Monoenyl hydrocarbons in female body wax of the yellow peach moth as synergists of aldehyde pheromone components

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Abstract

The non-polar components of female body wax and pheromone gland extracts of the yellow peach moth synergistically enhanced male behavioral responses from close to pheromone sources in wind tunnel tests when mixed with an aldehyde pheromone blend. When the non-polar fractions of female body wax were further separated by column chromatography, synergistic activities were found in the 3% and 50% ether in hexane fractions, and they additively increased male responses. The main components of the first fraction were (*Z*)-9-tricosene, (*Z*)-9-pentacosene, (*Z*)-9-heptacosene, (*Z*)-9-nonacosene and (*Z*)-9-hentriconten. Only (*Z*)-9-heptacosene showed a significant synergistic effect in enhancing male responses, but the other components had no effect. A mixture of the five monoenyl hydrocarbons lost activity at lower doses than 5 ng. Natural ratios of these hydrocarbons in the female body wax and pheromone gland extracts were similar, but amount of (*Z*)-9-heptacosene in the female body wax was significantly higher than in the pheromone gland extracts. We conclude that (*Z*)-9-heptacosene increase male responses to aldehyde pheromones, and unknown component(s) in the 50% ether in hexane fraction are required for full synergistic enhancement by the non-polar fractions of the female body wax and the pheromone gland extracts.

Keywords: Monoenyl hydrocarbons; body wax; yellow peach moth; pheromone

41 synergists

Introduction

The yellow peach moth, *Conogethes punctiferalis* (GUENÉE), seriously damages a number of crops, fruits and vegetables in Tropical Asia, Eastern Asia and Australian regions (Sekiguchi, 1974; Waterhouse, 1993). A binary mixture of (*E*) -10-hexadecenal (E10-16: Ald) and (*Z*)-10-hexadecenal (Z10-16: Ald) at a ratio of 90:10 was reported as an effective pheromone lure in field tests (Konno et al., 1982). However low performance of lures baited with the mixture was later experienced in monitoring and mating disruption in some regions of Japan (Kondo et al., 2008). Therefore, the possibilities of inconsistences in ratios of natural pheromone components of the previous lures and shortage of unknown additional component(s) were predicted. This led to the re-analysis of the natural ratio of these two aldehyde components and it was revised to 95.4: 4.5, which never improved performance of the new lure (Kimura, 2002; Lin and Honda, unpublished data). Furthermore, EAD active compounds (hexadecanal and (*E*)-10-hexadecenol) subsequently identified in the pheromone gland extract also did not improve the activity of previously identified aldehyde blend (Kimura, 2002).

In our previous report, we showed that non-polar components of the pheromone gland extracts and female body wax extracts synergistically enhanced male behavioral

60 responses during the final stages of response to the pheromone sources when mixed
61 with the aldehyde pheromone blend (Xiao and Honda, 2010).
62 In the present paper, we report the identification of monoenyl hydrocarbons as
63 enhancing factors in the female body wax extracts as well as the pheromone gland
64 extracts, and discuss their function in the pheromone system of the yellow peach moth.
65

66 ## Materials and methods

67 Insects

68 The larvae of the yellow peach moth were reared on an artificial diet and chestnuts or
69 corn (Honda et al., 1979). Adults were sexed at the pupal stage and kept separately in
70 cages at 25± 1°C, 40-60% R.H. and under a 15 L-9 D photoperiod. Adults were
71 provided with a 10% sugar solution from cotton pads. A 15 W red incandescent lamp
72 was used for observations during scotophase.

73

74 Extracts and fractionation

75 Abdominal distal segments including the sex pheromone glands were excised with
76 micro-scissors from 2 to 4-day-old virgin female moths anesthetized with CO_2 at 5-8 hr
77 after lights-off. Abdominal tips were extracted for 15 min at room temperature with
78 redistilled *n*-hexane (ca. 1 μl per insect) containing 0.01% butylated hydroxyl toluene
79 (Wako Pure Chemical Industries Co. Ltd., Osaka, Japan) as an anti-oxidant. The
80 pooled pheromone gland extracts were collected in screw vials (200 female equivalents
81 per vial) with Teflon®-lined caps and stored at –20°C until use. Two to 4 day old
82 virgin female moths and male moths were anesthetized with CO_2 during photophase
83 (4-6 hr after light on) to avoid contamination of the sex pheromone aldehydes (Konno et
84 al,. 1982), and then their whole bodies were extracted with ca. 0.5 ml per insect of

redistilled hexane for 2 min in a small glass vial. Female body wax extracts and male body wax extracts were passed through a cotton filter in a Pasteur pipette® to remove body scales, respectively, and the extracts of 200 insects were pooled in each vial, and stored at -20°C.

