

Electrophysiological and behavioral responses of *Helicoverpa assulta* (Lepidoptera: Noctuidae) to tobacco volatiles

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Abstract The Oriental tobacco budworm moth, *Helicoverpa assulta* (Guenée) (Lepidoptera: Noctuidae), is a serious pest on tobacco in China. The flowering stage of the host plant is one of the most attractive stages to *H. assulta* for feeding and oviposition. Nine electrophysiologically active compounds in tobacco headspace at flower stage were detected by gas chromatography–electroantennographic detection (GC–EAD). These compounds were subsequently identified by gas chromatography–mass spectrometry (GC–MS) as (*E*)- β -ocimene, octanal, (*Z*)-3-hexenyl acetate, (*Z*)-3-hexen-1-ol, nonanal, (*Z*)-3-hexenyl-2-methyl butyrate, decanal, linalool, and (*E*)- β -caryophyllene. The synthetic blend containing nine of the above compounds attracted mated *H. assulta* females from a distance by upwind oriented flight. Selected subtraction assays showed that the 4-component mixture of (*E*)- β -ocimene, (*Z*)-3-hexenyl acetate, nonanal, and (*E*)- β -caryophyllene elicited equivalent levels of attraction as the 9-component mixture. The removal of any of the four compounds from the 4-component blend resulted in a significant decrease in female upwind flight behavior.

Keywords *Helicoverpa assulta* · GC–EAD · GC–MS · Tobacco · Wind tunnel · Olfactory receptor neuron · Single sensillum recording

Introduction

The Oriental tobacco budworm moth, *Helicoverpa assulta*, (Guenée) (Lepidoptera: Noctuidae), is distributed in three continents, Asia, Africa, and Australia. (Boo et al. 1995). Like the other oligophagous herbivores, *H. assulta* utilizes a narrow range of host plants and mainly feeds on plant species in Solanaceae such as tobacco, hot pepper, and several *Physalis* species (Fitt 1989; Wang and Dong 2001). The larvae of *H. assulta* preferentially feed upon the buds and the reproductive organs of tobacco and other host plants, leading to severe economic loss. Because of their habit of feeding inside the reproductive organs, the larvae are difficult to control with conventional insecticides, and it is urgent to develop alternative control strategies (Boo et al. 1995).

The sex pheromone of *H. assulta* has already been identified by Sugie et al. (1991) and is highly attractive to male *H. assulta* (Cork et al. 1992). However, control strategies based on lures developed from female sex pheromones have one important drawback, which is that only male behavior is affected, and not the behavior of gravid females. The pheromone-based approaches are not efficient at high population densities or when the mated females immigrate in the control areas. Methods that could also manipulate the behavior of egg-laying females would enhance the efficacy of pheromonal techniques.

Chemical stimuli from plants play an important role in insect–plant interactions. Many phytophagous insects use odors as cues for orientation to host, for mate-finding, their

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own nutrition, or oviposition (Schoonhoven et al. 2005). Up to now, in the case of lepidopteran moths, attractant plant volatiles mediating female long-range responses to host plants have been identified, including *Argyresthia conjugella* (Bengtsson et al. 2006), *Cydia molesta* (Natale et al. 2004), *Cydia pomonella* L. (Hern and Dorn 1999, 2004; Light et al. 2001), *Mamestra brassicae* (Rojas 1999), *Paralobesia viteana* (Cha et al. 2008a, b), *Lobesia botrana* (Tasin et al. 2005, 2006a, b, 2007), *Helicoverpa armigera* (Hartlieb and Rembold 1996; Jallow et al. 1999), *Manduca sexta* (Fraser et al. 2003). More recently, in *H. assulta* and other closely related heliothine moths, some selective olfactory receptor neurons for particular volatile compounds have been found by using gas chromatography (GC) linked to single sensillum recording techniques (Stranden et al. 2003a, b; Røsteliën et al. 2005).

Despite the importance of role played by olfactory cues in host-plant location by *H. assulta*, little is known about long-range perceptible host-plant signals of this moth species. Tobacco is the primary host plant of *H. assulta*, and the flowering period of host plant is the most attractive stage for ovipositing or feeding (Wu 1990). In the present study, electrophysiologically active compounds from headspace samples of tobacco flowers were identified by gas chromatography–electroantennographic detection (GC–EAD), and their behavioral activity with female *H. assulta* was investigated in a wind-tunnel bioassay.

Methods and materials

Plants

Tobacco plants, *Nicotiana tabacum* L. (Solanaceae) var. NC89, obtained from Institute of Tobacco, the China Academy of Agricultural Sciences (CAAS), were kept in a climatized greenhouse at 26 ± 2 °C, 60 ± 10 % R.H. Potted plants that had fully expanded 4–5 true leaves were transferred from the greenhouse to the outdoor in early June in Beijing. Tobacco plants at full blossom were used for collection of volatiles in the field.

