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Field evaluation of the sex pheromone components of a Chinese population of *Paleacrita vernata*

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Abstract

The spring cankerworm, *Paleacrita vernata* Peck (Lepidoptera Geometridae), is a pest of trees and shrubs across North America and China. In Canada, the components of the sex pheromone in spring cankerworms have been identified as 3(Z),6(Z),9(Z)-nonadecatriene (3Z,6Z,9Z-19:Hy), 3(Z),6(Z),9(Z)-eicosatriene (3Z,6Z,9Z-20:Hy) and 6(Z),9(Z)-nonadecadiene (6Z,9Z-19:Hy). However, field evaluation of these compounds was not conducted in China. In this paper, the main components of the female sex pheromone were synthesized in the laboratory and long-term trapping experiments were tested for a thorough evaluation of the attractiveness of pheromone blends. It showed that the most attractive mixture appeared to be approximate 8:2:1 blend of 3Z,6Z,9Z-19:Hy, 3Z,6Z,9Z-20:Hy and 6Z,9Z-19:Hy. 3Z,6Z,9Z-19:Hy had considerable activity alone, while 3Z,6Z,9Z-20:Hy tested individually had very weak activity. The 6Z,9Z-19:Hy compound had hardly any attractive abilities by itself. But an addition of 6Z,9Z-19:Hy to a basic bait comprised of 3Z,6Z,9Z-19:Hy and 3Z,6Z,9Z-20:Hy in a 4:1 ratio significantly increased trap captures. The candidate compounds structurally related to sex pheromone were neither attractive nor inhibitory to male moths. It finds that traps baited with 3Z,6Z,9Z-19:Hy (800 µg septum^{-1}), 3Z,6Z,9Z-20:Hy (200 µg septum^{-1}) and 6Z,9Z-19:Hy (100 µg septum^{-1}) can be used to capture *P. vernata* males in the field in China.

Key words: *Paleacrita vernata*, sex pheromone, trapping.

Introduction

The spring cankerworm, *Paleacrita vernata* Peck (Lepidoptera Geometridae), is one of the most serious pests in shelter belts and shrubs across North America and China (Millar *et al*., 1990; Wang *et al*., 2010), especially in *Caragana korshinskii* Komarov. The plant is well known for its ability to resist drought in the north of China (Fang *et al*., 2008; Wu *et al*., 2009; Gerile *et al*., 2011). The spring cankerworm has one generation per year; the larvae emerge in April and cause serious damage to *C. korshinskii*.

At present, the only methods of control are the application of a systemic insecticide against the young larvae. However, this approach has created many environmental problems, a promising way to monitoring the appearance and population of the pest might by through utilization of sex pheromones. Sex pheromones are valuable tools for use in integrated pest management as they are non-toxic and do not represent a health risk to humans and animals. Indeed, the use of pheromones has been reported for a number of insect species, for purposes such as detection and monitoring (Leif, 2013; Santi *et al*., 2015), mass trapping (Jing *et al*., 2010; Yang *et al*, 2012; Hussain *et al*., 2014), mating disruption (Il'Ichev *et al*., 2006; Stelinski *et al*., 2007; Higbee and Burks, 2008), and attracting insects to bait which contains an insecticide (Ebesu, 2003).

The sex pheromone of the spring cankerworm was reported to consist of 3(Z),6(Z),9(Z)-nonadecatriene (3Z,6Z,9Z-19:Hy), 3(Z),6(Z),9(Z)-eicosatriene (3Z,6Z,9Z-20:Hy) and 6(Z),9(Z)-nonadecadiene (6Z,9Z-19:Hy) in Canada (Millar *et al*., 1990). Field evaluation of these compounds was not conducted in China.

In many Lepidoptera, the species-specific blends of pheromone components are responsible for reproductive isolation between closely related species (Roelofs and Brown, 1982; Löfstedt *et al*., 1991; Yang *et al*., 2009). For example, in Japan, the sex pheromone of *Glossosphecia romanovi* (Leech) has recently been identified as a mixture of Z3,Z13-18:OH, Z3,Z13-18:OAc and E3,Z13-18:OH in an 8:1:1 ratio. However, for Korean male moths, this pheromone lure was ineffective (Yang *et al*., 2011). Therefore, it is very important to understand the pheromone components in different species and populations.

