Sex Attractant of a Clearwing Moth Synanthedon Nashivora (Lepidoptera: Sesiidae)

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Abstract

A sex attractant of *Synanthedon nashivora* Naka and Yano, a pest of Asian pear discovered in Kyoto, Japan in 2014 and described as a novel species in 2019 was revealed. Pheromone lures baited mixtures with one or two of the seven compounds used as sex pheromones by Sesiidae species were prepared, and screening tests were conducted using these lures in pear orchards in Kyotango City, Kyoto Prefecture, Japan, from 2017 to 2018. Males were attracted to various mixture ratios, especially 7:3 mixture ratio, of (3*Z*,13*Z*)-3,13-octadecadienyl acetate (Z3,Z13-18:OAc) and (2*E*,13*Z*)-2,13-octadecadienyl acetate (E2,Z13-18:OAc) mixtures. This finding will enable monitoring of this species in Asian pear orchards. By attempting to search for males in the field using this pheromone lure, it will be possible to understand the distribution of this species in Japan and neighboring countries, its microhabitat, and the seasonal occurrence of this species.

Introduction

A clearwing moth *Synanthedon nashivora* Naka and Yano (Lepidoptera: Sesiidae) was found in 2014 as a pest of Asian pear [*Pyrus pyrifolia* (Burm.f.) Nakai] and then described as a novel species in 2019 (Naka et al. 2019). Currently, this species is found only in the Tango Peninsula, Japan. The larvae attack pear trees in pear orchards in the Tango Peninsula. The larvae burrow alone under the bark, and larval feces are observed at the site of larval attack. If the base of a thin branch is damaged, the branch is more likely to break off and new buds are less likely to develop from the damaged area. When the large part of the main branch is damaged, the bark becomes rough. When the dead part of the tree expands, the tree's vigor declines (Kyoto Prefecture 2017; Kukizaki unpubl.).

This species is a recently discovered pest. Therefore, the seasonal trend and domestic distribution are not known. The status of damage to Asian pears by this species in the Tango Peninsula can be determined by careful investigation of traces of damage caused by the larvae of this species. However, it is very difficult to know the seasonal trend and to reveal the distribution of this species except for the Tango Peninsula. Because clearwing moths are diurnal, they are not attracted to light traps. Moreover, populations might be very low. If we want to collect adult clearwing moths by only visually searching for and catching them, we will suffer from encounters with numerous wasps and bees (Naka 2018).

The sex pheromone of clearwing moths has been studied for a long time and is used for monitoring and mating disruption all over the world. In Japan, pheromone lures for monitoring *Synanthedon hector* (Butler), *S. tenuis* (Butler), and *Glossosphecia romanovi* (Leech) are commercially available (Japan Plant Protection Association 2020). Also, mating disruptant has been used to control *S. hector*, *S. tenuis* and *Nokona feralis* (Leech) (Japan BioControl Association 2020).

In the case of clearwing moths, monitor seasonal trends and/or distribution surveys using sex pheromones are effective. Only 7 compounds, (3*E*,13*Z*)-3,13-octadecadien-1-ol (E3,Z13-18:OH), (3*Z*,13*Z*)-3,13-octadecadien-1-ol (Z3,Z13-18:OH), (2*E*,13*Z*)-2,13-octadecadien-1-ol (E2,Z13-18:OH), and the acetate esters of these 3 compounds (E3,Z13-18:OAc, Z3,Z13-18:OAc and E2,Z13-18:OAc, respectively), and (2*E*,13*Z*)-2,13-octadecadienal (E2,Z13-18:Ald) have been found as female sex pheromones or sex attractants of clearwing moths so far (Ando and Yamamoto 2020). In general, the sex pheromone of clearwing moths consists of one or two compounds of the 7 compounds; E3,Z13-18:OAc, Z3,Z13-18:OAc, E2,Z13-18:OAc, E3,Z13-18:OH, Z3,Z13-18:OH, E2,Z13-18:OH, and E2,Z13-18:Ald (Ando and Yamamoto 2020). Males of clearwing moths tend not to stick to strict mix ratios, and some of them are attracted even if they deviate from the optimal mixing ratio for attracting males. Therefore, pheromone lures with 2 of the aforementioned 7 compounds in mixtures of 10:0, 9:1, 5:5, 1:9, and 0:10 can be used to effectively collect a variety of species (Naka 2018). Besides, species in the genus *Synanthedon* are very common in the use of acetate as a sex pheromone (Ando and Yamamoto 2020). Based on these findings, we thought that the probability of this species' sex pheromones falling into 2 of these 7 compounds is very high and that it is not difficult to identify the sex attractants before the identification of the sex pheromones.

