The role of tetradecane in the identification of host plants by the pest bugs Apolygus lucorum and Adelphocoris suturalis

Haichen Yin
Institute of Plant Protection and Soil Fertilizer, Hubei Academy of Agricultural Sciences
Wenjing Li
Institute of Plant Protection and Soil Fertilizer, Hubei Academy of Agricultural Sciences
Min Xu
Institute of Plant Protection and Soil Fertilizer, Hubei Academy of Agricultural Sciences
Dong Xu
Institute of Plant Protection and Soil Fertilizer, Hubei Academy of Agricultural Sciences
Peng Wan (wanpenghb@126.com)
Institute of Plant Protection and Soil Fertilizer, Hubei Academy of Agricultural Sciences

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Abstract

Apolygus lucorum and *Adelphocoris suturalis* are considered serious pests to many cultures in China. Safe alternatives are needed to manage these mirid pests. The current study tested the olfactory responses of mirid bugs to cotton leaves, *Phaseolus vulgaris* pods, and its ethanol extracts. The results showed that *P. vulgaris* pods extracts were attractive to mirid bugs, predominantly female *A. lucorum*. Seven compounds from volatiles of the extract were selected to measure electrophysiological (EAG) responses of mirid bugs. Tetradecane, 2-propyl-1-pentanol, and dodecanal showed strong EAG responses in mirid bugs and, thus, used for field experiments. The results exhibited that the tetradecane showed a significantly higher attraction than other attractants with 30.33 ± 2.19 mirid bugs trapped during seven days. Lab trials showed a significant female mirid bug attraction towards tetradecane. The selected-response rates were above 60%, and it was the most attractive to females *A. lucorum* at the concentration of 1.5 mg/mL. Among seven tetradecane derivatives, tetradecane and tetradecanoic acid were the best attractants to *A. lucorum* and *A. suturalis* at specific concentrations. GCMS detection showed that tetradecane was present in the volatiles of 10 common hosts, and the difference of relative content was significant. The presence of tetradecane could modulate the responses of mirid bugs towards host plants suggesting that tetradecane plays a vital role in the olfactory selection of mirid bugs, while host recognition of these pests depends on many kinds of sensory cues

Introduction

The mirid bugs *Apolygus lucorum* (Meyer-Dür), and *Adelphocoris suturalis* Jakovlev (Hemiptera: Myridae) attack a wide range of host culture plants, including such important cash crops as cotton, jujube, and the kidney bean (Pan et al. 2015a). These bugs were previously considered pests of secondary importance to cotton in China (Lu et al. 2010), but after the adoption of transgenic cotton in the late 1990s towards reducing insecticide use in cotton fields (Lu et al. 2012b), the population of mirid bugs soon increased rapidly and became an economical pest in the cotton areas of Yangtze and Yellow rivers (Lu &Wu 2011).

At present, synthetic pesticides are the primary method to manage *A. suturalis* and *A. lucorum* but the rapid onset of insecticide resistance (Snodgrass et al. 2009, Zhang et al. 2015, Zhen et al. 2016) coupled with the strong dispersal abilityof mirids (Blackmer et al. 2004) still sustain them as a serious threat to cotton crops. Therefore, there is an urgent need to explore and test alternative pest management methods.

Insects are remarkable for their olfactory organs, represented by countless sensilla linked to sensory neurons scattered on their antennae, maxillary palps, and even wings and legs. They employ olfactory organs in sensing their environment, as in the case of selecting suitable hosts based on their location and development stage. Plants provide odorous cues in the form of volatile emissions denouncing their physiological status in the natural environment, thus enabling the selection of suitable hostsand food sources or oviposition sites (Cha et al. 2017, Hosseini et al. 2017, Shiojiri &Karban 2008, Sun et al. 2012, Weiss et al. 2011) by herbivorous insects. Based on this unique "chemical conversation" with their surroundings, novel efficient pest control technologies have emerged focusing on kairomones, mainly targeting small-sized pests with vast dispersal like mirid bugs (Pan et al. 2019). For instance, several literature reports presented food lures for mirid bugs validated by laboratory and field trials spanning several years (Pan et al. 2015b). However, studies concerning mirid bugs are still quite limited compared with the body of knowledge about lepidopteran and coleopteran pests, especially regarding attractive plant volatiles (Pan et al. 2015a, Pan et al. 2015b).

The current study tested the attractiveness of ethanolic extracts from *P. vulgaris* pods to mirid bugs, where the main volatiles were identified by gas chromatography and mass spectroscopy (GCMS). the electrophysiological responses of *A.suturalis* and *A. lucorum* to these compounds were measured, and their relative attractiveness was tested in the field and by two-way competition experiments in the laboratory. Finally, the volatiles of host plants were compared in terms of the attractiveness responses. The key objective of this study was to understand the mechanism of host plant identification by mirid bugs as to develop specific trapping for controlling field infestations.

Materials And Methods

Reagents and instruments

Synthetic compounds from the volatiles of *P. vulgaris* and their derivatives were purchased from Shanghai Macklin Biochemical Co., Ltd., and Aladdin Industrial Corporation, China. Anhydrous ethanol was purchased from Sinopharm Chemical Reagent Co., Ltd. A three-

arm olfactometer (YMM3-150) coupled with an EAG measurement system, and a QP2010 SE GC-MS system were respectively purchased from Shanghai Yuming Instrument Co., Ltd, Syntech Limited, and the Shimadzu Corporation.The adsorption column consisted of a glass tube (15 cm long and 0.7 cm in diameter) with the airflow outlet and inlet equipped with 200 mg SuperQ adsorbent, purchased from Altec, USA.

Preparation of P. vulgaris **extracts**

Pods of *P. vulgaris*(5000g) were soaked in 12L anhydrous ethanol at room temperature. After 15 days, the ethanolic extract was concentrated in a rotary evaporator in a water bath at 45°C. This crude extract was stored at 4°C in the refrigerator.

The olfactory responses of mirid bugs to cotton leaves, P. vulgaris **pods and their extract**

Prior to experimentation, male and female adults of *A.suturalis* and *A. lucorum* were starved for 4 h and submitted to olfactory behavior assays using a three-arm olfactometer (Shanghai Yuming Instrument Co., Ltd, China) (Fig. 1). The arms were ventilated for 5 min before the experiment, the airflow rate set to 2 L/min. *P. vulgaris* pods extract (50 µL), one *P. vulgaris* pod, and 2–3 cotton leaves were placed inside separate 250 mL glass bottles, each connected to the olfactometer. This experiment was repeated 9 times for each species, where10 adults were placed at the response chamber from an opening at the top center, in each replicate. Thus totaling 90 *A. suturalis* and *A. lucorum* male and female adults were assayed respectively. The behavioral response assays where done in the dark, recorded for 30 min after the insects were introduced. Any insects entering over half of an arm's lengthwere considered to show a positive response to that specific odor. For each replication, the tested mirids as well as the end positions of odor sources, were randomized. The olfactometer parts were washed with ethanol betweenreplications.

