

# Identification and Field Evaluation of the Female Sex Pheromone of the Sand *Salix* Carpenterworm, *Holcocerus arenicola* Staudinger (Lepidoptera: Cossidae)

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Z. Naturforsch. **65** c, 403–411 (2010); received February 2, 2010

Extracts of female sex pheromone glands of the sand *Salix* carpenterworm moth, *Holcocerus arenicola*, a serious pest of desert thicket, were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Based on comparison of retention times and mass spectra of synthetic standards, four compounds were identified as *cis*-7-tetradecen-1-ol (*Z7*-14:OH), *cis*-5-tetradecen-1-yl acetate (*Z5*-14:OAc), *cis*-7-tetradecen-1-yl acetate (*Z7*-14:OAc), and *cis*-9-hexadecen-1-yl acetate (*Z9*-16:OAc) with the ratio of 24:39:100:43. Electroantennographic (EAG) analyses of these standard chemicals and their analogues showed that *Z7*-14:OAc elicited the largest male EAG response, followed by *Z5*-14:OAc and *Z9*-16:OAc. In field trials, traps baited with either *Z7*-14:OAc or *Z5*-14:OAc captured males while *Z7*-14:OH-, *Z9*-16:OAc- or solvent-baited traps caught no males. *Z7*-14:OAc as a single component was significantly more attractive than *Z5*-14:OAc alone. The combination of *Z7*-14:OAc and *Z5*-14:OAc showed an evidently synergistic effect and attracted much more males than the individual compounds in the field. Addition of *Z7*-14:OH to the blend of *Z7*-14:OAc and *Z5*-14:OAc enhanced slightly the trap catches. We conclude that the major components of the sex pheromone of *H. arenicola* are *Z7*-14:OAc and *Z5*-14:OAc. Currently, a triangle trap baited with the synthetic compounds *Z7*-14:OAc, *Z5*-14:OAc, and *Z7*-14:OH in a 1:0.4:0.25 ratio at 825 µg/trap dosage can be effectively used to monitor the *H. arenicola* population level and catch the males within the desert regions in China.

**Key words:** *Holcocerus arenicola*, *cis*-5-Tetradecen-1-yl Acetate, *cis*-7-Tetradecen-1-yl Acetate

## Introduction

The sand *Salix* carpenterworm, *Holcocerus arenicola* Staudinger (Lepidoptera: Cossidae), one of the serious root-boring forest pests in desert areas, is widely distributed mainly in Shaanxi, Inner Mongolia, Gansu, Xinjiang and Ningxia in China, and Russia, Mongolia, Asia minor and Caucasia. One generation of *H. arenicola* lasts four years, and mature larvae transfer to the sand-soil from the host roots and begin to pupate under the ground in May every year. The hosts of *H. arenicola* are desert thickets including *Salix psammophila*, *Heey aprum*, *Hippophae*, *Salix microstachya*, and *Caragana korskinskii* (Hu *et al.*, 1987).

Desert thickets are mainly used to control desertification, to conserve land and water resources, and to provide fodder and shelter for wild and domestic animals in the northwestern desert areas in China. On all host trees, aggregations of larvae are observed to bore into woody taproot, causing significant damage and frequent mortality. In Qinghai province, for example, infestation levels of sand *Salix* by *H. arenicola* reached 35% (Xie, 2008). The damage is so severe and extensive that the sand *Salix* carpenterworm is considered a major threat to the continued existence of sand *Salix* forests in China.

Because wormholes are not easily accessible, insecticides are not a practical measure for the control of *H. arenicola*. However, insect pherom-

ones produced by target species, as an integral part of integrated pest management (IPM) programs, have been effectively used to control the Lepidoptera insects (Delisle, 1992; Strong *et al.*, 2008; Wins-Purdy *et al.*, 2007; Norio *et al.*, 2008; Stelinski *et al.*, 2007). Combined with traps, sex pheromones are particularly appealing for insects that develop in protected places and thus are not easily controllable with insecticides.