This study aimed to develop a sex attractant that can effectively attract males of this species to reveal the seasonal trend and the distribution of this species. To this end, field screening tests using pheromone traps with seven compounds commonly used as sex pheromones in clearwing moths as an attraction source were conducted at Asian pear orchards in the Tango Peninsula.

Materials And Methods

Chemicals.

E3,Z13-18:OAc, Z3,Z13-18:OAc, and E2,Z13-18:OAc, which were supplied by Shin-Etsu Chemical Co., Ltd. with purity levels greater than 98%, were utilized as analytical standards and lures for field tests after purification to purify level > 99.5% by column chromatography on silver nitrate-impregnated silica gels. E3,Z13-18:OH, Z3,Z13-18:OH, and E2,Z13-18:OH were prepared by saponification of the corresponding acetates. E2,Z13-18:Ald was obtained by oxidation of E2,Z13-18:OH with pyridinium chlorochromate in CH_2Cl_2 (Islam et al. 2007).

Field trapping test.

The attraction of *S. nashivora* males to synthetic lures was examined using three tests (I-III). The test I, a preliminary screening test, was conducted at two Asian pear orchards in Kyotango City, Kyoto Prefecture, Japan (35˚36’24”N, 134˚57’31”E) from 28 August 2017 to 1 November 2017. Thirty-one types of pheromone lures showed in Table 1, which contain a totally of 1,000 µg compounds of one or two of E2,Z13-18:OH, E3,Z13-18:OH, Z3,Z13-18:OH, E2,Z13-18:OAc, E3,Z13-18:OAc, Z3,Z13-18:OAc or E2,Z13-18:Ald, were used for the test I (*N* = 2).

Based on the results of test I, test II was conducted at the same orchards from May 1 to June 13, 2018. In this test, 11 types of pheromone lures were used; 10:0, 9:1, 5:5, 1:9, and 0:10 mixtures of E2,Z13-18:OAc and Z3,Z13-18:OAc, 9:1, 5:5, and 1:9 mixtures of E2,Z13-18:OAc and E3,Z13-18:OAc, and 9:1, 5:5, and 1:9 mixtures of E2,Z13-18:OAc and E2,Z13-18:OH (*N* = 4) (Table 2).

Test III, which Z3,Z13-18:OAc and E2,Z13-18:OAc was finely assigned for the attractant, was conducted from June 13 to October 15, 2018. In this test, 8 types of pheromone lures were used; 100:0, 95:5, 09:10, 70:30, 50:50, 30:70, 10:90, and 0:100 mixtures of Z3,Z13-18:OAc and E2,Z13-18:OAc (*N* = 4) (Table 3).

In all field screening tests, the numbers of males captured by each trap were checked every about a week. Synthetic compounds were dissolved in hexane (20 mg/ml) and applied to rubber septa (white rubber, for o.d. 8mm, Sigma-Aldrich Co., St. Louis, MO, USA) as dispensers, and placed at the center of sticky board traps (SE Trap®, 30 × 27 cm bottom plate with a roof, Sankei Chemical Co., Kagoshima, Japan), and were hung separately 1.5 m above ground level at intervals of 10 m. The traps were rotated after counting the captured moths to eliminate any position effect. Identification of the attracted males was following Naka et al. (2019). The sternites 4, 5, and 6 in this species were covered with white scales, and these white scales are important to identify this species.

The numbers of captured males in tests II and III, excluding zeroes, were analyzed using a generalized linear model (GLM) with Poisson distribution and log link function, applying treatment as a fixed factor. Significant explanatory variables were compared by Tukey's test. These analyses were performed with R version 4.0.3 (R Development Core Team 2020).