Pheromone gland extracts, female and male body wax extracts were also subjected to open column chromatography (1 cm in diameter, 3 cm in length) loaded with 1 g of Florisil (60-100 μm particle size, Wako Pure Chemical Industries Co. Ltd., Osaka, Japan) impregnated with 7% distilled water (Carroll, 1961), to obtain non-polar fractions (NPFs) with elution of 10 ml hexane. The NPFs were further fractionated by column chromatography with an open column (1 cm diameter, 3 cm length) packed with 1 g silica gel (Wako gel C-200, particle size: 75 -150 μm) impregnated with 10% $AgNO_3$ (99.8%, Wako). Each NPF was successively eluted with 10 ml each of hexane (Fr. 1), 1% (Fr. 2), 3% (Fr. 3), 10% (Fr. 4), 30% (Fr. 5) and 50% ether in hexane (Fr. 6), and 10 ml ether (Fr. 7). The fractions were concentrated to 1 FE (female equivalent) /μl under a N_2 stream and stored at –20°C until use.

Bioassay

All bioassays were conducted in a transparent acrylic wind tunnel (2 m in length, 0.3 m in diameter), in which rectified wind was provided at 30 cm/s by an electric fan located

104 at the downwind outlet. Male behavioral responses to stimuli were observed at 25±
105 1°C and 40-60% RH under illumination with a red incandescent light at ca. 2 lux. One
106 μl of test materials dissolved in hexane were loaded on a triangular filter paper (0.5 cm
107 in base, 1.5 cm in height), and the filter papers were hung 15 cm high from the floor and
108 15 cm from the upwind end of the wind tunnel. Prior to tests, the streams of
109 stimulus plumes in the wind tunnel were simulated with $TiCl_4$. Newly emerged 2 to 4
110 day old males were individually transferred into small metal mesh cages (6 cm in
111 diameter, 6 cm in height) at least 1 hr before testing. The cage was hung on a
112 ring-holder at 15 cm from the ceiling and 15 cm from the downwind end of the wind
113 tunnel. Male moths were allowed to naturally leave the cage just after setting a
114 stimulus source at the upwind end, and the following four behaviors were recorded for 3
115 min: (1) starting flight, (2) catching plume (male moth flies upwind along the plume), (3)
116 close to source (hovering around within 10 cm of the bait) and (4) source contact (Xiao
117 and Honda, 2010; Mazor and Dunkelblum, 1992). The duration of remaining close to
118 the source and number of source contact was also recorded. After each test, the inside
119 wall of the wind tunnel was rinsed well with 70% ethanol and then completely
120 ventilated.
121

122 Dimethyl disulfide (DMDS) treatment

123 The fraction (s) which was active in the wind tunnel bioassays were derivatized to
124 DMDS adducts to determine the position of the double bonds in the compounds
125 (Francis and Veland, 1981). DMDS (200 µl, Wako) and 50 µl of I_2 solution (60 µg/µl,)
126 in diethyl ether were added to the fractions. The mixtures were sealed in glass
127 ampoules and stirred for 24 h at room temperature and then extracted three times with 1
128 ml hexane after addition of 5% $Na_2S_2O_3$ solution. The extracts were subsequently
129 dehydrated with anhydrous Na_2SO_4 and concentrated for GC/MS analysis.

130

131 Chemicals

132 E10-16: Ald and Z10-16: Ald were obtained from Shin-Etsu Chemical Co. Ltd. (Tokyo,
133 Japan), and a blend of these two aldehydes (ratio of 95.5: 4.5) was prepared after
134 purification of both isomers (99.5% in purity) by chromatography over 10%
135 $AgNO_3$-SiO_2. (*Z*)-9-Tricosene (Z9-23: HC) and (*Z*)-9-nonacosene (Z9-29: HC) were
136 given by S. Matsuyama. (*Z*)-9-Pentacosene (Z9-25: HC), (*Z*)-9-heptacosene (Z9-27:
137 HC) and (*Z*)-9-hentricontene (Z9-31: HC) were synthesized by *Z*-selective Wittig
138 reactions of corresponding aldehydes with C_9-ylide, generated by mixing potassium
139 hexamethyldisilazane (KHMDS) and nonyltriphenylphosphonium bromide. As an

example, synthesis of Z9-25: HC is described below.

