Antennal Sensillum Morphology and Electrophysiological Responses of Olfactory Receptor Neurons in Trichoid Sensilla of the Diamondback Moth (Lepidoptera: Plutellidae)

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Antennal sensillum morphology and electrophysiological responses of olfactory receptor neurons in trichoid sensilla of the diamondback moth (Lepidoptera: Plutellidae)

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Abstract

Plant chemical signals are important olfactory cues for the survival and reproduction of phytophagous insects. The diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) is a *Brassica* spp. (Brassicales: Brassicaceae) specialist pest, with most of its life events occurring on *Brassica* spp. hosts. We conducted a scanning electron microscopy study on the morphology and distribution of antennal sensilla of male and female *P. xylostella*. Seven morphological types of sensilla were identified in the antennae of *P. xylostella*: 3 types of sensilla trichodea (Tr I, Tr II and Tr III), sensilla chaetica, sensilla coeloconica, sensilla auricillica and sensilla styloconica. One particular type of trichoid sensillum (Tr III) was present only in the males. The presence of numerous pores or deep longitudinal grooves on the surfaces of 5 morphological types of sensilla indicated that their major function is olfactory. Single sensillum recordings were also carried out on the trichoid sensilla of the female diamondback moth to identify the olfactory receptor neurons (ORNs) and to determine the response spectra of the ORNs, using a panel of 39 host and non-host volatile compounds. Based on the response profiles, 42 responsive trichoid sensilla could be segregated into 4 sensillum classes. Each sensillum appeared to contain 3 co-compartmentalized ORNs, and therefore a total of 12 classes of ORNs were identified from these sensilla. Each ORN class showed a narrow response spectrum, with some ORNs specialized for green leaf volatiles and (±)-linalool that are present in brassicaceous hosts, while several other ORNs responded to 2 non-host volatile sesquiterpenes, (E)-β-farnesene and germacrene D, as well as (E)-β-caryophyllene, a host-related sesquiterpene volatile. The sensitivity and selectivity of the female diamondback moth towards certain host plant volatiles warrants further investigation for potential behavioral manipulation to control this pest.

Key Words: *Plutella xylostella*; olfaction*;* scanning electron microscopy; single sensillum recording; trichodea; volatile compounds

Resumen

Las señales químicas de las plantas son importantes señales olfativas para la sobrevivencia y reproducción de los insectos fitófagos. La polilla de la col, *Plutella xylostella* L. (Lepidoptera: Plutellidae) es una plaga especialista sobre *Brassica* spp. (Brassicales: Brassicaceae), que pasa la mayoría de los eventos de su vida sobre hospederos de especies de *Brassica*. Se realizó un estudio de microscopía electrónica de barrido sobre la morfología y distribución de la sensila antenal del macho y la hembra de *P. xylostella*. Se identificaron siete tipos morfológicos de sensilla en las antenas de *P. xylostella*: 3 clases de sensilas trichodea (Tr I, II y Tr Tr III), sensilas chaeticas, sensilas coeloconicas, sensilas auricilicas y sensilas stiloconicas. Un tipo particular de sensila trichoid (Tr III) estaba presente sólo en los machos. La presencia de numerosos poros o ranuras longitudinales profundas en la superficie de 5 tipos morfológicos de sensila indicó que su principal función es olfativo. También, se realizaron grabaciones individuales de la sensila en las sensilas trichoides de las hembras de la palomilla dorso de diamante para identificar las neuronas olfativas del receptor (NORs) y para determinar los espectros de respuesta de los NORs, utilizando un panel de 39 compuestos volátiles de hospederos y no hospederos. Basado en los perfiles de respuesta, 42 sensilas trichoides que respondieron podrían ser segregadas en 4 clases de sensilas. Cada sensila parecía contener 3 NORs co-compartimentada, y por lo tanto se identificaron un total de 12 clases de NORs de éstas sensilas. Cada clase de NOR mostró un espectro de respuesta estrecha, con algunas NORs especializados para sustancias volátiles de las hojas verdes y (±) -linalool que están presentes en los hospederos brassicaceosos, mientras que varios otros NORs respondieron a 2 no hospederos sesquiterpenos volátiles, (E) -β-farneseno y germacreno D, así como (E) -β-cariofileno, un volátil sesquiterpeno relacionado con el hospedero. La sensibilidad y la selectividad de las hembras de la polilla de la col hacia ciertos volátiles de las plantas hospederas justifica mas investigaciones adicionales para la manipulación potencial del comportamiento para control de esta plaga.

Palabras Clave: *Plutella xylostella*; olfato; microscopía electrónica de barrido; grabación sensillum individuales; trichodea; compuestos

During ontogeny and especially at the time of flowering and fruiting both vegetative and reproductive parts of plants emit hundreds of volatiles in a bouquet that serves to advertise and attract potential pollinators (Hartmann 1996; Raguso 2008). However, these chemical

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signals also serve as olfactory cues for phytophagous insects that help mediate mate finding, food foraging, host location, host recognition and eventually oviposition. In most cases, not all volatiles released from host plants are of biological or ecological significance but only those chemicals that convey vital and essential information about the plant to which the insect species has adapted through evolution for their survival and reproduction (Hansson et al. 1999; Kalinova et al. 2001; Bruce et al. 2005; Raguso 2008).

Most insect olfactory receptors are located on the antennae, which enables the insect to detect semiochemicals with high sensitivity and selectivity. For olfactory perception, insects have various morphological and morphological types of olfactory sensilla on the antennae, each of which contains one or more olfactory receptor neurons (ORNs). Each ORN has a specific molecular receptive range, showing either a specialized response spectrum to a narrow range of volatiles or a broadly tuned responsiveness to a larger number of chemicals. The ORNs receptive to pheromones (Larsson et al. 1999) and many other semiochemicals (Shields & Hildebrand 2001) appear to be highly specialized for a narrow range of chemicals. Numerous neuro-ethological studies have indicated that the ORN response profile of a given species is directly related to its behavioral significance, thus conveying reliable information about relevant plant odors (Kaissling et al. 1989; D'Ettorre et al. 2004; Røstelien et al. 2005). The responses of ORNs to these chemical signals can be monitored with electrophysiological recording techniques such as electroantennogram (EAG) and single sensillum recording (SSR) (Lee et al. 2006). The SSR, which measures the responses of individual ORNs, is an effective tool in mapping the receptive range of the ORNs (Hallem & Carlson 2006), and has been used to characterize the response profiles of various ORNs in various insects such as the pine engraver, *Ips pini* (Say) (Coleoptera: Scolytidae), the blow fly, *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae), mosquitoes such as *Aedes communis* (DeGeer) (Diptera: Culicidae), and the clover root weevil, *Sitona lepidus* Gyllenhal (Coleoptera: Curculionidae) (Mustaprata et al. 1979; Huotari & Lantto 2007; Park et al. 2013).

Research on insect-host interactions, particularly the host volatile organic compounds (VOCs), has flourished in recent decades, whereby knowledge of VOCs not only has benefited our fundamental understanding of plant-insect interactions, but also allowed the development of some plant-derived chemicals that are used for insect pest management (Dickens 2000; Light et al. 2001; Li et al. 2012).

The diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) is a destructive specialist pest infesting high value *Brassica* spp. (Brassicales: Brassicaceae) vegetables and oilseed crops. This pest causes annual economic damage estimated at US$ 5,000 million globally (Furlong et al. 2013). Gravid diamondback moth females prefer to oviposit on cabbage (*B. oleracea* L. subsp. *capitata*), followed by cauliflower (*B. oleracea* L. subsp. *botrytis*) and broccoli (*B. oleracea* L. subsp. *italica*) (Reddy & Guerrero 2000; Reddy et al. 2004). Because neonates of *P. xylostella* have limited dispersal activity and generally feed on tissues surrounding the ovipositions site, the adult females are largely responsible for their dispersal by selecting host plants for oviposition. Therefore, fundamental information on the sensitivity and selectivity of antennal ORNs of the female diamondback moth would enhance our understanding of how this specialist pest perceives and screens the relevant plant VOCs among the hundreds of plant odors they encounter.

In this study, we investigated the morphology and distribution of antennal sensilla using scanning electron microscopy (SEM). Also we measured the electrophysiological responsiveness of ORNs in trichoid sensilla to a panel of synthetic host and non-host volatile compounds by SSR to determine the types of ORNs and the sensitivity and selectivity of each trichoid sensillum found on the antennae of diamondback moth females.

Materials and Methods

SOURCE OF INSECTS

The initial diamondback moth colony was established from field-collected larvae in the Canterbury region, New Zealand. Larvae were fed on cabbage seedlings in mesh cages (0.5 × 0.5 × 0.5 m) in a laboratory glass house that was maintained at 25 ± 2 °C and 60% RH with a natural photoperiod. Two to 5 day-old male and female adults were used in our experiments.

