

Volatiles induced by the larvae of the Asian corn borer (*Ostrinia furnacalis*) in maize plants affect behavior of conspecific larvae and female adults

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Abstract Effects of maize (*Zea mays* L.) volatiles induced by larvae of the Asian corn borer, *Ostrinia furnacalis* (Guenée), on the orientation behaviors of Asian corn borer larvae and oviposition of the females were investigated. Nineteen volatile chemicals, with terpenes being the major components, were identified from maize plants attacked by third instar Asian corn borer larvae. Coupled gas chromatographic-electroantennographic detection (GC-EAD) analyses revealed some electroantennographic differences between female and male Asian corn borer antennae in response to larvae-induced maize volatiles; female responded to (E)-2-hexenal, nonanal, (Z)-3-hexen-1-ol and three unknown compounds while the male only responded to (E)-2-hexenal, nonanal and one unknown compound. In laboratory orientation bioassays, Asian corn borer neonate larvae were attracted to extracts collected from Asian corn borer-damaged plants as well as to synthetic farnesene, but were repelled by (Z)-3-hexen-1-ol. In laboratory oviposition bioassays, gravid females laid fewer eggs on plants damaged by larvae than on mechanically damaged plants or undamaged plants. Adult Asian corn borer females deposited fewer eggs on wax paper treated with (E)-2-hexenal or (Z)-3-hexen-1-ol than on wax paper treated with hexane (control). The results suggest that Asian corn borer can affect the behaviors of conspecific larvae and adults by changing host plant volatiles.

Key words behaviors, conspecific adults and larvae, induced volatiles, *Ostrinia furnacalis* (Guenée), *Zea mays* L.

Introduction

Plant volatiles, especially herbivore-induced volatile components, such as green leaf volatiles, terpenes, alkenes, carboxylic acids and alcohols (Holopainen, 2004), play important roles in mediating behaviors such as host plant searching and acceptance in phytophagous insects, in attracting parasitoids or predators of pest insects, in directing

insect oviposition as well as in influencing plant–plant interactions (Konstantopoulou et al., 2004; Steinbauer et al., 2004; Rassmann et al., 2005; Ruther & Kleier, 2005; for reviews, see Visser, 1986; Anderson & Alborn, 1999; Dicke & van Loon, 2000; Wink, 2003; Dudareva et al., 2004; Carroll et al., 2006; Raguso, 2008). In recent years, volatiles in maize (*Zea mays* L.) were extensively explored. The maize plant could release abundant volatile chemicals after herbivore feeding (Turlings et al., 1998a), which was a rather complicated event varying with many factors. The total quantity and qualitative composition of volatile blend were variable among maize cultivars and their wild ancestors (Gouinguénée et al., 2001). The components and concentrations of the volatile chemicals were

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changing with time after feeding (Turlings et al., 1998b), and it also changed with the herbivore species of different feeding habits (Turlings et al., 1998a). Some genes of key syntheses in volatiles biosynthesis were identified from maize (Frey et al., 2000; Schnee et al., 2002; Kllner et al., 2004). The intensity of the emissions was correlated with the number of larvae feeding on a plant, and with the amount of damage, while different instar larvae only made slight differences in minor compounds (Gouinguen et al., 2003). As the biosynthetic pathways of induced maize volatiles became clear, increasing focus has turned to the ecological or behavioral functions of these chemicals. Parasitoids of stem-borer *Cotesia flavipes* can respond to systemically induced maize plant volatiles in host locations (Potting et al., 1995). It was reported that herbivore-induced volatiles of maize repelled the corn leaf aphid (*Rhopalosiphum maidis*) (Bernasconi et al., 1998). Special herbivore-induced volatile β -caryophyllene emitted from root of maize recruits entomopathogenic nematodes (Rassmann et al., 2005). However, little information is available about the effects of induced maize volatiles on conspecific larvae and adults.

In the present paper, our objective was to identify volatile chemicals induced by third instar larvae of the Asian corn borer (ACB), *Ostrinia furnacalis* (Guenée) (Lepidoptera: Pyralidae), feeding on maize, and to investigate their function on conspecific interaction. ACB is a polyphagous insect pest found on over 70 plant species in China, and is especially important in agricultural crops and vegetables (Liu et al., 1992). There are 3–5 overlapping generations per year in different regions of China. For an insect species with overlapping generations like ACB, the behavior of larvae and adults may be affected by induced chemical and/or physical changes in host plants in response to feeding by conspecific larvae. Therefore, comparison of volatiles released from larvae-damaged, mechanically damaged and undamaged maize plants, and observations in the laboratory of the electroantennographic and behavioral responses of ACB adults and first instar larvae to these induced chemicals may contribute to the understanding of their roles in plant–insect interactions.

