

Sex Pheromone Components of the Female Black Cutworm Moth in China: Identification and Field Trials

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Abstract: The sex pheromone blend of a China strain of the black cutworm moth *Agrotis ypsilon* (Rotttemberg) (Lepidoptera: Noctuidae) was investigated. Chemical analysis of pheromone gland extracts of 3-day-old females showed that individual isolated glands contained only very small amounts of pheromone. The high-resolution gas chromatography combined with mass spectrometry (GC-MS) analysis showed the presence of *Cis*-7-dodecenyl acetate (Z7-12:Ac, A), *Cis*-9-tetradecenyl acetate (Z9-14:Ac, B), *Cis*-11-hexadecenyl acetate (Z11-16:Ac, C), *Cis*-8-dodecenyl acetate (Z8-12:Ac, E) and *Cis*-5-decenyl acetate (Z5-10:Ac, D) in biologically active pheromone gland extracts. An extract of one gland from a day 3 female gave the following values for the gland components: 0.245±0.098 ng for Z7-12:Ac, 0.080±0.031 ng for Z9-14:Ac, 0.089±0.033 ng for Z11-16:Ac, 0.085±0.031 ng for Z5-10:Ac, 0.105±0.065 ng for Z8-12:Ac per female. The percentages of Z7-12:Ac, Z9-14:Ac, Z11-16:Ac, Z5-10:Ac and Z8-12:Ac in pheromone gland extracts from individual females were (mean±SE) 40.451±13.66, 13.176±5.279, 14.943±5.142, 14.392±6.10 and 17.225±9.792 respectively, and the percentages of Z7-12:Ac, Z9-14:Ac and Z11-16:Ac were 58.75±9.429, 18.91±7.539 and 22.34±7.209. Field trials indicated that each single component of sex pheromone was non-effective and captured no males. The lures baited with duality compound of AB (3 : 1) had a certain attraction to males, the mean captured number was 2.6. The attraction ability of the lures baited with ternary compound of ABC (3 : 1 : 1) to males added significantly, the mean captured number per trap was 7.40, which was 2.8 times of duality compound of AB (3 : 1). The contents of sex pheromone could obviously affect its capture ability to males, the mean captured number was the highest at the dosage of 200 µg.

Key words: *Agrotis ypsilon*; Chemical analysis; Field trial; Gas chromatography/High-resolution mass spectrometry; Internal standard; Sex pheromone; Standard chemicals

小地老虎中国种群性信息素的成分鉴定及田间试验

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摘要: 研究了小地老虎中国种群的性信息素组分, 3 日龄处女蛾单腺体性信息素提取物中性信息素的含量非常低。GC 和 GC-MS 分析表明, 小地老虎性信息素含有 5 种成分: 顺-7-12 碳乙酸酯 (A)、顺-9-14 碳乙酸酯 (B)、顺-11-16 碳乙酸酯 (C)、顺-5-10 碳乙酸酯 (D) 和顺-8-12 碳乙酸酯 (E)。它们的含量分别为: (0.245±0.098)、(0.080±0.031)、(0.089±0.03)、(0.085±0.031) 和 (0.105±0.065) ng, 这 5 种物质的百分比分别为 40.451±13.66、13.176±5.279、14.943±5.142、14.392±6.10 和 17.225±9.792, 前 3 种物质的百分比为 58.75±9.429、18.91±7.539 和 22.34±7.209。田间试验表明, 性信息素单一组分均未诱到雄蛾, AB 以 3 : 1 的比例配成的诱芯对雄蛾有一定的引诱活性, 一个诱捕器平均诱捕到 2.6 头。ABC 组分以 3 : 1 : 1 的比例配成的诱芯对雄蛾引诱活性显著增强, 一个诱捕器平均诱捕量为 7.40 头, 是 AB (3 : 1) 诱芯的 2.8 倍。诱芯中性信息素的含量对诱蛾活性有明显的影响, 在剂量为 200 µg 时的平均诱捕量最高。