The selected-response rate was calculated by using the following formula.

$$\mathrm{Selectedresponserate} = \frac{\mathrm{No.\ adultsselectedtreatment}}{(\mathrm{No.\ adultsselectedtreatment} + \mathrm{No.\ adultsselectedcontrol})} \times 100$$

Identification of volatiles of P. vulgaris **pods extracts**

About 10 mL of any given extract was added to a glass jar (35 × 20 cm – height and diameter) provided with an airflow inlet and outletat the top. An adsorption column was attached to the outlet, while activated carbon filter (air flow rate: 600 L/min) was attached to the inlet. After collecting for 8 h, the adsorption column was eluted with chromatographically-pure hexane for GC-MS detection of samples.

Gas chromatograph settings included an Rtx-5 MS chromatographic column (30 m. 0.25 mm; 0.25 µm), injector temperature set to 250°C, the carrier gas was helium, and injections were made on splitless mode. The oven temperature program was set to three steps: (i) 40°C for 1 min; (ii) increased to 130°C at the rate of 4°C/min, maintaining for 5 min; (iii) increased to 250°C at the rate of 10°C/min and maintaining for 5 min. Mass spectrometer has its ion source temperature set to 250°C, and scanning speed at 2500, with a 0.3 s interval.

Mass spectra were compared with standards in the Labsolution software to predict the chemical structure of the main volatiles in *P. vulgaris* pods extracts.

EAG responses of A. suturalis **and** A. lucorum **to the volatiles of** P. vulgaris **pods extract**

The crude extract from *P. vulgaris* pods extract was serially diluted to 15 mg/mL, 1.5 mg/mL and 0.15 mg/mL in anhydrous ethanol; pure anhydrous ethanol was used as CK group. Droplets containing 15 µL of each solution were added to pieces of filter paper (30 mm×10 mm), which were placed inside a 1000 µL pipette-tip connected to the airflow inlet. Antennae of *A. suturalis* and *A. lucorum* were excised using a scalpel, and a small segment of the tip of the antennae was removed. The airflow outlet was placed vertically to insects' excised antennae at the distance of about 10mm. A silver electrode of a diameter of 0.2 mm was inserted into a capillary glass tube, into which 0.9 mol/L saline solution was injected. The excised antennae were connected to the two electrodes. Stimulation lasted for 0.5 s and the interval between the two stimuli was 30 s to ensure complete recovery of the antennasensory response. The antennae were alternatively stimulated by each volatile and CK, and the average value of CK before and after each volatile measurement was taken as the CK value. Nine males and females of both mirid species were used in the experiment, and their antenna was stimulated three times with each compound. The relative EAG was the ratio of the average value of EAG response to the tested compound and the pertaining CK value.

Attractive effect of volatiles of P. vulgaris pods extract under field conditions

Volatiles eliciting significantly stronger EAG responses compared with others were selected to attractiveness field tests. The field test was conducted from 28 Aug 2020 to 3 Sep 2020 in a cotton field of about 4000m^2 in Ezhou, Hubei province. Compounds were diluted to 150 mg/mL with ddH_2O, and 1 mL solution was added to an Eppendorf tube sealed with plastic film, finely punctured to keep it from evaporating too quickly. Tubes were taped to the middle of a yellow board (RAL 1016 Sulfur yellow, International Standard colorimetric card). Glue was spread on the yellow board to capturethe bugs. Each compound was tested by using 3 yellow boards. An equal number of boards was presented as controls, containing no attractants, where ddH_2O was taken as a CK substance. Each of the yellow boards was held suspended about 20 cm above the cotton plant. Treatment and control groups were randomly distributed, and the distance between each yellow board was over 20m. After seven days, the numbers and species of mirid bugs on the yellow boards were counted.

Attractiveness of volatiles in lab tests

Volatiles presenting a positive attractiveness response in field experiments were further tested by a two-way competition experiment in the laboratory. Volatiles were diluted to 1.5 mg/mL and 15 mg/mL with ddH_2O, added with 30μL anhydrous ethanol to assist in complete solubilization. One of the three arms of the olfactometer was closed with absorbent cotton, and the other two were connected to two glass bottles containing either 50μL volatile solution or ddH_2O with 30 μL anhydrous ethanol as CK group. Other methods and the selected-response rate were as described above. The selected coefficient was calculated as follows:

$$\mathrm{Selectedcoefficient} = \frac{(\mathrm{No.\ adultsselectedtreatment} - \mathrm{No.\ adultsselectedcontrol})}{\mathrm{No.\ adultsselectedtreatment} + \mathrm{No.\ adultsselectedcontrol}}$$

Attractiveness of tetradecane analogues to A. lucorum and A. suturalis adults

The tetradecane analogues 7-tetradecene, 1-tetradecene, tetradecanal, 2-tetradecanol, 1-tetradecanol, 2-tetradecenoic acid and tetradecanoic acid were selected for two-way competition experiment of female adults. The experimental methods were as described before.

Amounts of tetradecane in volatiles and the attractiveness of different host plants

Fruits of *Vitis vinifera*, *Zea mays*, *Pyrus bretschneideri*, *Malus pumila* and seedlings of *Pisum sativum* (100 g each) were added to a glass jar to collect their volatiles. Five different parts of cotton plants, including buds, bolls, leaves of seedlings, leaves on the bolls, leaves on the buds were collected from Xinzhou in 2019 and stored in a refrigerator at -80°C in the form of ground powders. The amount of 15g powder of each different sample were employed to collect volatiles. Volatile analysis and identification methods were as described above. This experiment was repeated three times, and the relative content of tetradecane is each sample was calculated according to the specific chromatogram peak area.

Further 15-20g fruits of *Vitis vinifera*, *Zea mays*, and 5–7 cotton leaves were used to test the attractiveness of these plants as hosts to mirid bugs, but tests included 'strictly olfactory' assays and normal assays using female adults. In these 'strictly olfactory' assays, three different plants were presented as host options in the separate glass bottles connected to one of the three arms of the olfactory response chamber, in a way that the mirid bugs could only smell the hosts but could not access them visually or physically. Other methods were as described above.

For normal assays, the samples of three different plants were offered as host options in different arms directly, so that mirid bugs could access them by smell, touch and visually. 20 adults of *A. suturalis* and *A. lucorum* were placed into the olfactory response chamber from an opening at the top center in each replicate, from the overnight period of 17:00–8:00. All lights were switched off to maintain dark conditions. Each assay was repeated 3 times, where the position of each host option was changed every time. The selected-response rate was calculated as those described before.

Olfactory responses of A. lucorum and A. suturalis to plants supplemented with tetradecane

Plants presenting the highest and lowest tetradecane contents were selected for this experiment. The host plant presenting the lowest tetradecane content was supplemented with synthetic tetradecane, to verify for any changes in its relative attractiveness. The same three-arm olfactometer was employed in this experiment. CK groups included 15g-20g of the host plants containing the highest and lowest tetradecane contents, while the treatment group was 15g-20g of the hostplants with the lowest tetradecane content

supplemented with 50μL of tetradecane solution. Three different concentrations of tetradecane supplementation were tested: original fluid, 15mg/mL and 1.5mg/mL. The treatment and two CK groups were allocated to the 3 glass bottles connected to the olfactometer and assayed as above described for 'strictly olfactory' selection.