Materials and methods

Plants and insects

Seeds of maize (*Zea mays* L.) variety Nongda-108, provided by China Agricultural University, Beijing, were sown in artificial soil (a mixture of vermiculite, cattle excrement, and topsoil in 1 : 1 : 6 ratio) in plastic pots

(12 cm diameter and 10 cm height), and then kept in a growth chamber (HPG-280B, Donglian Electronic Technique Co., Harbin, China) under $28 \pm 1^\circ\text{C}$ and 3 000 lux with a 16 : 8 h (L : D) photoperiod and approximately 75% relative humidity (RH). After the seedlings emerged, only one single plant was kept per pot. Plants at the four-leaf stage (3 fully expanded leaves and cotyledon) with about 35–45 cm height were used for experiments. The cotyledons were not used for larval feeding and mechanical damage tests.

Egg masses of the ACB were obtained from the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing. Larvae were reared on a semi-artificial diet (Zhou et al., 1980) at $28 \pm 1^\circ\text{C}$ under a 16 : 8 h (L : D) photoperiod and 75% RH in the growth chamber. The third instar larvae were used for infestation experiments, and newborn (neonate) larvae (<3 h old) for orientation bioassays. Mated female adults (2 days after emergence) were used for gas chromatograph-electroantennographic detection (GC-EAD) and oviposition assays.

Treatments

Plants were chosen randomly for each of the following three treatments: larvae-damaged plants (LDP), mechanically damaged plants (MDP), and undamaged plants (UDP). In the LDP group, two third instar larvae starved for 2 h were placed within a plastic clip cage (2.5 cm inner diameter (i.d.), 2.2 cm height) on the terminal part of the second fully extended leaf of each plant for 48 h. The third instar larvae are the most likely stage overlapping with the adults and the first instar larvae of the next generations in the field, so we used them as the inducer here. Before plant volatile collections and bioassays, the clip cage, larvae and larval frass were removed to avoid any confounding odor substances from these possible sources (Li & Ishikawa, 2004). In the UDP group, there were no larvae in the cage. In the MDP group, mechanical damage of larval feeding was crudely simulated by punching a hole of 4-mm diameter on the maize leaf four times a day for 2 days. Different plant treatment groups were kept in separate growth chambers to avoid possible plant–plant interactions. Four replicates were set for each LDP, MDP and UDP treatment.

Plant volatile collections and chemical analysis

Volatiles from aerial maize plant parts of different treatment groups were collected using an aeration sampling system as described in Wei et al. (2006). The collection trap was a glass outlet tube (0.5 cm \times 3 cm long)

containing 60 mg of Porapak Q adsorbent (mesh 50–80, Supelco, Bellefonte, PA, USA) and the air flow rate was 800 mL/min. Volatile collections were conducted for 6 h from 1:00 p.m. to 7:00 p.m. in an air conditioned room at 28°C and approximately 3 000 lux.

After 6-h collection, the adsorbent trap was rinsed with 500 µL hexane (HPLC grade, J&K Chemical Co., Ltd., Beijing, China.) into a vial, with 5 µL internal standard solution (400 ng/µL octane and 400 ng/µL ethyl nonanate in hexane) added. The samples were stored at –20°C in a freezer until used. Two microliters of each sample was injected into a gas chromatograph – mass spectrometer (GC-MS) (Agilent 6890GC couple 5973N, Agilent Technologies, Inc., Santa Clara, CA, US) with an HP1 column (30 m × 0.25 mm, 0.25 µm film). The temperature program was initiated at 40°C and maintained for 3 min, then raised at 5°C/min to 220°C (held for 6 min). The splitless injector temperature was 220°C. Helium (99.999%) was used as carrier gas at 1 mL/min. The identification and quantification of chemicals in samples were done according to retention times, peak areas, MS spectra, as well as in comparison with internal standards and authentic synthetic chemicals.

