Secretion of Thrips

II. Secretions of Three Gall-Inhabiting Thrips (Thysanoptera: Phlaeothripidae)¹

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The secretions of gall-thrips, *Varshneyia pasaniae*, *Liothrips kuwanai* and their gall-inquilinous species, *L. piperinus* (Thysanoptera: Phlaeothripidae) were analysed by GLC and GC-MS. Dodecane, tridecane, tetradecane, pentadecane and perillene were identified from these three species. Myristyl acetate, cetyl acetate and heptadecene were identified from *V. pasaniae* as new components which were also found from *L. kuwanai*. In addition, 2-methylbutyric acid, a main active component of defensive secretion, was identified from *V. pasaniae*. In this species, perillene may serve as an alarm pheromone which stimulates emission of an anal secretion containing 2-methylbutyric acid against other individuals of the same species. It was confirmed that the components except for perillene were secreted from the anus of *V. pasaniae*, but the gland emitting perillene remains unknown.

INTRODUCTION

Most thrips species of the interstitial-dwelling Phlaeothripidae aggregate in colonies comprised of all juvenile stages and both sexes of adults on living plants, dead wood and dried leaves. Some of them are known to secrete chemical substances which are suggested to have important roles in their habitats (HOWARD et al., 1983; SUZUKI et al., 1986). Many species among these interstitial-dwellers form various galls on their host plants. The thrips are abundant in tropical and adjacent areas (ANANTHAKRISHNAN, 1978), and only a few species including the present materials are distributed in Japan.

We reported in a previous paper (SUZUKI et al., 1986), titled "Anal secretion of thrips" in the present paper we have revised this phrase to "secretion of thrips" that one of these species, *Varshneyia pasaniae* (MUKAIGAWA) (*Leeuwenia pasanii*) secretes perillene and hydrocarbons. However, we could not detect pungent substances from the species, although this was speculated by MUKAIGAWA (1912) who examined an anal secretion which turned the test paper red.

Another gall-forming thrips in Japan is *Liothrips kuwanai* (MOULTON) which has a much shorter tube than *V. pasaniae* and belongs to the tribe Liothripini; *V. pasaniae* is of the tribe Leeuwenini. Moreover, the host plant of *L. kuwanai*, *Piper kadsura* of the

¹ Part I. See References.

family Piperaceae is quite different taxonomically from *Castanopsis cuspidata* of *V. pasaniae*. However, the structure of their galls is similar with rough surfaces having many inside hollows and incompletely rolled leaf-margins, and an inquiline thrips species, *Liothrips piperinus* PRIESNER, can be found from both species galls. Relations between gall-forming and inquilineous thrips including *L. kuwanai* in Taiwan were studied by TAKAHASHI (1934), but those of the present three species have not been studied.

In this paper, a further analysis of the secretion of *V. pasaniae* including pungent substances is reported. Secretions of *L. kuwanai* and *L. piperinus* are studied, and their roles are discussed.

MATERIALS AND METHODS

Thrips. *Varshneyia pasaniae* and an inquilineous species of its gall, *Liothrips piperinus*, were collected with the leaves of the host plants, *Castanopsis cuspidata*, in Ibaraki Prefecture in 1986 and 1983, respectively. *L. kuwanai* were collected from the rolled-leaf galls of the host plant, *Piper kadsura*, in December in 1986 at Mt. Kuno in Shizuoka Prefecture. The galls containing living thrips were kept at 5°C in a refrigerator to suppress movement of the thrips, then the thrips were submitted to solvent extraction.

Extraction of the secretions. The thrips were immersed in ether (200 μ l) in a sampling tube (1 ml volume) and kept for ten minutes. The ether extracts were directly applied to instrumental analyses.

The acidic fraction of the secretion of *V. pasaniae* was obtained from the thrips collected on July 7 (about 200 individuals were used) by the usual method.