Insects

Helicoverpa assulta were originally collected as larvae from the tobacco field in Zhengzhou, Henan Province of China, and maintained for over ten generations in controlled conditions under a 16L: 8D photoperiod cycle at 26 ± 1 °C and 55–65 % relative humidity. Larvae were fed on artificial diet described by Wu and Gong (1997). Pupae were sexed and placed in cages separately. After emergence, male and female adults mated in nylon cages. 3-day-old mated females were used in wind-tunnel tests. To verify the

mating status, tested females were dissected and checked for the presence of a spermatophore in the bursa copulatrix. Moths were provided with a 10 % honey solution.

Chemicals

Plant odorants (*Z*)-3-hexen-1-ol (98 %) and (*Z*)-3-hexenyl acetate (97 %) were purchased from Roth KG Company (Karlsruhe, Germany). Octanal (98 %), nonanal (97 %), decanal (97 %), racemic linalool (97 %), (*E*)- β -caryophyllene (99 %), and (*R*)-(+)-limonene (96 %) were obtained from Fluka Chemie Company (Buchs, Switzerland). (*Z*)-3-hexenyl-2-methyl butyrate (97 %) and (*Z*)-3-hexenyl butyrate (98 %) were from Kosher (Houston, USA). (*E*)- β -ocimene, obtained as a gift from Dr. J. J. A. van Loon. (Wageningen University, the Netherlands), was analyzed by gas chromatography and mass spectrometry (GC–MS), and its purity was 60 %, and the other fraction is mainly limonene (more than 30 %). Hexane was from Beijing Chemical Company, Beijing, China.

Collection of volatiles

Headspace sampling was used to collect volatiles from clusters at full bloom of intact tobacco. This method is similar to that described by Wei et al. (2006), but with minor modifications. Whole flowers were enclosed in a plastic oven bag (50 cm \times 60 cm) that was sealed with self-sealing strips around the stem. The compressed air was led through a water bubbler (500 ml) for humidification and a freshly activated charcoal filter for purification. The moisturized and filtered air was pushed into the bag at a rate of 500 ml/min. Volatiles were trapped in a glass tube (10 cm long, 6 mm in diameter) containing at 80 mg of 80/100 mesh Super Q adsorbent (Alltech Assoc., Deerfield, IL, USA) with polypropylene wool in both ends. The glass tube was connected with Teflon tubing to a small pump (Beijing Institute of Labor Instruments, China). Each collection lasted for 8 h and replicated for 5 times. Volatiles were desorbed by eluting the cartridge with 200 μ l redistilled hexane and stored at a freezer at -20 °C for subsequent use.

Gas chromatography and electroantennographic detection (GC–EAD)

The antenna of *H. assulta* was prepared as described by Zhao et al. (2006) with some modifications. The antenna of the 3-6-day-old mated females was cut at the base of the flagellum and the tip of terminal segment was removed. The excised antenna was mounted between two microelectrodes with some electrode gel. The electrodes were connected with a micromanipulator (Syntech MP-15, Germany). The signals from the antenna were amplified with a high impedance

amplifier (IDAC-4, Syntech, Germany) and were sent to a computer. Data storage and processing was conducted by the Autospike software package (Syntech, Germany). Linked GC–EAD analyses were conducted using an Agilent 7890A GC equipped with a polar HP-INNOWAX column (30 m × 0.25 mm × 0.25 μm). Nitrogen was used as carrier and makeup gas (30 ml/min). A 2 μl sample was introduced into the GC column in a splitless mode using a split/splitless injector at 220 °C with the Flame Ionization Detector (FID) at 250 °C. The oven temperature was programmed as follows: 40 °C for 2 min; 5 °C/min to 230 °C and hold for 5 min. The outlet of the GC column was split 1:2 ratio between FID of the GC and the moth antenna using a Microfluids Splitter with Makeup Gas (Agilent, Technologies, Palo Alto, CA, USA). Effluent to the antenna was mixed with a charcoal-filtered and humidified air stream that flowed through an 8-mm-diameter glass tube at a constant rate of 250 ml/min. The mounted antenna was placed inside the expanded end of the glass tube (8 cm from the EAD outlet on the GC). An approximate proportion of the tobacco volatiles injected on the GC was based on peak area.

Gas chromatography and mass spectrometry

Headspace collections were analyzed by using a gas chromatography–mass spectrometry system (Hewlett Packard 6890 N GC model coupled with 5973 MSD), equipped with a polar HP-INNOWAX column (30 m × 0.25 mm × 0.25 μm) and a nonpolar DB-5 column (30 m × 0.25 mm × 0.25 μm). Helium was used as carrier gas with a constant flow of 26 cm/s. Temperature programs followed those of the GC–EAD system described above. Major volatiles and GC–EAD-active volatiles in the collected extracts were identified by comparing their mass spectra with NIST library spectra (Agilent Technologies, Palo Alto, CA, USA) and confirmed with authentic reference compounds (Yan and Wang 2005). No attempt was made to determine the absolute configuration of linalool, and the relevant synthetic compounds were employed as racemates in behavioral and single sensillum recording experiments.