A solution of hexadecanal (0.37 g, obtained by oxidation of 1-hexadecanol with PCC) in THF was added to a cooled (–30°C) stirred suspension of nonylideneltriphenylphosphorane, generated by KHMDS and 2.14 g of corresponding phosphonium bromide in THF/HMPA (3: 1). The reaction mixture was held overnight with stirring, allowed to warm to room temperature, then poured into an aqueous NH_4Cl_2 solution and extracted with ether. The combined ether layers were washed with brine, dried over $MgSO_4$ and the solution was evaporated. The resulting crude material was once chromatographed over SiO_2 (eluent: hexane) and subsequently purified with a Sephadex® LH-20 (Pharmacia Fine Chemicals, particle size: 25-100μm) column (eluent: $CHCl_3$) to produce 0.22 g of (*Z*)-9-heptacosene (99% in purity).

Chemical analysis

Gas chromatography (GC) analyses were carried out on a Shimadzu GC-17A gas chromatograph equipped with an FID on an HP-5 column (30 m× 0.32 mm ID, film thickness 0.25 μm, Agilent Technologes, USA), using a temperature program of 190°C to 280°C at 10°C /min, with an initial 2 min hold at 190°C and a final 10 min hold at

158 280°C. Samples were injected in splitless mode at 280°C using He as the carrier gas
159 (24 cm/sec). The temperature of the detector was 280°C.
160 Gas chromatography-mass spectrometry analyses were conducted with a mass selective
161 detector (5972 Series, Hewlett-Packard) coupled with a HP 5890 Series Ⅱ GC equipped
162 with a HP-5MS column (30 m × 0.25 mm ID, film thickness 0.25 μm, Agilent
163 Technologes, USA). The oven temperature was held for 2 min at 140°C and
164 increased to 280°C at 5°C /min, and held at 280°C for 10 min. Samples were injected
165 in splitless mode at 280°C using He as the carrier gas (37 cm/sec). The temperature of
166 the interface was maintained at 280°C.
167 For quantification of monounsaturated hydrocarbons in body wax extracts and crude
168 pheromone extracts, a tetracosane was used as an external standard for Z9-23: HC and
169 Z9-25: HC, and an octacosane for Z9-27: HC, Z9-29: HC and Z9-31: HC.
170
171 Statistical analysis
172 Data on starting flight, catching plume and close to source were first analyzed by n× 2
173 Fisher's exact probability test using the actual number of insects. When probability
174 was significant ($p < 0.05$), multiple comparisons were performed employing Ryan's
175 method. Data on mean time for duration of close to source and mean number of

176 source contacts by male moths were analyzed by Tukey's test after ANOVA. Software
177 package R, version 2.10.0 (R Development Core Team, 2009), was used for statistical
178 analyses.
179

Results

Enhancing activity by female body wax hydrocarbon fractions

As reported by Xiao and Honda (2010), a non- polar fraction of female body wax extracts of the yellow peach moth enhanced male responses to a pheromone source in the final stage of mating behavior when mixed with an aldehyde blend consisting of E10-16: Ald and Z10-16: Ald in a ratio of 95.4: 4.5. To survey the active components, the non-polar fraction was further separated into seven fractions, Fr. 1 –Fr. 7, by $AgNO_3$-impregnated silica gel column chromatography and the synergistic activity was evaluated in wind tunnel tests (Table 1). The male response rate in the first three stages, starting flight, catching plume and close to source, never increased even when each of the seven fractions was tested as a binary mixture with the aldehyde blend. However, the mean time remaining close to source and the mean number of source contacts by male moths remarkably increased with Frs. 3, 5 and 6 when mixed with the aldehyde blend. No significant increase in these responses was observed in the other four fractions.

The synergistic effects of these seven fractions on male response to the pheromone source were further evaluated by mixing the seven fractions in different combinations (Table 2). In two mixtures with combinations of Frs. 3 and 5, and Frs. 5 and 6, significantly higher activities than the aldehyde blend as a control were observed in the

mean time remaining close to source and the mean number of source contacts, but the activity was not significantly different between the two combinations. The highest enhancing effects were observed with a binary mixture of Frs. 3 and 6, a ternary mixture of Frs. 3, 5 and 6 and a mixture of all seven fractions. However, the enhancing activities of these three mixtures were not significantly different in the mean time remaining close to source and the mean number of source contacts by male moths.

