

# Active compounds in *Populus nigra* L. wilted leaves responsible for attracting *Helicoverpa armigera* (Hübner) (Lep., Noctuidae) and new agarosectin formulation

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**Abstract:** The leaf extracts of *Populus nigra* were collected and identified by steam distillation, air entrainment and gas chromatographic-mass spectrometric analysis. Electroantennograms were recorded from *Helicoverpa armigera* adults in response to the chemicals identified. Both aromatic compounds and green-leaf volatiles elicited strong responses. Field experiments revealed that the active compounds responsible for attracting *H. armigera* moths are mainly short-side-chain aromatic alcohols and aldehydes. We, for the first time, used agarosectin as the controlled-release matrix of insect attractants. A five-component lure containing all the aromatics without phenolics, mixed in the proportions as found in the steam distillate of the leaves collected in August, produced the best trap catch. The results showed that the volatiles of wilted leaves of *P. nigra* can attract *H. armigera* adults by feeding attraction.

**Key words:** *Helicoverpa armigera*, *Populus nigra*, agarosectin, formulation, poplar bundle

## 1 Introduction

The cotton bollworm (CBW), *Helicoverpa armigera* (Hübner) (Lep., Noctuidae) is a polyphagous and proliferous insect pest in most regions of the world (REMBOLD et al., 1991). More than 75% of the insecticides used in cotton fields are being targeted towards CBW (BANERJEE et al., 2000). Besides, semiochemicals may prove to be an alternative to current control methods. Among semiochemicals, female sex pheromone was used to monitor the population, but not as a control tool for these multiple-mating species (KIRITANI and KANO, 1984).

The Lombardy poplar, *Populus nigra* 'Italica' L. (Salicaceae), is a typical wind-pollinated plant, the flowers of which do not secrete nectar, or support large number of insects or vertebrate pollinators (STEVEN et al., 2001); however, the bundles made of the branches and leaves of this woody plant have been used to trap and kill CBW adults in cotton fields in China since 1956 (LI, 1966). It has been demonstrated that poplar bundles had different attractiveness to different CBW generations. In cotton fields of the same acreage, the ratio of 1-4 generations of CBW caught by sex attractants was 1 : 1.38 : 0.74 : 1.72, while that caught using poplar bundles was 1 : 5.40 : 10.89 : 58.14 (SONG, 1998). The mechanism of this attraction or the function of poplar bundles has been reported. One hypothesis is physical

performance, such as providing a shelter (XIA, 1978) or a mating site (FU et al., 2001); another is olfactory performance, such as stimulating sex pheromone production of virgin females (FU et al., 2001), stimulating reproduction of mated females (GUO et al., 2003), attracting both sexes to feed (GUO, 1998; LU et al., 1998; WANG et al., 1999), being related to calling of virgin females for poplar wilted leaves attracted virgin females but not mated females or males in flight tunnel (XIAO et al., 2002) and enhancing male electroantennograms (EAGs) when some components in poplar leaf volatile were combined with sex pheromone, compared with sex pheromone alone (DENG et al., 2004). However, long-term recording revealed that noctuids comprised over 85.3% of total captures; the number of males captured was slightly less than that of females and among the latter 93.3% were mated females carrying eggs (YUAN et al., 1999; GUO et al., 2003).

The attractive compounds have been postulated as salicyl alcohol and monosaccharides (GUO, 1998), benzaldehyde (LU et al., 1998), salicylic acid (WANG et al., 1999) or a highly volatile mixture of alkenes (FU et al., 2001). To date, no synthetic attractant from poplar leaf volatiles has been reported to be applicable in the field. Additionally, plant chemicals often involve a wide range of volatiles, polarities and concentrations (ROBERT and BRUCE, 1998). There is thus considerable

impetus to develop a formulation appropriate for dispersing highly volatile plant odours and to carry out field evaluations.