Single sensillum recording (SSR)

The insect was placed inside a disposable Eppendorf pipette tip with the narrow end cut to allow the head and the antenna to pass through. The Eppendorf pipette tip was inserted in a Plexiglas holder that was mounted on a platform. The head and antenna were exposed and immobilized by dental wax under a stereomicroscope. Electrophysiological recordings from single receptor neurons were recorded by the use of sharpened tungsten microelectrodes according to Mustaparta et al. (1979). The reference electrode was inserted into the compound eye. The recording

electrode was inserted into the base of a sensillum and made sure it was in contact with a single receptor neuron. First, we stimulated with the various volatiles under the dose of 500 μg, and when the responding signal was sufficiently strong for one compound, we stimulated with different concentrations of the compound. All the tests were performed by blowing air (10 ml/s) through a Pasteur pipette (15 cm long) containing test compounds on filter paper strip (0.5 cm × 3.0 cm). Action potentials of the receptor neurons were amplified through a USB-IDAC interface amplifier (IDAC-4, Syntech, Germany). Successful recordings were stored and analyzed by means of Autospike version 3.4 software (Syntech, Germany). The total successful recordings were from thirty-five receptor neurons randomly selected in antennae of seventeen mated females.

Wind-tunnel bioassay

Nine synthetic blends were used in wind-tunnel tests. The composition of each blend is shown in Fig. 4. The components were dissolved in redistilled hexane with the same ratio as naturally emitted by tobacco volatiles (Table 2).

Behavior observations of the mated female *H. assulta* were carried out in a Plexiglas wind tunnel, 2.4 m long, 0.9 m wide, 1 m high, similar to that described by Ming et al. (2007). The conditions in the wind tunnel were 24–27 °C, 40–60 % relative humidity and 0.6 lux of red light. The wind speed was 50 cm/s. Females were placed individually into a mesh cage (10 cm long and 5 cm in diameter) and allowed to acclimate the wind-tunnel conditions for at least half an hour. The rubber septa loaded with test samples or solvent was placed at the upwind end of the tunnel, above 30 cm from the floor. At the end of the photophase, test females were introduced into the downwind end of the wind tunnel one by one, 200 cm from the odor sources. Three groups were run for each blend tested. The number of females tested in each group ranged from 7 to 16 depending on availability of mated females. The behavioral responses were recorded as upwind flight over at least 60 cm and landing response on the odor source during a 5-min bioassay period. The proportions of responding females were subjected to $\chi^2 2 \times 2$ test with the threshold of significance set at $P < 0.05$ using software SPSS 10.0.

Results

GC–EAD analyses and identification of host-plant volatiles

In GC analyses on a polar column with FID, 25 compounds were identified in volatiles from tobacco flowers as shown

in Table 1. Of these compounds, nine elicited consistent responses in female *H. assulta* antenna above background (Fig. 1). The EAD-active compounds were identified by GC–MS analysis according to their mass spectra and retention times, in comparison with synthetic or authentic standards. The nine chemicals in tobacco headspace volatiles that elicited antenna responses were (*E*)- β -ocimene, octanal, (*Z*)-3-hexenyl acetate, (*Z*)-3-hexen-1-ol, nonanal, (*Z*)-3-hexenyl-2-methyl butyrate, decanal, linalool, and (*E*)- β -caryophyllene. The electroantennogram (EAG) activities of natural compounds eluting from the GC and their relative abundances were not correlated (Fig. 1, Table 2). Nonanal elicited the strongest response though its

Table 1 Volatile compounds identified in headspace collections from tobacco flowers

Compounds	Retention time (min)
<i>Hydrocarbons</i>	
Undecane	6.27
Dodecane	8.59
Tridecane	11.14
Tetradecane	13.72
Pentadecane	16.23
Hexadecane	18.65
Heptadecane	20.95
<i>Aldehydes</i>	
Hexanal	5.94
Heptanal	8.23
Octanal ^a	10.84
Nonanal ^a	13.52
Decanal ^a	16.15
<i>Alcohols</i>	
(<i>Z</i>)-3-hexen-1-ol ^a	13.37
<i>Ketones</i>	
6-methyl -2-heptanone	9.53
6-methyl-5-hepten-2-one	12.1
<i>Esters</i>	
Ethyl butyrate	5.76
(<i>Z</i>)-3-hexenyl acetate ^a	11.58
(<i>Z</i>)-3-hexenyl butyrate ^a	15.22
(<i>Z</i>)-3-hexenyl-2-methyl butyrate ^a	15.53
<i>Aromatics</i>	
Styrene	10.02
1,4-dichlorobenzene	13.99
<i>Monoterpenes</i>	
Limonene ^a	8.45
(<i>E</i>)- β -ocimene ^a	9.87
Linalool ^a	17.41
<i>Sesquiterpenes</i>	
(<i>E</i>)- β -caryophyllene ^a	18.36

^a Compounds confirmed by authentic samples

quantity is much less than that of (*E*)- β -ocimene, which also elicited a strong response (Fig. 1).

