Journal of Chemical Ecology, Vol. 31, No. 1, January 2005 (© 2005)
DOI: 10.1007/s10886-005-0972-6

SEX PHEROMONE COMPONENTS OF THE SANDTHORN CARPENTERWORM, *Holcocerus hippophaecolus*

YU-LING FANG, JIANG-HUA SUN, CHENG-HUA ZHAO, and ZHONG-NING ZHANG*

State Key Laboratory of Integrated Management of Pest Insects and Rodents
Institute of Zoology, Chinese Academy of Sciences
Beijing 100080, P. R. China

(Received March 5, 2004; accepted September 22, 2004)

Abstract—Extracts of female sex pheromone gland of the carpenterworm moth, *Holcocerus hippophaecolus* Hua, a pest of sandthorn, *Hippophae rhamnoides* L. were found to contain (*E*)-3-tetradecenyl acetate (E3-14:Ac), (*Z*)-3-tetradecenyl acetate (Z3-14:Ac), (*Z*)-7-tetradecenyl acetate (Z7-14:Ac), the corresponding alcohols, E3-14:OH, Z3-14:OH, Z7-14:OH, and (*E*)-9-tetradecenyl acetate (E9-14:Ac). Electroantennographic (EAG) analysis of these chemicals and their analogs demonstrated that Z7-14:Ac elicited the largest male EAG response, followed by E3-14:Ac. In field trials, traps baited with either Z7-14:Ac or E3-14:Ac alone caught no male moths, whereas a combination of these two components in a 1:1 ratio caught more males than control traps. Addition of Z7-14:OH and Z3-14:OH or the alcohols plus E9-14:Ac did not enhance trap catches. We conclude that the sex pheromone of *H. hippophaecolus* is composed of Z7-14:Ac and E3-14:Ac. Optimal ratios and doses of these two components, and the possible role of other minor components, remain to be determined.

Key Words—*Holcocerus hippophaecolus*, sex pheromone, (*E*)-3-tetradecenyl acetate, (*Z*)-7-tetradecenyl acetate, Lepidoptera, Cossidae.

INTRODUCTION

The carpenterworm, *Holcocerus hippophaecolus* Hua (Lepidoptera: Cossidae) is a destructive forest pest, widely distributed throughout northern and western regions of China, including Inner Mongolia Autonomous Region, Ningxia Autonomous Region, Shanxi, Shaanxi, Gansu, and Liaoning provinces. Its primary host is the sandthorn plant (*Hippophae rhamnoides* L.). Sandthorns have been planted widely

* To whom correspondence should be addressed. E-mail: zhangzn@panda.ioz.ac.cn

in China (>1.4 million ha), often in single-species plantations, to prevent soil erosion and desertification (Tian and Tang, 1997). The fruits of sandthorn are processed for juice and traditional Chinese medicines. Currently, the sandthorn carpenterworm infests sandthorn plantations totaling 50,000 ha in area, often at high levels. In Liaoning Province, for example, infestation levels of sandthorns by *H. hippophaecolus* can reach 85% (Zhou, 2002; Luo et al., 2003). The damage is so severe and extensive that the sandthorn carpenterworm is considered a major threat to the continued existence of sandthorn plantations in China.

Larvae of *H. hippophaecolus* burrow in the root crowns of sandthorns, resulting in a high probability of plant mortality. Chemical insecticides are ineffective due to the cryptic nature of *H. hippophaecolus*. In addition, the indiscriminate use of insecticide can lower the quality of sandthorn. Thus, an alternative strategy for monitoring and controlling sandthorn carpenterworms is urgently needed.

Sex pheromones have been identified for three related Cossidae species: *Cossus cossus* L. (Capizzi et al., 1983), *Cossus mongolicus* Ersch (Qi et al., 1990), and *Holcocerus insularis* Staudinger (Zhang et al., 2001). Attractants for *H. hippophaecolus* have not been reported. In this paper, we report identification of the sex pheromone for *H. hippophaecolus*. Field trapping studies using blends of synthetic compounds were conducted to develop an efficient trap lure that can be used to monitor this important pest of sandthorns in China.