关键词: 小地老虎; 化学分析; 田间试验; GC-MS; 内标; 性信息素; 标准化合物
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The black cutworm moth, *Agrotis ypsilon* (Rottemberg), belongs to family Noctuidae of Lepidoptera, its common name is soil silkworm, land scissors and roots-cutting worm etc. It is an important cosmopolitan polyphagous agriculture pest, being found in Africa, Asia, Australia, Europe, and North and South America (Rings et al, 1974; Clement et al, 1982; Radcliffe et al, 1991). In China, it distributes in every place of the country, and its host plants are very wide, it can mostly harm 106 kinds of crops, such as vegetables, tobaccos, cottons, corns, broomcorn, hems and so on (Wei et al, 1989; Zeng, 1994). Due to high resistance to insecticides and nocturnal feeding habits of the larvae, the black cutworm is very difficult to control with insecticides (Han, 1986; Li et al, 2002, 2008). Application of the female-produced sex pheromone provides a promising alternative technique in controlling the pests, and it has been successfully used in population forecasting and mating disruption in many countries (Clement et al, 1981, 1982; Willson et al, 1981; Levine et al, 1982; Wakamura et al, 1986). Pioneering work with this species resulted in the identification of two pheromone components *Cis*-7-dodecenyl acetate (Z7-12 : Ac) and *Cis*-9-tetradecenyl acetate (Z9-14 : Ac) (Hill et al, 1979). Recent studies have provided evidence that *Cis*-11-hexadecenyl acetate (Z11-16 : Ac) should be considered a third pheromone component for both a European (Picimbon et al, 1997) and a North American (Gemeno & Haynes, 1998) population of *A. ypsilon*. However, there can be a large amount of variability in sex pheromone components and the optimal ratios among components in different geographic populations. Such as *Agrotis segetum*, a close relative of *A. ypsilon*, shows geographic variation in pheromone blend composition and male response specificity throughout its area of occurrence (Arn et al, 1983; Löfstedt et al, 1986; Wu et al, 1999). Therefore, it is very important to start studies on the Chinese black cutworm population. In the present study, the chemical constituents of the sex pheromone gland of the female black cutworm in China were investigated, and individual variations of chemical components in the sex pheromone gland were quantified by gas chromatography.

1 Material and methods

1.1 Insect rearing

Larvae of *A. ypsilon* were reared in the laboratory at (25±1)°C under a 14 : 10(L : D)h photoperiod. Larvae before the third instar were reared together for 1–2

weeks and then were reared individually to avoid cannibalism. Male and female pupae were placed in different boxes under the same photoregime and temperature. Pupae were observed every day, and the day on which an adult emerged was taken to be day 0 of adult life. Adult males and females were isolated at emergence, placed in 475 mL glass containers covered with nylon screen and were provided with 10% sucrose-water solution.

1.2 Sex pheromone gland extraction

The terminal abdominal segments, including the sex pheromone gland, of 25 females (3 d old after emerged) were dissected during the second half of the scotophase, which corresponds with the calling period in this species (Swier et al, 1977; Gemeno & Haynes, 2000). Fat and other tissues were carefully removed from the inner side of the integument using fine forceps. Individual gland was placed in 1.5 mL conical bottom glass vials containing 10 µL redistilled hexane and 1 ng tridecanyl acetate (13:Ac) as an internal standard. The vials were immediately sealed with Teflon-lined caps and kept for 30 min at room temperature. Then the glands were taken out and the vials were sealed with spirit lamp, finally, the extracts were stored at –20°C until analyzed.

1.3 Chemicals

The standard chemicals *Cis*-7-dodecenyl acetate (Z7-12:Ac), *Cis*-9-tetradecenyl acetate (Z9-14:Ac), *Cis*-11-hexadecenyl acetate (Z11-16:Ac), *Cis*-8-dodecenyl acetate (Z8-12:Ac), *Cis*-5-decenyl acetate (Z5-10:Ac) and tridecanyl acetate (13:Ac) were purchased from ISCA Technologies, Inc (America).

