Insect science

Insect Science (2024) 31, 1503–1518, DOI 10.1111/1744-7917.13367

ORIGINAL ARTICLE

Functional types of long trichoid sensilla responding to sex pheromone components in *Plutella xylostella*

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Abstract Sex pheromones, which consist of multiple components in specific ratios promote intraspecific sexual communications of insects. *Plutella xylostella* (L.) is a worldwide pest of cruciferous vegetables, the mating behavior of which is highly dependent on its olfactory system. Long trichoid sensilla on male antennae are the main olfactory sensilla that can sense sex pheromones. However, the underlying mechanisms remain unclear. In this study, 3 sex pheromone components from sex pheromone gland secretions of *P. xylostella* female adults were identified as Z11-16:Ald, Z11-16:Ac, and Z11-16:OH in a ratio of 9.4 : 100 : 17 using gas chromatography – mass spectrometry and gas chromatography with electroantennographic detection. Electrophysiological responses of 581 and 385 long trichoid sensilla of male adults and female adults, respectively, to the 3 components were measured by single sensillum recording. Hierarchical clustering analysis showed that the long trichoid sensilla were of 6 different types. In the male antennae, 52.32%, 5.51%, and 1.89% of the sensilla responded to Z11-16:Ald, Z11-16:Ac, and Z11-16:OH, which are named as A type, B type, and C type sensilla, respectively; 2.93% named as D type sensilla responded to both Z11-16:Ald and Z11-16:Ac, and 0.34% named as E type sensilla were sensitive to both Z11-16:Ald and Z11-16:OH. In the female antennae, only 7.53% of long trichoid sensilla responded to the sex pheromone components, A type sensilla were 3.64%, B type and C type sensilla were both 0.52%, D type sensilla were 1.30%, and 1.56% of the sensilla responded to all 3 components, which were named as F type sensilla. The responding long trichoid sensilla were located from the base to the terminal of the male antennae and from the base to the middle of the female antennae. The pheromone mixture (Z11-16:Ald : Z11-16:Ac : Z11-16:OH = 9.4 : 100 : 17) had a weakly repellent effect on female adults of *P. xylostella*. Our results lay the foundation for further studies on sex pheromone communications in *P. xylostella*.

Key words electrophysiological responses; long trichoid sensilla; *Plutella xylostella*; sex pheromone; single sensillum recording

Introduction

Sex pheromones, have evolved as important chemical signals to facilitate intraspecific sexual communication and isolation of interspecific reproduction in insect species, including moths (Wyatt, 2014; Allison & Cardé, 2016; Jiang *et al.*, 2020). The sex pheromones in moths

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are produced by the sex pheromone glands in female adults, generally via precursors of unsaturated fatty acid catalyzed by desaturases (Chow *et al.*, 1976; Bjostad & Roelofs, 1984; Tian *et al.*, 2021; Cheng *et al.*, 2023). During sexual communication, female moths release a blend of species-specific sex pheromones that attract male moths to mate, even over long distances (Ando *et al.*, 2004; Wyatt, 2014; Foster & Anderson, 2015). Male moths rely on olfactory systems to sense and recognize their own species' pheromone blends (Chow *et al.*, 1984; Hansson & Anton, 2000; Leal, 2013). The pheromonal components are detected by specific olfactory sensory neurons (OSNs) in special sensilla on the antennae of male moths and the information is relayed to the brain to elicit mating behavior (Berg *et al.*, 1995; Baker *et al.*, 2004; Hansson & Stensmyr, 2011; Fleischer *et al.*, 2018; Jiang *et al.*, 2020). As pheromones play a key role in intraspecific sexual communication, sex pheromone-mediated mating disruption and the use of sex pheromone-baited traps, which represent effective and eco-friendly biocontrol strategies, are globally used to manage agricultural pests (Benelli *et al.*, 2019; Benelli & Lucchi, 2021; Fleischer *et al.*, 2022).

The diamondback moth (DBM), *Plutella xylostella*, is a destructive pest of cruciferous vegetables worldwide which causes huge economic losses annually (Furlong *et al.*, 2013). Many new insecticides have been introduced to control *P. xylostella* in the past decades. However, *P. xylostella* developed resistance to these insecticides within a short time of their introduction (Zhao *et al.*, 2002; Furlong *et al.*, 2013; Pudasaini *et al.*, 2022). Nowadays, the moth has developed resistance to almost all insecticides used for its control (Wang *et al.*, 2022a). Therefore, new environmental-friendly and efficient strategies are urgently needed to control *P. xylostella*. Sex pheromone-mediated mating disruption and the use of sex pheromone-baited traps are ideal control methods to address the resistance challenges, because of the advantages they offer. A comprehensive study of the mechanisms underlying the functions of sex pheromones in *P. xylostella* is therefore needed for their effective application. The sex pheromone components of *P. xylostella* were first isolated and identified in the 1970s (Tamaki *et al.*, 1977). Thereafter, the components and relative contents of these sex pheromones in different locations have been reported (Table 1). Generally, the sex pheromones are released in specific ratios which enable male moths to accurately recognize female adults (Byer, 2006). The component of *P. xylostella* sex pheromone has been identified to include Z11-16:Ac, Z11-16:Ald, and Z11-16:OH (Ando *et al.*, 1979), the relative content of which varies with locations (Miluch *et al.*, 2014). In theory, this variation may affect the mating behaviors between *P. xylostella* from different geographical regions. For instance, while sex pheromone components can have a strong trapping or straying effect on a population of *P. xylostella* in one

Table 1 Sex pheromone components of different geographic populations of *Plutella xylostella*.

Geographic population	Z11-16:Ald	Z11-16:Ac	Z11-16:OH	Reference
Japan	1	1	–	Tamaki *et al.*, 1977
	4	6	–	
Taiwan, China	1	1	–	Chow *et al.*, 1977
Japan	1	1	0.1	Ando *et al.*, 1979
Canada	7	3	–	Chisholm *et al.*, 1979
Taiwan, China	5	5	0.1	Lin *et al.*, 1982
India	1	1	0.01	Reddy & Guerrero, 2000
New Zealand	40	60	–	Suckling *et al.*, 2002
	60	30	10	
	10	60	30	
USA	3	1	2	He *et al.*, 2003
Korea	10	90	1	Yang *et al.*, 2007
	10	90	10	
	8	100	18	
Suzhou, China	7	3	1	Deng *et al.*, 2007
Shanghai, China	7	3	1	Dai *et al.*, 2008
Guangdong, China	30	70	0.1	Dai *et al.*, 2016

region, they may not have ideal control effects on populations in other regions. It is therefore necessary to determine the precise contents of sex pheromone components of different geographical populations for effective control of *P. xylostella*.