In the family Cossidae, sex pheromones for *Cossus cossus* (Capizzi *et al.*, 1983), *Zeuzera pyrina* (Tonini *et al.*, 1986), *Holcocerus insularis* (Zhang *et al.*, 2001), *Holcocerus hippophaecolus* (Fang *et al.*, 2005), *Cossus insularis* (Chen *et al.*, 2006), and *Holcocerus artemisiae* (Zhang *et al.*, 2009) have been identified. Till now sex pheromones for *H. arenicola* have not been found and characterized. In the present paper, we report the identification of the sex pheromone of *H. arenicola*. Field trapping studies using blends of synthetic compounds were also conducted to develop a rational program that can be used to monitor and control *H. arenicola*.

## Materials and Methods

### Insects

Pupae of *H. arenicola* were collected from sand-soil (1 m in diameter, 15 cm in depth) around a stem of sand *Salix* damaged badly by larvae in the Balasu woodland, Yulin, Shaanxi, China, in mid-May 2009. 100 ~ 150 pupae were buried in sands (containing 5 ~ 8% water), 15 cm deep, in wooden boxes (40 cm × 60 cm × 30 cm) covered with a nylon mesh. These boxes were maintained at 26 ~ 30 °C, 70 ~ 80% relative humidity, and the natural photoperiod (14 h light/10 h dark).

After emergence, the adults were immediately segregated by sex and transferred into cages (80 cm × 80 cm × 80 cm, 20 moths per cage) containing a source of 8% sucrose solution. Males and virgin females were kept in different cages. The calling behaviour of adult females was observed continuously during the first 6 h of darkness each night for 5 d. The calling time was recorded according to the criteria for calling behaviour (Zhang and Meng, 2001). A single red incandescent light bulb controlled by a rheostat was used to illuminate for observing at night.

### Extracts of the sex pheromone

Observations of virgin females revealed that the calling behaviour occurred 1.5 ~ 2.0 h after the lights were off. Previous assays showed that the biosynthesis of most lepidopterous pheromones started some hours prior to the onset of calling, and that the content of pheromones reached a peak at the onset of calling but declined once females expressed calling behaviour (Delisle and Royer, 1994). Consequently, in the 30 min before calling, sex pheromone glands were extruded immediately by applying gentle pressure to the female's abdominal tips to force eversion of the ovipositor, and were excised with small scissors and put into a 2-ml glass vial containing 500  $\mu$ l of distilled *n*-hexane in which 1-undecanol was added as the internal standard. Glands were extracted for 20 ~ 30 min, and then the solution was transferred to a clean vial. Extracts were stored at -20 °C if not used immediately. Extracts which contained 10 FE (female equivalent) were concentrated to 10  $\mu$ l under a gentle stream of N<sub>2</sub> before analysis (Julio *et al.*, 2006).

### Chemicals

Semiochemicals (> 98% purity) used in analytical work, electroantennographic analyses, and lures for field trials were synthesized in our chemical ecology laboratory. Reagents and solvents were from Fisher Chemicals (Fair Lawn, NJ, USA) and Aldrich Chemicals (St. Louis, MO, USA).

### Chemical analysis of the sex pheromone

The gas chromatography (GC) analyses of sex pheromone gland extracts and standard compounds were performed on an Agilent 6890N gas chromatograph equipped with a flame ionization detector (FID) and a splitless injector. Two fused silica capillary columns, A (DB-23, 50% cyanopropyl-methylpolysiloxane, 30 m × 0.32 mm i.d. × 0.33  $\mu$ m film, Palo Alto, CA, USA) and B (BP-1, 100% dimethyl polysiloxane, 30 m × 0.32 mm i.d. × 0.25  $\mu$ m film, SGE Pty Ltd., Australia), were used with the following temperature program: 80 °C for 2 min, 4 °C/min to 180 °C, then 10 °C/min to 240 °C, isothermal for 15 min. Injector and detector were set at 250 °C. Pure nitrogen was used as the carrier gas at a linear flow rate of 46 cm/s. The retention

times (Rt) of selected peaks on both columns were compared to the corresponding data of synthetic standard compounds; the mean quantity of each component in a single gland was calculated based on comparison of the integrated area with that of the internal standard (1-undecanol).

