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Plant Approach-Avoidance Response in Locusts Driven by Plant Volatile Sensing at Different Ranges

Peng Wang[1] · Xuewei Yin[1] · Long Zhang[1]

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Abstract
Several hypotheses have been proposed to explain how herbivorous insects approach plants by sensing plant volatiles. Insect antennae and maxillary palps are believed to have crucial roles in the detection of host plant volatiles. However, few studies have assessed the roles of these olfactory organs in food selection in terms of the effects of individual volatile compounds from plants at various distances. Therefore, we assessed the palp-opening response (POR), biting response, and selection behavior of locust (*Locusta migratoria*) nymphs in response to volatile compounds from host and non-host plants at various distances. Thirty odorants were identified as the active volatiles to locust by the POR tests. At a distance of 3 m, locusts were attracted to a few common volatiles (1% *v*/v) of both host and non-host plants, while few components of volatiles acted as repellants at this distance. At a distance of 1 m, locusts responded more readily to volatile compounds. At a distance of 1 cm, locusts mainly used their palps to detect volatiles. However, some components that acted as attractants at long distances had no effect on the biting response at a short distance. Together, the results suggest that plant volatiles generally attract locust nymphs at long distances, but the effects are influenced by distance and concentration. Moreover, there are substantial functional differences in the use of antennae and palps for detecting volatiles at various distances. Overall, the mechanism of food selection by locusts via olfaction can be divided into several continuous ranges according to the sensitivities of the two chemosensory organs and the characteristics of the plant odorants.

Keywords Chemosensory organs · Distance · Food selection · Herbivorous insect · Locust · Plant volatiles

Introduction

Olfaction plays a major role in host plant selection by insects, and herbivorous insects may behaviorally distinguish host plants from non-host plants or even from other host plants based exclusively on olfactory cues (Carlsson et al. 2011; Ikeura et al. 2010). Plant volatiles have an important role in the long-range location of host plants by insects (Hopkins and Young 1990; Szentesi et al. 1996); most of these volatiles belong to several specific functional groups, such as alkanes, alcohols, aldehydes, ketones, and alkenes (Dudareva et al. 2006; Laothawornkitkul et al. 2009; Paré et al. 2000; Whitman and Eller 1992). Attracted by olfactory cues, insects approach potential food sources and perform a series of additional exploratory and gustatory behaviors to make further food choice decisions (Mulkern 1969). However, they must be able to recognize plant volatile blends within a complex, dynamic sensory environment (Atema 1996). Volatiles are emitted as plumes from the surface of plant foliage into the atmosphere, which then dissipate, carrying information over long distances until the signals are received by herbivorous insects (Beyaert and Hilker 2014). During this process, the concentrations and contexts of plant volatiles change with distance, underlining the importance of host tracing in insects. Some evidence suggests that the effective distance at which insects can recognize volatiles may be limited to within dozens of centimeters (Finch and Collier 2012; Schoonhoven et al. 2005). Moreover, insects could potentially detect specific compounds or ratios to gain precise information on host and non-host plants within short ranges (Clifford and Riffell 2013).

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✉ Xuewei Yin
stone856@163.com

✉ Long Zhang
locust@cau.edu.cn

[1] Department of Entomology, China Agricultural University, Beijing 100193, People's Republic of China

There are various hypotheses as to how insects trace host signals and make food choices from a distance. For example, insects can detect volatiles from long distances, but the odor molecules must meet a threshold concentration (Geier et al. 1999). Moreover, insects fly in zigzag patterns to orient to a host-plant odor, and only switch to the volatiles from higher quality resources (Beyaert and Hilker 2014). Some pollinators, such as the tobacco hornworm (*Manduca sexta*), follow fixed-frequency odor pulses and trace increasing odor concentrations during food discrimination until reaching the host plant (Riffell et al. 2014). However, these observations did not focus on specific volatile chemicals or the relationship between chemicals and the functions of the main chemosensory organs.

Herbivorous insects rely on both antennae and maxillary palps as the main chemosensory organs, which are sensitive to odors from plant tissues. Antennae are the primary organs that receive odors of hosts from the environment and have been researched extensively to clarify their role in foraging behavior. Some insects such as the desert locust (*Schistocerca gregaria*) and potato beetle (*Leptinotarsa decemlineata*) can follow food signals over long distances of up to kilometers (de de Wilde 1974; Haskell and Schoonhoven 1969). Palps also have an important role in food choice in locusts, particularly in the detection of and discrimination between host and non-host plant odors (Blaney et al. 1973; Blaney 1975; Chapman and Sword 1993). Compared to antennae, maxillary palps contain far fewer olfactory receptor neurons of a single category, sensilla basiconica (de Bruyne et al. 1999). A recent study reported that locusts cannot behaviorally respond to host plant odors during long-range olfactory detection with only the palps (Zhang et al. 2017). This suggests that antennae and maxillary palps have different chemosensory functions in sensing plant odorants; antennae recognize volatiles over long distances and help insects trace food cues, while palps detect volatiles over a short distance to help insects make food intake decisions. Overall, locusts employ different plant detection and food choice pathways based on distance from the plant and olfactory cues.

Most locusts will eat a wide range of foods but appear to have selective feeding habits. For instance, migratory locust (*Locusta migratoria*) nymphs show strong preferences for plants in the *Gramineae* and *Cyperaceae* families over other species. Research on surface extracts of host plants has demonstrated that locust palps can perceive chemicals, which drives the decision to bite at the plant substratum (Blaney and Chapman 1970). Because the connection between plant volatiles and maxillary palp olfaction in locusts remains unclear, biting behavior could be used to evaluate the detection of odorants by chemosensory organs and investigate their possible functions related to locust feeding behavior.