METHODS AND MATERIALS

Insects. Roots of sandthorn infested with larval *H. hippophaecolus* were collected from Jianping county, Liaoning Province, in the spring of 2003. The roots were kept at 25 ± 1°C and 20 ± 1°C during light and dark hours, respectively, at 65 ± 5% RH, under 16L:8D photoperiod. Pupae were collected from soil and kept under the same conditions until moth emergence. Upon emergence, male and female moths were held at room temperature under a 16L:8D photoperiod. The antennae of male moths were used for electroantennographic (EAG) analyses whereas the abdominal tips of females were used for pheromone extraction and identification.

Chemicals. Semiochemicals (>98% purity) used in analytical work, EAG analysis, and lures for field trials were supplied by Plant Research International (Wageningen, The Netherlands). Reagents and solvents were obtained from Fisher Chemicals (New Jersey, USA), and dimethyl disulfide (DMDS) was obtained from ACROS Organics (New Jersey, USA).

Pheromone Extracts. Observations of 1-d-old virgin female moths for 24 hr revealed that their calling period was generally 2.5–5.5 hr into the scotophase. During this period, 84% of females were calling. The pheromone glands of calling females were extruded by gently pressing the abdominal tips, and then excised with a small blade. The single excised gland was immersed in 10 μl hexane containing

delta13–14:OH (1 ng) as internal standard for 20 min at room temperature and then immediately analyzed by gas chromatography (GC) or gas chromatography–mass spectrometry (GC–MS). The remaining extract was transferred into a clean conical glass vial and kept at −20°C for additional analysis.

Derivatization with Dimethyl Disulfide. Pheromone extracts were reacted with DMDS (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 12 FE (female equivalents) and kept at 40°C overnight. After the addition of 10 μl hexane and 20 μl of aqueous sodium thiosulfate (5%), the mixture was stirred and the hexane layer was transferred into a small vial, and concentrated under nitrogen prior to analysis by GC–MS.

Chemical Analysis. GC analyses of sex pheromone gland extracts were performed on an HP 5890 II GC with a flame ionization detector (FID) and a splitless injector using nitrogen as carrier gas. The GC conditions were as follows: BP-20 column (50 m × 0.25 mm i.d., Scientific Glass Engineering Pty. Ltd., Australia); temperature program, 50°C for 1 min to 150°C at 25°C/min, then 4°C/min to 200°C, hold for 10 min, finally 5°C/min to 210°C and hold for 30 min. The GC–MS analyses of pheromone gland extracts were performed on an HP 6890 GC interfaced with an HP 5973 mass selective detector using a 60 m × 0.22 mm i.d. HP-INNOWAX column. The temperature program was the same as that used in the GC analyses. Analyses of the DMDS derivatives were carried out on the same GC–MS instrument with a DB-5MS column (30 m × 0.25 mm i.d., J&W Scientific, USA). The column temperature was 100°C for 1 min then programmed at 10°C/min to 230°C, and hold for 20 min.

Electroantennograms. Dissected antennae from 1- to 3-d-old males were used for EAG measurements. Previously described methods (Fang and Zhang, 2002) were used for determining EAG responses to a series of monounsaturated 14-carbon alcohols and acetates. An aliquot of hexane solution of each tested compound was deposited onto a filter paper strip (5 mm × 30 mm). After allowing the solvent to evaporate at room temperature, the filter paper was inserted into a Pasteur pipette. A stimulus was introduced as a short puff (2 ml air) into a purified, humidified air stream from the Pasteur pipette, through a stainless steel tube positioned 9 cm from the antennae. The duration of each puff was kept at about 0.1 sec. Solvent blank puffs (filter paper and hexane) served as the controls. To compensate for possible deterioration of the antennal preparation, a stimulation with a reference compound, (*E*)-5-tetradecenol (E5-14:OH) (1 μg), preceded each test stimulus puff. Relative response to a test compound was expressed as a percentage relative to the response to E5-14:OH.