Gas chromatography-mass spectrometry (GC-MS) analyses of pheromone gland extracts were performed on a Finnigan Trace DSQ GC/MS instrument (EI mode, 70 eV). Fused silica capillary columns (HP-1, 50 m × 0.22 mm i.d. × 0.33 μm film) were used with the following temperature program: 80 °C/min to 280 °C, isothermal for 20 min. Mass spectral data of selected peaks were compared to the corresponding data of reference standards in the NIST database (version 2.0 a, National Institute of Standards and Technology, USA).

#### Microanalytical reaction

Double bond positions and geometries in unsaturated compounds were determined from dimethyldisulfide (DMDS) adducts of the insect-produced compounds (Buser *et al.*, 1983; Leonhardt and Devilbiss, 1985). A mixture of 50 μl DMDS and 5 μl iodine/diethyl ether solution (0.06%) was added to an extract containing 10 FE and kept at 40 °C overnight, then quenched with 200 μl of 5% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution in distilled water, and the mixture was extracted with 100 μl *n*-hexane. The resulting solution was dried over magnesium sulfate and analyzed by GC-MS on a non-polar column (HP-1).

#### Electroantennograms

The electrophysiological responses of 1-day-old male antennae to several analogues of major components in the sex pheromone were determined on an electroantennography (EAG) apparatus (Syntech, Hilversum, The Netherlands). The EAG setup was described previously (Malo *et al.*, 2004; Kong *et al.*, 2005), and solvent blank puff (filter paper and *n*-hexane) was used as the control. The male antennae were replaced by fresh ones every 20 min and 6 replicates were determined.

#### Field trapping

Field trapping trials were performed in a sand *Salix* forest at Yulin, Shaanxi, China. Green rubber septa were treated with appropriate amounts

of semiochemicals in *n*-hexane. Controls were treated with 50 μl *n*-hexane. One-day-old virgin females in small mesh cages suspended in traps were used as positive controls. Delta sticky traps, spaced 50 m apart, were hung from branches of sand *Salix*, about 1 m above the ground. All field tests employed a complete randomized block design with 6 replicates. Lures were left unchanged for 3 d, and virgin females were renewed every day. Captured moths were recorded and removed daily.

Experiment 1 was carried out from June 15 to June 18, 2009 to investigate the attraction of *H. arenicola* males to the potential semiochemicals *cis*-7-tetradecen-1-ol (*Z*7-14:OH), *cis*-5-tetradecen-1-yl acetate (*Z*5-14:OAc), *cis*-7-tetradecen-1-yl acetate (*Z*7-14:OAc), and *cis*-9-hexadecen-1-yl acetate (*Z*9-16:OAc) as single components and in binary blends. Experiment 2 was conducted from June 20 to June 23, 2009 to test the individual effects of two minor components identified in female gland extracts, *Z*9-16:OAc and *Z*7-14:OH, as possible synergists, by adding them into a standard blend of the two major components.

#### Data analysis

Statistical analysis was performed using the SPSS 10.0 statistical package (SPSS Inc., Chicago, IL, USA). The data obtained were submitted to one-way analysis of variance (ANOVA) by the *F* test, and the means were compared by Tukey's test. Capture data were transformed to log(*x* + 1) before the differences between means were tested for significance by analysis of variance.

## Results

#### Analysis of the sex pheromone extracts

All mass data are listed in Table I. Obviously, peaks I–IV (Fig. 1) produced common ion fragments of *m/z* 194, 166, 152, 138 and 124, which indicated that they had the similar straight-chain structure. The mass spectrum of peak I had diagnostic ion fragments at *m/z* 194 [*M*<sup>+</sup>–H<sub>2</sub>O], *m/z* 31 [CH<sub>2</sub>OH<sup>+</sup>], and *m/z* 71 [C<sub>4</sub>H<sub>6</sub>OH<sup>+</sup>] of tetradecen-1-ol (Silverstein *et al.*, 1981) with a retention time of 23.20 min agreeing with that for the same ions and retention time of a synthetic sample of *Z*7-14:OH. Peaks II and III contained diagnostic fragments at *m/z* 194 [*M*<sup>+</sup>–CH<sub>3</sub>COOH], *m/z* 166

Table I. Mass spectral data of components of sex pheromone gland extract and standard compounds.

