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Field responses of *Isoceras sibirica* to synthetic blends of its sex pheromone

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Abstract

The carpenterworm, *Isoceras sibirica* Alpheraky (Lepidoptera Cossidae), is a destructive pest that affects *Asparagus officinalis* L. In an effort to develop a new and effective method for controlling the pest, the main components of its female sex pheromone were synthesized in the laboratory. We evaluated the effects of the following synthetic compounds, alone and in combination: (*Z*)-9-tetradecenyl acetate (*Z*9-14:Ac), (*Z*)-7-tetradecenyl acetate (*Z*7-14:Ac), and (*Z*)-9-hexadecadecenyl acetate (*Z*9-16:Ac). When provided alone, *Z*9-14:Ac was very attractive to *I. sibirica* males. Neither *Z*7-14:Ac nor *Z*9-14:Ac alone attracted any moths. Moth capture increased significantly when *Z*7-14:Ac was added to *Z*9-14:Ac. The optimum ratio of *Z*9-14:Ac to *Z*7-14:Ac was 500:400. Male moths were not attracted by *Z*9-16:Ac alone, and this compound did not affect the attractiveness of synthetic pheromone blends. The best field activity was obtained for lures baited with *Z*9-14:Ac and *Z*7-14:Ac (5:4 ratio) at a dose of 900 µg septum^{-1}. The findings presented here could facilitate safer and more environmentally friendly management of *I. sibirica*.

Key words: *Isoceras sibirica*, sex pheromone, trapping, *Z*9-14:Ac, *Z*7-14:Ac, *Z*9-16:Ac.

Introduction

The carpenterworm, *Isoceras sibirica* Alpheraky (Lepidoptera Cossidae), is a serious pest of *Asparagus officinalis* L. across the former Soviet Union (Siberia), Mongolia, and China. *I. sibirica* produces one generation per year and its larvae burrow into the root crowns of *A. officinalis*, causing significant damage to this crop (Duan *et al*., 2008).

To date, effective measures for the control of *I. sibirica* are not available because of the root-boring habit of the larvae. However, the use of synthetic sex pheromones that interfere with reproduction may offer an environmentally friendly means of controlling the adults. Sex pheromones are species-specific and highly selective. They are valuable tools for integrated pest management as they are nontoxic and do not present health risks to humans or other animals. The use of pheromones has been reported for a number of insect species, for purposes that include monitoring pest populations to determine when to apply insecticides (Kehat *et al*., 1992; Boddum *et al*., 2009), assessing insecticide resistance in pest populations (Haynes *et al*., 1986; 1987), luring and trapping adult males to suppress pest populations (Zhang *et al*., 2002; Jing *et al*., 2010; Yang *et al*., 2012), and disrupting mating (Il'Ichev *et al*., 2006; Stelinski *et al*., 2007; Higbee *et al*., 2008; Lanzoni *et al*., 2012).

We previously determined that the female sex pheromone of *I. sibirica* contained (*Z*)-9-tetradecenyl acetate (*Z*9-14:Ac), (*Z*)-7-tetradecenyl acetate (*Z*7-14:Ac), and (*Z*)-9-hexadecadecenyl acetate (*Z*9-16:Ac) (Zhang *et al*., 2011). In that work, identification of these compounds was based largely on electroantennography (EAG) and gas chromatography-mass spectrometry (GC-MS); field evaluation of these compounds was not conducted. The effects of age and diel rhythms on sex-pheromone production, calling behavior, and sexual activity have also been investigated to improve understanding of chemical communication in reproductive processes and of pheromone use for detecting and monitoring *I. sibirica* in asparagus fields (Liu *et al*., 2013). However, results of long-term trapping experiments are lacking. In this context, we conducted a thorough evaluation of the attractiveness of pheromone blends to *I. sibirica* from 2009 to 2011 in China.

Materials and methods

Insects

In late April, pupae were collected from soil (5-10 cm depth) around the roots of *A. officinalis* plants that were visibly damaged by *I. sibirica* larvae in Wenxi County, Shanxi Province. The collected pupae were separated by sex and buried 10 cm deep in sand containing 5-8% water in cages (150 × 100 × 100 cm) covered with nylon screens; the cages were buried near the asparagus field to allow for natural eclosion under a natural light cycle. Every 24 h, virgin female moths were transferred to separate containers to be used as traps.

Chemicals

The compounds *Z*9-14:Ac, *Z*7-14:Ac, and *Z*9-16:Ac were synthesized in the Chemical Ecology Laboratory at Shanxi Agricultural University (Zhang *et al*., 2001; Jing *et al*., 2010) and were subsequently purified by column chromatography on silica gels. The chemical and isomeric purities of all compounds were >97%. Reagents and solvents were obtained from Fisher Scientific (Fair Lawn, NJ, USA).