## 2 Materials and Methods

### 2.1 Insects and chemicals

*Helicoverpa armigera* moths used in EAG bioassays were collected from tobacco fields in Zhengzhou, China and maintained for successive generations on an artificial diet in laboratory conditions at 25–28°C and 70% relative humidity under a 14 : 10 h (light : dark) photoperiod. Sexes were separated at the pupal stage and the adults collected daily. Moths were provided with 10% sucrose solution. One- to 3-day-old moths were used.

All the aromatic compounds (ACs) were obtained from Beijing Chemical Reagents Co. Ltd, China, while other chemicals used were obtained from Sigma.

### 2.2 Extractions and chemical analysis

We collected *P. nigra* leaves in accordance with the time that CBW flight peaks occurred in northern China on 19 May, 15 June and 25 August in the schoolyard of Henan Agricultural University, Zhengzhou, China, then put the distilled water-washed leaves in a shed at  $26 \pm 2^\circ\text{C}$ , 90% relative humidity. After 48 h, the wilted leaves were extracted using the following two methods: (1) *steam distillation*: 0.5 kg leaves were extracted for 2 h; during this process, the distillate was regulated at 70–80 droplets per minute. Then the distillate was extracted with redistilled dichloromethane ( $4 \times 25$  ml). Combined extracts were concentrated with a rotating evaporator. (2) *Air entrainment*: charcoal-filtered air was pulled through a glass vessel ( $0.01 \text{ m}^3$ ) containing 1 kg leaves collected on 15 June and then through a dichloromethane-washed Porapak Q column [0.01 m internal diameter (ID); 0.001 kg adsorbent] with a pump, the air-flow rate was maintained at 5 ml/min for 12 h. Then the Porapak Q trap was removed and rinsed with redistilled dichloromethane ( $8 \times 25$  ml). Combined rinses were dried over sodium sulphate and concentrated with a rotating evaporator.

A Hewlett-Packard (HP) 5890A gas chromatograph combined with a quadrupole 5972A mass spectrometer was equipped with a HP 5 quartz capillary column,  $60 \text{ m} \times 0.25 \text{ mm}$  ID. Helium was used as the carrier gas at an inlet pressure of  $1.034 \times 10^5$  Pa. The injector temperature and the interface temperature were 220 and 280°C respectively. The column was held at 70°C for 2 min, 70–225°C at 11°C/min and maintained at 225°C for 10 min. Injection volume was 2  $\mu\text{l}$ . Mass spectra in the electron-impact mode (MS/EI) were generated at 70 eV and the ion source temperature was 177°C. Mass range was 20–450 mass units. Compounds were identified using a NBS 75K HP MS Chemical Station library search.

### 2.3 EAG tests

Candidate chemicals were diluted 100-fold in paraffin oil. (*E*)-2-hexenal was used as the standard and paraffin oil without volatiles served as the control. Cyclohexanol, cinnamaldehyde, (*E*)-caryophyllant and hexadecanoic acid were tested together with the chemicals identified. Three groups of moths were used: virgin females, virgin males and mated females. An excised antenna was fixed between the recording electrode and the reference electrode. Glass

capillary electrodes filled with  $1 \times 10^{-3} \text{ m}^3$  kaissling saline solution ( $1 \times 10^{-3} \text{ m}^3$  of this solution contained the following substances: 6.4 mmol KCl, 20.0 mmol  $\text{KH}_2\text{PO}_4$ , 12.0 mmol  $\text{MgCl}_2$ , 1.0 mmol  $\text{CaCl}_2$ , 354 mmol glucose, 12.0 mmol NaCl, and 9.6 mmol KOH) plus distilled water were used with a connection to a pre-amplifier (Syntech AC/DC UN-06) through Ag–AgCl silver wires. The EAG readings were monitored on a storage oscilloscope. The amplified signals were digitalized and stored in a PC with a data acquisition system (Syntech, Hilversum, the Netherlands). Stimulus and clean-air puffs were delivered via two separate series of valves and tubing, and were controlled by an electronic stimulator (modules Syntech CS-05). Tested samples were applied (dosage: 20  $\mu\text{l}$ ) on filter paper strips ( $6 \text{ cm} \times 0.5 \text{ cm}$ ) into a Pasteur pipette oriented towards the antenna from a distance of 1 cm and puffed (0.02 s) randomly over the antenna with clean air flowing continuously at 20 ml/s with approximately 30 s between puffs. Each sample was replicated with six different antennae. For data analysis, analysis of variance and least significant difference were used ( $P = 0.05$ ).

