Identification and Behavioral Assays of Alarm Pheromone in the Vetch Aphid Megoura viciae

Xuan Song1 · Yao-Guo Qin1 · Yue Yin1 · Zheng-Xi Li1

Received: 26 April 2021 / Revised: 14 June 2021 / Accepted: 30 June 2021 / Published online: 4 August 2021
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2021

Abstract

Aphids are destructive pests, and alarm pheromones play a key role in their chemical ecology. Here, we conducted a detailed analysis of terpenoids in the vetch aphid, Megoura viciae, and its host plant Pisum sativum using gas chromatography/mass spectrometry. Four major components, (-)-β-pinene (49.74%), (E)-β-farnesene (32.64%), (-)-α-pinene (9.42%) and (+)-limonene (5.24%), along with trace amounts of (+)-sabinene, camphene and α-terpineol (3.14%) were found in the aphid. In contrast, few terpenoids were found in the host plant, consisting mainly of squalene (66.13%) and its analog 2,3-epoxysqualene (31.59%). Quantitative analysis of the four major terpenes in different developmental stages of the aphid showed that amounts of the monoterpenes increased with increasing stage, while the sesquiterpene amount peaked in the 3rd instar. (-)-β-Pinene was the most abundant terpene at all developmental stages. Behavioral assays using a three-compartment olfactometer revealed that the repellency of single compounds varied in a concentration-dependent manner, but two mixtures [(−)-α-pinene: (−)-β-pinene: (E)-β-farnesene: (+)-limonene = 1:44.4:6.5:2.2 or 1:18.4:1.3:0.8], were repellent at all concentrations tested. Our results suggest that (−)-α-pinene and (−)-β-pinene are the major active components of the alarm pheromone of M. viciae, but that mixtures play a key role in the alarm response. Our study contributes to the understanding of the chemical ecology of aphids and may help design new control strategies against this aphid pest.

Keywords Alarm pheromone · Terpenoids · Behavioral assay · Mixture · Megoura viciae

Introduction

Insects use volatile chemicals to communicate during mating, aggregation, predation, alarm, and self-defense (Belén et al. 2015). Alarm pheromones play an important ecological role in insects, especially in aphids (Verheggen et al. 2010). Most aphids, both adults and nymphs, release alarm pheromones when attacked by natural enemies to warn conspecifics of danger (Kunert et al. 2008). Previous studies have shown 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 volatiles in 23 aphid species and found that (E)-β-farnesene was the main component in 16 species and a minor component in five; only two species, Euceraphis punctipennis and Drepanosiphum platanioides, did not release (E)-β-farnesene. They also reported in the vetch aphid, Megoura viciae (Aphididae: Hemiptera), a profile of volatiles composed of (E)-β-farnesene and several monoterpenes, 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) and found that (−)-α-pinene and (+)-limonene were the main active components of the alarm pheromone. They also tested a mixture of (E)-β-farnesene (14.2%), (−)-α-pinene (11.8%), and β-pinene (74%) (see Table S1), which showed repellent activity against M. viciae. Molecular studies showed that the recombinant odorant-binding protein MvicOBP3 could bind to all four alarm pheromone components of M. viciae albeit with a much higher affinity to (E)-β-farnesene (Kᵢ, 0.1 μM) than to β-pinene (Kᵢ, 2.3 μM), (−)-α-pinene (Kᵢ, 1.8 μM) or (+)-limonene (Kᵢ, 2.5 μM) (Northey et al. 2016). This suggests that the molecular
Megoura viciae feed exclusively on Fabaceae, 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 alarm pheromone components differentiates it from most other aphid species. It has been shown, by rearing aphids on artificial diets and antibiotics, that the cotton aphid, Aphis gossypii, synthesizes alarm pheromone itself (Sun and Li 2017). However, it is unclear whether other aphids also synthesize alarm pheromone de novo or sequester it from host plants or symbionts. Our group has been working on the biosynthesis of aphid alarm pheromone in species that utilize (E)-β-farnesene, including the green peach aphid Myzus persicae (Cheng and Li 2018; Zhang and Li 2008; 2012), A. gossypii (Ma et al. 2010; Sun and Li 2017; Sun and Li 2018) and the bird cherry-oat aphid Rhopalosiphum padi (Zhang and Li 2012; 2019; 2020). Thus, M. viciae provides an opportunity for a comparative study on species that use other compounds.