Gas liquid chromatography (GLC). GLC analyses were carried out on a Hitachi 263-30 gas chromatograph equipped with a FID using a fused silica CP-Sil 19 CB capillary column (0.22 mm $\times$ 25 m; Chrompack), a Yanaco G-180 gas chromatograph using a fused silica FFAP capillary column (0.22 mm $\times$ 25 m; Quadrex), or a Shimadzu GC-6A gas chromatograph packed with 5% SE-30 or 10% FFAP both on Chromosorb WAW (80–100 mesh) using glass columns (3 mm $\times$ 2 m).

Mass spectrometry (MS). The mass spectra were measured with a Hitachi double-focusing mass spectrometer model M-80B coupled with a gas chromatograph model 367-0200. The operating conditions were as follows: column, fused silica methyl-silicone capillary column (0.22 mm $\times$ 25 mm; Quadrex) or PEG-20M capillary column (0.22 mm $\times$ 25 m); ionization voltage, 70 eV; Interface temp., 250°C; Inj. temp., 250°C.

Chemicals. Perillene was synthesized according to the method of TAKAHASHI (1976). Myristyl acetate and cetyl acetate were easily prepared by the reaction of myristyl alcohol and cetyl alcohol, respectively, with acetic anhydride in the presence of pyridine. The standard acids were purchased from Wako Pure Chem. Co.

Response of ants to the secretion of *V. pasaniae*. Thirty individuals of *V. pasaniae* were placed in a glass petri dish (9 cm diameter). Three ants, *Pristomyrmex pungens*, were released in the dish, and the behavior of both ants and thrips was observed.

Response of *V. pasaniae* to the synthetic perillene. *V. pasaniae* (5 individuals) were released in a small glass petri dish (1.6 cm diameter). A piece of filter paper (5 mm square) treated with perillene was placed in the dish, and the behavior of the thrips was observed. Perillene was applied on the filter paper at amounts of 3 μ g, 20 μ g and 200 μ g per respective paper.

Fig. 1. Gas chromatogram of the secretion of *V. pasaniae* collected in October. column, CP-Sil 19 CB capillary column; col. temp., 80–200°C, 4°C/min. Numbers refer to the text.

RESULTS

Secretion of V. pasaniae. The gas chromatogram of the extract from *V. pasaniae* collected in October is shown in Fig. 1, in which at least nine peaks are observed. Peaks 1, 2, 3, 4 and 5 were perillene, dodecane, tridecane, tetradecane and pentadecane, respectively as reported by SUZUKI et al. (1986) (Table 1). However, peaks 6, 7, 8 and 9 were newly detected. The molecular ion (M^+) of peak 8 was not observed, however, the fragment at m/z 61 ($\text{CH}_3\text{COOH} + \text{H}$) and 196 strongly suggested this compound was an acetate. The latter fragment was regarded to be $M^+ - 60$ (acetate of higher alcohol) due to McLafferty rearrangement. Thus, the molecular weight of this compound was thought to be 256, corresponding to the formula $\text{C}_{16}\text{H}_{32}\text{O}_2$. Reduction of this compound with LiAlH_4 gave myristyl alcohol which was identified by GLC and GC-MS. The MS spectrum as well as GLC retention times of peak 8 was identical with that of synthetic myristyl acetate. Similarly, peak 9 was also identified to be ceytl acetate (hexadecyl acetate). The structure of peak 6 ($M^+ = 166$) is still unknown. Peak 7 was heptadecene ($\text{C}_{17}\text{H}_{34}$) as judged by its $M^+ (=238)$ and fragmentation pattern as well as GLC traces.

The content of each component varied greatly depending on the months that the thrips were collected. Peaks 6, 7 and 9 gradually increased in content from July to October, while the content of perillene gradually decreased during the same period.