Data analysis

The least significant difference (LSD) test was employed to check for differences in the selected-response rates among samples of cotton leaves, *P. vulgaris* pods, and their extract. We also employed the LSD test to check for differences in numbers of mirid bugs trapped in the field, EAG responses to volatiles, the content of tetradecane in each plant volatile bouquet, and relative attractiveness among different plants as hosts. The olfactory responses to the volatiles and analogues were analyzed using chi-square test. All analyses were conducted using SPSS software.

Results

The olfactory responses of mirid bugs to cotton leaves, P. vulgaris pods, and their extract

The results showed that the female and male adults of *A. suturalis* yielded the highest selected-response rates to *P. vulgaris* pods in three-choice olfactory tests, which were 37.85% ± 3.97% and 40.91% ± 6.94%, respectively. However, there was no significant difference among the responses to cotton leaves, *P. vulgaris* pods, and the extract ($P > 0.05$) (Fig. 2).

The selected-response rate of female *A. lucorum* to the extract from *P. vulgaris* pods was 53.39%±1.78%, which was significantly higher than that of *P. vulgaris* pods and cotton leaves. While the male adults preferred *P. vulgaris* pods, their selected response rate was 42.28%±6.19% and the difference was not significant ($P > 0.05$) (Fig. 3).

Identification of volatiles of P. vulgaris pods extracts

Nine compounds were identified among volatiles of *P. vulgaris* pods extract by GCMS. According to the market supplement, tetradecane, 2-propyl-1-pentanol, dodecanal, naphthalene, 1,2,4,5-tetramethyl benzene, (+)-2-Bornanone and eicosane were used inthe EAG response tests(Table 1).

Table 1
Components of volatiles from *P. vulgaris* pods extract

No.	Retention time (min)	Peak area	Peak Height	Volatiles
1	7.373	1205308	225841	2-Propyl-1-pentanol
2	9.159	317708	96675	Benzene, 1,2,4,5-tet
3	9.706	2930389	923692	(+)-2-Bornanone
4	10.437	591140	202253	Naphthalene
5	10.612	126659	48835	Dodecane, 2,6,11-trim
6	10.714	125093	53917	Dodecanal
7	14.076	349373	186600	Tetradecane
8	15.672	403079	220918	Eicosane
9	17.922	110688	82202	1,4-Methanobenzocycl

EAG responses of mirid bugs to P. vulgaris pods extracts

As shown in Table 2, female adults had stronger EAG responses to tetradecane, dodecanal, and 2-propyl-1-pentanol. The responses of female adults to tetradecane were significantly higher than to other compounds whenever the concentration of such volatile was above 1.5 mg/mL ($P < 0.01$). When the concentration was 0.15 mg/mL, the difference in the EAG responses to volatiles was not significant (*P*

> 0.05). Although dodecanal at the concentration of 0.15 mg/mL could induce a significant difference in the EAG responses of female *A. suturalis* compared with the other five compounds ($P < 0.05$), this relative EAG response was only 1.21 ± 0.06.

Table 2
Relative EAG responsesof mirid bugs to *P. vulgaris* pods extracts

Concentration of volatiles	volatiles	Male		Female	
		Adelphocoris suturalis	*Apolygus lucorum*	*Adelphocoris suturalis*	*Apolygus lucorum*
0.15mg/mL	1	1.02 ± 0.14Aa	1.04 ± 0.09Aa	0.99 ± 0.02ABb	1.11 ± 0.04Aa
	2	1.04 ± 0.06Aa	1.02 ± 0.04Aa	0.93 ± 0.04Bb	1.20 ± 0.2Aa
	3	1.13 ± 0.05Aa	1.14 ± 0.12Aa	1.00 ± 0.02ABb	0.96 ± 0.03Aa
	4	1.05 ± 0.09Aa	1.00 ± 0.04Aa	0.99 ± 0.04ABb	1.06 ± 0.03Aa
	5	1.02 ± 0.09Aa	1.04 ± 0.08Aa	0.94 ± 0.10Bb	1.07 ± 0.05Aa
	6	0.98 ± 0.03Aa	0.93 ± 0.04Aa	1.03 ± 0.10Aab	1.13 ± 0.07Aa
	7	1.08 ± 0.07Aa	1.09 ± 0.06Aa	1.21 ± 0.06Aa	1.06 ± 0.12Aa
1.5mg/mL	1	1.20 ± 0.11Aab	1.11 ± 0.03ABabc	1.06 ± 0.07Bc	1.06 ± 0.06Bb
	2	1.27 ± 0.12Aab	1.28 ± 0.07Aa	2.57 ± 0.36Aa	1.43 ± 0.10Aa
	3	1.02 ± 0.03Ab	0.94 ± 0.04Bc	1.04 ± 0.08Bc	1.09 ± 0.02Bb
	4	1.14 ± 0.11Aab	1.16 ± 0.09ABab	1.15 ± 0.14Bc	1.09 ± 0.02Bb
	5	1.02 ± 0.08Ab	1.08 ± 0.05ABbc	1.21 ± 0.07Bbc	1.03 ± 0.05Bb
	6	1.16 ± 0.04Aab	1.11 ± 0.04ABabc	1.25 ± 0.06Bbc	1.19 ± 0.02ABb
	7	1.37 ± 0.10Aa	1.23 ± 0.04Aab	1.6535 ± 0.0682Bb	1.07 ± 0.04Bb
15mg/mL	1	0.93 ± 0.02Bc	0.96 ± 0.01Cc	0.9605 ± 0.0422Cc	1.20 ± 0.12Bbc
	2	2.06 ± 0.22Aa	1.85 ± 0.12Aa	2.3670 ± 0.0974Aa	2.01 ± 0.18Aa
	3	0.97 ± 0.03Bc	1.00 ± 0.02Cc	1.0761 ± 0.0620Cc	0.99 ± 0.07Bc
	4	1.00 ± 0.07Bc	0.96 ± 0.05Cc	1.0378 ± 0.0171Cc	1.10 ± 0.11Bc
	5	1.04 ± 0.08Bc	1.02 ± 0.05Cc	1.0873 ± 0.0582Cc	1.37 ± 0.11Bbc
	6	1.03 ± 0.07Bc	1.10 ± 0.06Cc	1.0907 ± 0.0721Cc	1.01 ± 0.05Bc
	7	1.43 ± 0.07Bb	1.40 ± 0.01Bb	1.7100 ± 0.1379Bb	1.53 ± 0.13ABb

The number of volatiles represents 1. eicosane, 2. tetradecane, 3. 1, 2, 4, 5-tetbenzene, 4. (+)-2-bornanone, 5. 2-propyl-1-pentanol, 6.naphthalene, 7. dodecanal. Different uppercase and lowercase letters indicate extremely significant ($P < 0.01$) and significant ($P < 0.05$) differences of relative EAG responses of male or female mirid bugs between 7 compounds(LSD)

Male adults were less sensitive. Whenever the concentration of volatiles was below 1.5 mg/mL, their relative EAG values ranked less than 1.5, and the difference among volatiles was not significant ($P > 0.05$). Whenever at concentration 15 mg/mL, tetradecane yielded the strongest response and ranked significantly higher than other compounds ($P < 0.01$).