Chemical

The following chemicals were used in experiments: trans-2-hexenal (99%, Acros, Geel, Belgium), cis-3-hexen-1-ol (98%, Acros), α-caryophyllene (provided by Mr. Yongjun Zhang, Plant Protection Institute of Chinese Academy of Agricultural Sciences, Beijing, China), β-caryophyllene (98.5%, Fluka, Milwaukee, WI, USA), farnesene (mixture of isomers, Tokyo Kasei Kogyo, Tokyo, Japan; 85% in purity, α–73%, β–27%, confirmed by gas chromatography), indole (99%, Beijing Chemical Reagents Company, Beijing, China), ethyl nonanate (99%, Beijing Chemical Reagents Company) and n-octane (99%, Acros).

Electrophysiological experiments

Gas chromatograph-electroantennographic detection recording methods described by Wei and Kang (2006) were used with a few modifications. The antenna was cut just before use and two glass electrodes filled with electrolyte (Brand, 1995) were applied to the antenna during EAD recording. GC-EAD analyses were carried out with a gas chromatograph (Agilent 5890 II) coupled with electroantennography (Syntech, Hilversum, the Netherlands). The GC was equipped with an SGE AC5 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm, SGE International Pty. Ltd., Melbourne, Australia), a flame

ionization detector (FID) and a split/splitless injector. Two microliters of a sample was injected. The oven temperature was programmed at 40°C for 3 min, then rose to 220°C at 3°C/min, and held for 3 min. Injector and detector temperatures were set at 220°C and 250°C, respectively. The injector was operated in the splitless mode. Nitrogen (99.999%) was used as carrier gas with flow rate of 1 mL/min. Air flow through the Y-tube splitter of the GC-EAD was 3 : 7 (FID : EAD). Male and female antennae were subject to test for four replications, respectively.

Bioassays of first instar larvae

To determine the role of induced maize volatile components in orientation behaviors of first instar ACB larvae, larval bioassays were conducted in the laboratory at ≈25°C and 75% RH. A rectangular arena (32 cm long × 23 cm wide × 1.5 cm deep) with humidified filter paper on the bottom was divided into three equal parts, named the “treatment zone”, “releasing zone” (the intermediate section) and “control zone” (Fig. 3, top). Two pieces of filter paper (1 cm × 1 cm) with 5 µL of either hexane or test chemical solution (in hexane) were placed on the edges of the treatment zone and control zone, respectively. Fifty first instar larvae (< 3-h-old) were released in the “releasing zone” to mimic their dispersal from an egg mass, then the arena was covered with plastic film wrap to form an airtight cell. The whole arena with first instar larvae and volatile sample was covered by a carton and a piece of dark green cloth to prevent any light effect. The dosage of chemicals and testing time used in experiments were based on a series of preliminary tests. In order to determine the time when larvae became consistently distributed in the arena, two pieces of filter paper with hexane only were placed in both the control and treatment zones, and the numbers of larvae in the three zones were counted every 10 min. Preliminary work indicated that a duration of 40 min was suitable for larvae to obtain a stable distribution. Then the numbers of larvae in the three zones were counted after 40 min in all larval bioassays. Larvae showed their preferences by distributing among the different zones according to the chemical gradients from the filter paper. Farnesene, the most abundance component in LDP sample, was used to test the appropriate dosage in bioassay. In the preliminary test, larvae gave the best responses at 100 ng/µL (Fig. 1). Each chemical was tested six times.

Bioassays of gravid female adults

Bioassays on ACB adult oviposition preference to pairs of maize plants of LDP, MDP, and UDP groups as well

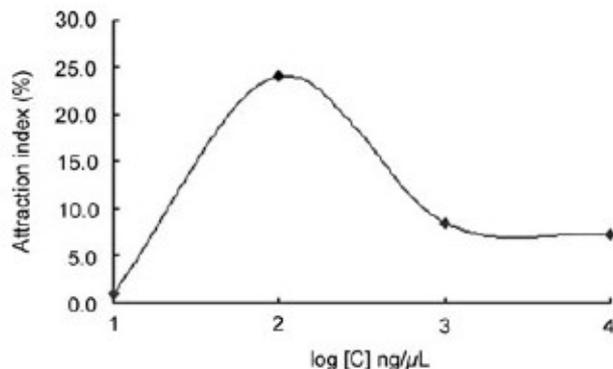


Fig. 1 Response curve of Asian corn borer (ACB) (*O. furnacalis*) larvae to farnesene at a series of concentrations. Attraction index = $(N_{\text{treatment zone}} - N_{\text{control zone}}) \times 100\% / N_{\text{total}}$.

as to herbivore-induced volatile compounds were carried out in the laboratory. For the female adult bioassays on the plants, two maize plants (LDP vs. UDP, MDP vs. UDP, or LDP vs. MDP) were placed in a nylon net cage (43 × 32 × 35 cm), and one newly mated female was released on the floor in the middle of the cage. Each cage was kept in a growth chamber (HPG-280B, Donglian Electronic Technique Co., Harbin, China) under 28 ± 1 °C, 3 000 lux, 16 : 8 h (L : D) photoperiod and ≈75% RH. The number of eggs on the plants was counted after 24 h. The experiment was replicated 10 times for each treatment pair.