SCANNING ELECTRON MICROSCOPY

Excised antennae from diamondback moth adults were individually fixed in 70% ethanol diluted in distilled water for at least 2 days. The fixed antennae were air-dried, mounted on aluminum stubs, and gold-coated with a sputter coater (SC502, Polaron, Quorum Technologies, United Kingdom). The antennae were then observed with a SEM (FEI Quanta 250 FEG, FEI, USA) and the sensilla on the antennae were classified according to their shape, size and surface morphology. The morphology, number and distribution of the sensilla were examined from 3 female and 5 male antennae. The number and distribution of each morphological type of antennal sensilla was examined from the 5th, 15th and 25th flagellomeres and several other flagellomeres along the antennae.

PREPARATION OF TEST CHEMICALS AND ODOR PRESENTATION

The responsiveness of ORNs in trichoid sensilla in female *P. xylostella* was investigated using a panel of 39 synthetic plant volatile compounds. These compounds included at least 25 volatiles produced by *Brassica* host species as well as several non-host plant volatiles that are commonly present in many plant species (Table 1). With the exception of 5 compounds, the chemicals tested had a minimum purity of 95% (Table 1). Each compound was dissolved in hexane as a 500 ng/μL solution, except the green leaf volatile compounds that were prepared in paraffin oil at the same concentration. Either hexane or paraffin oil was used as the solvent control stimulus.

The test chemicals were presented to the insect antennae in ways similar to those used in previous studies (Park & Baker 2002; Park & Hardie 2004; Park et al. 2013). A 20 μL aliquot of each test solution was applied onto a 5 × 30 mm piece of filter paper (Whatman No. 1, USA), and the filter paper strip was inserted into a glass Pasteur pipette (146 mm, Fisher Scientific, USA) after being evaporated for 10 s in air. The tip of the pipette was inserted into a small 2 mm diam hole in a glass tube at 10 cm from its outlet to the antennae. This arrangement allowed charcoal-filtered and humidified air at 600 mL/min to flow continuously over the antennal preparation. A 0.1 s-long pulse of charcoal-filtered air flowing at 10 mL/s was injected through the wide end of the Pasteur pipette odor cartridge for stimulation; this was accomplished by using an electronic airflow controller (CS-55, Syntech, Hilversum, The Netherlands). The wide end of the Pasteur pipette was covered with a piece of aluminum foil when not in use to reduce evaporation. Each odor stimulus cartridge was used less than 10 times.

SINGLE SENSILLUM RECORDING

Each experimental moth was mounted on a Plasticine® block using U-shaped thin copper wire restraints, and each antenna was

Table 1. Chemicals tested using single sensillum recordings with *Plutella xylostella*: their sources, purity and references of their presence in *Brassica* spp. hosts

Group	Compound	Solvent[a]	Purity	Source	Presence in *Brassica* hosts[b]
Mix-A	1-Nonanol	H	98%	Fluka	15, 16
	(*E*)-β-Caryophyllene	H	98.5%	Sigma	3, 4, 5, 8, 12, 15, 17
	(*E*)-β-Farnesene	H	98%	Bedoukian	
	Germacrene-D	H	40%	Treat & Co	5
Mix-B	(±)-Limonene	H	97%	Merck	1 ~ 8,10 ~ 14, 17
	Myrcene	H	95%	Aldrich	2 ~ 4, 7, 8, 10 ~ 12, 14, 15, 17
	(*E*)-β-Ocimene	H	70%	Fluka	5, 8, 11, 12, 15, 17
	(±)-α-Pinene	H	99%	Aldrich	2 ~ 5, 8,10 ~ 15,17
Mix-C	Geraniol	H	98%	Aldrich	15
	(±)-Linalool	H	97%	Aldrich	2, 4, 6, 8, 10 ~ 17
	Nerol	H	96%	Aldrich	
	2-Phenylethanol	H	99%	Fluka	12, 13, 17
	(±)-α-Terpineol	H	90%	Aldrich	
Mix-D	Benzaldehyde	H	99.5%	Aldrich	11 ,12, 13, 17
	Citral	H	96%	Aldrich	
	Phenylacetaldehyde	H	90%	Aldrich	11, 12, 13, 17
Mix-E	Benzyl acetate	H	99%	Aldrich	
	Diethyl malonate	H	99%	Aldrich	
	Geranyl acetate	H	98%	Aldrich	
	Isobutyl phenylacetate	H	98%	Aldrich	
	Methyl benzoate	H	99%	Aldrich	10, 17
	Methyl phenylacetate	H	99%	Aldrich	
	Neryl acetate	H	96%	Aldrich	
Mix-F	1,8-Cineole	H	98%	Aldrich	2 ~ 4, 8, 10, 12 ~ 14, 17
	(±)-Citronellal	H	95%	Aldrich	
	α-Phellandrene	H	95%	Aldrich	
	(±)-β-Pinene	H	99%	Aldrich	2, 4, 8, 10, 12, 14, 17
	γ-Terpinene	H	97%	Aldrich	10
	(±)-α-Terpinyl acetate	H	90%	Aldrich	
Mix-G	Hexane	H	99%	Aldrich	11
	1-Hexanol	P	99%	Aldrich	9, 10
	(*E*)-2-Hexenol	P	96%	Aldrich	15
	(*Z*)-2-Hexenol	P	95%	Aldrich	
	(*Z*)-3-Hexenol	P	98%	Aldrich	1 ~ 4, 9 ~ 12, 15, 16, 17
	Hexanal	P	98%	Aldrich	9, 10, 11
	(*E*)-2-Hexenal	P	98%	Aldrich	1, 9, 10, 16
	Hexyl acetate	P	99%	Aldrich	9, 10
	(*Z*)-3-Hexenyl acetate	P	98%	Aldrich	2 ~ 5, 7, 9, 10, 12, 15
	2-Heptanone	P	99%	Aldrich	10

[a]Solvent used: H (hexane); P (paraffin oil)

[b]Literature source: 1 (Smid et al. 2002); 2 (Blaakmeer et al. 1994); 3 (Mattiacci et al. 2001); 4 (Tollsten & Bergström 1988); 5 (Shiojiri et al. 2001), 6 (Jakobsen et al. 1994), 7 (McEwan & Smith 1998), 8 (Conti et al. 2008), 9 (Reddy & Gurrero 2000), 10 (Geervliet et al. 1997), 11 (Robertson et al. 1993),12 (Evans et al. 1992), 13 (Blight et al. 1997), 14 (Jakobsen et al. 1994), 15 (Han et al. 2001), 16 (Talavera-Bianchi et al. 2010), 17 (Kobayashi et al. 2012). No reference indicates tat the compound is not present in *Brassica* hosts.

further fixed using fine copper wires. The preparation was placed in the middle of the charcoal-filtered and humidified main airstream. A fine tip (tip diam < 10 µm) glass electrode (0.86 mm ID, A-M Systems Inc., USA) filled with 0.1 M KCl was inserted into a membranous part of the abdomen to serve as the reference electrode. An electro-chemically sharpened tungsten electrode (tip diam < 0.1 µm) was used as a recording electrode and the position of the electrodes was controlled with micromanipulators (Leitz, Germany; Sutter Instruments, USA). An Ag-AgCl junction was used to maintain electrical continuity between the reference electrode and the ground input of a high input impedance headstage preamplifier (Syntech, Hilversum, The Netherlands). The AC signals through the preamplifier were further amplified, digitized at 12,000/s sampling rate, and processed with a PC-based signal processing system (IDAC-4, Syntech, The Netherlands) and software (Autospike 32, Syntech, Hilversum, The Netherlands).