Trichoid sensilla are sensitive to species-specific pheromones in insects (Kaissling, 1986; Steinbrecht, 1997). Our previous study revealed that there were 17 types of sensilla on the antennae of *P. xylostella*: trichodea (2 subtypes), basiconica, coeloconica (3 subtypes), Böhm's bristles (2 subtypes), styloconica (2 subtypes), squamiformia, auricillica, furcatea (3 subtypes), cupuliform organs, and terminal sensory pegs (Yan *et al.*, 2017). Long trichoid sensilla are the most abundant of all sensillum types and the male adults have a higher number and size of the sensilla than female adults (Yan *et al.*, 2017). Trichoid sensilla on the male moth antennae contain different functional types, which detect different sex pheromonal components (Wu *et al.*, 2015; Chang *et al.*, 2016; Xu *et al.*, 2017; Jiang *et al.*, 2020). In Lepidoptera, the mechanisms underlying trichoid sensilla responses to sex pheromone components have been well described for *Helicoverpa armigera* and *Helicoverpa assulta*. (Z)-11-hexadecenal (Z11-16:Ald) and (Z)-9-hexadecenal (Z9-16:Ald) have been identified as pheromone components in *H. armigera* and *H. assulta*, but in nearly reversed ratios (Wu *et al.*, 2013; Wu *et al.*, 2015). Three types of trichoid sensilla (A-C) on the antennae of *H. armigera* have been identified to respond to (Z)-11-hexadecenal (Z11-16:Ald), (Z)-9-hexadecenal (Z9-16:Ald), and both Z11-16:Ald and Z9-16:Ald, respectively (Wu *et al.*, 2013; Wu *et al.*, 2015).

In China, until now, the components and relative contents of *P. xylostella* sex pheromones have all been identified from populations found in the south (Chow *et al.*, 1977; Lin *et al.*, 1982; Maa *et al.*, 1984; Dai *et al.*, 2008). However, the populations in northern China appear to have distinct origins compared to those in southern China, with the southern population originating from Yunnan and the northern population primarily deriving from Sichuan (Chen *et al.*, 2021). The variations among the different geographical populations are substantial. It is therefore essential to know the sex pheromone components from the different populations. In addition, how male adults detect these sex pheromone components are still unclear. To address these, this study was performed to identify sex pheromone gland secretions of *P. xylostella* female adults from Taigu, Shanxi Province (North China), using gas chromatography with electroantennographic detection (GC-EAD) and gas chromatography-mass spectrometry (GC-MS). Next, we then identified the long trichoid sensilla in male and female adults of *P. xylostella* and their antennal distributions based on their sensitivity to sex pheromones using single sensillum recording (SSR). Finally, we evaluated the responses of male and female adults to the sex pheromone components by behavioral assays and field trapping. The results from this study enrich our understanding of the mechanism underlying detection of *P. xylostella* to sex pheromones.

Materials and methods

Insects rearing

The larvae and pupae of *P. xylostella* were collected and reared in the Insect Neurobehavioral and Sensory Biology Laboratory, in Shanxi Agricultural University, Taigu campus. The larvae were fed with fresh *Brassica oleracea* leaves, and the adults were fed with 10% honey solution. The rearing conditions were temperature at 25 ± 1 °C, a photoperiod of 14 L : 10 D, and relative humidity at 70% ± 5%. Newly emerged (1−3-d-old) female and male adults of *P. xylostella* were used in the experiments.

Chemicals

Sex pheromone components, including Z11-16:Ald (≥99%), Z11-16:OH (≥99%), and Z11-16:Ac (≥98%), were purchased from Toronto Research Chemicals (Toronto, Canada). Hexane (≥98%) and paraffin oil were purchased from Sigma–Aldrich (St. Louis, MO, USA). These chemicals were diluted to 1, 10, and 100 μg/μL by paraffin oil for electrophysiological and behavioral assays.

Extraction of sex pheromone

Extraction of sex pheromone extracts were performed as described by Jiang *et al.* (2021) with minor modifications. The extracts were obtained from 2-3-d-old virgin female adults during 5:00 p.m. to 12:00 p.m. When female adults of *P. xylostella* calling behavior occurred, 60 pheromone gland-ovipositor complexes were excised using micro-scissors. They were immediately placed into brown vials, and immersed with 500 μL of hexane (>98% purity) for 20 min, then desiccated through a $MgSO_4$ column. The samples were then concentrated to 60 μL (1 μL per *P. xylostella* female adult) with high purity nitrogen gas flow. Concentrated samples were sealed and stored in 2 mL glass vials in a −20 °C refrigerator.

Each of the 60 pheromonal glands and ovipositors were grouped as a single repeat, totaling 3 sets of repeats.

GC-EAD

The electrophysiological response of male antennae to the pheromone gland extracts of female adults was evaluated using an Agilent 6890N gas chromatograph (Agilent, USA) with a HP-5 capillary column (30 m × 0.25 mm internal diameter [ID], 0.25 μm film thickness) coupled with an electroantennographic detector (Syntech, Hilversum, the Netherlands). An aliquot of pheromone gland extracts (2 μL) was injected into the GC column at 220 °C with a flame ionization detector (FID) at 250 °C. The GC column temperature was programmed to 50 °C for 1 min, and then raised at 10 °C/min to 180 °C, finally, the temperature was raised at 5 °C/min to 220 °C, and held for 5 min. Helium was employed as the carrier gas at a speed of 1.0 mL/min. The column effluent was divided into FID and EAD in a 1 : 1 ratio. For EAD preparation, a male antenna was cut at the base and tip with micro-scissors, which was immediately mounted on the antenna holder with 2 metal electrodes using conductive gel (Spectra 360, Parker Lab, NJ, USA), and then the electrode holder was inserted into the EAD probe. The antenna was positioned at 1 cm from the glass tube, and the purified and humidified supplemental air flow (500 mL/min) was delivered to the antenna using a stimulus flow controller (CS-55, Syntech, Buchenbach, Germany). The antennal analog signals were amplified and converted using an IDAC-2 amplifier (Syntech) and the data were analyzed using the GC-EAD 2014 software (version 1.2.5, Syntech). Eight replicates were performed.

GC-MS

The sex pheromone extracts were analyzed with an Agilent 5973 MS coupled to an Agilent 6890N GC (Agilent, USA). Aliquots of the extracts (1 μL) were injected into a splitless injector mounted onto an HP-5 capillary column (30 m × 0.25 mm ID, 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA). Helium was employed as the carrier gas at a speed of 1.5 mL/min. The temperature program followed the aforementioned GC-EAD system: the injector and detector temperatures were 250 °C and 300 °C, respectively. The ionization energy of the electron impact MS was 70 eV, the ion source temperature was 230 °C, and the mass range was 45−500 *m*/*z*. The EAD-active components in the pheromone gland extracts were identified by comparing their MS data with the NIST08 MS library, and the relative contents of these components were calculated from their peak areas.

Scanning electron microscopy

Scanning electron microscopy (SEM) was performed on antennal tissues (Yan *et al.*, 2017). Antennae from 5 healthy moths were cut off from the base, under a postural microscope and immersed in a 70% ethanol solution containing 0.5% Triton X-100 and stored in a 4 °C refrigerator for 24 h. Next, the antennae were cleaned with an ultrasonic cleaner for 5 s and rinsed in buffer solution. They were then dehydrated using a graded ethanol series of 30%, 50%, 70%, 80%, 95%, and 100% (fully dehydrated twice) for 15 min at each concentration. After critical point drying (ES-2030, Hitachi, Japan), the dried pheromone gland sample was pasted on a SEM sample holder with double-sided adhesive, sputter-coated with gold, then observed and photographed with a S-3400N (Hitachi, Japan) SEM.