In this study, we assess the male response to pheromone blends of different components and ratios of a Chinese population of *P. vernata* by conducting long-term field trapping experiments between 2013 and 2015. A series of related compounds were also tested, some of which have been found in the pheromone blends of other Geometridae or Noctuidae moths or structurally related to sex pheromones. These compounds include 3Z,6Z,9Z-21:Hy, 3Z,6Z,9Z-22:Hy, 6Z,9Z-20:Hy and 6Z,9Z-21:Hy.

Materials and methods

Insects

In March 2013-2015, pupae were collected by digging them from sand around the *C. korshinskii* shrubs that were damaged by the spring cankerworm in Yanchi County, Ningxia (37°38'N 107°40'E). The collected pupae were buried in sand containing 10% water in cages (100 × 80 × 80 cm) covered with nylon screens; the cages were buried near the shrubs to allow for natural emergence under a natural light cycle. Virgin female moths were collected by hand each morning to be used as lures.

Chemicals

The compounds were synthesized in the Chemical Ecology Laboratory at Shanxi Agricultural University by methods previously reported (Underhill *et al*., 1983; Wong *et al*., 1985; Millar *et al*., 1987; Millar, 2000). Before field tests, all compounds were purified by column chromatography using silica gel. The purity of these compounds exceeded >96% by gas chromatography. Reagents and solvents were obtained from Fisher Scientific (Fair Lawn, NJ, USA).

Experimental design

Experiment 1 investigated the attraction of the spring cankerworm moths to 3Z,6Z,9Z-19:Hy and 3Z,6Z,9Z-20:Hy independently and in various combinations, including the 2:8 ratio reported by Millar *et al*. (1990). The distribution of the different treatments was completely randomized and each treatment was replicated three times.

Experiment 2 tested the individual effects of 6Z,9Z-19:Hy identified in extractions from female glands and four candidate compounds (3Z,6Z,9Z-21:Hy, 3Z,6Z,9Z-22:Hy, 6Z,9Z-20:Hy and 6Z,9Z-21:Hy) as possible synergists or antagonists by using standard lures of the two major components. The distribution of the different treatments was completely randomized and each treatment was replicated three times.

Experiment 3 was conducted to estimate the optimum dosage of each active component required for male capture, six doses of a 8:2:1 blend of 3Z,6Z,9Z-19:Hy, 3Z,6Z,9Z-20:Hy and 6Z,9Z-19:Hy were tested: 200, 550, 1100, 1650, 2200 and 2750 μg. Each treatment consisted of 3 randomly distributed replicates of each dose among 18 traps.

Field tests

We conducted trapping experiments with the synthetic pheromone of *P. vernata* from 2013 to 2015. All tests were conducted in *C. korshinskii* shrubs in Yanchi County, Ningxia. Sticky traps were baited with rubber septa containing the synthetic compounds or the blends in hexane. The traps were placed about 1 m above the ground. The distance between traps within one block was at least 20 m. Two newly emerged virgin females were used as lures and placed in small screen cages inside sticky traps, for purposes of comparison. The moths were replaced daily. Hexane was used as a standard control (Jing *et al*., 2010). Trap catches were checked every morning; the captured moths were counted and removed daily or the sticky bottoms changed. The traps were moved one position within each block every time they were checked to minimize bias due to location.

Data analysis

Statistical analysis was performed using the Kruskal-Wallis method followed by the Mann-Whitney U test. The significance level was set to 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

Field tests of *P. vernata* were conducted to determine the most attractive blend of 3Z,6Z,9Z-19:Hy and 3Z,6Z,9Z-20:Hy between 2013 and 2015 (table 1). The most attractive mixture appeared to be an approximate a 4:1 blend of 3Z,6Z,9Z-19:Hy and 3Z,6Z,9Z-20:Hy. Moth capture rates using that blend were significantly greater than those of other ratios or of virgin females (2013, H = 25.5, d.f. = 8, P = 0.001; 2014, H = 25.7, d.f. = 8, P = 0.001; 2015, H = 25.4, d.f. = 8, P = 0.001). One of the components, 3Z,6Z,9Z-19:Hy had considerable activity alone, while 3Z,6Z,9Z-20:Hy, when tested individually, had very weak activity-significantly lower than those of virgin females.

