Journal of Asia-Pacific Entomology 21 (2018) 380–387

Contents lists available at ScienceDirect

Journal of Asia-Pacific Entomology

journal homepage: www.elsevier.com/locate/jape

The synergistic attractiveness effect of plant volatiles to sex pheromones in a moth

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ARTICLE INFO

Keywords:
Sex pheromones
Plant volatiles
Interaction
Olfactory response
Behavior

ABSTRACT

The effects of plant-derived chemicals (volatiles) on the attraction of the *Spodoptera litura* moth to sex pheromones were evaluated using an electroantennogram (EAG). Neuronal responses of male moths to sex pheromone mixtures (SPs) (a 9:1 mixture of synthetic (9*Z*,11E)-9,11-tetraddecadienyl acetate (Z9E11-14:OAc) and (9*Z*,12*E*)-9,12-tetradecadienyl acetate (Z9E12-14:OAc)) and to SPs mixtures with eight plant volatiles (benzaldehyde, (*E*)-β-caryophyllene, phenylacetaldehyde, 2,6-nonadienal, benzyl alcohol, racemic linalool, longifolene, and (*E*)-β-ocimene) were also measured. Then, wind tunnels and field trapping bioassays were conducted to determine the influence of plant volatiles on *S. litura* moth behavioral responses to SPs. The results indicated that benzaldehyde, phenylacetaldehyde, and benzyl alcohol significantly enhanced, and longifolene, (*E*)-β-caryophyllene, and (*E*)-β-ocimene had no significant effect on the attractions to SPs, whereas racemic linalool significantly decreased the attraction of male *S. litura* moths to SPs throughout the olfactory pathway. 2,6-Nonadienal significantly enhanced olfactory responses, but had no significant effect on output behavior. These findings provide foundations in utilization of plant volatiles and sex pheromones to manage the pest and other agricultural pests.

Introduction

Insects rely on the olfactory system to find food, habitation, and mates. Thus, olfactory senses are vital for insect survival and reproduction. Sex pheromones (SPs) are pheromones that are released by one organism to attract another organism of the opposite sex for mating and reproduction. In most insects, such as moths SPs are released by females and specifically focused on breeding, attracting the opposite sex, and conveying information to their species. Sp information is detected by pheromone olfactory receptor neurons (Phe-ORNs) in the male antenna and then transmitted to macroglomerular complex (MGC) within the antennal lobe for the pheromone processing. A latest study reported that both neuronal and behavioral responses to sex pheromones were altered when plant volatiles were present as a background (Dupuy et al., 2017). SPs can theoretically be used for the efficient control of pests by attracting the opposite sex. The use of SPs to lure and capture insects has been shown to control pests by reducing subsequent generations, and new insect SPs are continually being identified. For example, *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) were identified to be (9*Z*,11*E*)-9,11-tetraddecadienyl acetate (Z9E11-14:OAc) and (9*Z*,12*E*)-9,12-tetradecadienyl acetate (Z9E12-14:OAc) in 1973 (Tamaki et al., 1973). Field studies on different ratios of the two SP components found that a ratio of 10:1 resulted in an almost identical trap and bait outcome with ten virgin females and one male (Yushima and Tamaki, 1974).

Plants also represent a significant part of the natural environment for moths and emit many diverse volatile compounds. The volatile compounds released by plants are variable and depend on the plant species, and in some cases, an individual plant can emit different compounds depending on its physiological state (Niinemets et al., 2004) or the circadian rhythm (Kolosova et al., 2001). The host plant volatiles provide food sources, habitats, and oviposition sites for phytophagous insects (Bruce et al., 2005). Habitats create an unpredictable odorant background that can interact in various ways by perceiving specific signals and then synergizing or suppressing responses to the female-produced pheromones. For example, an early study of green leaf volatiles reported that the responses of the boll weevil, *Anthonomus grandis*, to an aggregated pheromone (grandlure) were enhanced compared with those exposed to the grandlure pheromone alone (Dickens, 1989). This kind of synergism by plant volatiles has been also reported