In this study, we investigated the volatile-sensing behaviors of nymphs of *L. migratoria* at different distances from host and non-host plants to clarify the mechanisms underlying locust foraging behavior.

Materials and Methods

Chemicals We selected information on volatile compounds from host and non-host plants based on previous studies (Buttery and Ling 1984; Buttery et al. 1985; Fu et al. 2014; Leppik and Frerot 2014; Michereff et al. 2011; Njagi and Torto 1996; Pan et al. 2010; Shibamoto et al. 2007; Zeringue and McCormick 1989). In total, 85 plant volatiles (Table S1) were purchased from Sigma-Aldrich (Shanghai, China) at the highest grade available (90–99.9%). Chemicals were grouped based on their functional type (e.g., aldehyde, alcohol, ketone, ester, and so forth), and organized into three sources: host plant volatiles, non-host plant volatiles and common volatiles (i.e., odorants emitted by both host and non-host plants). All tested chemicals were diluted in paraffin oil, which was used as the blank control.

Locusts Locusts (*L. migratoria*) were hatched and raised by feeding them daily with fresh wheat shoots in an insectarium at 28–30 °C with a relative humidity of 60% and a light:dark photoperiod of 18:6 h. Locusts in the fifth instar (4–6 days of the last nymph stage) were used for the experiments. Before the experiments, the locusts were starved for 12 h.

Locust Palp-Opening Response (POR) This experiment was performed according to the method of Zhang et al. (2017). Briefly, before the experiments, the antennae of locusts were removed with tweezers, and normal food was provided for 1 day to allow for recovery. Locusts were restrained within truncated Eppendorf tubes, leaving their heads and palps free to move. All tubes were immobilized on a tube panel to facilitate the rapid testing of 12 locusts for each chemical trial. Experiments were performed in a warm environment, temperature 26~28 °C, to support high palp activity. Before the POR test, all locusts were incubated for at least 10 min in the test area. For each group of 12 locusts, 10 μL diluted volatile compounds was applied to a filter paper strip (2 cm × 0.5 cm, L × W). Each strip was inserted into a Pasteur tube, each of which was labeled and used for only one compound. The strips were placed 1 cm from the locusts' mouthparts, and the chemicals were wafted with a stimulus controller CS-55 (Ockenfels Syntech, Kirchzarten, Germany) toward the locusts' mouthparts without contacting their palps. The airflow rate of the controller was set to 20 ml/min for a stimulation period of 1 s. There was a 2–3 s interval between two testing insects and 15 min interval between each test group to allow palp activity to recover. Palp movement, including the opening of at least one palp over the labrum sulcus, was observed after applying the airflow stimulus. All individuals that opened their palps were counted from each test group. The POR index was the response ratio of 12 tested locusts. All test chemicals were replicated five times at different concentrations (0.1%, 1%, and 5% *v*/v) in independent assays. The POR index was defined as the number of responsive individuals (N_r) divided by the total tested insects (N_t):

$$POR\ index = \frac{N_r}{N_t}$$

Locust Biting Response (BR) We selected 30 active chemicals with high POR index values to test for locust BR. Experiments were processed at room temperature (25 °C). Overhead illumination was provided by two 40 W light bulbs. Fifth-instar nymphs with antennae removed were placed in individual plastic Petri dishes and allowed to acclimatize to the area for 3 min. We tested 12 insects once per group. For the experiment, 10 μL 1% v/v diluted pure chemical was applied to a filter paper strip, and the strips were placed in the plastic Petri dish gently. The number of insects that bit the paper strips was documented for 5 min. After the first test, the dishes were cleaned by abluent and distilled water and dried for the next test, and three replicates were performed, using each insect only once. Paraffin oil was used as the blank control and wheat leaf juice was used as a positive control. The biting behavior of locusts was analyzed with a Wilcoxon matched-pairs signed-rank test. The insects' preference for volatile compounds was estimated with an excess proportion index (i.e., BR index) according to the formula:

$$BR\ index = \frac{P_t - P_c}{P_t + P_c}$$

where P_t and P_c represent the percentage of biting insects in the treatment and control traps, respectively (Sakuma and Fukami 1985). Significant differences in biting response between individual chemicals (P_t) and the blank control (P_c) were analyzed with a *t*-test to evaluate the roles of single odorants in insect feeding choice.

Locust Choice Response The main device used to determine the locust choice response was fabricated with sample bottles and T-maze tubes (Fig. S1). The T-maze tubes included several straight glass tubes (1 m in length, 8 cm in diameter), and T- and L-type tubes. The T tube was connected at each arm with one L tube and one or three straight tubes to create distances of 1 m or 3 m respectively. An air compressor (DOA-P504-BN, Gast) was attached to suck the air from the T tube and create wind tunnels at an airflow rate of 150 mL/min in each arm. Insects were tested in the wind tunnels in the tubes. Each tube was connected to one of two odor source bottles containing either a filter paper soaked in odorant or paraffin oil as a control. Pure or moist air was passed through the bottles before the odor source bottles. After each test, the olfactometer was separated into single glass tubes, and then cleaned by ethanol (95%) and distilled water and dried for the next test.