Synergists or antagonists tested

6Z,9Z-19:Hy, the third active compound in the female extract and a series of related compounds also were tested in 2015 (table 2). The 6Z,9Z-19:Hy compound had hardly any attractive ability by itself. But an addition of 6Z,9Z-19:Hy (100 μg) to a basic bait with 1 mg/septum of a 4:1 blend of 3Z,6Z,9Z-19:Hy and 3Z,6Z,9Z-20:Hy significantly increased trap captures

Table 1. Capture of *P. vernata* male moths in traps baited with mixtures of 3Z,6Z,9Z-19:Hy and 3Z,6Z,9Z-20:Hy in various combinations in Yanchi County, Ningxia, China.

Lure composition (μg)		Number of males captured (trap/3 days) (mean ± SD)		
3Z,6Z,9Z-19:Hy	3Z,6Z,9Z-20:Hy	2013	2014	2015
500	0	22.2 ± 5.8c	26.9 ± 5.0c	20.9 ± 4.1c
450	50	22.6 ± 4.0c	29.9 ± 0.6c	20.3 ± 3.0c
400	100	45.3 ± 6.2a	52.4 ± 8.5a	42.7 ± 4.0a
250	250	30.8 ± 8.6b	39.3 ± 9.6b	30.3 ± 2.6b
100	400	8.7 ± 2.6e	10.2 ± 6.0e	9.2 ± 6.5e
50	450	7.7 ± 1.5e	11.6 ± 6.0e	7.4 ± 3.2e
0	500	3.0 ± 1.0f	3.0 ± 1.7f	2.9 ± 1.2f
Virgin female (2 per trap)		14.6 ± 6.7d	18.7 ± 2.6d	14.4 ± 2.5d
Blank		0	0	0

Three replicates were used per treatment; SD, standard deviation; Values followed by the same letter are not significantly different ($P > 0.05$).

Table 2. Effect of adding 6Z,9Z-19:Hy and other related compounds to standard baits with 1 mg/septum of a 4:1 blend of 3Z,6Z,9Z-19:Hy and 3Z,6Z,9Z-20:Hy on males captured in 2015.

Lure composition (µg)	Number of males captured (trap/3 days)(mean ± SD)
Basic bait	52.0 ± 7.5b
6Z,9Z-19:Hy (500)	5.0 ± 3.6c
Basic bait + 6Z,9Z-19:Hy (100)	81.3 ± 2.5a
Basic bait + 6Z,9Z-19:Hy (200)	85.3 ± 5.5a
6Z,9Z-20:Hy (500)	0.33 ± 0.58c
Basic bait + 6Z,9Z-20:Hy (100)	50.0 ± 8.6b
Basic bait + 6Z,9Z-20:Hy (200)	51.3 ± 10.0b
6Z,9Z-21:Hy (500)	0
Basic bait + 6Z,9Z-21:Hy (100)	51.0 ± 10.1b
Basic bait + 6Z,9Z-21:Hy(200)	53.7 ± 6.4b
3Z,6Z,9Z-21:Hy (500)	0.7 ± 0.6c
Basic bait + 3Z,6Z,9Z-21:Hy (100)	53.0 ± 8.5b
Basic bait + 3Z,6Z,9Z-21:Hy (200)	51.3 ± 12.0b
3Z,6Z,9Z-22:Hy (500)	0
Basic bait + 3Z,6Z,9Z-22:Hy (100)	52.7 ± 2.5b
Basic bait + 3Z,6Z,9Z-22:Hy (200)	53.0 ± 20.8b
Blank	0

Three replicates were used per treatment; Traps were set out from April 1 to 3 in Yanchi County, Ningxia; SD, standard deviation; Values followed by the same letter are not significantly different ($P > 0.05$).