Single sensillum recording

Attempt was made to see whether there are olfactory receptor neurons (ORNs) selectively responding to the EAD-active compounds identified by GC–EAD. Of the thirty-five ORNs from *H. assulta* females by SSR, 4 were found to respond to linalool, 3 to (*E*)- β -caryophyllene, 2 to nonanal and (*Z*)-3-hexenyl acetate, and only one response to each of octanal and (*Z*)-3-hexen-1-ol. No ORN was found to respond to (*E*)- β -ocimene, (*Z*)-3-hexenyl-2-methyl butyrate, or decanal (Fig. 2). The remaining ORNs have no responses to the nine tested compounds. When stimulated with the plant odorants, all the related ORNs appeared to elicit an excitatory response and increased firing rate at increasing stimulatory concentrations (Fig. 3). No inhibition of responses by reduced firing rate or stop firing was observed.

Wind-tunnel bioassay

Behavioral responses of *H. assulta* females to the mixtures containing the main nine EAD-active compounds found in tobacco flowers were conducted in the wind tunnel. The proportion and amount of the ‘tobacco mimic’ are shown in Table 2. A significantly greater number of females were elicited upwind orientation flight when presented with the 9-component blend compared with a solvent control ($X^2 = 6.75$). The female moths clearly displayed upwind flight toward the 9-component blend, but not toward the solvent control (Fig. 4). However, there was no significant difference between the number of landings elicited by the 9-component blend and the solvent control.

The compounds that may be essential for female attraction were screened by a series of behavioral tests in the wind tunnel. We first tested the 9-component blend, which elicited 40.3 % of females to exhibit upwind flight responses (Blend A in Fig. 4). Few females responded to a blank stimulus. If one compound in the 9-component blend was removed without reducing the attractiveness relative to the full blend, this compound was omitted, if not, this compound was deemed as a key component. Removal of (*Z*)-3-hexen-1-ol, (*Z*)-3-hexenyl-2-methyl butyrate, octanal, decanal, and linalool to produce blends B, D, F, and H did not result in significantly lower flight responses from females. However, behavioral measurements evoked by the blends E, G, and I by the removal of (*Z*)-3-hexenyl acetate, nonanal, (*E*)- β -ocimene were not significantly different from those evoked by the 9-component blend. Although omission of (*E*)- β -caryophyllene (Blend C) did not give a significant reduction in upwind flight response of females,

Fig. 1 GC–EAD responses of mated female *H. assulta* to headspace volatiles from tobacco flower. EAD-active compounds: (1) (*E*)- β -ocimene; (2) octanal; (3) (*Z*)-3-hexenyl acetate; (4) (*Z*)-3-hexen-1-ol; (5) nonanal; (6) (*Z*)-3-hexenyl-2-methyl butyrate; (7) decanal; (8) linalool; and (9) (*E*)- β -caryophyllene

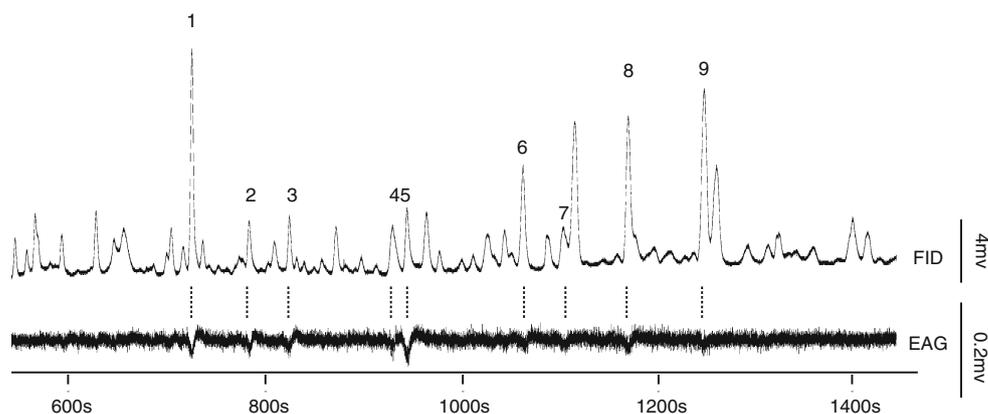


Table 2 The proportion and amount of the nine electrophysiologically active compounds identified from tobacco flowers in wind-tunnel bioassay

Compound ^a	Proportion ^b	EAG response ^c	Amount loaded on rubber septum (mg)
(<i>E</i>)- β -ocimene	4.80	+++++	0.25
Octanal	1.00	+++	0.05
(<i>Z</i>)-3-hexenyl acetate	1.22	+++	0.06
(<i>Z</i>)-3-hexen-1-ol	3.06	+++	0.15
Nonanal	1.92	+++++	0.1
(<i>Z</i>)-3-hexenyl 2-methyl butyrate	4.05	++	0.2
Decanal	1.04	++	0.05
Linalool	5.61	+++	0.28
(<i>E</i>)- β -caryophyllene	9.74	+	0.5

^a In order of elution during gas chromatography

^b Proportion relative to the chromatogram area of octanal expressed as 1.00

^c The relative EAG response strength of *H. assulta* to compounds indicated by the number of crosses (+++++ for the strongest reaction)

this compound resulted in a substantial decrease in landing response. We concluded that (*Z*)-3-hexenyl acetate, nonanal, (*E*)- β -ocimene, (*E*)- β -caryophyllene in Blend H were essential compounds for attracting *H. assulta* female.

