Glycyrrhiza Volatiles Mediate the Host Preference of *Bemisia tabaci* (Hemiptera: Aleyrodidae)[1]

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Abstract Licorice of the taxonomic genus *Glycyrrhiza* is a traditional Chinese medicinal plant. Its large-scale cultivation is threatened by insect pests, including the whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae). Our study (1) examined the preference of *B. tabaci* for *G. uralensis* Fischer ex de Candolle, *G. glabra* L., and *G. inflata* Batalin through caged trials; (2) identified the volatile organic compounds (VOCs) released by the three *Glycyrrhiza* species using headspace gas chromatography–mass spectrometry (HS-GC-MS); and (3) screened the olfactory behavior responses of *B. tabaci* to the key VOCs identified. The caged host preference trial showed that *B. tabaci* had a clear preference for *G. uralensis* over *G. inflata* and *G. glabra*. Through HS-GC-MS analysis, we identified 26, 33, and 31 VOCs from *G. uralensis*, *G. glabra*, and *G. inflata*, respectively. (3*Z*)-Hexen-3-ene-1-yl acetate, *trans*-3-hexen-1-ol, *cis*-3-hexen-1-ol, 3-carene, and β-pinene were selected for further olfactory response assays on the basis of variable importance projection values and quantity differences. From these, we learned that *cis*-3-hexene-1-ol and *trans*-3-hexene-1-ol, abundant in *G. uralensis*, attract *B. tabaci*, whereas 3-carene and β-pinene, abundant in *G. inflata*, repel *B. tabaci*. The different VOCs from these three species of licorice may be primary factors affecting the preference for and feeding on cultivated licorice by *B. tabaci*. Our results will facilitate studies on the potential of using selected VOCs for managing *B. tabaci*, perhaps in push–pull strategies of their use against the pest.

Key Words volatile organic compounds, *Bemisia tabaci*, *Glycyrrhiza*, host selection

The whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), is one of the most destructive agricultural pests, resulting in massive losses to various crops worldwide (Farina et al. 2022). *Bemisia tabaci* is a species complex consisting of at least 31 cryptic species (Qin et al. 2013) that have been reported on more than 900 plant species, including food, ornamental plants, and other economically important plants, in both field and greenhouse production systems (Gelman et al. 2005, Lee 2020). Losses result from direct feeding, transmitted viruses, plant physiological disorders, honeydew contamination, and associated fungal growth (Oliveira et al. 2001), with the greatest damage associated with the Mediterranean (Q biotype) and Middle East–Asia Minor 1 (B biotype) (Qin et al. 2013). Whiteflies

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are a devastating pest in agriculture in China, including the Xinjiang Uyghur Autonomous Region, where vegetable, fruit, and medicinal plant industries are prevalent (Gorman et al. 2007). Chemical pesticides remain the primary means of controlling *B. tabaci*; however, their extensive use poses health and environmental risks (Liu et al. 2016), including unacceptable levels of chemical residues on plants as well as in the environment and food chains, sublethal effects on nontargets, and ecosystem degradation (Sharma et al. 2020).

The “push–pull” management method is a widely used strategy for controlling insect populations. It is intended to encourage ecologically favorable biological techniques and reduce the dependency on chemical control (Alkema et al. 2019). Plant volatile organic compounds (VOCs) are potential ideal materials for implementing the push–pull strategy. VOCs, which are volatile secondary metabolites produced by plants, are ubiquitous organic compounds with specific allelochemicals ubiquitous in plants (Dudareva et al. 2013). VOCs are not directly involved in the growth, development, or reproduction of plants, yet they play a crucial functional role in the interactions between plants and insects (Schwery et al. 2023) in that they attract insect pollinators (Junker 2016, Muhlemann et al. 2014), repel herbivorous insect feeding or oviposition (Heil 2008, Zhou and Jander 2022), and recruit natural enemies of herbivores to prey upon or parasitize pests (Liu et al. 2019). In contrast, insects typically possess many extremely sensitive chemoreceptors (sensilla) and depend on olfactory communication for basic functions such as foraging, mating, and oviposition (Haverkamp et al. 2018). Insects prefer host plants with a specific composition, content, and ratio of plant volatiles (Ahmed et al. 2019). VOCs, therefore, are promising compounds for the push–pull management of agricultural pests.