1.4 Chemical analysis

1 µL extract was injected into the gas chromatograph (Hewlett-Packard HP6890GC, Made in Agilent Technologies, State of California, America) equipped with a 30 m×0.25 mm-ID Carbowax capillary column with a split sample injector (40 : 1) and a flame ionization detector, connected to a Hewlett-Packard (HP5973, Made in Agilent Technologies) mass selective detector (MSD). Helium was carrier gas. The injector was set at 250°C with the purge valve opening 0.75 min after a manual injection. The oven temperature was held at 80°C for 2 min, increased to 290°C at 8°C/min, and the final temperature was maintained for 2 min. The flow rate of helium through the column was held at 1 mL/min and the pressure was 7.62 psi. Electron impact (EI) mass spectra were collected at 70 eV with the separator and source at 230°C.

Each pheromone components were identified by

comparing their mass spectra with those of authentic standards, and identification was checked subsequently by comparing the GC retention time of the natural product with that of an authentic standard under the same GC conditions as those described above. The quantity of each component was determined by relating its peak area to the internal standard and correcting for the differential responsiveness of the MSD to the compound, which was established daily with the multicomponent standard.

1.5 Field trials

Field trials were carried out in March and April, 2007 in a green vegetable producing base at Huaxi, Guiyang. Laver type traps (made from green plastic basin with a diameter of 20cm and 10cm deep) were used. Traps were filled with water containing 0.2% detergent and set up in the field 1.5m above the ground. Chemicals tested were impregnated into natural rubber septa (0.9×0.5cm) and put in the center of the laver trap 1.0cm above the water level. Blank traps, traps baited with the extracts from 10 females and hexane were used for comparison. Traps were inspected at 8 O'clock every morning and the number of males captured were counted and recorded.

2 Results

2.1 Chemical identification

GC analysis showed 5 peaks in the *A. ypsilon* gland extract (Fig. 1). The retention times of the five peaks were 11.642, 14.818, 14.750, 17.557 and 20.174 min respectively. The mass spectra of these 5 components of female tips all gave a fragmentation ion at m/z 61, indicating that these 5 components are all acetates. By comparing the GC retention times and spectra of these components with specific synthetic standards, the 5 peaks were identified to be Z7-12:Ac, Z9-14:Ac, Z11-16:Ac, Z8-12:Ac, Z5-10:Ac (m/z , 166, 194, 222, 166, 138) as shown in Fig. 1.

2.2 Quantitative analysis

Tab. 1 shows the amount of sex pheromone components collected from females in midscotophase (mean±SE). An extract of 1 gland from a day 3 female gave the following values for the sex pheromone components: 0.245±0.098ng of Z7-12:Ac, 0.080±0.031 ng of Z9-14:Ac, 0.089±0.033 ng of Z11-16:Ac, 0.085±0.031 ng of Z5-10:Ac, 0.105±0.065 ng of Z8-12:Ac per female. The percentages of Z7-12:Ac,

Tab. 1 Average quantity (ng/female) of Z7-12:Ac, Z9-14:Ac, Z11-16:Ac, Z5-10:Ac and Z8-12:Ac produced by individual *Agrotis ypsilon* virgin females that initiated calling at 3 day old, respectively (n= 25)

Number	Amount of females	Contents of sex pheromone components				
		Z7-12:Ac	Z9-14:Ac	Z11-16:Ac	Z5-10:Ac	Z8-12:Ac
1	1	0.351	0.117	0.081	0.073	0.052
2	1	0.241	0.082	0.086	0.102	0.073
3	1	0.192	0.076	0.089	0.068	0.251
4	1	0.131	0.064	0.078	0.069	0.074
5	1	0.290	0.086	0.054	0.087	0.123
6	1	0.453	0.079	0.167	0.051	0.099
7	1	0.187	0.044	0.059	0.157	0.114
8	1	0.029	0.151	0.101	0.089	0.037
9	1	0.228	0.097	0.12	0.09	0.075
10	1	0.295	0.098	0.096	0.11	0.257
11	1	0.254	0.010	0.071	0.069	0.095
12	1	0.365	0.062	0.094	0.086	0.072
13	1	0.220	0.076	0.042	0.049	0.064
14	1	0.262	0.073	0.163	0.038	0.045
15	1	0.117	0.085	0.053	0.160	0.229
16	1	0.081	0.122	0.095	0.091	0.098
17	1	0.233	0.087	0.121	0.075	0.134
18	1	0.401	0.027	0.149	0.103	0.035
19	1	0.249	0.039	0.094	0.085	0.139
20	1	0.283	0.134	0.085	0.083	0.196
21	1	0.199	0.078	0.089	0.058	0.058
22	1	0.245	0.066	0.071	0.061	0.063
23	1	0.380	0.083	0.064	0.06	0.044
24	1	0.251	0.081	0.057	0.142	0.065
25	1	0.212	0.094	0.062	0.057	0.128
Mean±SE		0.246±0.098	0.080±0.031	0.089±0.033	0.085±0.031	0.105±0.065
F		0.40	0.39	0.37	0.37	0.62