### 2.4 Field-trapping experiments

Compounds derived from the same biosynthetic route formed clear 'peak clusters' on the GC column and the EAGs of green-leaf volatiles (GLVs) and ACs were fairly strong (see below). Hence, we designed three blends as follows: (1) *basic blend*: a four-component blend mixed in the proportion as found in the steam distillate of leaves collected on 25 August. Benzaldehyde (6.73%) + phenylacetaldehyde (4.33%) + salicylaldehyde (58.34%) + benzyl alcohol (30.6%). (2) *GLV6*: all GLVs mixed in the proportions as found in the headspace of leaves collected on 15 June. (3) *GLV8 blend*: all GLVs mixed in the proportions as found in the steam distillate of leaves collected on 25 August. Other blends were modified from the basic blend. The periods and fields of the four field-trapping tests are shown in table 3. During tests 1, 2 and 3, shaken blends diluted 10-fold in different solvents were applied to filter paper rolls in 8-ml penicillin vials just before sundown daily. Paraffin oil was used in tests 1 and 3 while soya bean oil used in test 2 except for basic blends, which were also diluted in paraffin oil. The dosages used, being expressed as basic blend loading, were 10, 50 and 50  $\mu\text{l}$  (more details in table 3). Lures used in test 4 were prepared as follows: 1 g agar in 200 ml water was held at 85–90°C until the agar dissolved completely. Then the gel-melting solution was filled in vials (5 ml per vial) as described above and then cooled to 40°C. After the test blends were added, the vials were sealed immediately and stirred to ensure uniform mixing until the gel-melting solution formed into agaropectin. The lures were then suspended in water traps without change during the test. Water basin (24 cm ID, green) traps were used with a trap spacing of more than 20 m, at random arrangement, and replicated three times with more than 500 m between replicates. Poplar bundles as the special type of lure were used for comparison, while blank traps were used as control. Fifteen cuttings (60–70 cm) of poplar branches with leaves were tied up in a bundle from the cut end, with a bamboo pole inserted in the middle. For field trapping, the bundle was stuck up by the bamboo pole driven into the ground just like an umbrella and renewed every 3 days. Every morning, the trapped insects were recorded and removed, the poplar bundles sprayed with water to maintain high humidity and the positions of the traps rerandomized. The trap data were transformed by  $(x + 0.5)^{1/2}$  and compared by DUNCAN'S (1955) multiple range test ( $P = 0.05$ ).

**Table 1.** Relative contents of main compounds of *Populus nigra* wilted leaves collected by steam distillation and air entrainment