Attractive effect of volatiles of P. vulgaris **pods extract under field conditions**

Based onto the results of relative EAG response, tetradecane, 2-propyl-1-pentanol, and dodecanal were selected for field tests. As shown in Fig. 4, tetradecane presented on the yellow board yielded high attractiveness: within7 days, the average amount of captured mirid

bugs was 30.33 ± 2.19, which was significantly higher than withother attractants ($P < 0.01$) and yellow board only (CK) ($P < 0.05$). Interestingly, the number of mirid bugs trapped by 2-propyl-1-pentanol and dodecanal was significantly lower than in the CK group ($P < 0.05$).

Verification of volatiles with attractive effect in the lab

Tetradecane proved attractive to bugs in the field tests, however as multiple factors can influence such trials, so we further verified the attractiveness of tetradecane in laboratory controlled conditions. Based on to the EAG readings, we opted for the volatile concentrations of 1.5 mg/mL and 15 mg/mL in two-choice olfactory tests, given there were no significant difference whenever volatiles concentration was 0.15 mg/mL. The results showed that tetradecane yields significant attractiveness to female mirid bugs: selected-response rates were above 60%. It was most successful in attracting female *A. lucorum* at the concentration of 1.5 mg/mL. While being was less attractive to males than females, it showed no significant olfactory response when the concentration was1.5 mg/mL (Table 3).

Table 3
The olfactory responses of mirid bugs to tetradecane

Gender	Specis	Concentration(mg/mL)	treatment	CK	NR	*P* value	χ^2	SRR(%)	SC
Female	*Apolygus*	15	38	24	28	0.012**	6.32	61.29	0.23
	lucorum	1.5	45	20	25	< 0.001**	19.23	69.23	0.38
	Adelphocorissuturalis	15	38	23	29	0.007**	7.38	62.30	0.25
		1.5	40	24	26	0.005**	8.00	62.50	0.25
Male	*Apolygus*	15	45	20	25	< 0.001**	19.23	69.23	0.38
	lucorum	1.5	31	30	29	0.8563	0.03	50.82	0.02
	Adelphocorissuturalis	15	41	22	27	< 0.001**	29.30	65.08	0.30
		1.5	31	29	30	0.715	0.13	51.67	0.03

** represents extremely significantly difference between the amount of mirid bugs that selected treatment group and CK group ($P < 0.01$) (Chi-square test). NR represents mirid bugs of no response, SRR represents selected response rate, SC represents selected coefficient

Attractiveness of tetradecane analogues to A. lucorum and A. suturalis adults

To check for the attractiveness of tetradecane analogues to *A. lucorum* and *A. suturalis*, seven chemical derivatives were selected for the two-way competition experiment, including alcohols, aldehydes, acids, and olefins. Because tetradecane provedrelatively less attractive to males, we only tested the olfactory response of females. In general, tetradecane derivatives were more attractive to *A. lucorum* than to *A. suturalis*, especially at low concentrations. Moreover, 4 analogues showed an extremely significant difference ($P < 0.001$) compared with the CK group at concentration 1.5 mg/mL. Among these compounds, tetradecanal was the most attractive to *A. lucorum* with a higher selected-response rate than tetradecane. With *A. suturalis*, only tetradecanoic acid yielded a higher attraction ($P < 0.001$), of which the selective reaction rate was also higher than that of tetradecane at 15 mg/mL (Table 4).

Table 4
The olfactory responses of mirid bugs to tetradecane derivatives

Species	Concentration(mg/mL)	Odor source	Treatment	CK	NR	*P* value	χ^2	SRR(%)	SC
Apolygus lucorum	15	7-tetradecene	43	22	25	< 0.001**	13.57	66.67	0.33
		1-tetradecene	40	25	25	0.001**	10.45	61.72	0.23
		Tetradecanal	29	34	27	0.37	0.79	45.83	-0.08
		2-tetradecanol	33	27	30	0.27	1.2	55.00	0.10
		1-tetradecanol	31	34	25	0.6	0.28	46.94	-0.06
		2-tetradecenoic acid	27	37	26	0.07	3.13	42.42	-0.15
		Tetradecanoic acid	35	25	30	0.07	3.33	58.97	0.18
	1.5	7-tetradecene	44	20	26	< 0.001**	18	68.18	0.36
		1-tetradecene	32	34	24	0.73	0.12	48.57	-0.03
		Tetradecanal	48	17	25	< 0.001**	29.57	73.33	0.47
		2-tetradecanol	42	25	23	0.003**	8.63	63.16	0.26
		1-tetradecanol	32	31	27	0.86	0.03	51.35	0.03
		2-tetradecenoic acid	33	24	33	0.09	2.84	57.89	0.16
		Tetradecanoic acid	43	23	24	< 0.001**	12.12	64.86	0.30
Adelphocorissuturalis	15	7-tetradecene	30	31	29	0.86	0.03	48.48	-0.03
		1-tetradecene	31	28	31	0.58	0.3	52.63	0.05
		Tetradecanal	33	29	28	0.47	0.52	53.85	0.08
		2-tetradecanol	32	28	30	0.47	0.53	52.78	0.06
		1-tetradecanol	29	35	26	0.29	1.125	44.83	-0.10
		2-tetradecenoic acid	35	28	27	0.21	1.56	54.84	0.10
		Tetradecanoic acid	49	14	27	< 0.001**	38.89	77.78	0.56
	1.5	7-tetradecene	33	28	29	0.37	0.82	54.55	0.09
		1-tetradecene	32	28	30	0.47	0.53	53.19	0.06
		Tetradecanal	32	30	28	0.72	0.13	51.85	0.04

** represents extremely significantly difference between the amount of mirid bugs that selected treatment group and CK group ($P < 0.01$) (Chi-square test). NR represents mirid bugs of no response, SRR represents selected response rate, SC represents selected coefficient.

Species	Concentration(mg/mL)	Odor source	Treatment	CK	NR	*P* value	χ^2	SRR(%)	SC
		2-tetradecanol	32	28	30	0.47	0.53	46.51	-0.07
		1-tetradecanol	32	31	27	0.86	0.03	51.16	0.02
		2-tetradecenoic acid	33	29	28	0.47	0.52	53.19	0.06
		Tetradecanoic acid	31	29	30	0.72	0.13	51.61	0.03

** represents extremely significantly difference between the amount of mirid bugs that selected treatment group and CK group ($P < 0.01$) (Chi-square test). NR represents mirid bugs of no response, SRR represents selected response rate, SC represents selected coefficient.

