Identification of (Z)-9-heptacosene and (3Z,6Z,9Z)-tricosatriene as new sex pheromone components of Korean Conogethes punctiferalis (Lepidoptera: Crambidae) population and field attraction testing

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Research Article

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Abstract

Recently, reduced attractiveness of *Conogethes punctiferalis* adult males to sex pheromone, (*E*)-10-hexadecenal and (*Z*)-10-hexadecenal, has been reported. To identify the other essential components of sex pheromone, male and female body extracts were analyzed. Two hydrocarbon components, (*Z*)-9-heptacosene (*Z*9-27:HC) and (3*Z*,6*Z*,9*Z*)-tricosatriene (*Z*3,*Z*6,*Z*9-23:HC), were identified from only female body extract. There was a significant difference in the electroantennogram (EAG) response of male antennae to *Z*3,*Z*6,*Z*9-23:HC and *Z*9-27:HC at all test concentrations compared to response to the hexane control. In field attraction testing, the addition of *Z*9-27:HC and *Z*3,*Z*6,*Z*9-23:HC to binary aldehyde pheromones significantly increased trap catches of *C. punctiferalis* male adults. Based on the female and male body extract analysis and field attraction test, *Z*-9-27:HC and *Z*3,*Z*6,*Z*9-23:HC were determined to be other essential sex pheromone components of the Korean *C. punctiferalis* population.

Introduction

Chestnut (*Castanea crenata*) is one of major forest products in Korea, with a cultivated area of about 20,000 ha, producing 51,000 tons annually, amounting to exports of more than $24 million/year (Korea Forest Service 2018). However, chestnut production continues to decline because of climate change, a shortage of the agricultural working population, and damage by insect pests (Korea Forest Service 2017). Among the insect pests of the chestnut, the yellow peach moth, *Conogethes punctiferalis*, has caused the most serious damage to chestnuts. The annual damage is estimated to be $30–50 million (Jeong et al. 2020). The yellow peach moth is a polyphagous pest that damages major fruit trees in Korea, such as peach, chestnut, walnut, and apple trees (Konno et al. 1982; Jeong et al. 2020). Yellow peach moths spend most of their larval stage inside of the fruit, so it is essential to monitor the exact flight phenology of yellow peach moths to gain information on the best timing of insecticide application. Pheromones have long been considered as one of the most efficient tools for the monitoring the flight phenology of insect pests. Since Konno et al. (1982) identified (*E*)-10-hexadecenal (*E*10-16:Ald) and (*Z*)-10-hexadecenal (*Z*10-16:Ald) as sex pheromone components of yellow peach moths, pheromone traps have been used to monitor the flight phenology of yellow peach moths in Korea and Japan over the past several decades (Kim et al. 2013; Kondo et al. 2008). However, low attraction of yellow peach moth male adults to traps baited with *E*10-16:Ald and *Z*10-16:Ald has been reported in prior studies, suggesting that other components are indispensable (Kondo et al. 2008; Xiao and Honda 2010). To determine essential components as sex pheromones, Xiao et al. (2011, 2012) analyzed female body extract of yellow peach moths, and monoenyl hydrocarbons such as (*Z*)-9-tricosene (*Z*9-23:HC), (*Z*)-9-pentacosene (*Z*9-25:HC), (*Z*)-9-heptacosene (*Z*9-27:HC), (*Z*)-9-nonacosene (*Z*9-29:HC), (*Z*)-9-hentriacontene (*Z*9-31:HC) and trienyl hydrocarbons such as (*Z*3,*Z*6,*Z*9)-tricosatriene (*Z*3,*Z*6,*Z*9-23:HC), (*Z*3,*Z*6,*Z*9)-pentacosatriene (*Z*3,*Z*6,*Z*9-25:HC), (*Z*3,*Z*6,*Z*9)-heptacosatriene (*Z*3,*Z*6,*Z*9-27:HC), and (*Z*3,*Z*6,*Z*9)-nonacosatriene (*Z*3,*Z*6,*Z*9-29:HC) were identified. Among the identified monoences and triences, the combination of *Z*9-27:HC, *Z*3,*Z*6,*Z*9-23:HC, and binary aldehyde pheromones (*E*10-16:Ald and *Z*10-16:Ald) enhanced male orientation and

remaining time near the source in laboratory wind tunnel tests. However, previous studies (Xiao and Honda 2010; Xiao et al. 2011; Xiao et al. 2012) did not conducted field attraction tests.

Low attraction of the yellow peach moth male adults to binary aldehyde pheromones has also been reported in Korea (Yang 2022). To determine the other essential components of sex pheromone, we analyzed the male and female body extracts of the Korean yellow peach moth population. In addition, field attraction tests of traps baited with several combinations of candidate pheromone components identified in female body extracts of the yellow peach moth were conducted.

Materials And Methods

Insect Chestnut damaged by yellow peach moths were collected from the Eocheon experimental forest (37°16'28"N 126°55'21"E) of the National Forest Research Institute, Hwaseong-si, Republic of Korea. Each mature insect larva was transferred into insects breeding cases (25 mm in lower diameter, 35 mm in upper diameter, and 40 mm in height) and cotton wool soaked with distilled water was provided every three days. They were kept at 26 ± 1°C and a relative humidity of 60 ± 5% under a 16:8 h light/dark cycle.

Whole body extraction Newly emerged 2–4 days old virgin female and male adults were anesthetized with CO_2 at 3–6 h into scotophase and the whole bodies of male and female adults were extracted with n-hexane for 15 minutes. Whole body extracts were filtered with a PTFE syringe (0.2 mm, 25 mm, CHMLAB, Barcelona, Spain) and the solvent was removed using a gentle flow of nitrogen gas. Concentrated body extracts were stored at -80 ℃ before analysis. A total of 220 female and 196 male adults were extracted.

Gas chromatography-mass spectrometer Whole body extracts of the males and females were diluted with 50 µL n-hexane, and analyzed by a gas chromatography-mass spectrometer (GC-MS; GC 7890B, MS 5977B, Agilent Technologies, Santa Clara, CA, USA). In the GC-MS, a HP-5MS column (30 m × 0.25 mm × 1 mm film thickness; Agilent Technologies) was used. The initial oven temperature was 60°C and it was increased at a rate of 15°C/min to 150°C and maintained for 5 minutes, followed by increasing to 300°C at 5°C/min, and maintaining for 15 minutes. The inlet temperature was 250 ℃ and samples were injected under split-less conditions. Helium gas was used as the carrier gas, and the flow rate was 1.0 mL/min.

Chemicals The chemicals used in this study are shown in Table 1. The chemicals were purchased from Daejung Chemicals (Siheung-si, Republic of Korea), Sigma-Aldrich (Milwaukee, WI, USA), Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), Samchun Chemicals (Seoul, Republic of Korea), and MP Biomedicals (Illkirch, France). (*E*)-10-Hexadecenal, (*Z*)-10-hexadecenal, *Z*-9-27:HC, and *Z*3,*Z*6,*Z*9-23:HC were synthesized in the laboratory as described below. ^{1}H NMR (at 400 MHz) and ^{13}C NMR (at 151 MHz) spectroscopic data were recorded on an Advance 400 MHz spectrometer (Bruker, Germany) in $CDCl_3$.

Table 1
Chemicals used in this study

Chemicals	Purity (%)	Source
n-Hexane	> 98.5%	Daejung Chemicals
1-Heptyne	98	Sigma-Aldrich
n-BuLi	2.5M in hexane	Sigma-Aldrich
1,9-Nonanediol	98	Sigma-Aldrich
3,4-Dihydro-2*H*-pyran	97	Sigma-Aldrich
$LiAlH_4$ (Lithium aluminum tetrahydride)	95	Sigma-Aldrich
Bromine	99.5	Sigma-Aldrich
$Pd/BaSO_4$	5% Pd basis	Sigma-Aldrich
Quinoline	98	Sigma-Aldrich
Pyridinium chlorochromate	98	Sigma-Aldrich
(*Z*)-Docos-13-enoic acid	90	MP Biomedicals
n-Pentylmagnesium bromide	2 M in diethyl ether	Sigma-Aldrich
Hexamethylphosphorus triamide	97	Sigma-Aldrich
(*Z,Z,Z*)-9,12,15-Octadecatrienoic acid	99	Sigma-Aldrich
Methanesulfonyl chloride	99.7	Sigma-Aldrich
p-Toluenesulfonyl chloride	99	Sigma-Aldrich
Triethylamine	99	Sigma-Aldrich
HCl	35–37	Samchun Chemicals
H_2SO_4	95	Samchun Chemicals
p-Toluenesulfonic acid	98	Sigma-Aldrich
NH_4Cl	99.5	Sigma-Aldrich
$NaHCO_3$	99.7	Sigma-Aldrich
$MgSO_4$	97	Sigma-Aldrich
Silicagel	40–63 mesh	Sigma-Aldrich
Florisil	100–200 mesh	Sigma-Aldrich
Tetrahydrofuran (THF)	97	TCI

Chemicals	Purity (%)	Source
Methylene chloride (DCM)	99	TCI
Ethyl acetate	98	Samchun Chemicals
Hexane	98	Samchun Chemicals
MeOH	99	Samchun Chemicals
EtOH	99	Samchun Chemicals

Table 2

The values L^*, a^*, and b^* values of the color bucket traps determined by chromometer analysis

Trap color	L^*[1]	a^*[2]	b^*[3]
Red	41.2577	46.7066	21.4851
Yellow	59.2927	6.8836	45.8015
Green	33.4178	-11.2822	0.4692
Black	26.0313	1.4991	-4.2059
White	94.8931	-1.092	-1.8659
[1] L^* reveals a measure of lightness; black (0) to white (100).			
[2] a^* reveals a red shade when greater than zero (+) and a green shade when lower than zero (-).			
[3] b* reveals a yellow shade when greater than zero (+) and a blue shade when lower than zero (-).			

