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(Z,E)-9,12-Tetradecadien-1-Ol: A Major Sex Pheromone Component of Euzophera pyriella (Lepidoptera: Pyralididae) in Xinjiang, China

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(*Z*,*E*)-9,12-TETRADECADIEN-1-OL: A MAJOR SEX PHEROMONE COMPONENT OF *EUZOPHERA PYRIELLA* (LEPIDOPTERA: PYRALIDIDAE) IN XINJIANG, CHINA

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ABSTRACT

The moth *Euzophera pyriella* (Lepidoptera: Pyralididae) is one of the important fruit pests in the pear orchards of Xinjiang, China. Extracts from the sex pheromone gland of virgin female moths were analyzed with gas chromatography-mass spectrometry (GC-MS). Two components, (*Z*)-8-Dodecenyl acetate (*Z*8-12:Ac) and (*Z*,*E*)-9,12-Tetradecadien-1-ol (*Z9E*12-14:OH), were identified in extracts. *Z9E*12-14:OH was the most active in electroantennogram (EAG) and field attraction studies. *Z*8-12:Ac and other common components identified in *Euzophera* (including *Z*9-14:Ac, *Z*9-14:OH and *Z9E*12-14:Ac) neither elicited significant EAG responses nor attracted many males in field tests. Binary mixtures of *Z9E*12-14:OH and *Z*8-12:Ac in different ratios were active in field tests, but the number of male moths trapped did not differ between these binary mixtures and *Z9E*12-14:OH alone. We conclude that *Z9E*12-14:OH is a major component of the female sex pheromone of *E. pyriella* and could be used as an attractant for monitoring the populations of this moth species.

Key Words: *Euzophera pyriella*, sex pheromone, identification, electroantennogram, EAG, field trapping

RESUMEN

La polilla *Euzophera pyriella* (Lepidoptera: Pyralididae) es una de las plagas frutales importantes en los huertos de pera en Xinjiang, China. Se analizó los extractos de la glándula de feromona sexual de las polillas hembras vírgenes mediante la espectrometría de masascromatografía de gases (EM- CG). Dos componentes, (*Z*)- 8 -dodecenilo de acetato (*Z*8-12:Ac) y (*Z*,*E*)-9,12 - Tetradecadien - 1 -ol (*Z9E*12-14:OH), fueron identificados en los extractos. *Z9E*12-14:OH fue el más activo en electroantenograma (EAG) y en los estudios de atracción del campo. *Z*8-12:Ac y otros componentes comunes identificados en *Euzophera* (incluyendo *Z*9-14:Ac, *Z*9-14:OH y *Z9E*12-14:Ac) no provocaron respuestas de EAG significativas tampoco atrajeron a muchos machos en las pruebas de campo. Las mezclas binarias de of *Z9E*12-14:OH y *Z*8-12:Ac en diferentes proporciones fueron activos en las pruebas de campo, pero el número de polillas macho atrapados no fue diferente entre estas mezclas binarias o de solo *Z9E*12-14:OH. Llegamos a la conclusión de que *Z9E*12-14:OH es un componente principal de la feromona sexual femenina *de E. pyriella* y podría ser utilizado como un atrayente para el monitoreo de las poblaciones de esta especie de polilla.

Palabras Clave: *Euzophera pyriella*, feromona sexual, identificación, electroantenograma, EAG, captura en el campo

Pyrus × *sinkiangensis* T. T. Yu (Rosales: Rosaceae), Xinjiang fragrant pear, is a major economic crop in Xinjiang, China (Li et al. 2007; Chen et al. 2007; Jia et al. 2009), and *Euzophera pyriella* Yang (Lepidoptera: Pyralididae) is one of the most important insect pests in Xinjiang fragrant pear orchards (Hou et al. 2011). *Euzophera pyriella* is widely distributed in Xinjiang (Yang 1994) and has caused serious damage to pear trees, especially in Korla and Aksu areas, in recent years. The larvae not only attack pear trees, but also damage other crops, including *Malus pumila* Mill, *Ziziphus jujuba* Mill, *Prunus armeniaca* Linn, *Amygdalus persica* Linn, *Ficus carica* Linn (Song et al. 1994; Yang et al. 2011). Thus, determining methods to monitor population dynamics of *E. pyriella* is a high priority for orchard managers.