Content of tetradecane in the volatiles and the relative attractiveness of different host plants

GCMS detection showed that tetradecane was present in the volatiles of 10 common host plants of mirid bugs, and the difference in theirrelative content was significant ($P < 0.05$). The content of tetradecane in the volatiles of *Vitis vinifera* fruits was the highest, significantly higher than in *Zea mays* fruits, reaching 1.0744%±0.0649% ($P < 0.05$). However, there was no significant difference in the contents of tetradecane among five different parts of cotton plants, which were all significantly lower than in *Zea mays* fruits ($P < 0.01$) (Fig. 5).

Fruits of *Vitis vinifera*, *Zea mays*, and seedling leaves of *Gossypiumher baceum* were employed to compare their attractiveness to *A. suturalis* and *A. lucorum* adults, representing significantly difference of tetradecane volatile concentrations. Since tetradecane proved highly attractive to females, we chose females for such experiments. Results varied wildly between host plant options in 'strictly olfactory' mode. We understand that, if pests selected for hosts strictly by olfaction, the variation in the selected-response rate would proportional to the relative content of tetradecane in volatiles of such plants. Under these circumstances, the response rates of mirid bug females to *G. herbaceum* leaves were significantly lower than to *V.vinifera* fruits ($P < 0.01$). While when mirid bugs were able to select hosts by multiple sensory cues in normal assays, their varied selected response rates were irrespective of their relative content of tetradecane. Females of *A. lucorum* preferred *Zea mays* fruits, while *A. suturalis* preferred *G.herbaceum* leaves, with respective selected-response rates of 56.33%±3.30% and 57.92%±5.61% (Fig. 6).

Olfactory responses of A. lucorum **and** A. suturalis **to host plants supplemented with tetradecane**

As shown in Fig. 7, supplementation with original fluid of tetradecane significantly increased the olfactory selected-response rate of *A. lucorum* and *A. suturalis* to *G. herbaceum* leaves, represented by selected response rates of 46.41% ±3.15% ($P < 0.01$) and 45.31 ± 3.86% ($P < 0.01$), which were significantly higher than those of *G. herbaceum* leaves in the control group, or to *Vitis vinifera* fruits. On the other hand, when tetradecane at concentrations of 15mg/mL and 1.5mg/mL were added to *G. herbaceum* leaves, the selected response rates of mirid bugs did not change significantly ($P > 0.05$).

Discussion

The ethanolic extract of *P. vulgaris* pods proved attractive to *A. suturalis* and *A. lucorum*, and the volatile tetradecane yielded the strongest EAG response among other volatiles in the extract. The attractive effect on mirid bugs was confirmed both by field trapping experiments and olfactory tests in the laboratory. Furthermore, the study proved that other tetradecane analogues display a robust attractive effect on mirid bugs. As this compound is a common volatile among host plants, it is likely a key olfactory recognition cue to suitable host plants. But host recognition of *A. suturalis* and *A. lucorum* depends on more than olfaction.

Plants produce multiple secondary metabolite volatiles including hydrocarbons, alcohols, aldehydes, ketones, esters, organic acids, and terpenes, which are important cues for herbivorous insects in identifying and locating their hosts (Defagó et al. 2016, Lu et al. 2012a, Najar-Rodriguez et al. 2013). For example, it has been demonstrated that seven compounds, e.g. *cis*-formic acid-3-hexene ester, m-xylene, and 3-ethyl benzene, among the volatiles of eighteen host plants will trigger a strong EAG response of *A. lucorum*. Moreover xylene, butyl acrylate, acrylic acid butyl ester and butyl butyrate showed a more significant attraction to mirid bugs and played an important role in host conversion (Pan et al. 2015a, Pan et al. 2015b). *P. vulgaris* is an important host of *A. suturalis* and *A. lucorum*, regarded as important to the growth and development of mirid bugs (Xiao et al. 2013), and its extract has no less attractive effect, which may greatly facilitate practical applications. The present investigation relied on ethanolic extract to characterize the host-localization cues used by mirid bugs on *P. vulgaris*. Our data strongly indicated tetradecane is central for host attraction, as illustrated by electrophysiological and behavioral results.

Tetradecane seems widely distributed among the volatiles of host plants exploited by mirid bugs, making it surprising that the attraction activity of the compound was rarely reported. When *A. suturalis* and *A. lucorum* was only allowed to asses hosts by olfaction, their attractiveness was closely related to their relative amounts of tetradecane in volatiles, indicating it is an important olfactory clue for mirid bugs. This was further confirmed by the increased selected-response rates after supplementation with tetradecane to host plants presenting a low concentration of that compound. Further studies have shown that some tetradecane analogues can be more attractive to mirid bugs than tetradecane, which opens an opportunity for the development of attractants with similar chemical structure.

Our data also suggests that the selection of hosts by mirid bugs will vary depending on whether they physically assess plants, implying other sensorial organs play important roles in host recognition. For example, some insects have a complex visual perception. Physical and chemical signals can synergistically compound attractiveness to host plants. For instance, color seems to play an important role: black and red traps on pine woods will capture more *Hylastesater* (Coleoptera: Scolytidae) and *Arhopalusferus* (Coleoptera: Cerambycidae) adults among other traps of different colors, regardless of whether attractants like alpha-pinene are included. Still, the addition of attractants can be conducive to trapping more pests (Kerr et al. 2017). This phenomenon has also been demonstrated in mirid pests, *A. lucorum* was more attracted to cotton plants with green lights, and the selective response was significantly higher than that of two signals alone (Pan et al. 2015c). For this reason, yellow boards with attractants may have compounded a synergistic effect during the field experiment.

Gustation and tactile cuesmayalso be critical to host recognition by arthropods. This has been demonstrated for a number of species. For example, direct contact involving chemical, visual, and tactile cues triggered stronger responses of *Tunicotheres moseri* to the hosts (Ambrosio &Brooks 2011). Gustatory sensilla have even been described from the prothoracic legs of female adults of the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae) which respond to sucrose, glucose, fructose, maltose, inositol, and 20 different amino acids, thus enabling adults to evaluate nectar upon perching, triggering feeding behavior (Zhang et al. 2010). In short, host recognition by mirid bugs is described as a complex process that may be affected by different sensory organs in different spatial levels (Henze et al. 2018).Future studies should thus focus on defining the role of sensorial organs, especially gustation and tactile receptors,in host recognition.

