The Sex Pheromone of the Sand Sagebrush Carpenterworm, *Holcocerus artemisiae* (Lepidoptera, Cossidae)

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(*Z*)-5-dodecen-1-ol (Z5–12:OH), (*Z*)-5-dodecenyl acetate (Z5–12:Ac), and (*Z*)-5-tetradecenyl acetate (Z5–14:Ac) were found in the extracts of the female sex pheromone gland of the carpenterworm moth *Holcocerus artemisiae* Chou et Hua, a pest of *Artemisia filifolia*. The average amounts of Z5–12:OH, Z5–12:Ac, and Z5–14:Ac in a single sex pheromone gland of a calling moth were (7.14 ± 0.73) ng, (54.20 ± 0.34) ng, and (38.70 ± 0.46) ng, respectively. Electroantennography (EAG) of these compounds and their analogues demonstrated that Z5–12:Ac excitated the largest male EAG response, followed by Z5–14:Ac. Traps baited with rubber septa impregnated with Z5–12:Ac (500 µg/septum) and Z5–14:Ac (300 µg/septum) were more effective than traps with other baits or virgin females. Addition of Z5–12:OH to rubber septa did not enhance the trap catches, but (*E,Z*)-3,5-dodecadienyl acetate (E3,Z5–12:Ac) enhanced the trap catch.

Key words: Sex Pheromone, (*Z*)-5-Dodecenyl Acetate, *Holcocerus artemisiae*

Introduction

The carpenterworm, *Holcocerus artemisiae* Chou et Hua (Lepidoptera, Cossidae), is a destructive pest for desert thicket. It is widely distributed throughout the northern and western desert regions of China, including Inner Mongolia Autonomous Region, Ningxia Autonomous Region, Xinjiang Autonomous Region, Shaanxi, and Gansu provinces. Its primary host is sand sagebrush (*Artemisia filifolia*). Planted and wild sand sagebrush grow widely in these areas of China to prevent soil erosion and desertification (Wang *et al.*, 2007). The fruits of sand sagebrush are processed for artemisia oil to be used as edible oil. Currently, the sand sagebrush carpenterworm has infested nearly all of the distribution areas of *Artemisia filifolia*, frequently at high levels. In Ningxia Autonomous Region, for example, about 60% of sand sagebrush is infested by *H. artemisiae*, *i.e.*, the sand sagebrush carpenterworm is a major pest in the distribution area of *Artemisia filifolia* in China.

The larvae of *H. artemisiae* burrow in the root crowns of sand sagebrush, which results in plant mortality. Chemical insecticides are ineffective due to the cryptic nature of *H. artemisiae*. In addition, other artificial controls are almost not available for thickeened sands in the desert. So there is an urgent need to establish an alternative strategy for monitoring and controlling sand sagebrush carpenterworms.

Sex pheromones and sex attractants have been reported for seven related Cossidae species, *i.e.* sex pheromones of *Prionoxystus robiniae* (Solomon *et al.*, 1972), *Cossus cossus* (Capizzi *et al.*, 1983), *Cossus mongolicus* (Qi *et al.*, 1990), *Holcocerus insularis* Staudinger (Zhang *et al.*, 2001), *Holcocerus hippophaecolus* (Fang *et al.*, 2005), and sex attractants of *Acossus centeresis* (Doolittle *et al.*, 1976a, b) and *Prionoxystus piger* (Landolt *et al.*, 1985). Sex pheromones of *H. artemisiae* have not been found and characterized. In the present paper, we report the identification of the sex pheromone of *H. artemisiae*. Field trapping studies using blends of synthetic compounds were conducted to develop an efficient trap lure that can be used to monitor and control this pest of sand sagebrush in China.