Change of odor concentration in this olfactometer was evaluated by absorption of solid phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS). Acetophenone was used as the standard compound, and four odor compounds, including hexanal, 2-heptanone, (*Z*)-3-hexenol, and benzaldehyde, were examined in this test. One compound and acetophenone, diluted with paraffin oil into 1% *v*/v respectively, were applied to filter paper strips. Strips were placed in sample bottle as odor source. A fibre from Supelco (100 μm polydimethylsiloxane, PDMS) was exposed in 1 m olfactometer or 3 m olfactometer respectively for 30 min at 30 °C. Three or four replicates were performed for each compound at each distance. SPME injections were performed in splitless mode at 250 °C for 2 min for the thermal desorption of analytes from the fibre. The GC-MS system used was Agilent 7890B gas chromatograph coupled to an Agilent 7200 mass selective detector (Agilent Technologies, Santa Clara, CA, US). A DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm) was used for the separation. The temperature program started at 50 °C for 1 min, increased at a rate of 5 °C/min to 180 °C, and 10 °C/min to 230 °C, then holding at 230 °C for 4 min. The carrier gas used was helium with a flowrate of 1 ml/min. The ion source temperature was maintained to 150 °C and the interface temperature was 280 °C. Molecular ionization took place with an energy of 70 eV. The acquisition range was 35–350 *m/z*. Identification and analysis of compounds were carried out with the Agilent MassHunter Workstation software (Qualitative Analysis B.07.00). Odor concentrations were evaluated using the relative peak area ratio at certain distance according to the formula:

$$RR1\&RR3 = \frac{AAc}{AA0}$$

where *RR1* or *RR3* represents the relative peak area ratio of test compound and standard compound, 1 m or 3 m respectively. AA_C represents the relative peak area of test compound, AA_0 represents the relative peak area of standard compound. The significant difference was examined by using t-tests.

To examine the efficacy of the system, sufficient fresh wheat leaves were ground and the supernatants were collected after centrifugation. Crude extract was prepared in one-tenth dilutions of the supernatant in paraffin oil. We used 100 μL of this plant extract on filter paper strips as the treatment or the same amount of solvent as the control (2 cm × 0.5 cm, L × W). The locusts responded significantly more to the treatment than the blank control at distances of 1 m and 3 m (Fig. S2). For the individual chemical odorants, 10 μL diluted volatile compound (1% *v*/v) was applied to filter paper strips. The strips were placed in the sample bottles, and each strip was used for one insect only. In addition, volatile tests were performed using leaf tissue of host plants (wheat and maize) and non-host plants (broad bean and mulberry). Plant leaves were cut into small pieces or used on the whole branch, as follows: wheat (cultivar 2427, 2 g, cut), maize (cultivar Z58, 10 g, uncut), mulberry (cultivar Gui25, 1.2 g, cut), and broad bean (Chongli faba bean, 4.5–5.0 g, uncut). The plant tissues were placed in the sample bottles as odor sources and an empty bottle was used as the control. When performing the behavioral experiment, the glass tubes of the T-maze olfactometer were covered with a black sheet to avoid the influence of light. For each chemical or plant leaf sample, total 45 to 60 individuals of locust nymphs were tested. Each test lasted for 10 min. The location of the locust was observed after each test: when a locust moved out of the red line circle, it was regarded as making a choice; when it was within the red line circle, no choice was regarded within the duration of experiment (Fig. S1). The responses of the locusts to the odor were evaluated using the responsive index according to the formula:

$$Responsive \qquad index = \frac{N}{N_t}$$

where N and N_t represent the number of insects making choices and total insect number of each replicate, respectively. Significant differences were analyzed using one-way ANOVA with Tukey's multiple comparisons test by software GraphPad Prism 6.0.

Results

Locust Palp-Opening Response to Plant Volatiles The POR is a behavior that insects perform in response to a chemical stimulus. Because locusts always exhibit palpations before or along with their biting behavior (Blaney and Chapman 1970), the POR is associated with their feeding choice and can be valuable for evaluating the sensitivities of locusts to plant volatiles. We tested locust PORs with 85 plant volatiles. The results indicated selective and dose-dependent PORs to various plant volatiles (Fig. 1a, b), and when odorant concentrations were decreased to 1% *v*/v, the POR index also decreased (Fig. S3). Active and non-active compounds can be distinguished based on the POR index (Zhang et al. 2017). In this study, we identified 30 active chemicals with POR index values >0.5 at a concentration of 5% v/v, including 10 aldehydes, 8 alcohols, 5 ketones, 5 esters, and 2 compounds from other functional groups. Aldehydes and alcohols appeared to particularly influence PORs. Moreover, half of these chemicals were common volatiles, whereas the other active volatiles were specific to host or non-host plants.

Biting Response to Plant Volatiles The active compounds selected based on the POR index were used to evaluate the preference of insects to volatile odorants by estimating the BR index (Fig. 2a). Some chemicals (e.g., hexanal, (*E*)-2-pentenal, (*E,E*)-2,4-hexedienal, (*Z*)-3-hexenol, and 2-heptanone) had a BR index similar to the positive control (wheat leaf juice). However, other chemicals (e.g., hexenol, 4-keto-isophorone, heptanal, and (*E*)-2-hexenol) elicited negative responses. These results suggest that the chemicals can act as either attractants or repellents in locusts. Among the active volatiles, 15 chemicals from three sources (three specific to host plants: (*E*)-2-pentenal, 2-methyl-2-pentenal, 4-keto-isophorone; three specific to non-host plants: heptanal, 1-heptanol, 2-octanone; and nine common volatiles: (*E*)-2-hexenol, 2-heptanone, (*Z*)-3-hexenol, hexanol, benzaldehyde, acetophenone, (*E*,*E*)-2,4-hexedianal, hexanal and (*E*)-2-hexenal) were selected for subsequent T-maze tests. And statistics analysis indicated that 12 of these chemicals had significant effects on the biting response at the short range, and the three with no effects were common volatiles (Fig. 2b).