Chemical analysis of hydrocarbon synergists

The combination of Frs. 3 and 6 enhanced the activity of the aldehyde blend the same as the mixture of all fractions, indicating the synergistic compounds exist in these two fractions. The Fr. 3 was first analyzed and the results are reported in the present paper. The major peaks (percent of total peak area: 91.61± 0.83%) in the gas chromatogram profile of the Fr. 3 showed five homologous compounds. Their retention times in GC were 19.81 min, 23.05 min, 26.10 min, 29.05 min and 31.74 min, respectively. GC/MS analysis of these compounds gave a common base peak at *m/z* 97 (100%), and molecular ions at *m/z* 322 (12%), 350 (10%), 378 (9%), 406 (8%) and 434 (8%), respectively, supposing these compounds to be tricosene, pentacosene, heptacosene, nonacosene and hentriacontene, respectively. After DMDS treatment, all corresponding adducts showed a same fragment ion at *m/z* 173, indicative of a double

218 bond located at the 9-position in the molecules. Additionally, in the GC/MS
219 chromatogram, each peak of pentacosene, heptacosene and nonacosene was closely
220 followed by a peak with a much smaller area, which formed three pairs of peaks.
221 Moreover the two peaks in each pair gave the same mass spectra. These paired peaks
222 were estimated to represent the geometrical isomers. By comparison of GC retention
223 times and mass spectra between the natural compounds and authentic chemicals, the
224 earlier peaks in each paired peaks were confirmed to be the Z isomers. Thus, the
225 dominant monounsaturated hydrocarbons were identified as Z9-23: HC, Z9-25: HC,
226 Z9-27: HC, Z9-29: HC and Z9-31: HC.
227 Not only NPF from the female body wax, but also NPFs from the pheromone gland
228 extracts and male body wax extracts gave the same monoenyl hydrocarbons in the Fr. 3
229 after $AgNO_3$-impregnated silica gel column chromatography. Moreover, the female
230 body wax extracts and pheromone gland extracts showed similar compositions of those
231 hydrocarbons, which were much different from those in male body wax extracts.
232 Additionally, the total amount of those hydrocarbons in the female body wax was about
233 25 times and 33 times higher than in the pheromone gland extracts and male body wax
234 extracts, respectively (Table 3).
235 **Bioassay of monounsaturated hydrocarbons**
236 Male moth responses in the last two behavioral stages, mean time remaining close to

source and mean number of source contacts, to the mixtures of five homologous monoenyl hydrocarbons from Z9-23: HC to Z9-31: HC and the aldehyde blend are shown in Fig. 1. The highest male responses were observed to Z9-27: HC, and increases and decreases in the number of carbons atoms in the molecule resulted in diminution of the male response to the mixture of monoenyl hydrocarbons and the aldehyde blend. The responses evoked by Z9-27: HC were as high as that of the all monoenyl hydrocarbons mixture, with the addition of the aldehyde blend, respectively. The synergistic effect of all monoenyl hydrocarbons mixture disappeared when Z9-27: HC was removed. The activities with Z9-27: HC and the mixture of all monoenyl hydrocarbons also corresponded to those of the Fr. 3 that was separated by $AgNO_3$-impregnated silica gel column chromatography from the NPF of female body wax extracts (Table 1).

The dose-response relationship of synergism with the mixture of all monoenes with the aldehyde blend at different doses is shown in Fig. 2. A similar dose-response relationship was found between the mean time remaining close to source and the mean number of source contacts. A remarkable increase in male responses was observed when the doses were 50 ng and 300 ng. However, at a dose of 5 ng and below, no significant increase in responses was observed.

Discussion

We reported synergism of the NPF of the pheromone gland extracts and female body wax extracts with aldehyde pheromone components (Xiao and Honda, 2010). To identify the active components, the NPF of female body wax extracts was separated into several fractions based on their degree of unsaturation. As shown in Table 1, when the fractions were mixed with the aldehyde blend, significant increases in responses were observed in Frs. 3, 5 and 6. This suggests that different types of unsaturated hydrocarbons in these fractions synergistically contribute to the increase in male responses during the last two behavioral stages of mating. Moreover, the activities of only the binary mixture of Frs. 3 and 6 was as high as the three-fraction mixture and NPF, indicating that components contributing to the activity in the NPF were mainly contained in the Frs. 3 and 6 (Table 2).

In the Fr. 3 of the female body wax extracts, the total amount (per insect) of the main monoenyl hydrocarbons was ca. 25 times higher than in the pheromone gland extracts, but the relative ratios showed no difference between the two sources (Table 3). Therefore, synergistic pheromonal activities of the hydrocarbons with the aldehydes in the yellow peach moth probably come from female body wax hydrocarbons. Additionally, the same hydrocarbons with a quite different relative ratio and a much

smaller total amount than female body wax extracts were also found in male body wax extracts, which contained ca. 7.4 ng/male equivalent of Z9-27: HC (Table 3). This amount was between those of Z9-27: HC in mixture of all monoenes at the dose of 5 ng which showed no synergism with the aldehyde blend, and the dose of 50 ng which showed the synergism (Fig. 2). Such amount of Z9-27: HC in male body wax was either inadequate to elicit the synergism with the aldehyde blend, or the synergism was covered by unknown factors, which resulted in no synergism found in male body wax extracts (Xiao and Honda, 2010).