Material and Methods

Insects

H. artemisiae larvae were collected from roots of sand sagebrush infected with *H. artemisiae* in Ningxia in May 2007~2008 before emergence, and were maintained in a screen cage (6 m × 4 m × 2 m) to allow natural eclosion. Virgin male moths were removed immediately after emergence. The females were left in the original cage for observation and extraction. The antennae of male moths were used for electroantennography (EAG) analyses whereas the abdomial tips of females were used for pheromone extraction and identification.

Collection of the sex pheromone from calling females

After 24 h of observation of female moths in the desert thicket or screen cage, it revealed that the moths began calling and mating ~2 h after sunset. Sex pheromone glands were extruded immediately by applying gentle pressure to the females' abdominal tips to force eversion of the ovipositor, and were excised with small scissors and immersed in re-distilled *n*-hexane (*ca.* 20 μl/tip) containing 1-undecanol as internal standard for 30 min at room temperature. The *n*-hexane extracts were transferred and pooled to a clean conical glass vial and kept in a freezer at –10 °C. The extracts were concentrated carefully before analysis.

Chemicals

Semiochemicals (>98% purity) used in the analytical work, EAG analysis, and lures for field trials were synthesized in our chemical ecology laboratory. Reagents and solvents were from Fisher Chemicals (Fair Lawn, New Jersey, USA).

Thin-layer chromatography of the sex pheromone

The active extracts were subjected to TLC on a silica gel G60 plate developed with petroleum ether/diethyl ether (1:1 v/v). A mixture of (*Z*)-5-dodecenyl acetate (Z5–12:Ac), (*Z*)-5-dodecen-1-ol (Z5–12:OH), and (*Z*)-5-tetradecenyl acetate (Z5–14:Ac) was run as a contrast on another silica gel G60 plate. Horizontal strips were scraped off from the plate and extracted with re-distilled *n*-hexane.

Chemical analysis

The GC analyses of sex pheromone gland extracts and standard compounds were performed on a HP 6890 gas chromatograph fitted with a flame ionization detector (FID) and a splitless injector. Two fused silica capillary columns, A (HP-1, 50 m × 0.22 mm × 0.33 μm, Hewlett Packard, Paloalto CA, USA) and B (BP10–0.5, 50 m × 0.42 mm × 0.33 μm, SGE, Australia), were used with the following temperature program: 80 °C for 1 min, then 4 °C/min to 180 °C and 10 °C/min to 240 °C, isothermal for 14 min for column A; 80 °C for 2 min, then 4 °C /min to 200 °C, isothermal for 25 min for column B. Gas chromatography-mass spectrometry (GC-MS) analyses were performed on a Finnigan Trace 2000 Voyage instrument interfaced with a Voyager mass spectrometer. Fused silica capillary columns (HP-1, 50 m × 0.22 mm × 0.33 μm) were used with the following temperature program: 80 °C/min to 280 °C, isothermal for 20 min. Mass spectral data and retention times of selected peaks on both columns were compared to the corresponding data of reference standards.

Electroantennography (EAG)

EAG assays were performed as previously described (Roelofs *et al.*, 1971; Zhang and Meng, 2000). Electrophysiological responses of antennae dissected from 1- to 2-d-old males to a series of C_{12} and C_{14} unsaturated alcohols and acetates and TLC extracts were measured. Solvent blank puffs (filter paper and hexane) were used as the controls.

Field tests

Trapping tests were carried out during the *H. artemisiae* flight season (June to August 2007~2008) in Lingwu city, Ningxia Autonomous Region, China. Semiochemicals were dissolved in petroleum ether and loaded onto green rubber septa. White delta sticky traps were hung on wooden support about 1 m in height, and 50-m intervals. The insect traps were checked every day. 1- to 2-d-old virgin females were put into a small screen cage in the centre inside the trap as bait for comparison. Petroleum ether was used as control. Each formulation was repeated 6 times in a randomized block. The experiments were designed to examine the attraction of different chemical compositions and

the optimum dosage of each active component. Captured moths were recorded and removed daily.