Licorice plants of the genus *Glycyrrhiza* (Rosales: Fabaceae) are highly valued medicinal and commercial herbs in China and have an unchallenged standing in the pharmaceutical sector (Batiha et al. 2020). Chinese medicine frequently uses the underground plant portions and roots of licorice, whereas the food and daily chemical industries make extensive use of the aerial stems and leaves (Ding et al. 2022, Rizzato et al. 2017). Among the several *Glycyrrhiza* species, *G. uralensis* Fischer ex de Candolle, *G. glabra* L., and *G. inflata* Batalin are the three main species of medicinal plants recognized by the Chinese Pharmacopoeia (National Pharmacopoeia Committee 2020). *Glycyrrhiza inflata* is also a national class II key protected plant species owing to a rapid decline in wild populations and serious degradation of its habitat. As a result, the therapeutic qualities of *Glycyrrhiza* have been well documented and investigated, and artificial cultivation is currently the primary method for obtaining *Glycyrrhiza* owing to growing market demand. However, large-scale cultivation also poses an increased risk of insect pests such as *B. tabaci* (Wu 2009), with densities of $>50/\text{cm}^2$ of *Glycyrrhiza* the field (Zhang et al. 2014).

Of interest, in our preliminary laboratory observations *B. tabaci* showed an obvious feeding preference for *G. uralensis* over *G. inflata* and *G. glabra*. There are differences in the composition and content of VOCs between different plant species (Matsui and Engelberth 2022). Therefore, we hypothesized that *B. tabaci* was either attracted by chemical substances emitted from *G. uralensis* or repelled by chemicals emitted from the other two *Glycyrrhiza* species, and that we could identify several specific compounds with exact regulatory roles (attraction or repulsion) in the behavior mediation of *B. tabaci.* To test our hypothesis, we systematically evaluated the selection behavior of *B. tabaci* among the three *Glycyrrhiza* species, collected and identified VOCs from the three species, and verified the specific roles of the compounds according to our screening criteria and preference tests. Little is known about how volatiles from different *Glycyrrhiza* species affect *B. tabaci* host selection. This study aimed to identify *Glycyrrhiza* cultivars with attractants or repellents that could be utilized to manage *B. tabaci*. We hypothesized that volatiles from *Glycyrrhiza* species influence host selection by *B. tabaci*. This change in selection would allow the identification of attractants or repellents for *B. tabaci* in *Glycyrrhiza* volatiles. The results of our study will provide a reference for controlling *B. tabaci* in the field using the push–pull strategy, a significant baseline for effective protection and management of the vulnerable *G. inflata* species.

Plant and insect colonies. Seeds of *G. uralensis*, *G. glabra*, and *G. inflata* were provided by the Key Laboratory of Xinjiang Phytomedicine Resource and Utilization, Ministry of Education, Shihezi University, Xinjiang, China. The seeds were first steeped with 98% H_2SO_4 for 40 min to break the seed coat, then rinsed three times with sterile water and disinfected for 7 min by soaking in 0.1% $HgCl_2$. Seeds were soaked in distilled water for 12 h at 26°C and then planted three to a pot (15-cm diameter) filled with preirrigated nutrient soil, vermiculite, and perlite (1:1:1). Seedlings and plants were maintained in nylon cages (80 mesh, 60 × 60 × 60 cm) in climate chambers at 25 ± 2°C, 40% relative humidity, and on a 14:10-h light:dark photoperiod. Plants were watered once a week and fertilized every month with Hoagland's nutrient solution (Kang and van Iersel 2002).

Bemisia tabaci colonies were maintained on susceptible tobacco plants in nylon cages (80 mesh, 60 × 60 × 60 cm) for several generations in the laboratory. Plants were periodically replaced or added to the cages to provide a consistent and healthy supply of host plants.

Chemical standards, such as *cis*-3-hexen-1-ol (98%, CAS 928-96-1), *trans*-3-hexen-1-ol (97%, CAS 928-97-2), (3*Z*)-hex-3-en-1-yl acetate (98%, CAS 3681-71-8), 3-carene (>90%, CAS 13466-78-9), β-pinene (98%, CAS 18172-67-3), and *N*-hexane (AR, CAS 110-54-3), were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd., Shanghai, China.

Host preference. One plant each of *G. uralensis*, *G. inflata,* and *G. glabra* with uniform blooms was placed in random arrangements in a nylon cage (60 × 60 × 60 cm). Two-hundred *B. tabaci* adults (female-to-male ratio of approximately 1:1) were released into the center of the cage after starving for 4 h. The number of landings of *B. tabaci* adults on each plant was counted at 3, 6, 12, 24, 36, 48, 60, and 72 h. This procedure was replicated three times.

VOC collection and analyses. The VOCs of the three *Glycyrrhiza* species were analyzed using an Agilent 19091S-433UI headspace gas chromatography and mass spectrometry (HS-GC-MS) instrument with three repetitions per species. Fresh leaves were first excised from a healthy plant, weighed accurately at 0.50 g, cut into pieces, and placed in 20-ml HS bottles that were capped and sealed in the sample tray. VOC emission from the sample was detected at an equilibrium temperature of 95°C, an equilibrium time of 25 min, a quantification loop temperature of 115°C, a transmission line temperature of 135°C, an injection volume of 1 ml, and an injection time of 1 min.

