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Identification of the Terpenoid Compounds and Behavioral Assays of Alarm Pheromones in the Vetch Aphid Megoura Viciae

Xuan Song
China Agricultural University
Yao-Guo Qin
China Agricultural University
Yue Yin
China Agricultural University
Zheng-Xi Li (zxli@cau.edu.cn)
China Agricultural University https://orcid.org/0000-0003-3611-8046

Research Article

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Abstract

Aphids are destructive insect pests worldwide, and alarm pheromones play a key role in their chemical ecology. However, the composition and key active components of alarm pheromone differentiate among aphid species. Here we conducted a detailed analysis of the terpenoid compounds in the vetch aphid *Megoura viciae* and its host plant *Pisum sativum* by using gas chromatography-mass spectrometry. The results showed that a variety of terpenoid compounds existed in the aphid, with four major terpene components, *i.e.*, (-)-β-pinene (49.74%), (*E*)-β-farnesene (32.64%), (-)-α-pinene (9.42%) and (+)-limonene (5.24%), in addition to a trace amount of minor terpenoid components (3.14%). In contrast, the terpenoid compounds were relatively scarce in the host plant, mainly consisting of squalene (66.13%) and its analogue 2,3-epoxysqualene (31.59%) in addition to some minor components. Quantitative analysis of the dynamics of four major terpene components during different developmental stages showed that the monoterpenes increased with continuous development, while the sesquiterpene reached peak at the 3rd -instar; all terpene components remained at a high level in the 4th -instar, with (-)-β-pinene accounting for the highest proportion during all developmental stages. Behavioral assays with single components and mixtures at different concentrations were conducted in a three-compartment olfactometer, revealing that the repellent activities of single components varied in a concentration-dependent manner, but two mixtures (1:44.4:6.5:2.2 and 1:18.4:1.3:0.8) prepared according to the proportions of four major components at the 3rd - and 4th -instar stages maintained a significant repellent activity at all concentrations tested. Our results suggested that (-)-α-pinene and (-)-β-pinene were the major active components of alarm pheromone in *M. viciae*, but the mixtures of single components play a key role in the alarm behavior of *M. viciae*. Our study helps to understand the chemical ecology of insects and design alternative control strategies against aphids.

Introduction

Insects use chemical volatiles to communicate in mating, aggregation, predation, alarm and self-defense (Belén et al., 2015). Among them, alarm pheromones as the second largest family of insect pheromones play an important ecological role in insects (Verheggen et al., 2010). Most aphids release alarm pheromones when they are attacked by natural enemies, and both aphid nymphs and adults utilize alarm pheromone to warn con-specifics of danger (Kunert *et al*., 2007). Aphids are among the most widespread and harmful agricultural pests in the world (Simon and Peccoud, 2018). Previous studies showed that the major component of alarm pheromone for most aphid species is the sesquiterpene (*E*)-β-farnesene (Edwards et al., 1973; Pickett and Griffiths, 1980). Francis et al. (2005) tested the composition of volatile molecules in 23 aphid species, finding that (*E*)-β-farnesene was the main component in 16 species and the minor component in five species; only two aphid species (*Euceraphis punctipennis* and *Drepanosiphum platanoides*) did not release (*E*)-β-farnesene. They also reported a particular profile of volatile molecules composed of not only (*E*)-β-farnesene but also several monoterpenes in the vetch aphid *Megoura viciae* Buckton (Aphididae: Hemiptera), including (-)-α-pinene, (-)-β-pinene and (+)-limonene, but no behavioral assays were performed. Nevertheless, Bruno et al. (2018) assessed the behavioral response of *M. viciae* to the compounds identified by Francis et al. (2005), indicating that (-)-α-pinene and (+)-limonene were the main active components of alarm pheromone in *M. viciae*. Moreover, they tested a mixture at the ratio of (*E*)-β-farnesene (14.2%), (-)-α-

pinene (11.8%) and β-pinene (74%) as reported (Table S1), showing a repellent activity against *M. viciae*. Additionally, molecular studies revealed that the recombinant odorant binding protein MvicOBP3 could bind to all four alarm pheromone components of *M. viciae*, displaying a much higher affinity for (*E*)-β-farnesene (K_i 0.1 μM) than for β-pinene (K_i 2.3 μM), (−)-α-pinene (K_i 1.8 μM) and (+)-limonene (K_i 2.5 μM) (Northey et al., 2016). It seems that the molecular binding affinity could not reflect the alarm activity of terpene components.

M. viciae feeds exclusively on the Fabaceae (Nuessly *et al*., 2004), causing serious damage to the broad bean *Vicia faba* and the pea *Pisum sativum* (Kunert et al., 2008; Leroy et al., 2011). Its unique composition of terpene components differentiate it from most other aphid species in alarm behavior. It has been shown that alarm pheromone was synthesized by the aphid itself in the cotton aphid *Aphis gossypii* by rearing aphids with artificial diets (Sun and Li, 2017). However, it is still unclear whether other aphids also synthesize *de novo* alarm pheromone. Our group has been working on the biosynthetic mechanisms of aphid alarm pheromone, yet the major component of alarm pheromone in all aphid species that have been investigated was (*E*)-β-farnesene, including the green peach aphid *Myzus persicae* (Cheng and Li, 2018; Zhang and Li, 2008; Zhang and Li, 2012), *A. gossypii* (Ma et al., 2010; Sun and Li, 2017; Sun and Li, 2018) and the bird cherry-oat aphid *Rhopalosiphum padi* (Sun and Li, 2012; Sun and Li, 2019; Sun and Li, 2020). Thus, *M. viciae* can provide a good opportunity for comparative study in this line of work. Here we first analyzed the composition of terpenoid compounds in *M. viciae* and its host plant *Pisum sativum* by using gas chromatography-mass spectrometry. Next, the dynamics of the major terpene components was investigated during different developmental stages of *M. viciae*. Moreover, we conducted a series of behavioral assays with single components and mixtures at different concentrations in a three-compartment olfactometer. Our study identified a novel set of alarm pheromones in *M. viciae*.

