

## EAG and behavioral responses of *Helicoverpa armigera* males to volatiles from poplar leaves and their combinations with sex pheromone<sup>\*</sup>

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**Abstract:** Electroantennogram (EAG) evaluation of selected compounds from wilted leaves of black poplar, *Populus nigra*, showed that phenyl acetaldehyde, methyl salicylate, (E)-2-hexenal elicited strong responses from male antennae of *Helicoverpa armigera*. When mixed with sex pheromone (Ph), some volatiles, e.g. phenyl acetaldehyde, benzyl alcohol, phenylethanol, methylsalicylate, linalool, benzaldehyde, (Z)-3-hexenol, (Z)-3-hexenylacetate, (Z)-6-nonenol, cineole, (E)-2-hexenal, and geraniol elicited stronger responses from male antennae than Ph alone. Wind tunnel bioassay demonstrated that various volatiles could either enhance or inhibit the effect of synthetic sex pheromone. (E)-2-hexenal, (Z)-3-hexenol and linalool in combination with Ph could not induce any male to land on source at all, whereas phenyl acetaldehyde, benzaldehyde, (Z)-6-nonenol and salicylaldehyde combined with Ph enhanced male response rates by 58.63%, 50.33%, 51.85% and 127.78%, respectively, compared to Ph alone. These results suggested that some volatiles should modify sex pheromone caused behavior and that some of them could possibly be used as a tool for disrupting mating or for enhancing the effect of synthetic sex pheromone in the field.

**Key words:** *Helicoverpa armigera*, Wilted leave volatiles, Chinese black poplar, Sex pheromone, EAG, Wind-tunnel bioassay

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### INTRODUCTION

Bundles of *Populus* sp. (Salicaceae) branches have been used to forecast and control cotton bollworm (CBW), *Helicoverpa armigera*, for more than 40 years since farmers attempted to trap-kill

adult CBW in fields with them in northern China in 1956 (Li, 1966). In the 1960s and 1970s, extensive studies were conducted on the attraction of CBW to poplar branches, on defining the application technology, on proving their controlling effect, and on determining the mechanisms for the powerful attraction of CBW to these non-host plants. Until recently, it was ruled out that the physical effect of poplar bundles was responsible for attraction to CBW (Yuan *et al.*, 1999) and proved that volatiles emitted from the poplar bundles played a major role

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in their attraction to the cotton bollworm (Ding *et al.*, 1997; Xiao *et al.*, 2002). According to Lin *et al.* (1998), the application of the poplar bundles as a bait was more effective than that of sex pheromone to detect the occurrence of the 3rd and 4th generations of CBW in fields. Xiao *et al.* (2002) also proved that volatiles from wilted leaves of Chinese wing-nut tree, *Pterocarya stenoptera*, could enhance the attraction of female sex pheromone to some extent. Isolation and identification of volatiles from wilted leaves of Chinese black poplar, *Populus nigra*, were carried out to develop new efficient lure for CBW in our laboratory in 1997 (unpublished data). This paper reports the results of both electroantennogram and wind-tunnel behavioral response of CBW male moths to volatiles identified from Chinese black poplar and their combination with the synthetic sex pheromone of CBW.

## MATERIALS AND METHODS

### Chemicals

(Z)-9-hexadecenal (Z9-16:Ald) was synthesized in our laboratory followed by purification by column chromatography and (Z)-11-hexadecenal (Z11-16:Ald) was provided by the Beijing Institute of Zoology, Chinese Academy of Sciences. The purity of these two compounds was confirmed by gas chromatography to be more than 95%. (Z)-3-hexenyl acetate, (Z)-3-hexen-1-ol, linalool, (E)-2-hexenal, geraniol, methyl-2-methoxybenzoate and cineole were provided by New England University, Australia, with the purity of these compounds being over 98%. Benzaldehyde (purity $\geq$ 98.5%) and phenylacetaldehyde (purity $\geq$ 94%) and other chemicals (purity $\geq$ 98.5%) were all purchased from Chinese Medical Chemical Company.