Role of Plant Volatiles in Locust Choice Responses at Different Distances As shown in Figure S4, the ratio of the relative peak area of (*Z*)-3-hexenol at 1 m (0.043 ± 0.008), was significantly higher ($P = 0.033$) than that at 3 m (0.029 ± 0.006). This indicates that the concentration of this compound decreased significantly during the dispersion process. 2-heptanone, however, showed no significant difference between the ratios at these two distances (RR1 = 0.062 ± 0.0005, RR3 = 0.047 ± 0.012, $P = 0.163$). Neither did that of benzaldehyde (RR1 = 0.428 ± 0.0108, RR3 = 0.442 ± 0.047, $P = 0.651$). Strangely, 1% *v*/v hexanal was not obtained by SPME at both distances. Thus, the concentrations of odors were changed differently when they traveled in the olfactometer.

The locust behavioral choice assay was performed with the T-maze olfactometer at a concentration of 1% v/v chemicals. The results were in agreement with those of Zhang et al. (2017), which indicates that only the antennae of locusts support long-range olfaction detection (Fig. S5). Only four common volatiles and one non-host odorant influenced choice at a distance of 3 m (Fig. 3a). When locusts were closer (1 m) to the odor, nine chemicals from various plant sources had either attractive or repellent effects on the insects (Fig. 3b). Besides, the ratios of no choice response for each odorant were also changed with distances. The response of locust at different distances (Fig. 2b, Fig. 3a, and b) suggests that insects might obtain more accurate olfactory information from plants the closer they get to them, first with their antennae and subsequently their palps, enabling them to perform specific responses.

Of the host plant volatiles, (*E*)-2-pentenal and 2-methyl-2-pentenal did not influence choice behavior at long distances (1 m and 3 m) but resulted in significantly higher BRs at a short distance. Another host odorant, 4-keto-isophorone, attracted insects at 1 m but repelled them from biting at a short distance. Of the non-host plant volatiles, heptanal and 1-

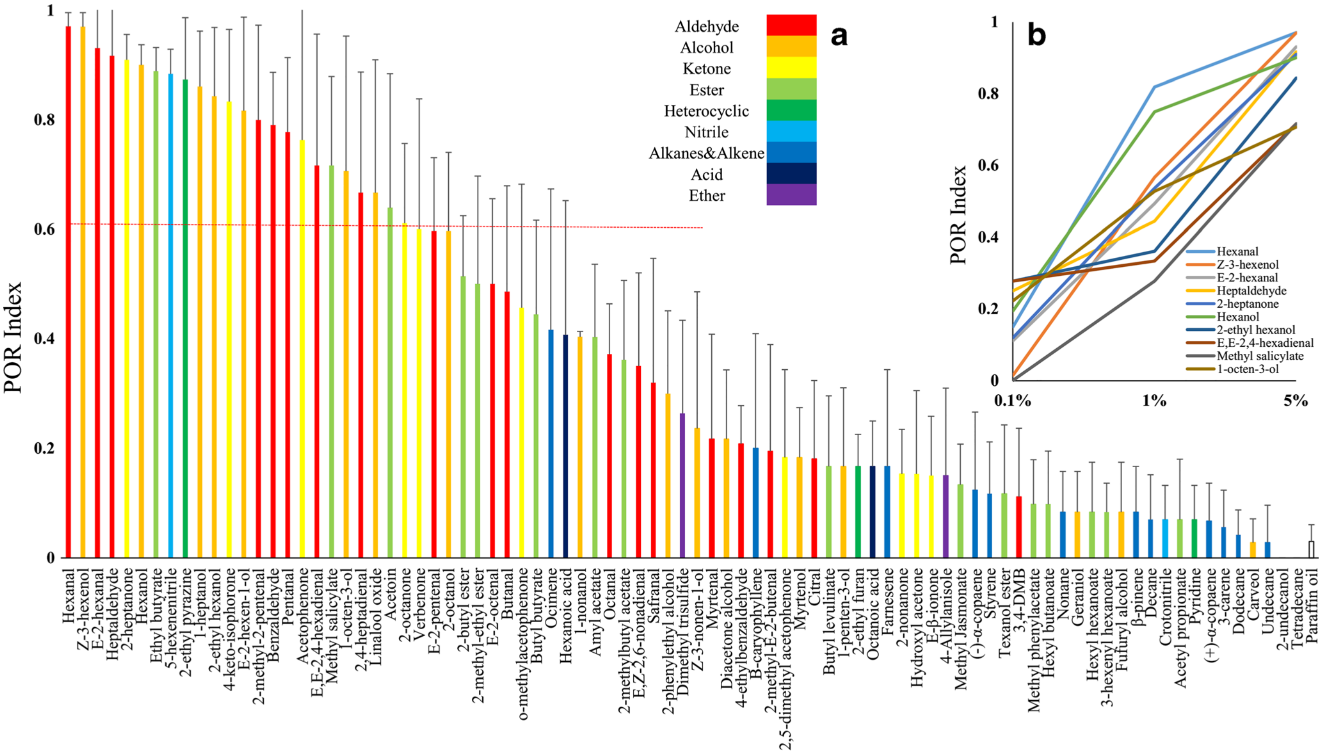

Fig. 1 POR index of plant volatile stimuli (5% *v*/v). **a** The colors represent different functional groups (e.g., aldehydes to ethers). **b** Dose-dependent response to individual plant odorants at concentrations of 0.1–5% v/v