Materials And Methods

Culture of aphids

The aphid *M. viciae* was provided by the Laboratory of Biological Control led by Dr. Tinghui Liu in Hebei Agricultural University and maintained on *P. sativum* in the Laboratory of Insect Molecular Ecology in China Agricultural University. The aphids were reared in a climate incubator (RXZ-300B, Ningbo, China) under the conditions of 19 ± 1 ℃, 70 ± 5% relative humidity and a photoperiod of 16L:8D.

Collection of terpenoid compounds fromM. viciaeand its host plantP. sativum

M. viciae aphids (overlapping developmental stages, *n* = 200) were collected and put into a 1.5-mL centrifuge tube containing 500 μL of *n*-hexane on ice, fully milled, centrifuged at 4 ℃ for 30 min. The supernatant was transferred into a 2-mL vial for gas chromatography-mass spectrometry (GC-MS) analysis. The same procedure was performed to collect volatile terpenoid compounds from *P. sativum* seedlings (2.0g) for GC-MS analysis.

Identification of terpenoid compounds fromM. viciaeandP. sativumby GC-MS

The samples collected from *M. viciae* and *P. sativum* were analyzed on an Agilent 6890 gas chromatographer coupled to an Agilent 5973 ion trap mass detector (Agilent Technologies Inc., California, USA). The instrument was equipped with a HP-5 capillary column (300 mm × 0.25 mm × 0.25 μm, Agilent, Santa Clara, USA). The program of GC-MS was set up as described (Huang et al., 2013). Briefly, after sample injection, the GC oven temperature was held at 40°C for 1 min, followed by a two-step temperature increase: the first increase was from 40°C to 130°C at a rate of 4°C/min and maintained for 5 min, and then the temperature was increased at a rate of 10°C/min to 250°C and held for 5 min. The temperatures of injector and ion source were 250°C. The mass spectrometer was operated under the electron impact ionization mode (El, 70 eV) with a *m/z* scan range of 35–650. Terpenes were identified by comparing their retention time and mass spectra with those of standards (SigmaAldrich, Oakville, Canada) under the same conditions. The quantity of each component was estimated based on the peak area ratio of sample to the chromatographically pure external standard (-)-β-pinene (Magdalena and Henryk, 2016). Three biological replicates were performed for each treatment. The proportions of single components were calculated as their percentages in total terpenoid compounds.

Quantitative analysis of terpene components at different developmental stages of aphid

M. viciae aphids of the same developmental stage (*n* = 30), including the 1st -instar, 2nd -instar, 3rd -instar, 4th -instar nymphs and adult, were ground in a 1.5-mL centrifuge containing 100 μL of hexane. The supernatant was transferred to a chromatography vial for GC/MS analysis as described above. (-)-β-Pinene (purity > 99%; Sigma-Aldrich, Oakville, Canada) was used as the external standard. Three biological repetitions were performed for each stage. The amount and proportion of each single terpene component were estimated based on the peak area ratio of sample to standard for different developmental stages.

Behavioral assays

Behavioral assay experiments were carried out in a three-compartment perspex olfactometer modified from the design based on an optimized behavioral selection model of aphid (Khashaveh et al., 2020; Satyajeet et al., 2021; Yu et al., 2019) (Fig. 1). The olfactometer was composed of three compartments (7 cm × 13 cm × 5 cm for each) connected by a door (3 cm × 3 cm) between two adjacent compartments. The test samples, *i.e.*, (-)-α-pinene, (-)-β-pinene, (+)-limonene and (*E*)-β-farnesene, were diluted into three different concentrations (0.1 μg/μL, 1 μg/μL and 10 μg/μL) with light mineral oil. Meanwhile, two mixtures (Mix I and Mix II) were prepared by mixing the test samples in that order in the ratios of 1:44.4:6.5:2.2 and 1:18.4:1.3:0.8 according to the proportions of the four major terpene components at the 3rd - and 4th -instar nymphal stages, respectively. Light mineral oil alone was used as a negative control. The test sample (B) and control (C) were placed in a petri dish (Φ3.0 cm) near the door of the side compartments, respectively, and five host plants fixed in smaller petri dishes (Φ1.0 cm) (covered with 10% agar) were placed in the far side of the lateral compartments. Twenty wingless 3rd - and 4th -instar nymphs were introduced into the petri dish in the middle (A) and allowed to move freely for 30 min. A total of 100 nymphs were used to test

their selection preference for each sample compound. All behavioral assays were performed under the same conditions and under a dark environment to avoid light interference. The numbers of aphids crawling close to the host plants in the two lateral compartments were counted. For each sample tested, the behavioral index value (BIV) was calculated according to the following formula: BIV = [(C − T)/ (C + T)] × 100, where C and T are the numbers of aphids in the control and treatment compartments, respectively.