Once a stable contact was made between the electrodes and a sensillum, showing spontaneous firing of action potentials, the antenna was stimulated with a series of 7 mixtures of test chemicals (Table 1). If any electrophysiological response was observed after the stimulation with mixtures, the antenna was further stimulated with the individual chemicals of the mixture that had elicited responses. The order of testing the chemicals was random and the time interval between successive stimulation was approximately 30 s. When a response lasted for a long time (e.g. > 30 s), sufficient time was allowed until spontaneous activity returned to initial levels before re-stimulation. The trichoid sensilla at the central regions on the flagellar segments (Fig. 1) of 7 female moths were investigated in this study.
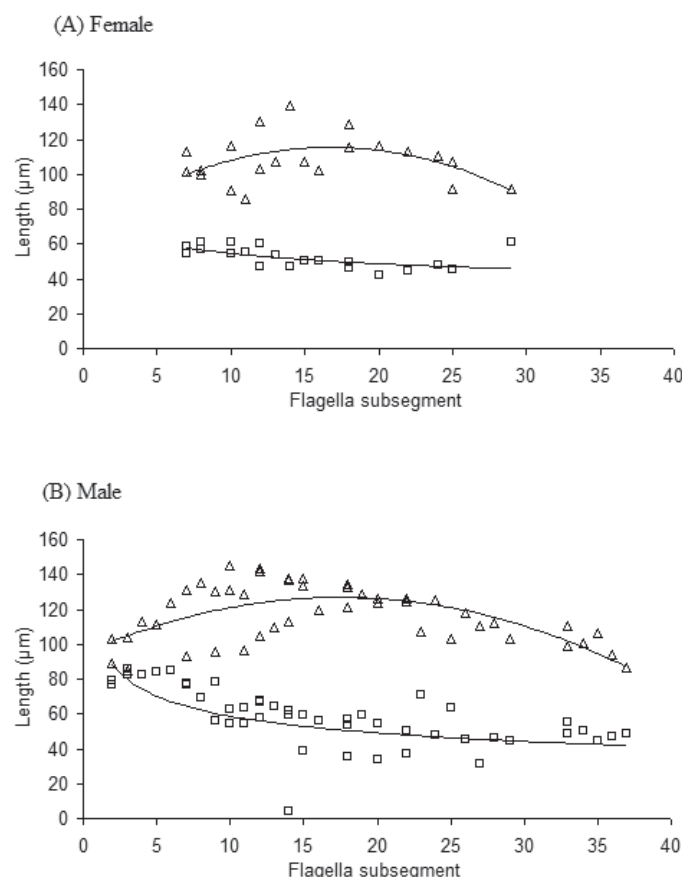
Fig. 1. The length (triangles) and diam (squares) of each flagellar subsegment of the antennae of female (A) and male (B) diamondback moth, *Plutella xylostella*. Data obtained from 3 females and 5 males.

SPIKE ANALYSIS, ORN CLASSES AND STATISTICAL ANALYSIS

The widths and lengths of the various types of trichoid sensilla were compared by one way ANOVA followed by Fisher's LSD; and between sexes using Student's *t*-test. The responsiveness of the ORNs was analyzed by comparing the number of spikes between 1,000 ms before and 1,000 ms after odor stimulation and sorted into 5 categories according to response strength, i.e., < 10 spikes = no response; 10–20 spikes; 21–30 spikes; 31–40 spikes and > 40 spikes, respectively. Different ORNs co-compartmentalized within the same sensilla were sorted into different ORN classes according to the spike amplitudes. Statistical analysis was carried out using one way-ANOVA followed by a Fisher's least significant difference (LSD) test when necessary (*P* = 0.05).

Results

MORPHOLOGY AND DISTRIBUTION OF ANTENNAL SENSILLA

The antennae of male moths were a little larger than those of females (Fig. 1). In both sexes, the largest diam of each antennal flagellomere was found in the proximal segments and the diam decreased gradually towards the distal end. In contrast, the longest flagellomeres were found in the middle segments (15th–25th), and the length of each flagellomere decreased gradually towards both ends (Fig. 1).

Seven morphological types of sensilla were identified in the antennae of *P. xylostella* (Fig. 2, Table 2): sensilla trichodea (3 types: Tr I, Tr II and Tr III), sensilla chaetica (Ch), sensilla coeloconica (Cc), sensilla auricillica (Ac) and sensilla styloconica (Fig. 2, Table 2). There were

more trichoid sensilla than any other type of sensilla on both male and female antennae across all segments (Fig. 3). The density of trichoid sensilla decreased toward the distal ends of female antennae (Fig. 3A), whereas the density of trichoid sensilla remained similar across most segments in male antennae (Fig. 3B). For both males and females, the estimated number of trichoid sensilla on each segment decreased towards the distal end (Fig. 3C, 3D).

The trichoid sensilla could be sorted into 3 types according to their diam, which averaged 1.3 ± 0.05, 1.8 ± 0.02 and 2.6 ± 0.05 µm, respectively (Table 2, Fig. 4 A-C). The sensilla could readily be separated into 3 distinct groups when their diam and lengths were plotted on different axes (Fig. 4D). The type Tr III trichoid sensilla that were the longest and had the largest diam were present only in male moths (Fig. 4, Table 2). Numerous pores, each approximately 30–50 nm in diam, were observed on the surfaces of the sensilla trichodea (Fig. 5). The distribution of these pores appeared to be regular, although their numbers gradually decreased towards the tips of the sensilla (Fig. 5C, D).

In contrast to the sensilla trichodea that were curved with a pointed tip and no basal socket, the sensilla chaetica (average basal width of 1.8 ± 0.04 µm in females and 2.0 ± 0.03 µm in males) (Table 2) were straight, and possessed a basal socket and a blunt tip (Fig. 5). No distinct pores were present on the fish-scale looking surface of these sensilla chaetica (Fig. 5E). We observed that female moths had more sensilla coeloconica on their antennae than males (Fig. 3). The difference in the number of sensilla coeloconica between males and females was larger in the proximal segments (Fig. 3), which appeared to be related to an increase in the number of sensilla coeloconica towards the distal segments in male flagella whereas they were evenly distributed on female flagella (Fig. 3). No pores were present on the surface of sensilla coeloconica in both male and female moths (Fig. 6A–C). Instead, deep longitudinal grooves were present on the surface of central and circumferential pegs in sensilla coeloconica (Fig. 6A–C).

A number of pores, approximately 30–80 nm in diam, were present on the surface of sensilla auricillica in both male and female diamondback moth (Fig. 6D–F). Sensilla auricillica were characterized by a rather flattened, often rabbit-ear like shape, except the basal area near the socket, which was short and cylindrical. The sensilla styloconica, present on the ventral side near the distal end of each flagellomere in both sexes of the diamondback moth, showed a small terminal sensory cone on its apex (Fig. 7).

SENSITIVITY AND SELECTIVITY OF OLFACTORY RECEPTOR NEURONS IN TRICHOID SENSILLA

We tested 57 trichoid sensilla that contained ORNs with spontaneous firing activities against 39 synthetic plant volatile compounds and found 42 sensilla (73.7 %) to be responsive to some of the chemicals tested. The ORNs in the 15 remaining sensilla (26.3%) did not respond to any of the chemicals tested although they showed spontaneous activity (Table 3). All of the responses to these olfactory stimuli appeared to be excitatory, resulting in an increase of action potentials after stimulation (Figs. 8 and 9). Based on the response profiles across the panel of test stimuli, the 42 sensilla could be sorted into 4 groups, i.e. A, B, C and D (Table 3). Three ORNs were located in each sensillum (Tables 3, 4 and 5) and the responses of the co-compartmentalized ORNs could be distinguished by their different spike sizes (Tables 4 and 5). Among the 12 different classes of ORNs identified in these sensilla, 10 classes of ORNs were responsive to some of our test stimuli, whereas ORN class B and C showed negligible response to the 39 chemicals tested (Tables 4 and 5). Olfactory receptor neurons A1, A2 and A3 showed specialized responses to the green leaf volatiles with the highest sensitivity
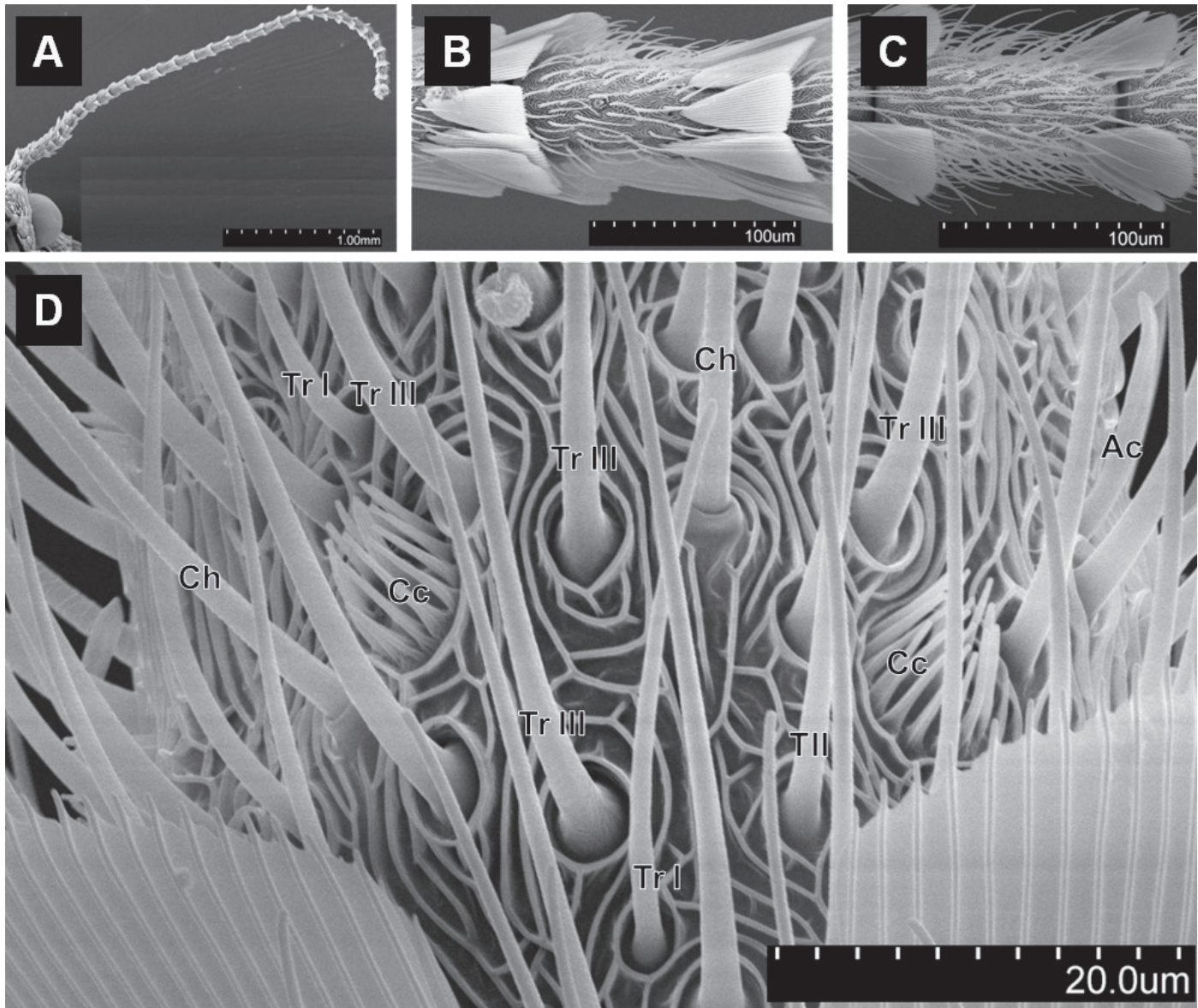
Fig. 2. Gross antennal morphology of female (A, B) and male (C) *Plutella xylostella*, and a part of a male antenna showing the presence of different morphological types of sensilla (D). Ac (sensilla auricillica); Cc (sensilla coeloconica); Ch (sensilla chaetica); Tr I (sensilla trichodea Type I); Tr II (sensilla trichodea Type II); Tr III (sensilla trichodea Type III).