Euzophera pyriella has 3 generations per yr. Adults of the first generation emerge in late April, those of the second generation in early June and the third generation adults emerge in late July. Adults eclose mainly during the day, but mate at night. Mated females lay eggs singly or in small groups in wounds and crevices of trees. Larvae

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feed on phloem and xylem by boring tunnels filled with yellow or black feces, resulting in hollow tree limbs and trunks. The larva surrounded by a thin, gray cocoon overwinters in feeding tunnels, fractures of the trunk, and tree holes (Ma et al. 2013). Sometimes fragrant pear fruits also are heavily damaged with crop losses exceeding 90%.

Larvae live beneath the tree bark, which makes them both difficult to detect and difficult to control by insecticide sprays. An alternate way to control *E. pyriella* may be to reduce the population by trapping adults or disrupting mating behavior. The motivation for this study was to identify key components of the *E. pyriella* sex pheromone with the ultimate goal of using this pheromone to manage populations.

Materials and Methods

Insects

Euzophera pyriella larvae were collected in fragrant pear orchards in Korla (N 86.06° E 41.68°) and Aksu (N 80.29° E 41.15°) (Xinjiang Uygur Autonomous Region, northwestern China) in spring (Mar-Apr, 2013). In the laboratory, larvae were transferred to plastic containers (4.5-cm diam × 4.0-cm high), and reared until pupation in an artificial climate box at 22 °C, 75-80% RH and 14:10 h L:D. Pupae were separated by sex: female pupae have a short (65~70μm long), smooth-sided suture in the middle of the 8th abdominal segment and 2 sides of the suture were smooth, whereas male pupae have a longer (100~110μm long) suture with a lump on its sides formed by the genital pore on the 9th abdominal segment. Emerging adults were collected daily and kept individually in plastic dishes (9.0-cm diam), and fed 10% sugar-water solution.

Pheromone Gland Extraction

Female moths have a large sex pheromone gland located at the intersegmental membrane between the 8th and 9th abdominal segment. Pheromone glands of 2-3 day-old virgin females were excised during the calling period (about 50 min into the scotophase, Fig. 1). Calling females were cooled to -20 °C for at least 3 min prior to gland excision. Pheromone glands were excised