Component	Standard	<i>m/z</i> (relative intensity of major ions) [assignment]
I	Z7-14:OH	194(20) [M <sup>+</sup> -H <sub>2</sub> O], 166(3) [M <sup>+</sup> -CH <sub>3</sub> CH <sub>2</sub> OH], 152(1), 138(11), 124(15), 123(15), 110(23), 109(28), 96(68), 95(65), 82(56), 81(53), 71(7) [C <sub>4</sub> H <sub>6</sub> OH <sup>+</sup> ], 69(40), 68(57), 67(100), 55(30), 54(16), 31(12) [CH <sub>2</sub> OH <sup>+</sup> ]
II	Z5-14:OAc	194(50) [M <sup>+</sup> -CH <sub>3</sub> COOH], 166(20) [M <sup>+</sup> -CH <sub>3</sub> COOC <sub>2</sub> H <sub>5</sub> ], 152(5), 138(29), 124(27), 96(93), 95(86), 82(62), 81(53), 68(28), 68(65), 67(100), 61(4) [CH <sub>3</sub> COOH <sub>2</sub> <sup>+</sup> ], 55(17), 54(14), 43(65) [O=CCH <sub>3</sub> <sup>+</sup> ]
III	Z7-14:OAc	194(46) [M <sup>+</sup> -CH <sub>3</sub> COOH], 166(4) [M <sup>+</sup> -CH <sub>3</sub> COOC <sub>2</sub> H <sub>5</sub> ], 152(4), 138(20), 124(20), 123(19), 110(25), 109(33), 96(100), 95(75), 82(59), 81(60), 61(3) [CH <sub>3</sub> COOH <sub>2</sub> <sup>+</sup> ], 55(20), 43(78) [O=CCH <sub>3</sub> <sup>+</sup> ]
IV	Z9-16:OAc	222(25) [M <sup>+</sup> -CH <sub>3</sub> COOH], 194(3) [M <sup>+</sup> -CH <sub>3</sub> COOC <sub>2</sub> H <sub>5</sub> ], 166(3), 152(3), 138(6), 124(11), 110(19), 96(39), 82(50), 69(32), 67(42), 55(54), 61(10) [CH <sub>3</sub> COOH <sub>2</sub> <sup>+</sup> ], 43(81) [O=CCH <sub>3</sub> <sup>+</sup> ]
V	Stearic acid	284(30) [M <sup>+</sup> ], 255(5) [M <sup>+</sup> -C <sub>2</sub> H <sub>5</sub> ], 224(1.5) [M <sup>+</sup> -CH <sub>3</sub> COOH], 185(12), 171(5), 143(4), 129(32), 115(11), 97(19), 85(24), 84(12), 83(25), 73(83), 60(81) [CH <sub>3</sub> COOH], 57(75), 43(100) [O=CCH <sub>3</sub> <sup>+</sup> ], 29(38)

[M<sup>+</sup>-CH<sub>3</sub>COOC<sub>2</sub>H<sub>5</sub>], and *m/z* 61 [CH<sub>3</sub>COOH<sub>2</sub><sup>+</sup>] of tetradecen-1-yl acetate (Brown *et al.*, 1988) with the retention times of 25.92 min and 26.02 min coinciding with those for synthetic Z5-14:OAc and Z7-14:OAc. Peak IV showed

diagnostic ions at *m/z* 222 [M<sup>+</sup>-CH<sub>3</sub>COOH], 194 [M<sup>+</sup>-CH<sub>3</sub>COOC<sub>2</sub>H<sub>5</sub>], *m/z* 61 [CH<sub>3</sub>COOH<sub>2</sub><sup>+</sup>], and *m/z* 43 [O=CCH<sub>3</sub><sup>+</sup>], suggesting hexadecen-1-yl acetate. The retention behaviour of peak IV (29.35 min) corresponded exactly with that of