Synthesis of aldehyde pheromones, (E **)-10-hexadecenal and (** Z **)-10-hexadecenal** The synthetic scheme of (*E*)-10-hexadecenal and (*Z*)-10-hexadecenal is shown in Fig. 1. ^{1}H and ^{13}C NMR spectra of (*E*)-10-hexadecenal and (*Z*)-10-hexadecenal are provided as supplementary data (Fig. S1 and S2).

1) Tetrahydropyranyloxy-hexadeca-10-yn (6) A solution of 1-heptyne (**8**) (5.0 g, 52.0 mmol) in dry tetrahydrofuran (THF, 100 mL) was added to n-BuLi (21.0 mL, 2.5 M in hexanes) at -78 ℃ under a nitrogen (N_2) atmosphere. After stirring at -78 ℃ for 1 hr, 1-bromo-9-tetrahydropyranyl nonane (**7**) (15.0 g, 48.8 mmol) dissolved in THF (20 mL) was added dropwise to the mixture at -78 ℃. The reaction mixture was allowed to warm to room temperature for 6 hr. The resulting mixture was quenched by a saturated NH_4Cl solution (10.0 mL) and THF solvent was evaporated using a rotary evaporator (N-1300 V-WB; Eyela Pte Ltb., Singapore). The residue was diluted with ethyl acetate (EtOAc, 100 mL), washed with water, dried, and concentrated using a rotary evaporator. The resulting oil was purified by flash chromatography (hexane/EtOAc, 90:10) yielding 8.50 g of tetrahydropyranyloxy-hexadeca-10-yn (54%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.55–4.53 (*m*, 1H), 3.87–3.85 (*m*, 1H), 3.83–3.81 (*m*, 1H), 3.73–3.45 (*m*, 1H), 3.38–3.32 (*m*, 1H), 2.13–2.09 (*m*, 4H), 1.83–1.71(*m*, 2H), 1.69–1.27 (*m*, 24H), 0.87 (*t*, 3H).

2) Hexadeca-10-yn-1-ol (5) *p*-Toluenesulfonic acid (*p*-TsOH, 0.6 g, 3.5 mmol) was added to a solution of 1-tetrahydropyranyl hexadeca-10-yn (**6**) (3.0 g, 9.3 mmol) in MeOH (30 mL), and the mixture was stirred for 6 hr at room temperature. MeOH solvent was evaporated using a rotary evaporator and the resulting oil was purified by flash chromatography (hexane/EtOAc, 70:30) yielding 1.9 g of hexadeca-10-yn-1-ol (86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.63–3.06 (*t*, 2H), 2.13–2.09 (*m*, 4H), 1.56–1.28 (*m*, 3H), 0.87 (*t*, 3H).

3) (E **)-10-Hexadecen-1-ol (4)** Hexadeca-10-yn-1-ol (**5**) (1,0 g, 4.2 mmol) in THF (5 mL) was added to a stirred solution of $LiAlH_4$ (160 mg, 4.2 mmol) in dry THF (20 mL). The reaction mixture was allowed to warm to reflux for 3 hr. The resulting mixture was quenched by a saturated NH_4Cl solution (10.0 mL) and THF solvent was evaporated in vacuo. The residue was diluted with ethyl acetate (EtOAc, 50 mL), washed with water, dried, and concentrated. The resulting oil was purified by flash chromatography (hexane/EtOAc, 70:30) yielding 0.70 g of (*E*)-10-hexadecen-1-ol (70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.36–5.35 (m, 2H), 3.64–3.60 (t, 2H), 1.95–1.93 (m, 4H), 1.56–1.52 (m, 4H), 1.40–1.20 (m, 17H), 0.86 (t, 3H).

4) (Z **)-10- Hexadecen-1-ol (3)** Quinoline (2 drops) and 5% Pd/$BaSO_4$ (50 mg) was added to a stirred solution of hexadeca-10-yn-1-ol (**5**) (0.8 g, 3.4 mmol) in EtOAc (20 mL). The mixture was stirred under a hydrogen atmosphere for 2 hr and filtered on a Florisil pad to remove the catalyst. EtOAc was evaporated in vacuo. The resulting oil was purified by flash chromatography (hexane/EtOAc, 70:30) yielding 0.70 g of (*Z*)-10- hexadecen-1-ol (87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.38–5.32 (m, 2H), 3.64–3.60 (t, 2H), 2.01–1.93 (m, 4H), 1.57–1.53 (m, 4H), 1.40–1.20 (m, 17H), 0.86 (t, 3H).

5) (E **)-10-Hexadecenal (2)** Pyridinium chlorochromate (PCC, 0.5 g) in DCM (5 ml) was added to a stirred solution of (*E*)-10-Hexadecen-1-ol (**4**) (0.5 g, 2.1 mmol) in methylene chloride (DCM, 10 mL). The mixture was stirred at room temperature for 5 hr. The reaction mixture was filtered through a Florisil pad. (*E*)-10-Hexadecenal was purified by flash chromatography (hexane/EtOAc, 90:10) yielding 0.36 g (72%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.76(s, 1H), 5.40–5.37 (m, 2H), 2.43–2.40 (m, 2H), 1.99–1.96 (m, 4H), 1.64–1.60 (m, 2H), 1.38–1.25 (m, 16H), 0.89 – 0.87 (m, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 202.96, 130.52, 130.17, 43.92, 32.43, 31.76, 29.71, 29.59, 29.53, 29.49, 29.44, 29.42, 29.38, 29.33, 29.27, 29.10, 27.18, 14.20. The purity of (*E*)-10-hexadecenal was 97.24% (Fig. S5a).

6) (Z **)-10-Hexadecenal (1)** PCC (0.5 g) in DCM (5 mL) was added to a stirred solution of (*Z*)-10-Hexadecen-1-ol (**3**) (0.5 g, 2.1 mmol) in DCM (10 mL). The mixture was stirred at room temperature for 5hr. The reaction mixture was filtered through a Florisil pad. (*Z*)-10-Hexadecenal was purified by flash chromatography (hexane/EtOAc, 90:10) yielding 0.35 g (70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.76(s, 1H), 5.42–5.32 (m, 2H), 2.43–2.40 (m, 2H), 2.05–1.94 (m, 4H), 1.68–1.60 (m, 2H), 1.38–1.25 (m, 16H), 0.94 – 0.88 (m, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 202.96, 130.73, 130.46, 43.92, 34.43, 32.47, 31.76, 29.69, 29.61, 29.57, 29.53, 29.48, 29.44, 29.38, 29.27, 27.18, 14.09. The purity of (*Z*)-10-hexadecenal was 100% (Fig. S5b).

Synthesis of (Z)-9-heptacosene (*Z*)-9-Heptacosene (*Z*9-27:HC) was synthesized according to the methods reported in prior studies (Masuda and Mori 2002; Xiao et al. 2011) and the synthetic scheme of *Z*9-27:HC is shown in Fig. 2. ^{1}H and ^{13}C NMR spectra of *Z*9-27:HC are provided as supplementary data (Fig. S3).

1) (Z)-18-Heptacosen-6-ol (10) n-Pentylmagnesium bromide (16.0 mL, 1.0 M in THF) was added to a solution of (*Z*)-13-docosenal (**11**) (5.0 g, 15.5 mmol) in dry THF (100 mL) at -78 °C under a nitrogen (N_2) atmosphere. The reaction mixture was allowed to warm to room temperature for 6 hr. The resulting mixture was quenched by a saturated NH_4Cl solution (10.0 mL) and THF solvent was evaporated in vacuo. The residue was diluted with ethyl acetate (EtOAc, 100 mL), washed with water, dried, and concentrated. The resulting oil was purified by flash chromatography (hexane/EtOAc, 70:30) yielding 3.10 g of (*Z*)-18-heptacosen-6-ol (51%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.34–5.31 (m, 2H), 3.57 (m, 1H), 2.82–2.70 (m, 4H), 2.01–1.97 (m, 6H), 1.40–1.21 (m ,31H), 0.89 – 0.84 (t, 6H).

2) (Z)-9-Heptacosene (9) Triethylamine (1.2 mL) was added to a solution of (*Z*)-18-heptacosen-6-ol (**10**) (3.0 g, 7.6 mmol) in DCM (30 ml) and then, methanesulfonyl chloride (0.9 g) dissolved in DCM (2 mL) was added to this solution at 0 °C. The reaction mixture was allowed to warm to room temperature for 10 hr. The mixture was poured into water and extracted with DCM. The organic phase was successively washed with 1N HCl, a saturated aqueous $NaHCO_3$ solution, water, and brine, and the solvent was evaporated in vacuo. The residue was dissolved in dry THF (30 mL). $LiAlH_4$ (290 mg) was added to this solution at 0 °C and then, the mixture was allowed to warm to room temperature for 12 hr. The reaction was quenched with water and THF solvent was evaporated in vacuo. The resulting oil was purified by flash chromatography (hexane/EtOAc, 95:5) yielding 1.34 g of (*Z*)-9-heptacosene (47%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.33–5.30 (m, 2H), 2.82–2.70 (m, 4H), 2.00-1.97 (m, 6H), 1.30–1.22 (m, 32H), 0.87 – 0.83 (m, 6H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 130.38, 129.91, 31.95, 31.93, 29.79, 29.72, 29.68, 29.58, 29.54, 29.38, 29.34, 27.22, 22.71, 14.13. The purity of *Z*9-27:HC was 97.24% (Fig. S5d).