In the current study, we observed a difference in the sensitivity to tetradecane between male and female mirid bugs. In fact, previous studies indicated that males usually respond more strongly to sex pheromones than females in locating mating partners, while host plant volatiles were more attractive to female adults. For example, male antennae of *Heliothis virescens* and *H.subflexa* were more responsive to the major sex pheromone compound, (Z)-11-hexadecenal, than were female antennae (Groot et al. 2005); female *Apantelest aragamae* responded significantly longer to the volatiles of host plants than to clean air, while male adults responded significantly longer to clean air rather than to host plants (Nurkomar et al. 2017). In this paper, female mirid bugs were found to be attracted by two concentrations of tetradecane; however, the compound elicited no attractiveness to male adults at lower concentrations. Therefore, we hypothesize that female mirid bugs will locate hosts using tetradecane, while male adults were more likely to be attracted to host plant fields by the sex pheromones of females, due to their low sensitivity to the volatiles. The differential olfactory response suggests there are differences between male and female mirid bugs in sensorial factors, like odor binding proteins (OBPs), chemosensory proteins (CSPs), and odor receptor protein (ORs), such as has been demonstrated with some other pests by transcriptome analyses (Große-Wilde et al. 2010, Li et al. 2015). Still, the molecular basis of host recognition in mirid bugs using tetradecane warrants further research.

Finally, we explored the recognition mechanism of mirid bugs towards multiple host plants and proposed controlling methods of mirid bugs for the cotton field. While the attractants surveyed in this study needed to be matched with yellow boards, it should be expected that they yield good attractiveness when used singly; nonetheless, combining with other attractants is usually a good strategy (Kendra et al. 2017, Tasin et al. 2018). Previous studies showed that sex pheromones have a significant attractive effect on male adults, making up for the poor attractiveness of tetradecane observed in males. Moreover, the volatiles of host plants can increase the EAG response of males to sex pheromones and promote mating behavior (Binyameen et al. 2013, Ian et al. 2017, Namiki et al. 2008). Therefore, we speculate that the combination of sex pheromones with tetradecane hold sample prospects for future research. In addition, olfaction recognition has been demonstrated as only a component of the host recognition, so that the shape and color of the traps also significantly impact attractiveness in the field. The commercial scope of attractants for mirid bugs remains quite limited compared with available options against Lepidoptera pests, although some products have already been pre-tested under field conditions. We hope that the current study will provide the possibility of developing more efficient and safe pest control measures againstmirid bugs in the future.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Written informed consent for publication was obtained from all participants

Availability of data and materials: The data has submitted in supplementary material

Competing interests: Not applicable

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Authors' contributions: Haichen Yin and Min Xu did the experiment. Haichen Yin wrote the main manuscript text. All authors reviewed the manuscript.

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Figures

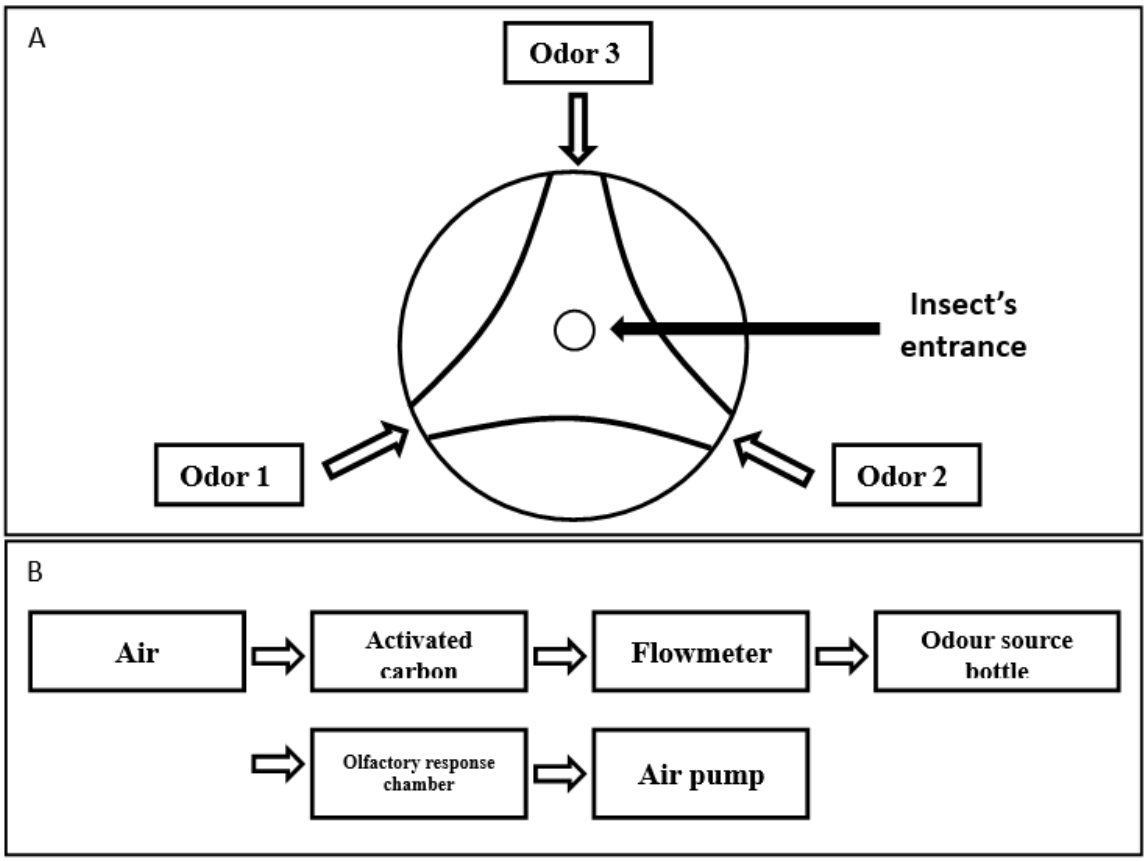

Figure 1

Schematic Diagram of YMM 3-150 Olfactometer. (A) Top view of olfactory reaction chamber of olfactometer, (B)connection diagram of olfactometer.

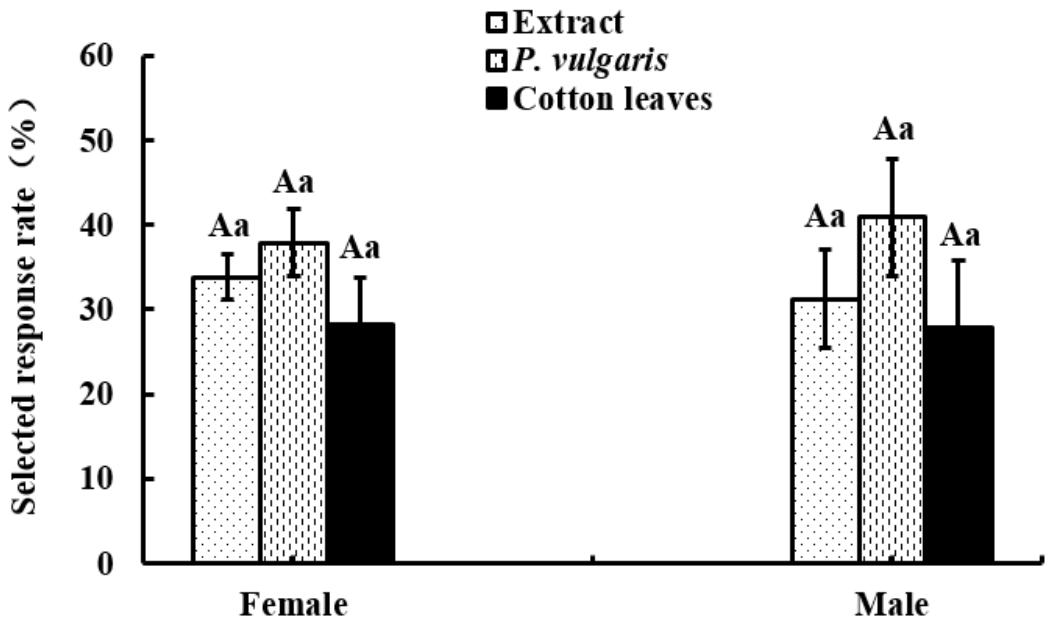

Figure 2

Selected response rate of *A. suturalis*tocotton leaves, *P. vulgaris* pods and their extract