For the ACB adult oviposition bioassays with individual volatile compounds, a metal mesh cage (16 × 8 × 16 cm) was used, modified from He et al. (2000), which consisted of an oviposition container (16 × 8 × 16 cm), an inner lid (15.9 × 7.9 × 3 cm) and a cover (16.1 × 8.1 × 3 cm). Two holes were cut in the inner lid (2.4 cm in diameter) and cover (2.7 cm in diameter) through which two glass cuvette tubes (2.5 cm i.d., 6 cm long) containing cotton with 10 μL of either a test chemical or hexane were inserted into the cage. The openings of the glass tubes were covered with pieces of wax paper that had been perforated seven times with a needle to allow volatiles to pass into the oviposition cage. ACB females would only lay eggs on the wax paper. One gravid female was used in each bioassay to avoid any possible influence on oviposition by conspecific females. Twenty-four hours later, the numbers of eggs on the wax paper were counted under a stereomicroscope. Each chemical was tested in 10 replicates.

Data analysis

The bioassays of larvae as well as female adults were conducted as bi-choice tests, so t-test was used in data

analysis. Excel and Minitab 13.2 software were applied in data collection and calculation. No data was transferred in analysis except that the farnesene concentrations were log transformed to draw the larvae responding curve in the preliminary test (Fig. 1).

Results

Volatile changes in UDP MDP and LDP,

Significant differences in volatile chemical profiles were found among ACB-damaged (LDP), mechanically wounded (MDP), and undamaged (UDP) maize plants of Nongda-18 (Table 1). Nineteen volatile compounds were detected in LDP and MDP, while only seven chemicals were found in UDP. The amounts of seven volatile compounds of LDP were significantly higher than those of MDP. The total chemical quantities released were 1249.2 ng/h in LDP, 332.9 ng/h in MDP, and 55.1 ng/h in UDP. High percentages of terpenoids were characteristics of the LDP volatile blend, with caryophyllene, farnesene and (E)-7,11-dimethyl-3-methylene-1,6,10-dodecatriene comprising 15%, 9% and 33% of the total amounts, respectively. In UDP, volatiles were dominated by indole and nonanal.

Antennal responses of the ACB adults

The gravid ACB female antennae responded to six compounds, that is, (E)-2-hexenal, (Z)-3-hexen-1-ol, nonanal, and three unknown compounds, in the volatiles of LDP. The ACB male antennae only responded to four components in LDP volatile collections, that is, (E)-2-hexenal, nonanal and two unknown compounds (Fig. 2).

Responses of ACB first instar larvae to volatile components

Five synthetic chemicals (100 ng/μL) and the natural volatile extracts collected from LDP (LDP mix sample) were used to test orientation of first instar ACB larvae. The neonate larvae showed variable responses to these chemicals (Fig. 3). The LDP headspace samples collected from infested maize plants attracted significantly more larvae than did the control (hexane) ($P = 0.048$). Neonates were also significantly attracted to farnesene (mixture of isomers) ($P = 0.036$) (41.7% in positive zone and 23.3% in negative zone). However, (Z)-3-hexen-1-ol ($P = 0.016$) and nonanal ($P = 0.003$) were repellents to the larvae. β-Caryophyllene and (E)-2-hexenal had no significant effects on larval orientation.

Table 1 Chemicals released from Asian corn borer (ACB) damaged, mechanically damaged and healthy maize plants of variety "Nongda 108".