Field Trials. Trapping tests were carried out in Jianping County, Liaoning Province from June 15 to July 7, 2003. Semiochemicals were dissolved in hexane and loaded onto gray rubber septa (The West Company, Phoenixville, PA) at a dose of 500 μg/septum. After the solvent had evaporated, 2 × 100 μl aliquots

of dichloromethane were added to each septum to help the chemical permeate into the septum. Sticky traps, similar to the Pherocon 1C trap (Trécé Inc., Salinas, CA), were constructed from two pieces of cardboard (42 × 28 cm). All traps were hung on branches of *Hippophae rhamnoides* at 1.5–1.8 m above ground level. Seven synthetic chemical blends and a blank control were tested in a completely randomized block design with five replicate blocks. Traps were spaced 50 m apart and the trap positions were rotated to minimize the effects of habitat heterogeneity. Captured mothes were recorded and removed daily.

Statistical Analysis. Differences among the EAG responses to different compounds were analyzed by one way ANOVA. Values for field traps were compared with the Bonferoni test. All data were analyzed with a statistical program for Windows 11.0 (SPSS Inc., 2001).

RESULTS

Analysis of Sex Pheromone Gland Extracts. Sex pheromone gland chemicals of female *H. hippophaecolus* were identified and quantified by GC–MS and GC. The GC–MS analyses of the gland extracts revealed that the mass spectra of peaks I, II, III, and IV were consistent with those of monounsaturated 14-carbon acetates with a comparatively strong fragment at m/z 194 (27–30% of the base peak, loss of acetic acid), and a small but distinct fragment at m/z 61 (1–8%, $CH_3COOH_2^+$) (Figure 1, Table 1). The mass spectra of peaks V, VI, and VII contained diagnostic ions at m/z 194 (10–12% of the base peak, loss of H_2O) and m/z 31 (12–14%, CH_2OH^+). This suggested that the compounds were monounsaturated 14-carbon primary alcohols. After the comparison of the relative intensities of five diagnostic ions $[(m_1/z)/(m_2/z)]$: 54/55, 67/68, 81/82, 81/95, and 95/96 with literature data on positional isomers of 14-carbon acetates and alcohols (Horiike et al., 1990, 1991; Zhang et al., 2001) we determined the identities of the seven compounds as follows: peak I and II were 3-tetradecenyl acetates (3-14:Ac), peak III was a 7-tetradecenyl acetate (7-14:Ac), peak IV was a 9-tetradecenyl acetate (9-14:Ac); peak V and VI were 3-tetradecenols (3-14:OH), and peak VII was a 7-tetradecenol (7-14:OH).

Double bond locations of the pheromone components were further confirmed by analyses of their DMDS derivatives. Analyses revealed the existence of DMDS adducts derived from 7-14:OH (diagnostic ions at m/z 145, 161, and 306 [M^+]); 3-14:OH (diagnostic ions at m/z 105, 201, and 306 [M^+]); 3-14:Ac (diagnostic ions at m/z 87, 147, 201, and 348 [M^+]); 7-14:Ac (diagnostic ions at m/z 143, 145, 203, and 348 [M^+]); and 9-14:Ac (diagnostic ions at m/z 117,171, 231, and 348 [M^+]). The DMDS derivatives of E3-14:Ac and Z3-14:Ac and the corresponding alcohols could not be separated efficiently by DB-5MS column in our GC–MS system.