Different uppercase and lowercase letters indicate extremely significant (P<0.01) and significant (P<0.05) differences of selected response rate of male or female adults to three odor source(Least significant difference test)

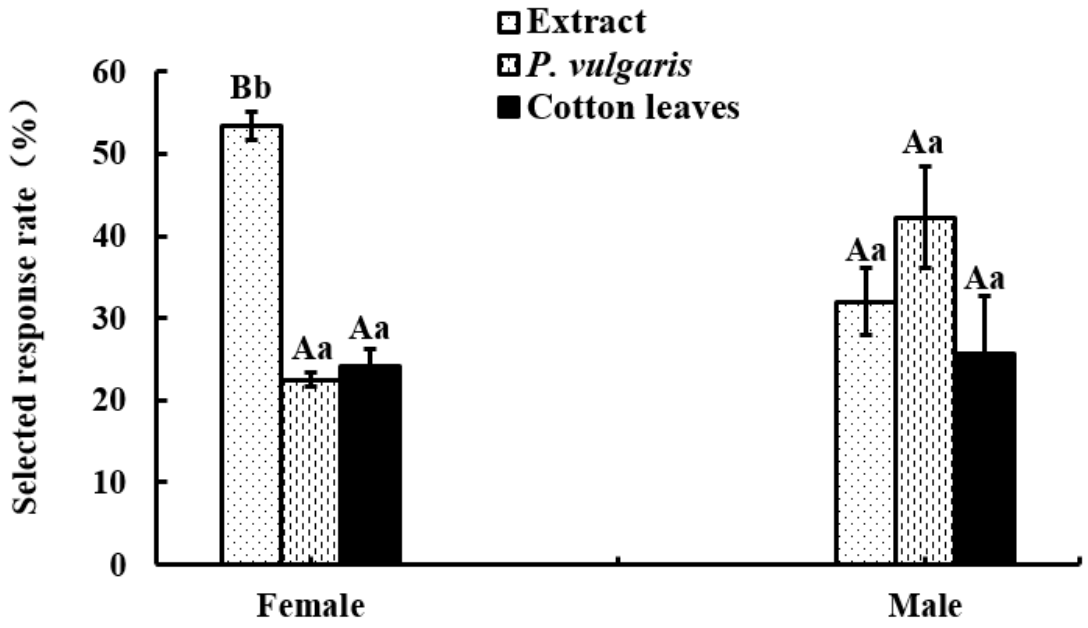

Figure 3

Selected response rate of *A. lucorum*tocotton leaves, *P. vulgaris* pods and their extract

Different uppercase and lowercase letters indicate extremely significant (P<0.01) and significant (P<0.05) differences of selected response rate of male or female adults to three odor source(Least significant difference test)

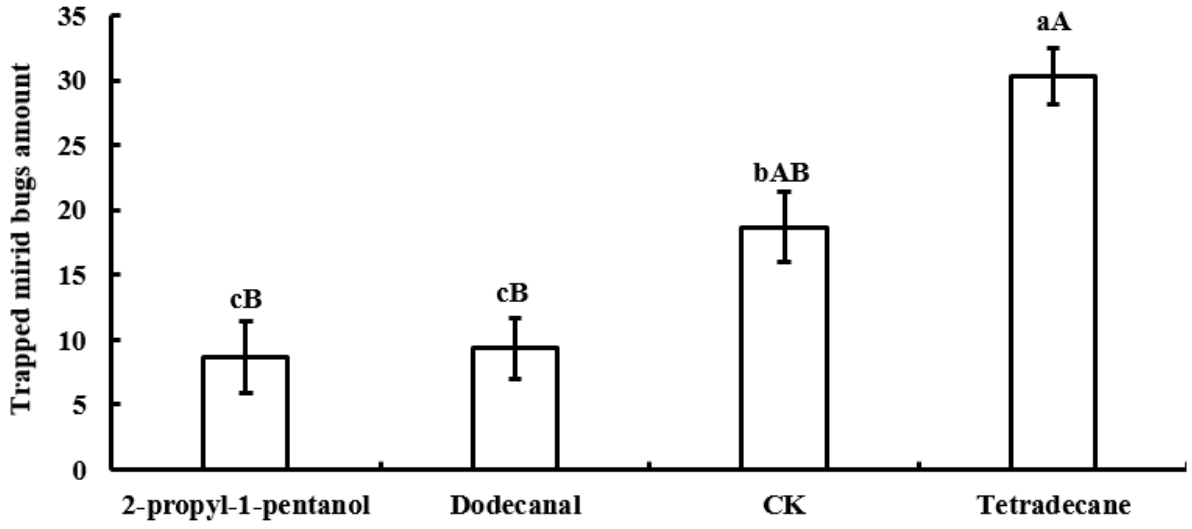

Figure 4

Mirid bugs trapped by yellow boards with different attractant

Different uppercase and lowercase letters indicate extremely significant (P<0.01) and significant (P<0.05) differencesof mirid bugs amount that trapped by yellow boards with different attractant (Least significant difference test).