Synthesis of Z5–12:OH, Z5–12:Ac, and Z5–14:Ac

The title compounds were synthesized via Wittig reaction (Horiike *et al.*, 1980) (Fig. 1).

The acetyl bromide **1a** reacted with tetrahydropyran **2a** to produce 5-bromo-*n*-pentyl acetate **3a** (key step), which was further converted into the triphenyl phosphonium salt **4a**. By Wittig reaction of **4a** with nonaldehyde Z5–14:Ac (**5a**) was produced. Also by Wittig reaction of **4a** with heptaldehyde Z5–12:Ac (**6a**) was produced. The acetate was transformed to Z5–12:OH (**7a**) by hydrolysis.

All products were purified by silica gel column chromatography; the purity of the compounds was more than 98%.

Results

Analysis of sex pheromone gland extracts

Extracts of 10 female equivalent (FE) sex pheromone glands were subjected to TLC and separated into four bands: Rf_{01} 0.05 (TLC_1), Rf_2 0.37 (TLC_2), Rf_3 0.70 (TLC_3), and Rf_4 0.73 (TLC_4) on the plate, of which TLC_2, TLC_3, and TLC_4 showed the same Rf values like the synthetic compounds Z5–12:OH, Z5–14:Ac, and Z5–12:Ac, respectively. The bands were scraped off from the plate and extracted with re-distilled *n*-hexane. The extract was concentrated to 40 μl. Male antenna responses to the extracts (5 FE/20 μl) were analyzed by EAG. The extract of TLC_4 caused high electrophysiological responses (4.52 mV) of antennae of males, whereas TLC_3 and TLC_2 caused moderate (2.73 mV) and small responses (0.52 mV), respectively.

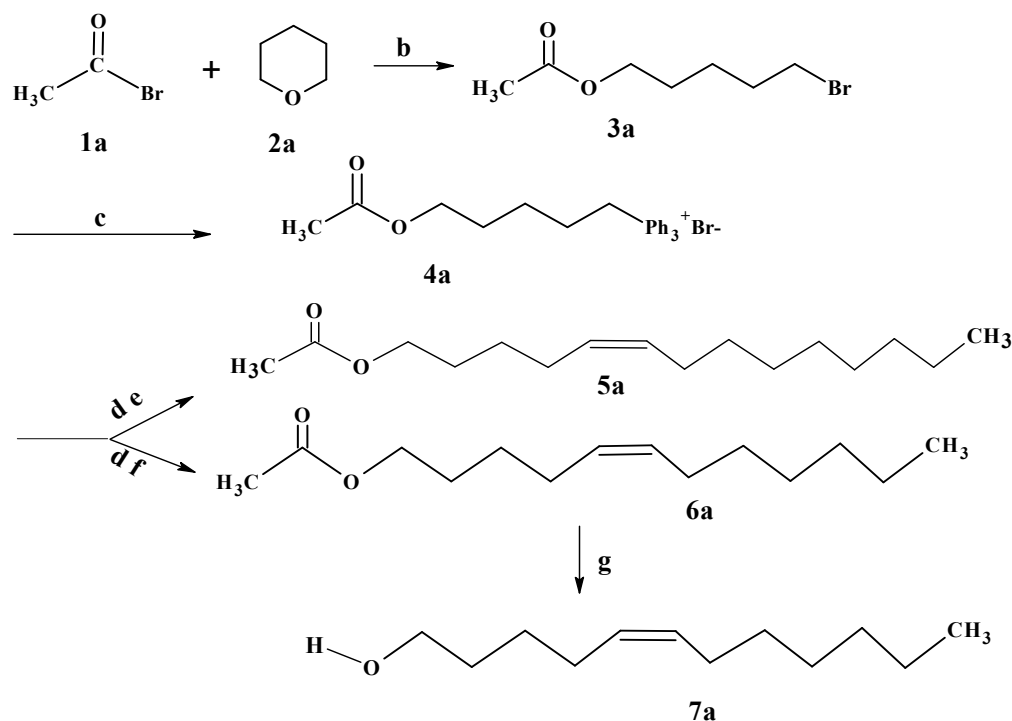GC of female sex pheromone gland extracts and a series of *Z* and *E* isomers of monounsaturated