(2015, H = 42.2, d.f. = 16, P = 0). However, trap catches of males to the binary blend were unaffected by the addition of 3Z,6Z,9Z-21:Hy, 3Z,6Z,9Z-22:Hy, 6Z,9Z-20:Hy and 6Z,9Z-21:Hy.

Optimum pheromone dose

A dose-response experiment with a 8:2:1 blend of 6Z,9Z-19:Hy, 3Z,6Z,9Z-19:Hy and 3Z,6Z,9Z-20:Hy revealed no significant difference in trap captures with 1100,1650, 2200 and 2750 µg (2015, H = 20.0, d.f. = 7, P = 0). Based on these tests, we selected 1100 µg as the standard dose per dispenser in subsequent tests, as a larger dose was not needed (table 3). Trap captures at the 550 µg was lower, but still significantly greater than virgin females and blank traps. There was no significant difference in trap captures with 200 µg and virgin females.

Table 3. Attraction of *P. vernata* males to lures baited with different dose of a 8:2:1 ratio of 3Z,6Z,9Z-19:Hy, 3Z,6Z,9Z-20:Hy and 6Z,9Z-19:Hy in 2015.

Dosage of sex attractant µg/septum	Number of males captured (trap/3 days) (mean ± SD)
200	6.7 ± 2.5c
550	31.7 ± 6.4b
1100	52 ± 11.3a
1650	54.3 ± 11.7a
2200	57.7 ± 11.0a
2750	55 ± 12.0a
Virgin females	14 ± 8.7c
Blank	0

Three replicates were used per treatment; Traps were set out from April 4 to 7, 2015 in Yanchi County, Ningxia; SD, standard deviation; Values followed by the same letter are not significantly different ($P > 0.05$).

Discussion

Field trapping experiments showed that 3Z,6Z,9Z-19:Hy and 3Z,6Z,9Z-20:Hy were necessary for optimal attraction of male *P. vernata*, and males were preferentially attracted to a 4:1 blend, which were more effective than traps containing other lures or virgin females, which was contrary to the results of Millar *et al*. (1990). They identified that the primary binary blend of 3Z,6Z,9Z-19:Hy and 3Z,6Z,9Z-20:Hy, in a 1:4 ratio was considered optimal for attracting male *P. vernata* from Canada. These results indicate that there is variation in male responses to the primary blend between Canadian and Chinese populations. Additional research to examines populations around the world is necessary to clarify intraspecific variation in pheromone systems.

The 6Z,9Z-19:H compound was not attractive by itself, but the addition of 6Z,9Z-19:Hy (100 µg septum^{-1}) to standard traps significantly increased the attraction of males. This result was consistent with Millar *et al* studies. In their research, 6Z,9Z-19:Hy was only tentatively identified from female *P. vernata* because there may be a positional isomer. It suggested that this compound acted synergistically and it is indeed a necessary component of the pheromone. Similar results were found in *Prays citri* Milliere (Sternlicht *et al*., 1978) and *Phyllocnistis citrella* Stainton (Lapointe *et al*., 2006).

This study also tested a series of related compounds, which were also unsaturated hydrocarbons and had been found in the pheromone and sex attractant blends of other Geometridae or Noctuidae moths. These components varied in either chain length or the degree of unsaturation. However, the candidate compounds structurally related to sex pheromone were neither attractive nor inhibitory to male *P. vernata* moths.

Field observations seem to suggest that the synthetic product can attract the male *P. vernata*. We did not capture any other species in traps baited over the course of this study. For the time being, the attractant mixtures defined in this study seem to provide a basis for monitoring this pest and for further behavioural studies. Thus, the 8:2:1 ratio of 3Z,6Z,9Z-19:Hy, 3Z,6Z,9Z-20:Hy and 6Z,9Z-19:Hy was found to be the most attractive to male moths. That is, the results obtained from our field-trapping experiments between 2013 and 2015 suggest that traps baited with 3Z,6Z,9Z-19:Hy (800 µg septum^{-1}), 3Z,6Z,9Z-20:Hy (200 µg septum^{-1}) and 6Z,9Z-19:Hy (100 µg septum^{-1}) can be used to capture *P. vernata* males in the field in China. More thorough studies will be necessary to define the true pheromone blend emitted by Chinese populations of *P. vernata*.

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