In this study, we first analyzed the composition of terpenoids in M. viciae and its host plant Pisum sativum using gas chromatography/mass spectrometry (GC/MS). Next, we investigated the quantities of the major terpenes in different developmental stages of M. viciae. Finally, we conducted a series of behavioral assays in a three-compartment olfactometer testing responses to single compounds and mixtures at different concentrations. Our study identified a novel set of alarm pheromone components in M. viciae.

**Materials and Methods**

**Culture of Aphids**

Aphids for our colony were provided by the Laboratory of Biological Control (Dr. Tinghui Liu, Hebei Agricultural University). They were maintained on P. sativum in the Laboratory of Insect Molecular Ecology at China Agricultural University and reared in a climate incubator (RXZ-300B, Ningbo, China) at 19 ± 1°C, 70 ± 5% RH with a photoperiod of 16L:8D.

**Collection of Terpenoids from M. Viciae and its Host Plant P. Sativum**

Aphids (overlapping developmental stages, n = 200) were collected in a 1.5 ml centrifuge tube, on ice, containing 500 μl of n-hexane. They were then homogenized and centrifuged at 4 °C for 30 min, and the supernatant transferred to a 2 ml vial for GC/MS analysis. A similar procedure was used to collect terpenoids from P. sativum seedlings (2.0 g).

**Identification of Terpenoids from M. Viciae and P. Sativum by GC/MS**

Samples were analyzed on an Agilent 6890–5973 (Agilent Technologies Inc., California, USA), equipped with a HP-5 capillary column (300 mm × 0.25 mm × 0.25 μm, Agilent, Santa Clara, USA). The column oven program was 40 °C for 1 min, followed by an increase to 130 °C at 4 °C.min⁻¹, maintained for 5 min, and then increased at 10 °C.min⁻¹ to 250 °C. The injector and ion source temperatures were set to 250 °C. The MS was operated with electron impact ionization (EI, 70 eV) and a scan range of m/z 35–650. Terpenes were identified by comparing retention times and mass spectra with standards (Sigma-Aldrich, Oakville, Canada). The quantity of each component was estimated by the peak area ratio of the sample to external standard of (-)-β-pinene (Magdalena and Henryk 2017). Three replicates were analyzed for each treatment. The proportions of single components were calculated as percentages of total terpenoids.

**Quantitative Analysis of Terpenes In Aphid Developmental Stages**

Aphids of the same developmental stage (n = 30), including 1st, 2nd, 3rd, 4th instars or adults, were homogenized in a 1.5 ml centrifuge tube containing 100 μl hexane. The supernatant was transferred to a vial for GC/MS analysis as described above. (-)-β-Pinene (purity > 99%; Sigma-Aldrich, Oakville, Canada) was used as the external standard. Three replicates were analyzed for each stage. The amount of each terpene was calculated based on the peak area ratio of the sample to standard.

**Behavioral Assays**

Behavioral assays were carried out in a three-compartment plexiglass olfactometer modified from a previous design (Khashaveh et al. 2020; Satyajeet et al. 2021; Yu et al. 2019) (Fig. 1). The olfactometer comprised three compartments (each 7 cm × 13 cm × 5 cm) connected by a door (3 cm × 3 cm) between two adjacent compartments. The samples, (-)-α-pinene, (-)-β-pinene, (+)-limonene and (E)-β-farnesene were each diluted to three concentrations (0.1, 1 and 10 μg/μl) with light mineral oil. Two mixtures (Mix I and Mix II) were prepared in ratios of 1:44.4:6.5:2.2 and 1:18.4:1.3:0.8 of the four terpenes, respectively. These mixtures corresponded to the ratios of the four terpenes in the 3rd and 4th instars, respectively. Light mineral oil was used as a negative control. Sample (B) and control (C) were placed in a petri dish (diam. 3.0 cm) near the door of the side compartments, with five host plants fixed in smaller
petri dishes (diam. 1.0 cm; covered with 10% agar) placed in the far side of the lateral compartments. Twenty wingless 3rd and 4th instars were introduced into the petri dish in the middle (A) and allowed to move freely for 30 min. A total of 100 nymphs was used to test preference to each sample. All behavioral assays were performed in the dark to avoid light interference. At the end of a test, the numbers of aphids crawling close to the host plants in the two lateral compartments were counted and a behavioral index value (BIV) calculated according to the formula

\[ BIV = \left( \frac{C - T}{C + T} \right) \times 100, \]

where C and T are the numbers of aphids in the control and sample compartments, respectively. Behavioral responses were categorized into 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).

