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ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

VOLATILE COMPOUNDS FROM UNDERSTORY AIR OF A FOREST ECOSYSTEM AND  
THEIR EFFECTS ON INSECTS IN DOI PHU KA NATIONAL PARK



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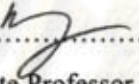
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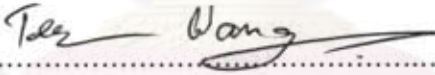
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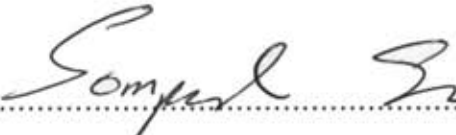
  
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ผกาเวียง ช่อกระดิน : สารระเหยง่ายจากอากาศป่าพื้นล่างของระบบนิเวศป่าและผลที่มีต่อแมลงในเขตอุทยานแห่งชาติดอยภูคา. (VOLATILE COMPOUNDS FROM UNDERSTORY AIR OF A FOREST ECOSYSTEM AND THEIR EFFECTS ON INSECTS IN DOI PHU KA NATIONAL PARK) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: อ.ดร.ธีรดา หวังสมบูรณ์ดี, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : รศ.ดร.วันชัย ดีเอ็กนามกุล, 74 หน้า.

พืชปลดปล่อยสารระเหยง่ายซึ่งมีบทบาทสำคัญต่อปฏิสัมพันธ์ระหว่างสิ่งมีชีวิตในระบบนิเวศและส่งผลกระทบต่อสารเคมีในบรรยากาศด้วย การศึกษาความสัมพันธ์ระหว่างสารกับแมลงถูกทดลองในป่าเขตร้อนบนพื้นที่อุทยานแห่งชาติดอยภูคา จังหวัดน่าน ประเทศไทย เนื่องจากเป็นพื้นที่ซึ่งมีความหลากหลายของพืชและแมลงสูง อุปกรณ์ดักจับสารอย่างง่ายถูกเตรียมขึ้นเพื่อใช้ในการดักจับสารระเหยง่ายในอากาศป่าพื้นล่าง โดยบรรจุ Super Q ในหลอดพลาสติกและต่อกับเครื่องปั๊มอากาศ จากนั้นวางเครื่องดักอากาศและแผ่นดักแมลงในพื้นที่ 15 ตำแหน่งที่เลือกไว้เพื่อศึกษาสารอินทรีย์ระเหยง่ายและผลของสารต่อแมลง จากผลการวิเคราะห์พบสารในกลุ่มโมโนเทอร์ปีนทั้งหมด 7 ชนิด ได้แก่  $\alpha$ -thujene camphene 3-carene *p*-cymene limonene (1,8)-cineole และ camphor นอกจากนี้พบสารระเหยง่ายชนิดอื่นด้วย จากผลของความสัมพันธ์ระหว่างสารระเหยง่ายที่มีต่อแมลง พบว่าแมลงในอันดับ Hymenoptera และ Homoptera ถูกดึงดูดด้วย camphene และ limonene ตามลำดับ ขณะที่แมลงในอันดับ Hymenoptera และ Coleoptera ถูกขับไล่ด้วย benzaldehyde และ 1-octen-3-ol ตามลำดับ สารในกลุ่มโมโนเทอร์ปีน คือ *p*-Cymene และ (1,8)-cineole สามารถขับไล่แมลงทั้งในอันดับ Coleoptera และ Homoptera ได้ นอกจากนี้แมลงในอันดับ Diptera ถูกขับไล่ได้ด้วย limonene จากผลการทดลองนี้ทำให้ได้ข้อมูลซึ่งจะเข้าใจความสัมพันธ์ของสารเคมีที่สิ่งมีชีวิตสร้างขึ้นในระบบนิเวศป่าเขตร้อนได้ดีขึ้น และอาจจะนำผลของความสัมพัทธ์ไปประยุกต์ใช้ในการจัดการพื้นที่การเกษตรได้ด้วย

สาขาวิชาเทคโนโลยีชีวภาพ

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## 4773860123 : MAJOR BIOTECHNOLOGY

KEYWORDS : Chemical Ecology / Volatile compounds / Attractant / Repellent

PAKAWIENG CHOKRATIN : VOLATILE COMPOUNDS FROM UNDERSTORY AIR OF A FOREST ECOSYSTEM AND THEIR EFFECTS ON INSECTS IN DOI PHU KA NATIONAL PARK. THESIS ADVISOR: TEERADA WONGSOMBOONDEE, Ph.D., THESIS CO-ADVISOR: ASSOC. PROF. WANCHAI DE-EKNAMKUL, Ph.D., 74 pp.

Plants emit volatile organic compounds (VOCs) that play important roles in their interactions with other organisms in the ecosystem and have a major impact on atmospheric chemistry. In order to obtain information on the relationships between VOCs and insects in tropical forests, the forest area of Doi Phu Ka National Park, Nan Province of Thailand was selected as a study site due to its high plant and insect diversities. A simple air trap was constructed to study the composition of VOCs in the understory air of the forest by using Super Q adsorbent packed in a plastic syringe and connected to a portable air pump. The air traps, together with insect traps, were both placed at selected 15 sites for simultaneous study of the VOC components and their effects on insects. The results revealed that seven VOCs identified as monoterpenes;  $\alpha$ -thujene, camphene, 3-carene, *p*-cymene, limonene, (1,8)-cineole and camphor and other VOCs from the understory air. The results on the correlation between the VOC composition and the effect on insect showed that the insect types of Hymenoptera and Homoptera were attracted by camphene and limonene, respectively. Whereas, the insects types of Hymenoptera and Coleoptera were repelled by benzaldehyde and 1-octen-3-ol, respectively. On the other hand, the monoterpenes that are *p*-Cymene and (1,8)-cineole repelled not only the insects of Coleoptera but also the insects of Homoptera. In addition, the insects of Diptera were repelled by limonene. These results of interactions may lead to the understanding of the chemical ecology of the tropical forests and may be applied to the area of agricultural management.

Field of Study : Biotechnology .....

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**LIST OF ABBREVIATIONS**

a.s.l.	above sea level
bp	boiling point
BVOCs	biogenic volatile organic compounds
°C	degree Celsius
EI	electron impact ionization
eV	electron volt
FID	flame ionization detection
GC	Gas Chromatography
GPS	Global Positioning System
hr	hour
HS	Headspace
I.D.	internal diameter
L	length
m	meter
min	minute
ml	milliliter (s)
mm	millimeter
MS	Mass Spectrometry
NIST	National Institute of Standard and Technology
No.	number
ppm	part per million
ppb	part per billion
r	correlation coefficient
SIM	selected ion monitoring mode
µm	micrometer
VOCs	volatile organic compounds

## CHAPTER I

### INTRODUCTION

Currently, several studies in ecology have focused on the relationship between an organism and both its biotic and abiotic environments which influence the organisms' distributions and populations. The studies of biotic factors are involved in the disciplines of physiology, behavior, genetics and evolution which are interrelated under the subject of ecology. Among the biotic factors, these natural products with biological origin and activities which mediate interactions between organisms are the subject of "chemical ecology". The interactions not only involve chemicals having direct physical effects, but also their existing signals which give these compounds potentials of being control agents as alternatives to pesticides.

Volatile organic compounds (VOCs) are very important as chemical composition of ambient atmosphere. Ozone is formed through the photochemical reactions of VOCs of both anthropogenic and biogenic origin with oxides of nitrogen ( $\text{NO}_x$ ) (Atkinson, 2000). VOC emissions from anthropogenic sources such as power plants and road traffic have been well studied for quality control of air pollution. However, little is known about VOC emissions from biogenic sources. Plant volatile organic compounds are constituents that can be found both in plants and in the atmosphere. The most abundant VOCs found in the forest air are phytochemical species such as isoprenes and monoterpenes (Fuentes et al., 1996; Moukhtar et al., 2006; Nunes and Pio, 2001). Since VOCs play important roles in the interactions among organisms in an ecosystem, a study on the relationship between VOCs emitted from particular plants and their effects on other organisms, especially insects, would lead to a better understanding of such interactions.

In Thailand, however, there have been no studies in the field of chemical ecology, although Thai forests have high degree of diversity of plants and insects. Therefore, this research work aimed to study the interactions between the emitted volatiles and some kinds of insects found in the forest. Emphasis was on the test on the efficiency of the attractant or repellent properties of a particular volatile compound on the insects. It is expected that the obtained knowledge can be applied for pest control in the future. Furthermore, the volatile compounds of plants in the study trail were identified and some volatile oils were extracted and tested for their effects on insects (Sonthimanotham, 2009).

## Objectives

1. To evaluate the efficiency of a new device of trapping and identification of volatile organic compounds present in the forest atmosphere.
2. To study the relationship between some detected volatile organic compounds constituents and some insects found in the target area.
3. To confirm the predicted effect of some volatile components to the insects by field trials using pure VOCs.



ศูนย์วิทยทรัพยากร  
จุฬาลงกรณ์มหาวิทยาลัย

## CHAPTER II

### LITERATURE REVIEW

#### 2.1 Doi Phu Ka National Park

##### 2.1.1 Location

Doi Phu Ka National Park is located in Nan province with Global Positioning System (GPS) at 19°10'34.33"N 100°54'59.94"E (Google Earth) and covers the area of eastern part of Northern Thailand near by Lao PDR border. According to a royal decree, Doi Phu Ka and surrounding areas have been established as a national park since 17 June 1999 (The Government Gazette, 1999). The area covers approximately 1,704 km<sup>2</sup> (170,400 hectares or 1,065,000 Rai) in eight administrative districts: Bo Kluea, Chalerm Pra Kiat, Chiang Klang, Mae Charim, Phu, Santisuk, Tha Wang Pha and Thung Chang. It situates in high-land area with 1,980 meters (6,600 feet) above sea level (a.s.l.) (National Parks, Wildlife and Plant Conservation Department [DNP], 2006).

##### 2.1.2 Climate

The seasons in the park are divided into three characteristics which are rainy season on May to October with the peak rainfall on August and September, cold season on November-February and summer on March to April. The most popular time to visit the park is the cold season because of the good weather and the blooming of Chompoo Phuka flowers (Srikosamatara and Suteethorn, 1994; DNP, 2006).

##### 2.1.3 Forest trees

There are six main different forest types in this National Park which are coniferous forest, montane scrub forest, mixed deciduous forest, deciduous dipterocarp forest, dry evergreen forest and hill evergreen forest (Srisanga, 2005). There is also a rare plant called Chompoo Phuka Tree (*Bretschneider sinensis*) in the park with white rose flower and bloom in long upright racemes. Furthermore, there is ancient palm, Tao Rang Yak (*Caryota gigas*), which is the endangered species. These trees grow well here due to the proper soil properties. These are found on the rocky mountain slopes. These two species only found at Doi Phu Ka National Park (Gardner, et al., 2007).

Thirty nine species of vascular plants on Doi Phu Ka National Park have been identified as endemic in Thailand, including 6 endemic to Doi Phu Ka and 60 species were classified as rare species (Srisanga, 2005). One new species is *Capparis trisonthiae* (Capparaceae) Srisanga & Chayamarit (Srisanga and Chayamarit, 2004) and additional 15 species are probably new species or at least new records for Thailand.

Doi Phu Ka National Park consists of a high plant diversity which is potentially the rich source of emitted volatile compounds. The area in this park was, therefore, chosen to study the emission of plant volatile compounds in the forest atmosphere in our research.

## 2.2 Plant volatile compounds

### 2.2.1 Biosynthesis of plant volatile compounds

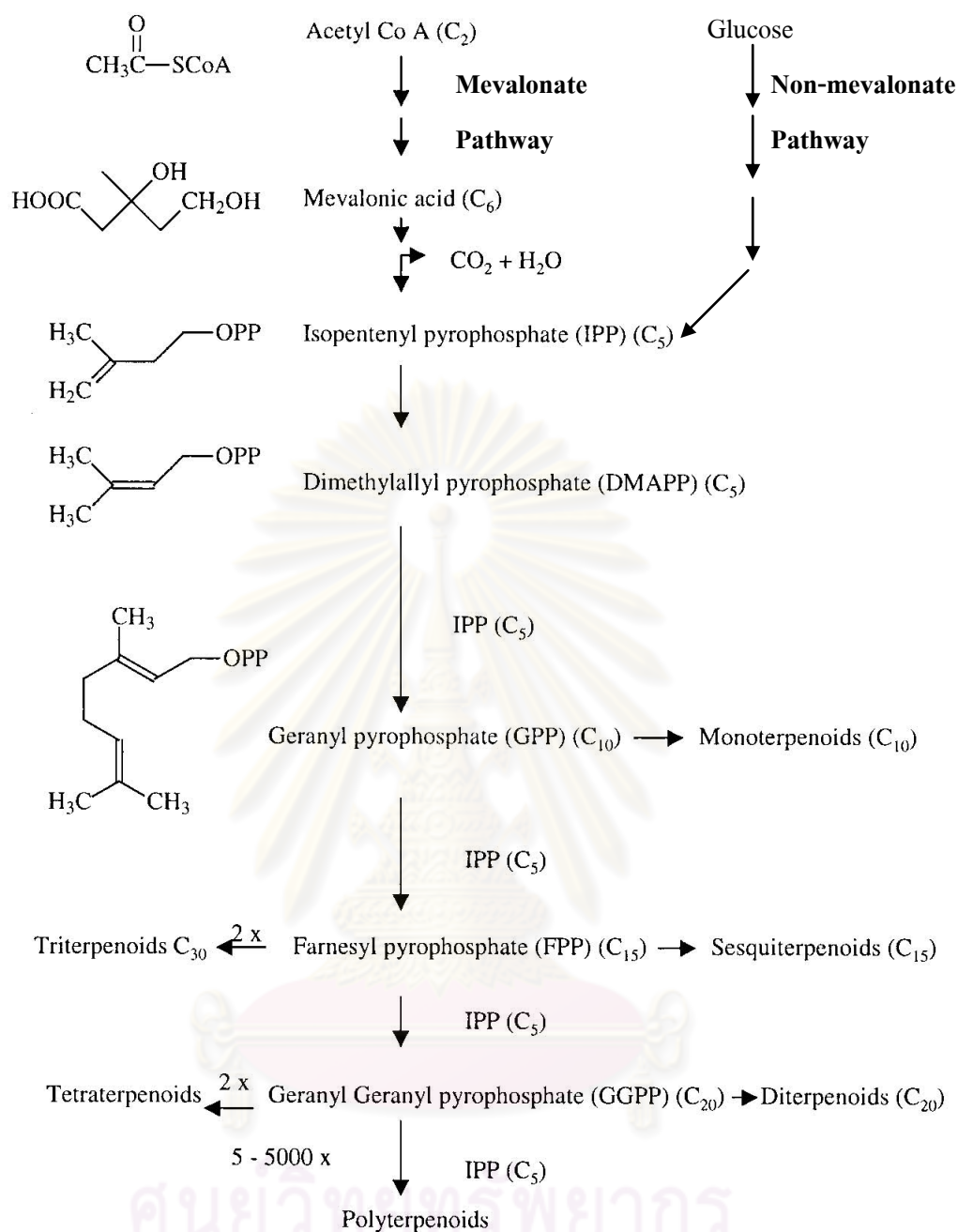
Terpenes are the largest class of plant secondary metabolites consisting of isoprene units and many of them are volatile representatives. The compounds with basic skeleton derived from either mevalonic acid, or non-mevalonic acid, are termed terpenoids. Terpenoids are all based on the isoprene molecule (2-methylbutadiene) and their carbon skeletons are built up from the union of two or more of these C<sub>5</sub> units. The classification and the schematic outline of terpenoids biosynthesis are shown in Table 2.1 and Fig 2.1.

**Table 2.1** The main classes of plant terpenoids

Number of isoprenes units	Carbon number	Name of class	Main types
1	C <sub>5</sub>	isoprene	detected in leaf
2	C <sub>10</sub>	monoterpenoids	plant essential oils, lactones, tropolones
3	C <sub>15</sub>	sesquiterpenoids	essential oils, lactones, abscisins
4	C <sub>20</sub>	diterpenoids	plant resins, gibberellins
6	C <sub>30</sub>	triterpenoids	sterols, triterpenes, saponins, cardiac glycosides
8	C <sub>40</sub>	tetraterpenoids	carotenoids
n	C <sub>n</sub>	polyisoprene	rubber

**Source:** Harborne (1984)





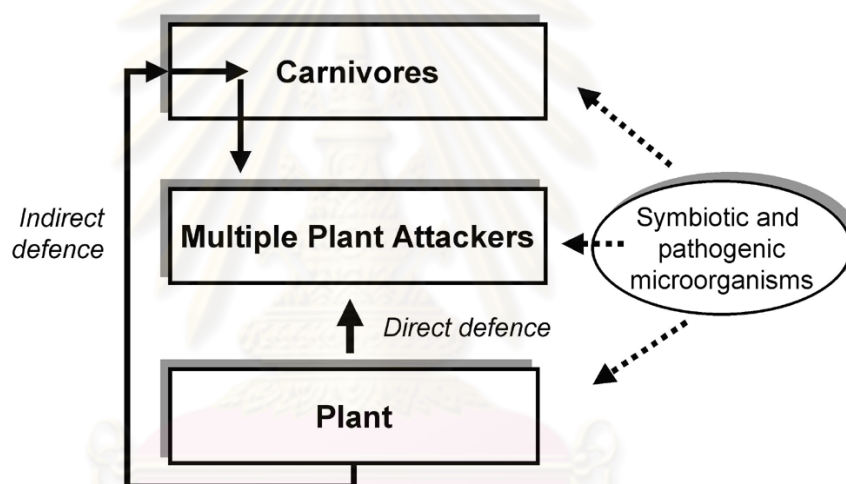
**Figure 2.1** Schematic outline of terpenoid biosynthesis (Langenheim, 1994)

### 2.2.2 Function of plant volatile compounds

Plants have affection for perfuming the atmosphere around them and it has been known that both floral and vegetative parts of many species emit substances with unique smells. The compounds emitted from flowers are generally presented as the attraction for guide pollinators. Theis (2006) have discussed the two components of Canada thistle (*Cirsium arvense*), benzaldehyde and phenylacetaldehyde, as both pollinators and florivores. Reinhard et al. (2004)

have shown that a familiar nectar scent can trigger specific memories of route and therefore expedite navigation to the food source of honeybees.

The release of volatiles from vegetative parts has been widely studied. The relatively new research field of Chemical Ecology has revealed an important role of plant-produced volatile organic compounds (VOCs) in mediating interactions between plants and other organisms (Agelopoulos and Pickett, 1998; D'Alessandro and Tyurlings, 2005). Plants emitting VOCs for direct plant defences against attackers while indirect plant defences may attract carnivores or predators of the plant attacker (Fig 2.2). The understanding in these interactions can be used to apply in agriculture such as pest control (Pickett et al., 1997) and medicine such as bioactive compounds for antimicrobial or aromatherapy.



**Figure 2.2** Multitrophic context of defence of a plant within a community (Dicke and Hilker, 2003)

### 2.2.3 Plant volatile compounds in the atmosphere

The plant volatile compounds are constituents that can be found both in plants and in the atmosphere. Much of the recent work on emissions of biogenic volatile organic compounds (BVOCs) has focused on isoprene (Geron et al., 2000) and monoterpenes (Vuorinen et al., 2004; Lee et al., 2005; Holzinger, 2006). Since high concentrations of biogenic compounds can be found in nature, many sites have been selected to study the biogenic compounds as shown in Table 2.2.

**Table 2.2** Plant volatile compounds in the forest atmosphere

Countries	Sites	Plants	Dominant compounds	References
Benin	Nangantchori	many plants	isoprene, monoterpenes and sesquiterpenes	Saxton et al., 2007
China	Xishuangbanna	8 tree species	$\alpha$ -pinene, $\beta$ -pinene, sabinene, myrcene, limonene, camphene, <i>p</i> -cymene, $\alpha$ -phellandrene, and $\gamma$ -terpinene	Wilske et al., 2007
Finland	Huhus and Hyytiälä	<i>Pinus sylvestris</i>	$\alpha$ -pinene, $\beta$ -pinene, 3-carene and myrcene	Rinne et al., 2000
France	Corineland	Forest trees, Coniferous	isoprene and monoterpenes	Solmon, 2004
India	-	51 local Indian plants	isoprene, $\alpha$ -pinene and other VOCs	Padhy and Varshney, 2005
Japan	Oshiba plateau	<i>Pinus densiflora</i>	$\alpha$ -pinene, limonene and $\beta$ -phellandrene	Tani et al., 2002
Mongolia	Inner grassland	grasses, sedge and forbs	isoprene and monoterpenes	Bai et al., 2006
Netherlands	Speulderbos	pine	$\alpha$ -pinene, $\beta$ -pinene, 3-carene and limonene	Peters et al., 1994
Spain	local urban vegetation	3 conifers and 9 angiosperms	$\alpha$ -pinene, $\beta$ -pinene, myrcene, $\alpha$ and $\beta$ - phellandrene, careen, limonene and eucalyptol	Noe et al., 2008
Sweden and Finland	Boreal forest	<i>Pinus sylvestris</i> and <i>Picea abies</i>	acetone, monoterpenes and isoprene	Janson and Serves, 2001
USA	Sierra Nevada Mountains	coniferous	21 terpenoids, <i>p</i> -cymene and longifolene	Helmig and Arey, 1992
USA	Blodgett in the Sierra Nevada Mountains	ponderosa pine, manzanita and ceanothus	monoterpenes, sesquiterpenes and methyl chavicol	Bouvier-Brown et al., 2009

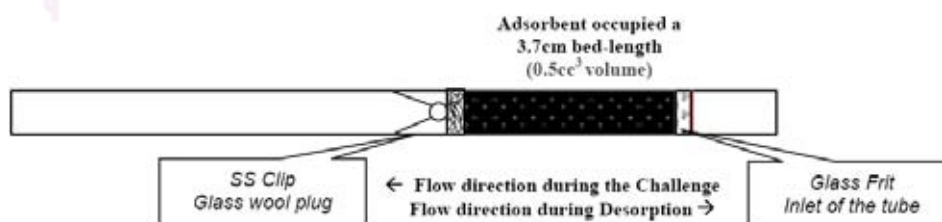
### 2.2.4 Factors affecting plant volatile emission

The emission of a particular volatile compound into the atmosphere depends on both the rate of its biosynthesis and the rate of its release. Environmental factors such as light, temperature, and moisture status can greatly influence the emission of volatiles, yield and composition of essential oils (Dudareva et al., 2004). Moreover, the measurement of isoprene and monoterpene above Amazonian rainforest has shown that the increase of emissions depends on light and temperature (Rinne et al., 2002).

