Feeding Stimulants Eliciting the Probing Behavior for *Peregrinator biannulipes* Montrouzier et Signore (Hemiptera: Ruduviidae) from *Tribolium confusum* (Jacquelin du Val)

Shin-ichi Tebayashi[a*], Takuya Kawahara[a], Chul-Sa Kim[a], Akinori Nishi[b], Keiichi Takahashi[c], Akihiro Miyanoshita[c], and Michiro Horiike[a]

[a] Department of Bioresources Science, Faculty of Agriculture, Kochi University, B200 Monobe, Nankoku 783-8502, Japan. Fax: +81-88-864-5219. E-mail: tebayasi@cc.kochi-u.ac.jp
[b] Tokyo University of Agriculture and Technology, 3-5-8 Saiwaicho, Fuchu, Tokyo, 183-8509, Japan
[c] Food Entomology Laboratory, National Food Research Institute, 2-1-12 Kannondai, Tsukuba, Ibaraki, 305-8642, Japan

* Author for correspondence and reprint requests.

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Four fatty acid methyl esters identified in the solvent extract of *Tribolium confusum* (Jacquelin du Val) larvae as kairomones were individually and collectively tested for probing behavior of *Peregrinator biannulipes* Montrouzier et Signoret. All identified fatty acid methyl eaters, methyl palmitate, methyl linolate, methyl oleate and methyl stearate, exhibited characterisitic kairomonal probing behavior of *P. biannulipe* toward the lure. These fatty acid methyl ester were active at 0.2 μg/lure but a synergistic effect was not observed among them. Commercially available C_8–C_{14} even-numbered fatty acid methyl esters that were not detected in the extract of *T. confusum* larvae also elicited a probing behavior but their activities were weaker than those of four fatty acid methyl ester ($C_{16:0}$, $C_{18:0}$, $C_{18:1}$ and $C_{18:2}$) identified in the extract. On the other hand, C_{17} and C_{19} odd-numbered fatty acid methyl esters did not show any activity at all.

Key words: Pergrinator biannulipes, Feeding Stimulant, Fatty Acid Methyl Ester

Introduction

Peregrinator biannulipes Montrouzier et Signoret world-wide distributed bug (Ghauri, 1962), and is known as predator for stored products pests including *Stegobium paniceum* (L.), *Lasioderma sericorne* (F.), *Anagasta kuhniella* (Zell), *Pyralis farinalis* L., and *Tribolium* spp (Awadallah *et al.*, 1990). The size of the reduviid imagines is 6–7 mm in length and 3–4 mm in width and their body, especially nymph, is usually covered with detritus for concealing (Takahashi and Romero, 2001). Some biological studies, such as effects of temperature, humidity and prey, have been conducted on this predator for controlling stored products pests (Tawfik *et al.* 1983a, b; Awadallah *et al.*, 1990), but the chemical ecology of this reduviid has not been reported so far. In this paper we report the identification of four fatty acid methyl esters from larvae of *Tribolium confusum* (Jacquelin du Val) as feeding stimulants eliciting the probing behavior of *P. biannulipe* toward the lure.

Abbreviations: fr, fraction; GLC, gas liquid chromatography; GC-MS, gas chromatography mass spectrometry; Rt, retention time.

Materials and Methods

Instruments

GLC was carried out using Hewlett-Packard HP6890 series equipped with FID and HP-5 capillary column (30 m × 0.32 mm ID, 0.25 mm film thickness). The column temperature initially programmed at 150 °C for 3 min and then increased at a rate of 1 °C/min to final isothermal period at 270 °C. Injector and detector temperatures were set at 250 °C. Sample was injected by the split technique into helium carrier gas. GC-MS was performed on a Jeol MS600 mass spectrometer using a fused silica HP-5 capillary column. The GC parameters were same as above.