### Data Analysis

Quantities of (-)-α-pinene, (-)-β-pinene and (+)-limonene and (E)-β-farnesene were compared using GraphPad Statistics version 8.0 (San Diego, USA) by one-way analysis of variance (ANOVA), followed by Tukey’s B multiple range test (\( P < 0.05 \)). Differences in BIVs were analyzed using SPSS Statistics version 21 (IBM) by ANOVA followed by Duncan’s test (\( P < 0.05 \)).

### Results

#### Identification of Terpenoids from M. Viciae and P. Sativum

Four major terpenes were detected by GC/MS in M. viciae, including three monoterpenes, (-)-α-pinene, (-)-β-pinene and (+)-limonene and the sesquiterpene (E)-β-farnesene (Table 1; peaks 1, 2, 3 and 6, respectively, Fig. S1). Stereochemistry was confirmed using reference standards and consistent with previous studies (Pickett and Griffiths 1980). Some minor peaks were also detected and tentatively identified as β-myrcene, (+)-sabinene, camphene, and α-terpineol, based on spectral matches with the NIST17s library. The proportions of compounds were calculated as (-)-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% of the mass (Fig. 2A). In contrast, terpenoids were relatively scarce in the plant and consisted mainly of squalene (66.13%) and its analog 2,3-epoxysqualene (31.59%) (Table 2 and Fig. 2B and Fig. S2). Some minor terpenoids were tentatively identified in P. sativum, including 4-isopropyl-5-methylhexa-2,4-dien-1-ol.

### Table 1

Gas chromatography/mass spectrometry analysis of terpenoids in Megoura viciae

| No | Retention time (min) | Name of terpenoid compound | Scan range (m/z) | % Peak area | Molecular formula | Kovats Index value |
|----|----------------------|----------------------------|------------------|-------------|-------------------|-------------------|
| 1  | 10.099               | (-)-α-Pinene               | 35–650           | 0.18        | C10H16            | 948               |
| 2  | 10.639               | Camphene                   | 35–650           | 0.01        | C10H16            | 943               |
| 3  | 11.562               | (+)-Sabinene               | 35–650           | 0.01        | C10H16            | 897               |
| 4  | 11.676               | (-)-β-Pinene               | 35–650           | 0.95        | C10H16            | 943               |
| 5  | 12.244               | β-Myrcene                  | 35–650           | 0.03        | C10H16            | 958               |
| 6  | 13.665               | (+)-Limonene               | 35–650           | 0.1         | C10H16            | 1018              |
| 7  | 19.977               | α-Terpineol                | 35–650           | 0.01        | C10H18O           | 1143              |
| 8  | 29.105               | (E)-β-Farnesene            | 35–650           | 0.62        | C15H24            | 1440              |

Fig. 1 Schematic showing the three-compartment olfactometer for behavioral assays of Megoura viciae. A group of 20 wingless 3rd and 4th instars 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.

Springer
2,4-pentadien-1-ol, 3-pentyl-, (2Z)-, limonene and cyclohexanol, 1-methyl-4-(1-methylethenyl)-, cis-, based on spectral matches with the NIST17s library.

Quantitative Analysis of Terpene Compounds in Aphid Developmental Stages

The contents of the four major terpenes in different developmental stages (1st, 2nd, 3rd, and 4th instar, and adult) of