Compounds	Retention time (min)				Relative content(%)			
	19 May	25 August	15 June <sup>1</sup>	15 June <sup>2</sup>	19 May	25 August	15 June <sup>1</sup>	15 June <sup>2</sup>
3-Methyl-butanol				6.21				0.68
2-Methyl-butanol				6.24				0.45
1-Pentanol				6.59				1.48
1-Penten-3-ol			6.60				1.43	
Hexanal	6.34	6.33	6.96	6.97	1.15	1.03	1.28	1.79
Furfural		7.11				1.83		
Unknown compound				7.61				2.04
( <i>E</i> )-2-hexenal	6.91	6.89	7.72	7.77	5.48	3.24	9.67	20.78
( <i>Z</i> )-3-hexen-1-ol	7.03	6.98	7.88	7.94	44.81	4.75	28.71	29.12
Unknown compound		7.11				1.82		
4-Methyl-1-pentanol	7.12				21.85			
( <i>E</i> )-2-hexenol			8.02	8.12			9.07	29.22
1-Hexanol			8.06				5.43	
( <i>E,E</i> )-2,4-hexenal				8.63				0.5
Benzaldehyde	8.41	8.39	9.56		2.53	3.31	1.32	
Phenol		8.54				0.96		
Hexanoic acid			9.84	9.85			2.00	0.72
Unknown compound				10.10				0.34
Ethyl hexanoate				10.29				0.38
( <i>Z</i> )-3-hexen-1-ol acetate	9.08	8.94		10.40	1.44	2.15		1.67
Unknown compound			10.10				0.98	
1,2-Cyclohexanedione			10.19				9.38	
Unknown compound			10.39				1.03	
3-Methyl-2,5-furandione		9.20	10.47			10.01	5.53	
1-Hexenol acetate				10.52				1.02
Benzyl alcohol	9.51	9.50	10.82		1.19	15.06	1.09	0.53
Phenylacetaldehyde	9.6	9.61			1.00	2.13		
Salicylaldehyde	9.75	9.73	11.05	11.05	15.19	28.71	10.35	3.48
4-Methyl-phenol		10.08				0.86		
1-Octanol	10.17				0.76			
<i>Cis</i> -Linaloloxide		10.39				4.22		
Unknown compound	10.70		11.91	11.93	0.66		2.81	1.85
Phenylethyl alcohol	10.84	10.83	12.23		0.94	13.03	1.28	
Eugenol		14.71	16.00	16.00		4.78	2.27	0.52
2,6-di- <i>tert</i> -butyl-4-methyl phenol				18.30			1.88	
Phytol			20.11				4.36	
Unknown compound	11.58	11.89			1.28	1.04		
Borneol acetate		12.00				0.97		
Hexadecanol	20.38		21.16		1.71		2.00	

<sup>1</sup>Steam distillate of poplar leaves collected on 15 June.

<sup>2</sup>Air-entrained extracts of poplar leaves collected on 15 June.

### 3 Results

#### 3.1 Chemical analyses

The components differed from each other on different collecting dates and with different collection methods (table 1). Eighteen compounds were identified in the headspace volatiles of the poplar leaves. Components from the same biosynthetic route have similar retention time on the GC column, forming into four 'peak clusters' based on their retention times: C5-chain alcohols, GLVs, short-chain esters and ACs. Green-leaf volatiles were the major group accounting for more than 86.89% of total emitted volatiles while ACs only 6.51%.

Three GLVs and four ACs existed commonly in the steam distillates. The contents of GLVs in the steam distillates of the leaves collected on 19 May, 15 June and 25 August were 74.74%, 56.72% and 11.17% while that of ACs 21.06%, 16.47% and 68.84% respectively. The most abundant AC is salicylaldehyde

accounting for 15.19% (19 May), 10.15% (15 June) and 28.71% (25 August) of the total extracts respectively. In the same period (15 June), most esters and C5-chain alcohols were lost during the process of steam distillation compared with air entrainment (table 1).

#### 3.2 EAG tests

Table 2 summarizes the results of EAG responses of three CBW groups. Both GLVs and ACs elicited strong responses (table 2). To virgin females, GLVs and short-chain alcohols were more stimulating than ACs; they were hexanoic acid, 1-pentanol, (*Z*)-3-hexen-1-ol, 1-hexanol, phenylacetaldehyde, hexanal and 2-methyl-butanol in order of decreasing values. To mated females, ACs with one to two side-chain carbon units and saturated GLVs were more stimulating than others; they were hexanal, benzaldehyde, salicylaldehyde, 1-hexanol, phenylacetaldehyde and hexanoic acid in decreasing order. Only hexanoic