to 1-hexanol, followed by (Z)-3-hexenol, with a characteristic response profile to the green leaf volatiles in each class of ORNs (Tables 3 and 4; Fig. 8). However, non-alcohol green leaf volatiles such as (Z)-3-hexenyl acetate and (E)-2-hexenal did not elicit any responses from the ORNs present in the trichoid sensilla examined in our study, except hexanal and 2-heptanone, each of which elicited a weak response from ORN A1 (Table 3).

Five non-green volatile compounds elicited responses from the ORNs present in 3 classes of sensilla in female moths (Table 3). Olfactory receptor neuron class B1 and B3 showed specialized responses but with different sensitivities to (E)-β-caryophyllene, (E)-β-farnesene and germacrene D (Tables 3 and 5). Olfactory receptor neuron class B2 showed specialized responses to 1-nonanol (Table 5). However, the responses of these ORNs to 1-nonanol appeared to be mild, compared with the responses of other ORNs to corresponding active stimuli. Olfactory receptor neuron class D1, D2 and D3 showed specialized responses to (±)-linalool and geraniol (except for D3), with each class showing different sensitivities to these compounds (Tables 3 and 5; Fig. 9).

Discussion

Sexual dimorphism of antennal morphology in diamondback moth was first reported by Yang (2001), followed by Yan et al. (2014), in which male moths were shown to have significantly higher number of trichoid sensilla than female moths. Here, we showed that the sexual dimorphism could be attributed to (i) longer antennae in males, (ii) larger number of trichoid sensilla in males, and (iii) a decreasing density of trichoid sensilla toward distal segments in females. Three morphological types of trichoid sensilla (Tr I, Tr II and Tr III) were identified in our study, whereas only 2 morphological types of trichoid sensilla were reported previously in female *P. xylostella* (Chow et al. 1984; Yang 2001; Yan et al. 2014).

Table 2. Antennal sensilla identified in male and female diamondback moth, *Plutella xylostella*: morphological types, distribution and putative functions. Trichoid sensilla were sorted into 3 subtypes, based on the diam near the sensilla base (Type I: ≤ 1.6 µm; 1.6 µm < Type II: ≤ 2.2 µm; Type III: > 2.2 µm).

Sensilla Type	Sex	Width	Length	N	Socket	Pores	Distribution	Suggested function[2]
		Mean[1] ± S.E. (µm)						
Trichodea I	female	1.3 ± 0.05c	26.7 ± 1.44c	24	no	yes	random	olfactory
	male	1.3 ± 0.03c	19.1 ± 1.30c	15	no	yes	random	olfactory
Trichodea II	female	1.8 ± 0.02b	32.2 ± 0.35b	41	no	yes	random	olfactory
	male	1.9 ± 0.04b	32.9 ± 7.67b	5	no	yes	random	olfactory
Trichodea III	female	—	—	—	—	—	—	—
	male	2.6 ± 0.05a	48.3 ± 1.63a	16	no	yes	regular	olfactory
Chaetica	female	1.8 ± 0.04	25.0 ± 0.78	18	no	no	regular	mechanical
	male	2.0 ± 0.03**	30.1 ± 0.86**	9	no	no	regular	mechanical
Coeloconica	female	6.8 ± 0.11	5.8 ± 0.14	23	no	no	random	olfactory
	male	8.2 ± 1.17**	7.7 ± 0.20**	10	no	no	random	olfactory
Auricillica	female	1.3 ± 0.02	21.4 ± 0.46	32	no	yes	random	olfactory
	male	1.5 ± 0.04*	14.8 ± 1.25**	8	no	yes	random	olfactory
Styloconica	female	4.9 ± 0.27	14.9 ± 1.27	4	yes	no	regular	gustatory
	male	4.6 ± 0.08	18.4 ± 1.12	7	yes	no	regular	gustatory

[1]Means of width or length of trichoid sensilla followed by different letters are significantly different at $P = 0.05$ (Fisher's LSD), and those with asterisks are significantly different between males and females at $P = 0.05$ (*) or at $P = 0.01$ (**) (Student's *t*-test).