Discussion

Foliar, floral, and fruit odors can emit hundreds of compounds, but studies of insect olfaction reveal that only a minority of the components in the complex odor blend are detected by the antennae of insects. Even fewer seem to be involved in eliciting behavioral responses from insects (Rojas 1999; Zhang et al. 1999; Fraser et al. 2003). Volatile compounds of hawthorn fruit (*Crataegus* spp.) acted as behavioral attractants for hawthorn-infesting *Rhagoletis*

pomonella flies (Nojima et al. 2003). Consistent EAD activity was obtained for six chemicals, but selected subtraction assays showed that the four-component mixture of 3-methylbutan-1-ol, 4,8-dimethyl-1,3(*E*),7-nonatriene, butyl hexanoate, and dihydro- β -ionone elicited levels of upwind flight equivalent to the six-component mix. Antennae of grapevine moth *L. botrana* responded to 27 compounds identified from grapevine as determined by GC–EAD, but these compounds were in part behaviorally redundant. The blend composed of (*E*)- β -caryophyllene, (*E*)- β -farnesene, and (*E*)-4,8-dimethyl-1,3,7-nonatriene at 100:78:9 was as attractive as green grape clusters in wind tunnel (Tasin et al. 2005, 2006a, 2007). Likewise, another pest of grape *P. viteana* flew to grape shoots in the flight tunnel (Cha et al. 2008a, b). Consistent EAD activity was obtained for 11 chemicals in volatile compounds from shoots of grape. Of these compounds, seven were found to be essential for attracting moths.

There are more than eighty volatiles identified from different varieties of tobacco (Loughrin et al. 1990; Yan and Wang 2005; Cunningham et al. 2006; Raguso et al. 2006). In this study, we identified 25 compounds from the collected volatiles of tobacco flowers, including 8 compounds previously reported (Loughrin et al. 1990; Yan and Wang 2005; Raguso et al. 2006). Nine compounds ((*E*)- β -ocimene, octanal, (*Z*)-3-hexenyl acetate, (*Z*)-3-hexen-1-ol, nonanal, (*Z*)-3-hexenyl-2-methyl butyrate, decanal, linalool, and (*E*)- β -caryophyllene) elicited consistent antenna responses in female *H. assulta*. The female moths exhibited unwind flight in the wind tunnel when presented with the 9-component blend at the same ratio as in natural tobacco floral collection. Based on the results of subtraction assays, a 4-component blend of (*E*)- β -ocimene, (*Z*)-3-hexenyl acetate, nonanal, and (*E*)- β -caryophyllene provided the same level of activity as the 9-component blend, which implies that the female moth of *H. assulta* can utilize only a few plant volatiles to locate its suitable host plant, tobacco. Six of the 9 components have been implicated in the host location of other lepidopteran moth species. (*Z*)-3-hexenyl