No.	Chemical compound	LDP		MDP		UDP		t-test results	Identification method
		ng/h	%	ng/h	%	ng/h	%		
1	β -farnesene	392.7 \pm 37.0	31.4	50.9 \pm 32.6	15.3	ND	0.0	abN	MS, Rt
2	β -caryophyllene	192.7 \pm 30.1	15.4	40.0 \pm 27.8	12.0	ND	0.0	abN	MS, Rt
3	Z-3-hexen-1-ol acetate	115.9 \pm 15.8	9.3	41.9 \pm 11.6	12.6	ND	0.0	abN	MS
4	α -farnesene	112.0 \pm 8.7	9.0	14.5 \pm 9.8	4.4	ND	0.0	abN	MS, Rt
5	Unknown 1	66.7 \pm 26.9	5.3	61.1 \pm 37.5	18.4	1.1 \pm 1.6	2.1	aab	
6	Indole	53.8 \pm 4.6	4.3	17.9 \pm 12.1	5.4	10.2 \pm 3.6	18.4	abb	
7	Nonanal	48.5 \pm 20.1	3.9	35.5 \pm 9.9	10.7	34.8 \pm 24.9	63.3	aaa	
8	2,6-dimethyl-octane	37.8 \pm 39.5	3.0	9.3 \pm 0.3	2.8	3.4 \pm 4.8	6.1	aaa	MS, Rt
9	Germacrene D	35.2 \pm 0.1	2.8	11.8 \pm 3.5	3.6	4.1 \pm 3.2	7.4	abb	MS, Rt
10	(1s-cis)-1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-naphthalene	28.3 \pm 22.2	2.3	3.7 \pm 4	1.1	0.5 \pm 0.7	0.9	aaa	MS MS MS
11	Unknown 3	27.5 \pm 32.0	2.2	6.1 \pm 1.2	1.8	ND	0.0	aaN	
12	4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	26.5 \pm 19.8	2.1	4.3 \pm 6.0	1.3	ND	0.0	aaN	MS
13	β -sesquiphellandrene	25.3 \pm 15.9	2.0	1.3 \pm 1.8	0.4	ND	0.0	abN	MS
14	Copaene	25.2 \pm 14.1	2.0	5.6 \pm 0.4	1.7	1.0 \pm 1.4	1.7	aaa	MS
15	(Z)-3-hexen-1-ol	18.0 \pm 15.5	1.4	6.7 \pm 2.5	2.0	ND	0.0	aaN	MS, Rt
16	Decanal	15.6 \pm 12.1	1.2	5.9 \pm 2.0	1.8	ND	0.0	aaN	MS
17	α -caryophyllene	11.5 \pm 2.1	0.9	4.5 \pm 0.7	2.	ND	0	aaN	MS, Rt
18	Unknown 2	9.4 \pm 9.3	0.8	4.1 \pm 2.0	1.2	ND	0.0	aaN	
19	(E)-2-hexenal	6.6 \pm 9.1	0.5	5.5 \pm 1.7	1.6	ND	0.0	aaN	
	Total	1249.2	100	332.9	100	55.1	100		MS, Rt

LDP = larvae-damaged plants; MDP = mechanically damaged plants; UDP = undamaged plants. ND = not detectable; MS = identified by mass spectrum; Rt = identified by standard chemical retention time. In the t-test results column, the same letter means no significant difference between plant groups at $P < 0.05$, and the letter "N" means not detectable.

Gravid female oviposition tests with maize plants and individual chemicals

Adult females laid more eggs on UDP in both the UDP versus LDP group ($P = 0.029$) and the UDP versus MDP group ($P = 0.037$); no significant difference was found in the MDP versus LDP group (Fig. 4). ACB females laid fewer eggs on wax paper treated with LDP volatile sample than on the control wax paper ($P = 0.045$). (E)-2-Hexenal ($P = 0.042$), (Z)-3-hexen-1-ol ($P = 0.030$) and nonanal ($P = 0.035$) inhibited oviposition of the gravid female, while indole, α -caryophyllene, β -caryophyllene, farnesene and the UDP sample had no effect (Fig. 5).

Discussion

There is considerable biological information regarding effects of plant volatiles upon herbivory. The dynamic in-

formation encoded by compositions, concentrations, and proportions of plant-induced chemical is perceived by phytophagous insects as well as natural enemies (Schnee et al., 2006). The ecological or behavioral functions of herbivore-induced maize volatiles on insects have been extensively studied. Effects of chemicals in corn leaf extracts on the oviposition behavior of European corn borer (ECB) *Ostrinia nubilalis* (H' bner) have been reported (Binder, 1999; Binder & Robbins, 1997; Derridj et al., 1986; Udayagiri & Mason, 1995, 1997). The oviposition behavioral responses of ACB to four volatile chemicals from maize, that is, hyacinthin, benzaldehyde, limonene and 3-hexen-1-ol, were observed in the laboratory (He et al., 2000). Upon damage by ACB larvae in our present experiments, the composition, concentrations and proportions dramatically changed in the volatile profile of maize variety "Nongda 108". Phytophagous insects, including conspecific and heterospecific individuals, might differentiate UDP, LDP and MDP by a single, or a