[2]Olfactory function was inferred from the presence of pores on the surface of the sensilla; other sensory functions were based on published information of other moth species.
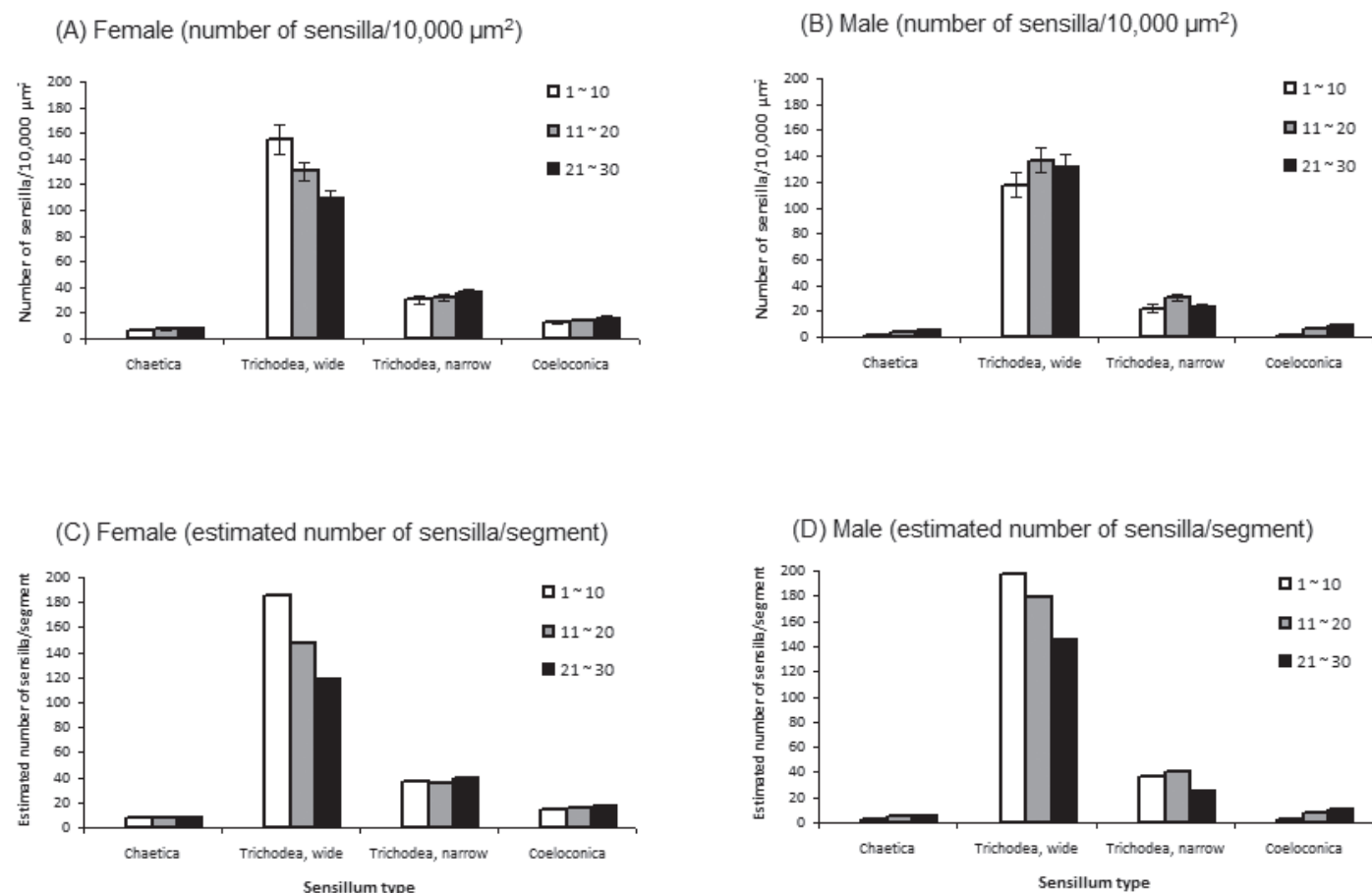
Fig. 3. The number of each of 4 types of sensilla along various antennal segments (1–10 = proximal end, 11–20 = middle, 21–30 = distal end) of female (A, C) and male (B, D) diamondback moths, *Plutella xylostella*. The estimated number of sensilla (C, D) was calculated based on the surface area of each segment section, and by assuming that sensilla were present on 2/3 of the surface (mean ± S.E., $n = 5$–15 in females and 9–18 in males).
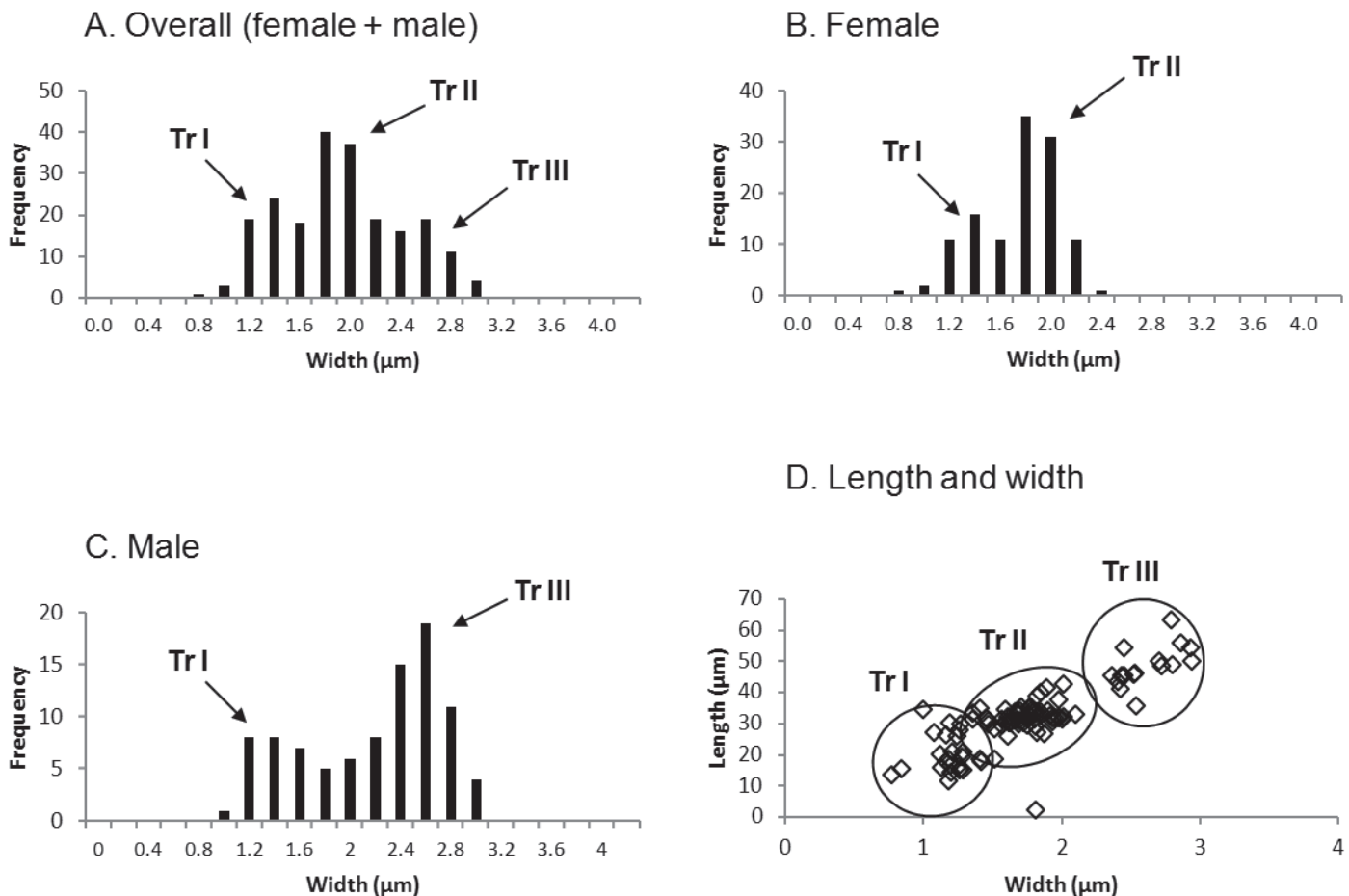
Fig. 4. The occurrence of 3 different antennal trichoid sensilla in relation to their widths (A, B and C) in male and female diamondback moth, *Plutella xylostella*. D shows the widths of the sensilla plotted against their lengths for the 3 trichoid sensilla types (Tr I, Tr II and Tr III). Data obtained from 3 females and 5 males.

The presence of pores, ranging from 30–50 nm in diam, on trichoid sensilla suggests their olfactory function as shown in other insects such as *Coleophora obducta* (Meyrick) (Lepidoptera: Coleophoridae) (Maitani et al. 2010; Faucheux 2011). Among the 3 types of trichoid sensilla, Tr III, which was the largest in diam and length, was found only in male *P. xylostella*. Such male-specific trichoid sensilla have been observed in a number of moth species such as the redbanded leafroller, *Argyrotaenia velutinana* (Walker) (Lepidoptera: Tortricidae) and the corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (Akers & O'Connell 1991; Cossé et al. 1998). Electrophysiological recordings indicated that these male-specific trichoid sensilla in moths contained ORNs responsive to conspecific female sex pheromones and related compounds (Akers & O'Connell 1991; Cossé et al. 1998). Therefore, it is likely that the ORNs in the male-specific trichoid sensilla are responsible for detecting the female sex pheromone and related compounds in *P. xylostella*.

In sensilla coeloconica, the presence of deep longitudinal grooves on the surface of central and circumferential pegs also indicates their olfactory function. A transmission electron microscope study in the tobacco hornworm, *Manduca sexta* L. (Lepidoptera: Sphingidae), suggested that olfactory molecules entered through the grooves in sensilla coeloconica (Shields & Hildebrand 1999). These results are further substantiated as at least some sensilla coeloconica in female *P. xylostella* appear to be related to oviposition because more sensilla coeloconica were present in female antennae than in male antennae, and a previous study showed that their numbers were highly correlated with ovi-

position preference (Yan et al. 2014). Single sensillum recordings from these sensilla with plant volatile compounds may elucidate if the ORNs in the sensilla coeloconica are specialized in detecting volatiles related to oviposition in *P. xylostella*. We also found that sensilla auricillica, sensilla chaetica and sensilla styloconica were present on the antennae of both male and female *P. xylostella*. The presence of pores (30–80 nm) on the surface of sensilla auricillica in both male and female *P. xylostella* also suggests their olfactory function. The olfactory function of sensilla auricillica has been shown through electrophysiological studies in some moths such as the Herald moth, *Scoliopteryx libatrix* L. (Lepidoptera: Noctuidae) (Anderson et al. 2000) and the codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae) (Ansebo et al. 2005).