heptanol repelled locusts at both long and short distances, while heptanal only repelled locusts at the greatest tested distance (3 m). Meanwhile, the non-host odorant 2-octanone elicited a biting response at a short distance but had no significant effects in the T-maze choice test. Of the common volatiles, hexanal, (*E*)-2-hexenal, and (*E*)-2-hexenol elicited no response or detection by locusts over long distances (1 m and 3 m). However, the chemicals had different effects on biting response, where hexanal acted as an attractant, (*E*)-2-hexenol was a repellant, and (*E*)-2-hexenal had no significant influence. Both (*E*,*E*)-2,4-hexedianal and 2-heptanone attracted locusts at 1 m and triggered significantly higher BRs, but there were no significant differences in choice-making at 3 m. (*Z*)-3-hexenol attracted locusts at both 1 m and 3 m at 1% *v*/v, and also elicited a significantly higher BR. Interestingly, hexanol changed from an attractant at 3 m to a repellent at 1 m and elicited a significantly lower short-range BR than the blank control. Both acetophenone and benzaldehyde affected long-range choice behavior, although acetophenone acted as a repellent and benzaldehyde was an attractant; however, neither yielded a significantly higher BR.

The results also indicated that the concentration of some common chemicals significantly influenced choice behavior. For example, 1% v/v hexanal and (*E*)-2-hexenal had no significant influence on locusts at 1 m but acted as an attractant at a concentration of 2% ($P < 0.05$) (Fig. S6). Furthermore, most of the tested aldehydes, except heptanal, had no influence on behavior at a distance of 3 m. By contrast, most of the aldehydes, except (*E*)-2-hexenal, significantly affected biting behavior at a short range.

Table 1 summarizes the responses of locusts to volatiles from host and non-host plants. We speculated that at long distances from odor sources, locusts may not be able to discriminate between host or non-host plants based on their volatiles. Therefore, we conducted the following experiment to test this hypothesis.

Long-Range Attraction to Host and Non-host Plant Volatiles

We tested the responses of locusts to the volatiles of wheat and maize (host plants) tissues or broad bean and mulberry (non-host plants) tissues at a distance of 3 m. All volatiles attracted fifth-instar nymphs at long distances (wheat, $P = 0.029$; maize, $P = 0.0115$; broad bean, $P = 0.0294$; mulberry, $P = 0.001$) (Fig. 4a). As such, insects may respond positively to the common volatiles of green leafy plants but cannot distinguish between host and non-host plants specifically over long distances. Furthermore, the locusts showed significantly different responses when the quantities of plant tissues and distances from source were changed (Fig. 4b), where the behavior changed from being attracted to being repelled ($P = 0.0015$) with an increase in the quantity of non-host leaves (from 4 g to 10 g) at 3 m. This could indicate that the concentrations and contexts of plant volatiles at shorter distances convey more specific information, which insects can use to make food choice decisions.