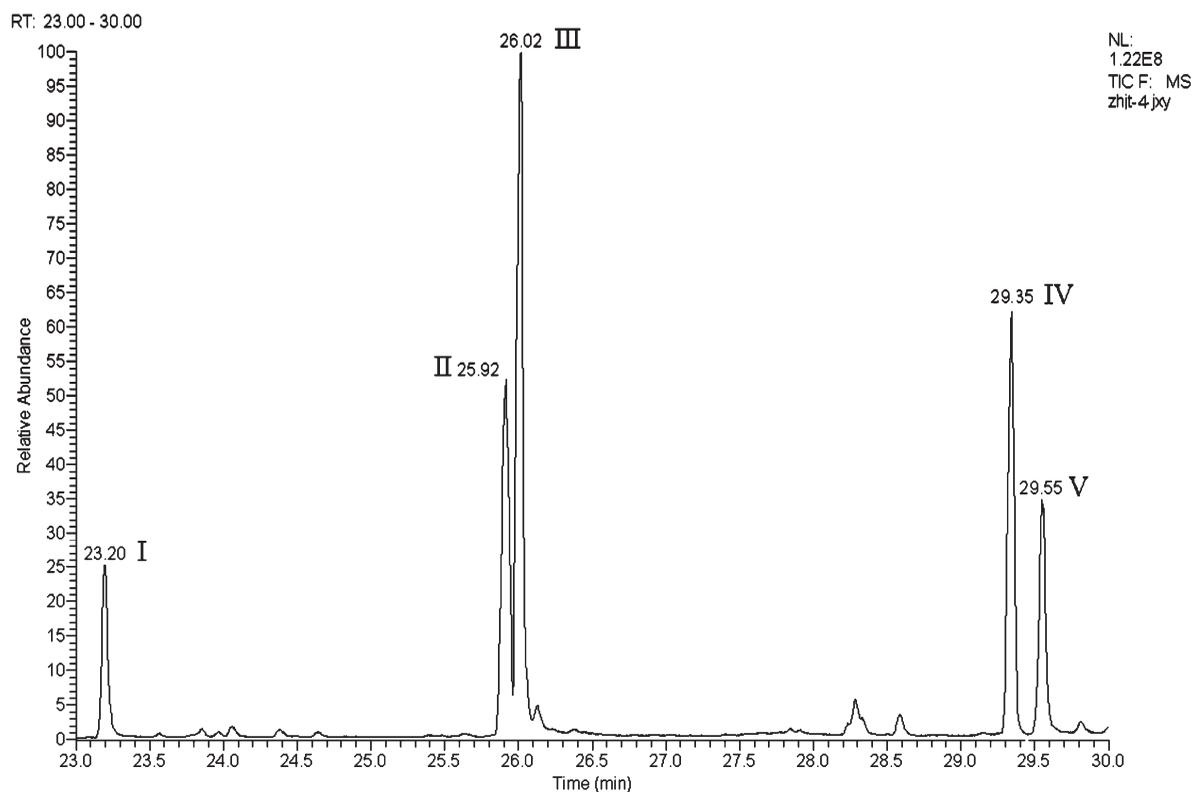
Fig. 1. Total ion chromatogram of GC-MS analysis of the sex pheromone gland extract of *H. arenicola*.

Table II. Retention times and relative quantities of gland components and synthetic standards on a BP-1 column.

Compound	Retention time [min]		Mean ( $\pm$ SD) quantities in a single gland [ng] ( $N = 10$ ) <sup>a</sup>
	Standard	In extracts	
Z7-14:OH	21.660	21.658	8.31 $\pm$ 3.45
E7-14:OH	21.742		
Z5-14:OAc	25.173	25.172	13.76 $\pm$ 2.86
E5-14:OAc	25.340		
Z7-14:OAc	25.241	25.239	34.96 $\pm$ 6.74
E7-14:OAc	25.356		
Z9-16:OAc	29.285	29.283	14.97 $\pm$ 1.06
E9-16:OAc	29.354		
Stearic acid	32.393	32.392	5.96 $\pm$ 0.58

<sup>a</sup> For temperature programs of GC column and determination of relative amounts of the gland components cf. "Materials and Methods".

synthetic Z9-16:OAc. All mass spectral data of peaks I-V in the extracts were compared to the corresponding data of reference standards in the NIST. The result indicated that peaks I-IV had the best matching with Z7-14:OH, Z5-14:OAc, Z7-14:OAc, and Z9-16:OAc, respectively. Peak V was possibly stearic acid ( $M_r$  284).