GC was conducted in a HP-5MS capillary column of 30 m $\times$ 250 μm $\times$ 0.25 μm with an inlet temperature of 250°C, a split ratio of 5 to 1, and a heating program with a starting temperature of 60°C, retained for 2 min, increased to 200°C at a rate of 5°C/min, and held for 5 min. The carrier gas was high-purity helium (purity $\geq$ 99.999%) with a flow rate of 1 ml/min and a pressure of 8.2317 psi. The MS electron ionization source was operated at an ion source temperature of 230°C, quadrupole temperature of 150°C, electron ionization energy of 70 eV, and scanning range of 30–500 m/z.

SIMCA18 software was used to perform orthogonal partial least-squares discriminant analysis (OPLS-DA), principal component analysis (PCA), and variable importance in the projection (VIP). IBM SPSS Statistics software (version 23.0) was used for one-way analysis of variance. The volatile components with $P < 0.05$ and VIP ≥ 1 were selected for downstream testing (Qin et al. 2012).

MS data were searched using the NIST14 and Flavor databases for graphical searches and matched with the NIST database for analysis. The relative percentage of each compound was calculated using peak area normalization. All data were analyzed three times for mean, standard deviation, and level of significance using Excel and SPSS 23.0.

Olfactometer behavioral responses. On the basis of the HS-GC-MS volatiles analysis, the candidate compounds were prepared as 1, 10, and 100 μl/ml solutions to determine the behavioral responses of *B. tabaci* adults to those solutions. The assays were conducted in the dark at ambient room temperature using a Y-tube (angle of 75°, length of each of the three walls of 10 cm, and inner diameter of 1 cm). The Y-tube was attached to two glass odor source containers at either end. One of the source containers contained 1 ml of the compound to be tested; the other contained *N*-hexane (the *N*-hexane concentration was the same as that of the tested compound) as a control. *Bemisia tabaci* adults were counted after 10 min of observation, during which they were free to select between the two volatiles. If *B. tabaci* entered an arm and remained there for at least 15 s, the response was recorded as a choice; if it was unresponsive after 10 min, it was deemed unresponsive (Khelfane-Goucem et al. 2014).

Each compound was tested on 50 adult whiteflies, and each individual was tested once. After testing 25 adults, the Y-tubes were replaced with fresh ones, and the orientation of the arms was reversed. Three replicate selections were performed for each experiment. All Y-tubes and glass chambers were cleaned with anhydrous ethanol and distilled water and then dried at 120°C for 1 h to minimize the effects of chemical residues before they were used further.

Data analysis. All statistical analyses were performed using IBM SPSS Statistics 23.0 software. The chi-squared test was used to compare the host preference responses among the three *Glycyrrhiza* species and the olfactory response assays among the selected compounds. Multiple comparisons among the treatments were made using the one-way Duncan's new compound extreme difference method ($P < 0.05$), which was used to create graphs in Origin 2018 (Chen et al. 2018).

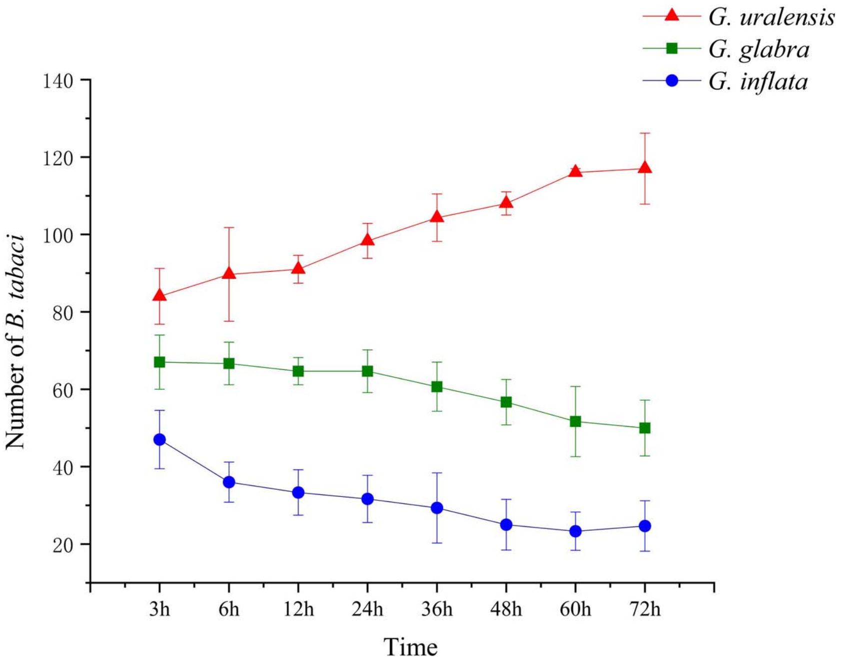

Fig. 1. Mean (± standard deviation) number of *Bemisia tabaci* adults on *Glycyrrhiza uralensis*, *G. glabra*, and *G. inflata* plants at different times after release of 200 *B. tabaci* in cages with plants.