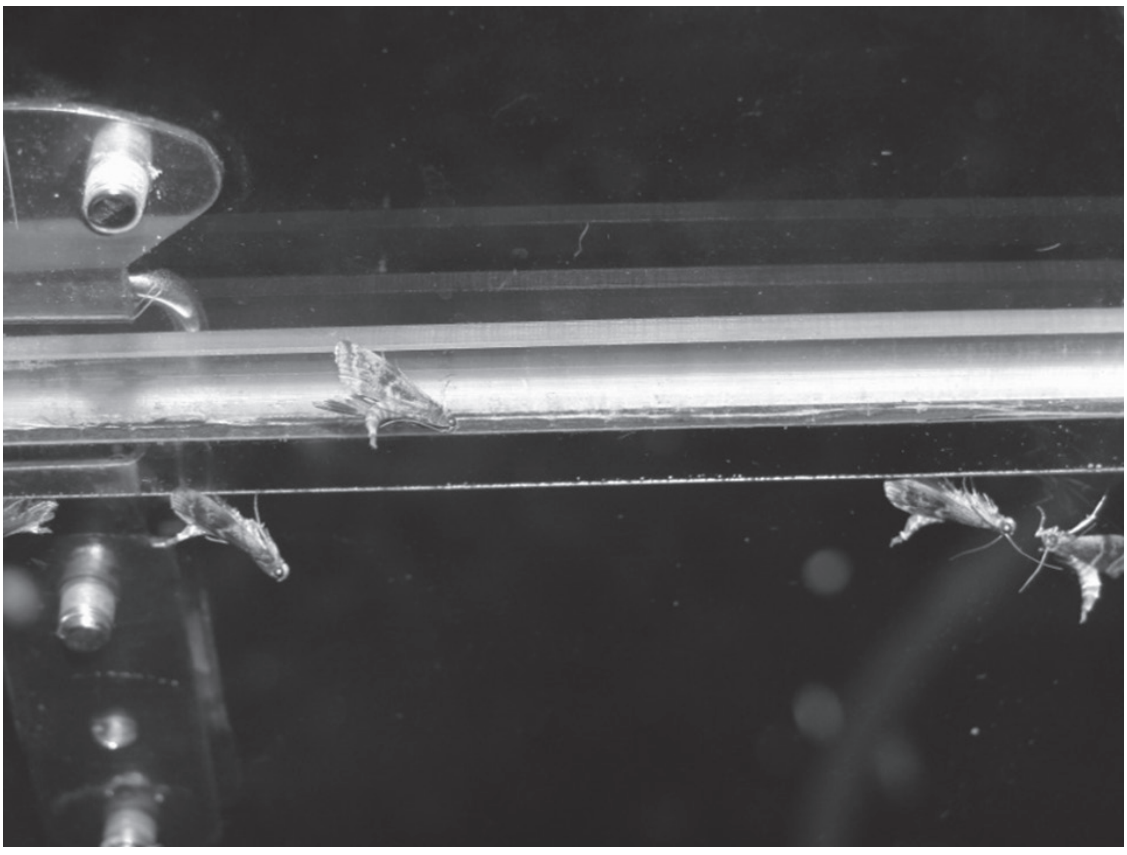


Fig. 1. Calling *Euzophera pyriella* female adults attract males by emitting a sex pheromone.

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and placed in hexane (10 µl hexane per abdominal tip) and extracted at room temperature for 50-60 min, and then transferred to a clean glass vial (Agilent, USA). Hexane extracts were stored at -20°C until they were analyzed. Sex pheromones were extracted from virgin females, while males were used for electroantennogram (EAG) studies.

Chemical Analysis

Pheromone gland extracts were analyzed on an Agilent 7890A GC interfaced to an Agilent 5975C mass-selective detector. Samples were run on DB-5 capillary columns (30 m × 0.25 mm ID, 0.25 µm film thickness, Agilent Technologies, USA). The column oven was maintained at 50 °C for 2 min, and then the temperature was increased to 270 °C at 10 °C /min, at which point it was held for 10 min. Helium was used as the carrier gas at a constant flow rate of 1 mL/min and GC inlet temperature was 260 °C. Electron ionization mass spectra were recorded from m/z 40 to 330 at 70 eV with the ion source temperature of 230 °C. GC retention times are quoted as retention indices relative to those of n-alkanes. Compounds from pheromone-gland extracts were identified by comparison of their retention indices and mass spectra with those of known standards, and a series of *n*-alkanes (C_{10}–C_{20}) were used to determine Kováts indices (*I*K) of analytes (Kováts 1965).

Electrophysiological Analyses

EAG recordings were made with a Syntech EAG, consisting of a probe/micromanipulator (MP-15), a data acquisition interface box (IDAC-2) and a stimulus air controller (CS-55). An excised male antenna was mounted onto an antenna holder for the EAG probe (PRG-2) with an electrode gel (Sigma). EAG responses (mV) to different stimuli were measured consecutively from the antenna. The process was repeated 3 times with the same antenna, for each of 5 antenna. Stimuli were applied to a strip of filter paper (0.5 × 3.0 cm), inserted into a 15-cm long Pasteur pipette. The tip of the pipette was introduced into the main airflow tube (8-mm diam), in which a moistened air stream (100 mL/min) was flowing continuously toward the antenna. Stimuli were applied to the antenna by puffing the humidified air for 0.3 s from the Pasteur pipette.

The antenna was stimulated by different sex pheromone standards, applied in random order, with at least 40 s between stimulations. Several sex pheromone standards diluted to 0.1% per µL with hexane were tested. The EAG values obtained from each standard were averaged.

Field Tests

Field trapping tests were conducted in April, and from 3 Jul to 2 Sep, 2013 in Korla (N 41.68° E 86.06°) and Aksu (N 41.15° E 80.29°) with delta traps (25-cm long × 20-cm wide) and blank dispensers (small green rubber septa, 8-mm diam) (Pherobio Technology Co. Ltd., China). Pheromone compounds were dissolved in hexane, and the solutions were pipetted into the groove of the blank dispenser. The solutions of the lure were completely infiltrated and stored at 4 °C until used. Each lure was placed at the center of a Delta trap 1 cm above the sticky board, and the traps were hung 1.5-2.0 m above the ground at intervals of 20-30 m in the fragrant pear orchards. Five replicates were arranged randomly at intervals of 100-200 m. The lure was replaced after 10 days. The number of captured males was counted every 2 days.