SSR

Recording of the responses of trichoid sensilla on the antenna of *P. xylostella* to sex pheromones by SSR followed Jiang *et al.* (2021). A 2-d-old moth was carefully inserted into a plastic pipette tip and moved forward to expose its head. It was fixed on a glass slide covered with double-sided adhesive. A tungsten wire reference electrode electrolytically sharpened with 10% KOH was inserted into the compound eye under a light microscope and grounded. The sensilla tips on its antennae were cut off using fine scissors and the action potential was recorded by inserting an Ag-AgCl electrode into a glass microtube filled with sensillum lymph ringer (Olsson & Hansson, 2013). The glass-recording electrode was placed on the tip of the cut sensilla using a micromanipulator (lintoninst ROE-200, U.K.). Z11-16:Ald, Z11-16:Ac, and Z11-16:OH were prepared with paraffin oil at concentrations of 1, 10, 100 μg/μL of solutions. A volume of 10 μL of each concentration was independently added to the filter papers (isosceles triangle with bottom side length of 0.5 cm and height of 3 cm) which were placed into Pasteur pipettes. An air stream from a stimulus flow controller CS-55 (Syntech, Buchenbach, Germany) was passed through at a flow rate of 500 mL/min. Signals were filtered with 100–10 000 Hz suppression using a cutoff filter and electrical signals were then recorded continuously for 15 s. Analog signals were sampled using a signal acquisition system

(IDAC-4, SynTech, Germany) and action potential frequencies (spikes/s) were counted offline using Autospike software 500 ms before and 500 ms during stimulation.

Y-tube olfactometer assays

Y-tube olfactometer bioassays were performed following Song *et al.* (2022). The Y-tube olfactometer made of glass consisted of a center (5 cm long × 2.5 cm ID) and 2 glass arms at an internal angle of 70° (15 cm long × 2.5 cm ID). The behavioral response of male and female adults of *P. xylostella* to single components of the sex pheromone and their mixture were then observed. Each component was diluted with paraffin oil to 100 μg/μL. Each of the 2 side arms of the olfactometer was connected to an odor source container. A volume of 10 μL of test solution was applied to a piece of Whatman® filter paper (5 mm × 50 mm) and placed in one of the source containers. A filter paper strip (5 mm × 50 mm) containing the same volume of paraffin oil was placed in the other source container. Moist air which was also passed through activated carbon for filtration was allowed to flow through each container at a flow rate of 350 mL/min. A single moth *P. xylostella* aged 1–2-d-old was then put into the long side of the olfactometer to make a choice to any of the arms within 3 min. If the moth entered more than 1/2 way upstream of the arm and stayed for more than 30 s, it was recorded as a "choice", otherwise, a "no choice" was recorded. Before the experiment, it was ensured that each moth had not encountered any components and was used only once. The positions of 2 test arms were swapped after 5 moth tests and the olfactometer was cleaned with 98% acetone after 10 moth tests. The period for the behavioral tests was from 17:00 to 22:00 hours.

Field trapping trials

Field trappings were carried out from late June to mid-July 2022 (Fu *et al.*, 2022), in a cabbage field (about 30 000 m^2) in Taigu District, Jinzhong, Shanxi Province, China (37°46′35″N, 112°50′54″E), without spraying any pesticides. Sex pheromone mixture (Z11-16:Ald : Z11-16:Ac : Z11-16:OH = 9.4 : 100 : 17) was prepared at a concentration of 100 μg/μL with paraffin oil. A volume of 100 μL was dispensed into a brown sustained-release bottle (1 mL, with a small hole of 1 mm diameter in the center of each cap for releasing odor). Another similar bottle contained paraffin oil, which served as a control. The slow-release bottles were hung in square holes (1 cm length, 1 cm width) made in the center of green and white sticky boards (25 cm length, 20 cm width) or in the center of white plastic triangle traps (17.8 cm of the triangle side, 26.5 cm length) for trapping moths. The traps were suspended about 15 cm above cabbage plants. Three groups of repeats were set and randomly placed in the field at intervals of 50 m. The number of trapped *P. xylostella* was counted after 48 h.

Statistical analysis

Data analyses were performed using SPSS version 25.0 (IBM, Armonk, NY, USA) and figures were drawn using Origin 2022. All results are presented as the mean ± standard error. The SSR response data were analyzed by one-way analysis of variance (ANOVA) and hierarchical cluster analysis (HCA) based on the maximum distance method was used to classify the sensilla (library *Superheat* in R) (Pérez-Aparicio *et al.*, 2022). The significant differences in the Y-tube olfactometer data of male and female adults were compared by Chi-square test. The data of the field trapping trials were subjected to one-way ANOVA followed by Tukey's multiple range test. Statistical significance was set at $P < 0.05$. Images obtained by SEM was processed and annotated using Adobe Photoshop CC 2018 software.

Results

Components and relative contents of P. xylostella sex pheromone

Three components, Z11-16:Ald, Z11-16:Ac, and Z11-16:OH in a ratio of 9.4 : 100 : 17, were identified from the pheromone gland extracts of female adults of *P. xylostella* using GC-EAD and GC-MS (Figs. 1, S1, and Table 2). The Z11-16:Ald, Z11-16:Ac and Z11-16:OH contents in the single pheromone gland extract were determined as 0.20, 2.08, and 0.35 ng, respectively (Table 2).

General morphology of long trichoid sensilla on the antennae of P. xylostella

The antennae consisted of 3 parts from base to top: scape (S, 1 segment), pedicel (P, 1 segment), and flagella (F, 31 segments) (Fig. 2A). Long trichoid sensilla were the main sensilla on the flagella (Fig. 2B) (Yan *et al.*, 2014; Yan *et al.*, 2017). To facilitate the study of the responses of long trichoid sensilla to the components of sex pheromones, the flagella was evenly divided into 4 sections: section I (the segments from the 1st to 8th), section

Table 2 Components and relative contents of sex pheromones of *Plutella xylostella*.

Sex pheromone component	Gland extract R.t (min)	Synthetic sample R.t (min)	Integrated area (mean ± SE)	Ratio	Content (per female/ng)
Z11-16:Ald	18.263	18.268	1 198 520 ± 398 292	9.4	0.2
Z11-16:Ac	22.309	22.306	12 646 645 ± 1 884 447	100	2.08
Z11-16:OH	22.96	22.95	2 229 281 ± 582 263.5	17	0.35

R.t: retention time; SE, standard error.

II (the segments from the 9th to 16th), section III (the segments from the 17th to 24th), and section IV (the segments from the 25th to last) (Fig. 2A).