Samples <sup>1</sup>	EAG relative to standard <sup>2</sup> (mean ± SE) <sup>3</sup>		
	Virgin females	Virgin males	Mated females
2-Methyl-butanol	1.40 ± 0.25 c	0.79 ± 0.14 cd	1.79 ± 0.13 bc
1-Pentanol	1.79 ± 0.34 ab	1.10 ± 0.12 c	0.62 ± 0.13 d
3-Methyl-butanol	0.64 ± 0.23 e	0.76 ± 0.36 cd	0.68 ± 0.19 d
(E)-2-hexanal	1.00 ± 0.00 d	1.00 ± 0.00 cd	1.00 ± 0.00 cd
Cyclohexanol	0.52 ± 0.11 e	0.57 ± 0.19 de	0.57 ± 0.17 de
Hexanal	1.50 ± 0.36 bc	0.76 ± 0.23 cd	2.78 ± 0.38 a
(Z)-3-hexen-1-ol	1.76 ± 0.32 ab	0.81 ± 0.32 cd	1.50 ± 0.22 c
Hexanol	1.74 ± 0.22 b	0.72 ± 0.34 d	2.43 ± 0.34 ab
Benzaldehyde	1.03 ± 0.23 d	1.09 ± 0.10 c	2.64 ± 0.27 a
Hexanoic acid	2.04 ± 0.12 a	2.00 ± 0.41 a	2.07 ± 0.28 b
Phenylacetaldehyde	1.55 ± 0.07 bc	1.51 ± 0.41 b	2.37 ± 0.17 ab
Salicylaldehyde	0.98 ± 0.04 d	0.75 ± 0.13 cd	2.60 ± 0.29 ab
1-Octanol	0.92 ± 0.09 de	0.80 ± 0.12 cd	1.12 ± 0.11 cd
Cinnamaldehyde	0.75 ± 0.12 de	0.88 ± 0.24 cd	1.00 ± 0.20 cd
Eugenol	0.45 ± 0.23 ef	0.62 ± 0.23 de	0.58 ± 0.12 d
(E)-caryophyllant	0.06 ± 0.14 f	0.15 ± 0.12 e	0.01 ± 0.00 e
Hexadecanol	0.02 ± 0.12 f	0.41 ± 0.16 de	0.01 ± 0.02 e
Hexadecanoic acid	0.02 ± 0.12 f	0.32 ± 0.23 e	0.02 ± 0.01 e

<sup>1</sup>The samples are presented in order of increasing molecular weight in this column.  
<sup>2</sup>20 µl of each volatile was tested and (E)-2-hexanal diluted 100-fold in paraffin oil was used as a standard.  
<sup>3</sup>Mean values within columns followed by the same letters are not significantly different (P > 0.05) by least significant difference test.  
EAG, electroantennograms; CBW, cotton bollworm.

**Table 2.** EAG profiles of virgin females, virgin males and mated females of CBW to compounds isolated from volatiles of *Populus nigra* wilted leaves

acid and phenylacetaldehyde significantly evoked EAG responses of males. Comparatively, the trend of EAGs of benzaldehyde is similar to that of salicylaldehyde and its EAGs were approximately 250% higher for mated females than for the two virgin groups. In addition, benzaldehyde is structurally similar to salicylaldehyde. Cyclohexanol and several compounds with high molecular weight such as eugenol, (E)-caryophyllant, hexadecanol hexadecanoic acid barely elicited EAG responses of all the CBW groups, and this may be due to the low volatility of these compounds. In summary, GLVs and ACs with one to two carbon units on the side chain of the benzyl ring elicited significant responses.

### 3.3 Field trapping

Table 3 summarizes the results of field trapping. In test 1, both basic blend and a binary blend (benzaldehyde + salicylaldehyde) produced significant and equivalent trap catches; the amounts of CBW moth and total moth caught by basic blends were 69% and 49% of that caught using poplar bundles respectively. The trap catch of CBW females of the binary blends was 1.78 times that of their male captures. Clearly, addition of eugenol to the two above-mentioned blends showed no synergistic effect. GLV6 produced catches equivalent to blank traps. All the blends tested did not catch armyworm and cutworm moths while poplar bundles caught them. In test 2, although the dosage was higher than that used in test 1, all the blends as well as poplar bundles gave poor captures. A possible explanation for the unsuccessful attraction may be that soya bean oil was not appropriate for use as a solvent under field conditions. Alternatively, a lack of capture could be the result of high environmental temperature,