### Insects

Larvae of *Helicoverpa armigera* were collected from Jiangxi Province in 1999 and reared in the laboratory at (25 $\pm$ 2) °C under a reversed 14:10 h light-dark photoperiod. Larvae were fed on a modified semi-artificial diet as described by Li *et al.* (1991). After sexing, male pupae were placed in 40

cm $\times$ 25 cm $\times$ 15 cm wooden cages. Moths were fed with 10% sugar water solution saturated on cotton balls.

### EAG responses

EAG responses from isolated male antennae were performed according to the methods described previously by Dong and Du (2001). An antenna from 2-day-old moth was excised at its base, and the distal part of the terminal segment was cut off. The antenna was mounted between two cotton balls, which were placed on separate electrodes and saturated with Beadle-Ephrussi solution (Zhang and Meng, 2000). Air-carrier speed was kept at 1 m/s. The potential range was 1 mV. Ten microliters of each solution containing 50  $\mu$ g plant volatile to be tested and/or 500 ng of sex pheromone components (Z11-16:Ald/Z9-16:Ald=97/3) was applied to a piece of filter paper (diameter=0.5 cm), which was inserted into a glass syringe. Solvent (hexane) alone was used as the control. An air-stream of 1-sec duration was blown over the antennae. Intervals between odorous stimuli were at least 2 min. Each solution was tested three times with six antennae of males.

### Wind-tunnel assays

In order to further confirm the screening of the behavioral responses, a wind tunnel bioassay was employed for such purposes. The tests were performed in a Plexiglas wind tunnel, 250 cm long, 96 cm wide, and 96 cm high under the conditions of (25 $\pm$ 2) °C, 70% $\pm$ 10% relative humidity, 0.3 Lux (red light) and air speed of 30 cm/s. Newly emerged moths were separated from the remaining pupae once a day. Only male moths on their third scotophase were tested since they had shown maximum response. Before the onset of the scotophase, males to be tested were transferred individually to test tubes (2.5 cm $\times$ 10 cm) and maintained until the 8th hour into the scotophase. The males were then allowed to be acclimated to the tunnel conditions for 30 min and then were introduced into the tunnel individually. Filter paper containing the pheromone and/or host plant volatiles was pinned on a steel jack, 20 cm high and 25 cm away from the up-wind

end at the middle line. Then the steel jack with the lure was caged with a cylindrical screen cage (25 cm high and 10 cm in diameter). For the test, a male moth was transferred from the test tube to a slightly larger cylindrical one-end-opened screen cage. The release cage was placed into the tunnel with the open end facing the lure direction, on a steel screen platform that was 28 cm high and 25 cm from the downwind end (this point was just in the center of the plume).

Males were allowed to respond for 3 min and scored for the following behaviors: takeoff (TF), orientation flight (OR), arrival at halfway (HW), approach to the lure cage (within 10 cm) (AP), and landing on the lure cage (LS). Sex pheromone solution and host plant volatile solutions were prepared in redistilled hexane and concentration of sex pheromone (Z11-16:Ald/Z9-16:Ald=97/3) solution was 50 ng/ $\mu$ l. For odor delivery, a piece of filter (triangular, 5 mm base $\times$ 10 mm height) was used as release substrate. Ten  $\mu$ l sex pheromone solution and 10  $\mu$ g host plant volatile were applied to filter papers prior to bioassay. The lure was used for the bioassay after all the solvent on the filter paper was evaporated. For each lure, responses of 8 to 20 males were recorded. The same experiment was at least duplicated three times.

### Statistical analysis

EAGs were compared statistically by using analysis of variance and Tamhane's T2 test ( $P < 0.05$ ) or Least significant difference ( $P < 0.05$ ). The number of responding insects in a wind tunnel was analyzed using a  $\chi^2$  test.