Seasonal variation has also effect on the concentration of VOCs. In summer, there is not only the highest isoprene emission observed but also sesquiterpenes. In addition to the seasonal changes, degradation of the monoterpenes by atmospheric radicals and the diffusion processes in the mixing layer, air temperature and the development of the leaves and needles appear to be important for the monoterpene concentration in coniferous forest (Hakola et al., 2003).

### 2.2.5 Analytical technique for plant volatile compounds

Gas Chromatography-Mass spectrometry (GC-MS) has been the method of choice for the separation, identification, and quantification of volatile components. Before the analysis, collection of the volatile component is considered important. A Volatile Collector Trap is a passive chemical filter designed for the collection of trace (extremely low-level, ppm-ppb) volatile organic compounds from sampled air or gas sources (ambient air, container/chamber air, head-space volatiles, chemical odors/fumes, etc...). The sampled gas is either pulled or pushed through the filter at a constant flow-rate and any VOCs in the gas stream are absorbed onto the filter media. However, just as important the adsorbent must also be able to release the analytes during desorption process for analyzing by laboratory analytical methods. The general traps were packed as shown in Fig 2.3.



**Figure 2.3** Drawing of packed adsorbent tube (Brown and Shirey, 2001)

After the collection, the collected VOCs are removed from the filter by either eluding them from the adsorbent media using some type of extraction such as a strong organic solvent or using gas which has a higher affinity for the adsorbent media than the collected compounds. Another method is by driving off the compounds by re-volatilizing them using heat (thermal desorption) with the optimization of parameters such as time and temperature (Penton, 1992).

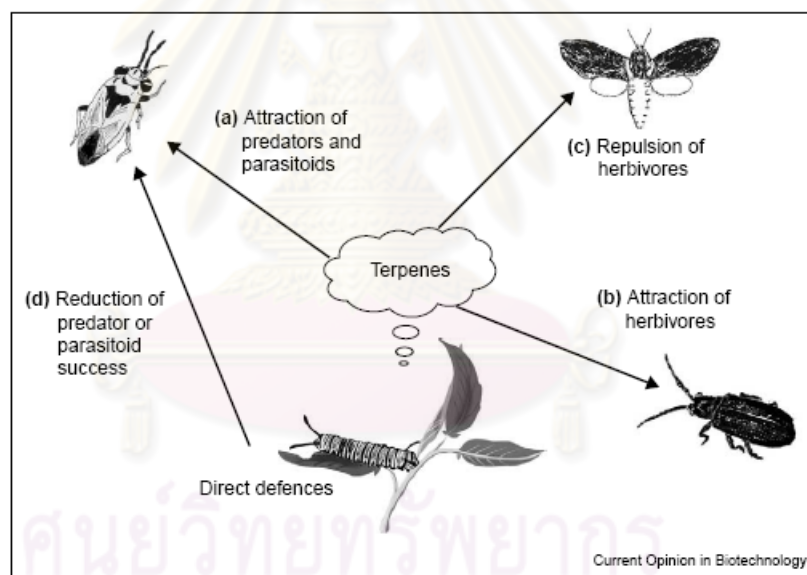
Ruther and Hilker (1998) have analyzed the volatile compounds emitted by living aerobic organisms using a modified thermal desorber and then utilize the purge and cold trap technique coupled with GC-MS. The volatiles are usually carried out by using a 30 m length (L) x 0.32 mm internal diameter (I.D.), DB-5ms (5% phenyl, 95% methylpolysiloxane) fused silica column, film thickness ( $d_f$ ) 1.0  $\mu\text{m}$  or 0.25  $\mu\text{m}$  with helium as carrier gas. A temperature program is started at 40°C, held for 1 min, and raised at 4°C/min to 280°C. The column effluent is ionized by electron impact ionization (EI) at 70 eV, and eluted compounds were identified by comparing obtained spectra with National Institute of Standard and Technology (NIST) library spectra.

Picone et al. (2002) have shown that the emission of floral volatiles from *Mahonia japonica* can be trapped by Porapak Q adsorbent, identified by GC-MS and confirmed by retention times of pure reference compounds. The column is an HP ultra2 (5% diphenyl, 95% dimethylpolysiloxane) 50 m L x 0.2 mm I.D., with film thickness 0.33  $\mu\text{m}$ . Carrier gas is Helium at 1.6 ml/min and the oven temperature can be programmed from 50–270°C at 2°C/min and injector temperature is 250 °C.

The monoterpene emissions in *Pinus sylvestris* dominated forests have been extensively studied using adsorption of air samples onto adsorbent. The samples have been analyzed using gas chromatograph with an HP-1 (100% dimethylpolysiloxane) column 50 m L x 0.32 mm I.D. and mass selective detector in selected ion monitoring (SIM) mode (Rinne et al., 2000). A number of monoterpenes have also been found in an old-growth Pacific Northwest coniferous forest using gas chromatography with DB-1 (100% dimethylpolysiloxane) capillary column 30 m L x 32 mm I.D., and flame ionization detection (FID) (Pressley et al., 2004).

### 2.3 Relationship between plant volatile organic compounds and insects

In an ecosystem, the plant substances can be involved in plant-insect interactions or chemical defense as attractants, stimulants or deterrents to feeding and oviposition. Plants normally release small quantities of VOCs, but when they are attacked by herbivorous insects, many more volatiles are released. These volatiles, dominated by terpenes, can attract predatory insects and/or repel herbivores (Fig 2.4). After a plant damaged by herbivore, it releases terpenes, the substances play role as chemical indirect defences by attracting predators and parasitoids that prey on the herbivores or use them as hosts for their larvae (Fig 2.4a). The same terpenes can also affect other herbivores, either attracting them to feed (Fig 2.4b) or repelling them from feeding or oviposition (Fig 2.4c). In addition, plant traits involved in direct defences against herbivores (e.g. glandular trichomes or toxins) can negatively affect predator or parasitoid success (Fig 2.4d) (Degenhardt et al., 2003).



**Figure 2.4** Interactions between plants, herbivores and herbivore enemies mediated by terpenes.

(Degenhardt et al., 2003)

Since Plant VOCs play important roles in the interactions among organisms in a forest ecosystem, the study on the relationship between plant VOCs emitted from particular plants and their effects on other organisms, especially insects, would lead to a better understanding of such interactions like attracting or repelling effect on insects.

### 2.3.1 Attracting effect

Plants can attract insects for pollination and indirect responses by which plants attract natural enemies of their herbivores that act as plant defending agents. The discoveries of plant volatiles that attract enemies may help harness the full potential of biological control in agroecosystems and applications. Some plant attractants tested with insects were shown in Table 2.3.

**Table 2.3** Attracting effect of plant volatile compounds on insects

Efficient plant VOCs	Insects	Insect Order	references
Volatile compounds from <i>Spondias mombin</i> fruits	fruit fly	Diptera	Lopez et al., 2006
(-)-camphene	<i>Eremostibes opacus</i> Koch	Coleoptera	Geiselhardt et al., 2006
$\alpha$ -pinene	conifer-feeding beetles	Coleoptera	Chenier and Philogene, 1989
$\alpha$ -pinene	female Japanese horntails	Hymenoptera	Sato and Maeto, 2006
1,4-dimethoxybenzene	females of bee	Hymenoptera	Dotterl et al., 2005
1,8-cineole	females of grape berry moth	Lepidoptera	Katerinopoulos et al., 2005

### 2.3.2 Repelling effect

In some cases, herbivore-induced volatiles may act as direct defense that concerned with repelling effect on insects. Many investigations reported that some plant essential oils not only repel insects, but also have contact and fumigant insecticidal actions against specific pests (Isman, 2000). Some plant repellants tested with insects were shown in Table 2.4.

**Table 2.4** Repelling effect of plant volatile compounds on insects

Efficient plant VOCs	Insects	Insect Order	references
Volatile compounds from <i>Artemisia vulgaris</i>	yellow fever mosquito	Diptera	Hwang et al., 1985
1,8-cineole	Mosquito	Diptera	Klocke et al., 1987
Linalool, limonene, and $\beta$ -pinene	<i>Drosophila melanogaster</i>	Diptera	Yamasaki et al., 2007

### 2.3.3 Correlation analysis

Correlation analysis means the method for measure the strength and direction of the linear relationship between two variables by correlation coefficient value (Buntinas and Funk, 2005). In statistics, the value of the correlation coefficient varies between +1 and -1 ( $-1 \leq r \leq 1$ ) with scaled rating are very strong ( $>0.8$ ), moderately strong (0.5-0.8), fair (0.3-0.5) and poor ( $<0.3$ ). There are three types of correlation: Pearson correlation, Kendall rank correlation and Spearman correlation.

Pearson correlation is widely used in statistics to measure the degree of the relationship between the linear related variables. For the Pearson correlation, both variables should be normally distributed. When outliers are present, Kendall rank correlation and Spearman rank correlation, non-parametric tests, are used. Kendall rank correlation does not assume any assumptions related to the distributions like Pearson correlation. Spearman rank correlation was developed by Spearman, thus it is called the Spearman rank correlation. Spearman rank correlation test does not assume any assumptions about the distribution. Spearman rank correlation test is used when the Pearson test gives misleading results (Chen and Popovich, 2000).

### 2.3.4 Bioassay for VOC-insect interaction

The responses of insects to volatile compounds have been studied by using various methods. In laboratory, olfactometers have been used to monitor the responses of insects by tracking and measuring upwind movements to odors from plant or specific odors. For example, four-arm olfactometer has been used for testing the multiple odors on *Trichogramma chilonis* Ishii by recording the distance travel and unit time (Ranjith, 2007). Y-tube olfactometer has also been used for comparing the responses of vine weevils to two choices between attractant and no compound (Nakamura et al., 2005).

However, in the field, the baited sticky traps were used to determine the effects of monoterpenes on the number of insects captured. For example, baited sticky stovepipe traps have been used to study the responses of certain forest coleoptera to conifer monoterpenes and ethanol by compare the number of captured insects per treatment with unbaited traps (Chenier and Philogene, 1989). Sticky panels traps and black pyramids traps baited with different synthetic host odors and synthetic aggregation pheromone have been used to examine the attractiveness of adult plum curculios (Pinero and Prokopy, 2003). In addition, yellow sticky cards baited with 2-ml glass vials of herbivore-induced plant volatile solutions and unbaited traps were tied to wooden poles 2 m above the ground for demonstrating the responses of green lacewing (James, 2003).



## CHAPTER III

### MATERIALS AND METHODS

#### 3.1 Sample sources

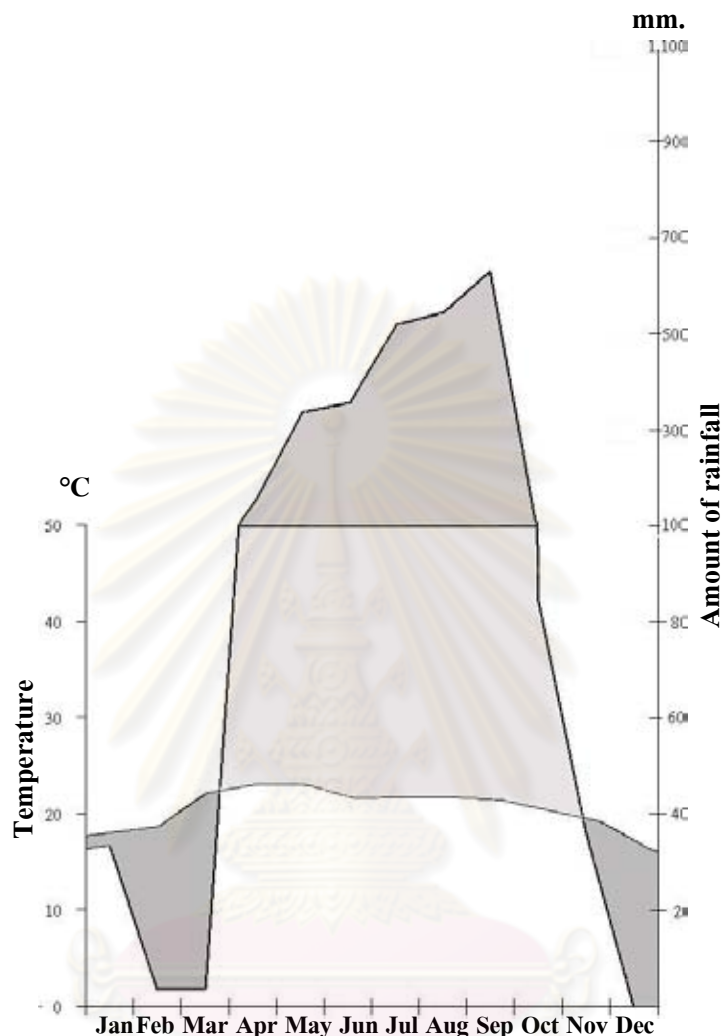
Forest understory air samples were collected from 15 permanent plots at Dong Ya Wai on hill evergreen forest, Doi Phu Ka National Park by GPS as follows:

No. of sites*	Global Positioning System		Elevation (m, a.s.l.)
01	19°11'00.10"N	101° 5'59.60"E	1,544
02	19°11'01.90"N	101° 5'59.90"E	1,632
03	19°11'08.00"N	101° 6'04.70"E	1,759
04	19°11'12.70"N	101° 6'19.30"E	1,806
05	19°11'14.70"N	101° 6'22.90"E	1,812
06	19°11'16.30"N	101° 6'24.90"E	1,876
07	19°11'17.10"N	101° 6'27.60"E	1,913
08	19°11'21.30"N	101° 6'33.20"E	1,926
09	19°11'30.50"N	101° 6'32.80"E	1,910
10	19°11'40.90"N	101° 6'28.90"E	1,849
11	19°11'50.70"N	101° 6'20.77"E	1,815
12	19°12'00.80"N	101° 6'16.80"E	1,804
13	19°12'06.50"N	101° 6'08.90"E	1,772
14	19°12'08.50"N	101° 6'04.40"E	1,712
15	19°12'13.10"N	101° 5'56.78"E	1,666

\* These sites were chosen based on the preliminary study of 35 sites which are 100 m apart from each side or had high volatiles quantity and some sites also had fragrance plants.

The area of Dong Ya Wai in Doi Phu Ka National Park was selected as the study site because it has high plant diversity (Srisanga, 2005) that potentially be a source of biogenic VOCs. From 35 sample sites preliminary specified in the survey trail, 15 sample sites were subsequently selected based on their high VOCs content. Some fragrance plants were also found in these selected sites such as *Cinnamomum iners*, *Litsea cubeba* and *Zanthoxylum* sp. (Sonthimanotham, 2009). Air samples were collected in February/March and October/November from 2005-2008 for representing the air VOC constituents in the summer and winter,

respectively. The experiments were started in late October 2005. We omitted the rainy season because the high rainfall (Fig. 3.1) causing difficulty in travelling and research operating.



**Figure 3.1** The amount of rainfall (mm) and temperature (°C) at Doi Phu Ka National Park in 2005

### 3.2 Chemicals and equipments

#### 3.2.1 Chemicals

(-)-trans-Caryophyllene	SIGMA Chemical Co
Eugenol	SIGMA Chemical Co
(±)-Linalool	SIGMA Chemical Co
(1S)-(-)- $\alpha$ -Pinene	SIGMA Chemical Co
Safrole	CHEM SERVICE
Eucalyptol (1,8-Cineol)	CHEM SERVICE and Fluka Chemika
(+)-3-Carene	Fluka Chemika

Camphor	Fluka Chemika
Benzaldehyde	Aldrich Chemical Co
Camphene	Aldrich Chemical Co
<i>p</i> -Cymene	Aldrich Chemical Co
Limonene	Aldrich Chemical Co
<i>E</i> -4-Octene	Aldrich Chemical Co
1-Octen-3-ol	Aldrich Chemical Co
1, 2, 4-Trimethylbenzene	Aldrich Chemical Co
Super Q (Divinylbenzene/Ethylvinylbenzene) polymer,	Alltech

### 3.2.2 Equipment

#### 3.2.2.1 Portable volatile collectors

The device of volatile collector were assembled using 5 ml a plastic syringe tube packed with 0.5 g Super Q and connected the collection tube to the portable air pump using battery (Fig 3.2).



**Figure 3.2** The applied portable volatile collectors.

#### 3.2.2.2 Headspace Gas Chromatography-Mass Spectrometry (HS/ GC-MS)

The HS/GC-MS was performed on Genesis<sup>®</sup> Headspace Autosampler connected to a Varian Saturn Ion Trap GC-MS system includes Varian 3400 GC coupled directly to Ion Trap Mass spectrometer at the central laboratory of faculty of Pharmaceutical Sciences, Chulalongkorn University

#### 3.2.2.3 Yellow sticky traps

The trap was applied by coating one side of yellow plastic board (22 cm x 22 cm) with glue traps for catching the population of insect. The yellow sticky trap for field-tests

was performed by putting the eppendorf tube without cap filled with absorbent cotton soaked with a pure chemical in the middle of the trap.

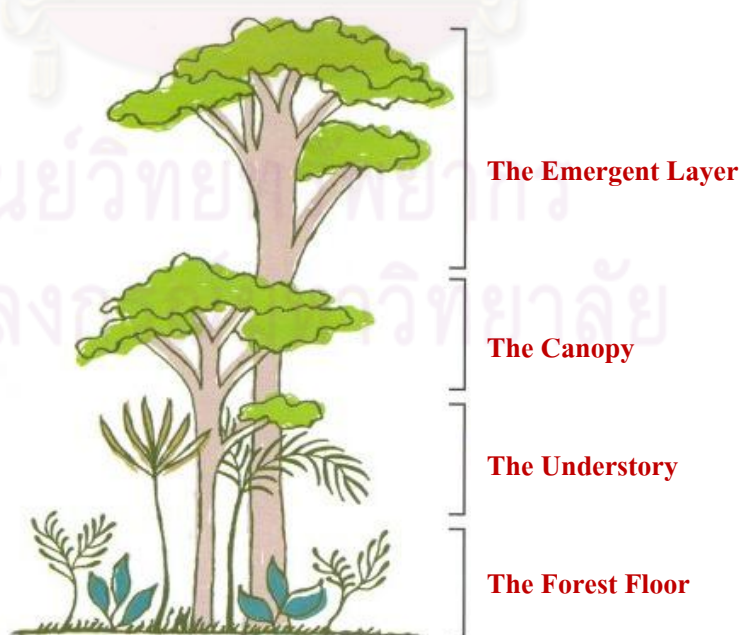
### 3.3 Optimization of a portable volatile collector in laboratory

The volatile trap was put into the enclosed chamber (30 cm x 30 cm x 30 cm) with standard volatile compounds. Ten microliters of seven pure oils:  $\alpha$ -pinene, 3-carene, eucalyptol, eugenol, linalool, safrole and caryophyllene were diluted with 10 ml methanol, and then 1ml of the mixed oils was placed in petri dish. Air sample was collected until the pumps run out of batteries (2 days). The optimization of headspace was performed to select parameters for the analysis, which are temperature at 120, 150, 180 and 200°C and equilibration time at 5, 10, 15, 20 and 30 mins.

### 3.4 Determination of understory air composition

#### 3.4.1 Sample collection

The portable volatile collectors were placed on big trees for sampling at the height of 2 m above the ground in the layer of understory (Fig. 3.3), and then the adsorbed components were extracted and concentrated by passing air simultaneously through the adsorbent tubes filled with solid trap, Super Q, for 48 hr by portable air pumps. The samplings were collected on October 2005, March 2006, February 2007, November 2007, March 2008 and November 2008. After the collection, the air traps were kept in dark until analysis was carried out in laboratory within one week.



**Figure 3.3** The layers of growth in the forest

### 3.4.2 Gas chromatography-mass spectrometry (GC-MS)

For identification of the composition of essential oil, a headspace technique coupled with GC-MS was used. The adsorbent from each collector was placed into a vial (22 ml) with aluminum cap seal. Then, the vial was heated at 200°C for 5 minutes in the Genesis<sup>®</sup> headspace-autosampler before the volatiles were automate-injected into GC-MS system. The condition of GC-MS was described below. The spectra were recorded and compared with the NIST and terpene library. GC-MS condition was modified from the method described by Ruther and Hilker (1998):

Column	DB-5ms fused silica column film thickness (d <sub>f</sub> ) 0.25 μm, 30 m length (L) x 0.25 mm internal diameter (I.D.)
Column programming	40°C hold for 5 minutes then rose to 230°C with the rate at 5°C per minute and hold for 10 minutes
Carrier gas	Helium, 12 psi
Injector	SPI injector holds at 250°C
Accelerating voltage	1700 volts
Sample size	250 ul

### 3.4.3 Identification of the components

Identification of the components based on GC retention time computer matching of NIST and terpene library, comparison of the fragment pattern with those reported in the library. The peak areas were used for quantitative analysis.