Data analysis

The quantities of (-)-α-pinene, (-)-β-pinene and (+)-limonene and (*E*)-β-farnesene were statistically analyzed and compared on the GraphPad Statistics version 8.0 (San Diego, USA) using One-way analysis of variance (ANOVA) followed by Tukey's B multiple range test ($P < 0.05$). The significance of differences in the behavioral index values were analyzed on SPSS Statistics version 21 (IBM) using ANOVA followed by Duncan's test ($P < 0.05$).

Results

Identification of the terpenoid compounds from M. viciae and its host plant P. sativum

The terpenoid compounds from *M. viciae* and its host plant *P. sativum* were identified by using GC-MS analysis. The results showed that four major terpene compounds were detected in *M. viciae*, including the sesquiterpene (*E*)-β-farnesene and three monoterpenes, *i.e.*, (-)-α-pinene, (-)-β-pinene and (+)-limonene (Table 1). The compounds (-)-α-pinene, (-)-β-pinene, (+)-limonene and (*E*)-β-farnesene were corresponding to the peaks nos.1, 2, 3 and 6, respectively, as characterized by GC-MS (Fig. 2 and Fig. S1). Interestingly, some additional minor peaks were detected, which were identified as β-myrcene, (+)-sabinene, camphene and α-terpineol, based on comparative analysis with the standard mass spectrometry library NIST17s. Moreover, the proportions of different components were calculated: (-)-α-pinene (9.42%), (-)-β-pinene (49.74%) and (+)-limonene (5.24%), and (*E*)-β-farnesene (32.64%), with the minor components accounting for 3.14% (Fig. 3A). In contrast, the types of terpenoid compounds were relatively scarce in the host plant compared to in the aphid, mainly consisting of squalene (66.13%) and its analogue 2,3-epoxysqualene (31.59%) (Table 2, Fig. 3B and Fig. S2). Some minor terpenoid components were also identified in *P. sativum*, such as 4-isopropyl-5-methylhexa-2,4-dien-1-ol, 2,4-pentadien-1-ol, 3-pentyl-, (2*Z*)-, limonene and cyclohexanol, 1-methyl-4-(1-methylethenyl)-, *cis*-, based on comparative analysis with the standard library.

Table 1
GC-MS identification of terpenoid compounds in *Megoura viciae*

No.	Retention time (min)	*m/z*	Peak area	% Peak area	Peak height	% Peak height	Name of terpenoid compound	Molecular formula
1	10.099	TIC	1203409	0.18	456923	0.24	(-)-α-Pinene	C10H16
2	10.639	TIC	57434	0.01	14470	0.01	Camphene	C10H16
3	11.562	TIC	75660	0.01	30386	0.02	(+)-Sabinene	C10H16
4	11.676	TIC	6325841	0.95	2149907	1.15	(-)-β-Pinene	C10H16
5	12.244	TIC	229816	0.03	68879	0.04	β-Myrcene	C10H16
6	13.665	TIC	658790	0.1	195955	0.1	(+)-Limonene	C10H16
7	19.977	TIC	40572	0.01	8983	0	α-Terpineol	C10H180
8	29.105	TIC	4102715	0.62	805659	0.43	(*E*)-β-Farnesene	C15H24

Table 2
GC-MS identification of terpenoid compounds in the host plant *Pisum sativum*

No.	Retention time (min)	*m/z*	Peak area	% Peak area	Peak height	% Peak height	Name of terpenoid	Molecular formula
1	13.674	TIC	138863	0.02	30615	0.02	Limonene	C10H16
2	22.52	TIC	42633	0.01	15660	0.01	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, *cis*-	C10H18O
3	27.43	TIC	174315	0.03	83769	0.05	2,4-Pentadien-1-ol, 3-pentyl-, (2*Z*)-	C10H18O
4	42.391	TIC	1057080	0.18	267448	0.15	4-Isopropyl-5-methylhexa-2,4-dien-1-ol	C10H18O
5	42.944	TIC	19187774	3.32	3786188	2.14	2,3-Squalene-epoxy	C30H50O
6	43.013	TIC	13851345	2.39	3818540	2.16	Squalene	C30H50
7	43.094	TIC	26363816	4.56	5569989	3.15	Squalene	C30H50

Quantitative dynamics of terpene compounds at different developmental stages of M. viciae

The contents of four major terpene components were investigated at different developmental stages of aphid: 1st -instar, 2nd -instar, 3rd -instar, 4th -instar and adult. The results showed that the content of (-)-β-

pinene increased rapidly from the 1st - to 2nd -instars, and the content of (*E*)-β-farnesene had a distinct increase from the 2nd - to 3rd -instars, while the contents of (+)-limonene and (-)-α-pinene had a substantial increase from the 3rd - to 4th -instars (Fig. 4 top). All components remained at a high level in the 4th -instar nymph. As a general trend, the contents of all monoterpene components increased with continuous development, while the content of (*E*)-β-farnesene displayed a different trend. The proportions of different terpenoid components were also calculated for different developmental stages (Fig. 4 bottom), showing that the proportion of (-)-β-pinene was the highest (> 81%) from the 2nd -instar to adult stages. The ratios of the four major terpene components at the 3rd - and 4th -instar stages were used for preparing the two mixtures for behavioral assays.