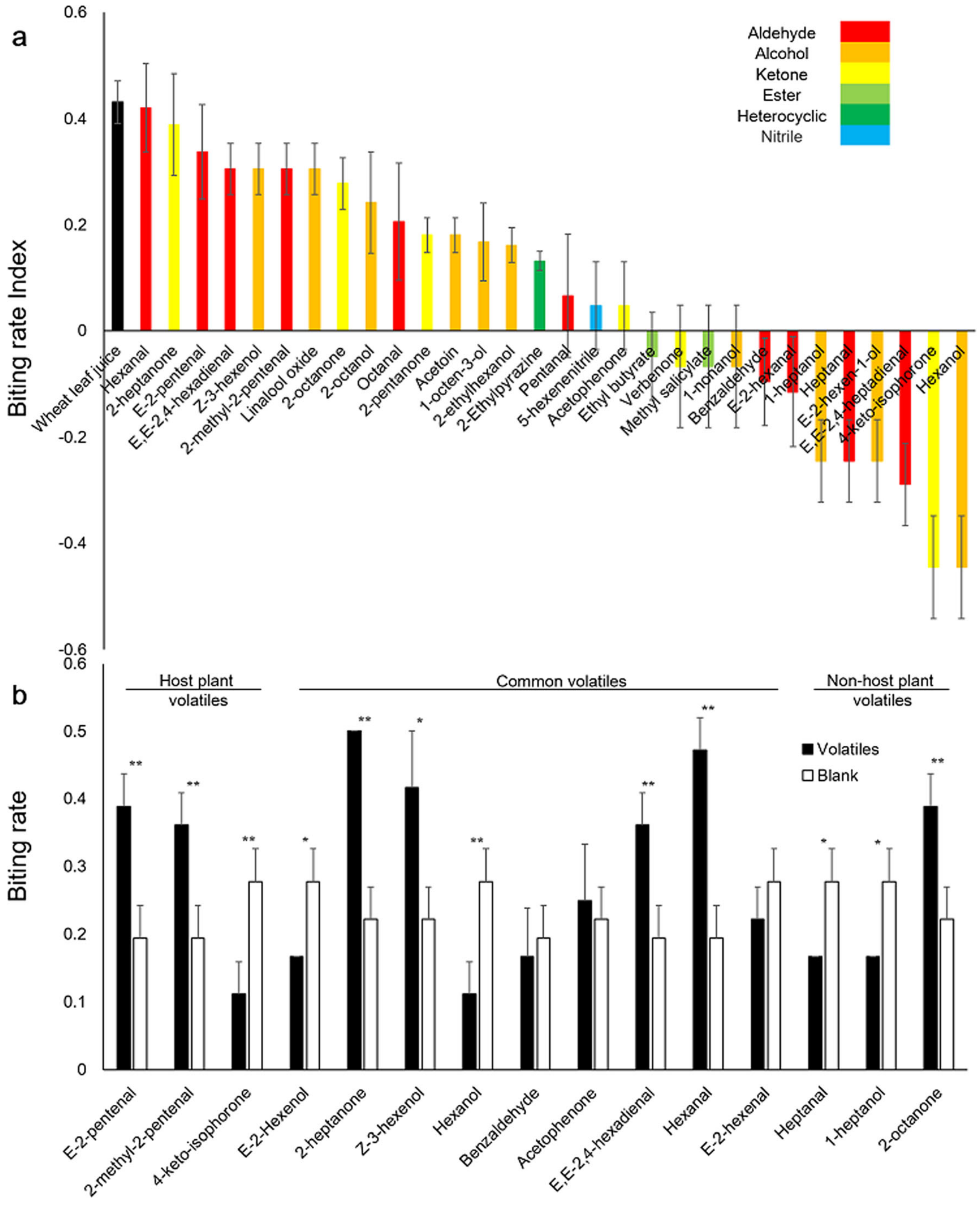

Fig. 2 **a** Locust BR index of volatile stimuli (1% v/v). The colors represent different functional groups (e.g., aldehydes to nitriles). **b** Significant difference of BR between volatiles and paraffin oil. Volatiles are grouped as host plant volatiles, non-host plant volatiles, and common volatiles. Analyzed using *t*-tests, where * represents $p < 0.05$, ** represents $p < 0.01$, and *** represents $p < 0.001$

Discussion

The locusts were sensitive to dozens of plant volatiles (Figs. 2, Fig. 3). Such odorants might have major influences on host plant detection by insects. Previous studies have found that most herbivorous insects with different feeding habits have strong responses to green-leaf volatiles as stimuli, which indicates that these chemicals help insects search for host plants (Hopkins and Young 1990). However, odorants are released in abundance by both host and non-host plants without specific signatures (Brilli et al. 2009; Heil 2004). This study revealed that locusts can sense and respond to some common chemicals at distances of 1 m and 3 m, which could reflect the effectiveness of these odorants in long-range