Results

Host preference. The number of *B. tabaci* that selected *G. uralensis* gradually increased over time, whereas the number that selected *G. glabra* and *G. inflata* decreased. After 3 h and 6 h, *B. tabaci* showed a significant preference for *G. uralensis* (3 h: $F = 19.538$; df $= 2$; $P = 0.002$; 6 h: $F = 32.034$, df $= 2$; $P = 0.001$). Statistical significance ($P < 0.001$) was seen at the other observation times. More *B. tabaci* adults were attracted to *G. uralensis* than to either *G. glabra* or *G. inflata* at any given time point (Fig. 1). This showed a significant preference of *B. tabaci* to *G. uralensis* over *G. glabra* and *G. inflata*.

VOC identification. A total of 44 volatile components was detected from the three *Glycyrrhiza* species using HS-GC-MS analysis. These included 9 alcohols, 6 aldehydes, 12 esters, 2 acids, and 15 terpenes (Table 1). Of these, 31, 33, and 26 volatile compounds were identified from *G. glabra*, *G. inflata,* and *G. uralensis*, respectively. The most abundant volatile compounds detected in *G. glabra* were esters (38.87%), whereas terpenoids (86.54%) and alcohols (39.95%) were detected in *G. inflata* and *G. uralensis*, respectively (Table 1).

Principal component analysis (PCA) was used to further delineate the relative quantity of the major VOCs obtained in these samples. The PCA score plots (Fig. 2) showed that two principal components, PC1 and PC2, contributed 58.1% and 28.9%, respectively, of the total variance of the data set. All three *Glycyrrhiza*

Table 1. Volatile organic compounds detected in *Glycyrrhiza glabra, G. inflata,* and *G. uralensis.**

Type of Compound	RT (min)	Compound	CAS	*G. glabra***	*G. inflata***	*G. uralensis***	*P* value	VIP
Terpene (15)	7.509	3-Carene	13466-78-9	3.6 4 ± 3.90b	25.44 ± 7.19a	2.43 ± 1.31b	0.002	2.230
	7.867	(+)-α-Pinene	7785-70-8	NA†	8.45 ± 0.97a	0.50 ± 0.17b	0.000	1.382
	8.649	β-Pinene	18172-67-3	5.78 ± 5.21b	30.37 ± 6.78a	6.03 ± 6.62b	0.001	2.325
	9.709	p-Mentha-1,4-diene	99-85-4	NA	0.54 ± 0.05a	0.29 ± 0.14b	0.001	0.352
	10.087	(+)-Limonene	5989-27-5	NA	NA	1.29 ± 0.15	0.000	0.670
	12.495	Perillen	539-52-6	0.29 ± 0.04b	0.57 ± 0.04a	NA	0.000	0.370
	19.802	α-Copaene	3856-25-5	0.69 ± 0.13b	2.29 ± 0.83a	0.18 ± 0.07b	0.005	0.653
	20.968	*trans*-Caryophyllene	87-44-5	1.62 ± 0.51b	6.49 ± 0.94a	0.42 ± 0.32c	0.000	1.154
	21.521	(-)-α-Gurjunene	489-40-7	NA	1.47 ± 0.27	NA	0.000	0.582
	21.819	(*Z*)-β-Ocimene	13877-91-3	0.1 7 ±0.05b	0.09 ± 0.03c	0.31 ± 0.03a	0.002	0.235
	22.001	Alloaromadendrene	25246-27-9	NA	1.04 ± 0.70	NA	0.031	0.449
	22.636	Valencene	4630-07-3	NA	1.34 ± 1.41	0.27 ± 0.07	0.183	0.441
	22.835	Cyperene	2387-78-2	0.24 ± 0.02b	1.52 ± 0.60a	NA	0.004	0.558
	22.900	(-)-α-Cubebene	17699-14-8	0.62 ± 0.19b	2.00 ± 0.10a	NA	0.000	0.668
	23.455	(+)-δ-Cadinene	483-76-1	0.24 ± 0.02c	5.2 ± 1.48a	0.83 ± 0.15b	0.000	1.109
Alcohols (9)	3.336	4-Methylcyclohexanol	589-91-3	1.18 ± 0.30	NA	NA	0.000	0.621
	4.628	*trans*-1, 2-Cyclopentanediol	5057-99-8	0.13 ± 0.03b	NA	0.61 ± 0.15a	0.000	0.420

Table 1. Continued.