background floral host odours, host-related dispersal of second-generation CBW (WANG et al., 1999) and missed time of adult appearance. In contrast to EAG tests, GLV6 and GLV8 containing only GLVs produced no catch and showed no synergistic effect on the basic blend. In test 3, all the blends and poplar bundles produced significant and equivalent total catches and CBW catches, indicating that the four components in the 'basic blends' were responsible for attraction while the additional components were dispensable. Poplar bundles produced noctuid catches two times that produced by every synthetic blend; this is mainly because no armyworm moths were captured in the traps of the latter. Cotton bollworm moths caught by the blends containing eugenol were relatively fewer than those without this component. Floral hosts of CBW adults were flourishing everywhere in this period, indicating that poor captures of test 2 was not because of the background floral host odours, but probably the inappropriate solvent. In test 4, basic blend plus phenylethyl alcohol produced the most catches for CBW moths and the total moths, which are two and 3.89 times that of the poplar bundle caught, respectively, indicating that phenylethyl alcohol as the fifth additional component to the basic blend is necessary. Cotton bollworm population was fluctuant during the four tests, but poplar bundles were used as comparison all along, so the dramatic increase in trap catch is due, at least in part, to the use of agaropectin formulation. Based on the remains of agaropectin, we tentatively conclude that the field longevity of lures formulated in agaropectin (0.5% in water coagulated) would be more than 2 weeks. No clear sex-specific effect was found during the whole testing period. The sex ratios of CBW captures may have relation to the time that the field trapping was conducted. For example, test 4 was

**Table 3.** Field trapping of CBW and other moths

Blends and poplar bundles	Dose ( $\mu$ l)	Total catches/three traps <sup>1</sup>			Sex ratios <sup>2</sup>
		CBW	Noctuids	Total	
Test 1. 13 and 19 June 2002. Cotton fields, Lankao, China					
Basic	10	48 a	59	66 b	1
Basic, eugenol	10 : 3	24 b	37	42 c	1.18
Benzaldehyde, salicylaldehyde	1 : 9	25 b	34	39 c	1.78
Benzaldehyde, salicylaldehyde, eugenol	1 : 9 : 1	18 b	13	37 c	1
GLV6	10	4 c	10	15 d	1
Poplar bundles	–	69 a	135	135 a	1.16
Blank traps	–	3 c	8	16 d	0
Test 2. 13 and 20 July 2002. Cotton fields, Lankao, China					
Basic, eugenol	50 : 5	6 a	12	18 a	1
Basic (diluted in paraffin oil)	50	6 a	10	13 ab	2
Benzyl alcohol, phenylethyl alcohol	23 : 27	1 a	11	12 ab	1
Basic, GLV6	50 : 25	3 a	5	7 c	2
Benzaldehyde, salicylaldehyde	5 : 45	0 a	2	5 c	–
Basic (diluted in soybean oil)	50	3 a	3	4 c	3
GLV8	50	0 a	0	1 c	–
GLV6	50	1 a	1	1 c	1
Poplar bundles	–	5 a	23	25 a	1.5
Blank traps	–	1 a	1	4 c	0
Test 3. 11 and 24 August 2002. Tobacco and cotton fields, Zhengzhou, China					
Basic	50	34 ab	48	90 a	1.83
Basic, pentanol	50 : 1	38 ab	58	90 a	1.11
Basic, phenylethyl alcohol, eugenol	50 : 8 : 5	27 b	44	87 a	1.08
Basic, phenylethyl alcohol	50 : 8	42 ab	35	83 a	1.21
Basic, eugenol	50 : 5	23 b	52	82 a	1.88
Basic, ( <i>Z</i> )-3-hexen-1-ol	50 : 18	35 ab	50	68 ab	1.92
Poplar bundles	–	51 a	96	110 a	1.68
Blank traps	–	1 c	6	6 c	1
Test 4. 4 and 10 September 2002. Tobacco and cotton fields, Zhengzhou, China					
Basic, phenylethyl alcohol	43 : 7	84 a	145	214 a	0.91
Basic, pentanol	49 : 1	58 ab	135	189 b	1.09
Basic	50	54 ab	124	164 b	0.74
Basic, eugenol	47 : 3	54 ab	101	148 b	0.69
Poplar bundles	–	42 ab	48	55 c	0.75
Blank traps	–	6 c	25	29 d	1