Double bond locations of the pheromone components were further confirmed by analyses of their DMDS derivatives. The mass spectrum of DMDS adducts derived from peak I (diagnostic ions at  $m/z$  145 [ $\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{SCH}_3^+$ ], 161 [ $\text{H}_3\text{CS}^+=\text{CH}(\text{CH}_2)_5\text{CH}_2\text{OH}$ ], and 306 [ $\text{M}^+$ ]) and peak III (diagnostic ions at  $m/z$  143 [ $\text{CH}_3\text{COO}(\text{CH}_2)_5\text{CH}_2^+$ ], 145 [ $\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{SCH}_3^+$ ], 203 [ $\text{CH}_3\text{COO}(\text{CH}_2)_6\text{CH}=\text{SCH}_3^+$ ], and 348 [ $\text{M}^+$ ]) confirmed that their double bonds were located at the 7-position. Peak II gave a derivative with strong ions at  $m/z$  115 [ $\text{CH}_3\text{COO}(\text{CH}_2)_3\text{CH}_2^+$ ], 173 [ $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{SCH}_3^+$ ], 175 [ $\text{CH}_3\text{COO}(\text{CH}_2)_4\text{CH}=\text{SCH}_3^+$ ], and 348 [ $\text{M}^+$ ], indicating that the double bond was in the 5-position. The DMDS adduct of peak IV showed diagnostic ions at  $m/z$  145 [ $\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{SCH}_3^+$ ], 171 [ $\text{CH}_3\text{COO}(\text{CH}_2)_7\text{CH}_2^+$ ], 231 [ $\text{CH}_3\text{COO}(\text{CH}_2)_8\text{CH}=\text{SCH}_3^+$ ], and 376 [ $\text{M}^+$ ], demonstrating that the double bond was in the 9-position.

Each component in the pheromone gland extracts was further identified by comparison of its retention time with those of *Z* and *E* isomers of monounsaturated 14-carbon-atoms standards on both capillary columns A and B under different temperature conditions. Four components consistently co-chromatographed with the synthetic

standard compounds Z7-14:OH, Z5-14:OAc, Z7-14:OAc, and Z9-16:OAc on column A. In addition, when 0.2  $\mu\text{l}$  extracts and 0.2  $\mu\text{l}$  blend of the four standards were injected simultaneously into column A, the integrated areas and heights of the peaks corresponding to these four components in the extracts increased. The pheromone components and their geometric isomers were also determined on column B (Table II). The close similarities in retention time between the four components and their corresponding standards on the two columns strongly suggested that Z7-14:OH, Z5-14:OAc, Z7-14:OAc, and Z9-16:OAc were present in the sex pheromone of *H. arenicola*. Moreover, quantitative analysis by GC showed that the ratio of them varied around a mean of 24:39:100:43 in a single calling female sex pheromone gland (Table II).

#### Electroantennographic analysis

EAG responses of male *H. arenicola* to the gland components and their analogues varying in double bond positions and configurations are summarized in Fig. 2. The results showed that Z7-14:OAc elicited the strongest response (4.73 mV), followed by Z5-14:OAc (3.48 mV), Z3-14:OAc (2.84 mV), and Z9-16:OAc (2.42 mV) among the compounds tested. It can be seen from Fig. 2 that the EAG response elicited from acetates were higher than those from the corresponding alcohols, and *Z* isomers elicited much stronger response than *E* isomers. This suggested indirectly that the active components in extracts were *Z*-configurational acetates. Though the EAG