Chemicals

Chemicals used in identification, electrophysiological analyses and field tests are provided by Sigma-Aldrich (America), Chemos GmbH (Germany), and Pherobio Technology Co. Ltd. (China).

Statistical Analyses

Kováts indices (*I*K) were calculated by the standard formula (Marques et al. 2000). Data Analyses for significant differences were conducted by one-way ANOVA (analysis of variance [ANOVA]), and multiple comparisons were conducted by Duncan's multiple range test. The level of significance in all tests was set at $\alpha = 0.05$.

Results

GC-MS Analysis of Sex Pheromone Extracts

GC-MS analyses on crude sex pheromone-gland extracts of *E. pyriella* females revealed the presence of 2 suspected pheromone components (peaks 1 and 2) found in other Lepidoptera (Fig. 2). Peak 1 and 2, respectively, represent *Z*8-12:Ac and *Z*9*E*12-14:OH, with a compatibility >97%. Retention times of these 2 peaks (Table 1), and the mass spectra of their diagnostic fragment ions were consistent with known standards (Fig. 3).

Mass chromatograms of M^+ at m/z 226 characteristic fragment ions of acetate (ions at m/z 67) indicated an occurrence of *Z*8-12:Ac, RT 15.324 min and M^+ at m/z 210 characteristic fragment ions of double-bond tetradecanol (ions at m/z 81, 95) indicated the occurrence of *Z*9*E*12-14:OH, RT 16.198 min in the extract.

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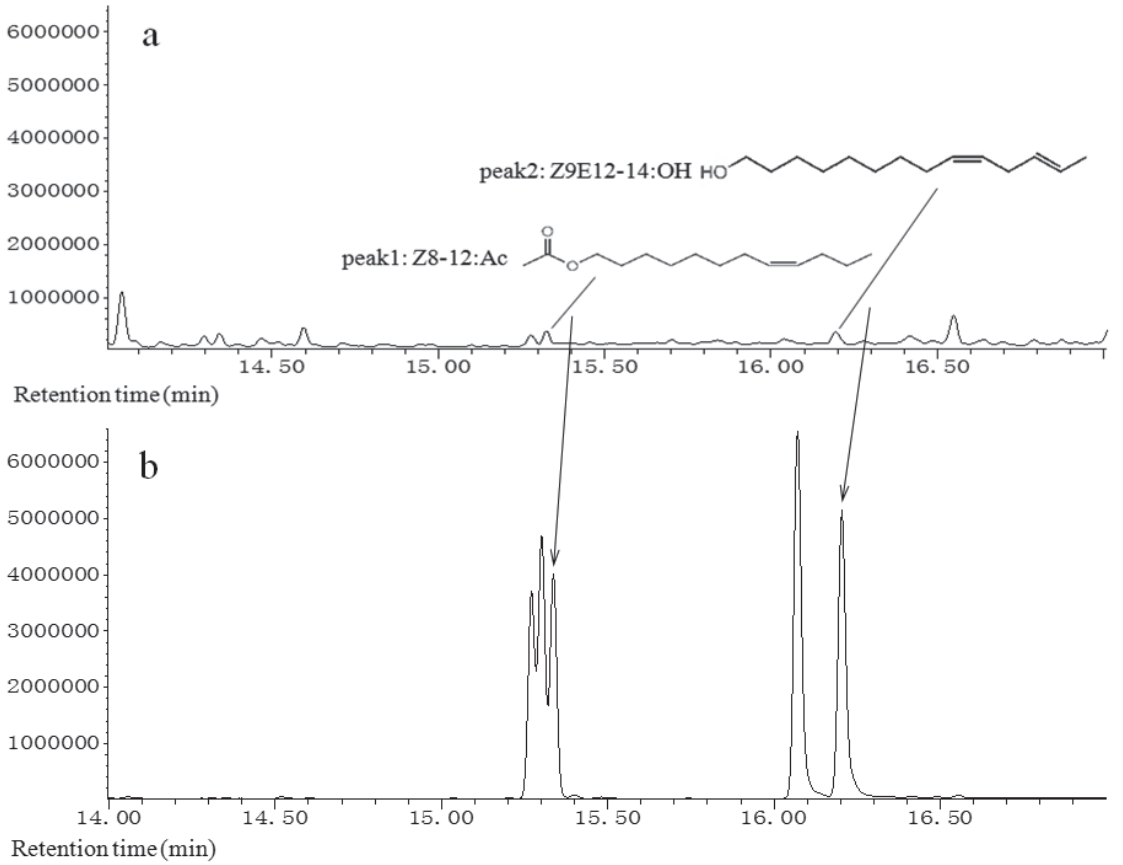


Fig. 2. GC-MS of pheromone gland extracts of *Euzophera pyriella* calling females and synthetic standards. (a) Total ion chromatogram of pheromone gland extracts. (b) Total ion chromatogram of synthetic standards.