### 3.5 Relationships between volatile composition and insect distribution

The peak areas of compounds were used for quantification of some VOCs which were then correlated to the types and numbers of insects caught by yellow traps posted nearby the sites of air samplings (Fig 3.4). Data of VOCs were collected twice per year from 2006 - 2008. This was analyzed by correlation analysis using SPSS 15.0 for windows program. (The insect data were obtained from “Studies on Biological Interactions and Biochemicals in the Ecosystems of Deciduous Dipterocarp and Deciduous Forests in Nan Province” project. The number of insects was obtained from the total counts of two yellow traps (22 cm x 22 cm) for each site).



**Figure 3.4** Set up of the volatile collector and the insect trap in the forest

### **3.6 The effect to insects of some volatile components**

The active components showing volatile-insect effects from the analysis were confirmed by addition of their pure compounds in particular sites of the forest. The treatment was separately by 3 doses of pure compounds (500  $\mu$ l, 200  $\mu$ l and lowest dose or about tenfold of compounds which detected in the forest air) on November 2007, November 2008 and March 2008, respectively. The traps with the pure compounds were set on 3 plot sites with 5 replicates. The numbers of insects were then compared with control (the traps without compounds which were set up 2-4 days before those tests).

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## CHAPTER IV

### RESULTS AND DISCUSSION

#### 4.1 Testing of a volatile collector device in laboratory

##### 4.1.1 Volatile trapping efficiency of the collector

A simple, inexpensive and effective volatile collector was constructed by using aquarium air pump. A 5-ml plastic syringe packed with 0.5 g Super Q resin was connected with a battery-operated aquarium air pump. When operated, the air pass through the column. In this study, Super Q and Porapak Q were considered as the possible adsorbents. These adsorbents are slightly polar porous polymer which were used for variety of VOCs sampling such as plant volatiles (Tholl et al., 2006), pheromones (Gemeno et al., 2003) and other volatiles (Pankow et al., 1996). When the chromatograms of the blank Super Q and porapak Q obtained by HS/GC-MS were compared, the results showed that the blank Super Q had no interference while porapak Q had 2 peaks of contaminants (Fig 4.1), owing to the prior elimination of contaminants in Super Q by washing with a selected program of organic solvents and acids and then conditioned in an oxygen-free atmosphere to removes residual monomers and polymerization catalysts (Althech catalogue).

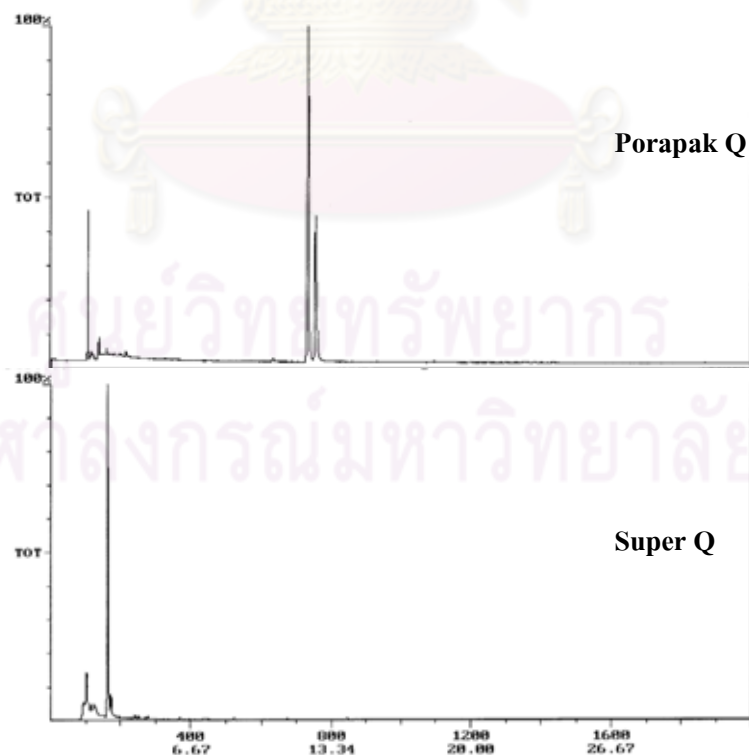
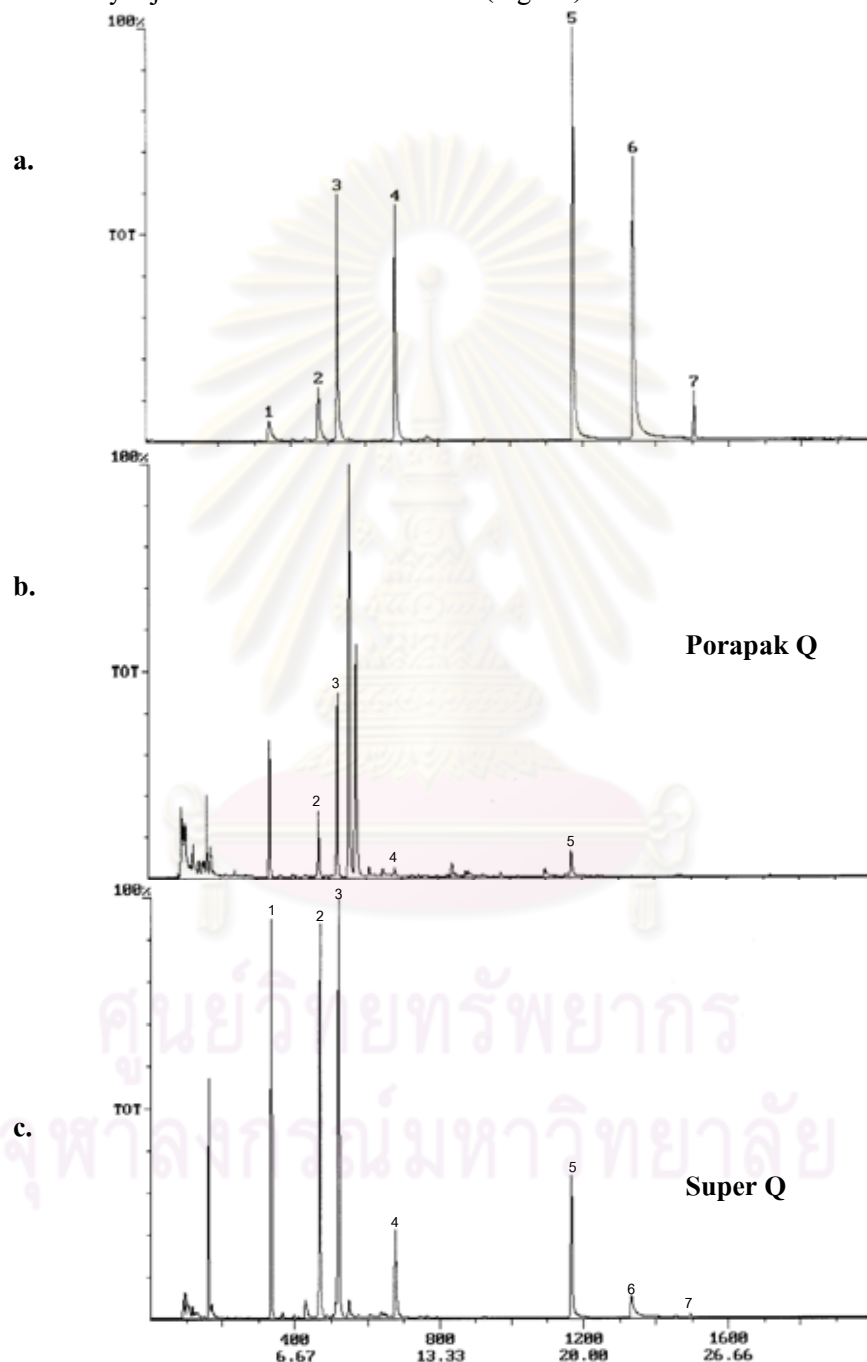


Figure 4.1 The chromatograms obtained from blank adsorbent

Subsequently, a new volatile collector device was tested for its adsorbing ability with the mixture of seven pure oils set up in laboratory and the analysis was performed with the operating condition that modified from Adams (1995). The chromatograms obtained from trapped volatiles on porapak Q and Super Q run with HS/GC-MS were compared with the chromatogram of manual directly injection of mixture to GC/MS (Fig.4.2).



**Figure 4.2** The chromatogram of mixture oils (a) manually by direct injection to GC-MS (b) trapped on porapak Q and (c) trapped on Super Q

(1)  $\alpha$ -pinene, (2) 3-carene, (3) eucalyptol, (4) eugenol, (5) linalool, (6) safrole and (7) caryophyllene



The comparison in Figure 4.2 shows the appearance of mixture oils by GC/MS with seven peaks of chromatogram arranged by the number of peak or retention time were  $\alpha$ -pinene, 3-carene, eucalyptol, eugenol, linalool, safrole and caryophyllene (fig 4.2a) which have boiling point (bp) at 155-156°C, 168-169°C, 176-177°C, 198°C, 232-234°C, 254°C and 262-264°C, respectively. Almost all compounds were detected in both adsorbents (Fig 4.2b and c) which showed similar patterns of peaks between the trapped samples and the direct injection samples. Eugenol, linalool, safrole and caryophyllene were rarely detected in porapak Q but well detected in Super Q. Comparing between the two adsorbents, it was obvious that interference peaks were detected in porapak Q but not in Super Q. Moreover, Super Q could be reused by washing with organic solvent such as acetone. For these reasons, Super Q was selected as adsorbent for VOCs sampling in the applied volatile collector for trapping of VOCs, particularly terpenes, in this study.

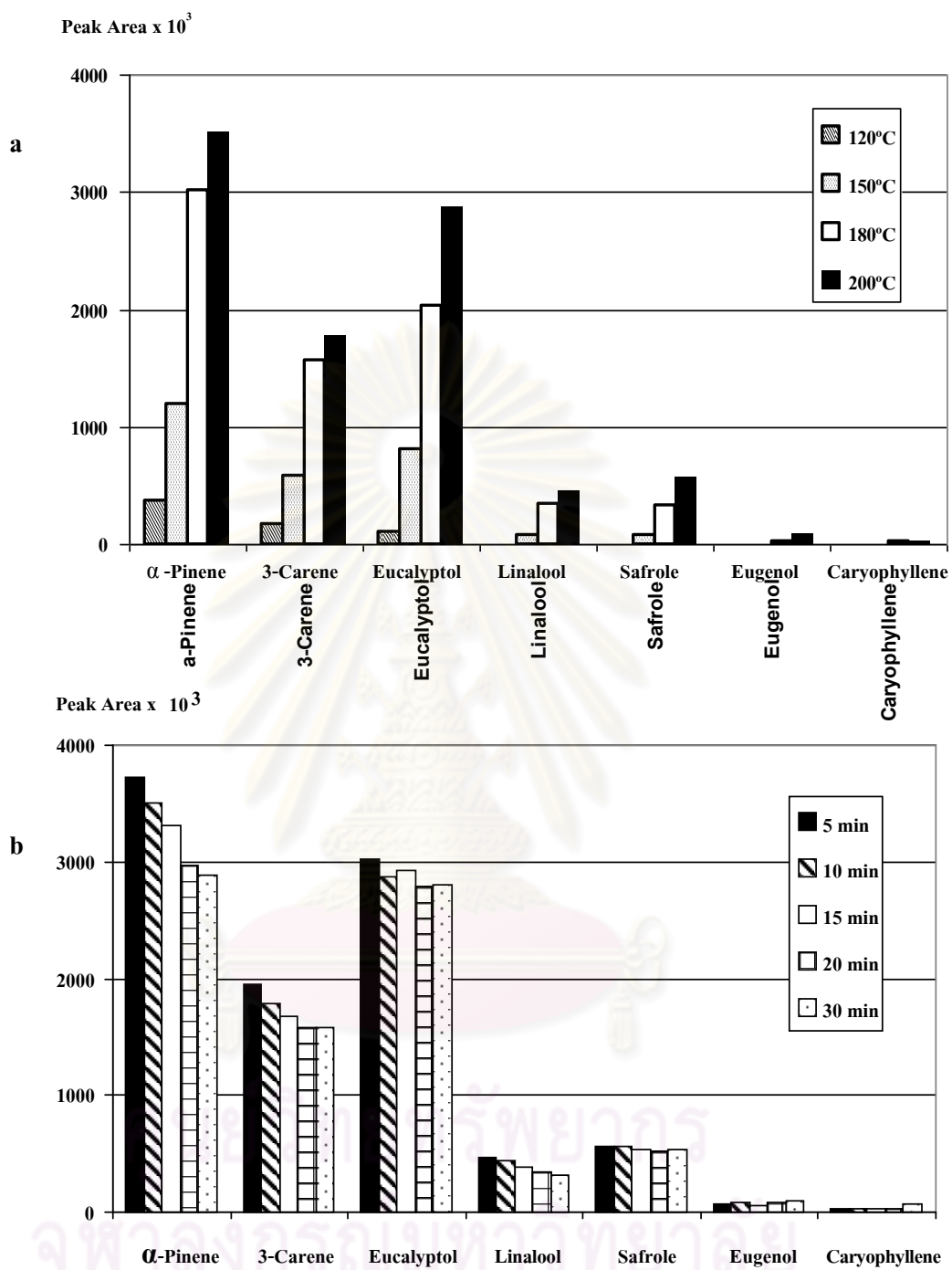
#### 4.1.2 Parameters affecting the headspace - GC/MS results

After sample correction, the trapped volatiles were desorbed from the adsorbent by using Genesis Headspace autosampler. The important parameters such as temperatures and equilibration time of the sample heating which are related to its sensitivity and maximize precision (Penton, 1992) were studied. It was found that these parameters could influence the GC/MS performance as presented by peak areas (Table 4.1). It can be seen that some components were detected only at high temperature such as eugenol and caryophyllene at 200°C, while the lower bp monoterpenes such as  $\alpha$ -pinene, 3-carene and eucalyptol were desorbed at all conditions. Optimization study showed that the highest quantity of volatile oils were established at 200°C which is the maximum temperature reachable by autosampler oven (Fig. 4.3a), and almost all volatile oils show high level of compounds at 200°C and 5 minutes (Fig 4.3b). So this condition was selected to evaporate the trapped volatiles from Super Q adsorbent for analysis of all experiments.

**Table 4.1** Optimization data of temperature and time to analyze mixture oils in Super Q

Parameter	Peak Area x 10 <sup>3</sup>						
	$\alpha$ -Pinene	3-Carene	Eucalyptol	Linalool	Safrole	Eugenol	Caryophyllene
120 / 10	377	176	111	-	-	-	-
120 / 20	327	154	106	-	-	-	-
120 / 30	334	144	87	-	-	-	-
150 / 10	1,202	584	806	84	75	-	-
150 / 20	1,175	575	803	75	71	-	-
150 / 30	1,036	522	770	80	68	-	-
180 / 10	3,022	1,574	2,028	348	326	33	29
180 / 15	2,883	1,485	1,997	307	291	10	28
180 / 20	2,697	1,330	1,961	292	275	2	20
180 / 30	2,658	1,266	1,934	305	289	-	23
200 / 5	3,719	1,950	3,030	474	570	74	22
200 / 10	3,508	1,782	2,873	446	570	88	23
200 / 15	3,313	1,683	2,925	388	530	50	28
200 / 20	2,963	1,574	2,794	339	519	86	30
200 / 30	2,883	1,581	2,807	319	531	97	72

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**Figure 4.3** Effects of temperatures and time on the response of volatile compounds

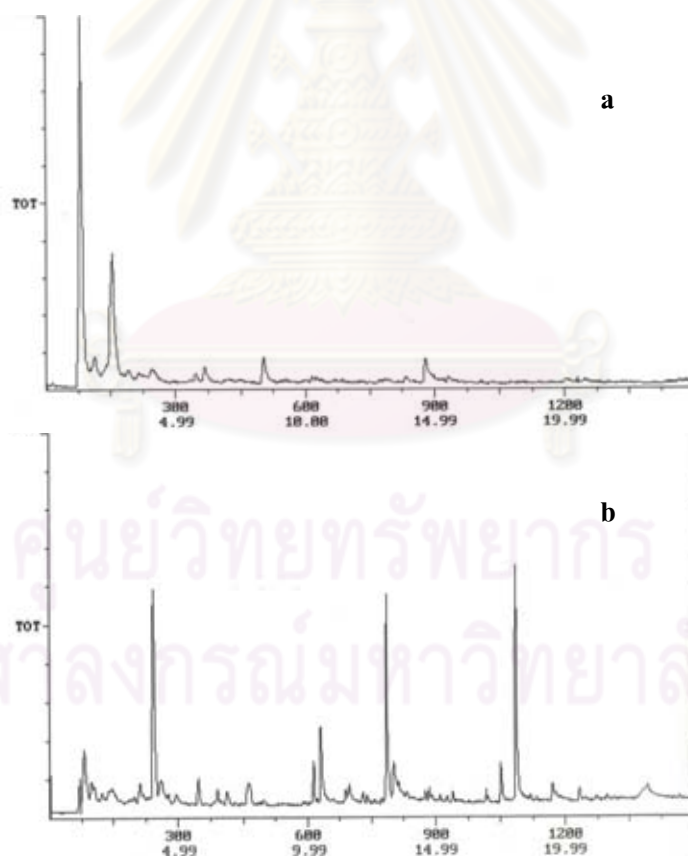
(a) vary temperatures at 10 min

(b) equilibration time at 200°C

## 4.2 Volatile compounds collected from the understory air in a forest at Doi Phu Ka National Park

### 4.2.1 Identification of VOCs

After collection of air samples in the forest, the trapped VOCs were desorbed from the adsorbent and analyzed for constituents by using optimal HS conditions and our modified GC-MS program, respectively. The analysis of the air VOCs was firstly performed by using the same GC method used previously in our laboratory (60°C for 3 min, then increased to 220°C at 3°C/min and held for 10 min). With these conditions, the chromatograms showed no clear appearance of peaks of VOCs (Fig 4.4a). Therefore, the separating conditions was changed to be as follows: initial oven temperature 40°C for 5 min, then increased from 40 to 230°C at a rate of 5°C/min and held for 10 min. Under these conditions, the obtained chromatogram of the same air sample showed the presence of much more peaks of VOCs (Fig 4.4b). Thus, in this study, the new program of GC operation was used to separate the VOC components in the forest air.