Behavioral responses of M. viciae to single and mixed terpene compounds

The behavioral responses of *M. viciae* to single terpene components and their mixture were measured in the three-compartment olfactometer. The two mixtures of (-)-α-pinene, (-)-β-pinene, (+)-limonene and (*E*)-β-farnesene were prepared in that order at the ratios of 1:44.4:6.5:2.2 (Mix I) and 1:18.4:1.3:0.8 (Mix II) according to the proportions of the four major terpene components at the 3rd - and 4th -instar stages. Behavioral responses were categorized as four types: NR (no response, BIV < 20%), W (weak, 20 < BIV < 40%), M (moderate, 40% < BIV < 60%) and S (strong, BIV > 60%) (Hieu et al., 2014; Khashaveh et al., 2020). The results showed that the repellent activities of the compounds tested varied at different concentrations (Table 3 and Fig. 5). Specifically, (-)-α-pinene, (-)-β-pinene, (+)-limonene and (*E*)-β-farnesene all displayed repellent activity to some extents at the concentration of 10.0 μg/μL: (-)-α-pinene had the strongest activity with a highest BIV value (Type S); (-)-β-pinene, Mix I and Mix II exhibited moderate repellent activity (M), and (+)-limonene and (*E*)-β-farnesene showed merely weak activity (W). Similarly, under the concentration of 1.0 μg/μL, (-)-α-pinene, (-)-β-pinene, Mix I and Mix II all showed moderate repellent activity, and (+)-limonene and (*E*)-β-farnesene had only weak repellent activity. In contrast, under the concentration of 0.1 μg/μL, no behavioral response or merely weak repellent activity were observed for all four single compounds. As a single component, (*E*)-β-farnesene displayed only weak repellent activity at all tested concentrations. Nevertheless, the two mixtures showed a significant repellent activity against *M. viciae* ($F_{17, 72}$=5.748, P< 0.05) at the low concentration of 0.1 μg/μL.

Table 3
Behavioral index values (BIV) of *Megoura viciae* in response to single and mixed terpene compounds

Compound	Concentration (μg/μL)	BIV (%)	BA
(-)-α-Pinene	10.0	67.85 ± 4.52a	Repellent (S)
(-)-β-Pinene		42.55 ± 9.52 abcde	Repellent (M)
(+)-Limonene		23.49 ± 6.37 cde	Repellent (W)
(*E*)-β-farnesene		20.33 ± 2.43 cde	Repellent (W)
Mix I		58.19 ± 2.69 ab	Repellent (M)
Mix II		43.55 ± 8.39 abcde	Repellent (M)
(-)-α-Pinene	1.0	34.81 ± 12.51 bcde	Repellent (M)
(-)-β-Pinene		38.67 ± 4.94 abcde	Repellent (M)
(+)-Limonene		23.49 ± 6.39 cde	Repellent (W)
(*E*)-β-farnesene		27.94 ± 6.28 bcde	Repellent (W)
Mix I		43.59 ± 4.80 abcde	Repellent (M)
Mix II		49.00 ± 7.14 abcd	Repellent (M)
(-)-α-Pinene	0.1	16.41 ± 8.73 de	NR
(-)-β-Pinene		12.89 ± 6.34 e	NR
(+)-Limonene		13.27 ± 2.45 e	NR
(*E*)-β-farnesene		22.53 ± 1.56 cd	Repellent (W)
Mix I		51.27 ± 3.94 abc	Repellent (M)
Mix II		40.95 ± 1.90 abcde	Repellent (M)

Different letters at the same concentration indicate significant difference between different compounds (Tukey's test, $P < 0.05$), while the same letters indicate no significant difference between the compounds. BA, behavioral activity. S, strong; M, moderate; W, weak; NR, no response. Mix I: (-)-α-pinene:(-)-β-pinene:(+)-limonene:(*E*)-β-farnesene = 1:44.4:6.5:2.2; Mix II: (-)-α-pinene:(-)-β-pinene:(+)-limonene:(*E*)-β-farnesene = 1:18.4:1.3:0.8.

Discussion

GC-MS analysis of the terpenoid compounds in *M. viciae* identified four major terpene components, including (-)-β-pinene (49.74%), (*E*)-β-farnesene (32.64%), (-)-α-pinene (9.42%) and (+)-limonene (5.24%), in addition to some minor terpenoid components (3.14%). Compared with the previously reported data (Francis et al., 2005), the proportion of (*E*)-β-farnesene measured here was much higher (32.64% vs 14.2%), while the proportion of (-)-β-pinene was much lower (49.74% vs 74.0%). The aphid samples used for GC-MS analysis

were composed of winged and wingless forms at different developmental stages in both studies. Thus the difference in the proportions of terpenoid components in *M. viciae* might be caused by either endogenous or exogenous factors. As a possible exogenous factor, the devices used in two studies were different; as the endogenous factor, the aphids analyzed were different: it was highly probable that the compositions of terpenoid components differentiated in the two different geographic populations of *M. viciae*. Therefore, the ecological significance of our results needs further investigation.