EAG Responses of Male *E. pyriella* to Synthetic Sex Pheromone Components

Figure 4 shows the EAG responses of *E. pyriella* males to the 2 sex pheromone components identified by GC-MS (*Z*8-12:Ac and *Z9E*12-14:OH) and 3 other common compounds previously identified in *Euzophera* (*Z*9-14:Ac, *Z*9-14:OH, *Z9E*12-14:Ac). *Z9E*12-14:OH elicited the strongest response, followed by *Z*9-14:OH and *Z*8-12:Ac. Two other acetates, *Z*9-14:Ac and *Z9E*12-14:Ac, did not elicit significant EAG responses in *E. pyriella* males.

Field Attraction of *E. pyriella* Males by Synthetic Dispensers

Figure 5 shows that of the 8 sex attractants tested, only the dispensers having *Z9E*12-14:OH attracted the most *E. pyriella* males. The dispensers with a mixture of *Z9E*12-14:OH and *Z*8-12:Ac also attracted males, but the number was much smaller than that of males attracted to using *Z9E*12-14:OH separately.

Lures baited with 200µg *Z9E*12-14:OH attracted more *E. pyriella* males than did either lower or higher concentrations (Fig. 6). When *Z*8-

TABLE 1. RETENTION TIMES AND KOVÁTS INDICES OF SUSPECTED SEX PHEROMONE COMPONENTS.

	Analytes		Standards	
	peak 1	peak 2	*Z*8-12:Ac	*Z9E*12-14:OH
Retention time (min)	15.324	16.198	15.326	16.198
Kováts index	1606	1944	1608	1944

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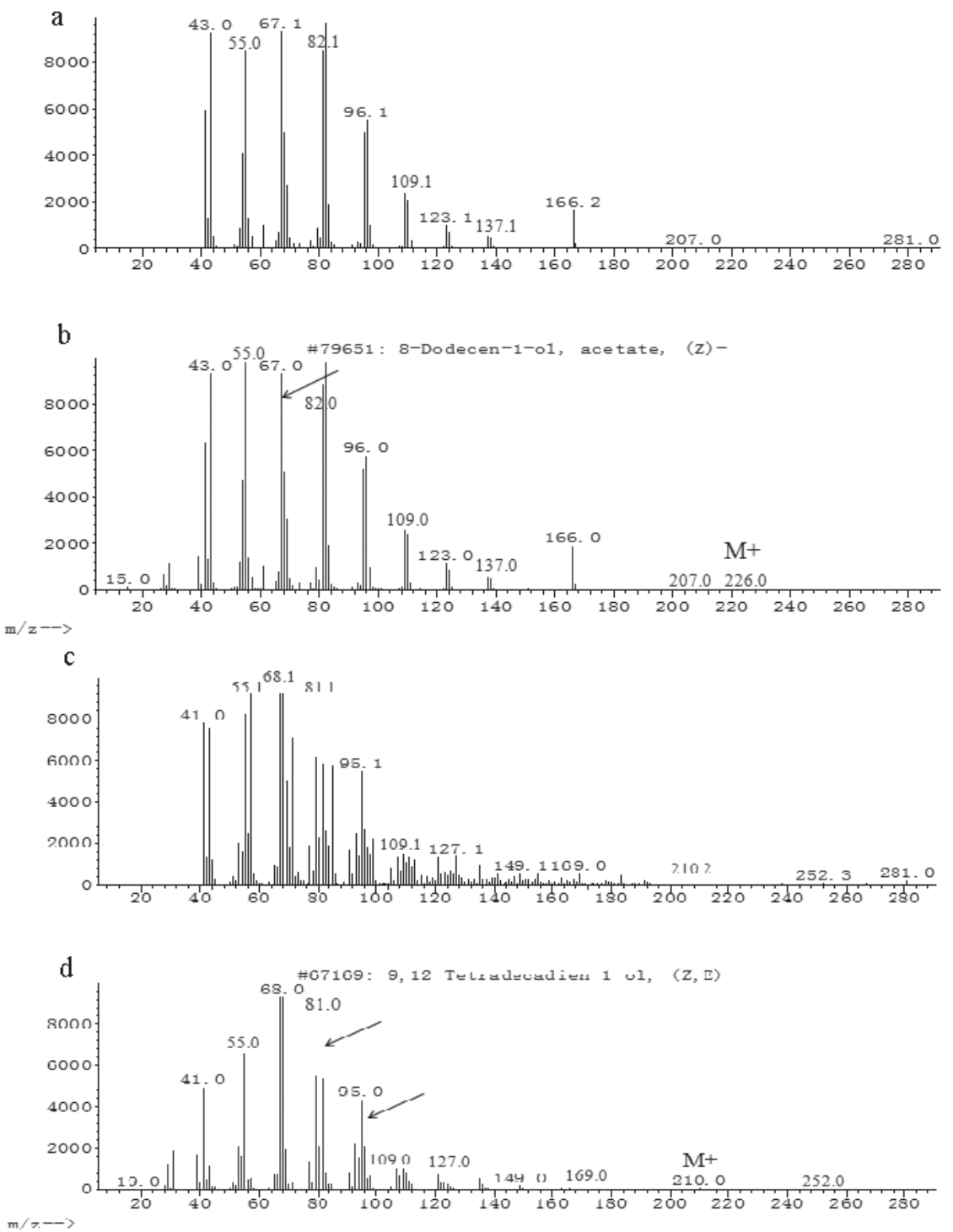