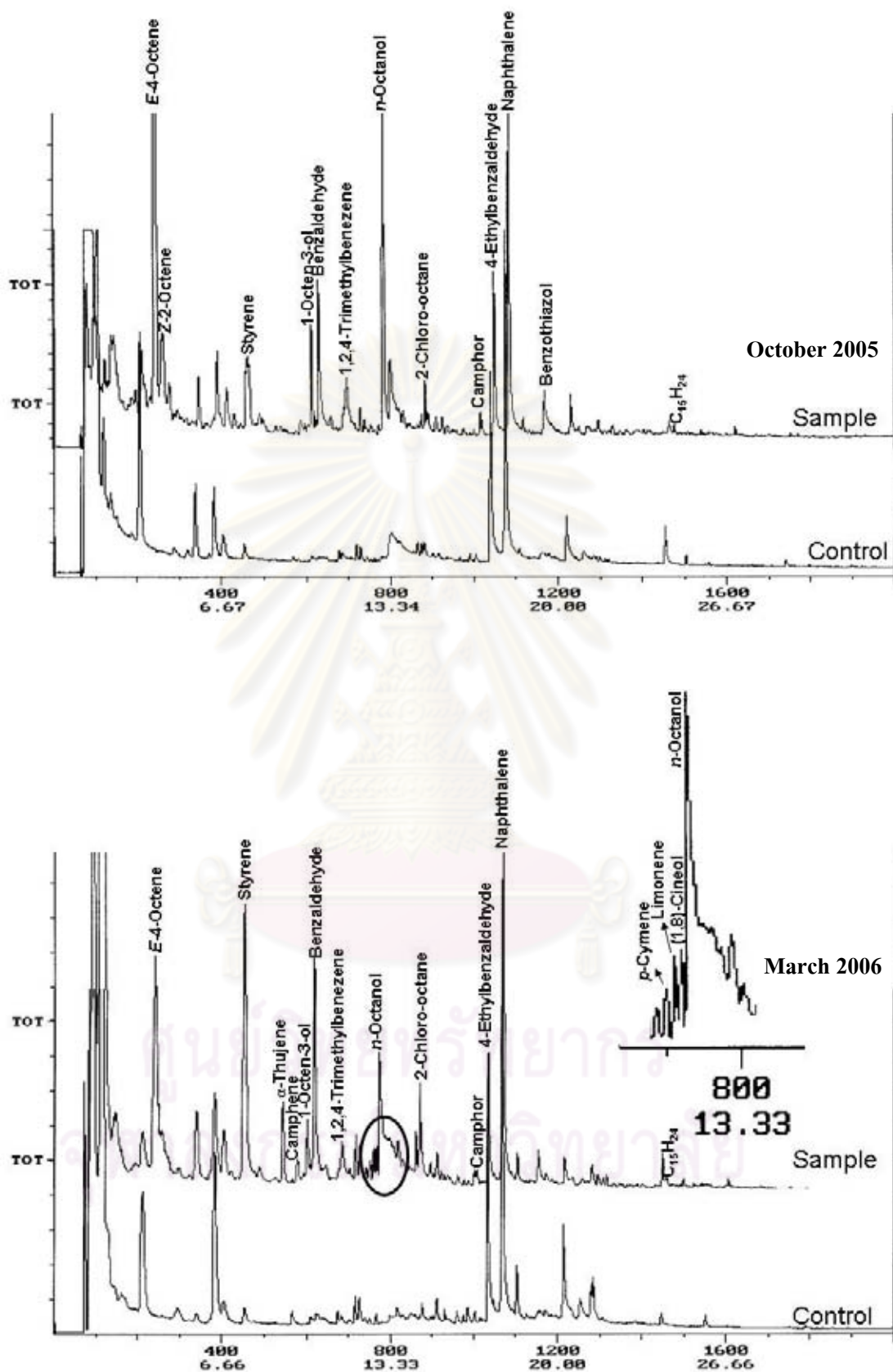
**Figure 4.4** Typical GC chromatograms of a forest air sample obtained by using two different GC programs

(a) 60 °C (3min) -> 220 °C (3 °C/ min) -> 220 °C (10 min)

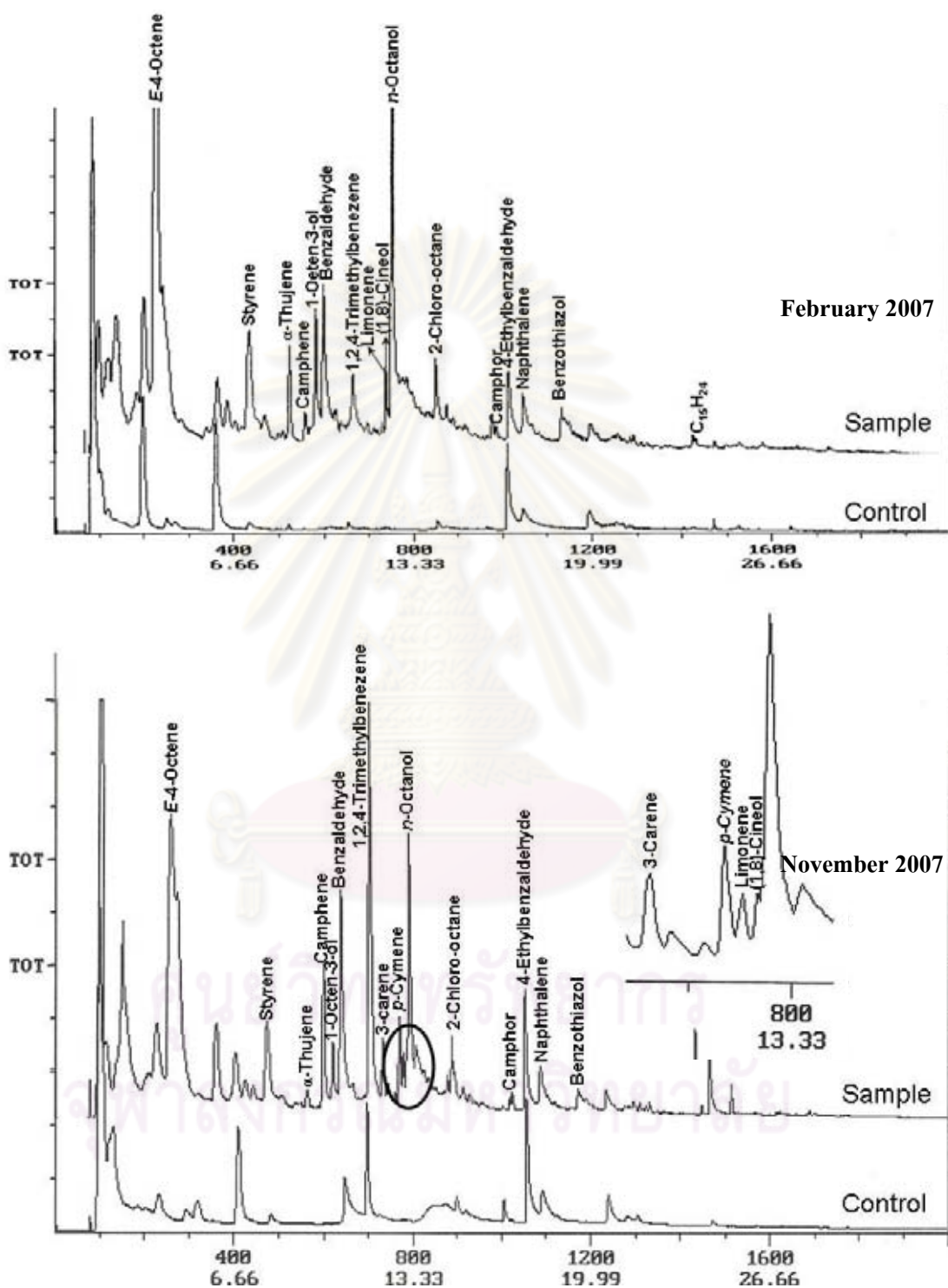
(b) 40 °C (5min) -> 230 °C (5 °C/ min) -> 230 °C (10 min)

Based on the GC analysis, chromatograms of various air samples collected in this study showed variation in the composition of the forest air VOCs (appendix A). Fig 4.5-4.7 show representative chromatograms of the air samples collected from site no. 5 compared with the controls (blank). In this experiment, different conditions of Super Q adsorbent were used for each sample collection. The VOCs trapped by newly purchased Super Q (October 2005 and March 2006) showed more contaminated constituents compared with the blank control (Fig 4.5). This contaminated problem was solved by washing the new Super Q with acetone and heated at 100°C for 1 hr before use. The washed Super Q allowed the samples collected in February and November 2006 be analyzed with clear peak separation (Fig 4.6). Thusfore, in practice, the used Super Q was cleaned by washing 3 times with acetone and then heated at 100°C for 1 hr before being reused. Although, the obtained GC chromatogram showed some background of peaks of constituents left in the adsorbent, different peaks of VOCs could be detected readily in these samples (Fig 4.7). As a result, structure identification of compounds in these analyses obtained could be performed by comparison between the mass spectra of the samples with those in the NIST and terpene library. Some comparisons of the mass spectra are shown in appendix B.

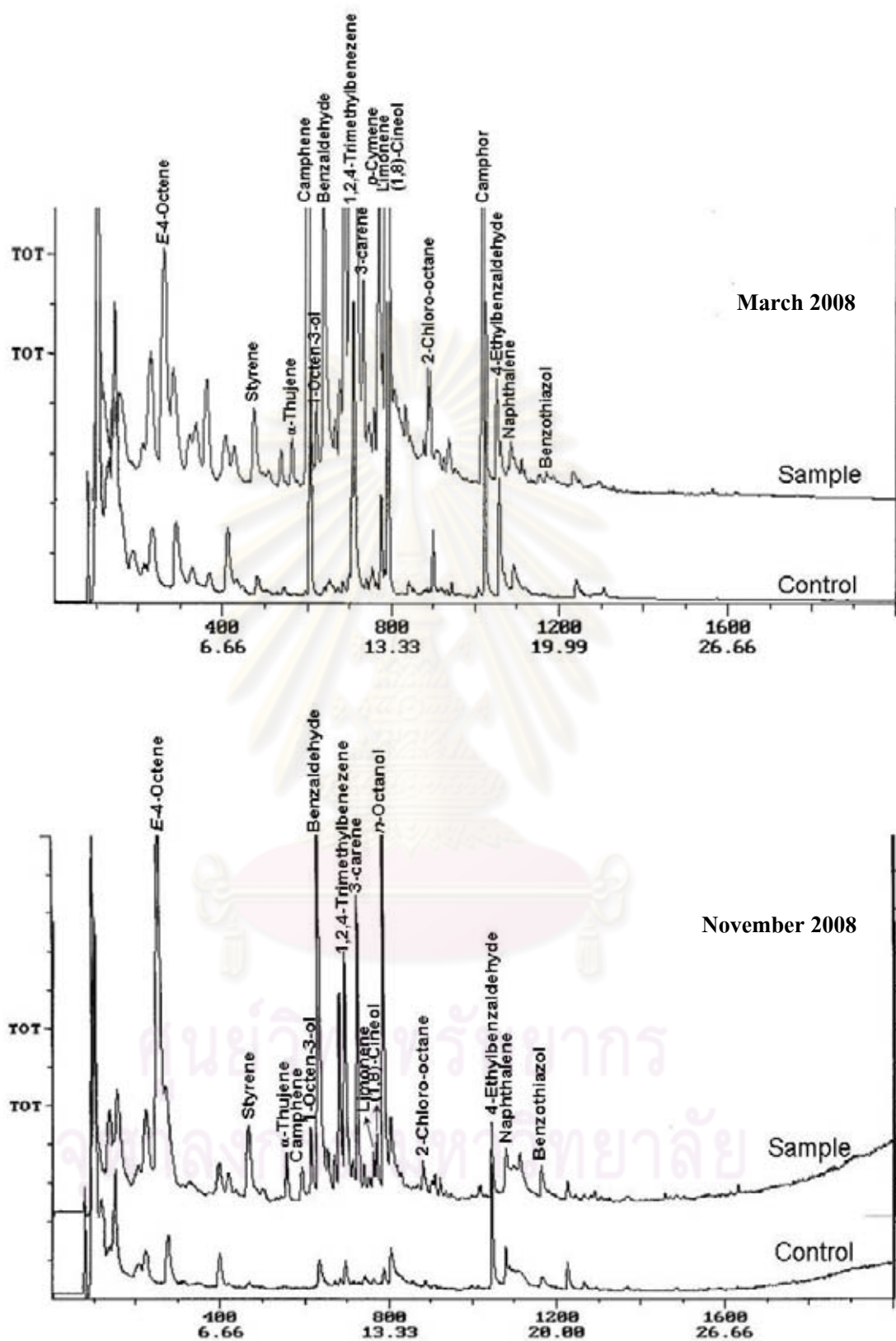
Fig 4.8 shows the overall picture of the chemical profiles of VOCs in each air collection obtained from various sampling site. It can be seen that the profiles showed similar pattern with peaks of contaminated compounds of 4-ethylbenzaldehyde and naphthalene which were utilized for relative peak area quantity. Even though these components were detected at high level in the new Super Q samples, they were decreased by washing with acetone as mentioned earlier. The detected compounds from the washed adsorbent were as good as the original Super Q. This confirmed that Super Q is a suitable adsorbent for trapping VOCs in the open field.



**Figure 4.5** The comparison between the sample and the control chromatograms based on new Super Q. The air samples were collected in October 2005 and March 2006.

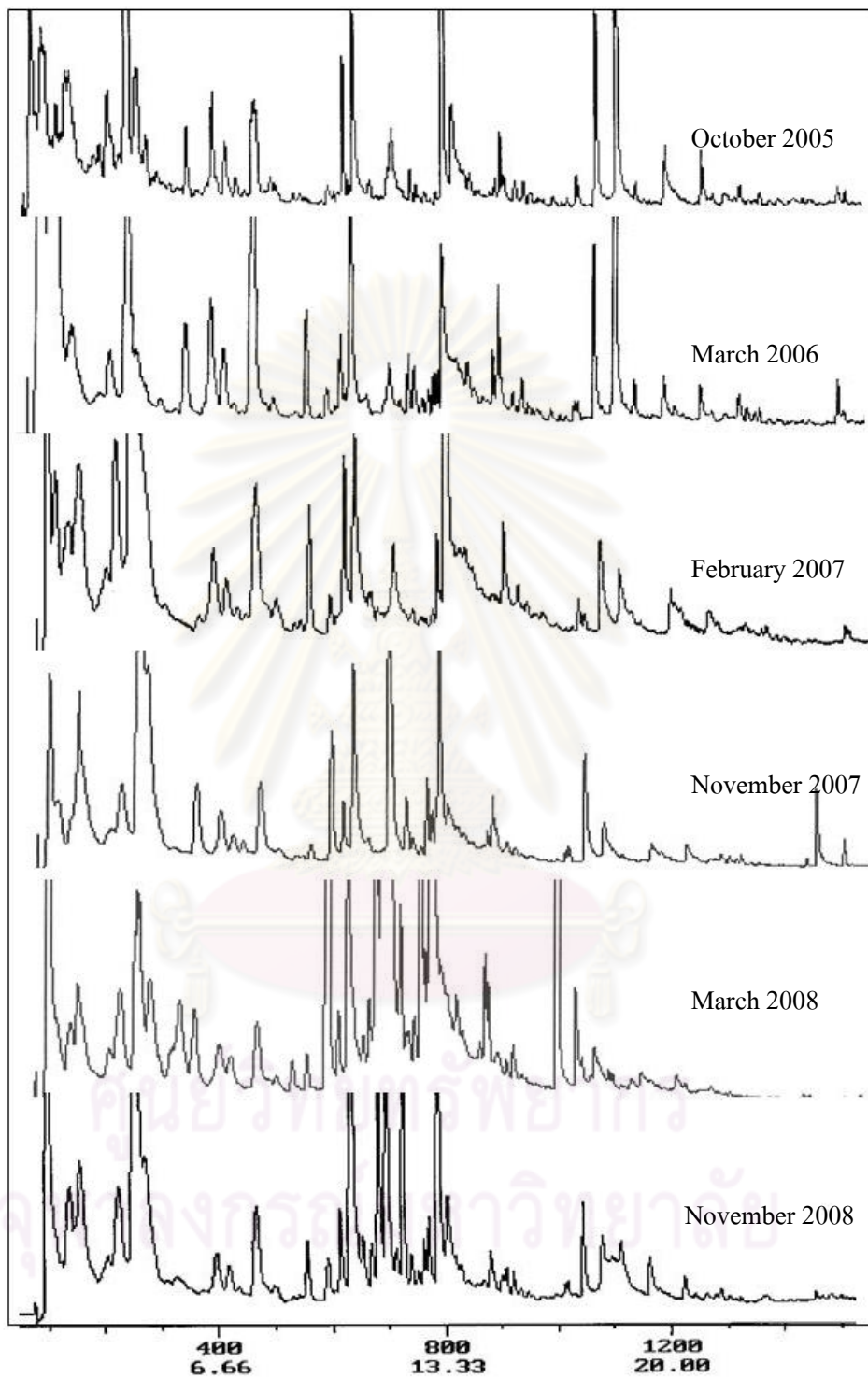


**Figure 4.6** The comparison between the sample and the control chromatograms based on washed new Super Q. The air samples were collected in February 2007 and November 2007



**Figure 4.7** The comparison between the sample and the control chromatograms based on reused Super Q. The air samples were collected in March 2008 and November 2008





**Figure 4.8** The overall picture of the GC chromatograms of understory air at Doi Phu Ka National Park from the site no. 5

From the results of GC-MS analysis, the VOCs in the forest air at different sites could be identified as two classes of terpenes, which are normally found in most of plant essential oils. Among these, seven compounds were classified as monoterpenes which are  $\alpha$ -thujene, camphene, 3-carene, *p*-cymene, limonene, (1,8)-cineole and camphor and one as sesquiterpene which could not be identified. Furthermore, other VOCs were also found in this study which are *E*-4-octene, *Z*-2-octene, styrene, 1-octen-3-ol, benzaldehyde, 1,2,4-trimethylbenzene, *n*-octanol, 2-chloro-octane and benzothiazole (Table 4.2). This pattern of these compounds emission was specifically detected in Hill ever green forest of Dong Ya Wai at Doi Phu Ka National Park with the new collector, which is the first information of VOCs in this area.

**Table 4.2** Composition of volatile compounds adsorbed by Super Q from the understory air.

Identification compounds	Formula	Oct 2005	Mar 2006	Feb 2007	Nov 2007	Mar 2008	Nov 2008
<b>Monoterpenes</b>							
$\alpha$ -thujene	$C_{10}H_{16}$	-	✓	✓	✓	✓	✓
Camphene	$C_{10}H_{16}$	-	✓	✓	✓	✓	✓
3-Carene	$C_{10}H_{16}$	-	-	-	✓	✓	✓
<i>p</i> -Cymene	$C_{10}H_{14}$	-	✓	-	✓	✓	-
Limonene	$C_{10}H_{16}$	-	✓	✓	✓	✓	✓
(1,8)-Cineole	$C_{10}H_{18}O$	-	✓	✓	✓	✓	✓
Camphor	$C_{10}H_{16}O$	✓	✓	✓	✓	✓	-
<b>Sesquiterpenes</b>							
Unknown sesquiterpene	$C_{15}H_{24}$	✓	✓	✓	-	-	-
<b>Other volatile compounds</b>							
<i>E</i> -4-Octene	$C_8H_{16}$	✓	✓	✓	✓	✓	✓
<i>Z</i> -2-Octene	$C_8H_{16}$	✓	-	-	-	-	-
Styrene	$C_8H_8$	✓	✓	✓	✓	✓	✓
1-Octen-3-ol	$C_8H_{16}O$	✓	✓	✓	✓	✓	✓
Benzaldehyde	$C_7H_6O$	✓	✓	✓	✓	✓	✓
1,2,4-Trimethylbenzene	$C_9H_{12}$	✓	✓	✓	✓	✓	✓
<i>n</i> -Octanol	$C_{10}H_{20}$	✓	✓	✓	✓	-	✓
2-Chloro octane	$C_8H_{17}Cl$	✓	✓	✓	✓	✓	✓
Benzothiazole	$C_7H_5NS$	✓	✓	✓	✓	✓	✓

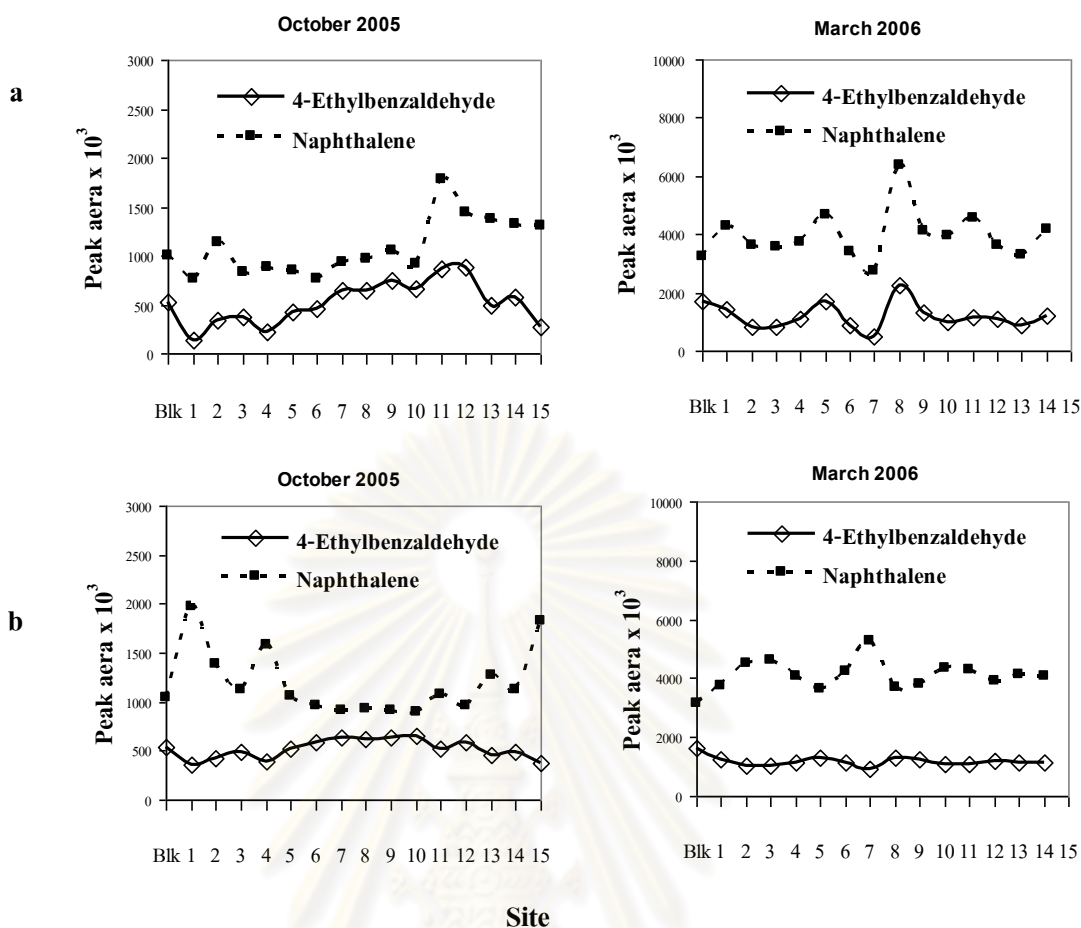
The sources of volatile emissions in the forest are many. Plants are the certain sources of biogenic volatile organic compounds (BVOCs), especially terpenes, assuming that their fate in the air is similar. In this study site, several plants were collected and analyzed for VOCs which the results showed that VOCs from plants also be detected in the atmosphere such as terpenes, *E*-4-octene, 1-octen-3-ol, and *n*-octanol (Sonthimanothum, 2009). Although, the sources of terpenes and some VOCs are natural plants, some of them may be released from several sources such as the plastic part and batteries of collector. Moreover some VOCs such as 1-octen-3-ol was not only emission from plants that also has been found as a major volatile produced by fungi (Kishimoto, 2007) and insect (McMahon, 2001). In addition, the anthropogenic VOCs found in ambient air such as styrene (synonym: vinylbenzene) and 1,2,4-trimethylbenzene (Hinwood, 2006) should be trapped by Super Q because the slightly polar property of Super Q (ethylvinylbenzene-divinylbenzene polymer) (Sturaro et al., 1992).