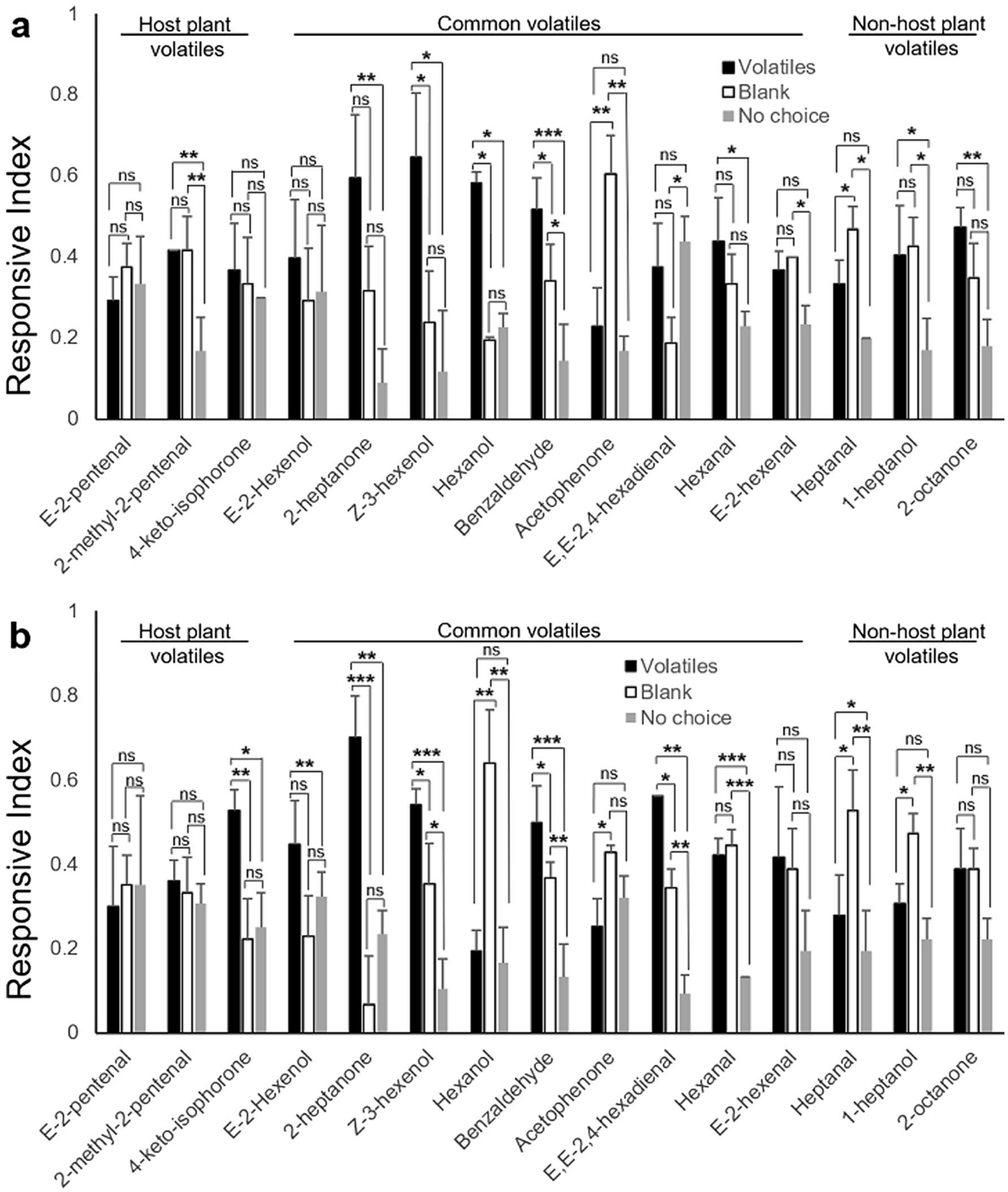

Fig. 3 Locust responsive index of individual odorants (1% v/v) at various distances, including the results of the insect T-maze choice at 3 m (**a**) and 1 m (**b**). Volatiles are grouped as host plant volatiles, non-host plant volatiles, and common volatiles. Significant difference between the positive and negative responses were analyzed by using one-way ANOVA with Tukey's multiple comparisons test, where * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$, and ^{ns} represents no significant difference

detection by insects (Table 1). Meanwhile, plant odors consisting of individual components, regardless of whether they were host or non-host plant volatiles, acted as attractants at 3 m (Fig. 4a). It indicated that certain common chemicals could act as the main cues for insects during the host-detection process, particularly during the initial search for plants. Kang and Hopkins (2004) viewed that plant volatiles could offer preliminary information on food resources for phytophagous insects and help them take the first step in their feeding process. However, the plant diversity could significantly influence insect host location detection (Randlkofer et al. 2010). As volatiles spread throughout the atmosphere, chemicals in plumes from different plants intermingle, creating a complex and dynamic spatiotemporal olfactory environment for herbivorous insects (Atema 1996). For example, insects can lose host plant signals due to low concentrations or interruption by

Table 1 Responses of locusts to 1% v/v plant volatiles in different ranges

Source	Volatiles	BR	POR	Behavioral Response	
			1 cm	1 m	3 m
C	Hexanal	A	+++	N	N
C	(*E*)-2-hexenal	N	++	N	N
C	2-heptanone	A	++	A	N
C	Hexanol	R	+++	R	A
C	(*Z*)-3-hexenol	A	++	A	A
C	(*E,E*)-2,4-hexadienal	A	++	A	N
C	Acetophenone	N	+	R	R
C	Benzaldehyde	N	+	A	A
C	(*E*)-2-hexen-l-ol	R	+++	N	N
H	(*E*)-2-pentenal	A	+	N	N
H	2-methyl-2-pentenal	A	+	N	N
H	4-keto-isophorone	R	+++	A	N
N	1-heptanol	R	++	R	N
N	Heptanal	R	++	R	R
N	2-octanone	A	+	N	N

H: host plant specific, N: non-host plant specific, C: common volatiles; A: attractive, R: repellent, N: no significant effects; +++: POR index >0.6, ++: POR index >0.3, +: POR index <0.3

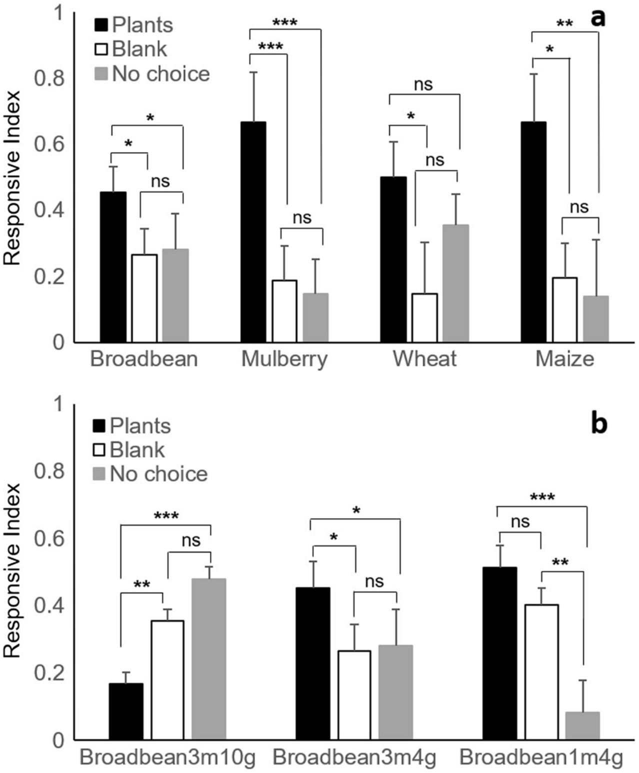

Fig. 4 **a** Locust T-maze choice to volatile stimuli from different plant sources at 3 m. **b** Locust response to volatiles from non-host plant leaf tissue (broad bean) at different distances (1 m and 3 m) and in different quantities (4 g and 10 g). Analyzed using one-way ANOVA with Tukey's multiple comparisons test, where * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$, and ^{ns} represents no significant difference

non-host plant signals (Murlis et al. 2000; Thiery and Visser 1987; Voskamp et al. 1998). Thus, insects may initially trace the common chemicals that are emitted in large quantities or can be transported over long distances.