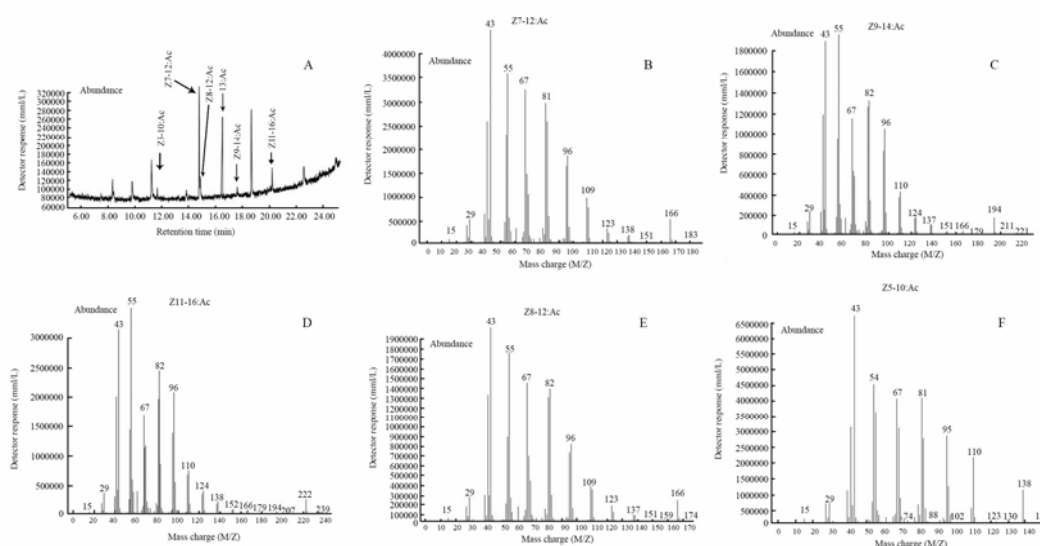


Fig. 1 GC/MS analysis of sex pheromone gland secretion of females from the China population of *Agrotis ypsilon*

A: Total ion current by electron impact of sex pheromone gland extract (10) from 3 day old *A. ypsilon* females at midscotophase; B: Mass spectrum of sex pheromone component I: *Cis*-7-dodecenyl acetate (Z7-12: Ac). C: Mass spectrum of sex pheromone component II: *Cis*-9-tetradecenyl acetate (Z9-14:Ac). D: Mass spectrum of sex pheromone component III: *Cis*-11-hexadecenyl acetate (Z11-16: Ac). E: Mass spectrum of sex pheromone component IV: *Cis*-8-dodecenyl acetate (Z8-12:Ac). F: Mass spectrum of sex pheromone component V: *Cis*-5-decenyl acetate (Z5-10:Ac). Synthetic 13: Ac was the internal standard.