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Insects

P. biannulipe were reared at 27 ± 2 °C and 70 ± 10% relative humidity in the polyplopyrene cage (32 × 22 × 11 cm) containing of larvae of *T. confusum* as food and crumpled kimwipers for hiding. Prior to bioassays, the reduviids had been isolated and starved for more than 7 days. *T. confusum* was reared at 25 ± 2 °C on a medium consisting of whole wheat flour supplemented with 5% dried brewer's yeast.

Bioassay procedure

In preliminary studies, probing behavior of the reduviids toward a lure treated with the methanol extract of *T. confusum* larvae have been observed. The behavior was constantly detected when the extract was applied on a bit of cotton thread in which its size and shape resembled to that of *T. confusum* larva. However, it was rarely observed when the extract was applied on a similar size and shape of piece of glass rod or silicon. Based on these observations, the bioassay was conducted as follows: a bit of cotton thread (1 mm in thickness × 5 mm in length) was used as a lure. The conditioned reduviid was introduced into a small glass petri dish (18 mm i. d. × 13 mm in height), in which a lure treated with test solution was placed in the bottom of it. Two μl of the test solution was applied uniformly on a bit of cotton thread and the solvent was removed quickly by air drying. Cotton thread treated with hexane or methanol were used as control. The petri dish was then covered with a watch glass. The tests were carried out under dim light and probing behavior was observed for 5 min in the room at 25 ± 2 °C and 70 ± 10% relative humidity. The number of reduviids which probed the lure at least one time during test period were recorded. Each test was replicated 16 times using new beetle, which was used for assay only one times a day, and the data were statistically analyzed using a Fisher's exact test ($P < 0.05$).

Extraction and purification of probing stimulants

T. confusum larvae (10.23 g) were extracted with 100 ml of hexane/acetone/methanol (1:2:1, v/v/v) mixture at 4 °C for 5 days. The extract was filtered and concentrated *in vacuo*, to yield 765 mg residue. After adsorbing on 2 g of silica gel, the residue (673 mg, 9.0 g larva equivalent) was chromatographed on a silica gel column (16.1 cm × 3.0 cm i. d.) eluted with hexane containing increasing concentrations of diethyl ether to obtain hexane (11.1 mg), 1% ether in hexane (0.6 mg), 3% ether in hexane (43.2 mg), 5% ether in hexane (341.0 mg), ether (24.3 mg) and methanol (171.6 mg) frs. Each fraction, after removing solvent *in vacuo*, was weighted, re-dissolved in hexane or metha nol, and assayed.

Results and Discussion

The reduviids repeatedly probed their proboses into a bit of cotton thread with grasping it by their both forelegs, when the cotton thread was treated with 2 mg larva equivalent of the crude extract of *T. confusum* larvae (Table I). The probing behavior was also observed at a dose of 0.2 mg larva

Table I. Kairomonal activities of larva extract, its fractions thereof and authentic fatty acid methyl esters.

Sample	Dose (mg larva equivalent/lure)		
	2.0	0.2	0.02
Crude extract	6[1] (+[2], $P < 0.001$[3])	3 (+, $P = 0.032$)	0 (−, $P = 1$)
Hexane fr.	5 (+, $P = 0.003$)	4 (+, $P = 0.009$)	0 (−, $P = 1$)
1% E/H fr.	0 (−, $P = 1$)		
3% E/H fr.	4 (+, $P = 0.009$)	6 (+, $P < 0.001$)	0 (−, $P = 1$)
5% E/H fr.	1 (−, $P = 0.333$)		
Ether fr.	0 (−, $P = 1$)		
Methanol fr.			
Methyl palmitate $C_{16:0}$	6 (+, $P < 0.001$)	4 (+, $P = 0.009$)	0 (−, $P = 1$)
Methyl stearate $C_{18:0}$	3 (+, $P = 0.032$)	1 (−, $P = 0.333$)	
Methyl linolate $C_{18:1}$	5 (+, $P = 0.003$)	5 (+, $P = 0.003$)	0 (−, $P = 1$)
Methyl oleate $C_{18:2}$	5 (+, $P = 0.003$)	4 (+, $P = 0.009$)	0 (−, $P = 1$)
Mixture	4 (+, $P = 0.009$)	3 (+, $P = 0.032$)	0 (−, $P = 1$)