Quantitative analysis of the dynamics of the four major terpene components in *M. viciae* during different developmental stages revealed different patterns of change trend in the monoterpene and sesquiterpene components: the monoterpenes increased with continuous development, while the latter reached peak at the 3rd -instar. We also found that all terpenoid components remained at a high level in the 4th -instar; the proportion of (-)-β-pinene remained the highest (> 81%) from the 2nd -instar to adult stages. This is the first report of the temporal dynamics in the composition of terpenoid components in an aphid species. These data formed the basis for the preparation of volatile mixtures for olfactory choice assays.

Behavioral assays revealed that the repellent activities of single components were concentration-dependent but the mixtures not: all single components showed a repellent activity against *M. viciae* to some extents at 1.0 μg/μL or above, but displayed no or merely weak activity at 0.1 μg/μL. (-)-α-pinene showed a strong repellent activity at 10.0 μg/μL, a moderate activity at 1.0 μg/μL, but no response at 0.1 μg/μL, while the two mixtures showed a moderate repellent activity against *M. viciae* at all tested concentrations. Under all concentrations tested, (+)-limonene and (*E*)-β-farnesene displayed only weak activity or no response. Our results suggested that (-)-α-pinene and (-)-β-pinene were the major active components of alarm pheromone in *M. viciae*, but the volatile mixtures of the single terpene components in a specific ratio play a key role in the alarm behavior of *M. viciae*, as a moderate repellent activity remained in the mixtures at 0.1 μg/μL although not in single components. In a previous study, five terpene compounds, *i.e.*, (-)-α-pinene, (±)-α-pinene, β-pinene, (+)-limonene and (*E*)-β-farnesene, were tested, showing that (±)-α-pinene, β-pinene and (*E*)-β-farnesene as single components were not repellent against *M. viciae*, although (-)-α-pinene, (+)-limonene and a mixture containing 14.2% (*E*)-β-farnesene, 11.8% (-)-α-pinene, 74% β-pinene showed a significant repellent activity against the aphids (Bruno et al., 2018). Their data indicated that (-)-α-pinene and (+)-limonene were the major active components of alarm pheromone in *M. viciae*, and similarly, the mixture in a ratio prepared according to the proportions of different terpenoid compounds determined by Francis et al. (2005) played a significant role in the alarm behavior of *M. viciae*. The finding that (-)-α-pinene was among the major alarm pheromone components was consistent between our study and the previous study; what's new in our study is that we found a concentration-dependent manner of the alarm pheromone components and the synergistic action mode of single components based on the formulae calculated according to the proportions of single components at the 3rd - and 4th -instar stages when the aphids might be most responsive to alarm pheromone. Additionally, the previous study used a Y-tube olfactometer, while we used a three-compartment olfactometer. Our device simulated the natural selection environment by adding the host plants in the lateral compartments (Yu et al., 2019).

Last but not least, our results revealed that the terpenoid compounds were relatively scarce in the host plant *P. sativum*, containing none of the major aphid alarm pheromone components. This result added a

biochemical evidence to the notion that alarm pheromone was synthesized *de novo* in the aphid (Sun and Li, 2017), and it is unlikely that aphid alarm pheromone is taken directly from the host plant.

In summary, we identified four major terpene components in addition to some minor terpenoid components in *M. viciae*. Different types of components exhibited different patterns of change trend across the developmental process of aphid: the monoterpenes increased with continuous development, while the sesquiterpene reached peak at the 3rd -instar; all terpene components remained at a high level in the 4th -instar, with (-)-β-pinene accounting for the highest proportion during all developmental stages. Behavioral assays revealed that the repellent activities of single components varied in a concentration-dependent manner, but the mixtures maintained a significant repellent activity at all concentrations tested. Our results suggested that (-)-α-pinene and (-)-β-pinene were the major active components of alarm pheromone in *M. viciae*, but the mixtures of single components play a key role in the alarm behavior of *M. viciae*. Our study helps to understand the chemical ecology of insects and design alternative control strategies against aphids.

Declarations

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Data Availability

The data sets generated during and/or analyzed during the present study are available from the corresponding author. Raw data are partially included in the Suppl. Info. file.

Code Availability

Not applicable.

Ethics Approval

The study used insects, but none of the experiments raise ethical issues. All animal care and experimentation complied with the guidelines provided by the Association for the Study of Animal Behavior (ASAB) and the Animal Behavior Society (ABS).

Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Conflicts of Interest/Competing Interests

The authors have no conflicts of interest or competing interests to disclose.