## RESULTS

The electro-physiological response of CBW male antennae to volatiles from withered leaves of Chinese black poplar is shown in Table 1. Of all volatiles tested, (E)-2-hexenal elicited the strongest EAG response at the dosage used. Phenyl acetaldehyde and methyl salicylate were ranked as the highest responsive group but were slightly weaker than (E)-2-hexenal (Table 1). In addition to the

above three components, relatively high EAG responses were also elicited by linalool, geraniol, phenylethanol, (Z)-6-nonenol, benzyl alcohol and eugeol, respectively. (Z)-3-hexenol, methyl-2-methoxybenzoate and (Z)-3-hexenylacetate elicited the lowest EAG responses from male CBW antennae.

**Table 1** EAG response of males *H. armigera* to volatiles from withered leaves of Chinese black poplar

Volatiles	EAG relative to standard <sup>a</sup> (mean $\pm$ SE) <sup>b</sup>
(E)-2-hexenal	1.03 $\pm$ 0.05 a
Phenyl acetaldehyde	1.00 $\pm$ 0.00 a
Methylsalicylate	0.88 $\pm$ 0.04 b
Linalool	0.61 $\pm$ 0.04 cde
Geraniol	0.66 $\pm$ 0.05 c
Phenylethanol	0.63 $\pm$ 0.04 cd
(Z)-6-nonenol	0.69 $\pm$ 0.06 c
Benzyl alcohol	0.74 $\pm$ 0.05 c
Eugeol	0.62 $\pm$ 0.04 cde
Cineole	0.50 $\pm$ 0.05 de
Benzaldehyde	0.50 $\pm$ 0.04 e
(Z)-3-hexenylacetate	0.31 $\pm$ 0.03 f
Methyl-2-methoxybenzoate	0.25 $\pm$ 0.02 f
(Z)-3-hexenol	0.27 $\pm$ 0.03 f
Hexane (Control)	0.24 $\pm$ 0.01 f

<sup>a</sup>50  $\mu$ g of each volatile was tested and phenyl acetaldehyde was used as a standard;

<sup>b</sup>Means with the same columns followed by the different letters are significantly different (Tamhane's T2 test,  $P < 0.05$ )

The combination of volatiles and CBW sex pheromone was also tested for better understanding of the volatile enhancement effects. It was proved that some volatiles added to sex pheromone components elicited significantly higher EAG responses compared with pheromone. Table 2 shows the different EAG responses when pheromone was mixed with various volatile compounds. Phenylacetaldehyde, benzyl alcohol, phenylethanol, methylsalicylate, linalool, benzaldehyde, (Z)-3-hexenol, eugeol, (Z)-6-nonenol showed strong enhancing effects. However, some other volatiles, such as (Z)-3-hexenylacetate, cineole, (E)-2-hexenal, (Z)-3-hexenyl benzoate, methyl-eugeol did not elicit EAG responses significantly different from Ph alone at the doses tested. Surprisingly, when methyl-2-methoxybenzoate was added to phero-

alone, the inhibition effect was seen. The EAG value was lower than that for pheromone tested alone.

**Table 2 EAG responses of *H. armigera* males to different blends of sex pheromone and plant volatiles from wilted leaves of Chinese black poplar**

Volatiles	EAG relative to standard <sup>a</sup> (mean±SE) <sup>b</sup>
Ph	1.00±0.00 fg
Phenyl acetaldehyde+Ph	1.61±0.10 a
Benzyl alcohol+Ph	1.47±0.09 ab
Phenylethanol+Ph	1.39±0.05 bc
Linalool+Ph	1.34±0.07 bcd
Benzaldehyde+Ph	1.34±0.10 bcd
Methylsalicylate+ Ph	1.32±0.06 bcd
(Z)-3-hexenol+Ph	1.31±0.12 bcd
Eugeol+Ph	1.28±0.07 bcde
(Z)-6-nonenol+Ph	1.24±0.07 bcde
(Z)-3-hexenylacetate+Ph	1.19±0.06 cdef
Cineole+Ph	1.15±0.06 def
(E)-2-hexenal+Ph	1.11±0.07 defg
Geraniol+Ph	1.01±0.05 efg
(Z)-3-hexenyl benzoate+Ph	0.98±0.05 fg
Methyl-eugeol+Ph	0.92±0.04 gh
Methyl-2-methoxybenzoate+Ph	0.72±0.06 h