Acidic components of the secretion of V. pasaniae. The acidic components were present in such small amounts that they could not be found in the gas chromatogram (Fig. 1) without separation of the acidic fraction, although they were detected by their odors. The gas chromatogram of the acidic fraction measured with 10% FFAP packed column

Table 1. Compounds of the secretions of three species of thrips

GLC peak No.	Content (%)			MS, M ⁺ (base peak)	Compound
	V. p. ¹	L. k.	L. p.		
1	2.0	8.3	22.6	150 (69)	perillene
2	0.9	0.3	trace	170 (57)	dodecane
3	51.3	31.8	53.0	184 (57)	tridecane
4	0.6	0.7	trace	198 (57)	tetradecane
	n.d.	6.3	trace	210 (69)	pentadecene
5	12.4	16.3	24.4	212 (57)	pentadecane
6	10.4	19.8		166 (41)	unknown
7	4.0	4.7		238 (69)	heptadecene
8	15.4	5.9		196 (M ⁺ -60) (43)	myristyl acetate
	n.d.	1.2		222 (M ⁺ -60?)	hexadecenyl acetate (?)
9	3.0	4.7		224 (M ⁺ -60) (43)	cetyl acetate

V. p.: *Varshneyia pasaniae*, L. k.: *Liothrips kuwanai*, L. p.: *Liothrips piperinus*.¹ Collected in October, n.d.: not detectedTable 2. Retention times (t_R) of standard acids and the acidic components

Compound	t_R (min)	
	10% FFAP	FFAP capillary
Acidic component		
Peak A	3.64	
Peak B	6.44	5.32
Peak C	10.30	
Standard acid		
Propionic	3.74	
Butyric	5.70	
2-Methylbutyric	6.20	5.00
3-Methylbutyric	6.28	5.10
Valeric	7.80	
2-Methylvaleric	9.20	
3-Methylvaleric	9.20	
co-GLC (peak B+2-methylbutyric)		5.08 (one peak)
co-GLC (peak B+3-methylbutyric)		4.95 { 5.11 { (two peaks)

10% FFAP (packed): col. temp., 150°C

FFAP capillary column: col. temp., 70°C

Fig. 2. Gas chromatogram of the acidic fraction of the secretion of *V. pasaniae*. column, 10% FFAP (packed); col. temp., 150°C.

revealed the presence of the three components (Fig. 2). The content of each component was 7.1% (peak A), 82.0% (peak B) and 10.9% (peak C), respectively. The retention times (t_R) of seven standard acids were measured under the same condition (Table 2). The t_R of peaks A, B and C agreed with those of propionic acid, 2- or 3-methylbutyric acid and 2- or 3-methylvaleric acid, respectively. The MS spectrum of peak B (main peak) is shown in Fig. 3. The fragment at m/z 87 was thought to be

Fig. 3. Mass spectra of peak B and 2- and 3-methylbutyric acids.

$M^+ - 15$, because the molecular weight of 2- or 3-methylbutyric acid was 102. The MS spectrum of peak B was identical with that of 2-methylbutyric acid (Fig. 3). In addition, co-GLC of the acid and peak B using FFAP capillary column gave a single peak, whereas co-GLC with 3-methylbutyric acid afforded two peaks. Thus, peak B was identified as 2-methylbutyric acid. The fragment at m/z 74 (base peak) was regarded as the result of MacLafferty rearrangement, whereas 3-methylbutyric acid gave the fragment at m/z 60 (base peak) due to this rearrangement (Fig. 3, below). The MS spectra of peaks A and C were not measured because of their small amounts.

Secretion of Liothrips piperinus. The gas chromatogram of *L. piperinus* measured with 5% SE-30 packed column was similar to that of *V. pasaniae* reported previously (SUZUKI et al., 1986). Each component of the secretion was identified as shown in Table 1.