Responses of long trichoid sensilla in different antennal regions of P. xylostella male adults and female adults to sex pheromone components

The functions of the long trichoid sensilla (581 male and 385 female sensilla) to sex pheromones were characterized using SSR experiments. Not all long trichoid sensilla responded to the sex pheromone components. Among them, 62.99% of male and 7.53% of female sensilla had different degrees of response to the 3 components (Fig. 4A, B). They were grouped into 6 distinct clusters by HCA, and therefore identified as 6 different types (Fig. 3). Long trichoid sensilla were the most abundant on the antennae of male adults. A proportion (59.72%) of the long trichoid sensilla responded to single components of the sex pheromones (Figs. 3, 4A, and S2A–S2C). In detail, 52.32%, 5.51%, and 1.89% of them showed electrophysiological responses to Z11-16:Ald (named as A type), Z11-16:Ac (B type), and Z11-16:OH (C type) dose-dependently. Only 3.27% of the long trichoid sensilla responded to 2 components of the sex pheromones (Figs. 3, 4A, 2D, and E). Of these, 2.93% responded to both Z11-16:Ald and Z11-16:Ac, named as D type, and 0.34% responded to Z11-16:Ald and Z11-16:OH, named as E type. Many A type and a few of B type sensilla were found in regions I–IV of male antennae. C type sensilla were evenly distributed in regions I–III. D type sensilla was found in regions II–IV. A thimbleful of E type sensilla was found only in region II (Fig. 4A).

On female antennae, long trichoid sensilla were also the most abundant, and their appearance and structure were similar to those on the antennae of males (Yan *et al.*, 2017). Therefore, further investigations were conducted to determine whether these were also sensitive to sex pheromones. The results showed that the majority of the long trichoid sensilla on the female antennae did not respond to the sex pheromone components; only a small number of them responded to the components. Only 4.68% of the long trichoid sensilla responded to a single component of the sex pheromones (Fig. 4B). Of these, 3.64% were A type sensilla, which only responded to Z11-16:Ald, and 0.52% were B or C type sensilla which responded only to Z11-16:Ac or Z11-16:OH (Figa. 3, 4B, and S3A–S3C). Additionally, 2.86% of the long trichoid sensilla responded to 2 or 3 components of the sex pheromones (Fig. 4B). In detail, 1.30% were D type sensilla which responded to both Z11-16:Ald and Z11-16:Ac (Figs. 3, 4B, and S3D), and 1.56% responded to all 3 components, which were named as F type sensilla (Figs. 3, 4B, and S3E). Surprisingly, E type sensilla were not found on the female antennae. The A type sensilla were relatively evenly distributed in regions I–III on the antennae of females, but none in region IV. The B type sensilla were evenly distributed in regions I–II. Besides A type, no other type of responding long trichoid sensilla was found in region III. However, types A, B, C, D, and F were found in region II (Fig. 4B).

Behavioral responses of male and female adults of P. xylostella to 3 sex pheromone components and their mixture

The behavioral responses of male and female adults of *P. xylostella* to single components and their mixture were evaluated in a Y-tube olfactometer (Fig. 5A). At a concentration of 100 μg/μL, male adults were attracted strongly to Z11-16:Ald, Z11-16:Ac, Z11-16:OH and the mixture. However, for female adults, the difference between response to control and any 1 of 3 components of the sex pheromone was not significant ($P > 0.05$). For the mixture of the sex pheromones (Z11-16:Ald : Z11-16:Ac : Z11-16:OH = 9.4 : 100 : 17), female adults significantly preferred the control arm, with the selection index being 66.00% ($P < 0.05$) (Fig. 5B).

Effects of the sex pheromone mixture on male and female adults of P. xylostella in the field

The field trapping results showed that single components and their mixture (Z11-16:Ald : Z11-16:Ac : Z11-16:OH = 9.4 : 100 : 17) of sex pheromones had strong

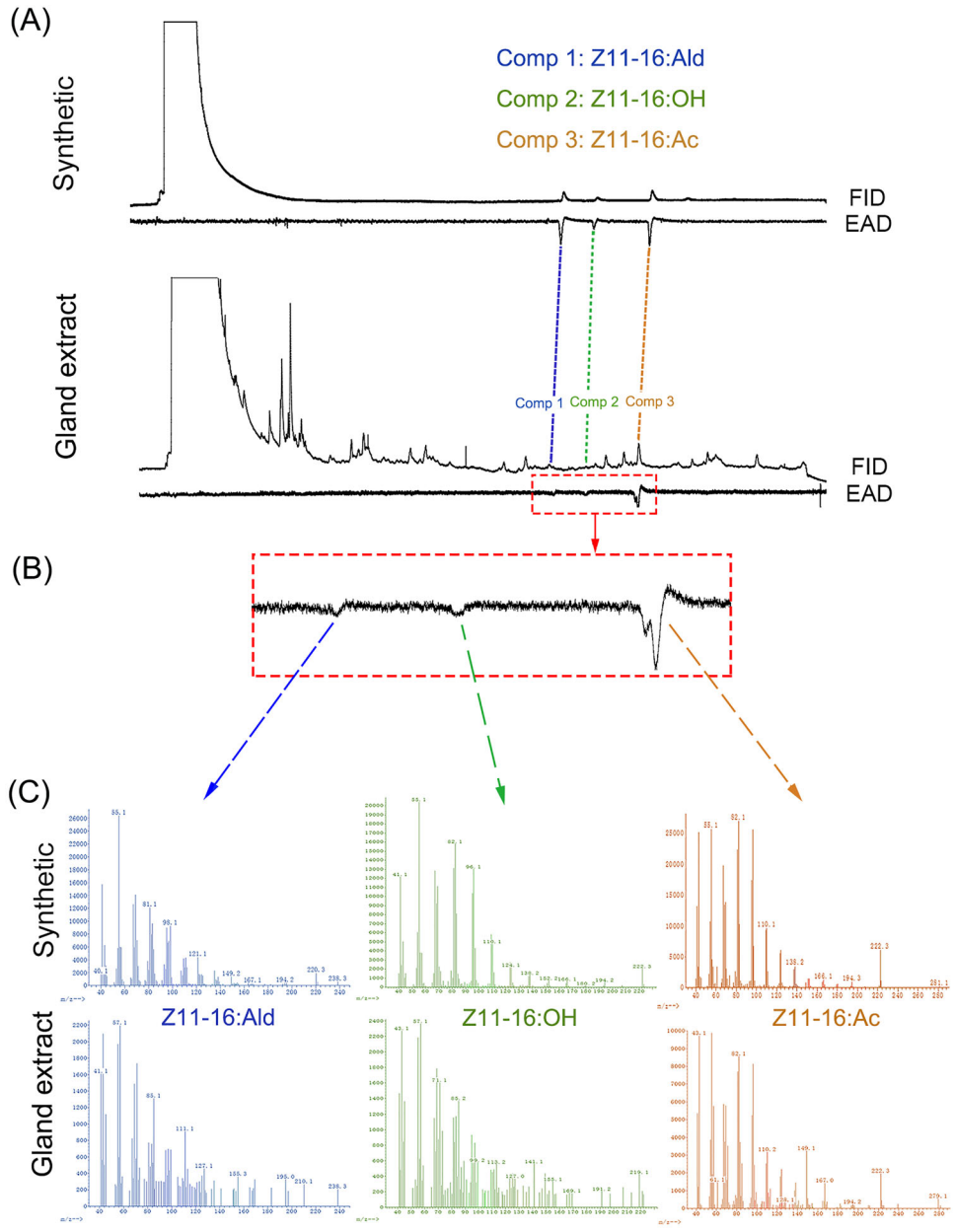

Fig. 1 Simultaneously recorded gas chromatography with electroantennographic detection (GC-EAD) using the antennae of *Plutella xylostella* male adults in response to gland extract. (A) GC-EAD profile of standard mixture and gland extract on antennae of *P. xylostella* male adults. (B) An enlarged image of the EAD trace detail. The active components in female pheromonal gland extracts identified by GC-EAD were Z11-16:Ald (Comp1), Z11-16:OH (Comp2), and Z11-16:Ac (Comp3). (C) Mass spectrometry of each GC-EAD-active component and its corresponding synthetic component.

attraction to male adults among the green sticky board, white sticky board, and triangle trap; however, they had no attraction on female *P. xylostella* in different traps (Fig. 6).