Fig. 1. Reaction scheme of the synthesis of (*Z*)-5-dodecenyl acetate, (*Z*)-5-dodecen-1-ol and (*Z*)-5-tetradecenyl acetate. (b) Zn; (c) Ph_3P/C_6H_6; (d) *n*-BuLi/DMSO; (e) $CH_3(CH_2)_7CHO$; (f) $CH_3(CH_2)_5CHO$; (g) NaOH, H_2O, CH_3OH.

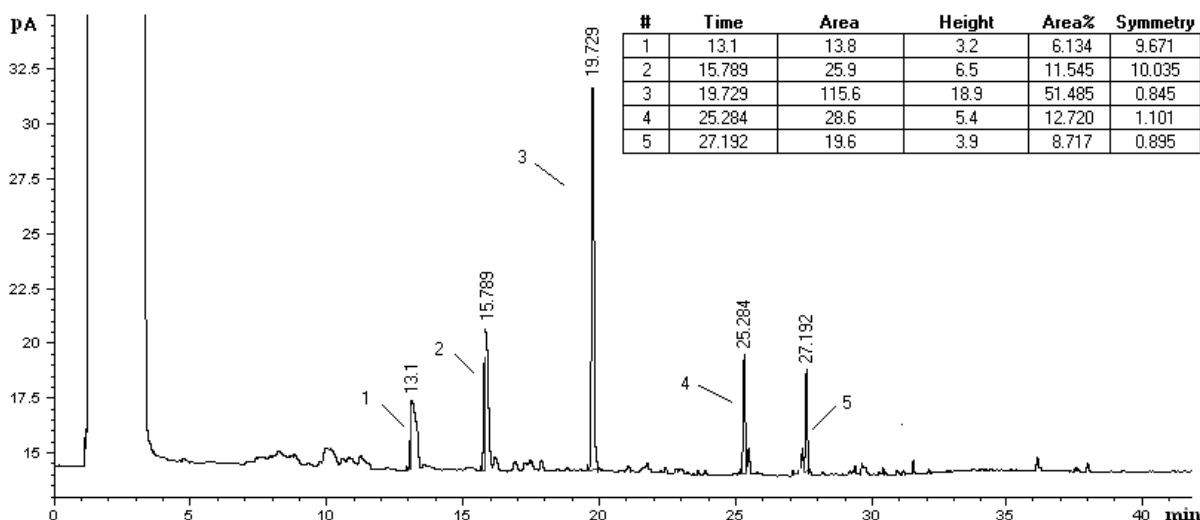| #	Time	Area	Height	Area%	Symmetry
1	13.1	13.8	3.2	6.134	9.671
2	15.789	25.9	6.5	11.545	10.035
3	19.729	115.6	18.9	51.485	0.845
4	25.284	28.6	5.4	12.720	1.101
5	27.192	19.6	3.9	8.717	0.895

Fig. 2. Gas chromatogram (HP-1) of the pheromone gland extracts (0.2 μl) of *H. artemisiae* and 1-undecanol (internal standard, 5 ng).