#### 4.2.2 Quantitative analysis

The quantitative analysis by relative peak areas of the obtained GC chromatograms was calculated. Since all chromatograms showed the presence of 4-ethylbenzaldehyde (**E**) and naphthalene (**N**) in both samples and blanks with the same peak sizes in October 2005 and March 2006 (Fig. 4.9a). The peak areas of these components were averaged and used to adjust the compounds quantity of all samples (Fig 4.9b) with the equation as follow:

$$\text{coefficient of each sites} = \frac{\text{coefficient of } \mathbf{E} \text{ at that site} \times \text{coefficient of } \mathbf{N} \text{ at that site}}{2}$$

- coefficient of **E** at that site as the mean of peak area of **E** / peak area of **E**
- coefficient of **N** at that site as the mean of peak area of **N** / peak area of **N**
- the means of peak area of compounds as the total of peak area from sample sites and blanks /the number of samples



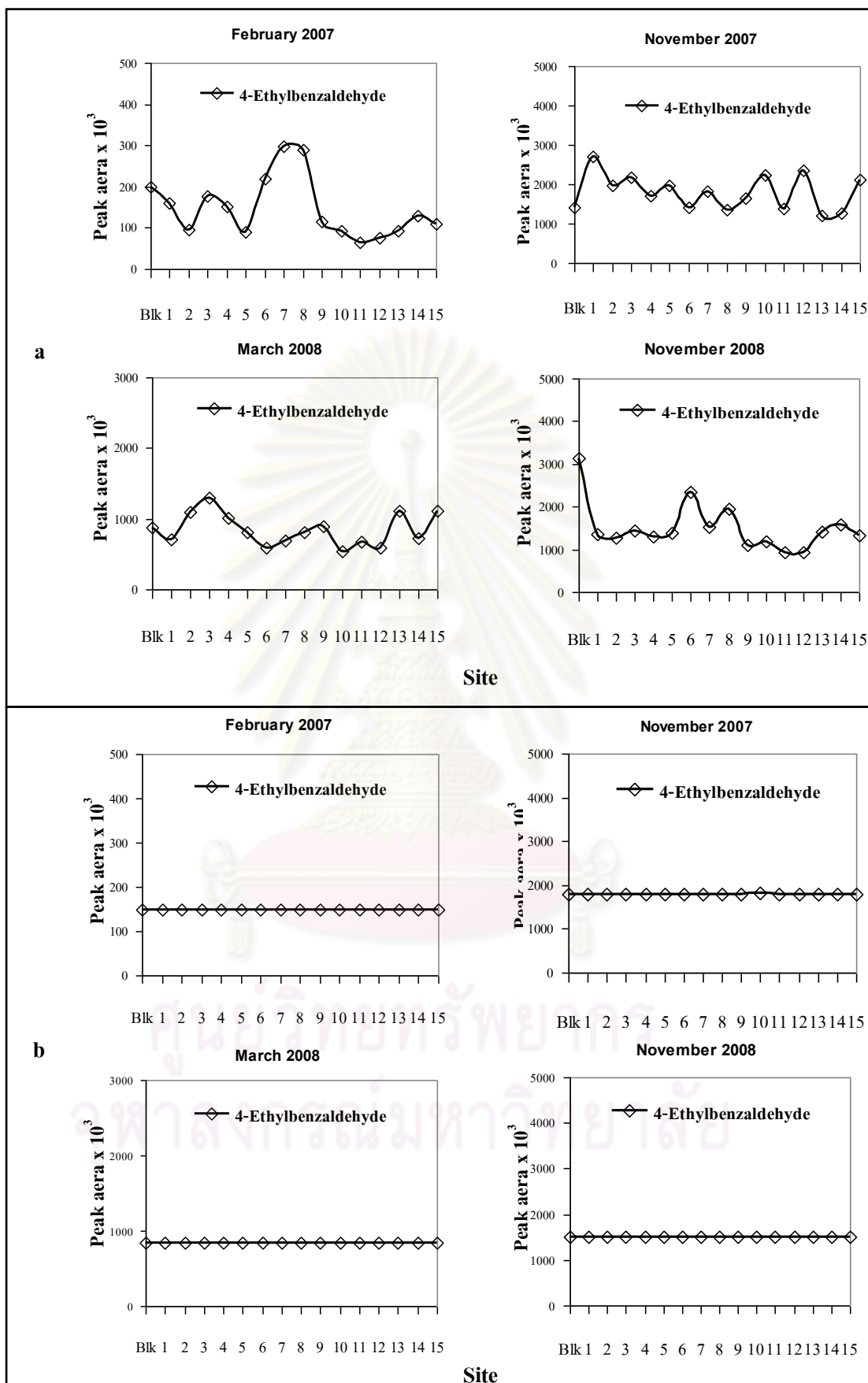
**Figure 4.9** Peak areas of 4-Ethylbenzaldehyde and Naphthalene (a) before and (b) after calculation,

Blk = Blank

However, naphthalene was sharply decreased in blank and samples from February 2007 and November 2007 because the adsorbent was washed with acetone. Thus the quantification of samples in the washed Super Q were adjusted by only peak areas of 4-ethylbenzaldehyde. This single compound adjusting was also used for samples in March 2008 and November 2008. The peak areas of these components were averaged and used to adjust the compounds quantity of all samples (Fig 4.10) with the equation as follow:

$$\text{coefficient of each sites} = \frac{\text{mean of peak area of E}}{\text{peak area of E at that site}}$$

- the mean of peak area of compounds as the total of peak area from sample sites and blanks /the number of samples



**Figure 4.10** Peak areas of 4-Ethylbenzaldehyde (a) before and (b) after calculation, Blk = Blank

The VOC composition from the forest air was calculated and is shown in table 4.3-4.8. The variation of compounds was detected at all 15 sites, possibly due to the diversity of plants at each site that emitted the volatile compounds into the forest air. It can be seen that seven monoterpenes including  $\alpha$ -thujene, camphene, 3-carene, p-cymene, limonene, (1,8)-cineol and camphor and one unknown sesquiterpene were the major constituents detected. These compounds have been reported as plant volatile compounds which may function to protect plants from pests or attract parasites of their pests in the defend mechanisms (Degenhardt et al., 2003; Dicke and Hilker, 2003). However other VOCs which are *E*-4-octene, styrene, 1-octen-3-ol, benzaldehyde, 1,2,4-trimethylbenzene, *n*-octanol, 2-chloro-octane and benzothiazole were detected in all times (except *Z*-2-octene). These VOCs may probably release from plants or others sources.



**Table 4.3** Composition of the volatiles emitted into the headspace on October 2005

RTime	Compounds	control*	Relative Peak Area x 10 <sup>3**</sup>														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
3.89	<i>E</i> -4-Octene	0	1394	2652	1798	1832	3336	2062	1865	1426	1742	1492	1492	1552	1341	2904	2288
4.18	<i>Z</i> -2-Octene	0	190	276	308	635	446	516	726	94	327	319	134	411	221	402	613
10.02	1-Octen-3-ol	0	286	245	240	308	275	187	253	191	212	165	151	145	124	247	221
10.28	Benzaldehyde	0	806	635	734	680	610	494	430	487	587	304	323	437	522	503	843
11.38	1,2,4-Trimethylbenzene	0	247	309	230	229	160	223	192	83	255	194	165	180	146	230	448
12.86	<i>n</i> -Octanol	0	1589	937	1532	2054	1038	1144	1209	1376	1368	1003	498	809	754	1238	819
16.69	Camphor	0	83	58	62	92	58	59	74	60	46	32	40	46	29	71	88
17.25	4-Ethylbenzaldehyde	546	356	423	496	392	528	589	635	616	636	653	519	595	454	496	368
17.84	Naphthalene	1036	1979	1392	1122	1580	1056	969	917	932	910	890	1072	963	1266	1131	1828
24.36	C <sub>15</sub> H <sub>24</sub>	0	0	0	29	20	29	28	0	0	18	31	0	0	15	0	28

\* Control means the adsorbent in the plastic syringe that was not set up in target area

\*\* Relative Peak Area x 10<sup>3</sup> means peak area x 10<sup>3</sup> of compounds calculated by interfere 4-ethylbenzaldehyde and naphthalene

**Table 4.4** Composition of the volatiles emitted into the headspace on March 2006

RTime	Compounds	control*	Relative Peak Area x 10 <sup>3**</sup>														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
4.03	<i>E</i> -4-Octene	0	7550	5855	1647	5668	2409	5211	8100	626	3526	4760	3243	4011	2087	3912	
9.08	$\alpha$ -Thujene	0	248	191	146	326	957	198	220	76	126	183	146	162	152	125	
9.64	Camphene	0	201	0	94	163	268	161	203	0	116	132	0	0	0	102	
10.04	1-Octen-3-ol	0	1007	861	218	612	583	563	845	334	456	634	580	640	522	745	
10.32	Benzaldehyde	0	3488	5185	2374	3172	2940	3544	4331	2599	3077	3620	2341	3086	3626	3517	
11.41	1,2,4-Trimethylbenzene	180	485	530	365	580	372	524	710	330	478	528	481	491	490	509	
12.55	<i>p</i> -Cymene	0	174	179	161	158	155	140	189	106	112	125	112	127	118	92	
12.68	Limonene	0	197	258	166	201	194	196	311	71	127	130	203	232	213	148	
12.79	(1,8)-Cineole	0	226	175	235	186	186	248	58	213	5	0	0	0	253	11	
12.88	<i>n</i> -Octanol	0	970	1974	530	1907	615	1775	4995	2444	2173	3222	4969	3580	4472	2784	
16.73	Camphor	0	13	140	74	111	113	128	139	67	0	24	61	129	77	95	
17.19	4-Ethylbenzaldehyde	1638	1253	1051	1041	1165	1314	1120	950	1299	1225	1084	1096	1196	1139	1157	
17.78	Naphthalene	3128	3736	4525	4622	4056	3653	4250	5290	3703	3814	4344	4313	3931	4133	4064	
24.35	C <sub>15</sub> H <sub>24</sub>	0	132	103	0	97	90	105	193	76	80	76	0	0	71	126	

\* Control means the adsorbent in the plastic syringe that was not set up in target area

\*\* Relative Peak Area x 10<sup>3</sup> means peak area x 10<sup>3</sup> of compounds calculated by interfere 4-ethylbenzaldehyde and naphthalene



**Table 4.5** Composition of the volatiles emitted into the headspace on February 2007

RTime	Compounds	control*	Relative Peak Area x 10 <sup>3**</sup>														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
3.82	<i>E</i> -4-Octene	0	790	2682	204	1422	1052	924	455	467	2345	2644	2615	2747	1058	1334	1861
8.79	$\alpha$ -Thujene	0	55	28	38	43	135	16	9	13	24	33	45	44	39	20	40
9.36	Camphene	0	23	36	18	20	34	16	9	11	24	25	36	38	24	26	26
9.74	1-Octen-3-ol	0	125	233	104	143	208	70	84	66	195	181	209	207	167	170	201
10.03	Benzaldehyde	0	281	437	369	253	373	257	291	221	400	411	552	435	478	469	410
11.11	1,2,4-Trimethylbenzene	0	51	146	102	62	93	38	39	37	122	105	130	98	85	120	169
12.36	Limonene	0	37	72	68	42	67	32	6	3	46	40	61	44	43	39	63
12.46	(1,8)-Cineole	0	0	0	61	0	8	5	2	63	0	0	18	13	0	10	15
12.55	n-Octanol	0	1049	1390	1103	833	641	215	421	372	433	665	468	512	677	540	419
16.34	Camphor	0	17	26	33	22	23	16	9	8	18	19	32	23	24	23	28
16.95	4-Ethylbenzaldehyde	148	148	148	148	148	148	148	148	148	148	148	148	148	148	148	148
17.50	Naphthalene	57	68	109	153	89	86	33	56	87	93	89	105	96	88	105	115
23.86	C <sub>15</sub> H <sub>24</sub>	0	5	6	17	9	8	0	0	4	8	6	9	0	22	12	16

\* Control means the adsorbent in the plastic syringe that was not set up in target area

\*\* Relative Peak Area x 10<sup>3</sup> means peak area x 10<sup>3</sup> of compounds calculated by interfere 4-ethylbenzaldehyde

**Table 4.6** Composition of the volatiles emitted into the headspace on November 2007

RTime	Compounds	control*	Relative Peak Area x 10 <sup>3**</sup>														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
4.36	<i>E</i> -4-Octene	0	2540	4288	6193	8720	8646	5756	4799	9870	7633	3569	8680	2730	18533	6310	6825
9.38	$\alpha$ -Thujene	0	179	150	133	190	245	375	326	0	0	0	0	0	174	0	75
9.98	Camphene	0	1969	2021	1176	1516	1951	6229	4202	1323	1923	3509	2064	1560	1263	2150	627
10.34	1-Octen-3-ol	0	164	531	544	517	545	722	495	948	697	451	860	295	681	713	600
10.61	Benzaldehyde	1027	2270	3486	3334	3330	3470	4354	9659	3877	4353	6030	11325	5055	3392	7181	2709
11.68	1,2,4-Trimethylbenzene	1362	5718	3383	5603	7565	6520	5003	5739	7485	12219	19139	5217	7030	3348	21157	2917
12.19	3-Carene	0	543	430	399	341	670	850	718	541	825	744	733	188	494	1007	258
12.81	<i>p</i> -Cymene	0	521	311	307	324	870	1072	1051	609	774	894	749	512	191	348	134
12.94	Limonene	0	120	217	233	254	357	235	750	105	171	0	159	0	81	185	36
13.08	(1,8)-Cineole	0	248	0	37	188	313	744	1538	203	506	707	124	201	126	74	73
13.14	<i>n</i> -Octanol	0	1976	6235	2803	4303	3911	4383	3452	5011	2809	3834	2376	2858	1350	5866	3401
16.94	Camphor	0	127	93	88	107	100	186	187	92	141	0	155	136	107	0	61
17.51	4-Ethylbenzaldehyde	1794	1795	1803	1801	1806	1795	1803	1800	1797	1803	1807	1796	1802	1803	1795	1788
18.09	Naphthalene	593	463	549	573	533	607	534	556	485	538	941	592	692	572	562	1100

\* Control means the adsorbent in the plastic syringe that was not set up in target area

\*\* Relative Peak Area x 10<sup>3</sup> means peak area x 10<sup>3</sup> of compounds calculated by interfere 4-ethylbenzaldehyde

**Table 4.7** Composition of the volatiles emitted into the headspace on March 2008

RTime	Compounds	control*	Relative Peak Area x 10 <sup>3**</sup>														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
4.34	<i>E</i> -4-Octene	0	2034	863	780	5962	1260	1738	863	1765	1185	1827	2545	998	725	1983	1589
9.30	$\alpha$ -Thujene	0	31	72	154	309	491	315	281	246	159	434	328	320	234	295	87
10.00	Camphene	1543	10397	5667	5246	13164	12390	16610	8226	8452	7136	12425	9765	9321	5866	7930	4281
10.34	1-Octen-3-ol	0	512	268	320	495	496	475	288	501	335	343	413	460	250	337	416
10.61	Benzaldehyde	112	3610	3222	2553	3384	3536	4244	2883	3456	2653	2334	2987	2469	1856	1836	1779
10.73	1,2,4-Trimethylbenzene	3249	84955	45035	44782	72600	89964	108899	67451	77936	42551	91055	65449	73734	51620	64262	47264
12.19	3-Carene	114	1524	687	678	1308	1246	1767	1118	1556	779	1453	1347	1372	662	1025	640
12.79	<i>p</i> -Cymene	622	5104	2726	2738	3762	3844	4167	3023	4567	2969	3024	4042	3647	2120	2739	1837
12.94	Limonene	0	724	285	189	413	276	281	188	640	196	217	564	189	76	110	123
13.06	(1,8)-Cineole	4693	12178	6868	6397	12170	8625	11065	9499	11119	7488	10645	11610	10563	6616	10369	6414
16.94	Camphor	3076	15736	10706	10386	15367	12621	18165	18052	17109	13085	15253	17762	17767	8177	15300	9855
17.46	4-Ethylbenzaldehyde	846	847	847	847	846	846	846	847	846	846	847	847	846	847	846	846
18.03	Naphthalene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

\* Control means the adsorbent in the plastic syringe that was not set up in target area

\*\* Relative Peak Area x 10<sup>3</sup> means peak area x 10<sup>3</sup> of compounds calculated by interfere 4-ethylbenzaldehyde

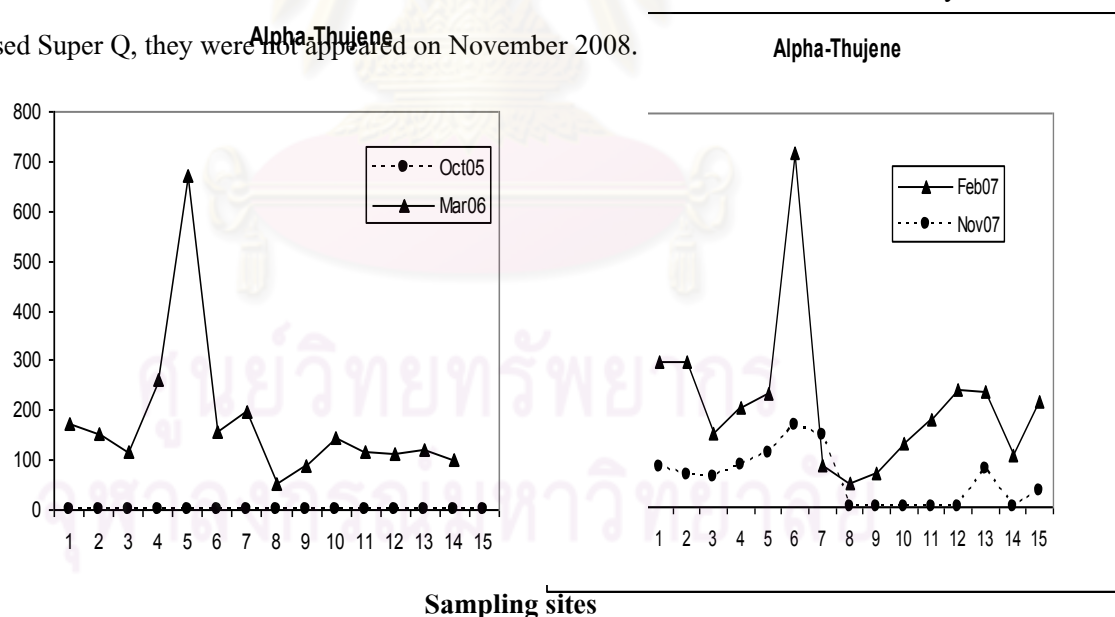
**Table 4.8** Composition of the volatiles emitted into the headspace on November 2008

RTime	Compounds	control*	Relative Peak Area x 10 <sup>3</sup> **														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
4.21	<i>E</i> -4-Octene	0	1196	11379	5864	8094	20557	6730	1482	2866	12821	3329	12865	14590	2789	2844	6407
9.24	$\alpha$ -Thujene	0	591	713	1137	726	1314	574	1374	899	1236	8879	3965	14768	477	379	569
9.84	Camphene	0	554	713	1005	627	786	642	1284	859	796	4716	2190	7906	479	379	569
10.23	1-Octen-3-ol	0	388	832	734	789	1463	585	634	696	1129	611	1358	1206	538	379	860
10.49	Benzaldehyde	0	12520	17278	17723	14285	14215	8998	20874	13118	32348	6045	21959	11801	8788	51529	40212
11.34	1,2,4-Trimethylbenzene	0	3378	5303	4058	4689	4826	2582	38527	30476	10964	3448	2298	4710	2341	2400	3864
12.08	3-Carene	0	1386	3056	3562	2492	5423	1497	3707	2630	3909	18046	8679	31307	1780	1591	1807
12.68	Limonene	0	386	570	635	585	382	140	857	331	808	2003	1255	2280	420	499	459
12.83	(1,8)-Cineole	0	223	570	746	473	1104	303	1726	513	595	4739	2120	8321	456	321	385
13.09	n-Octanol	212	5260	11443	13200	10922	9837	5505	14004	7453	12809	8809	6940	10164	8790	3919	7375
17.39	4-Ethylbenzaldehyde	1514	1514	1514	1514	1514	1514	1514	1514	1514	1514	1514	1514	1514	1514	1514	1514
17.98	Naphthalene	417	279	297	280	896	485	491	426	702	600	544	591	356	243	105	475

\* Control means the adsorbent in the plastic syringe that was not set up in target area

\*\* Relative Peak Area x 10<sup>3</sup> means peak area x 10<sup>3</sup> of compounds calculated by interfere 4-ethylbenzaldehyde

The VOCs after adjusting showed that terpenes were present with lower quantity than other VOCs in some samples or absent in some collections. For example, camphor was detected with average of peak area is  $100 \times 10^3$  from 2005-2007 in the exception of March 2008 and absent in November 2008. *E*-4-octene and *n*-octanol were found with the high quantity in all collections. In this study, emission of some terpenes such as  $\alpha$ -thujene might be depended on season and temperature. The high  $\alpha$ -thujene detections were found in March 2006 and February 2007 compared to in winter of both years (Fig 4.11). The reason for different amount of VOCs emission in the atmosphere may be because of many factors such as diurnal, seasonal, temperature, light and sources (Dudareva et al., 2004; Hakola et al., 2003). These may be the result of the unique VOC emissions profile in this research when compare with other forest. Although the diurnal behavior of VOC emissions was naturally observed from some living plants (Holzke et al., 2006; Pio et al., 2005), the volatile collectors were performed continuously for 48 hrs in this study which could be eliminated the diurnal behavior. Moreover benzaldehyde and 1,2,4-trimethylbenzene were irregular highly detected on November 2007 because the error of quantity in control that may be the contaminants from handle it in the field work. However many contaminants were found in the control on March 2008 because some errors that may cause of reused Super Q, they were not appeared on November 2008.