Conversely, the non-porous sensilla chaetica present in both male and female *P. xylostella* suggests that their function is not olfactory but mechanical. The ventral location of sensilla styloconica on the antennae and the absence of surface pores also indicate their non-olfactory function. Instead, their function appears to be gustatory, as shown in some other insects such as the cabbage stem flea beetle, *Psylliodes chrysocephala* (Coleoptera: Chrysomelidae) (Bartlet et al. 1999) and *C. obducta* (Yang et al. 2009).

Nocturnal insects such as the diamondback moth rely much on olfactory information for locating their mates and host plants. Our study indicates that a number of specialized ORNs in trichoid sensilla are designed to detect odor cues indicating the identity of host and non-host plants. Our SSR study indicates that each of the 10 classes of ORNs in diamondback moth has a narrow response spectrum to the plant
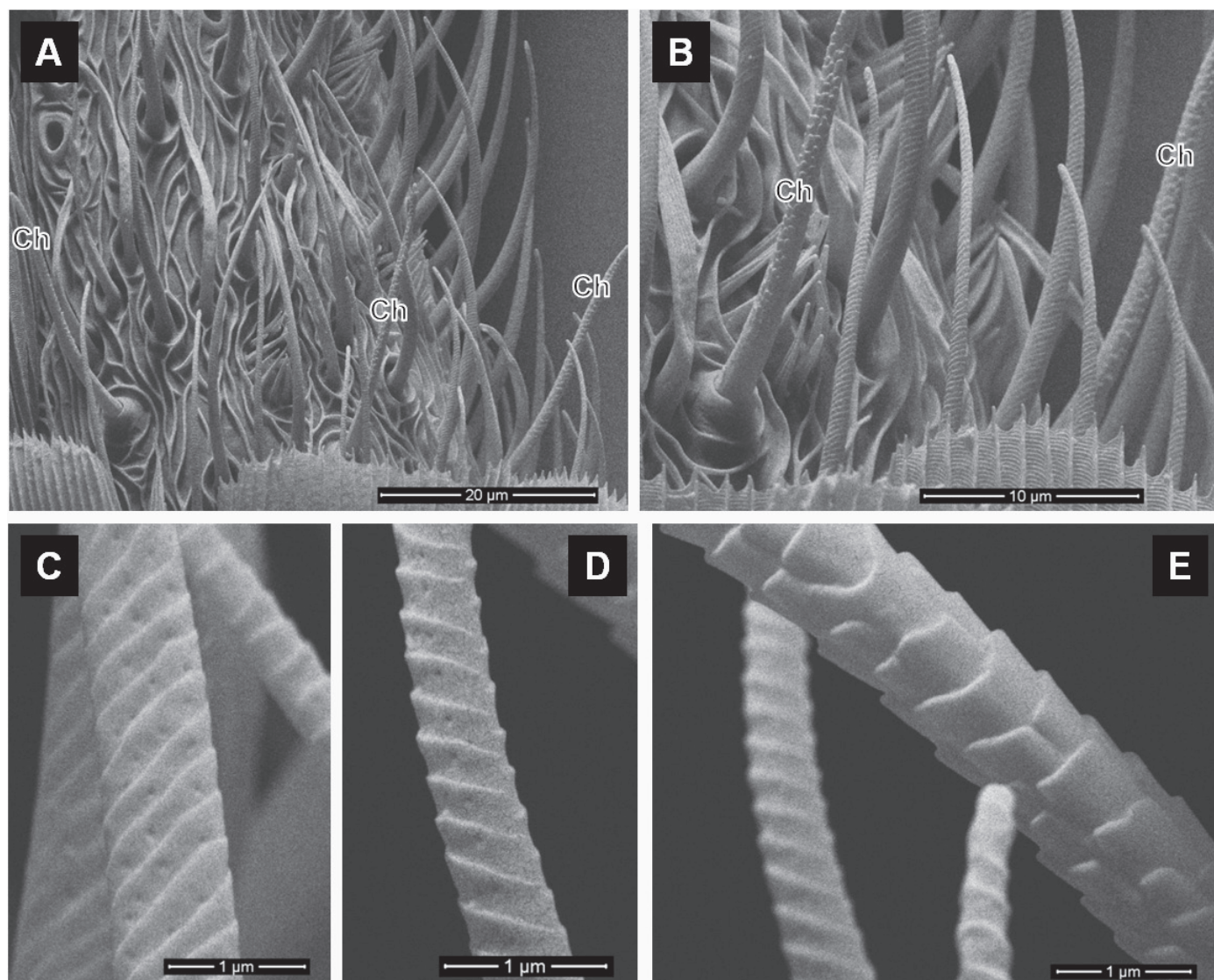
Fig. 5. Detailed surface morphology of the sensilla trichodea (A, B, C and D) and sensilla chaetica (A, B and E) of the antennae of *Plutela xylostella*.

volatile compounds tested. The presence of ORNs highly specialized for detecting green leaf volatiles with the highest sensitivity to 1-hexanol and (Z)-3-hexenol is in part corroborated by EAG recordings reported by Dai et al. (2008) and Li et al. (2012). Some other non-alcohol green leaf volatiles such as (Z)-3-hexenyl acetate and (E)-2-hexenal, which were shown to be behaviorally active in *P. xylostella* (Dai et al. 2008, Li et al. 2012), did not elicit any responses from the ORNs present in the trichoid sensilla examined in this study. It is likely that ORNs specifically for this compound are present in other non-trichoid sensilla such as sensilla coeloconica and sensilla auricillica in *P. xylostella*. The presence of antennal ORNs specialized for some green leaf volatiles has been reported in various insects such as the pale brownish chafer, *Phyllopertha diversa* Waterhouse (Coleoptera: Scarabaeidae) (Hansson et al. 1999) and the clover root weevil, *Sitona lepidus* Gyllenhal (Coleoptera: Curculionidae) (Park et al. 2013).

Apart from green leaf volatiles, *P. xylostella* also possesses ORNs B1 and B3 that are specialized for detecting some common plant sesquiterpenes such as (E)-β-caryophyllene, (E)-β-farnesene and germacrene D. In addition, ORN class C1 and C3 also displayed specialized responses to (E)-β-farnesene, but with slightly different sensitivities to this chemical between these ORN classes. (E)-β-Farnesene and germacrene D are

ubiquitous plant sesquiterpenes present in a number of plant species such as apple, *Malus domestica* (Bengtsson et al. 2001), and maize, *Zea mays* (Köllner et al. 2004). Germacrene D, considered a backbone molecule for synthesizing other sesquiterpenes, occurs widely in over 40 plant families (He & Cane 2004; Dudareva et al. 2006), but has not been found in *Brassica* spp with the exception of the report by Shiojiri et al. (2001). Similarly, (E)-β-farnesene, which has been reported to act either as an allomone, an attractant or a kairomone in various insects (Francis et al. 2004), as well as a pheromone, not only in insects (Pickett & Griffiths 1980) but also in African elephants and brown rats (Goodwin et al. 2006; Zhang et al. 2008), is not present in *Brassica* spp. The ability of the specialized ORNs to detect such non-host-plant species-specific volatiles may be used by the diamondback moth to discriminate between host and non-host plants as suggested previously by Park et al. (2013).