<sup>1</sup>Numbers in the same column with the same letter are not significantly different by DUNCAN'S(1955) multiple range test ( $P > 0.05$ ).  
<sup>2</sup>Only the sex ratios of CBW captures were considered.  
CBW, cotton bollworm; GLV, green-leaf volatile.

conducted at the end of the flight season of the fourth-generation CBW adults; so the ratio of females catches was relatively low.

#### 4 Discussion

Our results indicated that contents of GLVs and ACs decreased and increased, respectively, as the season progressed, while ACs was the major group accounting for 68.84% of total steam distillate when poplar bundles have their optimum attracting capacity in cotton fields (SONG, 1998). The result of EAGs also indicated that both saturated GLVs and short-side-chain ACs elicited significant responses of the three CBW groups tested. Electroantennograms of benzaldehyde and salicylaldehyde for mated females are approximately 250% higher than that for virgin sexes. Although benzyl alcohol and phenylethyl alcohol were not tested, earlier result of EAG bioassays indicated that they elicited significant EAGs (Guo et al., 2001). Field trapping revealed that only short-side-chain aromatic alcohols and aldehydes are responsible for

attracting CBW moths and agaropectin is appropriate for releasing the active compounds. A five-component lure (5.32% benzaldehyde + 3.42% phenylacetaldehyde + 24.2% benzyl alcohol + 20.94% phenylethyl alcohol + 46.13% salicylaldehyde) produced the best trap catch. Additionally, benzaldehyde and phenylacetaldehyde have previously been identified as attractants of CBW (BRUCE and CORK, 2001; CUNNINGHAM et al., 2004).

The results did not support the possibility that such odorants may affect on calling of virgin females on the ground that both poplar bundles and the synthetic blends caught many insect species and no clear sex-specific effect was found; additional field observations at intervals of 1 h indicated that most attraction occurred before midnight and most virgin females flew away after a short staying in the bundles (LU et al., 1998), while peak of calling and mating of CBW virgin females appeared from 3 : 30 to 5 : 00 hours (XIA, 1978). The stimulating effect of these odorants on the reproduction of mated females is also unclear, for the laboratory in which these bioassays were conducted lacked other necessary stimuli (Guo et al., 2003).

Among the possible mechanisms of attraction, feeding attraction may be the direct effect on the ground that the nature of the active ACs is just mimicry of floral volatiles. The emission rate of Lombardy poplar leaf odours may increase dramatically after wilting, so poplar bundles can be used in cotton fields, even when this larval host of CBW is at its optimum flowering states.