Moreover, some chemicals from specific functional groups (e.g., aldehydes) are difficult to detect or elicit little response in locusts over long distances, which indicates that they might mainly affect short-range foraging behavior. Some emitted volatile compounds are not stable in the atmosphere and can be transformed via reactions with O_3, -OH, and NO_3 or be absorbed by substrates when dispersed in urban areas (Helmig et al. 2004; Holopainen 2004). Therefore, the components of plant volatile plumes could vary by distance from the plant. In this research, the T-maze device was designed with large straws to simulate the transport of plant odors from short range to long range in distances. During the diffusion, the concentration of some odors gradually decreases, but different odorant has different dispersal characters (Fig. S4). For instance, hexanal at 1% *v*/v was not detected at long distances. A drastic drop in concentration of this odorant may occur and prevent insects from detecting and recognizing. This may explain, why in the behavioral experiments, the locust showed no directional reaction to 1% v/v hexanal, regardless of the test distance of 1 m or 3 m (Fig. 3a, b). It suggests that hexanal may not have the ability to travel or affect insect behavior over long distances. In contrast, benzaldehyde and acetophenone can travel and affect insect behavior over long distances. The reason that these chemicals showed differences in concentrations is possibly related to their physicochemical properties, such as chemical stability, evaporation rate and deposition or adhesion during the diffusion. For example, it has been reported that hexanal can readily react with air to give first peroxo acids, and ultimately carboxylic acids (USCG 1999), which may cause the decline of this compound when travelling in atmosphere. The results also revealed that insect responses are influenced by quantity and distance (Fig. 4b), and specifically by the concentration of odorants that the insect chemosensory organs can sense. A large quantity of volatile chemical can travel over longer distances than a small amount (Beyaert and Hilker 2014). Chapman (1982) noted that the quantities of specific volatiles emitted by host or non-host plants that can represent their specific signatures are much lower than the quantities of common volatiles. This suggests that the concentrations of specific chemicals may have to reach a threshold level to trigger detection by the insect olfactory system and elicit a behavioral response. Furthermore, most tested odorants associated with high locust POR index values acted as a significant attractant or repellant (Table 1), which indicates that the POR is well correlated with locust biting behavior. However, we found that some odorants that attracted locusts at long distances showed no effect on biting behavior. Thus, locust biting and food selection may depend on several cues, including smell, taste, and feel.

In this study, the functions of antennae and palps exhibited significantly different plant odorant-sensing capacities by distance (Fig. S5). Zhang et al. (2017) reported that locusts with mouthpart palps reserved but antennae removed could not behaviorally respond to long-range host plant odors. This suggests that antennae receive odors over long distances and influence locust choice behavior, whereas palps are presumably responsible for detecting odorants emitted near plant tissues to help insects make gustatory contact decisions (e.g., biting or touching). Although these two organs have somewhat different responsibilities in the continuous process of insect food selection, palps are used for the final decision. Therefore, the combination of different olfactory cues sensed with different chemosensory organs could improve the efficiency of food selection in insects.

This study focuses on how herbivorous insects choose and approach their host plants depending on the olfactory cues. During the foraging procedures, they will meet, detect and behaviorally respond to the plant odorants from long range to short range. According to the different responses of locust to plant volatiles via olfaction detection in distances, the changes in contexts and concentration of plant volatiles over long to short distances and their relationships with the functions of different chemosensory organs can affect insect host tracing behavior significantly. The herbivorous insect foraging process can be divided into several continuous choices by distance; over long distances, locusts detect only a few plant odors through their antennae and make decisions related to the approach direction based on limited and dynamic odor information. Such odor information is complex and variable (e.g., very low odor concentrations with little context); therefore, insects might initially follow any potential plant cue to trace food sources, regardless of whether they are from host or non-host plants. At this range, the main olfactory cues for insects include chemicals that can be transported a long distance. With decreasing distance between insects and food resources, the number of food-related odors and their concentrations increase, and information enabling the discrimination between host and non-host plants becomes increasingly accurate and reliable, allowing insects to make specific tracing decisions. Thus, combined with olfactory cues and other signals, insects can move toward food resources while making continuous decisions until arriving at host plants. Sensilla on palps or the mouthpart (e.g., sensilla basiconica) could help locusts decide whether to take bites of plant tissues as the final food consumption decision. At this time, specific chemicals, including some unstable volatiles that can only be detected over short distances, could be emitted in specific concentrations or ratios near the foliage surface, acting as the final signal to help herbivorous insects detect food while foraging.

Besides, the different performances of locusts to volatiles at ranges, i.e. their lack of behavioral responses to some compounds, could either be due to an inability of insects to detect these compounds or a lack of attraction, and it’s hard for us to figure out which it is. However, as a behavioral context, it is irrelevant which it is because the lack of attraction is the key factor in this research.

In addition, Bruce et al. (2005) reviewed that only a few insect species orient their hosts relying on the specific compounds, e.g. isothiocyanates are the key components for cabbage specialists, *Brevicoryne brassicae* and *Ceutorhynchus assimilis*, mediating host location. In fact, insects in natural environment normally use blends of volatiles during their host tracing. Blends with specific combinations or ratios play an important role in insect host/non-host odor recognition, and even different odors arrive simultaneously at the olfactory organs can change insect behavioral responses (Bruce 2015). Thus, it is possible that insects can be attracted to mixtures but repellent to one single odor. This paper only focused on the influence of individual odor to insect orientation in distances, and mixtures in specific ratios could be considered in further research. Our findings will give some helpful suggestions to understand the mechanism of the interaction between herbivorous insect and their host plants.

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