Baits and traps

To determine the optimum ratio of sex pheromone components for male capture, solutions of individual components and binary and ternary blends were prepared with hexane and loaded onto bell-shaped green

Table 1. Attraction of *I. sibirica* males to lures baited with synthetic pheromone components in asparagus fields in Shanxi Province, China.

Lure component (µg/ rubber septum)			Captured males (trap/3 days) (mean ± SD)		
*Z*9-14:Ac	*Z*7-14:Ac	*Z*9-16:Ac	2009	2010	2011
500	0	0	12.0 ± 1.4c	12.2 ± 2.2b	9.2 ± 1.5c
0	500	0	1.0 ± 0.8a	2.8 ± 1.7a	2.5 ± 2.4a
0	0	500	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
500	250	250	40.2 ± 4.6d	53.3 ± 5.3c	47.8 ± 2.7d
500	250	0	48.3 ± 3.0e	45.7 ± 8.2d	55.0 ± 4.9e
500	0	250	9.5 ± 1.3c	11.5 ± 1.3b	9.3 ± 1.3c
0	250	250	1.8 ± 0.9a	3.0 ± 0.9a	2.8 ± 1.7a
0	0	0	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
Virgin females			5.0 ± 1.8b	4.8 ± 3.3a	4.5 ± 1.3b

Four replicates were used per treatment; SD, standard deviation; numbers followed by different letters are significantly different at $P < 0.05$ (nonparametric Kruskal-Wallis analysis of variance followed by Mann-Whitney-U test).

rubber septa (190 × 80 mm, Baoji Guangren Biotechnology Co., Shaanxi, China) as bait. The distribution of the different treatments was completely randomized and each treatment comprised four replicates.

To estimate the optimum dosage of each active component required for male capture, four doses of a 5:4 blend of *Z*9-14:Ac and *Z*7-14:Ac were tested: 450, 900, 1350, and 1800 µg. Each treatment consisted of six randomly distributed replicates of each dose among 24 traps.

Field tests

All tests were conducted in an asparagus field in Wenxi, Taigu, Shanxi Province during the *I. sibirica* flight season (May and June) from 2009 to 2011. Traps were suspended from wooden supports (0.5 to 1 m tall) spaced at 30 m intervals for the dosage and ratio tests. For purposes of comparison, a net cage containing two 2-day-old virgin females was placed as bait beneath the roof of a delta sticky trap (28 × 20 × 11.5 cm, Baoji Guangren Biotechnology Co., Shaanxi, China). The moths were replaced daily. Hexane was used as a standard control (Jing *et al*., 2010). Trap catches were checked every morning and captured moths were recorded and removed daily. Trap locations were randomized at the site to minimize the potential effects of location.

Data analysis

Data were analyzed by non-parametric Kruskal-Wallis analysis of variance followed by Mann-Whitney-U tests (Sokal and Rohlf, 1995); catches were considered significantly different at $P < 0.05$. All analyses were conducted using SPSS version 16.0 for Windows software (SPSS Inc., Chicago, IL, USA).

Results

Optimum ratio of synthetic pheromones

The attraction of *I. sibirica* to field traps baited by lures containing three, two, or one component(s) are presented in table 1. Component *Z*9-14:Ac alone attracted significantly more moths than did the other singular components or virgin females (2009, H = 15.1, d.f. = 4, P = 0.005; 2010, H = 14.3, d.f. = 4, P = 0.006; 2011, H = 13.9, d.f. = 4; P = 0.007). A mixture of the three components in a 2:1:1 ratio and a mixture of *Z*7-14:Ac and *Z*9-14:Ac in a 2:1 ratio effectively attracted male *I. sibirica* (2009, H = 34.0, d.f. = 8, P = 0; 2010, H = 33.4, d.f. = 8, P = 0; 2011, H = 33.0, d.f. = 8; P = 0). The number of males attracted to the lure did not differ between these mixtures.

The optimum mixing ratio of *Z*7-14:Ac and *Z*9-14:Ac was examined (table 2). Moth capture rates for 5:4 and 1:1 mixtures of *Z*9-14:Ac and *Z*7-14:Ac were significantly greater than those of other ratios or of virgin females (2009, H = 38.0, d.f. = 9, P = 0; 2010, H = 37.5, d.f. = 9, P = 0; 2011, H = 37.9, d.f. = 9; P = 0). No significant difference was found between the 5:4 and 1:1 ratios, but the 5:4 ratio of *Z*7-14:Ac and *Z*9-14:Ac was considered optimal for attracting male moths.