Type of Compound	RT (min)	Compound	CAS	*G. glabra***	*G. inflata***	*G. uralensis***	*P* value	VIP
	5.223	Hexan-1-ol	111-27-3	NA	0.21 ± 0.01	NA	0.000	0.222
	5.661	*trans*-3-Hexen-1-ol	928-97-2	NA	NA	20.78 ± 1.36	0.000	2.691
	5.843	*cis*-3-Hexen-1-ol	928-96-1	13.54 ± 3.31b	NA	18.56 ± 0.52a	0.000	2.022
	10.209	1,8-Cineole	470-82-6	0.46 ± 0.05	NA	NA	0.000	0.395
	10.895	4-Aminopentan-1-ol	927-55-9	0.23 ± 0.02	NA	NA	0.000	0.279
	12.015	*trans*-2-Hexen-1-ol	928-95-0	0.83 ± 0.09	NA	NA	0.000	0.508
	22.829	10-epi-*g*-Eudesmol	15051-81-7	0.37 ± 0.19b	1.67 ± 0.55a	NA	0.002	0.589
Esters (12)	6.646	Phenethyl butyrate	103-52-6	0.83 ± 0.33	NA	NA	0.003	0.512
	7.286	Vinyl butyrate	123-20-6	NA	0.40 ± 0.22	NA	0.014	0.287
	9.371	(3*Z*)-Hex-3-en-1-yl acetate	3681-71-8	30.04 ± 3.70a	1.44 ± 0.26b	4.80 ± 1.31b	0.000	2.942
	10.945	γ-Caprolactone	695-06-7	0.70 ± 0.04	NA	NA	0.000	0.488
	12.036	(*R*)-Linalyl acetate	115-95-7	0.57 ± 0.28b	1.04 ± 0.09ab	1.52 ± 0.05a	0.002	0.540
	13.267	*cis*-3-Hexenyl isobutyrate	41519-23-7	1.15 ± 0.31a	0.14 ± 0.03c	0.68 ± 0.19b	0.001	0.578
	14.483	*cis*-3-Hexenyl Butyrate	16491-36-4	3.13 ± 0.68a	0.12 ± 0.01c	1.38 ± 0.41b	0.001	0.833
	15.775	*cis*-3-Hexenyl 2-Methylbutanoate	53398-85-9	1.59 ± 0.37a	0.07 ± 0.06c	0.92 ± 0.14b	0.001	0.568

Table 1. Continued.

Type of Compound	RT (min)	Compound	CAS	*G. glabra***	*G. inflata***	*G. uralensis***	*P* value	VIP
	16.582	Heptyl 2-methylpropanoate	2349-13-5	NA	0.17 ± 0.03	NA	0.000	0.201
	17.470	Hexyl tiglate	16930-96-4	NA	0.16 ± 0.02	NA	0.000	0.194
	17.990	Hexyl hexanoate	6378-65-0	NA	0.21 ± 0.02	NA	0.000	0.221
	20.159	Linalyl formate	115-99-1	0.89 ± 0.09a	0.71 ± 0.21b	NA	0.001	0.538
Aldehydes (6)	3.660	Heptaldehyde	111-71-7	0.34 ± 0.02	0.80 ± 0.04	1.36 ± 1.91	0.563	0.530
	4.380	Octanal	24-13-0	0.64 ± 0.02a	0.21 ± 0.04c	0.36 ± 0.04b	0.000	0.326
	4.627	Hexanal	66-25-1	1.43 ± 0.99	0.96 ± 0.79	0.74 ± 0.19	0.545	0.331
	6.519	Isovaleraldehyde	590-86-3	NA	1.18 ± 0.62a	0.59 ± 0.06b	0.021	0.482
	6.604	Valeraldehyde	110-62-3	0.36 ± 0.05	1.16 ± 0.18a	0.13 ± 0.03b	0.000	0.472
	12.152	Nonanal	124-19-6	2.25 ± 1.30a	0.28 ± 0.48b	0.29 ± 0.14b	0.039	0.739
Acids (2)	3.003	Oxalic acid	144-62-7	2.47 ± 1.25a	NA	0.45 ± 0.03b	0.013	0.792
	3.184	Malonic acid	141-82-2	1.32 ± 1.27	0.26 ± 0.03	0.15 ± 0.02	0.180	0.510

* RT = retention time; CAS = CAS registry number; VIP = variable importance in projection.

** Mean (± SD) amounts of compound detected followed by same lowercase letter are not significantly different.

† NA = compound not detected.