Table 2 Gas chromatography/mass spectrometry identification of terpenoids in the host plant *Pisum sativum*

| No | Retention time (min) | Name of terpenoid | Scan range (m/z) | % Peak area | Molecular formula | Kovats Index values |
|----|----------------------|-------------------|-----------------|-------------|------------------|---------------------|
| 1  | 13.674               | Limonene          | 35–650          | 0.02        | C10H16           | 1018                |
| 2  | 22.52                | Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, cis-         | 35–650          | 0.01        | C10H18O          | 1158                |
| 3  | 27.43                | (2E)-3-Pentyl-2,4-pentadien-1-ol | 35–650          | 0.03        | C10H18O          | 1233                |
| 4  | 42.391               | 4-Isopropyl-5-methylhexa-2,4-dien-1-ol | 35–650          | 0.18        | C10H18O          | 1164                |
| 5  | 42.944               | 2,3-Squalene-epoxy | 35–650          | 3.32        | C30H50O          | 2955                |
| 6  | 43.094               | Squalene          | 35–650          | 6.95        | C30H50           | 2914                |
the aphid were investigated. The amount of (-)-β-pinene increased from 1st to 2nd instar, the amount of (E)-β-farnesene increased from 2nd to 3rd instar, while the amounts of (+)-limonene and (-)-α-pinene increased from 3rd to 4th instars (Fig. 3 top). All components were at a high level in 4th instars. As a general trend, the amounts of the monoterpens increased with development, while the amount of (E)-β-farnesene increased from 1st to 3rd instar, and decreased slightly by the adult. The percentages of terpenes were calculated for different developmental stages (Fig. 3 bottom); the most abundant component throughout was (-)-β-pinene (> 81%). The ratios of the four major terpenes for 3rd and 4th instars were used for preparing the two mixtures used in the behavioral assays.

Behavioral Responses of m. Viciae to Single Terpenes and Mixtures

The responses of M. vicieae to single terpenes and mixtures were recorded in the olfactometer (Table 3 and Fig. 4). (-)-α-Pinene, (-)-β-pinene, (+)-limonene and (E)-β-farnesene all repelled aphids to some extent at 10.0 μg/μl: (-)-α-pinene had the strongest repellency (Type S, BIV), (-)-β-pinene, Mix I and Mix II had moderate repellency (Type M), while (+)-limonene and (E)-β-farnesene were weakly repellent (Type W). Similarly, at 1.0 μg/μl, (-)-α-pinene, (-)-β-pinene, Mix I and Mix II were all moderate repellent, with (+)-limonene and (E)-β-farnesene having weak repellency. In contrast, at 0.1 μg/μl, no or weak

| 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 abcd     | Repellent (M) |
| (-)-α-Pinene   | 1.0                   | 34.81 ± 12.51 bcde | Repellent (M) |
| (-)-β-Pinene   | 38.67 ± 4.94 abde     | 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 abcd     | 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 ec       | 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 abcd     | Repellent (M) |

Different letters at the same concentration indicate difference between different compounds (Tukey’s test, P < 0.05), while the same letters indicate no 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

![Fig 3](https://example.com/figure3.png) Quantities of four terpenes in different developmental stages of Megoura vicieae (top). The proportions of the four terpenes are shown underneath.
repellency was observed for all single compounds, but the two mixtures had moderate repellency ($F_{17, 72} = 5.748$, $P < 0.05$).

**Discussion**

GC/MS analysis of *M. viciae* identified four major terpenes, namely (-)-β-pinene (49.74%), (E)-β-farnesene (32.64%), (-)-α-pinene (9.42%) and (+)-limonene (5.24%), in addition to some minor components (3.14%). Compared with previously reported data (Francis et al. 2005), the proportion of (E)-β-farnesene in our study was much higher (32.64% vs 14.2%), while the proportion of (-)-β-pinene much lower (49.74% vs 74.0%). In our study, the samples used for GC/MS analysis contained both winged and wingless aphids at different developmental stages. Thus, differences in aphid developmental composition might explain the difference in chemical proportions. Alternatively, it is possible the difference may be explained by the use of different populations of aphids. Indeed, the terpenoid composition of two geographical populations (Herault and East Pyrenees) of the aphid *Pinus nigra* from two different locations, Herault and the East Pyrenees, were found to be different (Bojovic et al. 2005). The ecological significance of our results needs further investigation.

Quantitative analysis of the four major terpenes in *M. viciae* across different developmental stages revealed changes, with the relative amounts of monoterpenes increasing with development, while the sesquiterpene amount peaked at 3rd instar. All the terpenes remained at a high level in the 4th instar, with (-)-β-pinene being most abundant at all stages. This is the first report of developmental changes in the composition of terpenoids in an aphid species.