Tab. 2 Percentage of Z7-12:Ac, Z9-14:Ac, Z11-16:Ac, Z5-10:Ac, Z8-12:Ac and Z7-12:Ac, Z9-14:Ac, Z11-16:Ac

	Percentage of sex pheromone components (%)				
	Z7-12:Ac	Z9-14:Ac	Z11-16:Ac	Z5-10:Ac	Z8-12:Ac
Z7-12:Ac, Z9-14:Ac, Z11-16:Ac, Z5-10:Ac, Z8-12:Ac	40.451±13.66	13.176±5.279	14.943±5.142	14.392±6.10	17.225±9.792
Z7-12:Ac, Z9-14:Ac, Z11-16:Ac	58.75±9.429	18.91±7.539	22.34±7.209		

Z9-14:Ac, Z11-16:Ac, Z5-10:Ac and Z8-12:Ac in sex pheromone gland extracts from individual females were (mean±SE) 40.451±13.66, 13.176±5.279, 14.943±5.142, 14.392±6.10 and 17.225±9.792 respectively, and the percentages of Z7-12:Ac, Z9-14:Ac and Z11-16:Ac were 58.75±9.429, 18.91±7.539 and 22.34±7.209 (Tab. 2). From Tab. 1, we can see that the amount of sex pheromone components was different in different females, this owing to the difference between individual females. But this did not influence the calling behavior of the females and the attraction to the males, this indicated that the sex pheromone had a certain active range in the sex pheromone communication system of *A. ypsilon*.

2.3 The attraction effect of sex pheromone of *A. ypsilon* in the field trails

The result of field trials was shown in Tab. 3. From the table we can see that, each single component of sex pheromone was as less effective as hexane and captured no males. Based on the bioassay results in laboratory (published in other paper), we choose the more effective duality compound of AB (Z7-12:Ac+

Z9-14:Ac) at a ratio of 3:1 and ternary compound of ABC (Z7-12:Ac+Z9-14:Ac+Z11-16:Ac) at a ratio of 3:1:1 to proceed the field trial, the dosage of each treatment is 100µg. The results showed that the lures baited with duality compound of AB (3 : 1) had a certain attraction to males, and captured 13 males altogether, the mean capture number was 2.6 and the highest number was 4. The attraction ability of the lures baited with ternary compound of ABC (3 : 1 : 1) to males added significantly, and captured 37 males altogether, the mean captured number per trap was 7.40, which was 2.8 times of lure baited with duality compound of AB (3 : 1), and the highest number was 10. This was significantly higher than that of sex pheromone gland extracts (the mean capture number was 3.40).

2.4 The effects of sex pheromone dosages on its capture ability to males

We choose sex pheromone ternary compound of ABC (Z7-12:Ac+Z9-14:Ac+Z11-16:Ac) with a ratio of 3:1:1 at different dosages to proceed the field trial, the results were shown in the table 4. From the table we can see that, the contents of sex pheromone could

obviously affect its capture ability to males. When the contents of sex pheromone added, the capture numbers would change significantly. The mean capture number was the highest at the dosage of 200 µg, captured 11.40 males, and could capture 57 males altogether. The mean capture number at the dosage of 500 µg was slightly

lower, captured 9.20 males. When the dosage reached 1000 µg, the mean capture number was the lowest, captured only 2.40 males. So we can conclude that, the best dosage of sex pheromone for capture was around 200 µg.

Tab. 3 The capture numbers of male *Agrotis ypsilon* by sex pheromone in the field trial

Lures (100 µg/trap)	Seducing time	Total capture numbers	Mean capture numbers (males/trap)	Capture number scopes
Z7-12:Ac (A)	2007.3.22–2007.4.6	0	0.00±0.00 ^c	0
Z9-14:Ac (B)	2007.3.22–2007.4.6	0	0.00±0.00 ^c	0
Z11-16:Ac (C)	2007.3.22–2007.4.6	0	0.00±0.00 ^c	0
Z5-10:Ac (D)	2007.3.22–2007.4.6	0	0.00±0.00 ^c	0
Z8-12:Ac (E)	2007.3.22–2007.4.6	0	0.00±0.00 ^c	0
AB (3 : 1)	2007.3.22–2007.4.6	13	2.60±1.14 ^{bc}	1-4
ABC (3 : 1 : 1)	2007.3.22–2007.4.6	37	7.40±1.82 ^a	5-10
Extracts(10FE)	2007.3.22–2007.4.6	17	3.40±1.15 ^b	2-5
Hexane (CK)	2007.3.22–2007.4.6	0	0.00±0.00 ^c	0

The dates in the table are M±SE, the letters after them are the results of multiple comparison. The different letters in the same row means significant difference at 0.05 level, otherwise means no difference.