Fig. 3. The mass spectra of suspected *Euzophera pyriella* sex pheromone components: a, c - gland extracts; b, d - synthetic standards.

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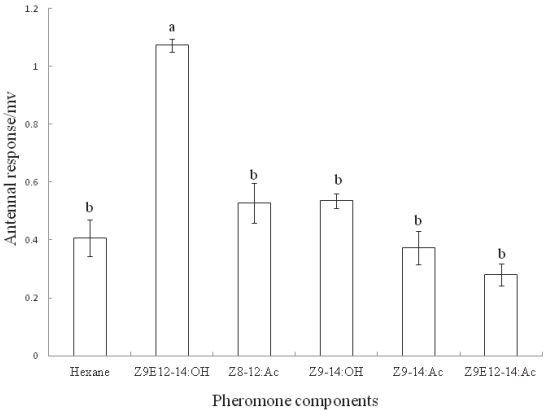


Fig. 4. EAG responses of *Euzophera pyriella* males to various sex pheromone standards. Means followed by the same lower case letters are not significantly different (Duncan's test, $P < 0.05$).

12:Ac was added to *Z*9*E*12-14:OH, decreasing the ratio of *Z*9*E*12-14:OH, the number of *E. pyriella* males caught decreased in the test (Fig. 7).

Discussion

Sex pheromones of Lepidoptera have been identified from nearly 630 species (Ando 2012); of these, close to 200 are from moths in the superfamily Pyraloidea (El-sayed 2012); Lepidopteran sex pheromones usually are straight-chain compounds. Their structures are determined by the degree of unsaturation, carbon chain length, functional group, and geometry and position of the double bond (Shibasaki et al. 2013). Lepidopteran pheromones are divided into 2 groups according to their chemical structure (Yamakawa et al. 2012). The more common Type I pheromones are comprised of C_{10} to C_{18} acetates, alcohols, aldehydes, and their derivatives. Type II pheromones, which have been detected from species in the families Lymantriidae, Arctiidae, Noctuidae, and Geometridae, are comprised of longer chain C_{17} to C_{23} compounds.

In our study, we detected 2 pheromones: *Z*8-12:Ac and *Z*9*E*12-14:OH, both Type I pheromones. *Z*9*E*12-14:OH, the main sex pheromone, is a prevalent pheromone component within other *Euzophera* species (Ando 2012), including *E. batangensis* Caradja (Wen et al. 2009), *E. pinguis* Haworth (Ortiz 2002), *E. punicaella* Moore (Bestmann et al. 1993b) and *E.semifuneralis* Walker (Biddinger et al. 1993). It also has been detected as a part of the sex pheromones of noctuid moths (*Rynchaglea scitula* [Butler] and *Spodoptera exigua* [Hübner]) and the crambid moth (*Anania verbascalis* [Denis & Schiffermüller]).