Behavioral assays revealed that the repellency of the individual components was concentration-dependent but not so for the mixtures. All single components were repellent to *M. viciae* at 1.0 μg/μl or above but had no or very weak repellency at 0.1 μg/μl. By contrast, the two mixtures were moderately repellent to *M. viciae* at all concentrations tested. Overall, our results suggest that (-)-α-pinene and (-)-β-pinene are the major active components of the alarm pheromone of *M. viciae*, but that a specific blend of terpenes can play a key role in the alarm response. In a previous study testing five terpenes, (-)-α-pinene, (±)-α-pinene, β-pinene, (+)-limonene and (E)-β-farnesene, the authors found that (±)-α-pinene, β-pinene and (E)-β-farnesene individually did not repel *M. viciae*, although did so when combined (Bruno et al. 2018).

Lastly, our results revealed that the host plant *P. sativum* did not contain any of the aphid alarm pheromone components. This suggests that alarm pheromone is not sequestered directly from the plant but is synthesized de novo in *M. viciae* (Sun and Li 2017).

In summary, we identified four terpenes in *M. viciae* that exhibited changes in amount across the developmental stage. Behavioral assays revealed that the single compounds repelled aphids in a concentration-dependent manner, while specific mixtures of the four compounds were repellent at all concentrations tested. In addition to increasing our understanding of the chemical ecology of aphids, our work should help the design of alternative control strategies for *M. viciae*.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s10886-021-01297-4.

**Acknowledgements** We kindly acknowledge Dr. Tinghui Liu (Hebei Agricultural University) for providing aphids for starting our colony. This work was supported by the National Natural Science Foundation of China (Grant nos. 31972267 and 31772169).

**Funding** Dr. Li ZX received funding from the National Natural Science Foundation of China (Grant nos. 31972267 and 31772169).
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.

Declarations

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.

References

Belén C, Linda Marie R, Maria B, Hans RN, Nicolai V, Meyling BR, Peter A (2015) Habitat selection of a parasitoid meditated by volatile信息 on host and intraguild predator densities. Oecologia 179:151–162. https://doi.org/10.1007/s00442-015-3326-2

Bojovic S, Jurc M, Drazic D, Pavlovic P, Mitrovic M, Djurdjevic L, Belén C, Linda Marie R, Maria B, Hans RN, Nicolai V, Meyling BR, Rahel F, Dietze K (2018) Sensilla morphology and complex expression pattern of odorant binding proteins in the vetch aphid Megoura viciea (Hemiptera: Aphididae). Front Physiol 9:777. https://doi.org/10.3389/fphys.2018.00777

Cheng YJ, Li ZX (2018) Spatiotemporal expression profiling of the farnesyl diphosphate synthase genes in aphids and analysis of their associations with the biosynthesis of the aphid alarm pheromone. Bull Entomol Res 109:398–407. https://doi.org/10.1017/S000708007000706

Edwards LJ, Siddall JB, Dunham LL, Uden P, Kislow CJ (1973) Trans-β-farnesene, alarm pheromone of the green peach aphid, Myzus persicae (sulzer). Nature 241:126–127. https://doi.org/10.1038/241126b0

Francis F, Vandermeuten S, Verheugen F, Lognay G, Haubrege E (2005) Is the (E)-β-farnesene only volatile terpenoid in aphids? J Appl Entomol 129:6–11. https://doi.org/10.1111/j.1439-4488.2005.00925.x

Hieu TT, Jung JW, Kim SI, Ahn YJ, Yoon HW (2014) Behavioural and electroantennogram responses of the stable fly (Stomoxys calcitrans L.) to plant essential oils and their mixtures with attractants. Pest Manag Sci 70:163–172. https://doi.org/10.1002/ps.3547

Huang XZ, Xiao YT, Kollner TG, Zhang WN, Wu JX, Wu J, Guo YY, Zhang YJ (2013) Identification and characterization of (E)-β-caryophyllene synthase and α/β-pinene synthase potentially involved in constitutive and herbivore-induced terpene formation in cotton. Plant Physiol Biochem 73:302–308. https://doi.org/10.1016/j.plaphy.2013.10.017

Khashaveh A, An XK, Shan S, Xiao Y, Wang Q, Wang SN, Li ZB, Geng T, Gu SH, Zhang YJ (2020) Deodorization of an odorant receptor revealed new bioactive components for green mirid bug Apolygus lucorum (Hemiptera: Miridae). Pest Manag Sci 76:1626–1638. https://doi.org/10.1002/ps.6582