Most lepidopteran pheromones are known to consist of multi-components that are classified into the Type I group including alcohols, acetates and aldehydes, the Type II group with unsaturated hydrocarbons or their epoxy derivatives, and miscellaneous pheromone components that are classified as neither Type I nor Type II (Ando et al., 2004). Meanwhile, both Type I and Type II compounds are also used together as the sex pheromones of several species (Hill and Roelofs, 1981; Hill et al., 1982; Cabrera et al., 2001; Leal et al., 2005; Millar et al., 2005; Gibb et al., 2007). Compounds in both the Type I and Type II groups of lepidopteran sex pheromones have been identified in pheromone extracts obtained by extracting the female's abdominal tips containing the sex pheromone glands.

292 On the other hand, it is well known that some monounsaturated hydrocarbons
293 originating from the body wax serve as contact sex pheromones in Diptera, Coleoptera,
294 and Hymenoptera (Uebel et al., 1975; Zhang et al., 2003; Mant et al., 2005; Ginzel et al.,
295 2006; Böröczky et al., 2009). However, in Lepidoptera, the pheromonal activity of
296 cuticular monoenyl hydrocarbons has rarely been studied. In the swallowtail
297 butterfly *Papilio polytes*, (*Z*)-7-tricosene, (*Z*)-7-pentacosene and (*Z*)-7-heptacosene were
298 identified in body waxes of both male and female adults. However, the pheromonal
299 activity of these compounds has not been investigated (Omura and Honda, 2005).
300 In the yellow peach moth, Z9-23: HC, Z9-25: HC, Z9-27: HC, Z9-29: HC and Z9-31:
301 HC were identified from female body wax extracts as well as pheromone gland extracts,
302 and at least Z9-27: HC was confirmed to have synergism with the aldehyde pheromone
303 components, E10-16: Ald and Z10-16: Ald. A similar phenomenon may also occur
304 widely in lepidopteran species. Especially in species that use both Type I and Type II
305 components as sex pheromones, unsaturated hydrocarbons (Type II) may be produced
306 not only in the pheromone gland but also from the body surface.
307 Increased activity was observed when each of the monoenyl hydrocarbons (tested in 50
308 ng) was mixed with the aldehyde blend. However, a remarkable increase was found in
309 the mixture of Z9-27: HC and the aldehyde blend when compared with the aldehyde

310 blend alone. Moreover, a mixture of all monoenyl hydrocarbons (total amount: 50 ng)
311 including Z9-27: HC and the remaining four monoenes also elicited synergism as high
312 as Z9-27: HC (Fig. 1). Together with the fact that the actual amount of Z9-27: HC in
313 the mixture of all monoenes was less than 50 ng, these results suggest that the
314 monoenes other than Z9-27: HC may also contribute to synergism in this species.
315 A mixture of monoenes was tested for a dose-response relationship and the dose range
316 of synergism was greater than 5 ng (Fig. 2). Considering that the natural amount of
317 those monoene hydrocarbons in body wax extracts is ca. 900 ng per one female (Table
318 3), we can calculate that the synergistic activity of monoene hydrocarbons with an
319 aldehyde blend may be lost after a 180 fold dilution of one female equivalent. A 1,000
320 fold dilution of 1 female equivalent of NPF from female body wax extracts showed
321 synergism in a previous report (Xiao and Honda, 2010). These results are not
322 contradictory because the synergism of NPF from female body wax extracts actually
323 represents a total synergism of active compounds in both Frs. 3 and 6.
324 As a summary, in the yellow peach moth, monoenyl hydrocarbons, at least Z9-27: HC,
325 in female body waxes synergistically enhanced the response of male moths to the
326 synthetic sex pheromone, the aldehyde blend. However, such synergism is not high
327 enough unless other synergistic compounds in the Fr. 6 of NPF from female body wax

328 extracts are added. Furthermore, identification of the synergistic monoenyl
329 hydrocarbons as well as other unsaturated hydrocarbons, probably polyenyl
330 hydrocarbons in the Fr. 6, will undoubtedly improve the understanding of the complete
331 sex pheromone system of the yellow peach moth. At present the identification and
332 synthesis of the compounds in the Fr. 6 are underway.

333 The pheromonal activity of cuticular monounsaturated hydrocarbons was demonstrated
334 in laboratory bioassays, but field tests are also needed for confirmation. The findings
335 of this study are expected to improve field performance of sex pheromone lures for the
336 yellow peach moth.

337

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342

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415 *Anoplophora glabripennis* (Coleoptera: Cerambycidae). *Naturwissenschaften.* 90:

410-413.