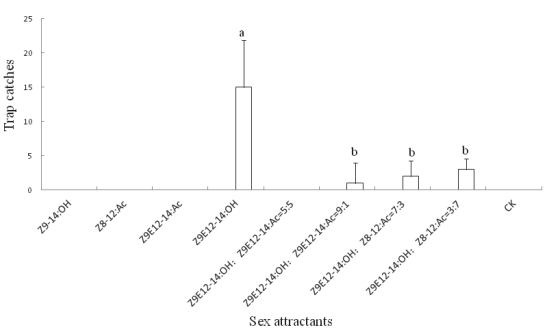


Fig. 5. Trap catches of *Euzophera pyriella* males baited with various sex attractants. Means followed by the same lower case letters are not significantly different (Duncan's test, $P < 0.05$).

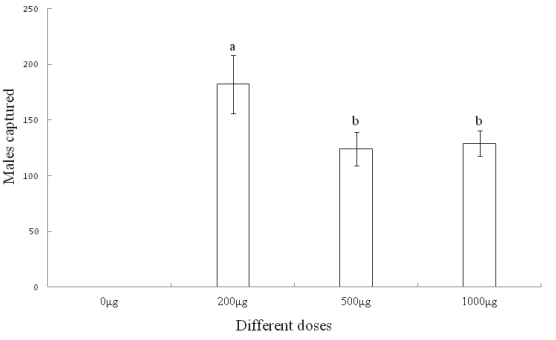


Fig. 6. Attraction of *Euzophera pyriella* males by traps baited with *Z*9*E*12-14:OH in different doses. Means followed by the same lower case letters are not significantly different (Duncan's test, $P < 0.05$).

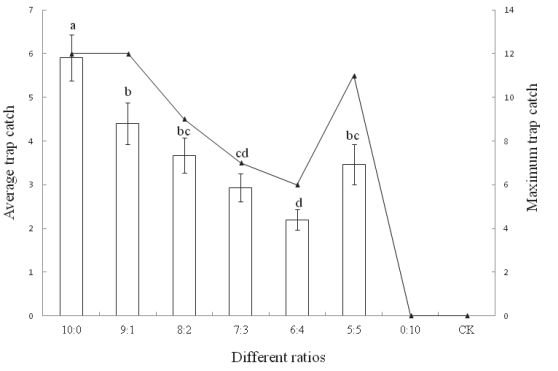


Fig. 7. Attraction of *Euzophera pyriella* males to traps baited with synthetic mixtures of the 2 pheromone components, *Z*9*E*12-14:OH and *Z*8-12:Ac, in different ratios. Means followed by the same lower case letters are not significantly different (Duncan's test, $P < 0.05$).

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In contrast, *Z*8-12:Ac was found in the sex pheromone-gland extracts by GC-MS, but alone it neither elicited antennal responses in EAG experiments nor attracted males in the field. Inhibitory effects of other compounds found in pheromone gland extracts of conspecific calling females have been reported for other moths (Gibb et al. 2007; Yang et al. 2009; Yang et al. 2013), but when *Z*8-12:Ac was added to *Z*9*E*12-14:OH to make a binary mixture, the trap catch was not significantly higher from the trap catch with *Z*9*E*12-14:OH alone. However, it remains unknown whether *Z*8-12:Ac is actually emitted by *E. pyriella* calling females or whether is simply a precursor compound to other acetates, and it may be active as a sex pheromone component over short distance. Behavioral function over short distance in chemical communication needs to be further studied (Hu & Du 2005).

In summary, we conclude that *Z*9*E*12-14:OH is a major sex pheromone component of *E. pyriella* calling females. When applied to rubber septum at a dose of 200 µg, *Z*9*E*12-14:OH is a potent lure that may be used as a convenient and reliable tool for monitoring, mass trapping, or possibly mating disruption in integrated pest management. Further chemical and electrophysiological analyses, including GC-EAD (Zhang et al. 2012), GC × GC/TOFMS (Kalinová et al. 2006), and GC/FT-IR (Leal et al. 2001; Visser 2002) with gland extracts and entrained volatile collections from *E. pyriella* females are needed to determine if other pheromone components are released by calling females. These could be used in subsequent behavioral and field tests to establish the optimum composition and dose for lures.

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