Discussion

Most Lepidopteran moth species, including *P. xylostella*, use multi-component sex pheromones of specific

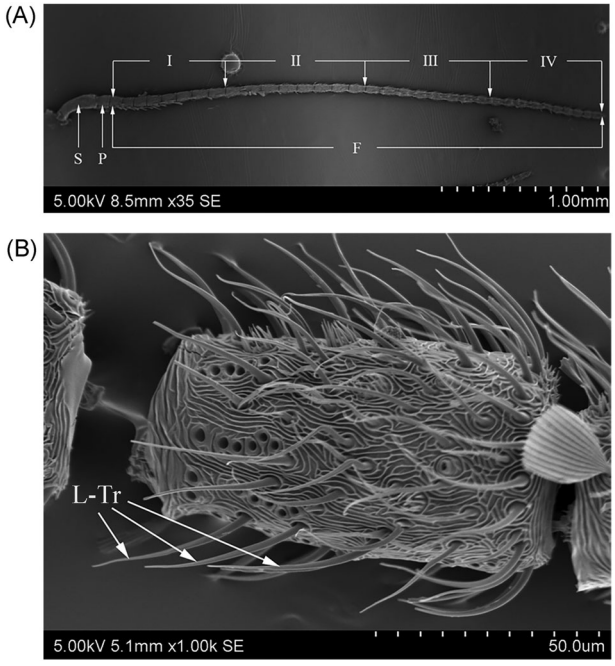

Fig. 2 Scanning electron microscopy (SEM) photomicrographs of *Plutella xylostella* antennae. (A) Schematic diagram of antennal regional division of *P. xylostella*. S: scape; P: pedicel; F: flagellum. The entire flagellum is divided into 4 regions, with 1–8 segments being region-I, 9–16 segments being region-II, 17–24 segments being region-III, and after 25 segments being region-IV. (B) Close-up views of long trichoid sensilla on the antennae of the *P. xylostella*. L-Tr: long trichoid sensilla.

ratios to perform calling and mating behaviors. However, the relative amounts of the sex pheromones of *P. xylostella* vary with location (Miluch *et al.*, 2014). In China, the components and relative amounts of *P. xylostella* sex pheromones were all identified previously in southern China, with little information about them in northern China. In this study, the components of the sex pheromone of the *P. xylostella* from northern China (Taigu, Shanxi province) were identified as Z11-16:Ald, Z11-16:Ac, and Z11-16:OH in a ratio of 9.4 : 100 : 17. The components are similar to that identified from many areas (Miluch *et al.*, 2014). The ratio of the sex pheromone components in this study (Taigu, North China) was most similar to that identified in Korea with a ratio of 8 : 100 : 18 (Yang *et al.*, 2007), but very different from that identified in southern China (Chow *et al.*, 1977; Lin *et al.*, 1982; Maa *et al.*, 1984; Dai *et al.*, 2008). This difference may be due to genetic differences between the northern and southern populations of *P. xylostella* in China.

Generally, sex pheromones are released by female moths to attract conspecific male adults for mating (Groot, 2014; Benelli & Lucchi, 2021; Wang *et al.*, 2022b). The antennae of male moths typically bear multiporous trichodea sensilla, which detect these sex pheromone components (Almaas & Mustaparta, 1991; Ammagarahalli & Gemeno, 2014; Wu *et al.*, 2015; Xu *et al.*, 2017). However, not all of them respond to the pheromones. For example, in *Manduca sexta* male adults, 59% of the trichoid sensilla showed a response to sex pheromone components (Kalinová *et al.*, 2001) and about 29% of the trichoid sensilla in *Grapholita molesta* males could not sense sex pheromone components (Ammagarahalli & Gemeno, 2014). In our study, 62.99% of the tested antennal long trichoid sensilla of male *P. xylostella* adults responded to sex pheromone components as expected. The results were consistent with previous studies in other moths.

The peripheral coding of sex pheromones has been studied in many insect species by electrophysiological recordings, and the performances of pheromone-sensitive trichoid sensilla are different (Wu *et al.*, 2015; Chang *et al.*, 2016; Xu *et al.*, 2017; Jiang *et al.*, 2020; Liu *et al.*, 2023). For example, 2 types of trichoid sensilla were classified in *Mythimna separata* male adults: A type sensilla responded to Z11-16:Ald and Z9-14:Ald, and B type sensilla mainly to Z9-14:Ald, and also to Z11-16:Ac, Z11-16:OH, and Z9-16:Ald (Jiang *et al.*, 2020). The sympatric species, *H. armigera* and *H. assulta* use the same sex pheromone components, Z11-16:Ald and Z9-16:Ald, but in ratios of 98 : 2 and 5 : 95, respectively (Wang *et al.*, 2005). In *H. armigera* male adults, the most represented sensilla, A type trichoid sensilla, respond to the major pheromone component (Z11-16:Ald), B type sensilla respond to Z9-14:Ald, C type sensilla responds to another major pheromone component (Z9-16:Ald), and also responded to Z9-14:Ald. However, the numbers of the A, and C type sensilla, responsive to Z11-16:Ald and Z9-16:Ald, respectively, are reversed in *H. assulta* male adults (Wu *et al.*, 2015; Chang *et al.*, 2016; Xu *et al.*, 2017). Similar to the above results, long trichoid sensilla on antennae of *P. xylostella* male adults were of 5 different functional types; the majority of the long trichoid sensilla were of the A type sensilla which responded to Z11-16:Ald, the B and C type sensilla responded to Z11-16:Ac and Z11-16:OH, respectively, D type sensilla responded to both Z11-16:Ald and Z11-16:Ac, and E type sensilla responded to both Z11-16:Ald and Z11-16:OH.