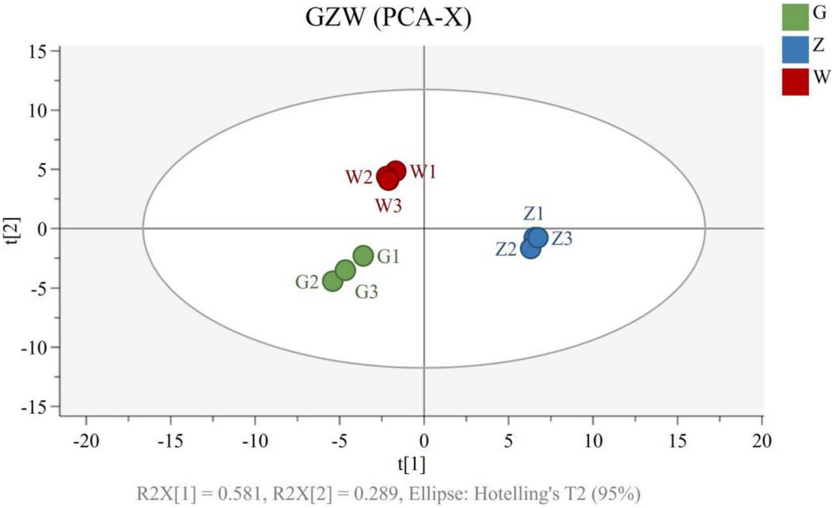

Fig. 2. Principal component analysis of the volatile organic compounds of *Glycyrrhiza uralensis* (W), *G. glabra* (G), and *G. inflata* (Z). Numbers after letters represent experimental repeats.

samples were within 95% of Hotelling's *T*-squared ellipse and were significantly separated into clusters. No outliers were found in these samples, indicating that the VOCs emitted by the three *Glycyrrhiza* species were significantly different.

VIP scores were used to calculate the contribution of each component. Using the 44 volatile components as dependent variables and the three *Glycyrrhiza* species as independent variables, effective differentiation of the three *Glycyrrhiza* species was achieved using OPLS-DA (Fig. 3). The explanatory rates of the model for the X and Y matrices are denoted by R^2x and R^2y, respectively, and the predictive power of the model is denoted by Q^2. The closer R^2 and Q^2 are to 1.0, the better the model's fit. Both values are close to 1, indicating that the model has a good degree of explanation. The fit index of the independent variable (R^2x) in this analysis was 0.925, the fit index of the dependent variable (R^2y) was 0.999, and the model prediction index (Q^2) was 0.977. R^2 and Q^2 exceeded 0.5, which is close to 1, indicating that the model fit results were acceptable. The reliability of the OPLS-DA model was tested using 200 cross-substitution tests. After the 200-time permutation test (Fig. 2), the intersection of the Q^2 regression line with the vertical axis was less than zero, which indicated that the model was not overfitted and was validated. The results were considered useful for the analysis of volatiles from different *Glycyrrhiza* species. Five differential volatile components were identified: *cis*-3-hexen-1-ol (VIP = 2.022), β-pinene (VIP = 2.325), *trans*-3-hexen-1-ol (VIP = 2.691), 3-carene (VIP = 2.230), and (3*Z*)-hex-3-en-1-yl acetate (VIP = 2.942).

Olfactometer assays. On the basis of our observations using synthetic compounds, *B. tabaci* adults were attracted to *cis*-3-hexen-1-ol, *trans*-3-hexen-1-ol, and (3*Z*)-hex-3-en-1-yl acetate (Fig. 4). At these three concentrations tested, *B. tabaci* was significantly attracted to *cis*-3-hexen-1-ol (Fig. 4a). At low