In an EAG study, linalool elicited moderate responses from *P. xylostella* antennae (Dai et al. 2008). Although the terpene alcohol is present in various *Brassica* spp., this chemical, in combination with limonene and α-terpinene, has been reported as a repellent and oviposition deterrent to adult diamondback moths (Zhang et al. 2004). In our study, none of the trichoid sensilla had ORNs that were responsive
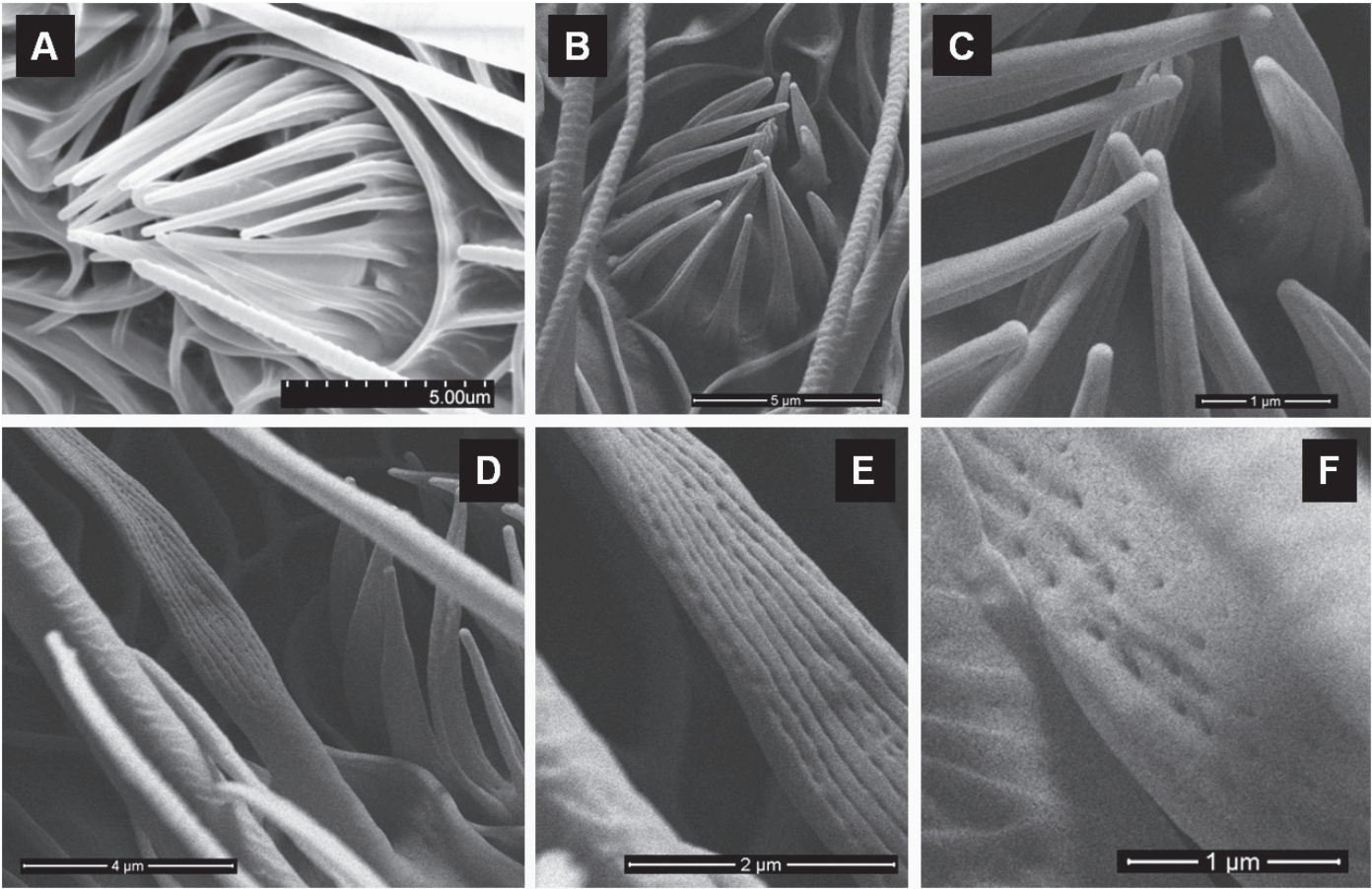
Fig. 6. Detailed surface morphology of sensilla coeloconica (A, B and C) and sensilla auricillica (D, E and F) of the antennae of *Plutela xylostella*.

Table 3. Classes of sensilla trichodea (A, B, C and D) and their olfactory receptor neurons (ORNs) (A1, A2, etc.) in female *Plutella xylostella*, identified electrophysiologically based on the strength of response to a series of host and non-host plant volatile chemicals. Only compounds eliciting responses from these ORNs are listed. Total number of sensilla investigated = 57; responsive sensilla = 42; non-responsive sensilla = 15.

Stimuli	ORN trichoid sensillum class and strength of response[a]									
Sensillum class	A			B		C		D		
ORN class	A1	A2	A3	B1	B3	C1	C3	D1	D2	D3
Number identified	18	18	18	11	11	3	3	10	10	10
Hexane†										
Mineral oil†										
1-Nonanol				○						
(E)-β-Caryophyllene				●	○					
(E)-β-Farnesene*				●	○	•	○			
Germacrene-D				●	○					
Geraniol								○		
(±)-Linalool								○	○	●
1-Hexanol	●	○	●							
(E)-2-Hexenol	•									
(Z)-2-Hexenol*	○									
(Z)-3-Hexenol	•	○	•							
Hexanal	○									
2-Heptanone	•									

[a]The size of each circle indicates the strength of the response of each ORN class to the corresponding stimuli: Blank < 10; ○ 10 ~ 20; • 21 ~30; • 31 ~ 40; ● > 40 spikes/s, i.e., the increase in the number of spikes/s after stimulation.
†Solvent control
*Non-host volatile chemical.
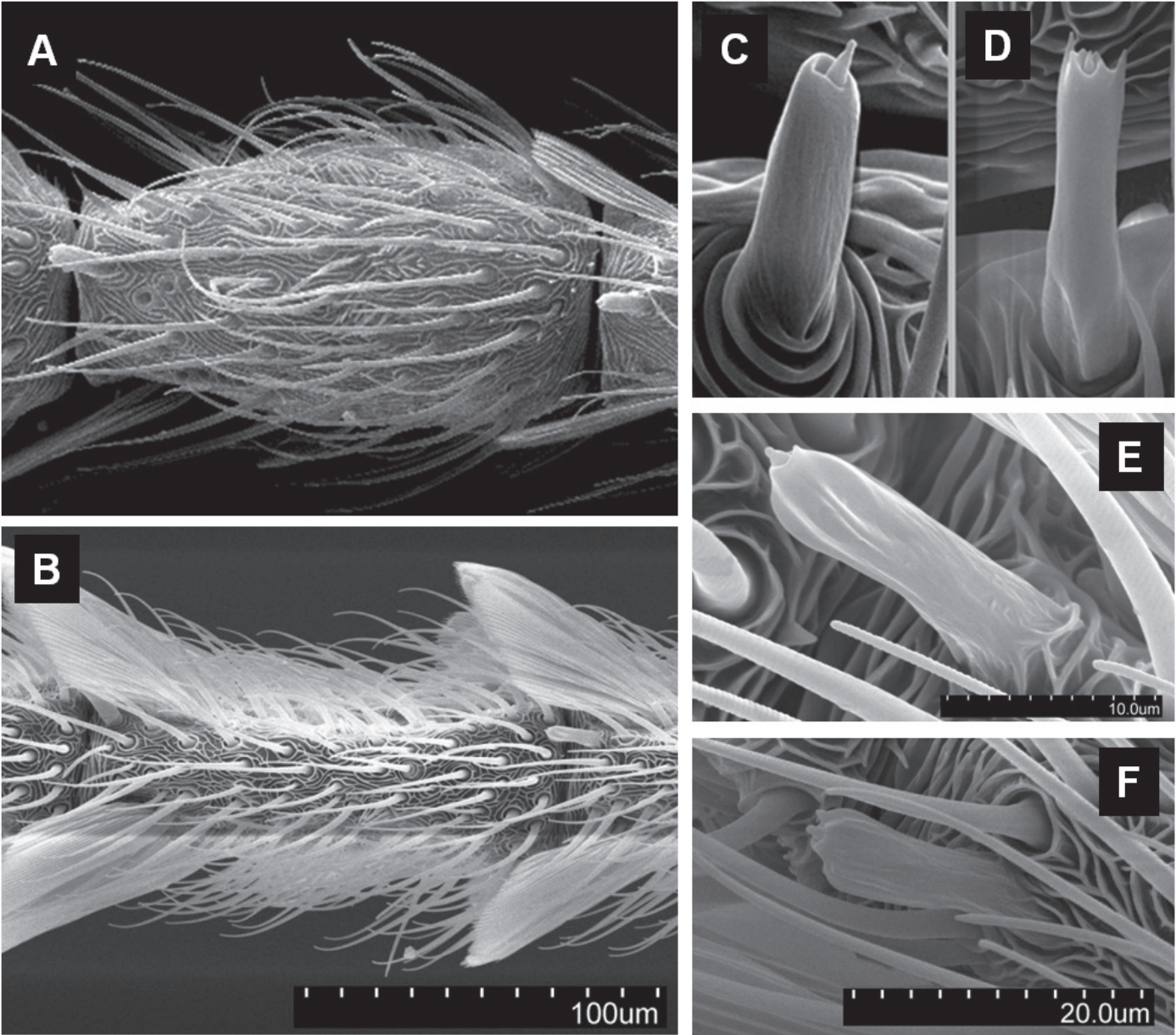
Fig. 7. Sensilla styloconica identified on the antennae of female (A, C and D) and male (B, E and F) *Plutela xylostella*. One sensillum styloconicum is located near the anterio-ventral end in each flagella subsegment (A, B). Usually 1 terminal sensory cone is present at the distal end in each sensillum styloconicum (C, D, E, F).