Results

In the preliminary screening test performed in 2017 (test I), a total of 15 males of *S. nashivora* were captured to the traps (Table 1). Single Z3,Z13-18:OAc attracted 12 of the 15 males. Single E2,Z13-18:OAc, 5:5 mixture of Z3,Z13-18:OAc and E3,Z13-18:OAc, 9:1 mixture of Z3,Z13-18:OAc and Z3,Z13-18:OH also attracted each 1 male, respectively. The following screening test in 2018 (test II), all males were attracted to mixtures contain Z3,Z13-18:OAc and E2,Z13-18:OAc, and the 9:1 mixture of Z3,Z13-18:OAc and E2,Z13-18:OAc attracted significantly the largest number of males (15.25 ± 2.32 males/trap, *N* = 4) (Table 2). In a test in which the ratio of E2,Z13-18:OAc and Z3,Z13-18:OAc was finely assigned (test III), the largest number of males were attracted to 3:7 mixture of E2,Z13-18:OAc to Z3,Z13-18:OAc (6.00 ± 1.47 males/trap, *N* = 4) (Table 3). However, males were attracted to mixtures of various ratios, and the lures contain E2,Z13-18:OAc and Z3,Z13-18:OAc mixed at a ratio of 9:1 attracted only a few males.

Discussion

In the three field attraction tests conducted from 2017 to 2018 with 7 compounds used as sex pheromones by clearwing moths, males of *Synanthedon nashivora* were attracted to various mixture ratios, especially the 7:3 mixture ratio, of Z3,Z13-18:OAc and E2,Z13-18:OAc mixtures (Tables 1-3). These results suggest that the sex pheromone components of this species contain at least two compounds, Z3,Z13-18:OAc and E2,Z13-18:OAc, which are essential for attraction.

Both Z3,Z13-18:OAc and E2,Z13-18:OAc are used as components of sex pheromones by many clearwing moths. In Japanese clearwing species, Z3,Z13-18:OAc was found in *S. hector* (Yaginuma et al. 1976; Naka et al. 2008), *S. tenuis* (Tamaki et al. 1977; Naka et al. 2013), and E2,Z13-18:OAc was found from *S. tipuliformis* (Clerck) (Szöcs et al. 1985; James et al. 2001) and *S. haitangvora* Yang (Yang et al. 2009) as sex attractants or sex pheromones with attractant activity. Furthermore, *Milisipepsis takizawai* (Arita & Špatenka) is attracted to mixtures of E2,Z13-18:OAc and E3,Z13-18:OAc (Bûda et al. 1993). Of these species, *S. haitangvora* has been thought to be distributed in the Korean Peninsula and China (Arita et al. 2004) but was only recently discovered in Hokkaido, Japan (Yata et al. 2019). As the same as this species, males of *S. haitangvora* are strongly attracted to mixtures of Z3,Z13-18:OAc and E2,Z13-18:OAc (Yang et al. 2009). To the best of our knowledge, no screening tests have been conducted in Japan using a mixture of Z3,Z13-18:OAc and E2,Z13-18:OAc as sex attractants. This may be one of the reasons why the presence of *S. nashivora* and *S. haitangvora* were not known until 2014.

Both the sex attractant of this species and the sex pheromone of *S. haitangvora*, a very closely related species, are composed of Z3,Z13-18:OAc and E2,Z13-18:OAc. The fact that both males of *S. nashivora* and *S. haitangvora* are attracted to a mixture of these two components reminds us of two hypotheses for the relationship between the two species. The first is that *S. nashivora* may be a vicarious species of *S. haitangvora*. As mentioned above, *S. haitangvora* is widely distributed in the Korean Peninsula to China and Japan (Hokkaido), but so far there is no co-distribute with *S. nashivora*. This reinforces the possibility that *S. nashivora* is a vicarious species in Honshu (Tango Peninsula) derived from *S. haitangvora* by geographical isolation. The second possibility is that *S. nashivora* and *S. haitangvora* are not sibling species (despite overlapping host plants and similar external morphology), but have different distributions as a result of reproductive interference (Ribeiro and Spielman 1986; Kuno 1992). Although *S. nashivora* is closely related to *S. haitangvora*, a closely related species, *S. unocingulata* Bartel, whose host plants are Elaeagnaceae, also occurs in Japan, China, and the Korean Peninsula. Although *S. unocingulata* occurs sympatrically in the habitats of *S. nashivora* and *S. haitangvora*, no *S. unocingulata* males were attracted in this study, Yang et al (2009), or monitoring conducted in various areas of Japan using pheromone lures containing these two components (Ohno et al. in prep.). This means that the sex pheromone of *S. unocingulata* may be different from the sex attractant of *S. nashivora* and the sex pheromone of *S. haitangvora*. If *S. nashivora* or *S. haitangvora* were sympatrically speciated from *S. unocingulata* by using different components of the host plant or sex pheromone, reproductive interference could explain why *S. nashivora* and *S. haitangvora* are not sympatrically distributed. In any case, a nationwide monitoring study using pheromone traps with Z3,Z13-18:OAc and E2,Z13-18:OAc as attractants would allow us to know the detailed distribution of *S. nashivora* and *S. haitangvora* simultaneously. This will reveal which of the two hypotheses for the relationship between the two species is more plausible.