Kunert G, Schmook-Ortlepp K, Reissmann U, Creutzburg S, Weiser WW (2008) The influence of natural enemies on wing induction in Aphis fabae and Megoura viciea (Hemiptera: Aphididae). Bull Entomol Res 98:57–62. https://doi.org/10.1017/S000708007005391

Leroy PD, Wathelet B, Sabri A, Francis F, Verheugen FJ, Capella Q, Thonart P, Haubrege E (2011) Aphid-host plant interactions: does aphid honeydew exactly reflect the host plant amino acid composition? Arthropod-Plant Inte 5:193–199. https://doi.org/10.1007/s11829-011-9128-5

Ma GY, Sun XF, Zhang YL, Shen ZY (2010) Molecular cloning and characterization of a prenyltransferase from the cotton aphid, Aphis gossypii. Insect Biochem Mol Biol 40:561. https://doi.org/10.1016/j.ibmb.2010.05.003

Magdalena K, Henryk HJ (2017) In-tube extraction for the determination of the main volatile compounds in Physalis peruviana L. J Sep Sci 40:532–541. https://doi.org/10.1002/jssc.201600797

Northev T, Venturh H, Biasio FD, Chauvic FX, Cole A, Ribeiro KAL, Grossi G, Falabella P, Field LM, Keep LH, Zhou JJ (2016) Crystal structures and binding dynamics of odorant-binding protein 3 from two aphid species Megoura viciea and Nasonovia ribisnigri. Sci Rep 6:24739. https://doi.org/10.1038/srep24739

Pickett JA, Griffiths DC (1980) Composition of aphid alarm pheromones. J Chem Ecol 6:349–360. https://doi.org/10.1007/BF01402913

Satyajeet G, Anusha LKK, Kaveri D, Jean-Marie B, Renee MB (2021) The scent of life: Phoretic nematodes use wasp volatiles and carbon dioxide to choose functional vehicles for dispersal. J Chem Ecol 47:139–152. https://doi.org/10.1007/s10886-021-01242-5

Sun ZJ, Li ZX (2017) Host plants and obligate endosymbionts are not the sources for biosynthesis of the aphid alarm pheromone. Sci Rep 7:6041. https://doi.org/10.1038/s41598-017-06465-9

Sun ZJ, Li ZX (2018) The terpenoid backbone biosynthesis pathway directly affects the biosynthesis of alarm pheromone in the aphid. Insect Mol Biol 27:824–834. https://doi.org/10.1111/imb.12521

Sun XF, Li ZX (2012) In silico and in vitro analyses identified three amino acid residues critical to the catalysis of two aphid farnesyl diphosphate synthase. Protein J 31:417–424. https://doi.org/10.1007/s10930-012-9421-x

Sun CX, Li ZX (2019) Production of alarm pheromone starts at embryo stage and is modulated by rearing conditions and farnesyl diphosphate synthase genes in the bird cherry-oat aphid Rhopalosiphum padi. Bull Entomol Res 109:821–830. https://doi.org/10.1017/s0007080019000154

Sun CX, Li ZX (2020) Biosynthesis of aphid alarm pheromone is modulated in response to starvation stress under regulation by the insulin, glycolysis and isoprenoid pathways. J Insect Physiol 128:101474. https://doi.org/10.1016/j.jinsphys.2020.101474

Verheugen FJ, Haubrege E, Mescher MC (2010) Alarm pheromone-chemical signaling in response to danger. Vitam Horm 81:63–76. https://doi.org/10.1016/b978-012-370037-0.00009-2

Yu XD, Jia DY, Duan PF (2019) Plasmid engineering of aphid alarm pheromone in tobacco seedlings affects the preference of aphids. Plant Signal Behav 14:1–4. https://doi.org/10.1080/15592324.2019.1588669

Zhang YL, Li ZX (2008) Two different farnesyl diphosphate synthase genes exist in the genome of the green peach aphid, Myzus persicae. Genome 51:501–510. https://doi.org/10.1139/G08-037

Zhang YL, Li ZX (2012) Functional analysis and molecular docking identify two active short-chain prenyltransferases in the green peach aphid, Myzus persicae. Arch Insect Biochem Physiol 81:63–76. https://doi.org/10.1002/arch.21032