Synthesis of (Z,Z,Z)-3,6,9-tricosatriene (*Z,Z,Z*)-3,6,9-Tricosatriene was synthesized according to the methods reported in prior studies (Huang et al. 1983; Kuenen et al. 2010; Wang and Zhang 2007; Xiao et al. 2012) and the synthetic scheme of *Z*3,Z6,Z9-23:HC is shown in Fig. 2. The ^{1}H and ^{13}C NMR spectra of *Z*3,Z6,Z9-23:HC are provided as supplementary data (Fig. S4).

1) (Z,Z,Z)-14,17,20-Tricosatrien-6-ol (13) n-Pentylmagnesium bromide (20.0 mL, 1.0 M in THF) was added to a solution of (*Z,Z,Z*)-9,12,15-Octadecatrienal (**14**) (5.0 g, 19.1 mmol) in dry THF (100 mL) at -78 °C under a nitrogen (N_2) atmosphere. The reaction mixture was allowed to warm to room temperature for 6 hr. The resulting mixture was quenched by a saturated NH_4Cl solution (10.0 mL) and THF solvent was evaporated in vacuo. The residue was diluted with ethyl acetate (EtOAc, 100 mL), washed with water, dried, and concentrated. The resulting oil was purified by flash chromatography (hexane/EtOAc, 70:30) yielding 3.20 g of (*Z,Z,Z*)-14,17,20-tricosatrien-6-ol (50%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.43–5.32 (m, 6H), 3.57 (m, 1H), 2.80–2.79 (m, 4H), 2.06–2.02 (m, 6H), 1.41–1.22 (m, 18H), 0.89 – 0.85(m, 6H).

2) (Z,Z,Z**)-3,6,9-Tricosatriene (12)** Methanesulfonyl chloride (1.1 g) dissolved in DCM (2 mL) was added to a solution of (*Z*,*Z*,*Z*)-14,17,20-tricosatrien-6-ol (**13**) (3.0 g, 9.0 mmol) in DCM (30 ml) and triethylamine (1.3 ml) at 0 ℃, and the reaction mixture was allowed to warm to room temperature for 10 hr. The mixture was poured into water and extracted with DCM. The organic phase was successively washed with 1N HCl, a saturated aqueous $NaHCO_3$ solution, water, and brine, dried with $MgSO_4$, and the solvent was evaporated in vacuo. The residue was dissolved in dry THF (30 mL). $LiAlH_4$ (290 mg) was added to this solution at 0℃ and then, the mixture was allowed to warm to room temperature for 12 hr. The reaction was quenched with water and THF solvent was evaporated in vacuo. The residue was diluted with ethyl acetate (EtOAc, 50 mL), washed with water, dried, and concentrated. The resulting oil was purified by flash chromatography (hexane/EtOAc, 95:5) yielding 1.50 g of (*Z*,*Z*,*Z*)-3,6,9-tricosatriene (52%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.42–5.31 (m, 6H), 2.82–2.80 (t, 4H), 2.11–2.03 (m, 6H), 1.36–1.25 (m, 20H), 0.86 (m, 6H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 131.96, 130.43, 128.56, 128.31, 128.25, 127.62, 127.13, 31.93, 29.70, 29.66, 29.57, 29.43, 29.37,29.34, 29.20, 27.26, 26.18, 26.07, 22.70, 20.56, 14.28, 14.13. The purity of *Z*3,6,9–23:HC was 93.83% (Fig. S5c).

Electroantennogram recordings The antennae of two day old male (*N* = 6) and female (*N* = 6) yellow peach moths were used in the electroantennogram (EAG) analysis. The antennae were cut at the line between the scape and pedicel using anatomical scissors. Glass electrodes filled with a 0.1 M KCl solution were connected to a signal amplifier (Syntech IDAC4, Buchenbach, Germany) with titanium lines (Baretungstenwire, 0.005 in., 0.18 ft; A-M Systems, Washington, USA). The antennae were connected to both ends of the two glass electrodes. Two aldehydes, (*E*)-10-hexadecenal and (*Z*)-10-hexadecenal, and two hydrocarbons, *Z*9-27:HC and *Z*3,6,9–23:HC, were dissolved in hexane and loaded onto paper discs (8 mm; Advantec MF, Inc., Dublin, CA, USA). The solvent was removed for 30 s and then, a treated paper disc was placed into a micro-pipet tip (1 mL, L = 72 mm, I.D.=8 mm; Eppendorf AG, Hamburg, Germany). The head of the pipet tip was connected vertically with a hole in the tube (I.D.=13 mm). The continuous flow rate and pulse flow rate controlled by a Stimulus Controller CS-55 V2 (Syntech, Buchenbach, Germany) were 6 mL/sec and 3.5 mL/sec, respectively. Stimulation of the test compound on each antenna was conducted three times for 1.5 s at 30 s intervals. The width of the glass tube towards the antennae was 13 mm, and a specially designed glass tube was used. The amounts of the test compound used were 0.1, 0.5, and 1 µl. A paper disc treated with only hexane was used as a control. The experiments were conducted in the order of low to high concentrations. Each signal was digitized using signal acquisition (IDAC-4; Ockenfels Syntech CmbH) and recorded by Autospike v.3.9 (Syntech, Buchenbach, Germany). The EAG amplitude values (mV) were analyzed by repeated measures ANOVA and compared by applying Bonferroni's HSD (R studio ver. 3.5.1, R development Core Team, 2013).

Color spectrometry analysis The bucket trap surface color values ($L^*a^*b^*$) were measured using a color spectrophotometer (ColorMate, SCINCO, Seoul, Republic of Korea). L^* reveals a measure of lightness from black (0) to white (100). a^* reveals a red shade when greater than zero (+) and a green shade when lower than zero (-). b^* reveals a yellow shade when greater than zero (+) and a blue shade when lower than zero (-).

Field experiment Pheromones and the same amount of 2,6-di-tert-butyl-4-methyphenol (BHT) as antioxidant dissolved in n-hexane were loaded on rubber septa (Sigma-Aldrich, Milwaukee, WI, USA) and used in the pheromone traps. The trap interval in the same block was approximately 15 m. Each block was 50 m apart. Traps were arranged in a randomized complete block design, counted and relocated every two weeks to minimize the effect of the trap position. The pheromone rubber septa were changed monthly. The numbers of male *C. punctiferalis* caught in the traps (Experiments 1–3) were analyzed by the two-way analysis of variance (ANOVA) and the differences between the mean catches for each treatment were compared by Tukey's HSD test. The numbers of male *C. punctiferalis* caught in the traps (Experiment 4) were analyzed by a *t*-test (R studio ver. 3.5.1).

1) Experiment 1: ratios of (E**)-10-hexadecenal and (**Z**)-10-hexadecenal** The attraction of male *C. punctiferalis* to various ratios of (*E*)-10-hexadecenal and (*Z*)-10-hexadecenal was investigated at a chestnut farm (35°05'51"N 127°40'17"E) in Gwangyang, Republic of Korea from 1 June to 31 October in 2017. The rubber septa were loaded with (*E*)-10-hexadecenal:(*Z*)-10-hexadecenal (0.9 mg:0.1 mg, 0.8 mg:0.2 mg, 0.7 mg:0.3 mg and 0.6 mg:0.4 mg). Control traps received only hexane. White delta traps (Green Agrotec, Gyeonsan, Republic of Korea) were used. The traps were randomly assigned within five replicate blocks (n = 5).

2) Experiment 2: addition of Z**3**,Z**6**,Z**9-23:HC and** Z**9-27:HC to the binary blend** The synergistic effect of *Z*3,*Z*6,*Z*9-23:HC and *Z*9-27:HC identified from *C. punctiferalis*'s female body extracts on the attraction of sex pheromone was investigated at the Eocheon experimental Forest (37°16'28"N 126°55'21"E) of the National Forest Research Institute, Hwaseong-si, Republic of Korea from 1 August to 8 October in 2019. The rubber septa were loaded with (*E*)-10-hexadecenal:(*Z*)-10-hexadecenal (0.9 mg:0.1 mg), (*E*)-10-hexadecenal:(*Z*)-10-hexadecenal:*Z*3,6,9–23:HC (0.9 mg:0.1 mg:1 mg), (*E*)-10-hexadecenal:(*Z*)-10-hexadecenal:*Z*9-27:HC (0.9 mg:0.1 mg:1 mg), (*E*)-10-hexadecenal:(*Z*)-10-hexadecenal:*Z*3,Z6,Z9-23:HC:*Z*9-27:HC (0.9 mg:0.1 mg:1 mg:1 mg), and *Z*3,Z6,Z9-23:HC:*Z*9-27:HC (1 mg:1 mg). The yellow delta traps used in this experiment were purchased from the Korea Institute of Insect Pheromone (KIP, Daejeon, Republic of Korea). The traps were randomly assigned within four replicate blocks (n = 4).