A pheromone lure containing a mixture of Z3,Z13-18:OAc and E2,Z13-18:OAc would be an effective tool for monitoring *S. nashivora*. By attempting to search for males in the field using this pheromone lure, it will be possible to understand the distribution of this species in Japan and neighboring countries, its microhabitat, and the seasonal occurrence of this species.

Declarations

Funding

Not applicable

Conflicts of interest/Competing interests

Not applicable

Availability of data and material

The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

Code availability

Not applicable

Authors' contributions

Hideshi NAKA: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing, Visualization, Supervision, Project administration; Takahiro KUKIZAKI: Validation, Investigation, Resources; Susumu TOKUMARU: Methodology, Resources; Satoshi OHNO: Investigation; Yuki MATSUI: Resources.

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Tables

Table 1. Attraction of *S. nashivora* by traps baited with E2,Z13-18:OH, E3,Z13-18:OH, Z3,Z13-18:OH and their acetate, and E2,Z13-18:Ald in different ratios (Test I)[a].

Acetates			Alcohols			Aldehyde	*N*	Total captured
E2,Z13-18:OAc	E3,Z13-18:OAc	Z3,Z13-18:OAc	E2,Z13-18:OH	E3,Z13-18:OH	Z3,Z13-18:OH	E2,Z13-18:Ald		
1,000							2	1
900	100						2	
900			100				2	
900						100	2	
500	500						2	
500			500				2	
500						500	2	
100	900						2	
100			900				2	
100						900	2	
	1,000						2	
	900	100					2	
	900			100			2	
	500	500					2	1
	500			500			2	
	100	900					2	
	100			900			2	
		1,000					2	12
		900			100		2	1
		500			500		2	
		100			900		2	
			1,000				2	
			900			100	2	
			500			500	2	
			100			900	2	
				1,000			2	
				900	100		2	
				500	500		2	
				100	900		2	
					1,000		2	
						1,000	2	

a Tested at Asian pear orchards in Kyotango City, Kyoto Prefecture from August 28 to November 1, 2017.

Table 2. Attraction of *S. nashivora* by traps baited with synthetic E2,Z13-18:OAc, E3,Z13-18:OAc, Z3,Z13-18:OAc, and E2,Z13-18:OH in different ratios (Test II)[a].

Compounds (µg)				*N*	Captured males (mean ± SE)[b]	
E2,Z13-18:OAc	E3,Z13-18:OAc	Z3,Z13-18:OAc	E2,Z13-18:OH			
1,000				4	0.25 ± 0.25	c
900		100		4	0.25 ± 0.25	c
500		500		4	6.5 ± 1.19	b
100		900		4	15.25 ± 2.32	a
		1,000		4	3.25 ± 0.75	b
900	100			4	0	
500	500			4	0	
100	900			4	0	
900			100	4	0	
500			500	4	0	
100			900	4	0	
Control				4	0	

a Tested at Asian pear orchards in Kyotango City, Kyoto Prefecture from May 1 to June 4, 2018.

b Males/trap. Values not zero followed by a different letter are significantly different by the Tukey's test ($P < 0.05$) after GLM (family = Poisson, link = log) performed with R version 4.0.3.

Table 3. Attraction of *S. nashivora* by traps baited with synthetic Z3,Z13-18:OAc and E2,Z13-18:OAc in different ratios (Test III)[a].

Compounds (μg)		N	Captured males (mean ± SE)[b]	
Z3,Z13-18:OAc	E2,Z13-18:OAc			
1000	0	4	2.50 ± 1.50	a b
950	50	4	2.25 ± 1.03	a b
900	100	4	2.00 ± 0.41	b
700	300	4	6.00 ± 1.47	a
500	500	4	3.50 ± 1.26	a b
300	700	4	1.50 ± 0.87	b
100	900	4	4.00 ± 1.58	a b
0	1000	4	0	
Control		4	0	

a Tested at Asian pear orchards in Kyotango City, Kyoto Prefecture from June 13 to October 15, 2018.

b Males/trap. Values not zero followed by a different letter are significantly different by the Tukey's test ($P < 0.05$) after GLM (family = Poisson, link = log) performed with R version 4.0.3.