Sex pheromone components are recognized by different trichoid sensilla on antennae of male adults, mainly because these sensilla possess pheromone-responsive OSNs, which inhabits specific pheromone receptors

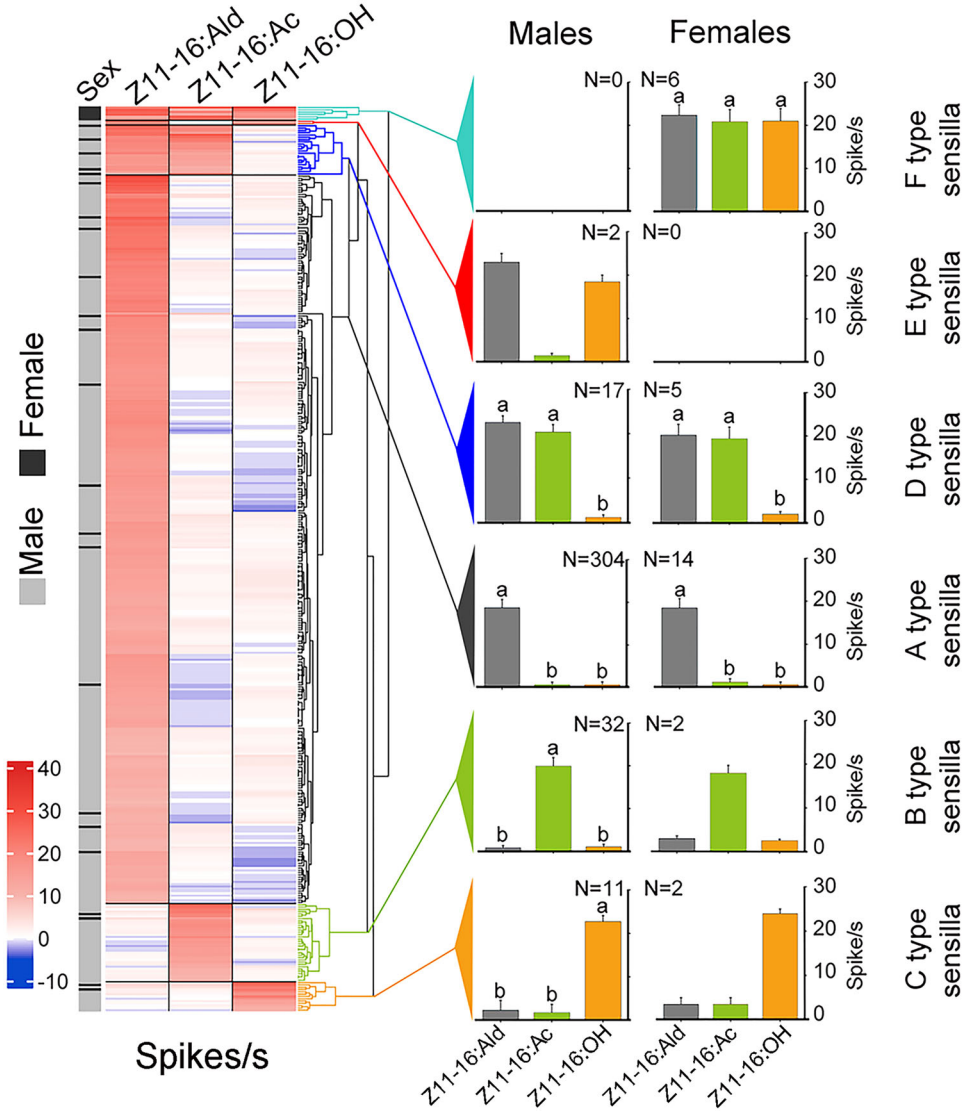

Fig. 3 Classification of sensilla types. The left panel shows a hierarchical cluster analysis (HCA) grouping of *Plutella xylostella* male and female adult sensilla based on their responses to the 3 components of sex pheromone. Each entry in the y-axis indicates a different sensilla color-coded by sex (light gray for male, black for female). The horizontal lines separate HCA clusters which have been color-coded in the dendrogram on the right side and represent 6 physiological sensilla types. The right panel shows average responses for each of the 6 HCA clusters: A type sensilla responded to Z11-16:Ald; B type sensilla responded to Z11-16:Ac; C type sensilla responded to Z11-16:OH; D type sensilla responded to Z11-16:Ald and Z11-16:Ac; E type sensilla responded to Z11-16:Ald and Z11-16:OH; F type sensilla responded to all 3 components. "N" indicates the number of each sensilla type. Different letters indicate significant differences in different components (one-way analysis of variance, Tukey's test, $P < 0.05$).

(PRs) involved in the sensing of pheromones (Berg *et al.*, 1995; Baker *et al.*, 2004; Fleischer *et al.*, 2018). For example, using a *Xenopus* expression system and 2-electrode voltage-clamp, MsepOR2 and MsepOR3 found in *M. separata* tuned to Z9-14:Ald and Z11-16:Ald, respectively, and 2-color fluorescence *in situ* hybridization validated that they were localized in the B type and A type sensilla (Jiang *et al.*, 2020). In *H. armigera* and *H. assulta*, OR13 (which responds to Z11-16:Ald specifically) was localized in A type sensilla in both species; OR6 (which responds to Z9-14:Ald and Z9-16:Ald) was localized in C type sensilla in both species; OR14b (which responds to Z9-14:Ald) was localized in B type sensilla *in H. armigera* (Jiang *et al.*, 2014; Chang *et al.*,