Table 4. Electrophysiological responses to green leaf volatiles of olfactory receptor neurons (ORNs) belonging to Class A sensilla of female *Plutella xylostella*. These ORNs did not respond to 29 other chemicals tested.

	Increased # of spikes/s (mean ± S.E., $n = 10$)[a] in the 3 ORNs in a Class A sensillum trichodea		
Compound	A1	A2	A3
Solvent control	0.22 ± 1.33d	0 ± 0.83c	0.11 ± 0.48b
1-Hexanol	51.33 ± 13.74a	19.89 ± 5.83a	49.00 ± 10.16a
(E)-2-Hexenol	33.33 ± 12.75abc	9.00 ± 2.40bc	7.00 ± 2.36b
(Z)-2-Hexenol	17.67 ± 9.36bcd	6.33 ± 2.12c	7.00 ± 3.37b
(Z)-3-Hexenol	37.56 ± 13.90ab	16.89 ± 5.56ab	37.22 ± 13.56a
Hexanal	15.22 ± 8.65bcd	0.56 ± 1.38c	1.56 ± 1.07b
(E)-2-Hexenal	4.78 ± 2.90cd	0.22 ± 0.91c	1.00 ± 0.33b
Hexyl acetate	1.89 ± 2.42d	0.56 ± 0.78c	0.33 ± 0.62b
(Z)-3-Hexenyl acetate	3.11 ± 1.55d	0.78 ± 0.89c	0.22 ± 0.22b
2-Heptanone	22.00 ± 9.79bcd	3.56 ± 3.27c	7.22 ± 3.74b

[a]Different letters indicate significant differences within a column (Fisher's LSD test, $P = 0.05$).
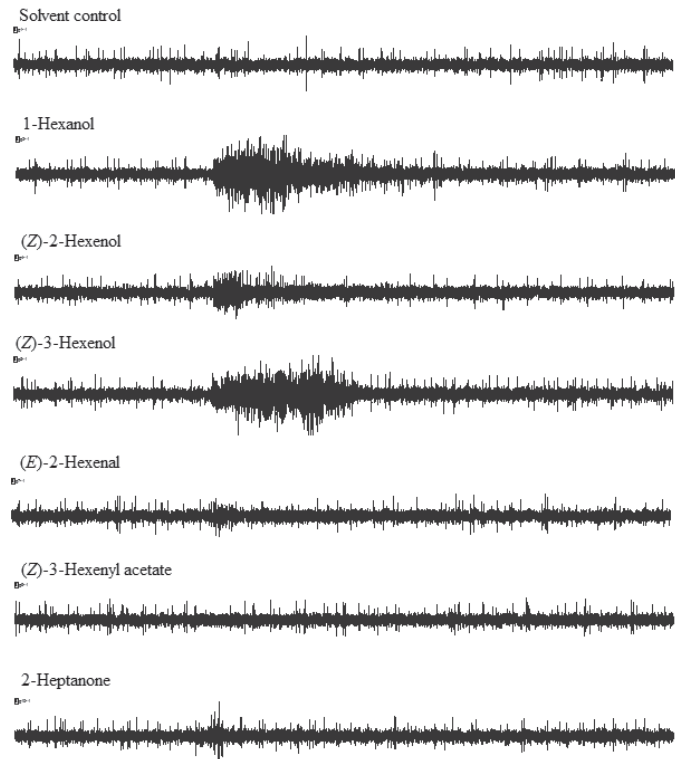
Table 5. Electrophysiological responses to plant volatile compounds of olfactory receptor neurons (ORNs) belonging to Class B, C and D sensilla of female *Plutella xylostella*. These ORNs did not respond to 32 other chemicals tested.

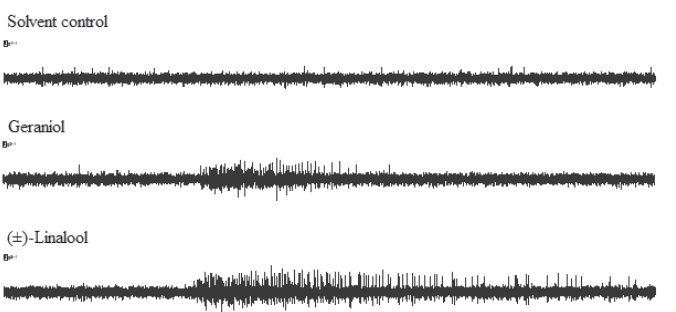| | Increase in the number of spikes/s (mean ± S.E.)[a] after stimulation | | | | | | | | |
| | Class B (*n* = 11) | | | Class C (*n* = 3) | | | Class D (*n* = 10) | | |
Compound	B1	B2	B3	C1	C2	C3	D1	D2	D3
Solvent control	0.0 ± 1.18b	0.5 ± 0.22b	0.0 ± 0.00b	3.7 ± 1.20b	0.0 ± 1.00a	0.3 ± 0.33b	1.8 ± 0.58b	0.4 ± 0.40b	0.0 ± 0.00b
(*E*)-β-Caryophyllene	42.8 ± 2.27a	0.7 ± 0.33b	12.7 ± 1.02a	1.0 ± 0.00b	0.0 ± 0.00a	0.7 ± 0.33b	—	—	—
(*E*)-β-Farnesene	44.0 ± 2.53a	1.7 ± 0.21b	17.7 ± 2.26a	31.3 ± 3.48a	0.7 ± 0.67a	14.3 ± 1.20a	—	—	—
Germacrene D	43.5 ± 3.15a	0.8 ± 0.17b	12.3 ± 3.31a	3.7 ± 2.19b	2.0 ± 0.00a	1.7 ± 1.67b	—	—	—
1-Nonanol	11.8 ± 1.76b	4.8 ± 1.54a	1.5 ± 0.85b	5.7 ± 0.88b	1.7 ± 0.67a	0.0 ± 0.00b	—	—	—
Geraniol	—	—	—	—	—	—	17.2 ± 7.27ab	9.8 ± 5.03ab	4.8 ± 3.62b
(±)-Linalool	—	—	—	—	—	—	18.6 ± 2.86a	14.4 ± 2.23a	58.0 ± 3.18a

[a]Different letters within a column indicate significant differences (Fisher's LSD test, *P* = 0.05).

to benzaldehye and phenylacetaldehyde, although these compounds showed strong EAG responses (Dai et al. 2008) and strong inhibition of behavioral attraction to green leaf volatiles in diamondback moths (Reddy & Gurrrero 2000). As we only examined the responsiveness of the ORNs present in trichoid sensilla, it is likely that in the diamondback moth, these chemicals are detected by the ORNs present in some of the non-trichoid olfactory sensilla such as sensilla coeloconica and sensilla auricillica.

In summary, we showed that there are 7 morphological types of antennal sensilla in diamondback moth, with a group of male-specific sensilla trichodea (Tr III) which may be responsible for sex pheromone detection. All 3 types of sensilla (sensilla trichodea, sensilla coeloconica and sensilla auricillica) had pores on the surface, indicating their involvement in olfactory perception. Electrophysiological recordings

Fig. 9. Traces of the response spikes of the olfactory receptor neurons (ORNs) in class D trichoid sensilla of female *Plutella xylostella* in response to geraniol and (±)-linalool. Each trace shows the extracellular signals for a period of 5 s. The scale bar indicates the stimulation for 0.1 s with corresponding test stimulus.

from the sensilla trichodea demonstrated that at least 12 classes of specialized ORNs are present in these trichoid sensilla in female *P. xylostella*. The response profiles of these ORNs indicate that female *P. xylostella* are able to detect and discriminate specific volatiles from host and non-host plants, using the combined inputs from these ORNs. Because our morphological observations indicate an olfactory function for sensilla coeloconica and sensilla auricillica, it is likely that the complete odor profile of either a host species or a non-host species is readily 'readable' by the combined effort of ORNs in all olfactory sensilla, such as sensilla trichodea, sensilla coeloconica and sensilla auricillica at the sensory periphery, which then induce both broadly tuned, and specific responses. Further these various responses are then conveyed to the brain which allows the moth to assess plant suitability and quality. Future investigation on the molecular receptive range of the ORNs in sensilla coeloconica and sensilla auricillica should certainly help to understand how plant odor information is encoded in the ORNs in a specialist pest like diamondback moth for potential behavioral manipulation in pest management.

Acknowledgments

Fig. 8. Traces of the response spikes of the olfactory receptor neurons (ORNs) in class A trichoid sensilla of female *Plutella xylostella* in response to various green leaf volatiles. Each trace shows the extracellular signals for a period of 5 s. The scale bar indicates the stimulation for 0.1 s with corresponding test stimulus.

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