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### References

- BANERJEE, S. K.; TURKAR, K. S.; WANJARI, R. R., 2000: Evaluation of newer insecticides for the control of bollworms in cotton. *Pestology* **24**, 14–16.
- BRUCE, T. J.; CORK, A., 2001: Electrophysiological and behavioral responses of female *Helicoverpa armigera* to compounds identified in flowers of African marigold, *Tagetes erecta*. *J. Chem. Ecol.* **27**, 1119–1131.
- CUNNINGHAM, J. P.; MOORE, C. J.; ZALUCKI, M. P.; WEST, S. A., 2004: Learning, odour preference and flower foraging in moths. *J. Exp. Biol.* **207**, 87–94.
- DENG, J. Y.; HUANG, Y. P.; WEI, H. Y.; DU, J. W., 2004: EAG and behavioral responses of *Helicoverpa armigera* males to volatiles from poplar leaves and their combinations with sex pheromone. *J. Zhejiang Univ. Sci.* **5**, 1577–1582.
- FU, X. H.; LEI, C. L.; WANG, X. P.; ZHOU, Y., 2001: Attracted effects of the substances extracted from *Populus canadensis* (Moench) on *Helicoverpa armigera*. *Nat. Enem. Insects* **23**, 22–25 (in Chinese with English summary).
- GUO, Y. Y., 1998: Trap and kill control of *Helicoverpa armigera*. In: *Study on Helicoverpa armigera*. Ed. by GUO, Y. Y. Beijing: Chinese Agriculture Press, 360–363.
- GUO, X. R.; YUAN, G. H.; LU, S. H., 2001: Electrophysiological and behaviour responses of *Helicoverpa armigera* elicited by volatiles from withered black poplar leaves. *Acta. Agric. Boreal-Sin.* **16**, 22–27 (in Chinese with English summary).
- GUO, X. R.; YUAN, G. H.; LI, W. Z.; MA, J. S.; SHEN, Z. R., 2003: The effects of withering poplar branch on the reproductive behavior of cotton bollworm. *Acta Phyto. Sin.* **30**, 148–152.
- KIRITANI, K.; KANO, M., 1984: Influence of delay in mating on the reproduction of the oriental tea tortrix, *Homona magnanima* (Diakonoff) (Lepidoptera: Tortricidae), with reference to pheromone-based control. *Prot. Ecol.* **6**, 137–144.
- LI, Y. H., 1966: Evaluation of effect of occurrence prediction and control of cotton bollworm, *Heliothis armigera* by trapping with poplar bundles in field. *Entomol. Knowl.* **10**, 67–69 (in Chinese with English summary).
- LU, Y. Y.; YIN, C. D.; TAO, Q. H., 1998: Controlling effect of poplar bundles on *Helicoverpa armigera*. *China Cotton* **25**, 18–19 (in Chinese with English summary).
- REMBOLD, H.; KOHNE, A. C.; SCHROTH, A., 1991: Behavioral response of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctidae) moths on a synthesis chickpea (*Cicer arietinum* L.) kairomone. *J. Appl. Ent.* **112**, 254–262.
- ROBERT, J. B.; BRUCE, W. Z., 1998: A versatile and quantitative volatile-delivery system for laboratory bioassays. *J. Chem. Ecol.* **24**, 535–558.
- SONG, X. L., 1998: Effect comparison of sex attractants of *Helicoverpa armigera* used for monitoring. *Plant Prot.* **35**, 348–352 (in Chinese with English summary).
- STEVEN, H. S.; STEPHEN, P. D.; RICHARD, M., 2001: Genetically modified poplars in context. *Forest. Chron.* **77**, 271–279.
- WANG, H. Z.; MOU, J. Y.; HUA, Y. N., 1999: Technology of trap and kill cotton bollworm with poplar bundles. In: *Monitor and IPM of Helicoverpa armigera*. Ed. by WANG, H. Z., MOU, J. Y., HUA, Y. N. Beijing: Chinese Agriculture Press, 542–545.
- XIA, B. Y., 1978: Field observation of *Heliothis armigera* moth-trapping with poplar bundles. *Agr. Sci. Tech. Commun.* **11**, 29 (in Chinese).
- XIAO, C.; GREGG, P. C.; HU, W. L.; YANG, Z. H.; ZHANG, Z. N., 2002: Attraction of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctidae), to volatiles from wilted leaves of a non-host plant, *Pterocarya stenoptera*. *Appl. Entomol. Zool.* **37**, 1–6.
- YUAN, G. H.; ZHENG, Q. W.; MA, J. S., 1999: Research on spectrum and mechanism of attracting insects with poplar branch. *Acta. Agr. Univ. Henan.* **33**, 147–150 (in Chinese with English summary).

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