3) Experiment 3: effect of the pheromone dose The effect of the pheromone dose on male capture of *C. punctiferalis* was investigated at the Eocheon experimental Forest from 3 August to 5 October in 2020. First, to investigate the effect of dose of two aldehydes, rubber septa were loaded with (*E*)-10-hexadecenal:(*Z*)-10-hexadecenal:*Z*3,*Z*6,*Z*9-23:HC:*Z*9-27:HC (0.09 mg:0.01 mg:1 mg:1 mg; 0.9 mg:0.1 mg:1 mg:1 mg; 1.8 mg:0.2 mg:1 mg:1 mg; and 2.7 mg:0.3 mg:1 mg:1 mg). Second, rubber septa loaded with (*E*)-10-hexadecenal:(*Z*)-10-hexadecenal:*Z*3,Z6Z,9–23:HC:*Z*9-27:HC (0.9 mg:0.1 mg:0.1 mg:1 mg; 0.9 mg:0.1 mg:1 mg:1 mg; 0.9 mg:0.1 mg:2 mg:1 mg; and 0.9 mg:0.1 mg:3 mg:1 mg) were prepared to determine the effect of the *Z*3,Z6,Z9-23:HC's dose on the capture of male *C. punctiferalis*. Third, rubber septa loaded with (*E*)-10-hexadecenal:(*Z*)-10-hexadecenal:*Z*3,Z6,Z9-23:HC:*Z*9-27:HC (0.9 mg:0.1 mg:1 mg:0.1 mg; 0.9 mg:0.1 mg:1 mg:1 mg; 0.9 mg:0.1 mg:1 mg:2 mg; and 0.9 mg:0.1 mg:1 mg:3 mg) were prepared to determine the effect of the *Z*9-27:HC dose on the capture of male *C. punctiferalis*. Red delta traps (Green Agrotec, Gyeonsan, Republic of Korea) were used. The traps were randomly assigned within

each of four replicate blocks (n = 4). An additional field experiment was conducted in the Eocheon experimental Forest from 2 August to 6 October in 2021, because we could not determine the optimal does of Z9-27:HC. Rubber septa were loaded with (*E*)-10-hexadecenal:(*Z*)-10-hexadecenal:*Z*3,Z6,Z9-23:HC: *Z*9-27:HC (0.9 mg:0.1 mg:1 mg:0 mg; 0.9 mg:0.1 mg:1 mg:1 mg; 0.9 mg:0.1 mg:1 mg:3 mg ; 0.9 mg:0.1 mg:1 mg:5 mg; and 0.9 mg:0.1 mg:1 mg:10 mg). Red delta traps (Green Agrotec, Gyeonsan, Korea) were used and the traps were randomly assigned within each of five replicate blocks (n = 5).

4) Experiment 4: trap designs The efficacies of the delta and bucket traps were investigated in the Eocheon experimental Forest and chestnut farms (36°12'25"N 126°51'26"E), Buyeo County, Republic of Korea from 3 August to 5 October in 2020. Rubber septa were loaded with (*E*)-10-hexadecenal:(*Z*)-10-hexadecenal:*Z*3,Z6,Z9-23:HC:*Z*9-27:HC (0.9 mg:0.1 mg:1 mg:1 mg). Yellow bucket traps and yellow delta traps were purchased from KIP. The traps were randomly assigned within six replicate blocks (n = 6).

5) Experiment 5: trap colors The effect of five different bucket trap colors (black, green, red, yellow, and white) on the capture of *C. punctiferalis* males was investigated in chestnut farms (36°12'25"N 126°51'26"E), Buyeo County, Republic of Korea from 3 August to 5 October in 2020. Rubber septa were loaded with (*E*)-10-hexadecenal:(*Z*)-10-hexadecenal:*Z*3,Z6,Z9-23:HC:*Z*9-27:HC (0.9 mg:0.1 mg:1 mg:1 mg). All of the different color bucket traps were purchased from KIP (Fig. 8a). The traps were randomly assigned within each of four replicate blocks (n = 4). R studio (ver. 1.1.456, R Studio Inc. MA, USA) was used for the linear regression analysis of the effect of trap surface color on trap capture (De Mendiburu, 2014).

Results

Analyses of C. punctiferalis**'s whole body extraction** The gas chromatography-mass spectrometer (GC-MS) analysis of the whole bodies of the yellow peach moth female and male adults indicated the existence of *Z*3,*Z*6,*Z*9-23:HC and *Z*9-27:HC with retention times of 27.874 min and 34.396 min only in female body extracts (Fig. 3a,b). The ratio of *Z*3,*Z*6,*Z*9-23:HC to *Z*9-27:HC was 1:38.7. The major electron ionization (EI) ions of *Z*3,*Z*6,*Z*9-23:HC and *Z*9-27:HC were identical to those of synthetic *Z*3,*Z*6,*Z*9-23:HC and *Z*9-27:HC (Fig. 3c,d). The EI-MS m/z ratios of the *Z*3,*Z*6,*Z*9-23:HC (female body extracts) were: 318(2), 262(5), 163(3), 149(5), 135(8), 121(10), 108(33), 95(34), 79(58), 67(49), 55(84), and 43(100), and (synthetic): 318(2), 262(5), 163(1), 149(3), 135(7), 121(12), 108(33), 95(38), 79(100) 67(69), 55(55), and 43(84). The EI-MS m/z values of the *Z*9-27:HC (female body extracts) were: 378 (9), 208 (2), 167 (6), 125 (32), 111 (55), 97 (90), 83 (80), 69 (78), 57 (100), 43 (89), and 43 (89), and (synthetic): 378 (12), 208 (1), 167 (4), 125 (26), 111 (48), 97 (83), 83 (76), 69 (67), 57 (96), and 43 (100).

Electroantennogram recordings The mean EAG responses of yellow peach moth to (*E*)-10-hexadecenal, (*Z*)-10-hexadecenal, *Z*3,*Z*6,*Z*9-23:HC, and *Z*9-27:HC are shown in Fig. 4. There was a significant difference in the EAG response of male antennae to (*E*)-10-hexadecenal, (*Z*)-10-hexadecenal, *Z*3,*Z*6,*Z*9-23:HC, and *Z*9-27:HC at all test concentrations compared to the response to the hexane control (Bonferroni's HSD, (*E*)-10-hexadecenal, $F_{3,12}$=146.500, $p < 0.0001$; (*Z*)-10-hexadecenal, $F_{3,12}$=36.650, $p < 0.0001$; *Z*3,*Z*6,*Z*9-

23:HC, $F_{3,12}$=14.770, p < 0.001; *Z*9-27:HC, $F_{3,12}$=9.338, p = 0.0018). However, no significant difference was observed in the EAG responses among the concentrations (0.1 mg – 1 mg) of all test compounds.

Color spectrometry analysis The surface color values of bucket traps including *L**, *a**, and *b** are shown in Table. 2. The highest *L**, *a**, and *b** values were obtained from white, red, and yellow bucket traps, respectively.

Field experiments

1) Experiment 1: ratios of (E)-10-hexadecenal and (Z)-10-hexadecenal The numbers of adult male moths caught in traps baited with different ratios of (*E*)-10-hexadecenal and (*Z*)-10-hexadecenal are shown in Fig. 5a. More male adults were attracted to the traps baited with 0.9:0.1 mg of (*E*)-10-hexadecenal to (*Z*)-10-hexadecenal compared to traps baited with other ratios of (*E*)-10-hexadecenal and (*Z*)-10-hexadecenal (Tukey's HSD, $F_{4,16}$=23.629, p < 0.0001). No yellow peach moth male adults were captured in the control traps.

2) Experiment 2: addition of Z3,Z6,Z9-23:HC and Z9-27:HC to the binary blend The numbers of adult male moths caught in traps baited with different combinations of binary pheromones, *Z*3,*Z*6,*Z*9-23:HC, and *Z*9-27:HC, are shown in Fig. 5b. More male adults were attracted to traps baited with binary pheromones (1 mg) + *Z*3,*Z*6,*Z*9-23:HC (1 mg) + *Z*9-27:HC (1 mg) and binary pheromones (1 mg) + *Z*3,*Z*6,*Z*9-23:HC (1 mg) compared to traps baited with other combinations (Tukey's HSD, $F_{4,12}$=5.819, p < 0.01).

3) Experiment 3: effect of pheromone dose The effect of the binary aldehyde pheromones dose on the male capture is shown in Fig. 6a. Relatively small numbers of *C. punctiferalis* male adults were attracted to the traps baited with 0.1 mg of binary aldehyde pheromones compared to traps baited with 1, 2, and 3 mg of binary aldehyde pheromones (Tukey's HSD, $F_{3,9}$=5.633, p = 0.018). No significant difference was observed in the number of male captures among traps baited with 1, 2, and 3 mg of binary aldehyde pheromones. The effect of the *Z*3,*Z*6,*Z*9-23:HC dose on the male capture is shown in Fig. 6b. No significant difference was observed in the number of male capture among traps baited with 0.1, 1, 2, and 3 mg of *Z*3,*Z*6,*Z*9-23:HC. The effect of the *Z*9-27:HC dose on the male capture is shown in Fig. 6c. Significantly more yellow peach moth male adults were attracted to the traps baited with 3 mg of *Z*9-27:HC than traps baited with 0.1, 1, and 2 mg of *Z*9-27:HC (Tukey's HSD, $F_{3,12}$=6.688, p < 0.01). In an additional field experiment (Fig. 6d), significantly more yellow peach moth male adults were attracted to the traps baited with 10 mg of *Z*9-27:HC compared to traps baited with 3 mg of *Z*9-27:HC (Tukey's HSD, $F_{2,8}$=4.654, p = 0.046).

4) Experiment 4: trap design The effect of the trap design on the male capture is shown in Fig. 7. No significant difference was observed in the number of male captures between the bucket trap and delta trap [t = 0.311, p = 0.766, Buyeo County (a); t = 0.870, p = 0.416, Hwaseong-si (b)].

5) Experiment 5: trap colors The linear regression analysis results of the effect of the trap surface color values (*L**, *a**, and *b**) on the capture of yellow peach moth male adults are shown in Fig. 8b-d. A positive

relationship was evident between the *b** value and trap capture (R^2 = 0.8835, p = 0.004). No relationship was found between the other values (*L** and *a**) and trap capture.

Discussion

The yellow peach moth is the main pest of fruits such as chestnuts, walnuts, plums, apples, and peaches in Asian countries such as China, Korea, Japan, and India (Du et al. 2016; Lee and Chung 1997; Konno et al. 1982; Stanley et al. 2018). It is very difficult to control yellow peach moths because they spend most of their lives inside of fruit (Stanley et al. 2018). To improve the control effect, the exact monitoring of adult occurrence is essential for optimal timing application of insecticides (Jeong et al. 2020). Sex pheromone has been considered as the most effective monitoring method for the flight phenology of insect pests (Leonhardt et al. 1990).