**Figure 4.11** The comparison of the amount of  $\alpha$ -thujene on four collections

### 4.3 The relationship between volatile composition and insect distribution

The relationship between volatile emissions and insect distribution in the understory air of the forest on five sampling times; October 2005, March 2006, November 2007, March 2008 and November 2008 was analyzed by using Spearman rank correlation analysis. This method is generally used to measure the strength and direction relationship of two numerical variables that are non-parametric (Chen and Popovich, 2000). The peak area of each compound obtained from 4.2 was paired and correlated to the numbers of four orders of the insects; Diptera, Hymenoptera, Homoptera and Coleoptera.

Table 4.9 shows the effect on VOC composition and insect distribution. It expresses in forms of (+) for attractant or (-) for repellent based on the value of Spearman's rank correlation coefficient ( $\rho$ ). It can be seen that the values showed relatively strong correlations in most pairs of the compound and insect;  $\alpha$ -thujene-Hymenoptera ( $\rho = +0.554$  and  $+0.541$ ),  $\alpha$ -thujene-Homoptera ( $\rho = +0.561$ ), camphene-Hymenoptera ( $\rho = +0.779$ ), and limonene-Homoptera ( $\rho = +0.603$ ). For different periods of time, the result also showed similar relationship for  $\alpha$ -thujene-Hymenoptera. Other correlation coefficient data are shown in appendix C and the trend of all relations are shown in Table 4.10. It showed that there are seven identical effects of the repell activity on insects of all sampling times; limonene-Diptera, benzaldehyde-Hymenoptera, *p*-cymene-Homoptera, (1,8)-cineol-Homoptera, 1-octen-3-ol-Coleoptera, *p*-cymene-Coleoptera and (1,8)-cineol-Coleoptera. Some relationships between VOCs and the insects by calculation showed similar effects in only some sampling times probably caused by the environment factors.

The results in this study lead to understand the complex relationships between the volatile emissions and the existence of the insects. All of the trapped insects found these experiments were the flying insects that were trapped by yellow traps. Therefore, their behaviors responding to the actual volatile compounds are of interest and were studied in the next experiments.

**Table 4.9** Correlation coefficient ( $\rho$ ) values of some volatile compounds detected and the insects trapped

Compounds	Relationship	Correlation coefficient		Time
		$\rho$	Sig.	
$\alpha$ -Thujene	+ Hymenoptera	+ .554*	.040	March 2006
		+ .541*	.037	November 2007
	+ Homoptera	+ .561*	.030	November 2007
Camphene	+ Hymenoptera	+ .779**	.001	March 2006
Limonene	+ Homoptera	+ .603*	.017	November 2007

\* and \*\* mean the correlation is significant at the 0.05 level and 0.01 level, respectively.

+ represent compound attract to insect.

**Table 4.10** The observed effect of some VOCs on the insect existence

Compounds	Diptera					Hymenoptera					Homoptera					Coleoptera				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
<b>Monoterpenes</b>																				
$\alpha$ -Thujene	n	A	A	A	R	n	A*	A**	R	R	n	R	A*	R	R	n	R	R	A	R
Camphene	n	A	A	A	R	n	A*	A	R	R	n	A	R	R	R	n	R	A	R	R
<i>p</i> -Cymene	n	R	A	R	n	n	A	A	R	n	n	R	R	R	n	n	R	R	R	n
Limonene	n	R	R	R	R	n	A	A	R	R	n	R	A*	R	R	n	R	A	R	R
(1,8)-Cineol	n	A	A	R	R	n	R	A	R	R	n	R	R	R	R	n	R	R	R	R
Camphor	R	A	A	R	n	R	A	A	A	n	A	R	R	R	n	R	A	R	A	n
<b>Other VOCs</b>																				
<i>E</i> -4-Octene	R	R	A	A	R	R	A	R	R	R	A	A	R	R	A	A	R	R	R	R
1-Octen-3-ol	R	R	A	A	R	R	A	R	R	R	R	A	R	R	A	R	R	R	R	0
Benzaldehyde	A	A	R	R	A	R	R	R	R	R	A	A	R	R	A	A	A	R	R	A
1,2,4-Trimethylbenzene	A	R	R	A	A	R	A	R	R	R	A	A	R	R	A	A	A	A	R	A
<i>n</i> -Octanol	R	R	R	n	A	R	A	A	n	R	R	A	A	n	A	R	A	A	n	A

1 = October 2005, 2 = March 2006, 3 = November 2007, 4 = March 2008 and 5 = November 2008

\* and \*\* mean the correlation is significant at the 0.05 level and 0.01 level, respectively.

n means no data and 0 means no correlation.

A and R mean trend of compound that attract and repel to insect, respectively.

#### 4.4 Test of some volatile components in the forest field

In order to confirm the potential effect of volatiles on insects, further experiments were performed by emitting certain amount of pure volatiles into the understory air that could be detected from this forest trail. The yellow sticky traps baited with pure volatile oils were placed for observing the responses of insects comparing with unbaited traps. Synthetic volatile oils were tested with three doses in various times, the dosage of 500 ul, lowest doses and 200 ul were tested on November 2007, March 2008 and November 2008, respectively. The amounts of the lowest doses were estimated based on the total peak area of each compound from 15 sites in summer and winter (February 2007 and November 2007) to peak area of VOCs in first treatment which is the emissions of 500 ul per one baited trap with 5 replicates (total = 2.5 ml per site) by the equation as follow:

$$\text{amount of each compound per site (ml)} = \frac{2.5}{\text{peak area of 2.5 ml emission}} \times \frac{A+B}{30}$$

Where A is the total of each compound on February 2007 and B is the total of each compound on November 2007.

After the calculation, the amount of each compound was multiplied by ten for the appearance of effects of volatile oils on insects in the forest and then divided by five and the absolutely values were used for a treatment per one trap (Table 4.11).

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**Table 4.11** Estimation of the low dose of each volatile oil

Compounds	Emissions of 2.5 ml/site	Peak area x 10 <sup>3</sup>			ml/1site	ten fold (ml)/site (5 traps)	ul/trap	dose/trap
		A	B	A+B 30				
<i>E</i> -4-Octene	276555	22601	105093	4256.47	0.038	0.38	76.96	100 ul
Camphene	656708	367	33484	1128.37	0.004	0.04	8.59	0.07 (g)*
1-Octen-3-ol	23397	2365	8763	370.933	0.040	0.40	79.27	100 ul
Benzaldehyde	96151	5636	74851	2682.9	0.070	0.70	139.52	100 ul
1,2,4-Trimethylbenzene	171593	1396	119404	4026.67	0.059	0.59	117.33	100 ul
<i>p</i> -Cymene	24599	0	8668	288.933	0.029	0.29	58.73	50 ul
Limonene	153224	662	2902	118.8	0.002	0.02	3.88	5 ul
(1,8)-Cineole	161948	196	5082	175.933	0.003	0.03	5.43	5 ul
Camphor	30361	321	1580	63.3667	0.005	0.05	10.44	0.05 (g)*

A = total peak area of each compound on February 2007 and B = total peak area of each compound on November 2007

\* The solid of camphene and camphor were calculated by  $D = m / V$ , D of camphene is 1.65 g/ml and camphor is 0.992 g/ml, m is mass (g) and V is volume (ml).

Dose of solid compounds at 200 ul; camphene = 0.33 g and camphor = 0.20 g

Dose of solid compounds at 500 ul; camphene = 0.82 g and camphor = 0.50 g

The increase of pure volatile compounds in the understory air showed some effects on the four groups of insects in the area; Diptera such as flies and mosquitoes, Hymenoptera such as bees and wasps, Homoptera such as leaf hoppers and Coleoptera such as beetles. The results showed that all insects were repelled by the use of highest dose (500  $\mu$ l) of volatile oils, except the pair of 1-octen-3-ol and Diptera (Fig 4.12; Table 4.12). This compound was detected in several living organisms and plays role the attractant of Diptera (McMahon et al., 2001; Pates et al., 2005). The medium and lowest doses of all compounds differentiate between the numbers of insects from atmospheric compounds and volatile oils treatments. These findings are discussed in the different respond of insects to the concentrations of compounds (Whitman and Eller, 1992). Moreover, the field trapping of pure compounds showed some similarities to VOC-insect correlation that can confirm the correlation analysis.

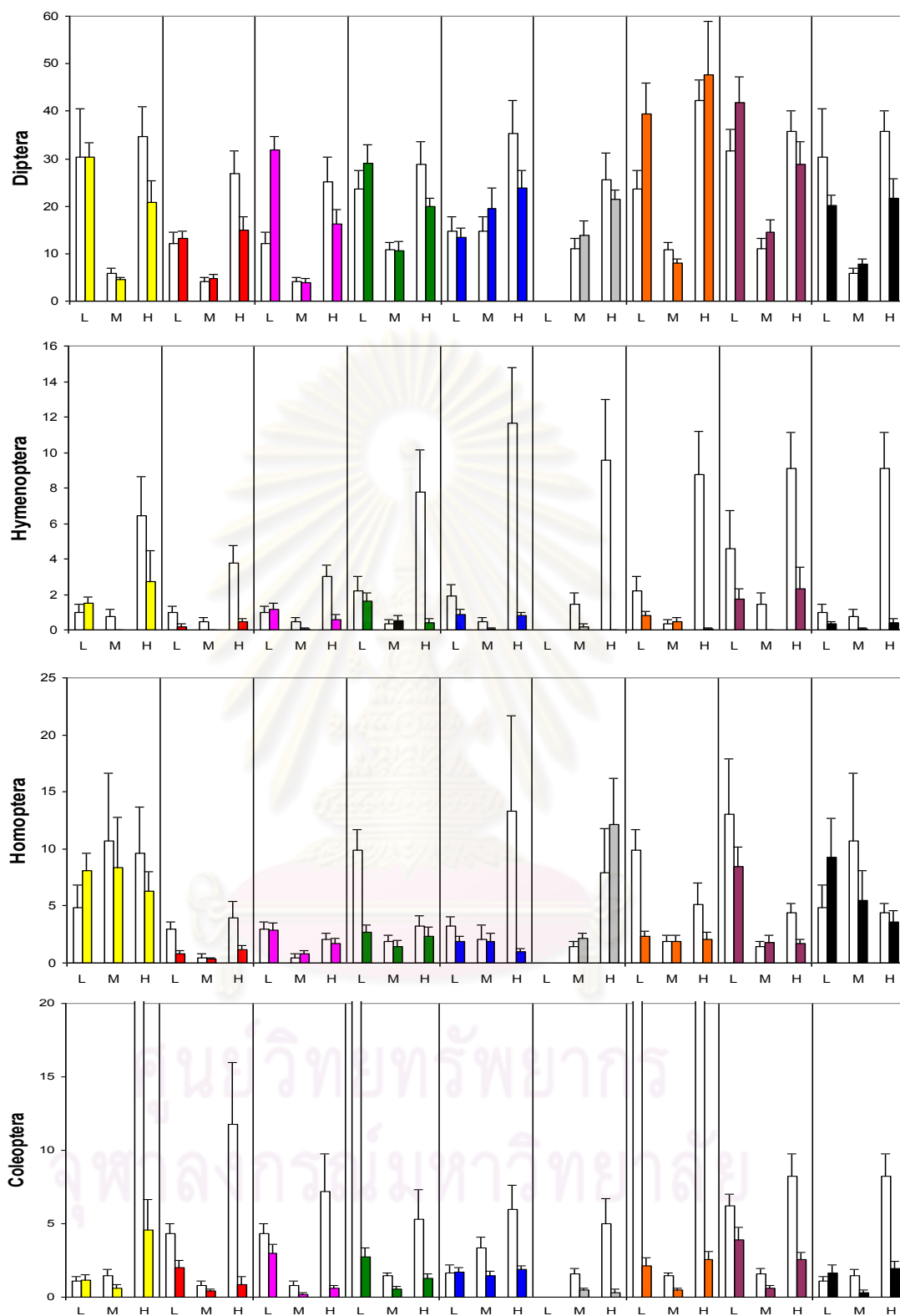
From the data obtained from the correlation analysis, the insect group of Hymenoptera was moderately strong attracted to camphene ( $\rho = +0.779$ , Sig. = 0.001) that correspond to low dose of camphene (0.07 g) in the field experiment. Hymenoptera were caught in traps baited with synthetic camphene more than in unbaited traps. This result was similar to the previous work showing that (-)-camphene is an important component of attractant to wasps (Tooker et al. 2005). Camphene at low dose that was not only attractive to Hymenoptera but also Homoptera and Coleoptera. Although camphene was attractive to Coleoptera at low dose, it was repellent at moderate and highest dose (0.33 and 0.83 g) during the response of Coleoptera in the field test. It is possible that these insects respond differently to the concentrations of compounds. Previously, Geiselhardt et al. (2006) tested the response of *E. opacus* (Coleoptera) to synthetic blend and single volatile and reported that only (-)-camphene was attractive to this beetles. Furthermore, Abdelgaleil et al. (2009) studied the fumigant toxicity of some monoterpenes on rice weevil and rust red flour beetle (Coleoptera) and reported that camphene were significantly less toxic with  $LC_{50}$  value (the lethal concentration causing 50% mortality after 24 hr) greater than 100 mg/l against both Coleoptera.

Abdelgaleil et al. (2009) suggested that (1,8)-cineol and (-)-limonene could be effective biocontrol agents against rice weevil and rust red flour beetle with low  $LC_{50}$  values. The identical of trend relationships of all sampling times show that (1,8)-cineol and limonene may be the repellent of Coleoptera. Therefore, it is not surprising that the traps baited with these volatile oils were completely repelled to Coleoptera in the field trapping testes. Moreover, the correlation of

limonene-Homoptera ( $\rho = +0.603$ , Sig. = 0.17) was confirmed by the attraction of medium doses (200  $\mu$ l) in this experiment. From the same trend of relationship, (1,8)-cineol and limonene were considered as repellent to Homoptera and Diptera, respectively. The experiments of pure compounds in the field showed similar effect on these insects. Klocke et al. (1987) reported the avoidance of mosquito (Diptera) to (1,8)-cineole in the feeding and oviposition behavior. However, the results of field testing show the different response on various concentrations.

From the trend of such a relationship, *p*-cymene may repel to Homoptera and Coleoptera. The field testing showed that *p*-cymene is the potential compound that can repel not only these insects but also the group of Hymenoptera. However, *p*-cymene appeared to repel to Diptera at highest dose of pure compound, while there are dissimilar activity at lowest dose. Besides, the field trapping testes also confirm the repellent effect on the pairs of Benzaldehyde-Hymenoptera and 1-octen-3-ol-Coleoptera. In the case of Coleoptera, host plant volatile compounds were often discussed for insect feeding frequency in beetle species (Faccoli et al., 2005; Kalberer et al., 2001; Martel et al., 2007). The actual numbers of insects captured in this forest were low indicated that there are none host plants of them around the traps. Although, the correlation analyses of camphor were not cleared, the repellent activity of camphor to all four orders of insects was exhibited in the field testing. The most abundance of camphor in dried leaves can repel beetles (Weaver et al., 1995) and camphor had weak activity toward both rice weevil and rust red flour beetle (Abdelgaleil et al., 2009).

For the usage of these compounds, the concentration of the compound should be considered to get good responses. Although the relationships between VOCs and insects were not significantly found in all relations, this study tested the field responses of certain insects to monoterpene emissions in a forest ecosystem that may be apply in the field. For example, use the attractant of Hymenoptera for attracting these insects into the surrounding of damage plants, then Hymenoptera will play role as the predator of plant pests. Since the discovery of chemically mediated communication in insects, the science of chemical ecology is extensively attempted in multidisciplinary. Almost topics are emphasized about the pheromones or plant volatiles that influence insect behaviors (Aluja and Fleischer, 2006; Blackmer et al., 2004; Sureda et al., 2006) by direct testing. This research presented simple method for verify the relationships between emission compounds and insects in the forest sample site that is useful for the chemical ecology study.



**Figure 4.12** Mean numbers of insects in traps with the effective pure compounds and control, atmospheric compounds ( $\square$ ) in the forest. L = lowest dose, M = 200  $\mu$ l and H = 500  $\mu$ l  
 monoterpenes: ■ camphene ■ *p*-cymene ■ limonene ■ (1,8)-cineol ■ camphor  
 other VOCs: ■ *E*-4-octene ■ 1-octen-3-ol ■ benzaldehyde ■ 1,2,4-trimethylbenzene

**Table 4.12** Correlation between various volatile contents on insect existence

Compounds	Diptera			Hymenoptera			Homoptera			Coleoptera		
	L	M	H	L	M	H	L	M	H	L	M	H
<i>E</i> -4-Octene	n.t.	+	-	n.t.	-	-	n.t.	+	+	n.t.	-	-
Camphene	<b>0</b>	-	-	+	-	-	+	-	-	+	-	-
1-Octen-3-ol	+	-	+	-	+	-	-	+	-	-	-	-
Benzaldehyde	+	+	-	-	-	-	-	+	-	-	-	-
1,2,4-Trimethylbenzene	-	+	-	-	-	-	+	-	-	+	-	-
<i>p</i> -Cymene	+	+	-	-	-	-	-	-	-	-	-	-
Limonene	<b>0</b>	-	-	+	-	-	<b>0</b>	+	-	-	-	-
(1,8)-Cineol	+	<b>0</b>	-	-	+	-	-	-	-	-	-	-
Camphor	-	+	-	-	-	-	-	-	-	<b>0</b>	-	-

n.t. means no testing; +, - and 0 mean attract, repel and no action, respectively.

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## CHAPTER V

### CONCLUSION

A new volatile collector assembled in this work is an effective device to collect volatile samples in the field. Preparation of the collector is simple only using 5-ml plastic syringe packed with 0.5 g Super Q and connected with a portable air pump modified from a fish-tank air pump. Super Q adsorbent can be replaced by other adsorbents for absorbing different types of VOCs and the glass tube can be replaced by the plastic syringe to eliminate the interference of target compounds. In addition, the rechargeable batteries of portable air pump can be used to minimize waste and the cost of experiment. In this study, one method used to reduce cost is to recycle adsorbent by washing with 3 times of acetone and then heat at 100°C for 1 hr. The adsorbent was more carefully handled both before and after sampling by separating it from the other volatiles and kept in the dark place, respectively.

The HS/GC-MS method was able to analyze the trapped volatile from the trapped Super Q by heating it up to 200°C for 5 min, and the evaporated components were separated by GC with DB-5ms column that was programmed at 40°C hold for 5 minutes, then rose to 230°C with the rate at 5°C per minute and hold for 10 minutes, finally, chromatograms were analyzed by comparing mass spectra of each peak with a database. This HS condition could evaporate the highest volatiles from Super Q adsorbent. The results show the identified compounds from the ecosystem of Doi Phu Ka National Park which were trapped by Super Q are seven monoterpenes ( $\alpha$ -thujene, camphene, 3-carene, *p*-cymene, limonene, (1,8)-cineole and camphor) and one unknown sesquiterpene. Moreover, the other VOCs were also found in this study which are *E*-4-octene, *Z*-2-octene, styrene, 1-octen-3-ol, benzaldehyde, 1,2,4-trimethylbenzene, *n*-octanol, 2-chlorooctane and benzothiazole. The diluted volatile property makes a trouble for standard volatile preparation in quantity analysis, however, the peak area was used for comparative quantity of volatile compounds.

The relationship between volatile emission and insect was also evaluated based on correlation analysis. This type of relationship can lead to a better understanding of chemical ecology in a forest ecosystem. The correlation analysis is a simple useful method for this purpose, while the results could be confirmed the influence of these volatiles. The results show that the insects of Hymenoptera, Homoptera could be attracted by camphene and limonene, respectively.

Besides, This interaction was confirmed by testing its pure compound in the field which showed that the insects of Coleoptera were repelled by *p*-cymene, (1,8)-cineol and 1-octen-3-ol, whereas Homoptera was repelled by *p*-cymene and (1,8)-cineol. Hymenoptera and Diptera were repelled by benzaldehyde and limonene, respectively. Consequently, these compounds are the possible substances for application in pest control such as using for the attractant components in trap-baited or repellent components in insecticide.

In summary, a new equipment and analytical procedures are useful as a simple tool for chemical ecology study. The interaction of chemically mediated communication between plants and insects was observed. This knowledge is useful for understand chemical signaling which can be applied in the field of biocontrol in the future.