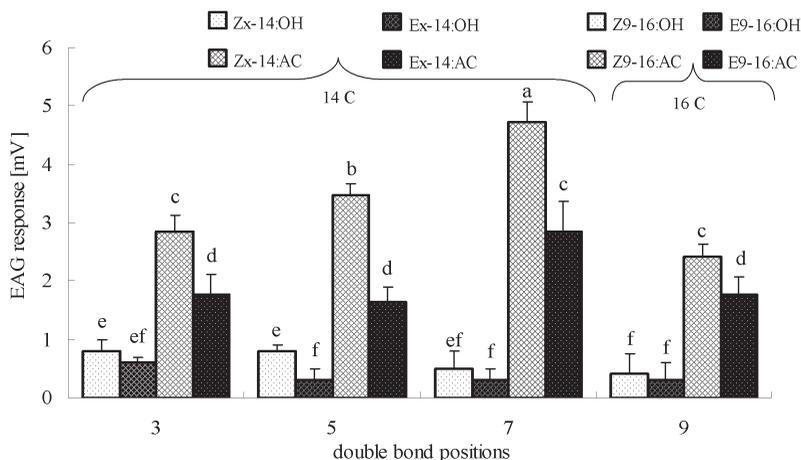


Fig. 2. EAG responses of male *H. arenicola* to a series of monounsaturated 14-carbon alcohols and acetates at doses of 10 ng ( $N = 6$ ). Bars followed by the same letter are not significantly different at the 5% confidence level by Tukey's test ( $F = 334.528$ ,  $df = 15.80$ ,  $P < 0.001$ ).

response of Z3-14:OAc was higher than that of Z9-16:OAc, it was not detected in the extracts of the glands. Stearic acid could not elicit an evident EAG response and was probably a metabolite of biochemical reaction in the sex glands.

#### Field trapping

In experiment 1, traps baited with either virgin females, Z7-14:OAc or Z5-14:OAc captured males while those containing Z7-14:OH, Z9-16:OAc or solvent did not (Table III). Z7-14:OAc as a single component was significantly more attractive than Z5-14:OAc alone. Traps baited with a two-component blend of Z7-14:OAc and Z5-14:OAc in a 10:4 ratio significantly enhanced catches compared with traps baited with individuals or other blends of the two components. All the lures lacking Z7-14:OAc caught few males, and the differences among these treatments were not significant.

In experiment 2, addition of Z7-14:OH to the binary blend of Z7-14:OAc and Z5-14:OAc enhanced the attraction of *H. arenicola* males, whereas Z9-16:OAc showed a slight inhibiting effect on male moth catches. Moreover, the full component blend did not significantly increase the trap catches compared with traps baited with the binary blend of the major components or the ternary blend of Z7-14:OH, Z5-14:OAc, and Z7-14:OAc.

#### Discussion

All the analytical data indicated that the extracts from the sex pheromone glands of *H. arenicola* contained four compounds, Z7-14:OH, Z5-14:OAc, Z7-14:OAc, and Z9-16:OAc. We have demonstrated that Z7-14:OAc and Z5-14:OAc were the major components of the sex pheromone of *H. arenicola*. These two compounds, produced by adult females, were indispensably required for the attraction, and the antennae of male *H. arenicola* were highly sensitive to both of them. In the field, it has been shown that males responded preferentially to blends containing these two components rather than to the individuals. The ternary blend of Z7-14:OH, Z5-14:OAc, and Z7-14:OAc showed a maximum catch, indicating that all three components were necessary for optimal attraction. Moreover, the existence of trace co-attractants in pheromones of some species, such as *Pseudaletia unipuncta* (Steck *et al.*, 1982), could enhance the attractiveness of either the natural female odour or its synthetic major components. If a certain trace component in *H. arenicola* plays the similar role, a series of experiments is needed to be conducted in the future research.

Even though Z9-16:OAc, with a low level EAG response of male moths, was detected in the extracts from the sex pheromone glands, it could hardly catch the male moths in field tests. More-

Table III. Catches of male *H. arenicola* in traps baited with potential pheromone components as single components, binary blends, and ternary blends.