<sup>a</sup>500 ng of Ph was used as a standard and 10 µg of each volatile was added;

<sup>b</sup>Means with the same columns followed by different letters are significantly different (Least significant difference,  $P<0.05$ )

Male behavioral responses were found to be different when volatiles were combined with pheromone in the wind tunnel bioassay (Table 3). At the TF stage, pheromone with some volatiles, phenylethanol, (Z)-3-hexenylacetate, (E)-2-hexenal, (Z)-3-hexenol, geraniol and linalool decreased the response rates compared to that of sex pheromone (Ph) alone. At the stage of OR, HW, AP and TS, phenyl acetaldehyde, benzaldehyde, (Z)-6-nonenol and salicylaldehyde mixed with pheromone significantly enhanced the behavioral responses, whereas, (E)-2-Hexenal, (Z)-3-hexenol and linalool reduced response rates. At the stage of TS, (E)-2-Hexenal, (Z)-3-hexenol and linalool when mixed with Ph could not elicit any male to land on the source at all, whereas phenyl acetaldehyde, benzaldehyde, (Z)-6-nonenol and salicylaldehyde when combined with Ph enhanced male response rates by 58.63%, 50.33, 51.85% and 127.78%, respectively, compared to that of Ph alone.

## DISCUSSION

Our EAG and behavioral results showed that males of *Helicoverpa armigera* responded to most volatiles identified from black poplar leaves. Some of the plant volatiles at the dose tested added to phero-

**Table 3 *H. armigera* male responses to different lures in a wind tunnel<sup>a,b</sup>**

Lure	N	TF (%)	OR (%)	HW (%)	AP (%)	LS (%)
Ph	82	100.00	73.17	45.12	19.51	14.63
Phenyl acetaldehyde+Ph	56	100.00	89.29***	85.71****	35.71***	23.21*
Benzyl alcohol+Ph	43	100.00	79.07	58.14*	30.23*	18.60
Methylsalicylate+Ph	30	100.00	63.33*	40.00	6.67***	6.67*
Benzaldehyde+Ph	50	100.00	94.00****	84.00****	38.00***	22.00*
Eugeol+Ph	24	100.00	50.00****	29.17**	4.17****	4.17****
Phenylethanol+Ph	50	88.00***	56.00***	32.00*	24.00	8.00*
(Z)-3-hexenylacetate+Ph	25	92.00***	64.00*	52.00	20.00	8.00*
(E)-2-Hexenal+Ph	33	75.76***	48.48****	18.18****	0.00****	0.00****
(Z)-3-hexenol+Ph	42	85.71***	85.71**	61.90**	19.05	0.00****
(Z)-6-nonenol+Ph	27	100.00	77.78	66.67****	33.33**	22.22*
Cineole+Ph	35	97.14	71.43	48.57	25.71	14.29
Geraniol+Ph	30	93.33**	60.00*	46.67	13.33	13.33
Salicylaldehyde+Ph	48	100.00	97.92****	83.33****	41.67****	33.33****
Linalool+Ph	36	88.89***	72.22	55.56*	5.56***	0.00****
Methyl-2-methoxybenzoate+Ph	38	100.00	73.68	63.16***	31.58	10.53

<sup>a</sup> Asterisks indicate differences ( $\chi^2$  test) from Ph: \*  $P<0.25$ ; \*\*  $P<0.05$ ; \*\*\*  $P<0.01$ ; \*\*\*\*  $P<0.005$

<sup>b</sup> TF=take off, OR=orientation flight, HW=arrival at halfway, AP=approach to the lure cage, LS=landing on the lure cage

more significantly enhanced the male EAG and behavioral responses compared with those when sex pheromone was used solely. These evidences indicated that the non-host plant volatiles could be also applied to modifying insect behavior.