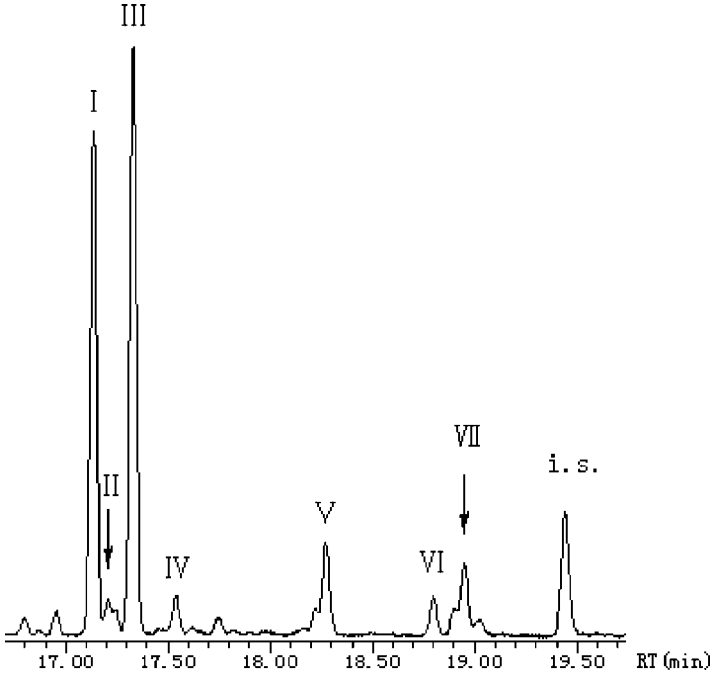

FIG. 1. Total ion chromatogram of GC–MS analysis of a single-gland extract from a female *H. hippophaecolus*. Identities of components are as follows: (I) E3-14:Ac; (II) Z3-14:Ac; (III) Z7-14:Ac; (IV) E9-14:Ac; (V) E3-14:OH; (VI) Z3-14:OH; and (VII) Z7-14:OH. Internal standard (i.s.): delta-13-14:OH (1 ng).

The configurations of monounsaturated 14-carbon compounds in the pheromone gland were first determined by a comparison of their retention times with those of standard compounds on a BP-20 GC column. However, the separation for Z7-14:Ac and E7-14:Ac was still poor, whereas the other components were well separated and were determined to be Z3-14:OH, E3-14:OH, Z3-14:Ac, E3-14:Ac, and E9-14:Ac. In GC–MS analyses, a more polar column, HP-INNOWAX was used. With this column, all possible isomers in female extracts were separated and the configurations of all monounsaturated 14-carbon compounds, including Z7-14:Ac, in the gland extracts were determined (Table 2). In the analyses of the single gland extracts, we found that the two gland components, E3-14:Ac and Z7-14:Ac were detected in calling females but not in noncalling ones. In calling females, the titer of Z7-14:Ac varied from 8.3 to 52 ng, whereas the titer of E3-14:Ac varied from 6.4 to 60.9 ng. Relative amounts of E3-14:Ac and Z7-14:Ac were equal, whereas for other components, the relative amounts were much lower than that of Z7-14:Ac (Table 2).

TABLE 1. MASS SPECTRAL DATA FOR CHEMICALS IN SEX PHEROMONE GLAND EXTRACTS

Component	Identity	Relative intensity of major ions (m/z)
I	3-14:Ac	194 (27) [M^+–60], 152 (10), 110 (23), 96 (66), 95 (37), 82 (71), 81 (57), 68 (67), 67 (65), 61 (1) [$CH_3COOH_2^+$], 55 (39), 54 (60), 43 (100) [$O{=}C^+CH_3$]
II	3-14:Ac	194 (27) [M^+–60], 152 (11), 110 (24), 96 (62), 95 (39), 82 (71), 81 (60), 68 (74), 67 (62), 61 (1) [$CH_3COOH_2^+$], 55 (39), 54 (57), 43 (100) [$O{=}CCH_3^+$]
III	7-14:Ac	194 (30) [M^+–60], 152 (3), 110 (31), 96 (85), 95 (69), 82 (100), 81 (89), 68 (46), 67 (92), 61 (8) [$CH_3COOH_2^+$], 55 (62), 54 (42), 43 (82) [$O{=}CCH_3^+$]
IV	9-14:Ac	194 (30) [M^+–60], 152 (3), 110 (37), 96 (84), 95 (65), 82 (100), 81 (92), 68 (45), 67 (78), 61 (10) [$CH_3COOH_2^+$], 55 (87), 54 (42), 43 (72) [$O{=}CCH_3^+$]
V	3-14:OH	194 (10) [M^+–H_2O], 166 (6) [M^+–C_2H_5OH], 109 (23), 96 (56), 95 (43), 82 (87), 81 (66), 68 (100), 67 (72), 55 (94), 54 (34), 31 (14) [CH_2OH^+]
VI	3-14:OH	194 (8) [M^+–H_2O], 166 (6) [M^+–C_2H_5OH], 109 (19), 96 (48), 95 (40), 82 (80), 81 (70), 68 (100), 67 (71), 55 (74), 54 (30), 31 (12) [CH_2OH^+]
VII	7-14:OH	194 (11) [M^+–H_2O], 166 (4) [M^+–C_2H_5OH], 109 (33), 96 (65), 95 (65), 82 (94), 81 (87), 68 (57), 67 (100), 55 (79), 54 (44), 31 (14) [CH_2OH^+]