C_{12} and C_{14} standards was conducted on either of the capillary columns A or B under different temperature conditions. In comparison with the retention times (Rt), peaks 2, 3 and 4 (Fig. 2) consistently co-chromatographed with the synthetic standard compounds Z5–12:OH, Z5–12:Ac, and Z5–14:Ac. The extracts TLC_2, TLC_3, and TLC_4 gave peak 2, peak 4 and peak 3, respectively. In addition, when 0.2 μl extract and 0.2 μl blend of each 1-undecanol (internal standard, 5 ng), Z5–12:OH, Z5–12:Ac, and Z5–14:Ac were injected simultaneously into column A, the peaks 2, 3, 4 corresponding to the compounds increased. These results, together with those above mentioned, strongly suggest that Z5–12:OH, Z5–12:Ac, and Z5–14:Ac are present in the sex pheromone of *H. artemisiae*. Moreover, quantitative analysis by GC showed that the ratio of them varied around a mean of (7.14 ± 0.73) ng, (54.20 ± 0.34) ng, and (38.70 ± 0.46) ng, respectively in a single calling female sex pheromone gland.

TIC showed the presence of four peaks (Fig. 3, peaks I, II, III, IV) in the extracts, which were identical with those of synthetic standard compounds. All mass data are listed in Table I. Although peaks I and II produced a common ion fragment of *m/z* 166, which indicated that they have a similar straight-chain structure, peak I had characteristic ion fragments of *m/z* 71 ($C_4H_6OH^+$) of alcohol (Silverstein *et al.*, 1981), peaks II and III

had *m/z* 61($CH_3COOH_2^+$) of acetate (Brown *et al.*, 1988). This confirmed that peaks I, II and III were those of a monounsaturated alcohol and acetate. The comparison of indices (*m/z* of characteristic ion fragments) of the extracts and standard compounds indicated that peaks I–III were those of 5-dodecen-1-ol, 5-dodecenyl acetate and 5-tetradecenyl acetate, peak IV (Fig. 3) was that of a saturated C_{16} fatty acid (MW 256). Finally, based on the computation according to the fussy reasoning double band positional isomers method (Horiike *et al.*, 1990, 1991) and the comparison of the Rf (TLC), Rt (GC), and spectral data (GC-MS) with those of authentic synthetic compounds, we concluded that peaks I–III were those of Z5–12:OH, Z5–12:Ac, and Z5–14:Ac, respectively.

Electroantennography

EAG responses of male *H. artemisiae* to the gland components and their analogues varying in double bond positions and configurations are summarized in Fig. 4. Results show that Z5–12:Ac elicited the strongest response (5.21 mV), followed by E3,Z5–12:Ac [(*E,Z*)-3,5-dodecadienyl acetate; 3.52 mV] and Z5–14:Ac (3.13 mV) among the compounds tested. The EAG response elicited from each acetate was higher than that from the corresponding alcohol. Though the EAG response of E3,Z5–14:Ac was higher than that of
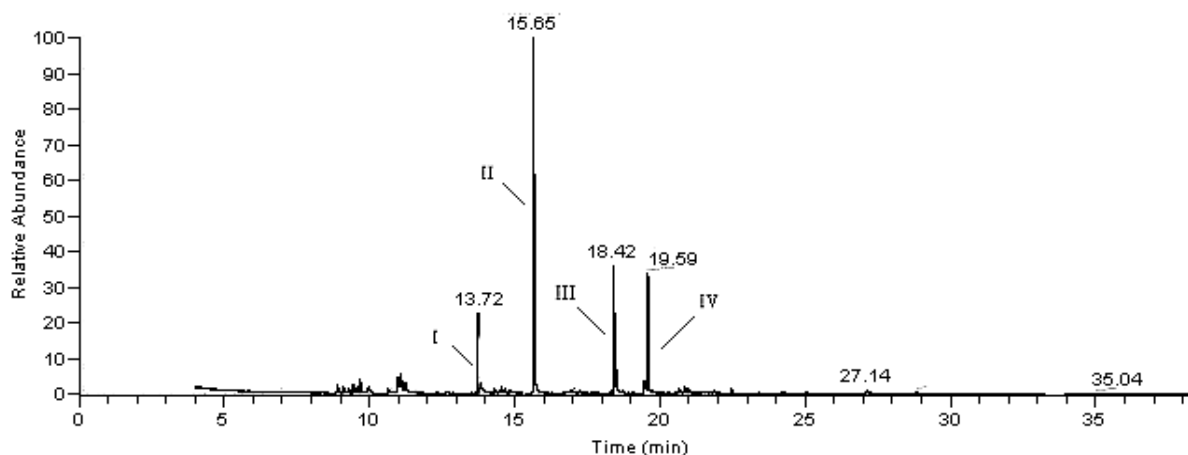
Fig. 3. EI (70 eV) mass spectrum showing the TIC of sex pheromone gland extracts of *H. artemisiae*.