[1] The number of reduviids probing into the treated lure (N = 16).
[2] +, Significant at $P < 0.05$; −, Significant at $P < 0.05$.
[3] P value was calculated by using Fisher's exact test (all reduviids did not probe the control lure. (N = 32)).

equivalent/lure but not at the lower concentration of 0.02 mg larva equivalent/lure. Thus these results revealed the presence of probing stimulants in the extract. Then the extract was chromatographed on a silica gel column eluted with hexane containing increasing concentrations of diethyl ether. By the results of the bioassay using reduviids, the hexane and the 3% ether-hexane frs showed activities at 0.2 mg larva equivalent/lure (Table I). GLC analysis of the active frs revealed that the 3% ether-hexane fr contained only four compounds (compound **1**; Rt = 26.8, compound **2**; Rt = 39.1, compound **3**; Rt = 39.5, compound **4**; Rt = 41.5) but there were several compounds present in the hexane fr. The 3% ether-hexane fr was then analyzed by GC-MS without further purification. The mass spectra of **1** gave ions at *m/z* = 270 (8.8), 239 (4.3), 227 (5.0), 199 (2.4), 117 (3.0), 143 (13.5), 129 (5.9), 101 (5.5), 97 (5.6), 87 (65), 73 (7.6), 74 (100), 59 (8.1) and 57 (15.6). In EI mass spectra of compound **1**, the fragment ions at *m/z* 59, 74 and 87 represents a typical pattern of ions for fatty acid methyl ester. The highest ion at *m/z* 270 could be the molecule ion. Therefore compound **1** could be identified as methyl palmitate. From similar assignments, compounds **2**, **3** and **4** could be assigned to methyl linolate, methyl oleate and methyl stearate, respectively. Finally compounds **1**, **2**, **3** and **4** were comfirmed as methyl palmitate (1.4 mg/g larva), methyl linolate (2.6 mg/g larva), methyl oleate (1.5 mg/g larva) and methyl stearate (0.67 mg/g larva), respectively, by comparing their retention times with the authentic methyl esters, and by coinjection as well. The activities of identified methyl esters were measured using standard compounds. When the four compounds were mixed according to their relative natural abundance, the mixture was active at the lowest dose of 0.2 mg larva equivalent/lure which was same dose of the crude extract and the hexane fr (Table I). Thus we concluded that four fatty acid methyl ester consisting of two saturated fatty acid methyl esters, methyl palmitate and methyl stearate, and two unsaturated fatty acid methyl esters, me thyl linolate and methyl palmitate, were one group of the feeding stimulants eliciting the probing behavior of *P. biannulipe*. The lowest dose for activities of methyl palmitate, methyl linolate and methyl oleate was 0.2 mg larva equivalent/lure but that of methyl stearate was 2.0 mg larva equivalent/lure, thus synergistic effects were not observed among the four compounds.

Fatty acid methyl esters in various degree of carbon chain length were also tested for their probing activities (Table II). In the series of even-numbered fatty acid methyl esters, methyl dodeca-

Table II. Kairomonal activities of the various fatty acid methyl esters.

