene	1.15	-	-
	Caryophyllene	12.63	-	-
Aldehydes	2-Methyl pentanal	20.83	-	1.73
	Heptanal	-	-	1.46
	Octanal	0.84	0.85	4.17
	Nonanal	3.71	3.54	17.03
	Decanal	3.69	1.79	7.81
Esters	Pentyl methoxyl acetate	-	7.16	-
	Isobutyl acetate	-	3.53	2.37
	Isopentyl acetate	-	4.15	6.69
	Ethyl hexanoate	-	3.99	6.03
	3-Hexenyl acetate	1.81	-	-
	Hexyl acetate	-	2.20	4.32

Compounds	Relative per cent of volatiles of hostplants (%)			
	C.	D.	P.	
	<i>spinosa</i>	<i>volubilis</i>	<i>laevigata</i>	
Alcohols	Eucalyptol	-	3.20	3.37
	3-Hexen-1-ol	-	-	1.82
	2-Methylundecanol	3.39	-	-
	2-Decen-1-ol	-	-	1.02
Ethers	2-(2-Butoxyethoxy) ethanol	10.48	7.56	6.93
	Pentane	-	3.28	1.99
Hydro-carbons	2-Ethenyl-1,1-dimethyl-3-methylenecyclohexane	-	3.72	-
	pentadecane	-	-	0.84
Aromatic compounds	Ethenyl benzene	-	-	3.82
	Naphthalene	-	-	0.97
Acids	Acetic acid	9.15	-	-
“-” meant no detected				

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