418 **Figure legends**

419 Fig. 1 Activities of mixtures of synthesized monoenyl hydrocarbons and the aldehyde
420 blend as mean time remaining close to source (open columns) and mean number of
421 source contacts (closed columns) in wind tunnel tests. Values are shown as the mean ±
422 standard error. Numbers in parentheses are replications of different individual insects.
423 For the mixtures, 10 ng of the aldehyde blend was mixed with either 50 ng monoenes or
424 50 ng of a mixture in which all monoenes were mixed at the natural ratio. Values
425 accompanied by the same letter were not significantly different by Tukey's test after
426 ANOVA at $p<0.05$.

427

428 Fig. 2 Dose-response relationship of the synergism of the synthetic monoenyl
429 hydrocarbons mixture to the aldehyde blend in the mean time remaining close to source
430 (open columns) and mean number of source contacts (closed columns) in wind tunnel
431 tests. Values are shown as the mean ± standard error. Numbers in parentheses are
432 replications of different individual insects. For the mixtures, 10 ng of the aldehyde
433 blend was mixed with each dose of a mixture in which all monoenes were mixed at the
434 natural ratio. Values accompanied by the same letter were not significantly different
435 by Tukey's test after ANOVA at $p<0.05$.

436

Tables and figures

Table 1 Synergistic activity in wind tunnel tests with an aldehyde blend of fractions separated by $AgNO_3$-silica gel chromatography from the non-polar fraction of female body wax extracts of *C. punctiferalis*.

Treatment [a,b]	N	Male responses (%) [d]			Mean time remaining close to source (sec) [e]	Mean number of source contact [e]
		Starting flight	Catching plume	Close to source		
Fr. 1	16	100.0 a	100.0 a	87.5 a	26.7± 3.0 e	6.9± 1.1 c
Fr. 2	16	93.8 a	93.8 a	93.8 a	34.8± 3.5 e	11.3± 1.3 c
Fr. 3	20	100.0 a	100.0 a	100.0 a	56.0± 5.6 cd	23.8± 2.7 b
Fr. 4	16	93.8 a	93.8 a	87.5 a	37.8± 4.5 de	12.7± 1.8 c
Fr. 5	15	100.0 a	100.0 a	93.3 a	61.7± 10.6 bc	24.2± 3.9 b
Fr. 6	17	94.1 a	94.1 a	94.1 a	79.4± 7.2 b	31.2± 2.9 b
Fr. 7	13	100.0 a	92.3 a	92.3 a	25.6± 5.9 e	4.1± 1.4 c
NPF(FBW) [c]	17	100.0 a	100.0 a	100.0 a	100.3± 7.8 a	43.7± 3.3 a
Control	27	100.0 a	88.9 a	88.9 a	23.5± 2.2 e	8.3± 1.0 c

[a] Ten ng (1 female equivalent, FE) of the mixture of E10-16: Ald and Z10-16: Ald at a ratio of 95.5: 4.5 was added to all treatments of 1 FE.

[b] Fr. 1 (100% hexane fraction), Fr. 2- 6 (1%, 3%, 10%, 30% and 50% ether in hexane fractions) and Fr. 7 (100% ether fraction) separated by $AgNO_3$-silica gel chromatography from the non-polar fraction of female body wax extracts.

446 [c] One FE of the non-polar fraction of female body wax extracts in Florisil column

447 chromatography.

448 [d] Data with different letters in the same column are significantly different at $p<0.05$ by

449 Ryan's multiple comparisons after Fisher's exact probability test ($P<0.05$).

450 [e] Data with different letters in the same column are significantly different at $p<0.05$ by

451 Tukey's multiple comparisons after ANOVA ($P<0.05$).

452

453 Table 2 Synergistic activity in wind tunnel tests of an aldehyde blend of mixed fractions
454 separated by $AgNO_3$-silica gel chromatography from the non-polar fraction of female
455 body wax extracts of *C. punctiferalis*.