TABLE 2. RETENTION TIMES AND RELATIVE QUANTITIES OF GLAND CHEMICALS AND KNOWN STANDARDS[a]

Retention time (min)				Mean (± SE) percentage relative to quantity of Z7-14:Ac ($N = 17$)
Synthetic compound		Component in female extracts		
17.135	E3-14:Ac	Peak I	17.135	102.5 ± 21.6
17.212	Z3-14:Ac	Peak II	17.211	17.6 ± 5.1
17.303	E7-14:Ac			
17.338	Z7-14:Ac	Peak III	17.336	100
17.445	E9-14:Ac	Peak IV	17.445	9.3 ± 5.6
17.541	Z9-14:Ac			
18.275	E3-14:OH	Peak V	18.277	28.1 ± 10.2
18.805	Z3-14:OH	Peak VI	18.801	21.2 ± 9.8
18.877	E7-14:OH			
18.953	Z7-14:OH	Peak VII	18.957	18.5 ± 7.7

[a] Temperature programs of GC column and determination of relative amounts of the gland components are described in "Methods and Materials."

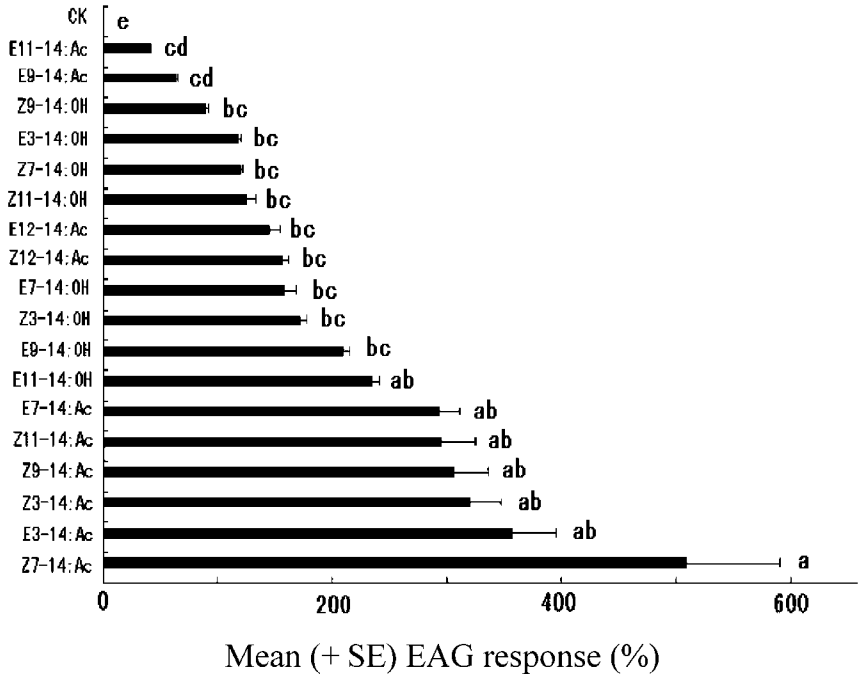

FIG. 2. EAG responses of male *H. hippophaecolus* (relative to responses to E5-14:OH) to a series of monounsaturated 14-carbon alcohols and acetates at doses of 10 μg ($N = 30$). Bars followed by different letters are significantly different at $P = 0.05$. Check control (ck). Response to standard application of 10 μg (*E*)-5-tetradecenol (E5-14:OH) was 4.56 $\pm$ 1.77 mV.

Electroantennographic Analyses. Male EAG responses to the gland components and their analogues varying in double bond positions and configurations are summarized in Figure 2. Results show that Z7-14:Ac elicited the strongest response (29.59 mV), followed by E3-14:Ac. Among tested compounds, the EAG response elicited from each acetate was higher than that from the corresponding alcohol, except for E11-14:Ac, which elicited a much lower EAG response than the corresponding alcohol, E11-14:OH.