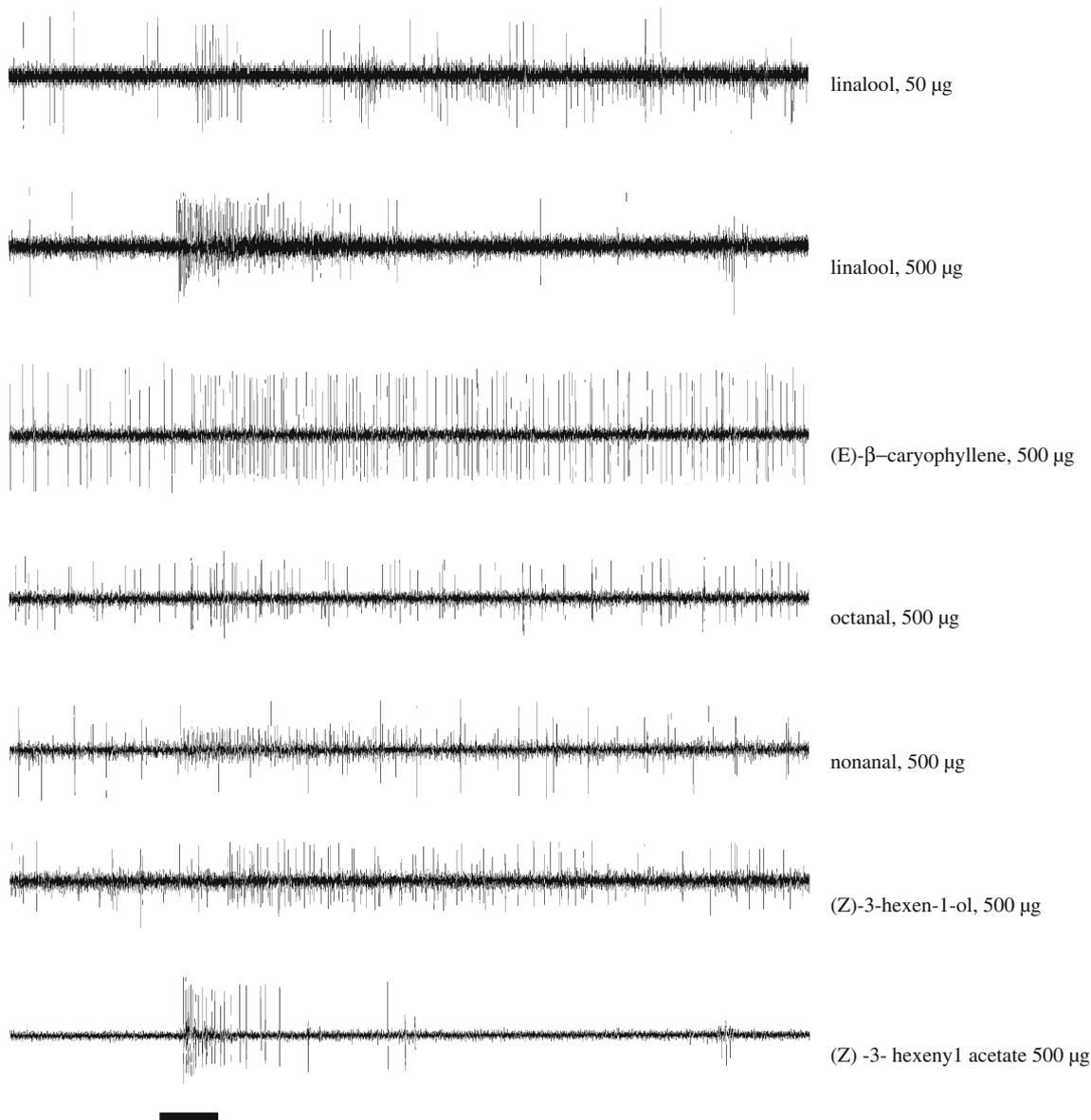


Fig. 2 Responses of different selective receptor neurons in *H. assulta* females tuned to linalool, (*E*)- β -caryophyllene, octanal, nonanal, (*Z*)-3-hexen-1-ol, and (*Z*)-3-hexenyl acetate, respectively. The horizontal bar indicates the stimulation period (300 ms)

acetate in chrysanthemum has been reported to elicit mated *M. brassicae* female upwind flight (Rojas 1999). (*E*)- β -caryophyllene identified from pigeonpea plants acted as an attractant and oviposition stimulant for *H. armigera* (Hartlieb and Rembold 1996). Linalool, in blends containing (*E*)- β -caryophyllene, was tested as kairomone for *L. botrana* (Tasin et al. 2007). *A. conjugella* was attracted to a blend containing decanal in the field (Bengtsson et al. 2006). A 7-component blend including nonanal, decanal, and (*E*)- β -caryophyllene elicited the oriented flight of *P. viteana* females (Cha et al. 2008b). (*E*)- β -ocimene in combination with nonanal and decanal attracted female *M. sexta* (Fraser et al. 2003). To our knowledge, there is no

report on behavioral response to (*Z*)-3-hexenyl-2-methyl butyrate by other lepidopteran insect species other than *H. assulta*.

GC-EAD is an effective method for screening the potential active compounds in insect behavior, but it has its limitations because EAG recording is just the summation of responses by all neurons on the antenna (Anderson et al. 1993; Burguiere et al. 2001). It is better to find more active compounds by using gas chromatography linked to electrophysiological recordings from single receptor neurons in addition to GC-EAD method (Wibe 2004; Bichão et al. 2005), since sometimes there was no apparent correlation between EAG response and behavioral activity of test