Treatment [a, b]	N	Male responses (%) [d]			Mean time remaining close to source (sec) [e]	Mean number of source contact [e]
		Starting flight	Catching plume	Close to source		
Fr. 3+ Fr. 5	24	87.5 a	87.5 a	87.5 a	53.3± 6.3 b	20.4± 3.0 b
Fr. 5+ Fr. 6	20	90.0 a	90.0 a	90.0 a	64.0± 7.0 b	26.1± 3.4 b
Fr. 3+ Fr. 6	16	100.0 a	100.0 a	100.0 a	112.8± 5.6 a	48.1± 2.6 a
Fr. 3 + Fr. 5+ Fr. 6	16	100.0 a	100.0 a	93.8 a	117.6± 9.8 a	52.7± 5.0 a
Fr. 1+ Fr. 2+ Fr. 4+ Fr. 7	18	100.0 a	83.3 a	83.3 a	24.0± 2.7 e	3.0± 1.2 c
Recombined [c] fractions mixture	15	100.0 a	100.0 a	100.0 a	108.0± 6.4 a	47.3± 3.2 a
Control	28	92.9 a	92.9 a	89.3a	28.6± 2.4 c	8.6± 1.9 c

456 [a] Ten ng (1 female equivalent, FE) of the mixture of E10-16: Ald and Z10-16: Ald at a
457 ratio of 95.5: 4.5 was added to all treatments of 1 FE.

458 [b] Fr. 1 (100% hexane fraction), Fr. 2- 6 (1%, 3%, 10%, 30% and 50% ether in hexane
459 fractions) and Fr. 7 (100% ether fraction) separated by $AgNO_3$-silica gel
460 chromatography from the non-polar fraction of female body wax extracts.

461 [c] A mixture of 1 FE each of Fr. 1- Fr. 7 separated by $AgNO_3$-silica gel chromatography
462 from the non-polar fraction of female body wax extract were tested.

[d] Data with different letters in the same column are significantly different at $p<0.05$ by Ryan's multiple comparisons after Fisher's exact probability test ($P<0.05$).

[e] Data with different letters in the same column are significantly different at $p<0.05$ by Tukey's multiple comparisons after ANOVA ($P<0.05$).

468 Table 3 The percentage of each (*Z*)-9-monoene area in all dominant (*Z*)-9-monoenes
469 areas in the GC profile and the amount of each (*Z*)-9-monoenes in the 3% ether in
470 hexane fraction separated by $AgNO_3$-silica gel chromatography from the non-polar
471 fraction of female body wax extracts, pheromone gland extracts and male body wax
472 extracts of *C. punctiferalis*.

Monoenes	Ratio (%) [a]			Amount (ng) [a, b]		
	FBW [c]	PG [d]	MBW [e]	FBW [c]	PG [d]	MBW [e]
Z9-23: HC	2.0± 0.1	3.7± 0.4	14.0± 0.3	16.2± 1.1	1.4± 0.1	3.2± 0.2
Z9-25: HC	7.5± 0.1	7.9± 0.2	24.3± 0.2	50.0± 4.3	2.4± 0.2	5.1± 0.4
Z9-27: HC	29.6± 0.1	27.2± 0.4	24.9± 0.5	272.0± 27.3	10.3± 0.8	7.4± 0.7
Z9-29: HC	51.5± 0.7	50.7± 0.6	26.4± 0.8	470.3± 48.7	18.7± 1.5	7.8± 0.8
Z9-31: HC	9.5± 0.1	10.6± 0.4	10.4± 0.5	90.5± 10.0	4.3± 0.3	3.5± 0.3
Total	-	-	-	899.0± 91.3	37.0± 2.8	27.0± 2.2

473 [a] Values are shown as the mean± standard deviation.

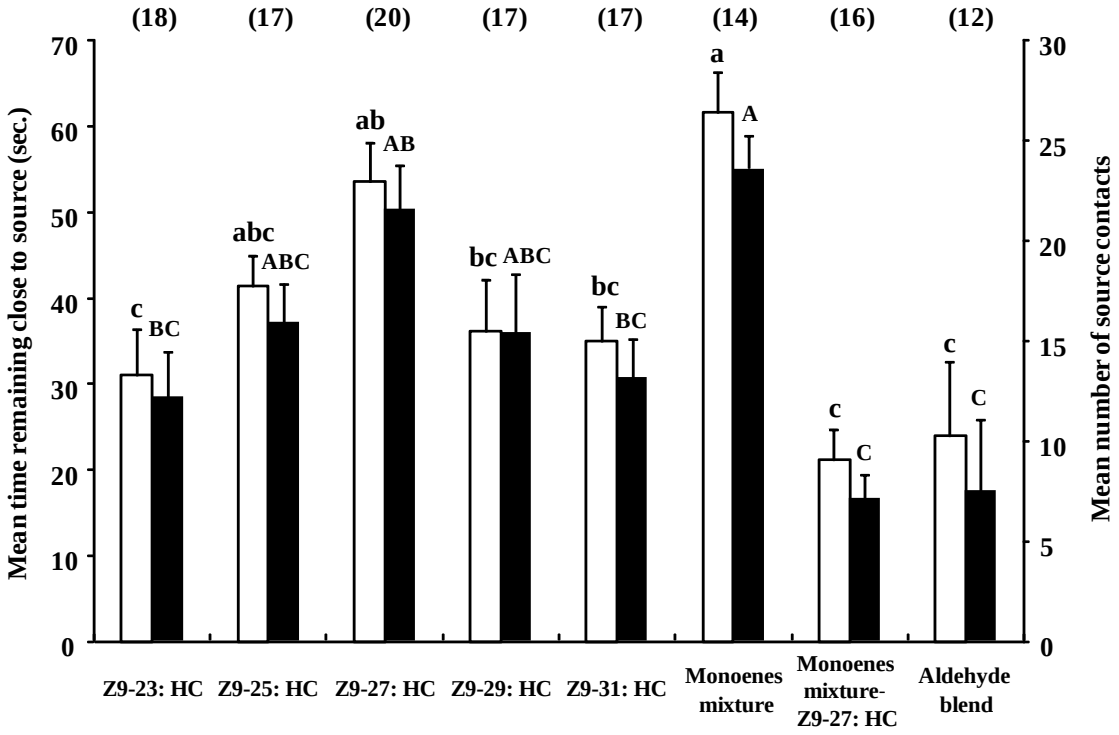
474 [b] Monoenyl hydrocarbons in one female equivalent of female body wax extracts and
475 pheromone gland extracts, and one male equivalent of male body wax extracts were
476 calculated.