Optimum pheromone dose

The greatest numbers of males were captured in traps baited with 900, 1350, and 1800 µg of the two components (5:4 ratio) (2011, H = 17.4, d.f. = 4; P = 0.002), with no significant differences in capture rates between the doses (table 3). Based on these tests, we selected 900 µg as the standard dose per dispenser in subsequent tests, as a larger dose was not needed.

Discussion

I. sibirica males were most strongly attracted to a 5:4 blend of *Z*9-14:Ac and *Z*7-14:Ac. Field tests indicated a discrepancy between the contents and emissions of pheromone glands in *I. sibirica* females. Different extracts from the female sex pheromone gland contained (*Z*)-7-tetradecen-1-ol (*Z*7-14:OH), (*Z*)-9-tetradecen-1-ol (*Z*9-14:OH), *Z*7-14:Ac, *Z*9-14:Ac, and *Z*9-16:Ac; however, traps baited with rubber septa impregnated with *Z*9-14:Ac (500 µg $septum^{-1}$) and *Z*7-14:Ac (400 µg $septum^{-1}$) were more effective than traps containing other baits or virgin females. The addition of *Z*7-14:Ac to *Z*9-14:Ac increased the mean male capture rate 4-fold, suggesting that these two compounds acted synergistically.

However, *Z*9-16:Ac was neither attractive nor inhibi-

Table 2. Attraction of *I. sibirica* males to lures baited with two synthetic pheromone components (*Z*9-14:Ac and *Z*7-14:Ac) in asparagus fields in Shanxi Province, China.

Lure component (µg/ rubber septum)		Captured males (trap/3 days) (mean ± SD)		
*Z*9-14:Ac	*Z*7-14:Ac	2009	2010	2011
500	50	14.3 ± 2.5c	15.2 ± 2.0b	12.3 ± 0.1c
500	100	30.7 ± 3.5e	37.8 ± 3.8e	27.5 ± 3.1e
500	200	26.8 ± 3.3de	30.0 ± 2.6d	22.8 ± 3.3e
500	300	48.2 ± 3.0f	57.8 ± 8.3f	45.0 ± 5.0f
500	400	80.3 ± 4.6g	73.3 ± 5.3g	67.8 ± 2.8g
500	500	78.3 ± 5.7g	75.5 ± 5.4g	69.8 ± 3.6g
400	500	22.8 ± 1.9d	21.0 ± 2.2c	17.3 ± 1.7d
200	500	7.7 ± 2.2b	13.8 ± 2.8b	7.0 ± 1.8b
50	500	1.5 ± 1.3a	1.8 ± 1.7a	2.0 ± 0.8a
Virgin females		5.3 ± 2.0b	3.8 ± 3.3a	4.0 ± 1.8ab

Four replicates were used per treatment; SD, standard deviation; numbers followed by different letters are significantly different at $P < 0.05$ (nonparametric Kruskal-Wallis analysis of variance followed by Mann-Whitney-U test).

Table 3. Attraction of *I. sibirica* males to lures baited with different doses of a 5:4 ratio of *Z*7-14:Ac and *Z*9-14:Ac in 2011.

Dosage of sex attractant µg/septum	Captured males (trap/3 days) (mean ± SD)
450	20.5 ± 3.4b
900	75.4 ± 5.8c
1350	79.0 ± 2.2c
1800	78.5 ± 6.2c
Virgin females	7.3 ± 2.1a

Four replicates were used per treatment; SD, standard deviation; numbers followed by different letters are significantly different at $P < 0.05$ (nonparametric Kruskal-Wallis analysis of variance followed by Mann-Whitney-U test).

tory to male *I. sibirica* moths. This chemical causes an EAG response in male moths of similar magnitude to that of *Z*7-14:Ac (Zhang *et al*., 2011), and probably acts as a pheromone 'mimic' considering its related chemical structure. Similar results were found in *Prays citri* (Milliere) (Sternliche *et al*., 1978) and *Phyllocnistis citrella* Stainton (Lapointe *et al*., 2006). The differences between the present results and the findings of our previous work (Zhang *et al*., 2011) were a result of the lack of a bioassay in our earlier research.

Results obtained from our field-trapping experiments in 2009-2011 suggest that traps baited with *Z*9-14:Ac (500 µg septum^{-1}) and *Z*7-14:Ac (400 µg septum^{-1}) can be used to capture *I. sibirica* males for pest control in the field. Further experimentation is required to optimize the various parameters affecting male attraction, including the optimal spacing of pheromone traps and the type and shape of the trapping devices.

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