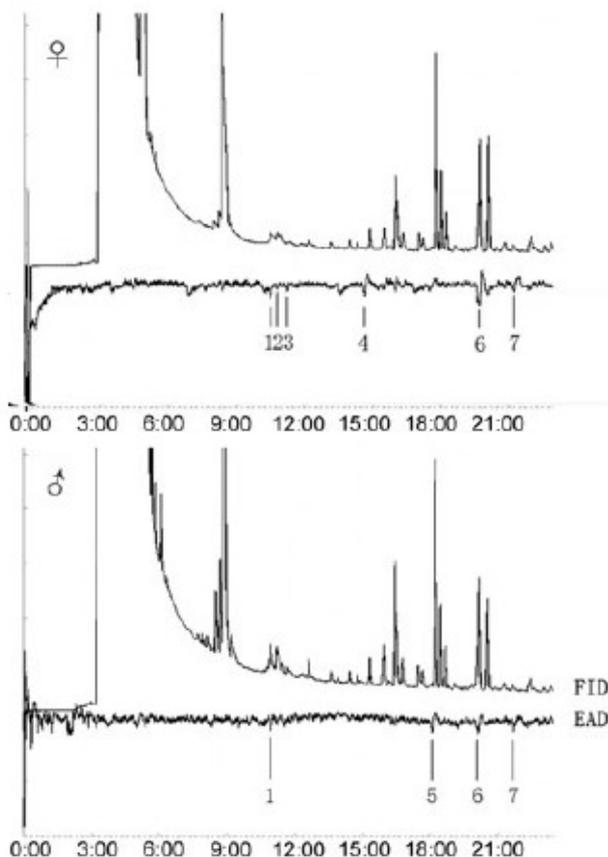


Fig. 2 Gas chromatograph-electroantennographic detection responses of female and male Asian corn borer (ACB) (*O. furnacalis*) antennae to larva-damaged plant volatiles. Ch1(EAD): 5 mV/div(fit:0); Ch2(FID): 0.5 mV/div(Fit:0); Horz: 3 min/div. 1: (E)-2-hexenal, 2: (Z)-3-hexen-1-ol, 3: unknown, 4: unknown, 5: unknown, 6: nonanal, 7: unknown.

combination of the above, volatile signals from these plants. In comparison to the volatiles blend induced by ACB with that by ECB (Turlings et al., 1998b), several major components, that is, indole, β -caryophyllene, α -farnesene, β -farnesene, were the same, but the minor compounds were quite different. These might result from gene diversity of the maize cultivars, difference of insect oral secretion, or the time of infestation.

According to our bioassay results of chemicals, the larvae and adults had different sensitivity and perception to the volatile chemicals induced by ACB larvae in Nongda 108. Some components of maize volatiles were bioactive to both adults and larvae of ACB, for example, (Z)-3-hexen-1-ol and nonanal were repellents to both ACB larvae and female adults (Figs. 3 and 5). Some volatile components were active on adults, but had no effects on larvae, and vice versa. For instance, (E)-2-hexenal was a

repellent to ACB female adults but it did not affect ACB larval orientation. Farnesene attracted larvae, but adults did not significantly respond to it, although farnesene can stimulate oviposition in ECB (Binder & Robbins, 1997). We were not able to obtain chemical standards of all components in the LDP sample, so there may be other functional compounds induced in maize that have behavioral effects on both ACB adults and larvae.

Punching holes (MDP) (4 times a day) crudely resembling continuous insect feeding, without applying ACB regurgitant, can induce almost the same components as larval damage (LDP), but the amounts were much lower. Continuous mechanical damage could induce volatile components that might become the prime cause of volatile release (Mithofer et al., 2005). Concentration differences between LDP and MDP probably resulted from absence of ACB elicitors in MDP. In the two-choice oviposition test, no significant difference in egg numbers was found between LDP and MDP in spite of the remarkably different concentrations of volatiles between these two groups. This implies that gravid ACB females determined the oviposition sites depending on volatile components rather than concentrations of the compounds from plants.