In many insect species, such as *Plutella xylostella* L. (Reddy and Guerrero, 2000), *Larra bicolor* (Meagher and Frank, 1998), *Gnathoyrichus sulcatus* (Deglow and Borden, 1998a; 1998b), *Heliothis virescens* (Dickens et al., 1993), *Heliothis zea* (Light et al., 1993) and *Helicoverpa armigera* (Fang and Zhang, 2002), the plant volatiles induced enhancement of attraction to sex pheromone was reported for their host plants. However, the non-host plant was also reported to show attraction to *H. armigera* (Xiao et al., 2002). Xiao et al.'s EAG analysis showed that male cotton bollworm EAG response to a combination of crude extract of female sex pheromone and Chinese wing-nut tree volatiles was stronger than that to either alone. Our results further support the above mentioned phenomenon.

Up to now, the behavioral mechanisms underlying the enhancement of attraction have not been well studied. The enhanced male behavioral responses to mixtures of pheromone plus plant volatiles are due at least in part to enhancement of pheromone specific pathways (Ochieng et al., 2002). On the other hand, since plant volatiles may selectively stimulate pheromone receptor neurons, thus affecting the quality of perceived pheromone blend, plant odors may be involved in cessation of pheromone-mediated flight as previously shown for individual pheromone components (Dickens et al., 1993; Baker et al., 1988). For many species of phytophagous insects, host plants influence insect pheromone communication with different effects reflecting different strategies by males and females of different species (Landolt and Phillips, 1997). Some aphid species may possess mechanisms to use combined sex pheromone/plant volatiles for mate location (Landolt and Phillips, 1997; Zhang et al., 2000). The interaction of host-plant cues with sex pheromone may enhance and improve success in mate location, and more important is that it may contribute to species-specific mate location and

thus species isolation (Zhang et al., 2000). The latter is very important for many species of aphids to use the same sex pheromone components. Phytophagous insects often meet, court, and mate on plants and their sexual behavior may occur principally or exclusively on the host plant. For a phytophagous species, host plants are an appropriately attractive resource. A combination of sex pheromone with host plant volatiles may provide more complete information for insects to search for mating, which suggests existence of not only the opposite sex but also suitable host. Host plant volatiles can induce insects to orient to host and sex pheromone can introduce the opposite sex insects to orient to mate. Host odor enhancement or synergism of sex attraction is of great importance in the development of pheromone-based lures. If insects in natural setting signal from food or plants from which biologically active odorants emanate, human-made lures based solely on pheromone are not likely to be competitive when placed in the field. These considerations were of great significance in the development of lures for many insect pests (Landolt and Phillips, 1997).

If the male sense is proved to detect the pheromone and plant volatiles at the same level, it is not surprising to find that some plant volatiles disrupt or inhibit insect pheromone responses. For instance, blends of (E)-2-hexen-1-ol, (Z)-2-hexen-1-ol, and (Z)-3-Hexen-1-ol with lineatin, the aggregation pheromone of the striped ambrosia beetle, caused a 63%–78% reduction in trap catches (Borden et al., 1997). Similarly, blends of 1-hexanol, (Z)-3-hexen-1-ol, and (E)-2-hexen-1-ol reduced *Ips typographus* catches by 85%, while ca. 70% trap reduction was achieved by blends of (E)-3-hexen-1-ol, (Z)-2-hexen-1-ol, and linalool (Zhang et al., 1999). In the field experiments, nonanal from aspen trees and ( $\pm$ )- $\alpha$ -pinene from coniferous trees were effective in reducing captures of male *Enarmonia formosana* in pheromone-baited traps (McNair et al., 2000).

It seems likely that some volatiles from black poplar leaves at certain dose or concentration could be used to enhance the effect of synthetic sex pheromone on CBW or disrupt male response to

calling female in the field; although it should be noted that release rates or concentrations of some plant volatiles may play a pivotal role in enhancing or disrupting the insect pheromone in a field test. Enhancement or disruption of the effect of insect pheromone by plant volatiles is of very great importance in practical applications since the cost of insect pheromone is often very high. The use of inexpensive and relatively environmentally safe plant volatiles could increase the effectiveness of the pheromone and some plant volatiles could effectively disrupt male response to calling insects in the field.

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