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

Compo-nent	Standard	m/z (relative intensity of major ions) [assignment]
I	Z5–12:OH	166(8) [M$^+$-18], 138(8) [M$^+$-(60+28)], 124(9), 109(26), 96(43), 95(57), 82(73), 81(82), 71(12), 68(51), 67(100), 55(50), 54(41)
II	Z5–12:Ac	166(28) [M$^+$-60], 138(24) [M$^+$-(60+28)], 124(13) [M$^+$-(60+42)], 110(39), 96(75), 95(74), 82(86), 81(85), 68(56), 67(100), 61(3), 55(37), 54(36)
III	Z5–14:Ac	194(22)[M$^+$-60], 166(10) [M$^+$-(60+28)], 152(2) [M$^+$-(60+42)], 96(80), 95(72), 82(100), 81(80), 68(48), 67(86), 61(3), 55(32), 54(34)

Z5–14:Ac, it was not detected in the extract of glands.

Field trapping

Traps baited with a single gland component, Z5–12:OH, Z5–12:Ac or Z5–14:Ac, failed to attract male *H. artemisiae* in the field. However, traps baited with the two gland components Z5–12:Ac and Z5–14:Ac in a 1:0.6 ratio captured more males than control traps (Table II). The catch activity was enhanced by combination with E3,Z5–12:Ac.

Discussion

The analytical data, electrophysiology and field trapping tests indicated that the extracts from the sex pheromone glands of *H. artemisiae* contained three compounds, Z5–12:OH, Z5–12:Ac, and Z5–14:Ac. We have demonstrated that Z5–12:Ac and Z5–14:Ac are the major components of the sex pheromone of *H. artemisiae*. These two com-pounds are produced by adult females, and the antennae of male *H. artemisiae* are highly sensi-tive to both of them. In field tests, traps baited with these two compounds in a 1:0.6 ratio were attractive to adult males (Table II). Both com-pounds were required for the attraction, and had been identified as Lepidoptera Cossidae sex phe-romone before. *H. artemisiae* belongs to the Cos-sidae, in which the common structures of sex phe-romones identified consist of monounsaturated dodecenyl acetates or tetradecenyl acetates with the double bond in position 5 or diunsaturated tetradecadienyl acetates with the conjugated dou-ble bond in positions 3 and 5.

Although a significant amount of Z5–12:OH was detected in the extracts from the sex phe-romone gland, and it resulted in a low-level EAG response of male moths, it could hardly catch male moths. Moreover, we did not observe any significant difference in the catches when Z5–12:OH was add to the mixture of Z5–12:Ac and Z5–14:Ac. In contrast, when E3,Z5–12:Ac

was added to the mixture, the trap catches of male moths were slightly increased. This showed that E3,Z5–12:Ac likely has a synergistic effect.