Sex pheromone has been widely used for monitoring of yellow peach moths in Japan and Korea (Konno et al. 1982, Kim et al. 2013), since (*E*)-10-hexadecenal and (*Z*)-10-hexadecenal were identified as sex pheromone components (Konno et al. 1982). However, weak attractiveness of pheromone traps baited with binary aldehyde pheromones has been reported in Japan (Kondo et al. 2008) and Republic of Korea (Yang 2022), indicating the existence of other essential components (Kimura 2002).

Xiao and Honda (2010) reported that when *C. punctiferalis* female body wax extracts were added to binary aldehyde sex pheromones, the time of remaining close to the source and the number of source contact of male *C. punctiferalis* was increased in a wind tunnel experiment. They analyzed female and male body-wax extracts, and several monoenes (*Z*9-23:HC, *Z*9-25:HC, *Z*9-27:HC, *Z*9-29:HC, and *Z*9-31:HC) and trienes (*Z*3,*Z*6,*Z*9-23:HC, *Z*3,*Z*6,*Z*9-25:HC, *Z*3,*Z*6,*Z*9-27:HC, and *Z*3,*Z*6,*Z*9-29:HC) were identified as main components (Xiao et al. 2011, 2012). However, there are differences of the chemical compositions of body wax extracts between Japanese and Korean yellow peach moth populations. Only *Z*9-27:HC and *Z*3,*Z*6,*Z*9-23:HC were identified from female body extracts of the Korean population, whereas these two hydrocarbons were not detected in male body wax extracts of the Korean population. Furthermore, there was a difference of the ratios of *Z*9-27:HC to *Z*3,*Z*6,*Z*9-23:HC from female body wax extracts between the Japanese and Korean populations. The ratios of *Z*3,*Z*6,*Z*9-23:HC to *Z*9-27:HC in Japanese and Korean populations were 1:113.3 and 1:38.7, respectively.

To investigate the effect of *Z*9-27:HC and *Z*3,*Z*6*Z*,9–23:HC on Korean *C. punctiferalis* male adults, we measured the EAG response of male antennae to *Z*9-27:HC, *Z*3,*Z*6,*Z*9-23:HC, and two aldehyde sex pheromones. Our study indicated that all test compounds elicited a strong EAG response of Korean *C. punctiferalis* male adults compared to the control. However, (*E*)-10-hexadecenal and (*Z*)-10-hexadecenal elicited 3 times stronger responses of *C. punctiferalis* male antennae than *Z*3,*Z*6,*Z*9-23:HC and *Z*9-27:HC, even though *Z*3,*Z*6,*Z*9-23:HC and *Z*9-27:HC were found to be other essential components of *C. punctiferalis* sex pheromones in a field test. This result implies the different role of the binary aldehyde compounds and two hydrocarbons. Chakravarthy et al. (2015) argued that (*E*)-10-hexadecenal and (*Z*)-10-hexadecenal might play a role as long-range attraction substances that distant males could use to

detect mating partners. They also suggested that two female body wax extracts, *Z*3,*Z*6,*Z*9-23:HC and *Z*9-27:HC, might be short-range attraction substances that, after attraction due to the two recognized binary aldehyde sex pheromones, males finally identify female adults before mating. The low volatility of two hydrocarbons with long carbon chains compared to aldehyde pheromones supports this assumption.

In the wind tunnel test, the addition of *Z*3,*Z*6,*Z*9-23:HC and *Z*9-27:HC to binary aldehyde sex pheromones significantly increased the remaining time close to source and the number of source contact of male *C. punctiferalis* (Xiao et al. 2012). However, it remains unclear if the addition of these two hydrocarbons enhances the activity of binary aldehyde sex pheromones in field conditions. The addition of *Z*3,*Z*6,*Z*9-23:HC and *Z*9-27:HC to the binary blend showed that few *C. punctiferalis* male adults were attracted to traps baited with only binary pheromones (1 mg), *Z*3,*Z*6,*Z*9-23:HC (1 mg) + *Z*9-27:HC (1 mg), binary pheromones (1 mg) + *Z*9-27:HC (1 mg) but were highly attracted to traps baited with binary pheromones (1 mg) + *Z*3,*Z*6,*Z*9-23:HC (1 mg), and binary pheromones (1 mg) + *Z*3,*Z*6,*Z*9-23:HC (1 mg) + *Z*9-27:HC (1 mg). This result confirmed that *Z*3,*Z*6,*Z*9-23:HC is another essential component of *C. punctiferalis* sex pheromone. In addition, *Z*3,*Z*6,*Z*9-23:HC was found only in female body extracts of the Korean *C. punctiferalis* population. Based on these results, we confirmed that *Z*3,*Z*6,*Z*9-23:HC is a new sex pheromone component of Korean *C. punctiferalis*.

A field attraction test of pheromone does showed that traps baited with 0.1 mg of binary aldehyde pheromones [(*E*)-10-hexadecenal (0.09 mg)+(*Z*)-10-hexadecenal (0.01 mg)] were not attractive to *C. punciferalis* males compared to traps baited with 1, 2, and 3 mg of binary aldehyde pheromones. No significant difference was observed in the number of males captured in traps baited with 1, 2, and 3 mg of binary aldehyde pheromones. In the case of *Z*3,*Z*6,*Z*9-23:HC, no significant difference was observed in the number of males captured among traps baited with 0.1, 1, 2, and 3 mg. However, significantly more *C. punctiferalis* male adults were attracted to the traps baited with 3 mg of *Z*9-27:HC than traps baited with 0.1, 1, and 2 mg of *Z*9-27:HC in a 2020 field test. We further investigated the effect of a Z9-27:HC dose over 3 mg on male capture in 2021, where traps baited with 10 mg of *Z*9-27:HC attracted more *C. punctiferalis* male adults than traps baited with 3 mg of *Z*9-27:HC. This result indicates that *Z*9-27:HC is another essential components of *C. punctiferalis* sex pheromone. However, a high amount of *Z*9-27:HC is required to act as a sex pheromone component. This could be well explained from the results of the female body extracts analysis. The amount of *Z*9-27:HC was 38.7 times more higher than *Z*3,*Z*6,*Z*9-23:HC in female body extracts. Based on the results of the female body extract analysis and field attraction test, we confirmed that *Z*3,*Z*6,*Z*9-23:HC and *Z*9-27:HC are new sex pheromones components of Korean *C. punctiferalis.*

The trap design has an greatly affected the trap capture of many insect species (Kim and Park 2013; Kwon et al. 2021; Malo et al. 2001). Kim et al. (2013) compared the effect of various trap design such as delta, bucket, and wing traps on trap capture of yellow peach moth male adults. They reported that delta traps were superior to bucket and wing traps for capturing *C. punctiferalis* males. However, no significant difference was observed in the number of male captures between bucket and delta traps in this study. The difference of the effect of trap design on trap capture between previous work and this study might be

attributed to the different pheromone components used. The previous study used only two binary aldehyde pheromones whereas two aldehydes and two hydrocarbons were used in this study.

Several studies have shown the effect of trap color on traps capture for many insect species (Gadi and Reddy 2014; Judd and Eby 2014; Abuaglala et al. 2012). Ren and Guo (2015) investigated the effect of the trap surface color value on trap capture of Chinese yellow peach moths. They reported that white, blue, and green traps with lower *b** values were superior to yellow traps with a high *b** value. However, our study showed the opposite result where there was a strong relationship between *b** and trap capture in the Korean yellow peach moth population. These results indicate that Korean and Chinese yellow peach moth might use difference visual cues to search for female adults.

In this study, *Z*3,*Z*6,*Z*9-23:HC and *Z*9-27:HC were determined as new sex pheromones components of Korean *C. punctiferalis* population based on the body extract analysis and field attraction test. Using a yellow bucket trap baited with binary pheromones and two hydrocarbons could improve the efficacy of monitoring of *C. punctiferalis.*

Declarations

Availability of data and material Not applicable

Code availability Not applicable

Author Contributions IN and IKP conceived and designed the research; IN performed the experiments. IN, JHP, JWL, and WL conducted field experiments. DHL and WJ synthesized the sex pheromones. IN and DHL prepared the figures. IN and DHL analyzed the data. IN, DHL, and IKP wrote, edited, and reviewed the manuscript. All authors accepted the final version of the manuscript.

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Ethical approval This article does not contain any studies with human participants or vertebrates performed by any of the authors.