Sample		Dose (μg /lure)			
		20	2.0	0.2	0.02
C	Methyl hexanoate	0[1], –[2] ($P = 1$)[3]			
C_8	Methyl octanoate	3,+ ($P = 0.032$)	0, – ($P = 1$)		
C_{10}	Methyl decanoate	3,+ ($P = 0.032$)	1, – ($P = 0.333$)		
C_{12}	Methyl dodecanoate	3,+ ($P = 0.032$)	9,+ ($P < 0.001$)	1, – ($P = 0.333$)	
C_{14}	Methyl tetradecanoate	7,+ ($P < 0.001$)	6,+ ($P < 0.001$)	2, – ($P = 0.106$)	
C_{16}	Methyl hexadecanoate	10,+ ($P < 0.001$)	5,+ ($P = 0.003$)	3,+ ($P = 0.032$)	0, – ($P = 1$)
C_{17}	Methyl heptadecanoate	1, – ($P = 0.333$)			
C_{18}	Methyl octadecanoate	3,+ ($P = 0.032$)	5,+ ($P = 0.003$)	3,+ ($P = 0.032$)	0, – ($P = 1$)
C_{19}	Methyl nonadecanoate	2, – ($P = 0.106$)			
C_{20}	Methyl icosanoate	2, – ($P = 0.106$)			

[1] The number of reduviids probing into the treated lure (N = 16).
[2] +, Significant at $P < 0.05$; –, Significant at $P < 0.05$.
[3] P value was calculated by using Fisher's exact test (all reduviids did not probe the control lure. (N = 32)).

noate (methyl laurate) and methyl tetradecanoate (methyl myristate), which were not detected in the extract of *T. confusum* larvae, were active at 2.0 μg/lure that is ten times lower than the minimal dose of activity of methyl hexadecanoate (methyl palmitate) present in the extract. Activity of esters with a carbon chain below fourteen was decreased with the shortening of carbon chain, and methyl hexanoate did not elicit probing behavior. In contrast, fatty acid methyl esters over eighteen carbon chain, methyl icosanoate (methyl behenate), and the odd-numbered fatty acid methyl esters did not show any activities with the tested concentration. Thus we conclude that the fatty acid methyl esters commonly found in insect show the probing activity toward *P. biannulipe* although the methyl esters identified form *T. confusum* larvae extract are most effective.

The identified fatty acids, methyl palmitate, methyl linolate, methyl oleate and methyl stearate, are long-chain fatty acids which were commonly found as triacylglycerols in the plants and animals. The free long-chain fatty acids and its derivatives were utilized as kairomones; the free acids in the cabbage butterfly (*Pieris rapae*) larvae were used for host recognition by the parasitic wasp (*Cotesia glomerata*) (Horikoshi *et al.*, 1997), methyl and ethyl esters of long-chain fatty acids in dorne larvae of the honey bees (*Apis melifera*) were utilized as attractants by the parasitic mite (*Varroa jacobsoni*) (Conete *et al.*, 1989), diacylglycerols identified in azuki beans infested by the azuki bean weevils (*Callosobruchus chinensis*) elicited the stinging behavior of the parasitic wasp (*Dinarmus basalis*) toward the larvae of the bean weevils (Kumazaki *et al.*, 2000). The fatty acids and its derivatives as kairomone have been mainly studied on parasitic wasps and other parasites. This is the first report that a predator utilizes fatty acids derivatives as a kairomone.

The predatory stink bug (*Eocanthecona furcellata*) is a generalist predator which attack larvae of various phytophagous species on field. (*E*)-Phytol, which is obtained by hydrolysis of chlorophyll included in the food plant of the herbivore, is utilized by *E. furcellata* as a kairomone eliciting prey- locating behavior (Yasuda, 1997). Fatty acid methyl ester is one of the metabolites of triacylglycerol that is abundantly found in the food source, especially in grains. Thus *P. biannulipes* also utilizes the metabolite derived from the food plants of prey as well as *E. furcellata*.

The hexane fraction obtained from silica gel column chromatography also showed probing activities toward the reduviids, and it was shown by GC-MS analysis that many kinds of hydrocarbons were present in this fr. Because of the small amount of each hydrocarbon, it was not possible to conduct further studies on this fr. It has been reported that parasite wasps used hydrocarbons for their host recognition (Jones *et al.*, 1971; Ohara *et al.*, 1996; Kumazaki *et al.*, 2000). It is therefore needed to conduct further studies on the hydrocarbons consisted in the hexane fr.

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