Tab. 4 The effects of the sex pheromone dosages on the captures of male *Agrotis ypsilon*

Dosages (µg)	Total capture numbers	Mean capture numbers (males/trap)	Capture number scopes
50	21	4.20±1.30 ^d	3-6
100	37	7.40±1.82 ^{bc}	5-10
200	57	11.40±3.36 ^a	7-15
500	46	9.20±2.77 ^{ab}	5-12
700	24	4.80±1.92 ^{cd}	3-8
1000	12	2.40±1.14 ^d	1-3

The dates in the table are M±SE, the letters after them are the results of multiple comparison. The different letters in the same row means significant difference at 0.05 level, otherwise means no difference.

3 Discussion

The sex pheromone blend of *A. ypsilon* had been reported to be a mixture of *Cis*-7-dodecenyl acetate (Z7-12:Ac) and *Cis*-9-tetradecenyl acetate (Z9-14:Ac)(Hill & Roelofs, 1977; Hill et al, 1979). Several reports indicated that the addition of *Cis*-11-hexadecenyl acetate (Z11-16:Ac) to the original 2-component blend increased male captures in Japan (Wakamura et al, 1986), France (Causse et al, 1988), and Ukraine (Buleza,1991). Recently, all three components (Z7-12: Ac, Z9-14: Ac, and Z11-16: Ac) were identified from sex pheromone gland extracts of female *A. ypsilon* from France, and all three were involved in male attraction as determined from laboratory wind-tunnel tests (Picimbon et al, 1997). French *A. ypsilon* moths produced Z11-16: Ac in addition to Z7-12:Ac, Z9-14:Ac, *Cis*-11-tetradecenyl acetate (Z11-14:Ac) and *Cis*-11-hexadecenol (Z11-16:OH) (Picimbon et al, 1997). However, of all these compounds, only Z7-12: Ac, Z9-14: Ac, and Z11-16: Ac were important in eliciting male attraction in the wind tunnel (Picimbon et al, 1997). Additional components may be

involved in the chemical communication system of North American *A. ypsilon*. These results suggested the existence of some polymorphism among different strains of *A. ypsilon* throughout the world, in particular, among the European, Asian, and North American populations.

Our GC-MS analysis showed that the sex pheromone gland of the China strain of *A. ypsilon* contains a multi-component blend consisting of at least five components: Z7-12:Ac, Z9-14: Ac, Z11-16: Ac, Z8-12:Ac, Z5-10:Ac. We proceeded the EAG bioassay experiment and behavior response in a wind tunnel (published in other papers), finding that all these components can trigger EAG response when tested on male antennae of the *A. ypsilon*, and Z7-12:Ac triggered the largest EAG response. But a ternary mixture of Z7-12:Ac, Z9-14:Ac, and Z11-16:Ac is active in attracting the male moth. Our data support the use of the ternary mixture which has already been used for field trapping (Causse et al, 1988). Z8-12: Ac and Z5-10:Ac are present in gland extracts but may not be involved in attraction of males. Some olfactory neurons tuned to these two components have been detected on the male antennae

(Renou et al, 1996).

Lack of Z5-10:Ac in the blend does not reduce attraction of the sex pheromone mixture. Z5-12:Ac was recently found to be a fourth sex pheromone component of *A. segetum*, (Wu et al, 1995). The presence of sensory cells tuned to Z5-10: Ac in both *A. ypsilon* and *A. segetum* antennae suggests that it could act as an inhibitor of male *A. ypsilon*. Z5-10: Ac is a powerful antagonist of the peach twig borer pheromone (Millar & Rice, 1996) and of *Coleophora laricella* (Priesner & Witzgall, 1984). The possible presence and the role of this component need to be elucidated in further studies of the sex pheromone communication system between *A.*

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