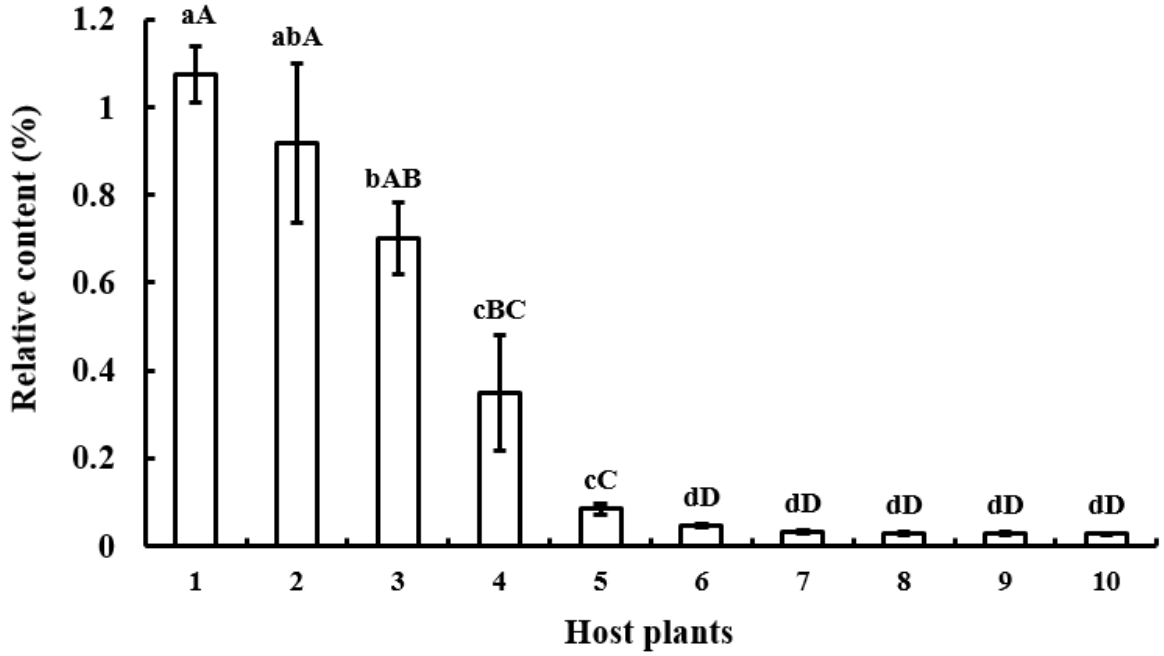

Figure 5

Relative content of tetradecane in volatiles of host plants

Different uppercase and lowercase letters indicate extremely significant (P<0.01) and significant (P<0.05) differencesof relative content of tetradecane (Least significant difference test). Numbers on the horizontal axis represent: 1. Fruits of *vitis vinifera*, 2. Seedling of *Pisum sativum*, 3. Fruits of *Zea mays*, 4. Fruits of *Pyrus bretschneideri*, 5. Fruits of *Malus pumila*, 6. Buds of *Gossypiumherbaceum*, 7. Bolls of *G.herbaceum*, 8. Leaves at seedling stage of *G.herbaceum*, 9. Leaves on the bolls of *G.herbaceum*, 10. Leaves on the buds of *G.herbaceum*

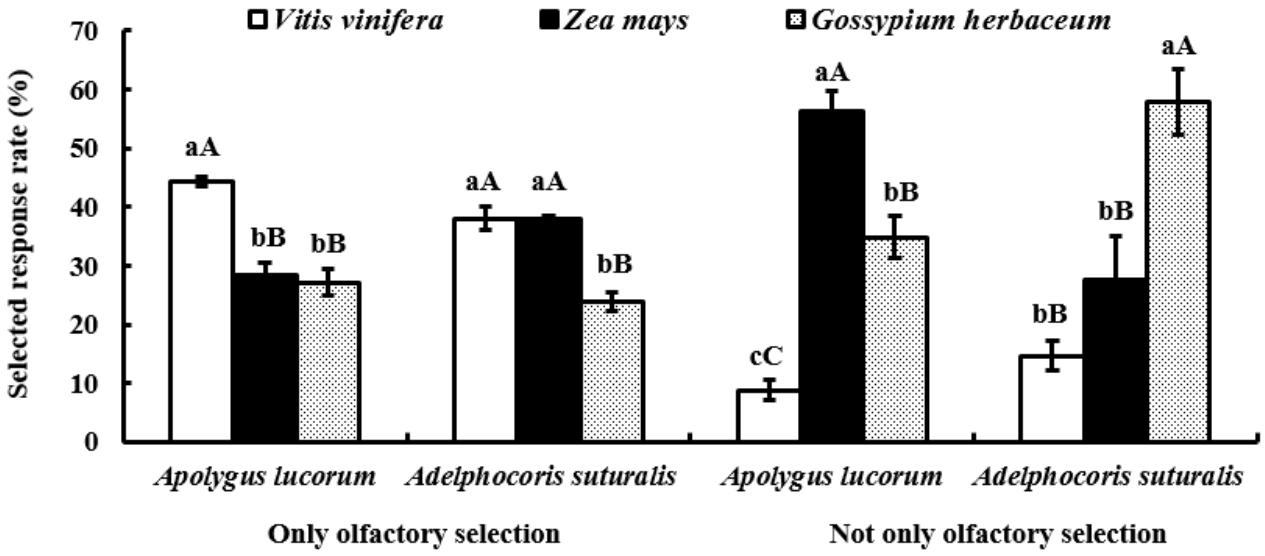

Figure 6

Attractive effect of different host plants on *A. suturalis* and *A. lucorum*

Different uppercase and lowercase letters show extremely significant ($P<0.01$) and significant ($P<0.05$) differencesofthe selected responserate of mirid bugs to different hosts

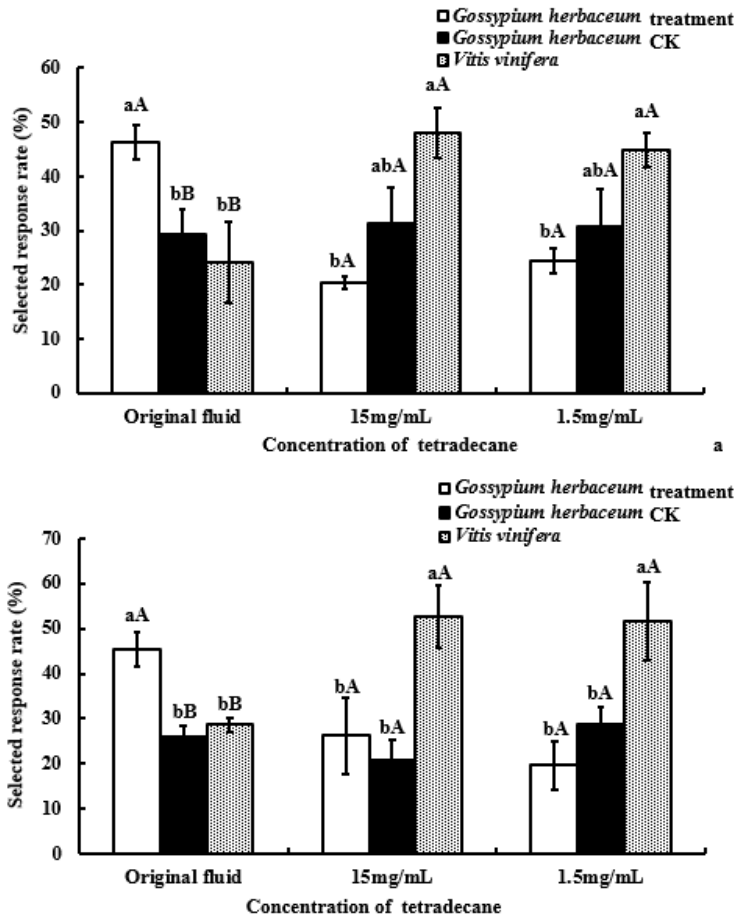

Figure 7

Selected response rates of *A. lucorum* and *A. suturalis* to host plants supplemented with tetradecane

Supplementary Files

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- volatilesfromhostsGCMS.xlsx