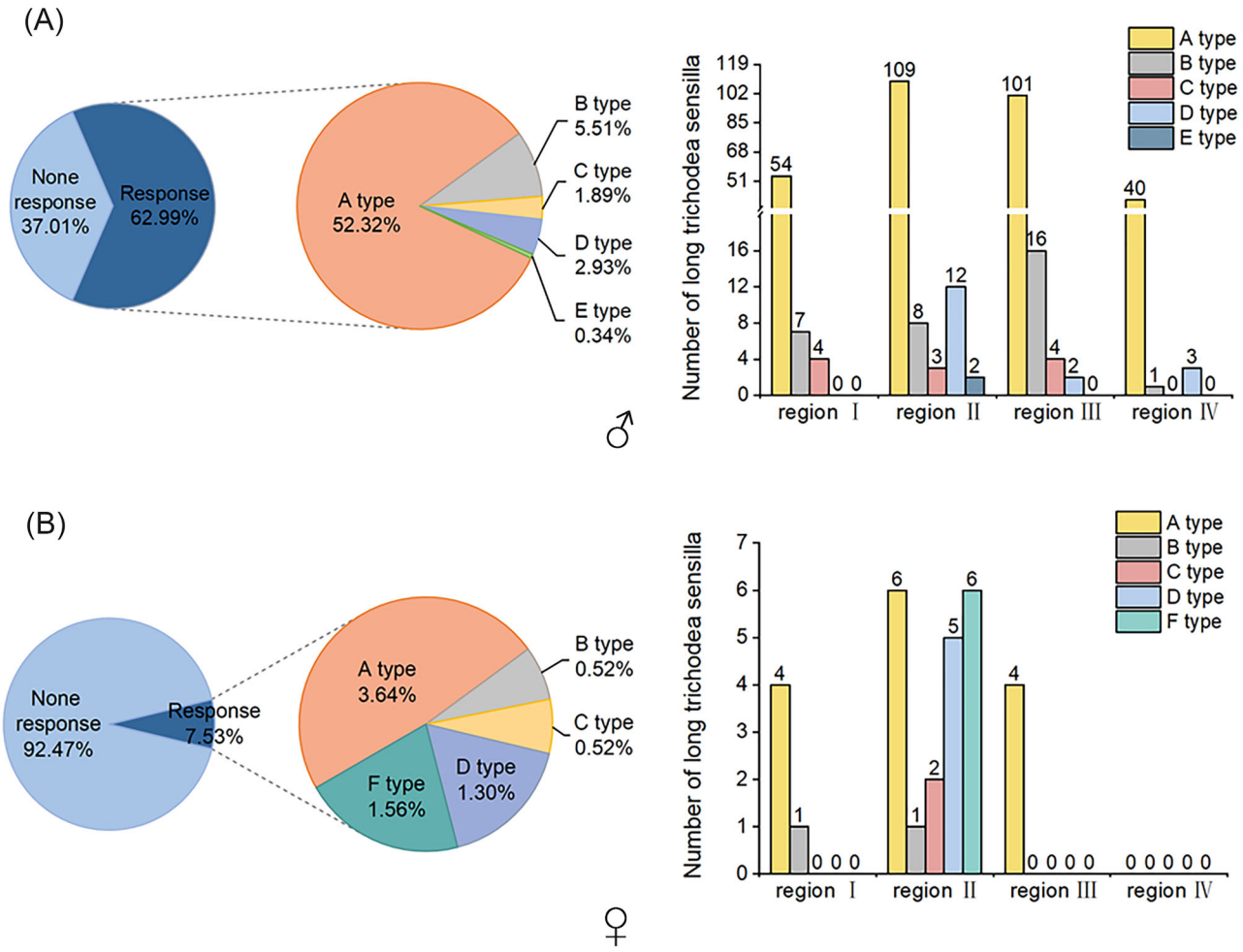

Fig. 4 The proportion of observed long trichoid and the distribution of the 6 types of pheromone-responding sensilla on the antennae (A) Statistics on the number of male sensillum and sensilla distribution of male adults (♂) of *Plutella xylostella*. (B) Statistics on the number of female sensillum and sensilla distribution of female adults (♀) of *P. xylostella*. The left image showed the proportion of antennal long trichoid sensilla of the male (♂) and female (♀) *P. xylostella* adults which responded to the sex pheromones. The right image shows the number of the 6 types of long trichoid sensilla that responded to sex pheromones in different regions of *P. xylostella* antennae.

2016). The PRs of *P. xylostella* have been identified and studied (Mitsuno *et al.*, 2008; Sun *et al.*, 2013; Liu *et al.*, 2018). Nine genes (*PxylOR1*, *PxylOR3*, *PxylOR4*, *PxylOR5*, *PxylOR6*, *PxylOR7*, *PxylOR8*, *PxylOR41*, and *PxylOR45*) have been cloned and identified as candidate PR genes through phylogenetic analysis (Sun *et al.*, 2013; Liu *et al.*, 2018). Through receptor expression in *Xenopus oocytes* and 2 electrode voltage-clamp electrophysiological recordings analysis, *Pxyl*OR1 was identified as a pheromone receptor which was tuned to Z11-16:Ald (Mitsuno *et al.*, 2008; Sun *et al.*, 2013). Therefore, we speculated that *Pxyl*OR1 was localized in the A type sensilla in *P. xylostella* which were responsive to Z11-16:Ald. The PRs for Z11-16:OH and Z11-16:Ac have not been deorphanized. Additionally, the localizations of PRs in the different types of long trichoid sensilla in *P. xylostella* are still unclear. Among noctuid species such as *H. armigera* and *H. assulta*, the majority of the population of trichoid sensilla respond strongly to the major component in sex pheromones. However, this phenomenon is reversed in *P. xylostella*, with fewer trichoid sensilla (5.51%) responding to the major component Z11-16:Ac, while the majority (52.32%) respond to the minor component Z11-16:Ald. It could be that the pheromone communication systems of different moths has adopted different evolutionary strategies. The

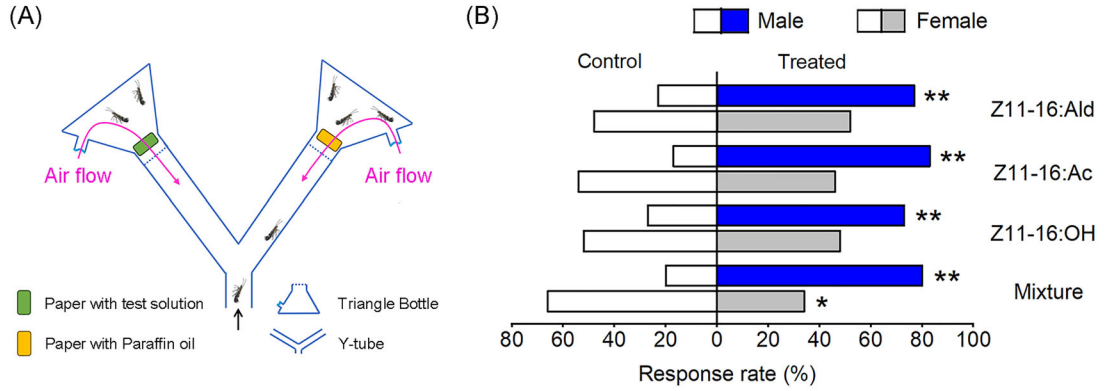

Fig. 5 Behavioral responses of *Plutella xylostella* adults to the sex pheromone components. (A) Illustration of the Y-tube olfactometer. (B) Behavioral responses of male and female adults of *P. xylostella* to Z11-16:Ald, Z11-16:Ac, Z11-16:OH and the mixture (Z11-16:Ald : Z11-16:Ac : Z11-16:OH = 9.4 : 100 : 17) at a concentration of 100 μg/μL. One asterisk indicates significant difference at $P < 0.05$; ** indicate significant difference at $P < 0.01$ (χ^2-test).

strategy in *P. xylostella* may balance the detection efficiency of each component in the sex pheromone blend. However, the relevant mechanisms remain to be elucidated by studying the regulation of pheromone receptor expression.

Olfactory communication research on sex pheromones is mainly focused on male insect reception of pheromones, while that on female sensilla responses to sex pheromone components is limited. Many pheromone detection studies have showed that most female moths cannot perceive their own pheromones (Schneider, 1957; Schneider, 1962; Hansson *et al.*, 1989; Maida & Ziesmann, 2001; Daimon *et al.*, 2012; De Silva *et al.*, 2013), or that the sensitivity of female antennae to pheromone was considerably lower than that of males (Ljungberg *et al.*, 1993; Holdcraft *et al.*, 2016). Only a few studies have reported that female sensilla were sensitive to their own pheromones (Ljungberg *et al.*, 1993; Larsson *et al.*, 1999). For example, in *Spodoptera littoralis*, antennal trichoid sensilla on females were reported to have responded to the 2 known pheromone components, Z9, E11-14:OAc and Z9, E12-14:OAc (Ljungberg *et al.*, 1993). Accordingly, in our study, a few antennal long trichoid sensilla in female adults also sensed sex pheromonal components. Furthermore, female adults showed slight repellent behavioral responses to the pheromonal blend. However, the pheromones had not significant trapping effects in the field, even at a higher concentration of 100 μg/μL. The repellent behavioral responses to pheromones have also been observed in other moths. For example, female *H. armigera* and *Helicoverpa zea* were repelled by their pheromones in olfactometer tests (Saad & Scott, 1981). Also, female *Ephestia kuehniella* were repelled by a pheromone-baited trap (Trematerra & Battaini, 1987). This implies that the repellent behavior away from pheromones could drive females from areas of high population density to reduce resource competition among individuals and increase the mating success rate of the population (Trematerra & Battaini, 1987; Pearson *et al.*, 2004).