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Figures

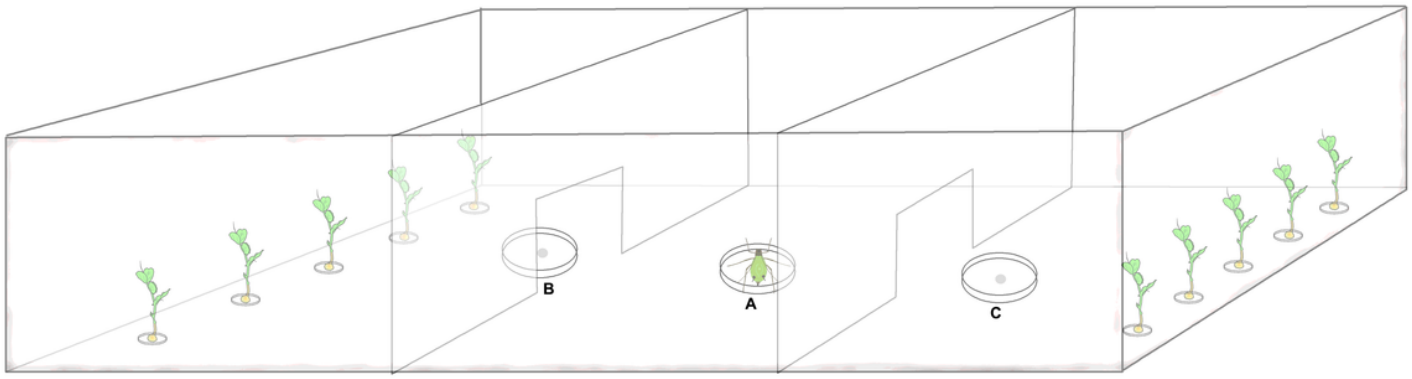

Figure 1

Composition of terpenoid compounds from M. viciae (A) and its host plant P. sativum (B). The terpenoid components are identified by GC-MS analysis. The proportions of different components are calculated based on the percentages of peak areas.

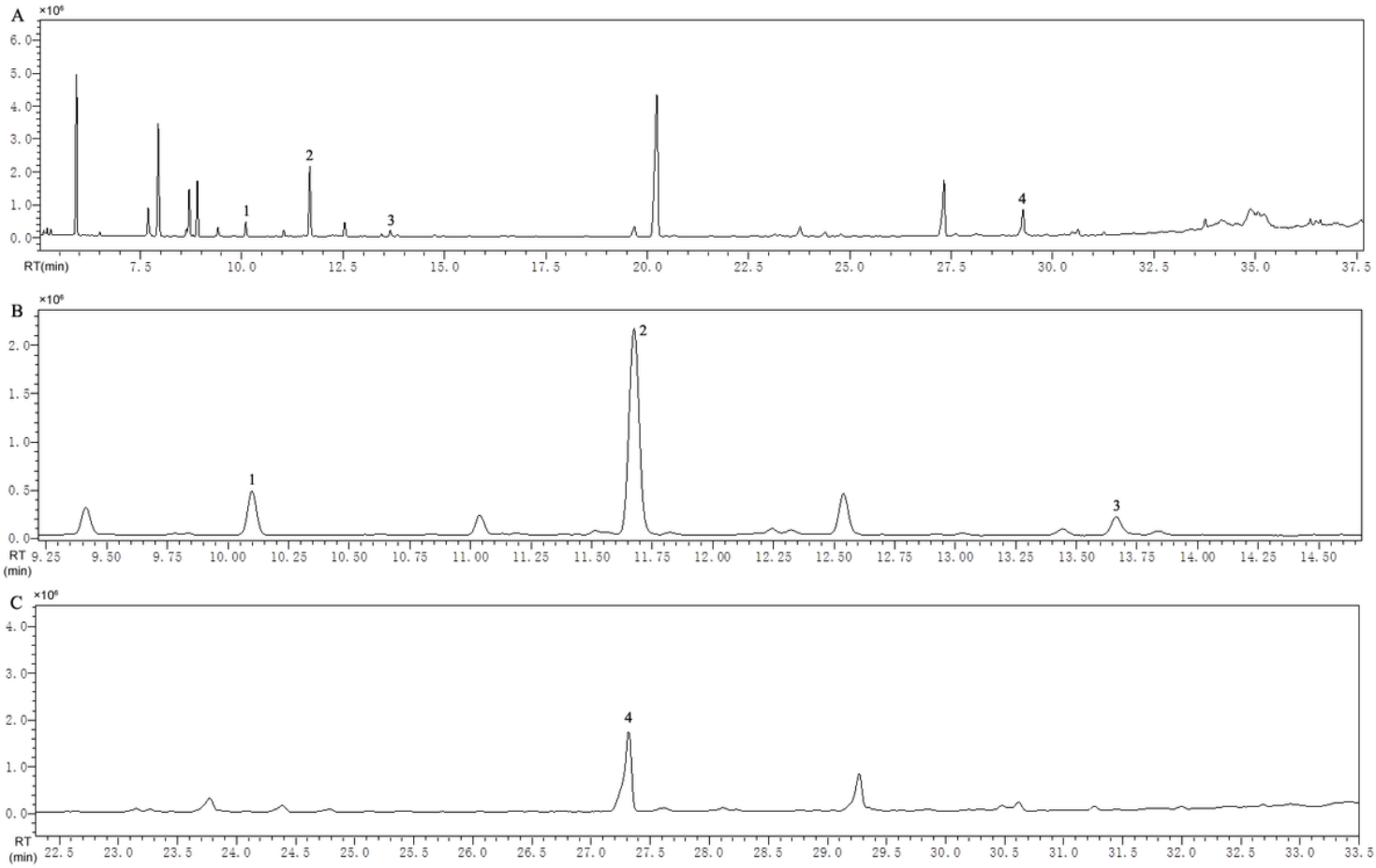

Figure 2

Gas chromatograms of four major terpene compounds from M. viciae. (A) The gas chromatogram of whole component analysis. (B) The local gas chromatogram of monoterpene analysis. (C) Local amplification of the gas chromatogram of (E)-β-farnesene. The peaks nos. 1, 2, 3 and 4 indicate (-)-α-pinene, (-)-β-pinene, (+)-limonene and (E)-β-farnesene, respectively.