Insects might accept a host based on the existence of attractants, or absence of repellents (Miller & Strickler, 1984; Byers et al., 1998, 2004). The differences in responses to the LDP volatiles between ACB larvae and female adults may result from different adaptation strategies to chemical information from host plants damaged by conspecific larvae. Selection pressures on larvae and adults may make them adapt different strategies so they respond quite differently to the same host plant conditions and volatiles. In spite of the negative responses to (Z)-3-hexen-1-ol and nonanal, ACB larvae still crawled to the odor source of the LDP sample. Farnesene may play an important role in the process of positive orientation to hosts by larvae because farnesene was attractive to ACB neonates and was a large proportion of the total volatile chemical amount of the LDP sample. Landolt (2000) reported similar observations in codling moth neonates attracted to larvae-infested apple fruits with increased amount of (E,E)- α -farnesene. Fall armyworm larvae were also attracted to volatiles induced by conspecific larvae in maize, and in this case the linalool in the blend played a key role (Carroll et al., 2006). Larvae-induced volatiles may contain both positive and negative information to conspecific ACB neonate larvae. Positive aspects may include: (i) there is an edible host plant although it may be occupied; and (ii) there may be other healthy host plants nearby (Carroll et al., 2006), especially in a maize field. The negative aspects may include: (i) other herbivore competitors may already be present;

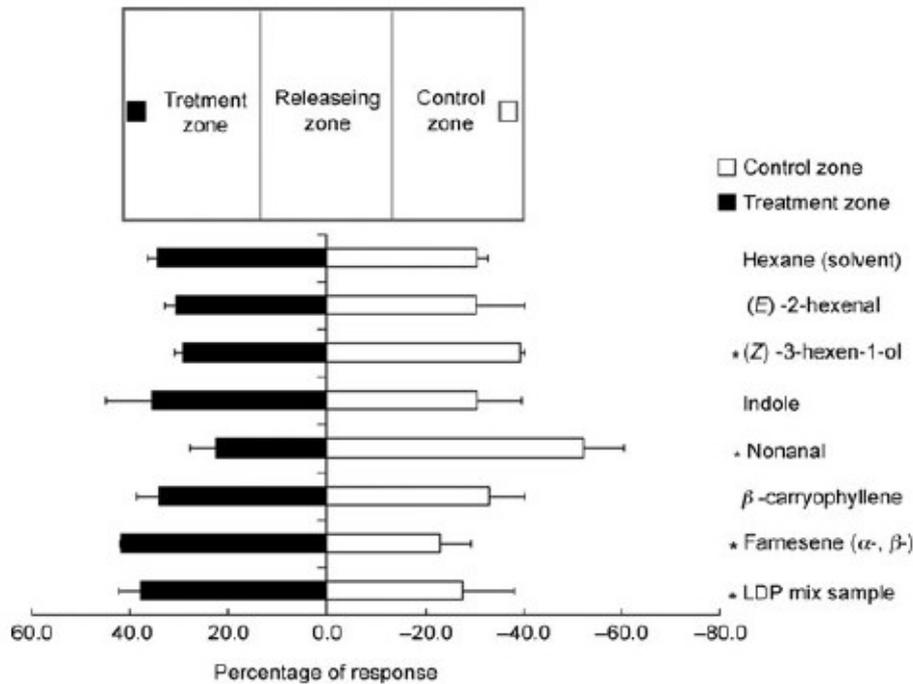


Fig. 3 Asian corn borer (ACB) (*O. furnacalis*) neonate larval orientation to different volatile chemicals. Five microliter solution of the larvae-damaged plant mix sample or any of the other five authentic chemicals in hexane, or hexane alone, were applied to filter paper and placed in the treatment zone. Filter paper with hexane (solvent) was placed in the control zone as control in each test. Larvae were released in the releasing zone. The asterisk mark (□) indicates a significant difference between percentages of larvae control and active zones at $P < 0.05$.

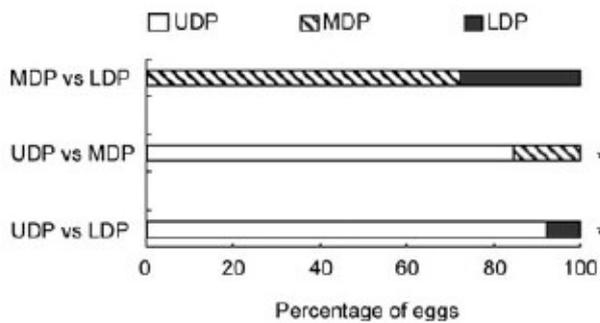


Fig. 4 Oviposition of Asian corn borer (ACB) (*O. furnacalis*) gravid females on undamaged plants (UDP), mechanically damaged plants (MDP) and larvae-damaged plants (LDP) in different two-choice tests. Egg numbers were counted and data changed to percentages. The asterisk mark (□) indicates a significant difference between egg percentages on different plants at $P < 0.05$.