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https://doi.org/10.1016/j.aspen.2018.01.009
Received 27 October 2017; Received in revised form 18 December 2017; Accepted 5 January 2018
Available online 06 January 2018

in the male longhorn beetle (*Anaglyptus subfasciatus*) (Nakamuta et al., 1997), American palm weevil (Saïd et al., 2011), the male tobacco budworm (*Heliothis virescens*) (Dickens et al., 1993), the codling moth males (Yang et al., 2004), and the grapevine moth (*Lobesia botrana*) (Sans et al., 2016). In addition, host plant volatiles significantly synergized SP-specific olfactory receptor neurons (Phe-ORN) responses in male *Helicoverpa zea* insects (Ochieng et al., 2002).

Recently, however, a greater number of studies have discovered that host plant volatiles can inhibit responses to pheromones. For example, the odor from the host plant of the noctuid moth *Agrotis ipsilon*, heptanal, reduced the olfactory receptor neuron (ORNs) and the macroglomerular complex (MGC) calcium responses to SPs (Deisig et al., 2012). Another noctuid moth, *H. virescens*, was attracted to SPs in the presence of natural background plant odors. However, the attraction was significantly reduced when a single plant odor was present in the background compared with only SPs being present (Badeke et al., 2016). (Collignon et al., 2016 found that the influence of plant odors on longhorn beetles responses to pheromones were not only dependent on the beetle species but were also dependent on the plant species and the release rates of plant volatiles.

The tobacco cutworm, *S. litura*, is a polyphagous pest of diverse vegetable and field crops and is native to Asia. This cutworm is found throughout the tropical and temperate regions of Asia, Australia, Africa, the Middle East, southern Europe, and the Pacific Islands and has damaged > 120 plant species worldwide. This insect is a concern from a regulatory perspective because of the potentially significant economic losses that result from direct agricultural crop damages. For example, the larva can cause 100% root damage in the sugar beet and consume up to 85.5% of leaf surfaces, resulting in huge yield reductions (Chatterje and Nayak, 1987; Ram et al., 1989). Thus, controlling *S. litura* is a critical issue for Integrated Pest Management (IPM) systems in agriculture. In previous decades, pest control depended mostly on insecticides, but many pests including *S. litura* have developed resistance to most pesticides available (Naeem et al., 2014). The use of female-derived SPs could help to solve this problem. Although synthetic pheromone lures derived from *S. litura* have been commercially available in China for more than a decade, the interactions between plant volatiles and sex pheromones from central nervous to peripheral levels have not studied.

Therefore, in this study, we first investigated the responses of male *S. litura* moths to single component and a 9:1 mixture of SPs, and to host plant volatiles with electroantennogram (EAG) recordings. The olfactory responses at the central nervous system and the behavioral responses to these mixtures were then detected using intracellular recordings and wind tunnels. Field bioassays were conducted to confirm the ability of these plant volatiles to influence male *S. litura* attraction to the SPs, and to find effective plant volatiles interacting with sex pheromones.

Materials and methods

Insects

The eggs and larvae of *S. litura* were collected in crop fields around Chashan Town, Wenzhou City, and then cultured in an artificial intelligence climate room at 28 °C, 65% relative humidity (RH) and a 14:10 (light:dark) photoperiod. The larvae were fed an artificial diet (Gupta et al., 2007). After emergence, adults were fed 10% sucrose. Three-day old adults were used for all experiments.