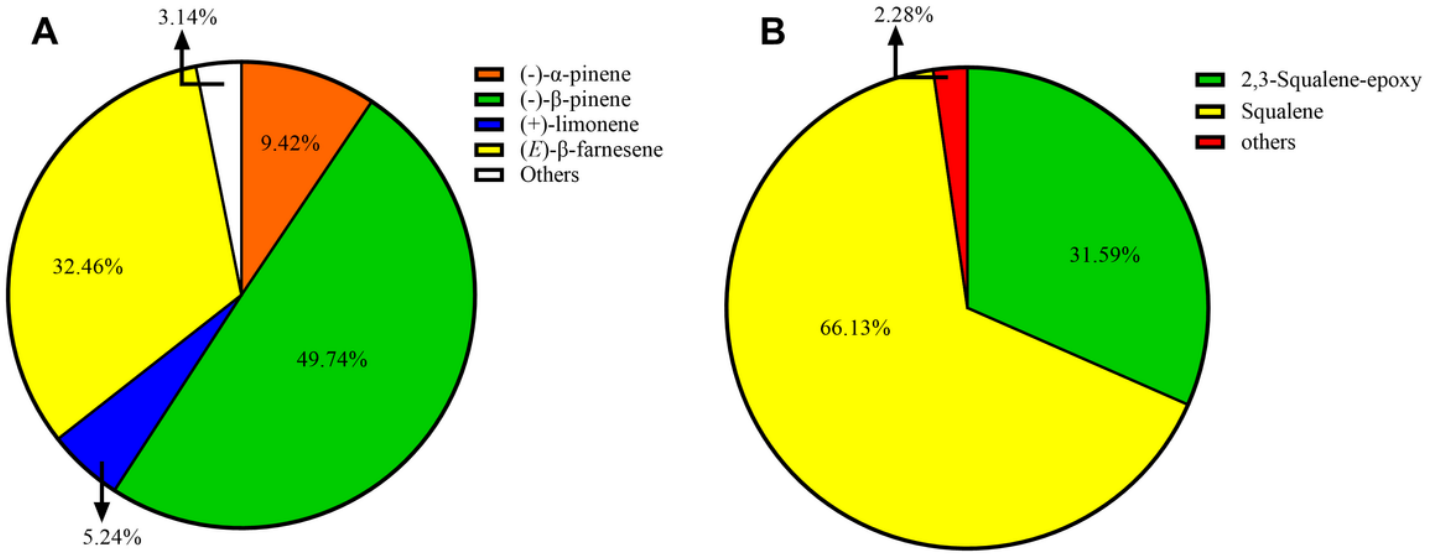

Figure 3

Temporal dynamics of terpene compounds at different developmental stages of M. viciae (top). The proportions of four major terpene components at the 1st-instar, 2nd-instar, 3rd-instar, 4th-instar nymphal and adult stages are also shown (bottom). The proportions of different components are calculated based on the percentages of peak areas.