During the course of our studies, we found that *H. artemisiae* lacks instrumenta suctoria. There-

fore, adults are unable to eat during their short lifespan. They mate only once and lay eggs within their short period of adult life. These facts suggest that it may be possible to control this pest by focusing on mass trapping or mating disrup-
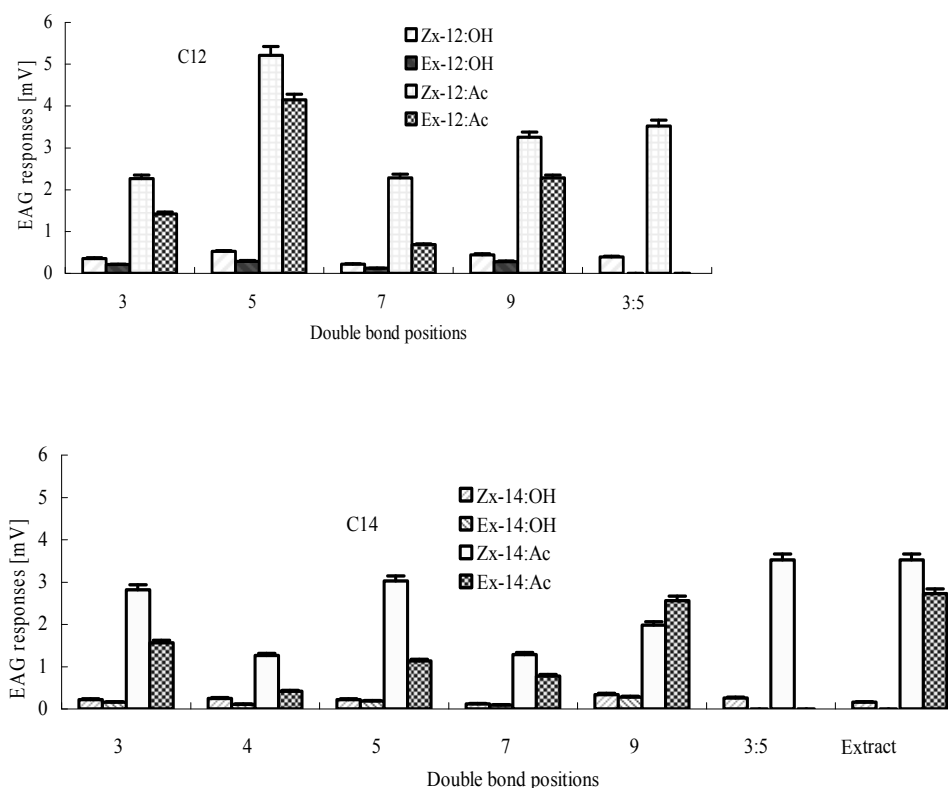

Fig. 4. EAG responses of male *H. artemisiae* to compounds (1 μg) and sex pheromone extract; 6 replicates. Bars indicate mean ± SE; extract: TLC$_2$, TLC$_4$, TLC$_3$.

Table II. Field attraction of male *H. artemisiae* in traps baited with various chemicals[a].

Treatment	Composition of baits [μg]				Mean (± SE) trap catch[b]	
	Z5–12:Ac	Z5–14:Ac	E3,Z5–12:Ac	Z5–14:OH	2007	2008
1	500				0.00c	0.00c
2		500			0.00c	0.00c
3			500		0.00c	0.00c
4				500	0.00c	0.00c
5	500	300	0	0	188 ± 2.68d	124 ± 4.25d
6	500	300	0	500	184 ± 5.10d	121 ± 5.37d
7	500	300	25	0	213 ± 3.16e	176 ± 3.58e
CK (hexane)	0	0	0	0	0.00c	0.00c

[a] Caught in Lingwu city, Ningxia Autonomous Region, China, July 3–July 17, 2007 and July 20–August 4, 2008.
[b] Six replicates; means followed by the same letter are not significantly different by Duncan's multiple range test ($p = 0.05$).

tion. However, development of a mass trap or mating-disruption system requires identification of the optimum pheromone dosage on lures and determination of any other potential synergists. Currently, a triangle trap baited with the synthetic compounds Z5–12:Ac, Z5–14:Ac, and E3,Z5–12:Ac in a 1:0.6:0.05 ratio at 825 µg/trap dosage can be used to monitor the *H. artemisiae* population level and catch the males within the desert regions in China.

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