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Figures

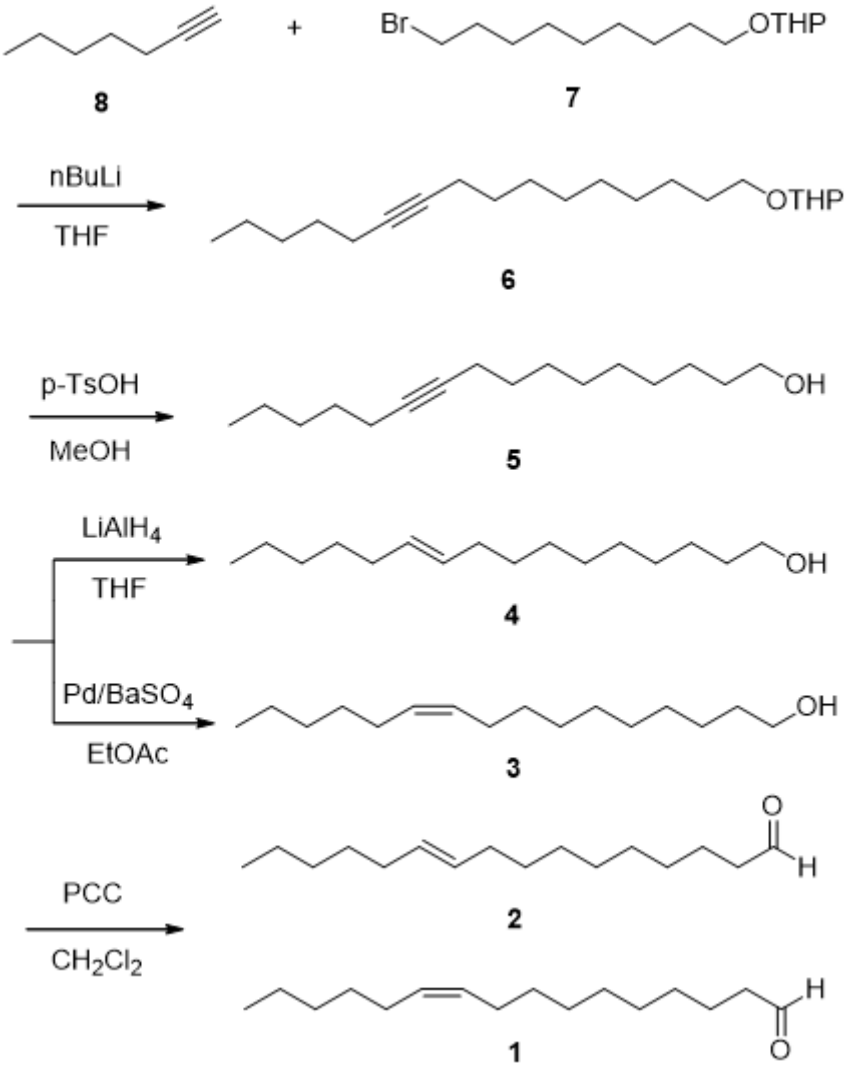

Figure 1

Synthetic scheme of (*E*)-10-hexadecenal (1) and (*Z*)-10-hexadecenal (2).

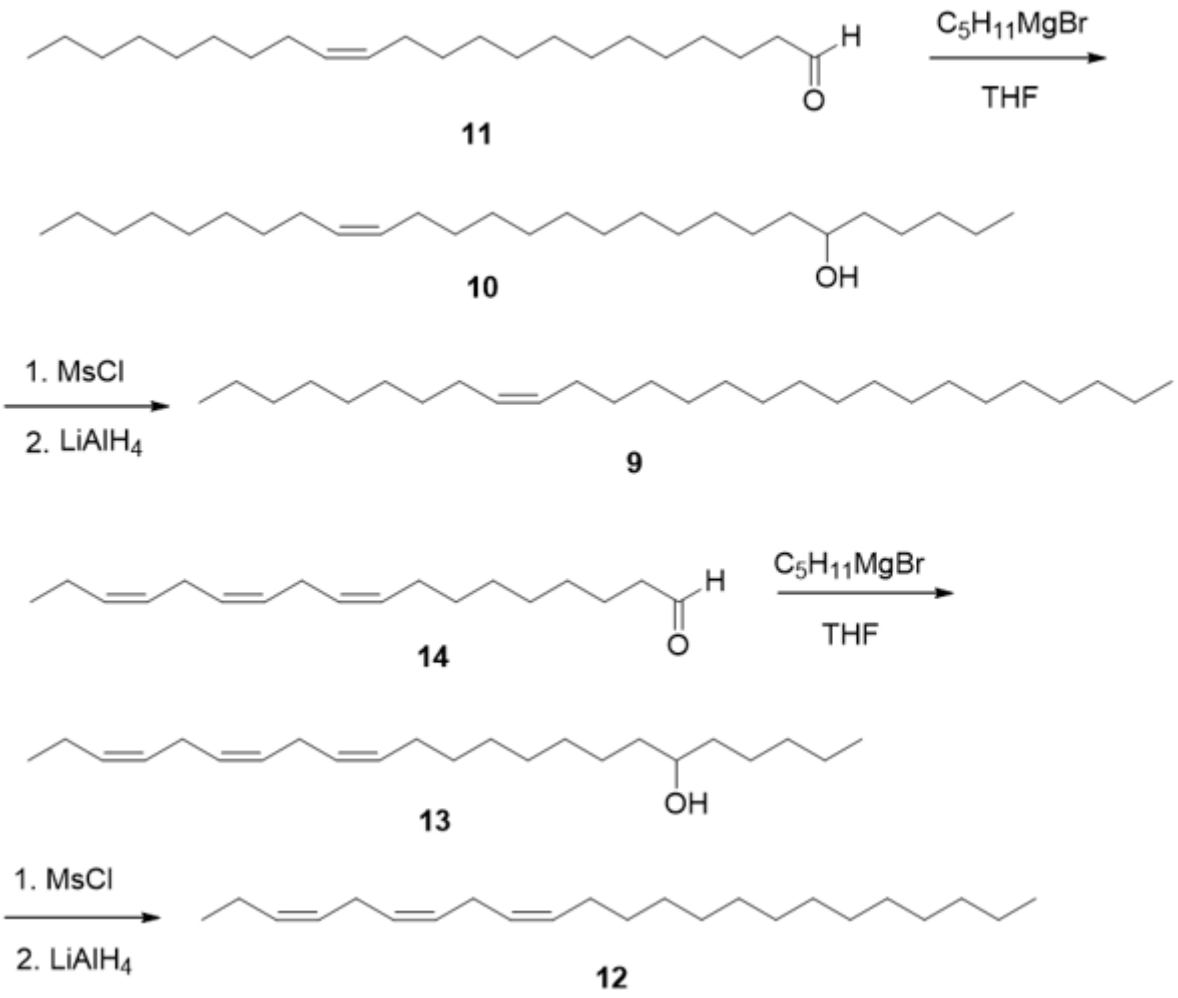

Figure 2

Synthetic scheme of (*Z*)-9-heptacosene (9) and (3*Z*,6*Z*,9*Z*)-tricosatriene (12).

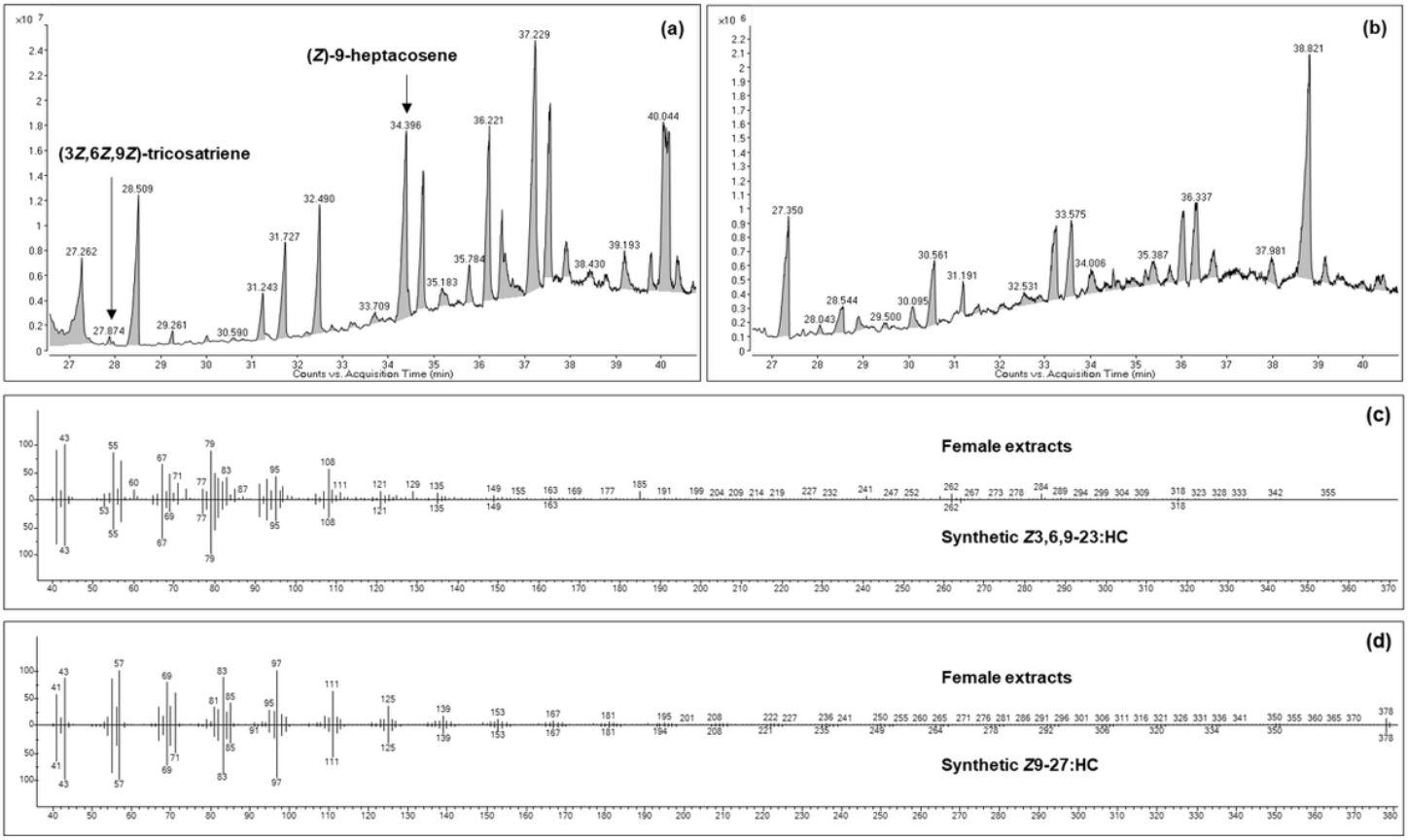

Figure 3

Total ion chromatograms of (a) female and (b) male yellow peach moth's body extracts, and ionization spectra of female-specific compounds and synthetic (c) (3*Z*,6*Z*,9*Z*)-tricosatriene and (d) (*Z*)-9-heptacosene.