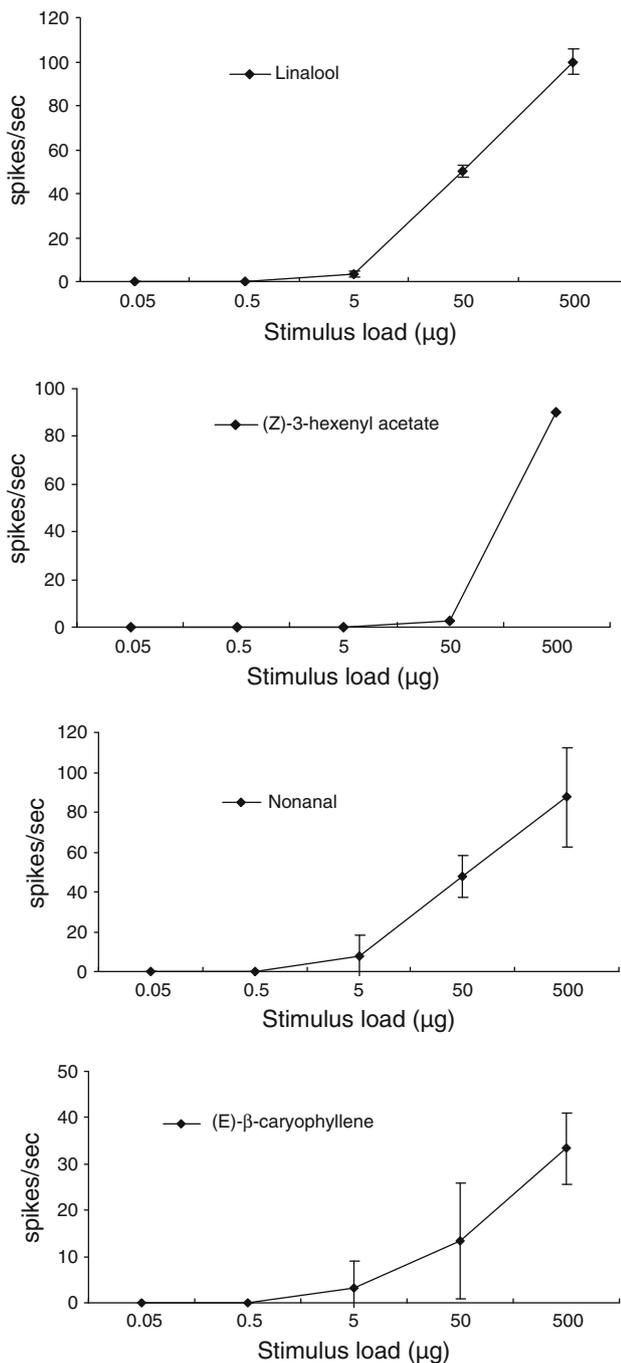


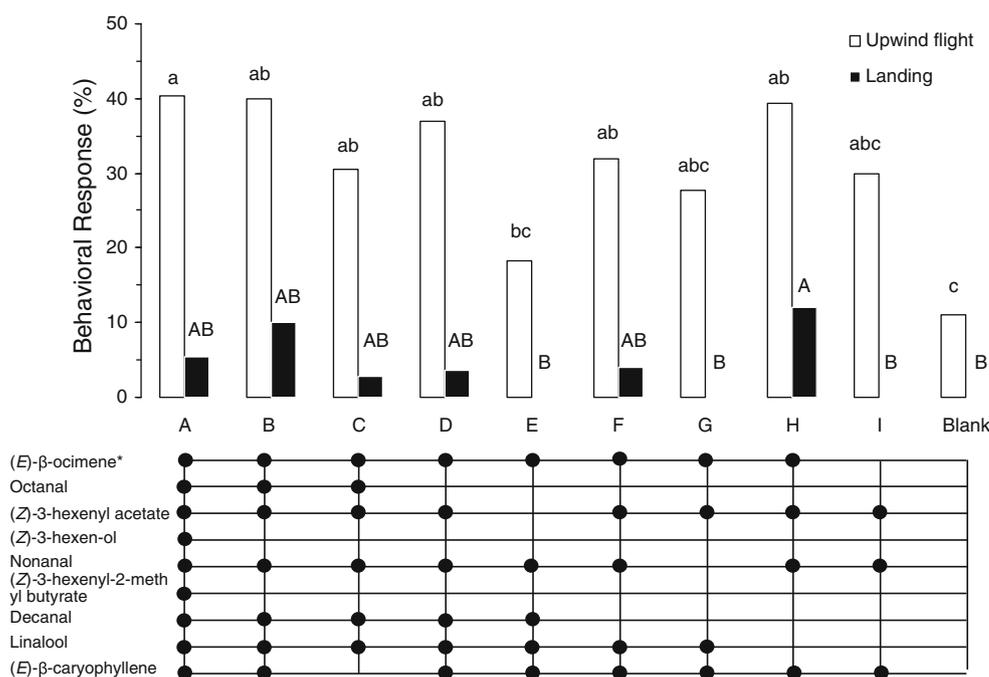
Fig. 3 Dose–response curves of ORNs in *H. assulta* females stimulated with linalool, (Z)-3-hexenyl acetate, nonanal, and (E)-β-caryophyllene. The curves with error bars were obtained from 3 neurons, and other curves showed the mean of 2 responses

compounds. Rojas (1999) working with cabbage moth, *M. brassicae* (L.), found that allyl isothiocyanate was an effective compound in stimulating upwind flight and landing of the females, despite lack of EAG response to the female antennae. In *H. armigera*, a closely related species to *H. assulta*, the amplitude of the antenna response elicited

by (*S*)-(-)-limonene was difficult to discern above the background responses, but its importance was demonstrated in the wind-tunnel bioassay (Bruce and Cork 2001). The discrepancy between antenna response and behavioral activity was also found in other insects, such as codling moth, *C. pomonella* (Ansebo et al. 2004); the almond moth, *Ephestia cautella* (Olsson et al. 2005); and the mirid, *Lygus hesperus* (Williams et al. 2010). In this study, we found (E)-β-caryophyllene to elicit a particularly weak EAG response despite the high levels present in tobacco headspace, but it is an essential component for attracting the female in wind tunnel. In contrast, compounds such as octanal elicited larger EAG responses even in trace amounts, but had only weak stimulatory effect (Figs. 1, 4).