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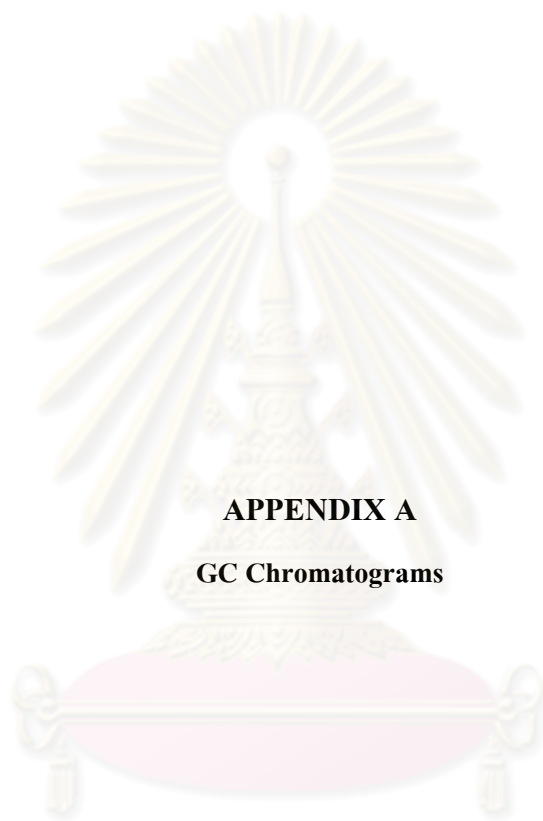
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**APPENDICES**

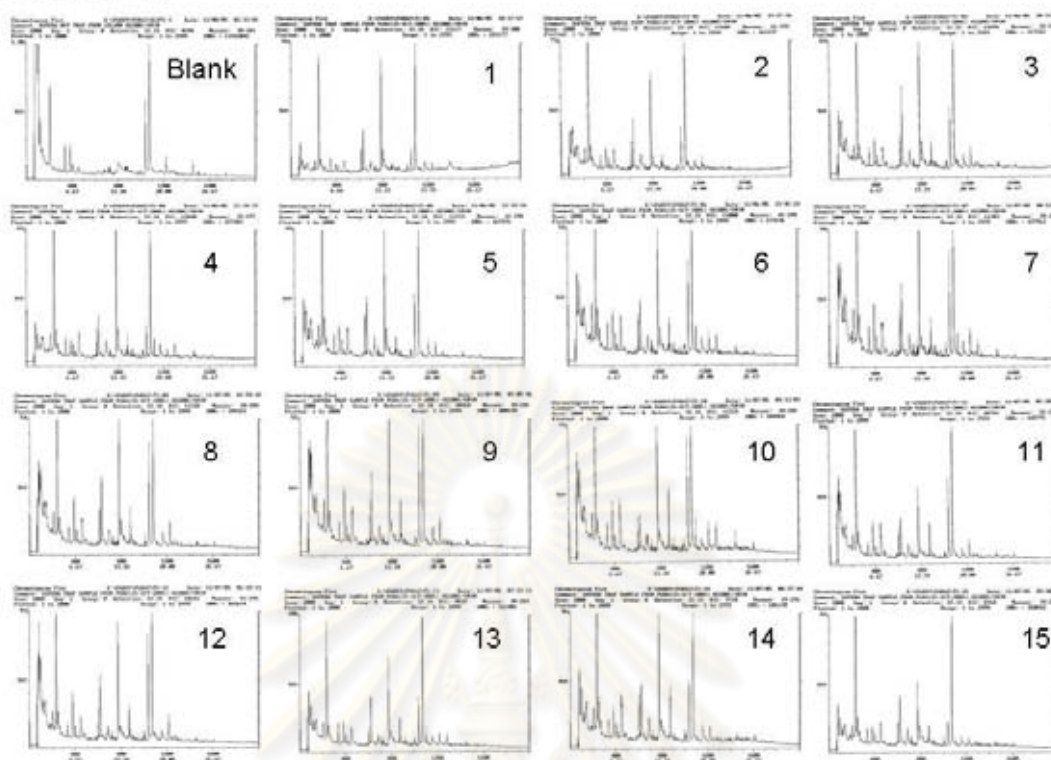
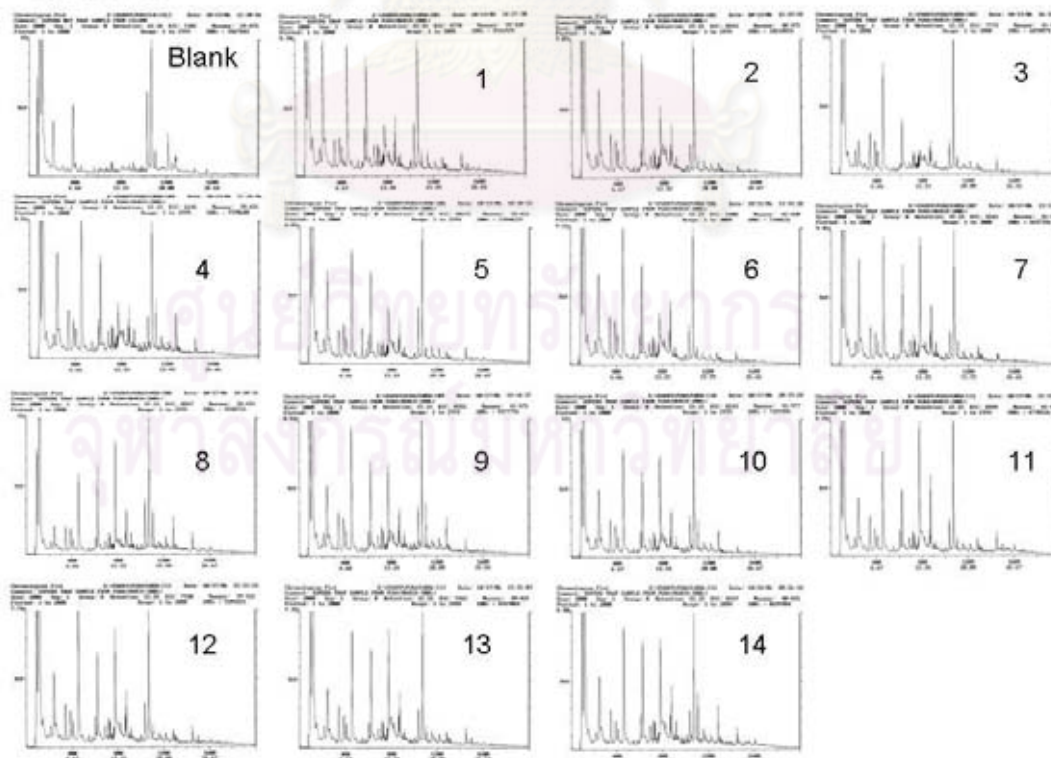
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**APPENDIX A**

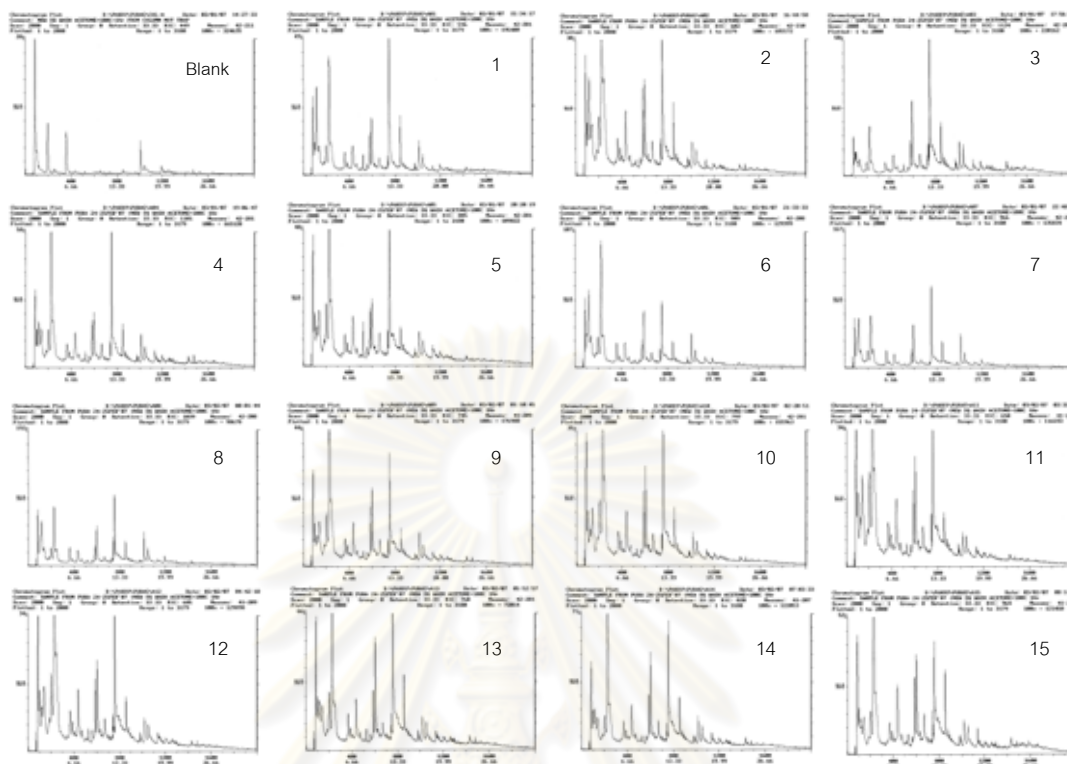
**GC Chromatograms**

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**October 05****March 06****Figure A 1** The GC chromatograms of 15 sites with new Super Q



## February 07



## November 07

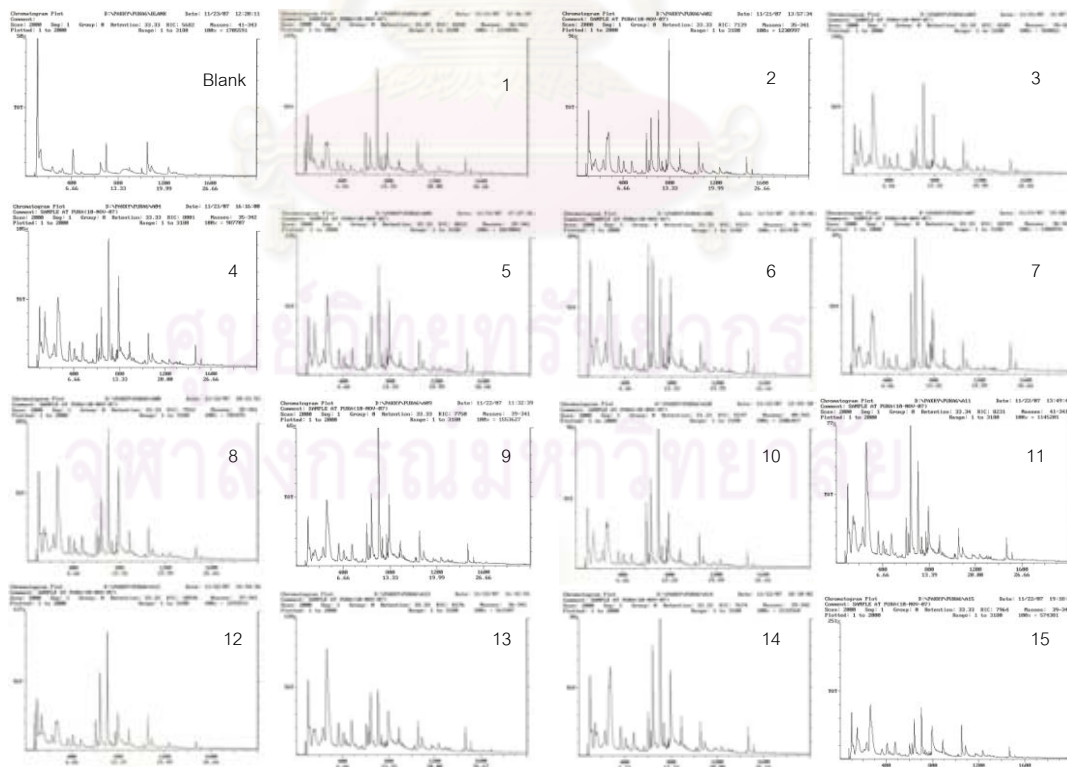
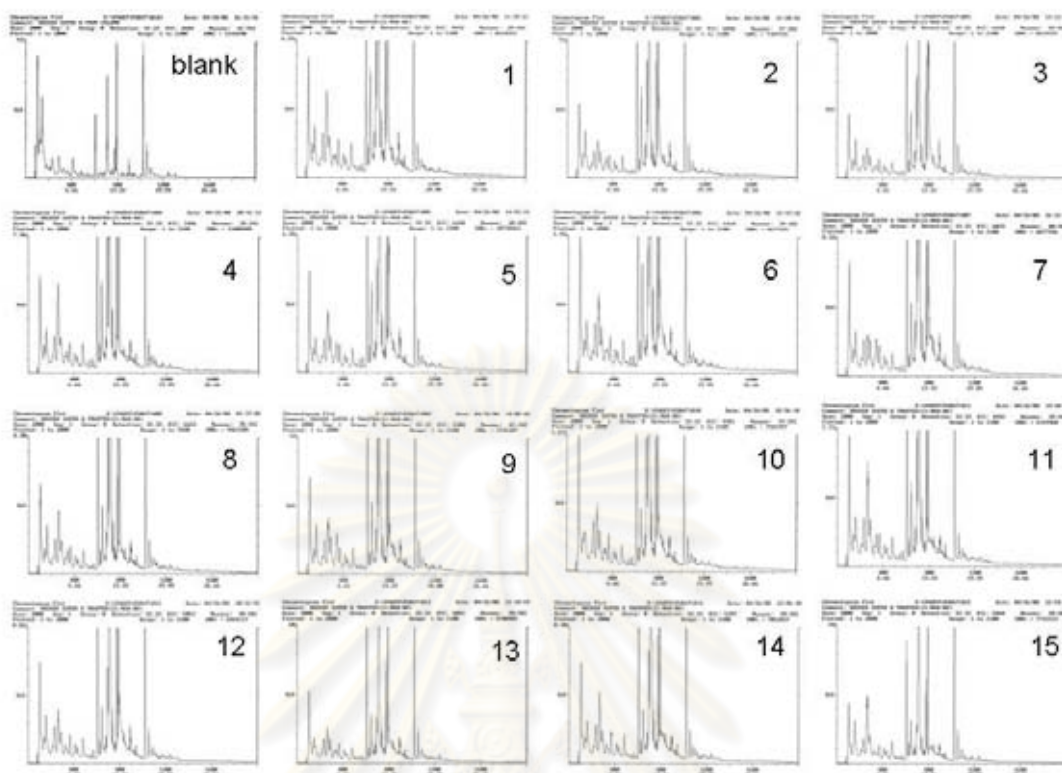
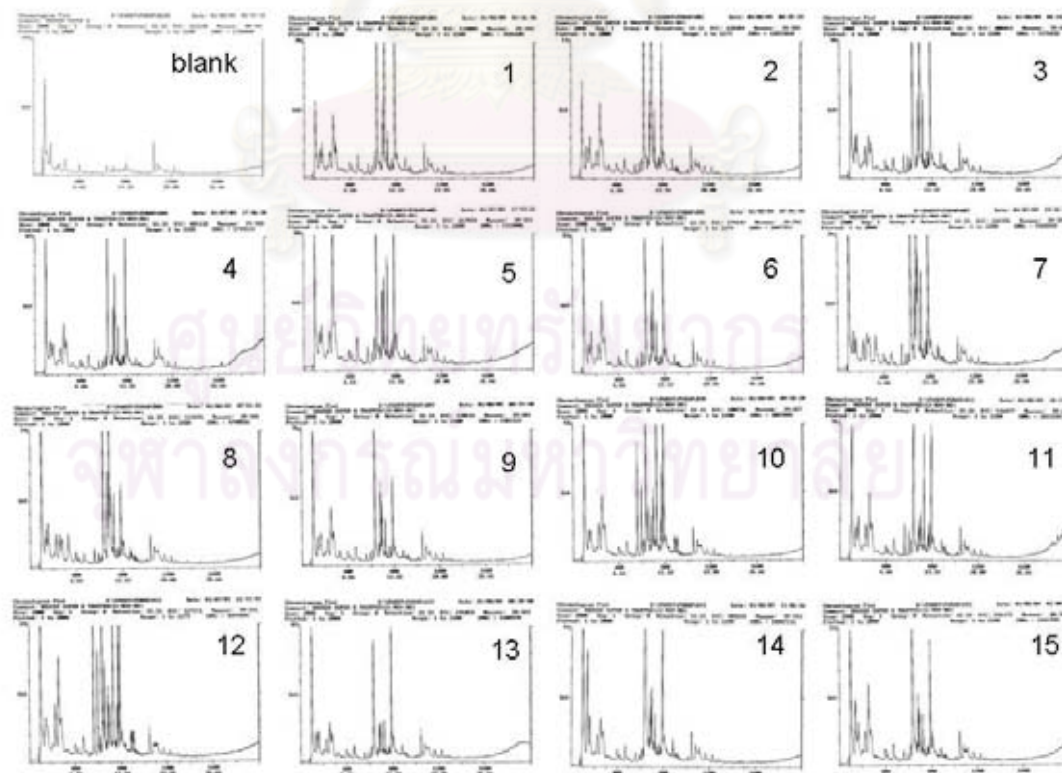


Figure A 2 The GC chromatograms of 15 sites with washed new Super Q

**March 08**



**November 07**



**Figure A 3** The GC chromatograms of 15 sites with reused Super Q

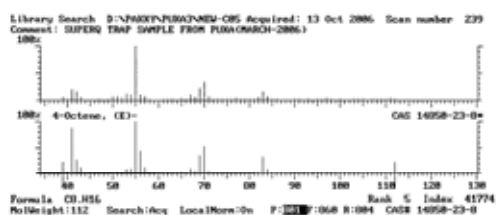
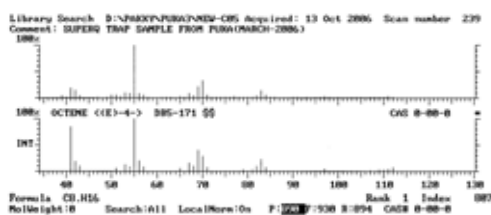


**APPENDIX B**

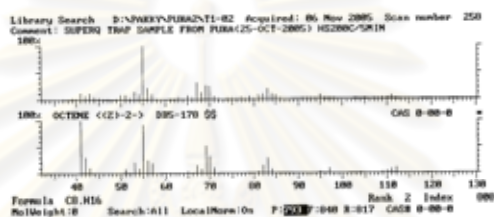
**Mass spectrum**

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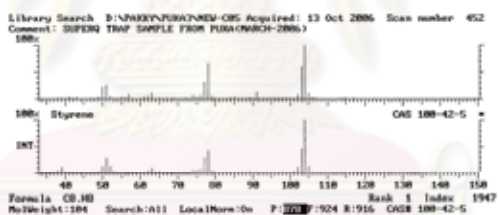
### E-4-Octene



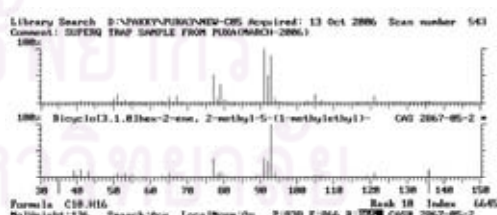
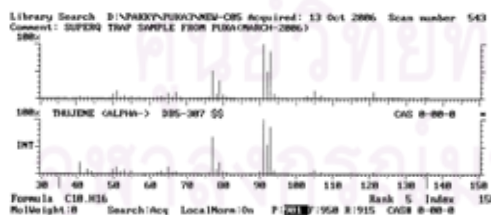
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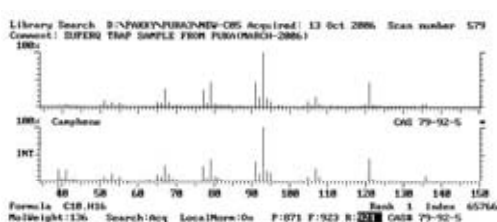
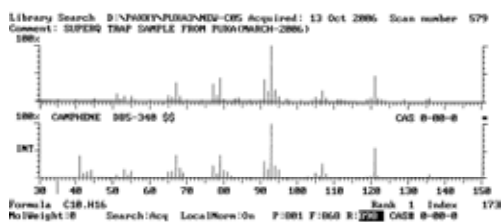
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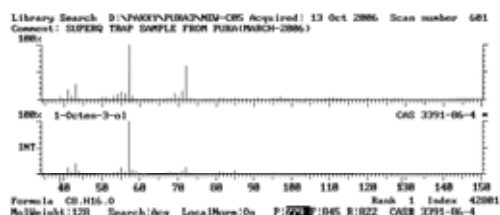
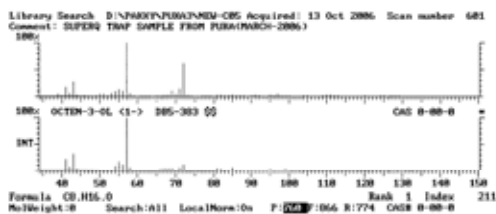
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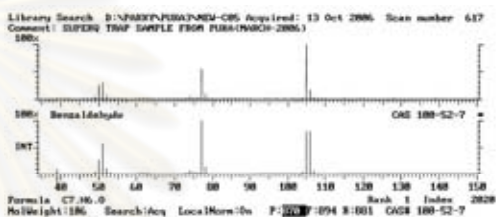
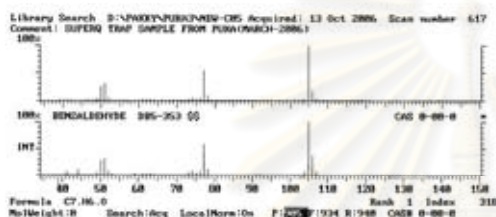
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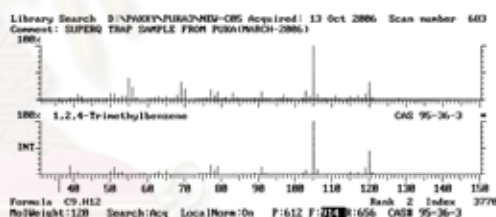
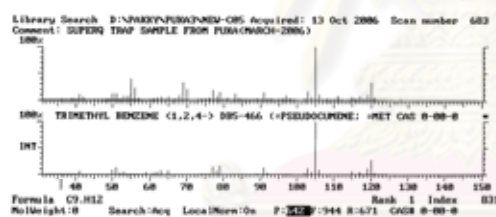
## 1-Octen-3-ol