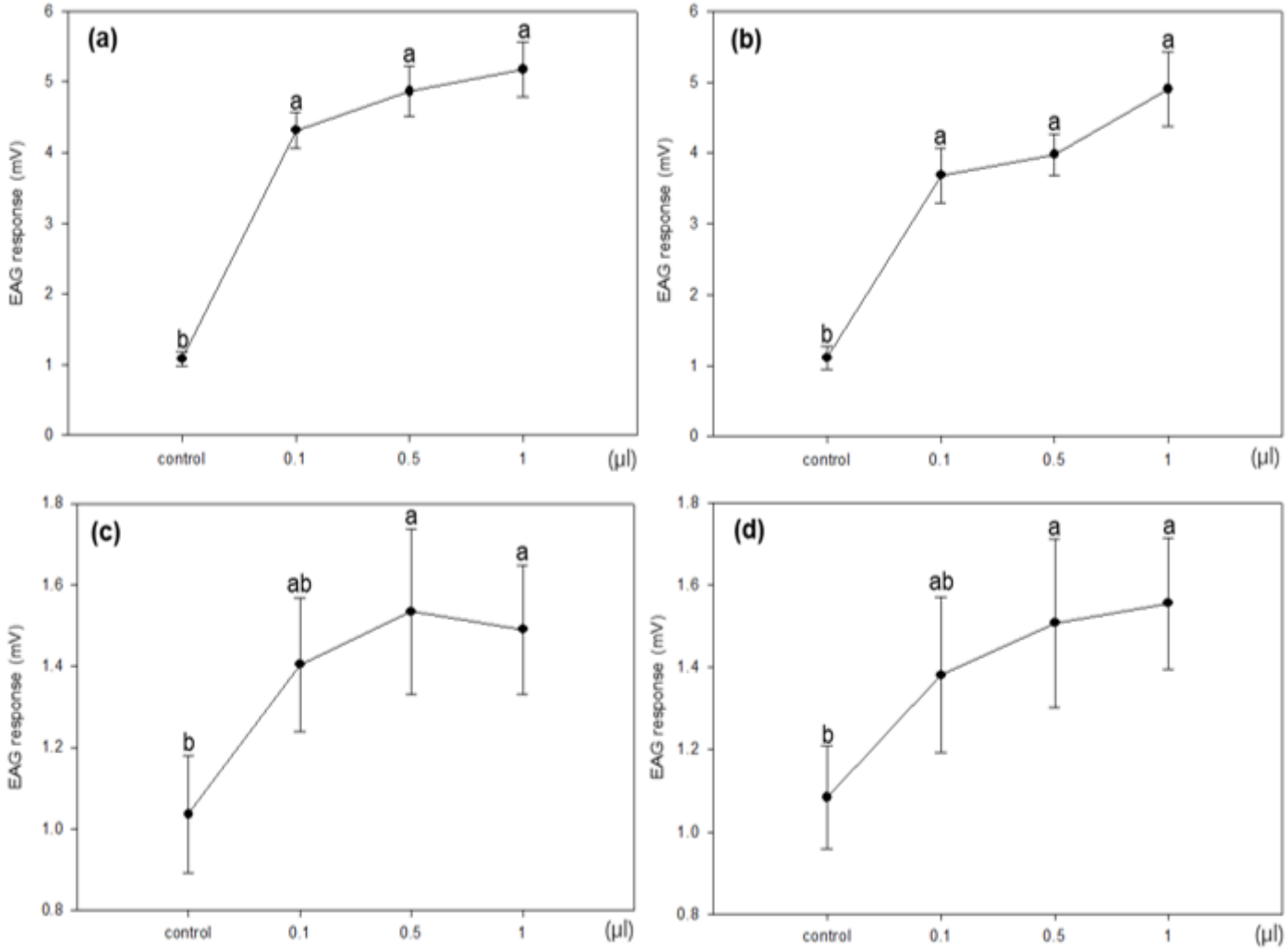

Figure 4

Mean electroantennogram (EAG) responses of male yellow peach moth to (a) (*E*)-10-hexadecenal (Bonferroni's HSD, $F_{3,12}$=146.500, p<0.0001), (b) (*Z*)-10-hexadecenal (Bonferroni's HSD, $F_{3,12}$=36.650, p<0.0001), (c) *Z*3,*Z*6,*Z*9-23:HC (Bonferron i's HSD, $F_{3,12}$=14.770, p<0.001), and (d) *Z*9-27:HC (Bonferroni's HSD, $F_{3,12}$=9.338, p=0.0018).

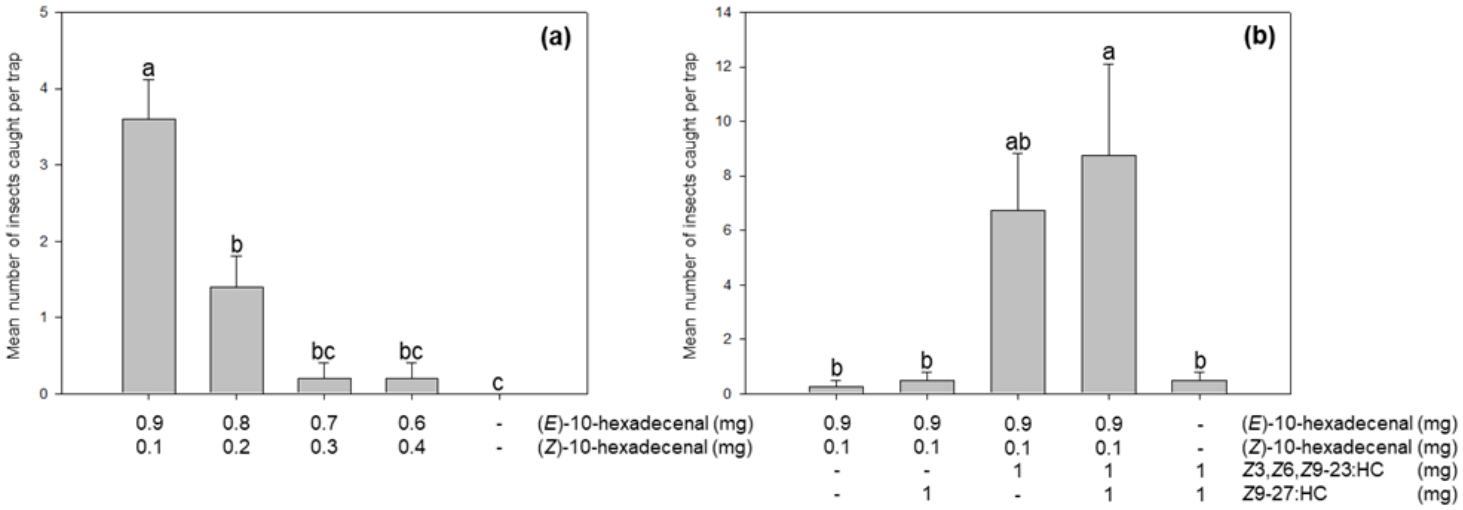

Figure 5

Number of *C. punctiferalis* adult males captured in traps baited with different ratios of (a) binary aldehyde pheromones (Tukey's HSD, $F_{4,16}$=23.629, p<0.0001) and (b) aldehyde+hydrocarbon pheromones (Tukey's HSD, $F_{4,12}$=5.819, p<0.01).