Secretion of Liothrips kuwanai. At least eleven peaks were detected in the gas chromatogram of the secretion of *L. kuwanai* collected in December, in which nine peaks were identified as shown in Table 1. The GLC pattern was very similar to that of *V. pasaniae*, however, two peaks which were not found in *V. pasaniae* were detected in *L. kuwanai*. One was identified as pentadecene ($C_{15}H_{30}$), as judged by its M^+ ($=210$) and t_R . The second peak had a somewhat smaller t_R than that of cetyl acetate. The M^+ of this peak was not observed, but the fragment at m/z 222 was regarded as $M^+ - 60$. Therefore, this compound may be hexadecenyl acetate; this was supported by the fact that the peak was found in the same fraction of myristyl acetate and cetyl acetate by preparative TLC (Kiesel gel 60 F_{254} ; dev. solv., hexane-ether 9:1; myristyl acetate, $R_f=0.39$).

Response of the ants to the secretion of V. pasaniae. When the ants came near, the thrips turned the tips of their abdomen toward them and sprayed anal secretion containing 2-methylbutyric acid around their body, causing the ants to run about crazily in the dish.

Response of V. pasaniae to perillene. It was observed that all the thrips were excited and showed the secreting behavior when they were treated with 20 μg or 200 μg of perillene, indicating that the compound acts as an alarm pheromone against other thrips.

DISCUSSION

In a previous paper (SUZUKI et al., 1986), we reported the identification of perillene and the four saturated hydrocarbons (dodecane, tridecane, tetradecane and pentadecane) from the secretion of *V. pasaniae* (= *Leeuwenia pasanii*).

In the present study, we identified 2-methylbutyric acid, myristyl acetate, cetyl acetate and heptadecene as new components from the same species. MUKAIGAWA (1912) studied the life history of this species and described that the anal secretion was stimulative and turned blue paper red. 2-Methylbutyric acid has the same pungent odor and is regarded to be the stimulative component of the anal secretion (defensive secretion) that Mukaigawa pointed out. This acid showed strong repellency against the ants, however, it showed no effect against a predatory bug (unidentified reduviid) which was sometimes found in the rolled-leaf galls containing the thrips. In fact, we observed that the body liquids of the thrips were sucked up by this predatory bug.

2-Methylbutyric acid is well known to be the main active component of osmeterial secretions of many species of papilionid caterpillars (EISNER and MEINWALD, 1965; HONDA, 1980). It was also identified from the defensive secretion of the carabid beetle, *Pterostichus sulcitaris* (KANEHISA and KAWAZU, 1982).

On the other hand, perillene and the four saturated hydrocarbons were also identified from the secretions of *L. kuwanai* and *L. piperinus*. Thus, these five components were identified from all three of these species. *V. pasaniae* live in aggregating colonies in rolled-leaf galls like the other two species, and when they meet an attacker they cannot run away quickly because of their inactive movement, so they emit an anal secretion containing 2-methylbutyric acid as defensive secretion. Perillene appears to serve as an alarm pheromone which stimulates emission of their anal secretion containing 2-methylbutyric acid against other thrips of the same species, considering the assay result of perillene against *V. pasaniae*.

It is interesting that the anal secretions of the two species were composed of almost the same components although they belong to different tribes. *V. pasaniae* belongs to the tribe Leeuwenini whereas *L. kuwanai* belongs to the tribe Liothripini. The inquiline species, *L. piperinus* may utilize perillene as an alarm pheromone. However, it is at present unknown whether *L. kuwanai* secrete acidic components or not, because they were collected during their inactive season.

Perillene was not detected from the steam-distillate of the hexane extract of the host plant, *Castanopsis cuspidata*, so it must be biosynthesized by the thrips themselves.

It was confirmed that eight components except for perillene identified from *V. pasaniae* were secreted from their abdominal tips (components of anal secretion), because these components were detected by GLC from the anal secretion absorbed by a filter paper. 2-Methylbutyric acid was detected by its odor on the filter paper, although it was not detected by GLC because of its small amount. It is unknown however, whether perillene is secreted from the anus or not.

The role of the acetates is also still unknown. 2-Methylbutyric acid was detected by its odor from the anal secretion of *V. pasaniae* from May to July, but not in October.

The characterization of the unidentified component and the gland secreting perillene are now in progress.

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