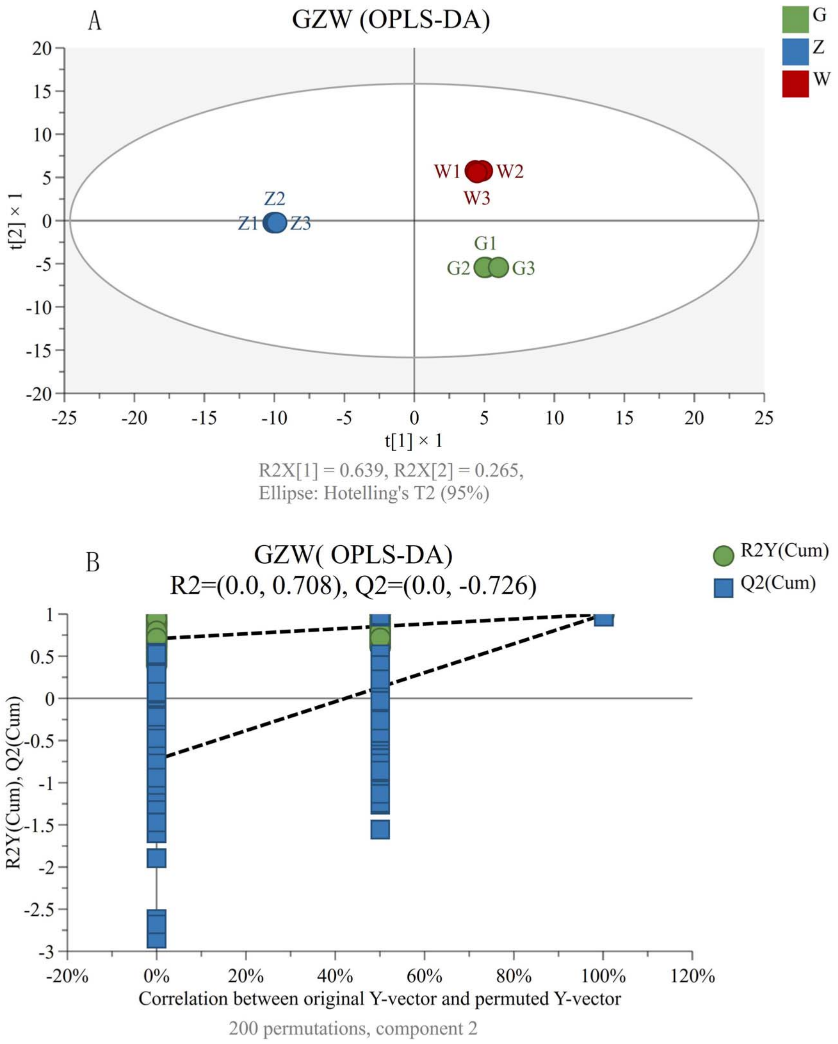

Fig. 3. Orthogonal partial least-squares discriminant analysis (OPLS-DA) (A) and permutation test of OPLS-DA model (B) of volatiles of *Glycyrrhiza uralensis* (W), *G. glabra* (G), and *G. inflata* (Z). Numbers after letters represent experimental repeats.

concentration (1 μl/ml), *B. tabaci* did not show response to *trans*-3-hexen-1-ol, but at medium (10 μl/ml) and high concentrations (100 μl/ml), notable response was observed (Fig. 4b). At low concentrations, (3*Z*)-hex-3-en-1-yl acetate exhibited a strong attraction to *B. tabaci*. At medium concentrations, there were no statistical differences; however, at high concentrations, it exhibited a significant repellent effect (Fig. 4c). 3-Carene and β-pinene also exhibited a repellent effect. 3-Carene was significantly repellent at all three concentrations tested (Fig. 4d). At the low

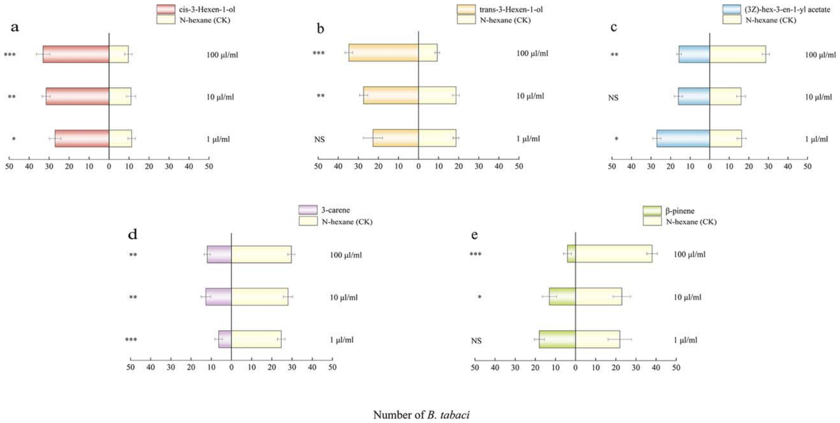

Fig. 4. Mean (± standard error) number of *Bemisia tabaci* responding to standard volatile organic compounds *cis*-3-hexen-1-ol vs. *N*-hexane (a), *trans*-3-hexen-1-ol vs. *N*-hexane (b), (3*Z*)-hex-3-en-1-yl acetate vs. *N*-hexane (c), 3-carene vs. *N*-hexane (d), and β-pinene vs. *N*-hexane (e) in Y-tube olfactometer behavioral assays performed with three concentrations. * indicates $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; NS = not significant.

concentration, β-pinene showed no significant effect but demonstrated significant repellent effects at medium and high concentrations (Fig. 4e).

Discussion

Plants have developed a range of direct or indirect self-defense mechanisms in response to insect attack and feeding (Wari et al. 2022). Plants release distinct volatiles or toxic secondary metabolites to repel insects. Indirect protection can also be established by recruiting natural enemies attracted to plant volatiles (Allmann and Baldwin 2010). We report, for the first time, the VOCs from *Glycyrrhiza* plants. The number and classes of VOCs from the three *Glycyrrhiza* species, including alcohols, aldehydes, esters, acids, and terpenes (Table 1), were similar to those of other Leguminosae plants (Khelfane-Goucem et al. 2014, Naghiloo et al. 2012). Most of the compounds were present in all three *Glycyrrhiza* species, and certain of the specific compounds were emitted from only one or two of the species. The relative abundances of the five major VOCs varied significantly.