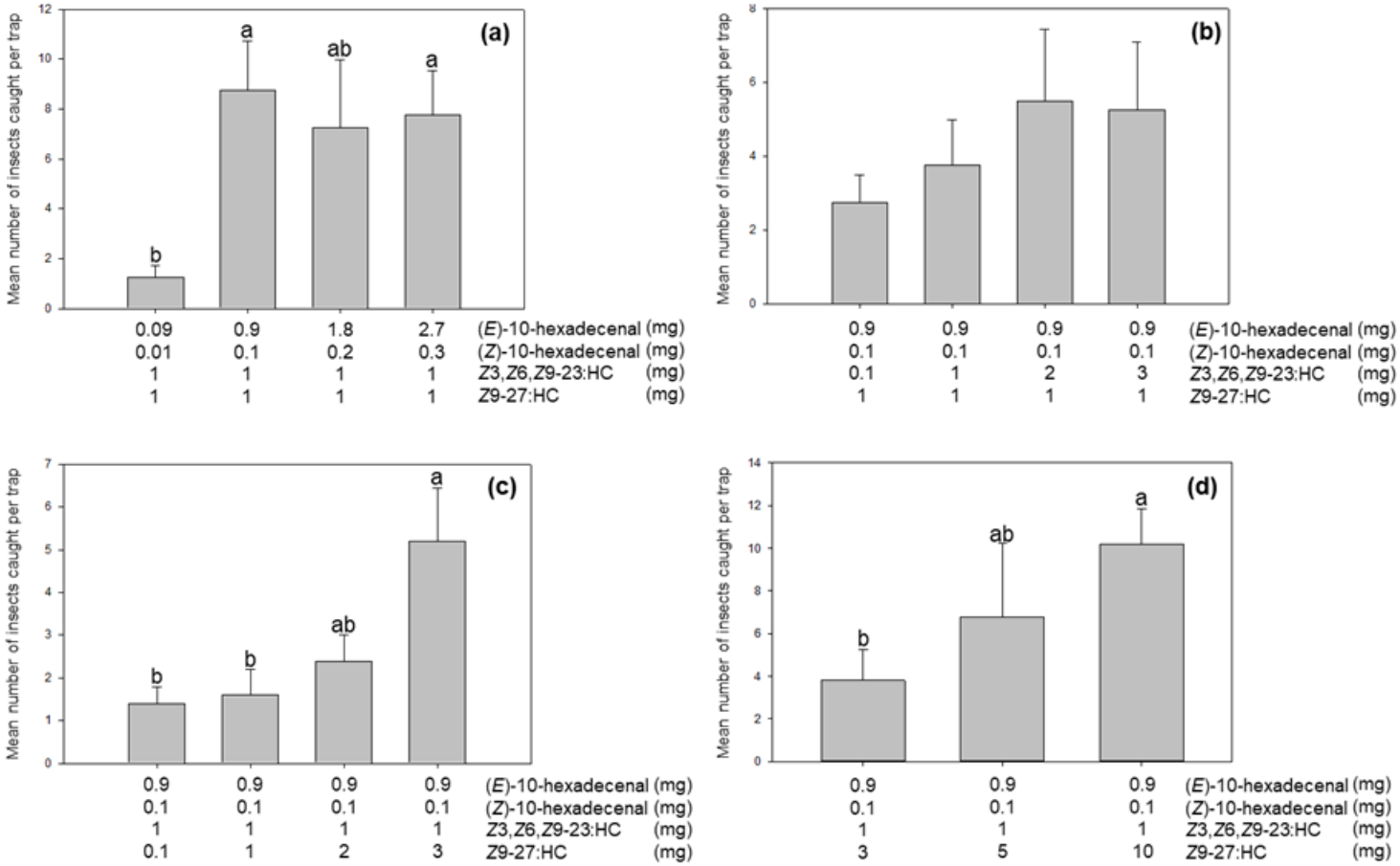

Figure 6

Number of *C. punctiferalis* adult males captured in traps baited with different amounts of aldehyde+hydrocarbon pheromones: (a) aldehyde pheromones (Tukey's HSD, $F_{3,9}$=5.633, p=0.018), (b) *Z*3,Z6,Z9-23:HC ($F_{3,9}$=1.141, p=0.384), (c) *Z*9-27:HC (Tukey's HSD, $F_{3,9}$=6.688, p<0.01), and (d) *Z*9-27:HC (Tukey's HSD, $F_{2,8}$=4.654, p=0.046).

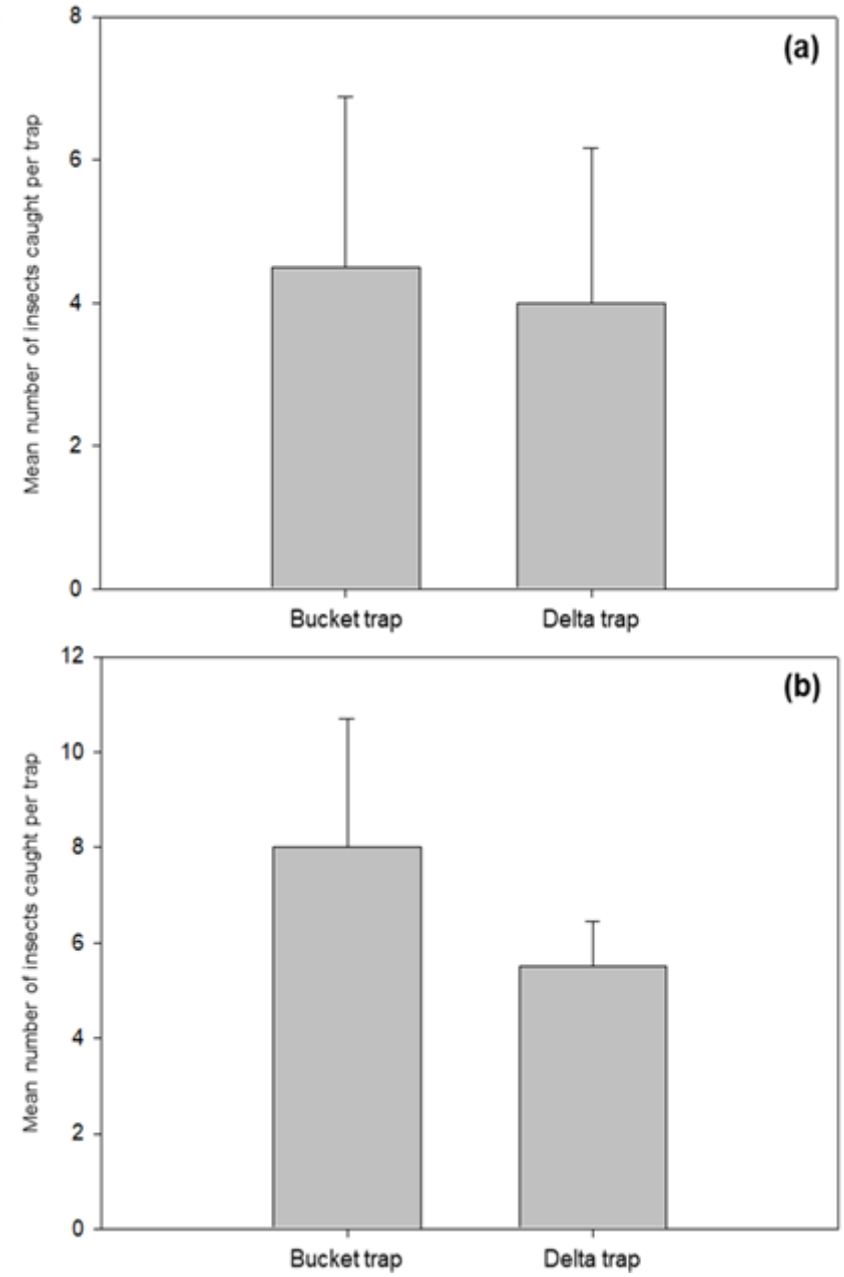

Figure 7

Number of *C. punctiferalis* adult males captured in bucket and delta traps: (a) Buyeo County (t=0.311, p=0.766) and (b) Hwaseong-si (t=0.870, p=0.416).

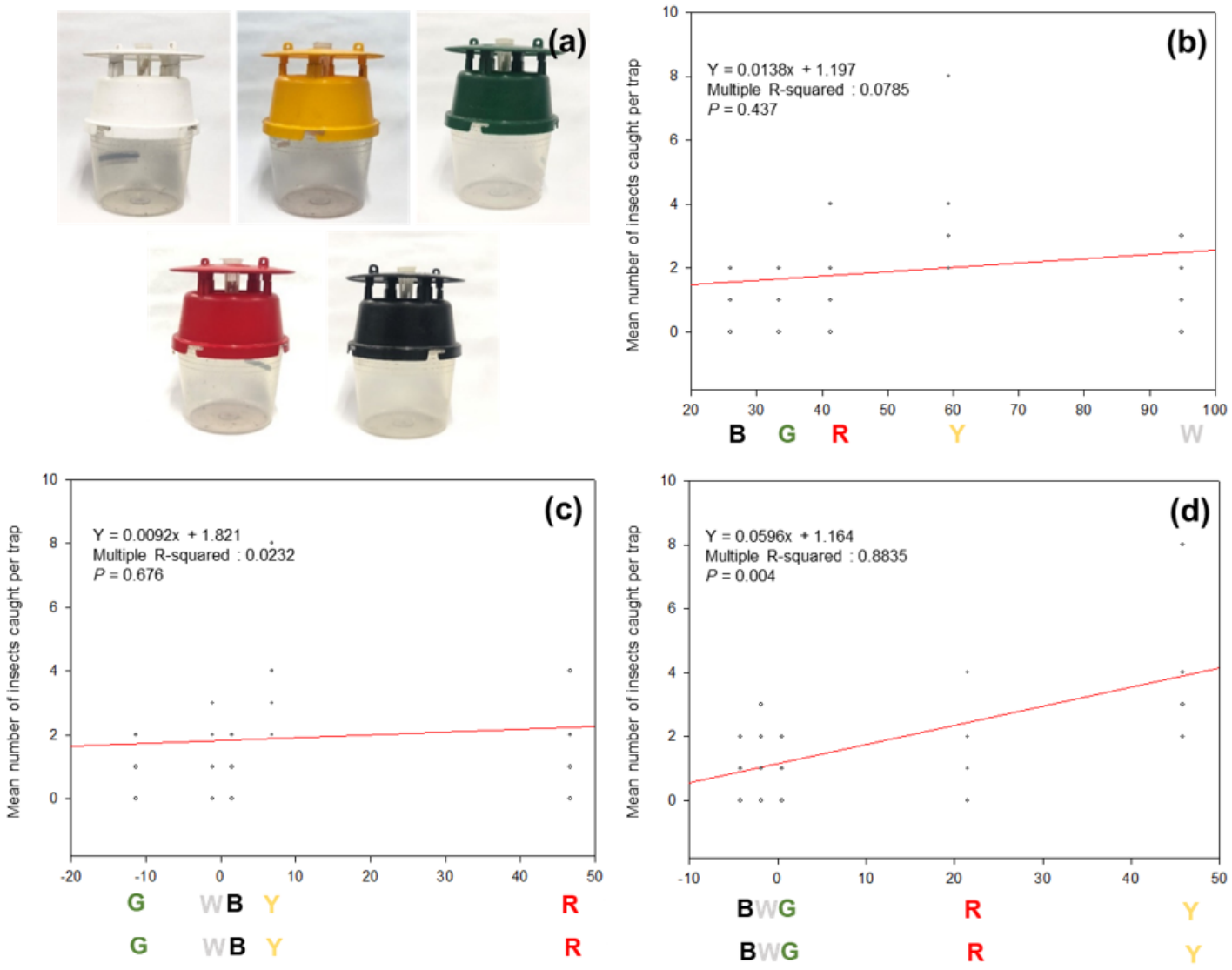

Figure 8

(a) The colored bucket traps used in the field experiment. Linear regression analysis of the effect of trap surface color values: (b) L^*, (c) a^*, and (d) b^* on trap capture.

Supplementary Files

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- Supplementarydata20220401.docx