(ii) natural enemies might aggregate on this plant due to specific volatile components, as in the cases of *Cotesia flavipes* (Potting et al., 1995) and *Ostrinia nubilalis* (Turlings et al., 1998a); and (iii) some allelochemic/toxic compounds may have begun to accumulate in the host plant (Bernasconi et al., 1998; Dicke & van Loon, 2000).

Newborn ACBs need to find suitable host plants rapidly because of high larval densities (potential competition; $\approx 20\text{--}100$ eggs/egg mass and sometimes more than one egg mass/plant), high mortality rate during the first few hours (Liu et al., 1992), and poor locomotive ability. At this time, a high level of LDP volatiles in the air might aid larvae in locating a nearby host plant. Furthermore, a damaged plant with one or a few competitors should be better than a plant with 50 or more larvae emerging from an egg mass. The food odors may encourage larval dispersal and would thereby decrease competition.

Insects of different feeding habits may have different responses to damaged plants or induced chemicals. Fifth instar *Lygus hesperus* responded to all plant/insect combinations, whereas female *L. hesperus* only responded preferentially to vegetative and flowering alfalfa where conspecifics had fed for 24–72 h (Blackmer et al., 2004). However, ACB gravid female adults avoided ovipositing on damaged plants or induced chemicals, as in the case of caterpillar-induced volatiles of tobacco on *Heliothis virescens* (De Moraes et al., 2001). In fact, ACB females avoided ovipositing on conspecific larvae-damaged plants, or responded negatively to LDP volatiles even though there were some components

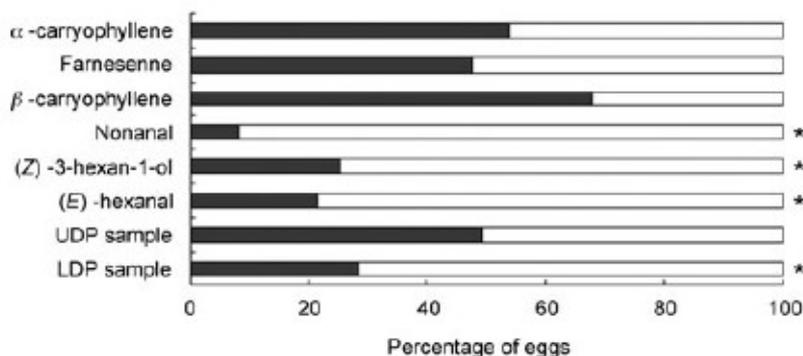


Fig. 5 Responses of Asian corn borer (ACB) (*O. furnacalis*) gravid females to different volatile chemicals in oviposition tests. The black bars indicate the percentage of eggs laid on wax paper with each test chemical. Larvae-damaged plants (LDP) and undamaged plants (UDP) samples were collected from larval-damaged and healthy plants, respectively. The asterisk mark (*) indicates a significant difference ($P < 0.05$).

stimulating oviposition. Gravid ACB females were more EAG-sensitive to the plant volatiles than were males, most likely due to females' eagerness for a plant for oviposition. ACB is a polyphagous pest with more than 70 host plants. Among the attractants and repellents, adults seemed to be more behaviorally sensitive to repellents. For example, female ACB adults did not respond to farnesene in oviposition tests, but were more sensitive to (E)-2-hexenal than larvae. In addition, the ability of ACB adults to fly ensures they can find a healthy plant more easily than newborn larvae and this may be another reason why ACB gravid female adults avoided LDP and induced volatiles while the larvae did not.

In conclusion, the feeding of ACB larvae could affect host searching or oviposition behaviors of the co-existing conspecific gravid female adults and newborn larvae. In plant-insect interactions, our work supports the interesting biological phenomenon that the same host volatiles can have different functions depending on the life stage of insects (Holopainen, 2004). Therefore, application of chemical blends that mimic conspecific larvae-induced chemicals may be a complementary strategy in ACB pest management, but care should be taken since the same induced chemical may have different effects on larvae and adults.

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