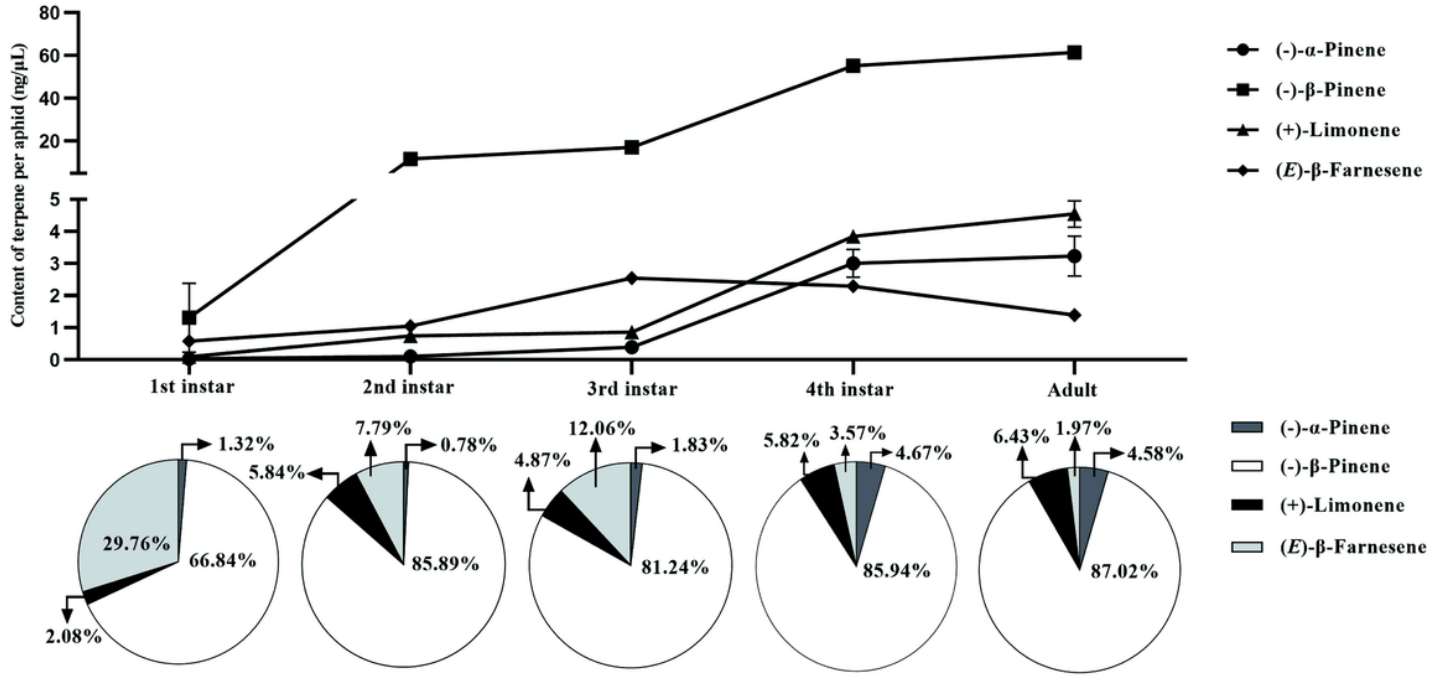

Figure 4

A schematic diagram showing the three-compartment olfactometer for behavioral assays of M. viciae. The olfactometer is composed of three compartments (7 cm × 13 cm × 5 cm for each). A group of 20 wingless 3rd-4th nymphs are released in Dish A (middle). Filter papers dipped with test compounds and mineral oil (negative control) are placed in Dish B and C, respectively. The numbers of aphids moving near to the host plants in the side compartments are recorded. Five biological repetitions are performed.

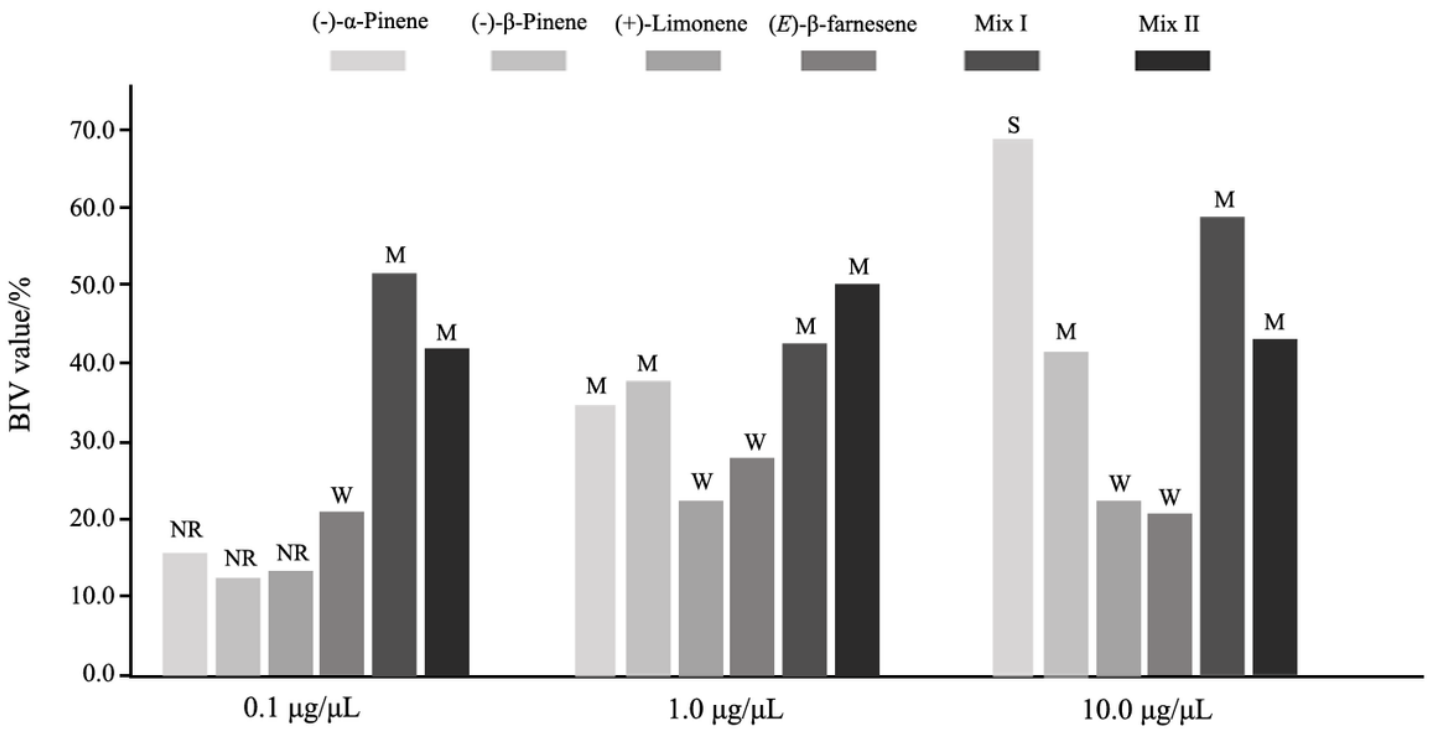

Figure 5

Behavioral responses of Megoura viciae to single terpene compounds and their mixtures. BIV, behavioral index value. S, strong; M, moderate; W, weak; NR, no response. Mix I: (-)-α-pinene:(-)-β-pinene:(+)-limonene: (E)-β-farnesene=1:44.4:6.5: 2.2; Mix II: (-)-α-pinene:(-)-β-pinene:(+)-limonene:(E)-β-farnesene=1:18.4:1.3:0.8.

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