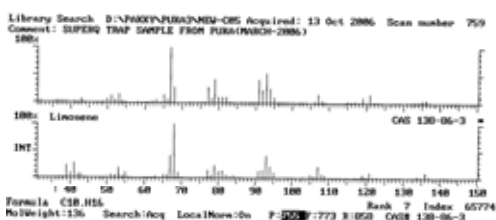
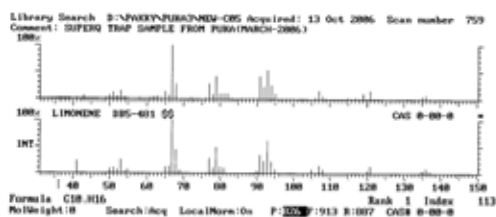
## Benzaldehyde



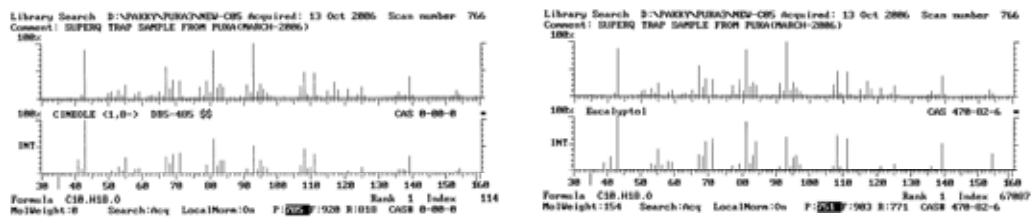
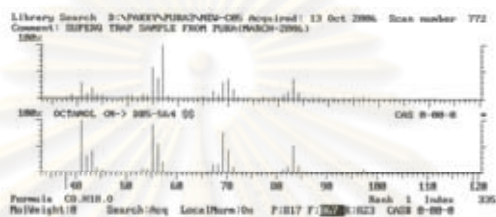
## 1,2,4-Trimethylbenzene

*p*-Cymene

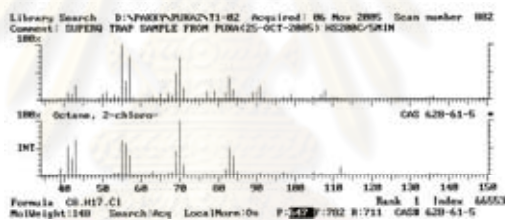
## Limonene



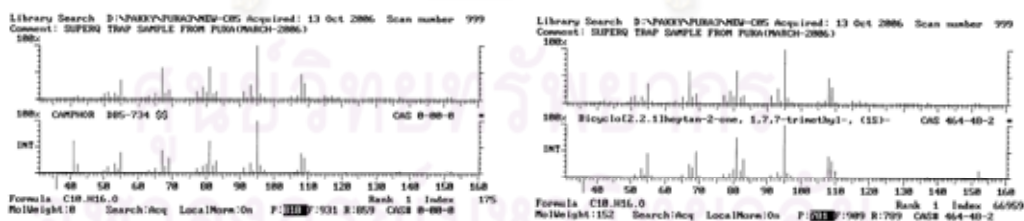
## (1,8)-Cineole = Eucalyptol

*n*-Octanol

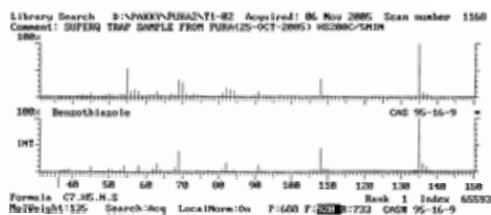
## 2-Chloro-octane

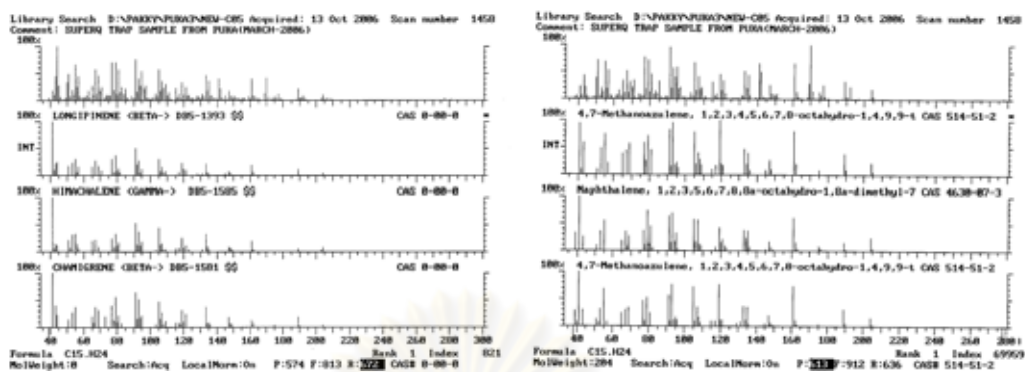


## Camphor

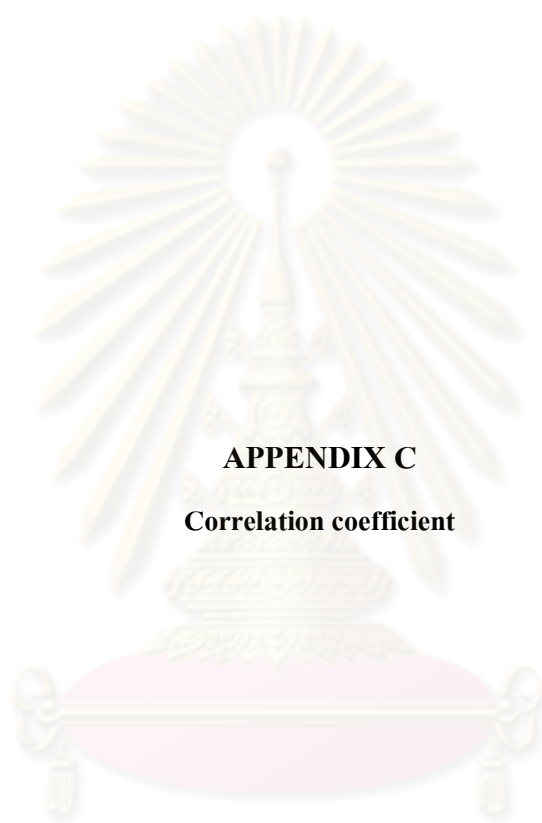


## Benzothiazole





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**APPENDIX C**

**Correlation coefficient**

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**Table C 1** Correlation coefficient of volatile compounds and insects on October 2005

Paired Samples Correlations		N	$\rho$	Sig.
Pair 1	<i>E</i> -4-Octene & Diptera	15	-.254	.361
Pair 2	Octen3ol & Diptera	15	-.189	.499
Pair 3	Benzaldehyde & Diptera	15	+.043	.879
Pair 4	Trimethylbenzene & Diptera	15	+.122	.666
Pair 5	<i>n</i> -Octanol & Diptera	15	-.461	.084
Pair 6	Camphor & Diptera	15	-.159	.571
Pair 7	<i>E</i> -4-Octene & Hymenoptera	15	-.227	.417
Pair 8	Octen3ol & Hymenoptera	15	-.465	.080
Pair 9	Benzaldehyde & Hymenoptera	15	-.187	.505
Pair 10	Trimethylbenzene & Hymenoptera	15	-.380	.162
Pair 11	<i>n</i> -Octanol & Hymenoptera	15	-.234	.402
Pair 12	Camphor & Hymenoptera	15	-.224	.422
Pair 13	<i>E</i> -4-Octene & Homoptera	15	+.049	.863
Pair 14	Octen3ol & Homoptera	15	-.009	.974
Pair 15	Benzaldehyde & Homoptera	15	+.080	.778
Pair 16	Trimethylbenzene & Homoptera	15	+.036	.898
Pair 17	<i>n</i> -Octanol & Homoptera	15	-.232	.406
Pair 18	Camphor & Homoptera	15	+.026	.926
Pair 19	<i>E</i> -4-Octene & Coleoptera	15	+.092	.745
Pair 20	Octen3ol & Coleoptera	15	-.018	.949
Pair 21	Benzaldehyde & Coleoptera	15	+.326	.236
Pair 22	Trimethylbenzene & Coleoptera	15	+.178	.525
Pair 23	<i>n</i> -Octanol & Coleoptera	15	-.094	.740
Pair 24	Camphor & Coleoptera	15	-.005	.985

**Table C 2** Correlation coefficient of volatile compounds and insects on March 2006

Paired Samples Correlations		N	$\rho$	Sig.
Pair 1	<i>E</i> -4-Octene & Diptera	14	-.112	.703
Pair 2	$\alpha$ -Thujene & Diptera	14	+.125	.669
Pair 3	Camphene & Diptera	14	+.196	.503
Pair 4	Octen3ol & Diptera	14	-.371	.191
Pair 5	Benzaldehyde & Diptera	14	+.108	.714
Pair 6	Trimethylbenzene & Diptera	14	-.024	.935
Pair 7	<i>p</i> -Cymene & Diptera	14	-.092	.753
Pair 8	Limonene & Diptera	14	-.222	.446
Pair 9	(1,8)-Cineol & Diptera	14	+.267	.355
Pair 10	<i>n</i> -Octanol & Diptera	14	-.209	.474
Pair 11	Camphor & Diptera	14	+.200	.493
Pair 12	<i>E</i> -4-Octene & Hymenoptera	14	+.308	.284
Pair 13	$\alpha$ -Thujene & Hymenoptera	14	+.554	.040
Pair 14	Camphene & Hymenoptera	14	+.779	.001
Pair 15	Octen3ol & Hymenoptera	14	+.152	.604
Pair 16	Benzaldehyde & Hymenoptera	14	-.040	.892
Pair 17	Trimethylbenzene & Hymenoptera	14	+.257	.376
Pair 18	<i>p</i> -Cymene & Hymenoptera	14	+.104	.724
Pair 19	Limonene & Hymenoptera	14	+.045	.880
Pair 20	(1,8)-Cineol & Hymenoptera	14	-.114	.697
Pair 21	<i>n</i> -Octanol & Hymenoptera	14	+.027	.928
Pair 22	Camphor & Hymenoptera	14	+.078	.791
Pair 23	<i>E</i> -4-Octene & Homoptera	14	+.264	.361
Pair 24	$\alpha$ -Thujene & Homoptera	14	-.009	.976
Pair 25	Camphene & Homoptera	14	+.437	.118
Pair 26	Octen3ol & Homoptera	14	+.099	.736
Pair 27	Benzaldehyde & Homoptera	14	+.247	.395
Pair 28	Trimethylbenzene & Homoptera	14	+.187	.522
Pair 29	<i>p</i> -Cymene & Homoptera	14	-.079	.787
Pair 30	Limonene & Homoptera	14	-.421	.134
Pair 31	(1,8)-Cineol & Homoptera	14	-.002	.994
Pair 32	<i>n</i> -Octanol & Homoptera	14	+.015	.958
Pair 33	Camphor & Homoptera	14	-.009	.976
Pair 34	<i>E</i> -4-Octene & Coleoptera	14	-.174	.552
Pair 35	$\alpha$ -Thujene & Coleoptera	14	-.495	.072
Pair 36	Camphene & Coleoptera	14	-.518	.058
Pair 37	Octen3ol & Coleoptera	14	-.038	.898
Pair 38	Benzaldehyde & Coleoptera	14	+.337	.239
Pair 39	Trimethylbenzene & Coleoptera	14	+.016	.958
Pair 40	<i>p</i> -Cymene & Coleoptera	14	-.432	.123
Pair 41	Limonene & Coleoptera	14	-.076	.796
Pair 42	(1,8)-Cineol & Coleoptera	14	-.331	.248
Pair 43	<i>n</i> -Octanol & Coleoptera	14	+.411	.145
Pair 44	Camphor & Coleoptera	14	+.011	.970

**Table C 3** Correlation coefficient of volatile compounds and insects on November 2007

Paired Samples Correlations		N	$\rho$	Sig.
Pair 1	<i>E</i> -4-Octene & Diptera	15	+0.039	.889
Pair 2	$\alpha$ -Thujene & Diptera	15	+0.161	.567
Pair 3	Camphene & Diptera	15	+0.016	.955
Pair 4	Octen3ol & Diptera	15	+0.341	.213
Pair 5	Benzaldehyde & Diptera	15	-0.114	.685
Pair 6	Trimethylbenzene & Diptera	15	-0.245	.379
Pair 7	<i>p</i> -Cymene & Diptera	15	+0.191	.495
Pair 8	Limonene & Diptera	15	-0.082	.771
Pair 9	(1,8)-Cineol & Diptera	15	+0.332	.226
Pair 10	<i>n</i> -Octanol & Diptera	15	-0.130	.643
Pair 11	Camphor & Diptera	15	+0.122	.666
Pair 12	<i>E</i> -4-Octene & Hymenoptera	15	-0.395	.145
Pair 13	$\alpha$ -Thujene & Hymenoptera	15	+0.541	.037
Pair 14	Camphene & Hymenoptera	15	+0.320	.245
Pair 15	Octen3ol & Hymenoptera	15	-0.064	.820
Pair 16	Benzaldehyde & Hymenoptera	15	-0.100	.723
Pair 17	Trimethylbenzene & Hymenoptera	15	-0.291	.292
Pair 18	<i>p</i> -Cymene & Hymenoptera	15	+0.250	.368
Pair 19	Limonene & Hymenoptera	15	+0.352	.199
Pair 20	(1,8)-Cineol & Hymenoptera	15	+0.311	.259
Pair 21	<i>n</i> -Octanol & Hymenoptera	15	+0.054	.849
Pair 22	Camphor & Hymenoptera	15	+0.234	.402
Pair 23	<i>E</i> -4-Octene & Homoptera	15	-0.107	.703
Pair 24	$\alpha$ -Thujene & Homoptera	15	+0.561	.030
Pair 25	Camphene & Homoptera	15	-0.021	.939
Pair 26	Octen3ol & Homoptera	15	-0.324	.239
Pair 27	Benzaldehyde & Homoptera	15	-0.404	.135
Pair 28	Trimethylbenzene & Homoptera	15	-0.157	.575
Pair 29	<i>p</i> -Cymene & Homoptera	15	-0.292	.292
Pair 30	Limonene & Homoptera	15	+0.603	.017
Pair 31	(1,8)-Cineol & Homoptera	15	-0.299	.279
Pair 32	<i>n</i> -Octanol & Homoptera	15	+0.363	.183
Pair 33	Camphor & Homoptera	15	-0.199	.477
Pair 34	<i>E</i> -4-Octene & Coleoptera	15	-0.456	.087
Pair 35	$\alpha$ -Thujene & Coleoptera	15	-0.041	.886
Pair 36	Camphene & Coleoptera	15	+0.002	.995
Pair 37	Octen3ol & Coleoptera	15	-0.481	.069
Pair 38	Benzaldehyde & Coleoptera	15	-0.016	.955
Pair 39	Trimethylbenzene & Coleoptera	15	+0.020	.945
Pair 40	<i>p</i> -Cymene & Coleoptera	15	-0.234	.401
Pair 41	Limonene & Coleoptera	15	+0.022	.937
Pair 42	(1,8)-Cineol & Coleoptera	15	-0.284	.304
Pair 43	<i>n</i> -Octanol & Coleoptera	15	+0.029	.919
Pair 44	Camphor & Coleoptera	15	-0.393	.147

**Table C 4** Correlation coefficient of volatile compounds and insects on March 2008

	Paired Samples Correlations	N	$\rho$	Sig.
Pair 1	<i>E</i> -4-Octene & Diptera	15	+.263	.344
Pair 2	$\alpha$ -Thujene & Diptera	15	+.186	.508
Pair 3	Camphene & Diptera	15	+.050	.860
Pair 4	Octen3ol & Diptera	15	+.246	.376
Pair 5	Benzaldehyde & Diptera	15	-.139	.621
Pair 6	Trimethylbenzene & Diptera	15	+.064	.820
Pair 7	<i>p</i> -Cymene & Diptera	15	-.096	.732
Pair 8	Limonene & Diptera	15	-.159	.571
Pair 9	(1,8)-Cineol & Diptera	15	+.032	.909
Pair 10	Camphor & Diptera	15	-.357	.191
Pair 11	<i>E</i> -4-Octene & Hymenoptera	15	-.035	.901
Pair 12	$\alpha$ -Thujene & Hymenoptera	15	-.235	.399
Pair 13	Camphene & Hymenoptera	15	-.413	.126
Pair 14	Octen3ol & Hymenoptera	15	-.070	.804
Pair 15	Benzaldehyde & Hymenoptera	15	-.228	.414
Pair 16	Trimethylbenzene & Hymenoptera	15	-.115	.683
Pair 17	<i>p</i> -Cymene & Hymenoptera	15	-.131	.642
Pair 18	Limonene & Hymenoptera	15	-.243	.384
Pair 19	(1,8)-Cineol & Hymenoptera	15	-.158	.574
Pair 20	Camphor & Hymenoptera	15	+.004	.990
Pair 21	<i>E</i> -4-Octene & Homoptera	15	-.206	.461
Pair 22	$\alpha$ -Thujene & Homoptera	15	-.021	.939
Pair 23	Camphene & Homoptera	15	-.369	.176
Pair 24	Octen3ol & Homoptera	15	-.304	.270
Pair 25	Benzaldehyde & Homoptera	15	-.279	.313
Pair 26	Trimethylbenzene & Homoptera	15	-.419	.120
Pair 27	<i>p</i> -Cymene & Homoptera	15	-.319	.247
Pair 28	Limonene & Homoptera	15	-.377	.166
Pair 29	(1,8)-Cineol & Homoptera	15	-.211	.450
Pair 30	Camphor & Homoptera	15	-.063	.824
Pair 31	<i>E</i> -4-Octene & Coleoptera	15	-.235	.400
Pair 32	$\alpha$ -Thujene & Coleoptera	15	+.061	.829
Pair 33	Camphene & Coleoptera	15	-.442	.099
Pair 34	Octen3ol & Coleoptera	15	-.392	.148
Pair 35	Benzaldehyde & Coleoptera	15	-.478	.071
Pair 36	Trimethylbenzene & Coleoptera	15	-.380	.163
Pair 37	<i>p</i> -Cymene & Coleoptera	15	-.269	.333
Pair 38	Limonene & Coleoptera	15	-.386	.155
Pair 39	(1,8)-Cineol & Coleoptera	15	-.197	.482
Pair 40	Camphor & Coleoptera	15	+.050	.859

**Table C 5** Correlation coefficient of volatile compounds and insects on November 2008

	Paired Samples Correlations	N	$\rho$	Sig.
Pair 1	<i>E</i> -4-Octene & Diptera	15	-.476	.073
Pair 2	$\alpha$ -Thujene & Diptera	15	-.319	.247
Pair 3	Camphene & Diptera	15	-.299	.279
Pair 4	Octen3ol & Diptera	15	-.172	.540
Pair 5	Benzaldehyde & Diptera	15	+.149	.597
Pair 6	Trimethylbenzene & Diptera	15	+.186	.506
Pair 7	Limonene & Diptera	15	-.211	.450
Pair 8	(1,8)-Cineol & Diptera	15	-.274	.323
Pair 9	<i>n</i> -Octanol & Diptera	15	+.263	.343
Pair 10	<i>E</i> -4-Octene & Hymenoptera	15	-.048	.864
Pair 11	$\alpha$ -Thujene & Hymenoptera	15	-.152	.588
Pair 12	Camphene & Hymenoptera	15	-.052	.854
Pair 13	Octen3ol & Hymenoptera	15	-.091	.747
Pair 14	Benzaldehyde & Hymenoptera	15	-.059	.834
Pair 15	Trimethylbenzene & Hymenoptera	15	-.043	.880
Pair 16	Limonene & Hymenoptera	15	-.349	.203
Pair 17	(1,8)-Cineol & Hymenoptera	15	-.241	.387
Pair 18	<i>n</i> -Octanol & Hymenoptera	15	-.380	.162
Pair 19	<i>E</i> -4-Octene & Homoptera	15	+.147	.600
Pair 20	$\alpha$ -Thujene & Homoptera	15	-.126	.655
Pair 21	Camphene & Homoptera	15	-.151	.591
Pair 22	Octen3ol & Homoptera	15	+.268	.335
Pair 23	Benzaldehyde & Homoptera	15	+.337	.166
Pair 24	Trimethylbenzene & Homoptera	15	+.435	.105
Pair 25	Limonene & Homoptera	15	-.239	.391
Pair 26	(1,8)-Cineol & Homoptera	15	-.013	.965
Pair 27	<i>n</i> -Octanol & Homoptera	15	+.365	.181
Pair 28	<i>E</i> -4-Octene & Coleoptera	15	-.101	.720
Pair 29	$\alpha$ -Thujene & Coleoptera	15	-.202	.470
Pair 30	Camphene & Coleoptera	15	-.204	.465
Pair 31	Octen3ol & Coleoptera	15	+.000	1.000
Pair 32	Benzaldehyde & Coleoptera	15	+.316	.251
Pair 33	Trimethylbenzene & Coleoptera	15	+.343	.210
Pair 34	Limonene & Coleoptera	15	-.262	.346
Pair 35	(1,8)-Cineol & Coleoptera	15	-.193	.490
Pair 36	<i>n</i> -Octanol & Coleoptera	15	+.170	.545

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