Treatment	Composition of baits [ $\mu\text{g}$ ] <sup>a</sup>				Mean ( $\pm$ SE) trap catch <sup>b</sup>
	Z7-14:OAc	Z5-14:OAc	Z7-14:OH	Z9-16:OAc	
Experiment 1					
1	500	–	–	–	6.50 $\pm$ 0.62 bc
2	–	500	–	–	1.33 $\pm$ 0.42 d
3	–	–	500	–	0.00 $\pm$ 0.00 d
4	–	–	–	500	0.00 $\pm$ 0.00 d
5	500	200	–	–	18.50 $\pm$ 0.76 a
6	500	–	125	–	8.17 $\pm$ 0.65 b
7	500	–	–	200	5.83 $\pm$ 0.60 c
8	–	200	125	–	0.67 $\pm$ 0.33 d
9	–	200	–	200	0.50 $\pm$ 0.34 d
10	–	–	125	200	0.00 $\pm$ 0.00 d
Virgin females	–	–	–	–	7.50 $\pm$ 0.56 bc
CK (hexane)	–	–	–	–	0.00 $\pm$ 0.00 d
Experiment 2					
11	500	200	–	–	19.00 $\pm$ 0.57 ab
12	500	200	125	–	21.33 $\pm$ 0.76 a
13	500	200	–	200	16.83 $\pm$ 0.60 b
14	500	200	125	200	19.50 $\pm$ 0.67 a

<sup>a</sup> The ratios of the components in baits were similar to those found in the pheromone gland.

<sup>b</sup> Experiments were conducted in Yulin City, Shaanxi Province ( $N = 6$ ). For ANOVA, data were transformed to  $\log(x + 1)$ . Means followed by the same letter are not significantly different at the 5% confidence level by Tukey's test (Experiment 1: June 15–18, 2009,  $F = 104.486$ ,  $df = 11.60$ ,  $P < 0.001$ ; Experiment 2: June 20–23, 2009,  $F = 8.332$ ,  $df = 3.20$ ,  $P < 0.001$ ).

over, we did not observe any significant difference in the catches when Z9-16:OAc was added in the mixture of Z7-14:OAc and Z5-14:OAc, suggesting that Z9-16:OAc was not important for attraction.

In this study, identification was accomplished using the classical method of sampling gland components, solvent extraction. However, this procedure could not avoid contamination with the living tissues, causing some unwanted components such as stearic acid originated from the insects or the glands to be extracted. Recently, a technique for solid-phase microextraction (SPME) of airborne volatiles has been developed and used for the collection of lepidopteran female pheromones (Ando *et al.*, 2004). Compared to the classical solvent extraction method, the optimized SPME method is easier to be performed and suffers much less contamination from the living tissues. Moreover, the SPME sampling results are in good agreement with those obtained by solvent extractions (Chu *et al.*, 2005). Thus, the further verification of componential ratio by SPME may be warranted to find a more effective blend.

In addition, Z7-14:OAc and Z5-14:OAc as the major components for attraction of *H. arenicola* males had been identified as one of the components in sex pheromones of *Elasmopalpus lignosellus* (Lynch *et al.*, 1984), *Ctenopseustis servana* (Foster and Dugdale, 1988), *Autographa nigrisigna* (Sugie *et al.*, 1991), *Holcocerus hippophaecolus* (Fang *et al.*, 2005), and *Holcocerus artemisiae* (Zhang *et al.*, 2009), etc. But the pheromonal composition and componential ratio in these species are not identical to those in *H. arenicola*. In the family Cossidae, the combination (Z5-14:OAc + Z7-14:OAc) as the major components of the sex pheromone is firstly reported in the present paper.

It may be possible to control this pest by focusing on mass trapping or mating disruption. Thus, extensive field trials need to be carried out to determine the best blend ratio and optimum pheromone dosage for the most effective lure. Currently, a triangle trap baited with the synthetic compounds Z7-14:OAc, Z5-14:OAc, and Z7-14:OH in a 1:0.4:0.25 ratio at 825  $\mu\text{g}$ /trap dosage can be used to monitor the *H. arenicola*

population level and catch the males within the desert regions in China.

#### Acknowledgements

This work was funded by the National Science and Technology Support Projects in the 11th Five-

year Plan of China (No. 2006BAD08A10) and the Creative Research Foundation of Shanxi Agricultural University (No. 2004052). We greatly appreciate Professor Xiangbo Kong for his invaluable assistance. We are also grateful to Junjiao Lu for assistance in data analysis.

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