Field Trapping. Traps baited with a single gland component, Z7-14:Ac or E3-14:Ac failed to attract male *H. hippophaecolus* males in the field. However, traps baited with the two gland components in a 1:1 ratio captured more males than control traps (Table 3). Catches were not affected by addition of Z7-14:OH and Z3-14:OH or the mixture of Z7-14:OH, Z3-14:OH, and E9-14:Ac.

TABLE 3. MEAN CATCHES OF MALE *H. hippophaecolus* IN TRAPS BAITED WITH VARIOUS SEX PHEROMONE GLAND CHEMICALS

	Composition of baits/μg[a]							
	Acetates					Alcohols		Mean ($\pm$ SE)
Treatment	Z7-14:Ac	E3-14:Ac	E7-14:Ac	Z3-14:Ac	E9-14:Ac	Z3-14:OH	Z7-14:OH	trap catch[b]
A	500							0
B		500						0
C	250	250						30.0 ± 4.8*
D		250	250					0
E	250			250				0
F	250	250				65	50	12.7 ± 4.1*
G	250	250			16	65	50	34.0 ± 7.5*
CK	0	0	0	0	0	0	0	0

Notes. Mean difference: C–F = 1.7700, Sig.: C–F = 0.091; mean difference: C–G = 0.1500, Sig.: C–G = 1.000; mean difference: C–A, C–B, C–D, C–E, C–CK = 2.1429, Sig.: C–A, C–B, C–D, C–E, C–CK = 0.0000; mean difference: F–A, F–B, F–D, F–E, F–CK = 0.8714, Sig.: F–A, F–B, F–D, F–E, F–CK = 0.004; mean difference: G–A, G–B, G–D, G–E, G–CK = 2.4286, Sig.: G–A, G–B, G–D, G–E, G–CK = 0.0000.

[a] The ratios of the components in baits were similar to that found in the pheromone gland.

[b] The mean difference is significant at the $P = 0.05$ level.

DISCUSSION

We have demonstrated that E3-14:Ac and Z7-14:Ac are the major components of the sex pheromone of *H. hippophaecolus*. The two compounds are produced by adult females, and the antennae of male *H. hippophaecolus* were highly sensitive to both compounds. In field trials, traps baited with the two compounds in a 1:1 ratio were attractive to adult males (Table 3).

Both compounds were required for attraction to occur. Sex pheromones and attractants identified to date for carpenterworms have been mono- or di-unsaturated straight chain acetates, most being 14-carbon acetates. Although Z7-14:Ac has been reported as a pheromone component for species in many families of Lepidoptera, E3-14:Ac has been reported only in *Symmetrischema tangolias* (Gyen) (Gelechiidae) (Griepink et al., 1995).

During the course of our studies, we found that *H. hippophaecolus* lacks *instrumenta suctoria*. Adults are, therefore, unable to feed during their short lifespan. They mate and lay eggs within a short period of time, with female moths mating only once during their adult life (Tian and Tang, 1997). These facts suggest that it may be possible to control this important pest by mass trapping through focused trap efforts or by mating disruption. However, development of a mass trapping or mating-disruption system will require identification of the optimum pheromone dosage on lures and determination of any other potential synergists. Currently, a wing trap baited with the synthetic compounds Z7-14:Ac and E3-14:Ac in a 1:1 ratio at 500 μg dosage can be used to monitor population levels of *H. hippophaecolus* within plantations of sandthorn in China.

Acknowledgments—We greatly appreciate the through review and editing of the manuscript by Nancy Gillette and Dan Miller (USDA, Forest Service) and the helpful comments on the manuscript by M. Renou (INRA, Unite de Phytopharmacie et Mediateurs Chimiques, Versailles), Michael Smith (USDA, ARS) and Xun Liu (Institute of Zoology, Chinese Academy of Sciences). Thanks are also extended to Wu Jian, State Forestry Administration, and Zhang Liansheng, Jianping County Forest Pest Control Station, Liaoning Province for collecting insects used in the study and assistance in field trapping. This work was partially supported by the CAS Knowledge Innovation Program (KSCX1-SW-13).

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