Over a long period of coevolution, insects and plants have formed a variety of connections, and host selection by phytophagous insects is reflected in a range of behaviors, including identification and location (Qin and Wang 2001). Specific plant VOCs are significant stimuli that attract insects to locate their hosts and feed or oviposit on them (Lu et al. 2008). As expected, *B. tabaci* had a significant feeding choice for *G. uralensis*, which became more evident over time (Fig. 1). The morphology and growth time of the three *Glycyrrhiza* species were similar and did not impede *B. tabaci* feeding. Much of the selective differences were associated with plant VOCs, and the PCA and OPLS-DA results showed that the VOCs released from the three *Glycyrrhiza* species differed. On the basis of the VIP and content differences, we selected five VOCs with the highest contributions. Olfactometer behavioral experiments clearly showed that *cis*-3-hexen-1-ol, *trans*-3-hexen-1-ol, and (3*Z*)-hex-3-en-1-yl acetate attract *B. tabaci*, whereas β-pinene and 3-carene had repellent effects (Fig 4). Therefore, *B. tabaci* exhibits different selection preferences for the three *Glycyrrhiza* species.

First, *cis*-3-hexen-1-ol was found only in *G. uralensis* and *G. glabra* (although its proportion was higher in *G. uralensis*) and attracted *B. tabaci* more strongly with increasing concentrations. As the most common green leafy volatile, *cis*-3-hexen-1-ol is widely found in a variety of plants and plays a key role in plant–insect communication. It has a significant attractive effect on a wide range of insects, such as *B. tabaci*, *Plutella xylostella* L., leafhoppers, *Aphidius gifuensis* Ashmead, and *Empoasca flavescens* (Goethe) (Chen et al. 2019, Hasanuzzaman 2016, Niu et al. 2022, Song et al. 2021, Yan et al. 2023). Second, like observations with *Aphis craccivora* Koch (Webster et al. 2010), *trans*-3-hexen-1-ol was emitted only by *G. uralensis*. Higher concentrations were more attractive to *B. tabaci*, but low concentrations had no significant effect. This may be due to the possibility that the low concentrations did not reach the minimum threshold for insect detection. Finally, (3*Z*)-hex-3-en-1-yl acetate was present in the three *Glycyrrhiza* species, but its content varied (*G. inflata* > *G. uralensis* > *G. glabra*). However, (3*Z*)-hex-3-en-1-yl acetate attracted *B. tabaci* only at low concentrations (Fig 4c). Likewise, emitted by cotton, (3*Z*)-hex-3-en-1-yl acetate also showed strong attraction to *B. tabaci* at high concentrations (Cheng 2021). It has also been found to attract *Bactrocera dorsalis* and *Asias halodendri* (Wu et al. 2018, Yang et al. 2016). Together, these results indicate that the distribution and content of the three VOCs in *Glycyrrhiza* explain the attraction of *Be. tabaci* to *G. uralensis*.

On the contrary, certain VOCs can repel *Be. tabaci* adults. For example, β-pinene and 3-carene showed significant repellency at all three concentrations tested, except at a low concentration of β-pinene (Fig. 4d, e). β-Pinene extracted by Li et al. (2022) from the aromatic plants *Thymus pulegioides* L. and *Artemisia absinthium* L. repelled *Be. tabaci*, an outcome that corroborates our findings. In addition, 3-carene also showed repellent effects against *Eupithecia abietaria* (Goeze), *Eupithecia abietaria debrunneata* Staudinger, and *Dendroctonus pseudotsugae* Hopkins (Ross et al. 2022, Wang 2023), suggesting that β-pinene and 3-carene are broad-spectrum repellents that can be used for controlling a variety of insects. It also has been noted that a mixture of several repellent volatiles did not show stronger repellency than single volatiles, suggesting that volatile repellency is not simply cumulative but may even have an attractive effect (Zhao et al. 2012). Although attractant or repellent volatiles were present in the three *Glycyrrhiza* species, this led to different behavioral responses in *Be. tabaci*.

In conclusion, our study showed that *cis*-3-hexen-1-ol, *trans*-3-hexen-1-ol, and (3*Z*)-hex-3-en-1-yl acetate attract *Be. tabaci*, whereas β-pinene and 3-carene repel *Be. tabaci*. More important, we showed, for the first time, that *trans*-3-hexen-1-ol has strongly attractive effects on *Be. tabaci*. Nevertheless, all these experiments were conducted under laboratory conditions, and whether the compounds could be used to control *Be. tabaci* in the field requires further investigation.

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