Our previous study demonstrated that successful mating rate decreased and mating peak was delayed if the antenna was shortened gradually from the terminal (Yan *et al.*, 2014). However, the regional responses of long trichoid sensilla distributed on the antennae of this species to sex pheromones was unaddressed in our previous study. Herein, we addressed it and interestingly found that the regional responses of both sexes were remarkably different. The pheromone-sensitive long trichoid sensilla were mainly found from the base to the middle of the female moth's antennae, but was found from the base to the terminal of the male moth's antennae, with many found in the middle of the antennae. This suggests that sensilla of the same shape, but located in different regions of the antennae may have different physiological functions. Differences in the spatial distribution of different functional trichoid sensilla have also been observed in other moths, such as *S. littoralis* (Ljungberg *et al.*, 1993), *H. armigera*, and *H. assulta* (Chang *et al.*, 2016). In *S. littoralis*, pheromone-sensitive sensilla were distributed on the ventral surface of male antennae and on the lateral surface of female antennae (Ljungberg *et al.*, 1993). In *H. armigera* and *H. assulta*, pheromone-sensitive and pheromone-insensitive trichoid sensilla were clustered in the proximal and distal regions of each annulus,

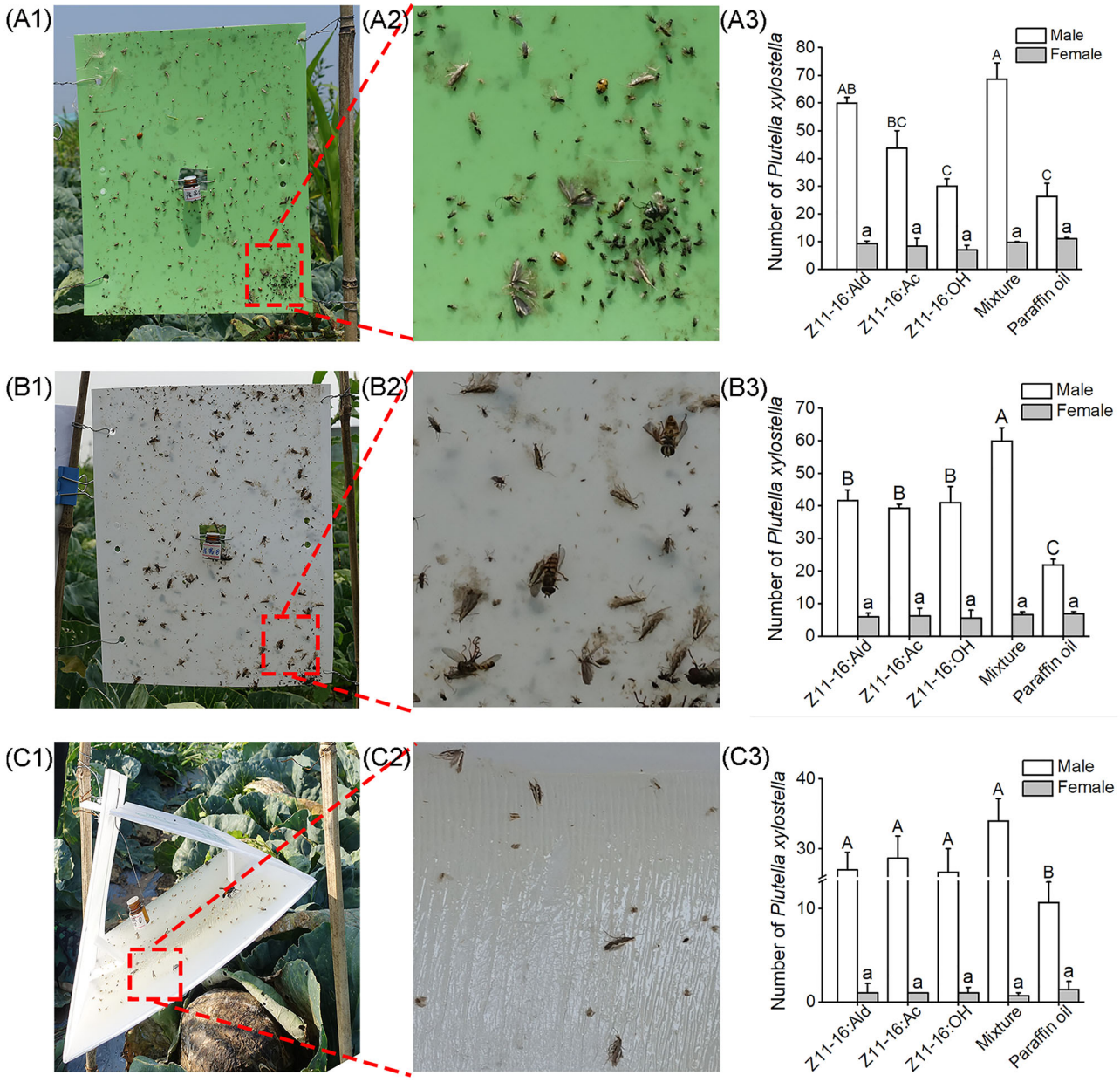

Fig. 6 Field trapping assay of male and female adults of *Plutella xylostella* with the Z11-16:Ald, Z11-16:Ac, Z11-16:OH and the mixture (Z11-16:Ald : Z11-16:Ac : Z11-16:OH = 9.4 : 100 : 17) ($n = 3$). Diagram of the trapping of *P. xylostella* adults with green sticky board trap (A1), white sticky board trap (B1), triangular trap (C1), and their close-up views (A2, B2, C2). The average number of male and female adults of *P. xylostella* trapped by green sticky board trap (A3), white sticky board trap (B3), triangular trap (C3). Values are means ± standard error. Different letters indicate significant differences in different components (one-way analysis of variance, Tukey's test, $P < 0.05$).

respectively (Chang *et al.*, 2016). These spatial organizations of functional trichoid sensilla are convenient for research efforts to unravel the mechanism underlying insect olfactory recognition of sex pheromones.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (Grant No. 32130090, 32300403),

the Distinguished and Excellent Young Scholar Cultivation Project of Shanxi Agricultural University (2023YQPYGC05), the Leading Industrial and Engineering Research Project of Shanxi Agricultural University (Grant No. CYYL23-25), and the Cultivation and Innovation Project of Plant Protection College of Shanxi Agricultural University (Grant No. ZBXY23B-13).

Disclosure

The authors declare they have no competing interests associated with this work.

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Manuscript received October 23, 2023
Final version received February 17, 2024
Accepted March 11, 2024

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Linear regression of Z11-16:Ac content and integral area in gas chromatography – mass spectrometry.

Fig. S2 The electrophysiological responses of 5 types of long trichoid sensilla in the antennae of *P. xylostella* male adults (♂) to the 3 components of sex pheromones.

Fig. S3 The electrophysiological responses of 5 types of long trichoid sensilla in the antennae of *P. xylostella* female adults (♀) to the 3 components of sex pheromones.