477 [c] FBW: Female body wax extracts.

478 [d] PG: Pheromone gland extracts.

479 [e] MBW: Male body wax extracts.

480


(18)
(17)
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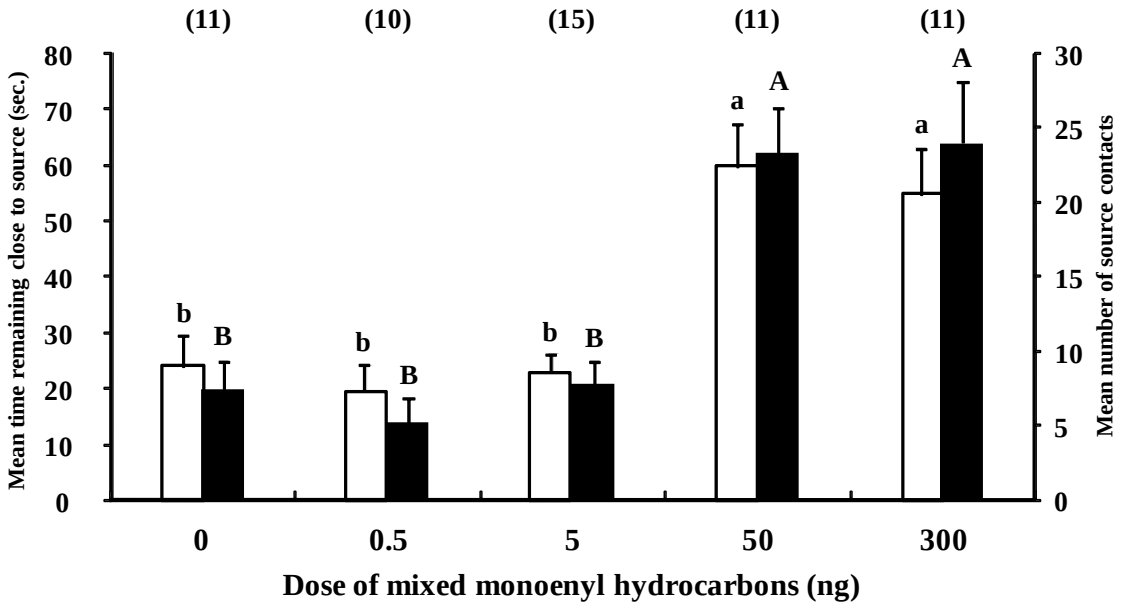
(17)
(14)
(16)
(12)
Mean time remaining close to source (sec.)
Mean number of source contacts
c
BC
abc
ABC
ab
AB
bc ABC
bc
BC
a
A
c
C
c
C
Z9-23: HC
Z9-25: HC
Z9-27: HC
Z9-29: HC
Z9-31: HC
Monoenes mixture
Monoenes mixture-Z9-27: HC
Aldehyde blend
Mixture of monoenyl hydrocarbon (s) and the aldehyde blend

481

482 **Fig. 1**


(11)
(10)
(15)
(11)
(11)
Mean time remaining close to source (sec.)
Mean number of source contacts
0
10
20
30
40
50
60
70
80
0
5
10
15
20
25
30
b
B
b
B
b
B
a
A
a
A
0
0.5
5
50
300
Dose of mixed monoenyl hydrocarbons (ng)

483

484 **Fig. 2**