Electrophysiological studies that use single sensillum recordings may show more correspondence with behavioral trials than EAG recordings (Bengtsson et al. 2009; Williams et al. 2010). By this method, five types of selective ORNs have been identified in the antennae of *H. assulta*, responding to (E)-β-ocimene, (E,E)-α-farnesene, (E,E)-TMTT, geraniol, and germacrene D, respectively (Stranden et al. 2003a, b). We found more types of ORNs specifically responding to linalool, (E)-β-caryophyllene, octanal, nonanal, (Z)-3-hexen-1-ol, and (Z)-3-hexenyl acetate (Fig. 2). Out of the 6 new odorants we have identified for *H. assulta*, 4 have previously been identified in *Heliothis virescens*, and 2 in *H. armigera* (Røsteliën et al. 2005). These same functional receptor neurons in heliothine species suggest that they evolve from a common origin independent on their different host ranges. Linalool sensitive neuron type was found to be the most frequent in female *H. assulta*, however, it is in part behaviorally redundant. The linalool ORN type was also found on the antenna of *H. virescens* and *H. armigera* (Røsteliën et al. 2005). It has been reported that *M. sexta* and *Anthonomus rubi* have antennal odor receptors tuned to linalool that are responding selectively to the enantiomers (Reisenman et al. 2004; Bichão et al. 2005). It is interesting to know whether the linalool ORNs in *H. assulta* have the same molecular receptive range to single enantiomers as those in other insect species. Linalool is thought to be a typical constituent released from many plants including tobacco (Røsteliën et al. 2005; Cunningham et al. 2006). In *M. brassicae*, there is one neuron type for receiving the information about two enantiomers of linalool, but with different intensity by the use of gas chromatography linked to electrophysiological recordings from single cells (GC-SSR) (Ulland et al. 2006). However, Rojas (1999) showed that mated *M. brassicae* females did not exhibit upwind flight when stimulated with linalool in wind tunnel. This suggested that *M. brassicae* may use linalool as a cue in nectar feeding but not in oviposition (Ulland et al. 2006). In the study by Skiri et al. (2005), *H. virescens*, another closely related species to

Fig. 4 Behavioral response of mated *H. assulta* females to the compounds in tobacco flowers headspace volatiles eliciting antennal response. Columns with the same letters are not significantly different ($P > 0.05$). The dots indicated the composition of the blend tested. * ocimene blend contains 60 % (*E*)- β -ocimene and about 30 % limonene



H. assulta, showed the ability to learn racemic linalool at a much lower concentration (100 times lower) than β -ocimene and β -myrcene. We speculate that linalool may play a more important role in feeding than in host-plant searching behaviors for *H. assulta*.

Knowledge of the role of kairomones in host-finding by insects is increasing. However, two contrasting hypotheses have been proposed to understanding mechanisms of olfactory cues for plant volatiles in insect–plant interactions (Bruce et al. 2005). One hypothesis is that host recognition relies on key compounds that are not found in unrelated plant species. For example, allyl isothiocyanate is produced only in five dicotyledonous families, and it is the most prominent isothiocyanate in several cruciferous plants (Rojas 1999). *M. brassicae* (L.) females have evolved the ability to recognize the characteristic chemical, and are evoked the strongest behavioral response in wind tunnel. A series of studies have indicated that many compounds such as (*E,E*)- α -farnesene, butyl hexanoate, and ethyl (2*E*, 4*Z*)-2,4-decadienoate can attract *C. pomonella* moths or stimulate oviposition in wind tunnel or in field trapping (Hern and Dorn 1999, 2004; Light et al. 2001). Recently, a study on *Pachnoda interrupta* showed that racemic 2,3-butanediol is a powerful novel attractant in the field captures (Bengtsson et al. 2009). Another hypothesis is that some insects require ratio-specific odors to be attracted to their host. Five esters composed of propyl hexanoate, butyl hexanoate, hexyl butanoate, butyl butanoate, and pentyl butanoate at a fixed ratio were attractive to the apple maggot fly, *R. pomonella* (Zhang

et al. 1999). Birkett et al. (2004) showed that of the six electrophysiologically active compounds identified from wheat volatiles, none was behaviorally attractive to female *Sitodiplosis mosellana* when present individually. The synthetic mixture composed of nine compounds identified from *Datura wrightii* flowers evoked foraging behavior in *M. sexta*, but feeding responses elicited by single odorants were not significantly different from those elicited by the control (Riffell et al. 2009). The results reported in this work support the second hypothesis. Host location of gravid *H. assulta* females appears to be mediated by a blend of compounds. These compounds are quite common in many plant species. We suggest that ratio of these compounds play an important role in host-plant selection of *H. assulta*, and maybe also in other heliothine moth species.

In conclusion, this study is the first report of host location of *H. assulta* being mediated by plant-derived volatiles. Nine compounds were identified from tobacco extract by using GC–EAD and GC–MS, and a 4-component blend played an essential role in attracting *H. assulta* females. These chemical cues used in host-plant selection by *H. assulta* could be useful in developing potent attractants for females other than pheromones for males. In the near future, it is necessary to clarify the trapping efficiency of the compound mixtures in the field. Also, further electrophysiological studies by using single sensillum recordings are needed to identify more selective olfactory receptor neurons, tuned to plant volatiles detected by *H. assulta*.

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