EAG recordings

Whole-antennae electrical activity recordings in response to volatile stimuli were made according to standard techniques. Male and female moths were stabilized in a 1 mL plastic pipette with a cut tip that allowed only the antennae to project through the opening. The tip of one of the antennae was cut, and a recording electrode filled with Beadle-Ephrussi Ringer (7.5 g NaCl, 0.35 g KCl, and 0.279 g $CaCl_2 \cdot 2H_2O$ dissolved in 1 L ultrapure water) was placed in contact with the cut surface of the antenna. An Ag/AgCl wire was inserted into the insect's abdomen and served as a ground electrode. A glass tube (8 mm i.d.) was used to wash the antenna continually with moistened air purified with a charcoal filter. The tube outlet was approximately 20 mm from the antenna and the stimulus was injected into the air stream 15 cm upstream from the antenna using a Pasteur glass tube. A stimulation device was used to deliver stimulation at a flow rate of 5 mL/s in 0.5 s puffs (Syntech, The Netherlands). Signal amplification with a high impedance amplifier was performed, and data were stored and analyzed with EAG2000 software. *S. litura* antennae were stimulated with the SPs mixture, Z9E11-14:OAc and Z9E12-14:OAc, at a ratio of 9:1, based on our preliminary work (Fig. S1) and previously published study (Sun et al., 2003), which showed a ratio that most efficiently attracted males. In addition, combinations of SPs with eight plant volatiles to which *S. litura* male moths high responded including benzaldehyde, (E)-β-caryophyllene, phenylacetaldehyde, 2,6-nonadienal, benzyl alcohol, racemic linalool, longifolene, and (E)-β-ocimene (Fig. S2; Table 1) were added to the EAG tests. Antennae of *S. litura* males were stimulated with filter paper (4 cm × 1 cm) containing 20 μL of each mixture with 20 ng SPs and 10 ng SPs plus 100 ng of volatiles (dissolved in paraffin oil). Twenty microliters of paraffin oil on filter paper was used as control. For each mixture, the responses of antennae from six male moths were individually tested. The subtraction of absolute EAG responses of treatments and controls were used for comparisons. Recordings were performed under red lights because the males were in scotophase at the start of the EAG recordings.

Intracellular recording

Male moths were mounted in plastic tubes and immobilized with dental wax (Kerr Corporation, Romulus, MI, USA). Brains were dissected in a saline solution (150 mM NaCl, 3 mM $CaCl_2$, 3 mM KCl, 25 mM sucrose $C_{12}H_{22}O_{11}$ and 10 mM TES buffer, pH 6.9) until the whole antennal lobe was exposed. Borosilicate glass capillary electrodes (1.0 mm o.d., 0.5 mm i.d.; World Precision Instruments) with a resistance of 250 MΩ were pulled using a Flaming-brown Puller (P-2000, Shutter Instrument). The electrode shaft was filled with a filtered (0.2-μm pore size) 2.5 mol/L KCl solution and the glass microelectrode was inserted into the male-specific macroglomerular complex (MGC). Moth antennae were stimulated with trains of 5 air puffs (50 ms each; 2 s interpulse intervals) from a Pasteur tube containing SPs, SP mixtures, and volatiles with the same doses as those used in the EAG test.

Table 1
The list of chemicals used in the experiments.

Classification of chemicals	Chemicals	Purity	Source
Sex pheromone compounds	(9Z,11E)-Tetradecadienyl acetate (Z9E11-14:OAc) (Main impurity: E9Z11-14:OAc)	≥ 92.0%	Bedoukian Research
	(9Z,12E)-Tetradecadienyl acetate (Z9E12-14:OAc) (Main impurity: E9Z12-14:OAc)	≥ 93.0%	Bedoukian Research
Floral aromatic compounds	Benzaldehyde	≥ 99.0%	Sigma Aldrich
	Phenylacetaldehyde	≥ 95.0%	Sigma Aldrich
	Benzyl alcohol	≥ 98.0%	Sigma Aldrich
Terpenes	(−)-β-Caryophyllene	≥ 80.0%	Sigma Aldrich
	β-Ocimene	≥ 99.0%	Sigma Aldrich
	longifolene	≥ 98.0%	Sigma Aldrich
	LINALOOL	≥ 98.0%	Sigma Aldrich
Aliphatic compound	2,6-Nonadienal	≥ 95.0%	Sigma Aldrich