Electroantennogram and Olfactory Behavioral Responses of *Trabala vishnou gigantina* (Lepidoptera: Lasiocampidae) to Six Pest-induced *Hippophae rhamnoides* volatiles

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Abstract: The moth *Trabala vishnou gigantina* Yang is a leaf-eating pest, and there have been severe outbreaks of it in *Hippophae rhamnoides* plantations in North China. The aim of this study is to investigate the influence of volatiles emitted by *T. vishnou gigantina*-infested *H. rhamnoides* on the behavioral responses of *T. vishnou gigantina*, with the ultimate goal of laying the groundwork for the development of plant-based elicitors. In this study, we investigate the chemical basis of pest–host dynamics between these species to inform novel pest control methods. After identifying differentially produced compounds between healthy and infested plants, we identified six critical volatile compounds and explore their attractant effects on *T. vishnou gigantina* imagoes using electroantennogram (EAG) and olfactory behavioral response experiments. The results showed that the EAG responses of *T. vishnou gigantina* imagoes differed not only among the six different pest-induced volatiles but also between different concentrations of the same volatile. The EAG responses to the pest-induced volatiles peaked at a stimulus concentration of 100 μg/μL, with Hexyl 2-methyl butyrate eliciting the highest EAG response. The test results of olfactory behavioral responses revealed significant differences in the olfactory behavioral responses of female and male imagoes to the six pest-induced volatiles. Hexyl 2-methyl butyrate produced the strongest attracting effect on both female and male imagoes, followed by Hexyl 2-methyl butyrate and longifolene. Hexyl 2-methyl butyrate produced a significant attracting effect on female imagoes, although there was no significant attraction to males.

Key words: *Trabala vishnou gigantina*; *Hippophae rhamnoides*; Volatiles; Electroantennogram; Olfactory behavioral

1. Introduction

Hippophae rhamnoides, a member of the Elaeagnaceae family, is distributed across Northwest China, Southwest China, North China, and other regions. It is highly resistant to cold, drought, wind, and sandy conditions. In Northwest China, it is widely used in desert greening. Its root system is developed in association with nitrogen-fixing bacteria, and it reduces wind speed and erosion, with positive effects on soil ecosystem (Yang et al. 2024). Consequently, it has extremely high practical value in soil and water conservation. Larvae of the moth *Trabala vishnou gigantina* Yang (Lepidoptera: Lasiocampidae) feed on *H. rhamnoides* leaf blades and leave only petioles, resulting in the failure of normal plant physiological functioning (Liu et al. 2021a). *H. rhamnoides* is used not only for food production but also for soil improvement. In recent years, *T. vishnou gigantina* outbreaks have occurred in sea buckthorn planting areas in Wuqi County, Shaanxi Province, and Zhidan County, Shaanxi Province, China, severely reducing plant vigor and threatening the healthy and sustainable development of the local *H. rhamnoides* industry (Liu et al. 2013).

Host volatiles are a major and important regulator in plant–pest nutrient systems (Zang 2021). Pest infestation can induce host plants to generate new volatiles or cause changes in the composition of host volatiles in order to lure or repel insects of the same or different species, thus affecting the growth, reproduction, migration, and population of pest species (Qian et al. 2024; Liu et al. 2021b). Elucidating the interactions between pests and pest-induced host volatiles provides an important basis for developing sustainable solutions to pest control (Arriola et al. 2020; Mitra et al. 2020). For example, Qiao et al. (2020) found that aphid-induced wheat volatiles can attract *Harmonia axyridis*, a natural enemy of aphids, making them an effective solution for pest control in agricultural fields. Therefore, studying the interactions between pests and host volatiles can promote the identification of compounds with attractant or repellent activity and provide a theoretical basis for further research (Turlings and Erb 2018). To date, several studies have investigated the host selection mechanism for *T. vishnou gigantina*, its bioecological characteristics, and prevention and control methods (Liu et al.2013, 2021a). However, no studies have examined the mechanism underlying the chemical communication between *T. vishnou gigantina* and *H. rhamnoides*. Hence, we

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analyzed the composition of *T. vishnou gigantina*-induced *H. rhamnoides* volatiles and identified a total of 34 compounds, with significant changes in volatile composition between healthy and infested *H. rhamnoides*. Six compounds that may significantly affect *T. vishnou gigantina* were identified, and electroantennogram (EAG) and olfactory behavioral response experiments were performed, to provide a theoretical basis for the prevention and control of *T. vishnou gigantina* using plant-derived attractants or repellents.

2. Materials and Research Method

2.1 Test insects

In mid-August 2023, approximately 200 cocoons of *T. vishnou gigantina* were collected from *H. rhamnoides* forests in Wuqi County, Shaanxi Province, China, and placed in an insect cage (100 × 40 × 50 cm). After the cocoons hatched, ~~and~~ male and female imagoes were distinguished (based on what characters any ref. please), healthy, equally sized imagoes were selected for experimental use.

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2.2 Collection and analysis of *H. rhamnoides* volatiles

2.2.1 Collection of *H. rhamnoides* samples

Fresh healthy and infested *H. rhamnoides* leaf blades were collected from the experimental site, placed in centrifuge tubes, quickly frozen with liquid nitrogen, labeled, and brought back to the laboratory for testing, with three sets of replicates for each group. The time for collecting the samples was the same as that for behavioral testing through a Y-tube olfactometer.

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2.2.2 Extraction and analysis of *H. rhamnoides* volatiles

The *H. rhamnoides* leaf samples were stored in a refrigerator at –80 °C; they were ground with liquid nitrogen during the operational test and mixed evenly. A 500 mg subsample was taken from each sample and placed into a headspace vial. A saturated NaCl solution was then added to prepare a 20 μL internal standard solution. Fully automated headspace solid phase microextraction HS-SPME (CTC Analytics AG) was used to extract samples for GC-MS analysis. Then, the internal standard solution was vibrated for 5 min at 60 °C, and a 120 μm Agilent SPME Fiber head（DVB/C-WR/PDMS）extraction head was inserted into the sample headspace vial for 5 min at 250 °C in a Fiber Conditioning Station (CTC Analytics AG) before sampling. The headspace was extracted for 15 min and resolved for 5 min at 250 °C, and then separation and identification were conducted by a GC-MS. The chromatographic column was a DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm, Agilent J&W Scientific, Folsom, CA, USA); the carrier gas was high-purity helium. Splitless sampling was conducted at a flow rate of 1.2 mL/min. The sample inlet port was heated to 250 °C, and the solvent was delayed for 3.5 min. The temperature was programmed as follows: 40 °C for 3.5 min, 10 °C/min to 100 °C, 7 °C/min to 180 °C, 25 °C/min to 280 °C, and hold at 280 °C for 5 min. The MS test conditions were as follows: EI ion source, ion source temperature of 230 °C, electron energy of 70 eV, quadruple rod temperature of 150 °C, MS interface temperature of 280 °C, and ion detection mode (SIM). The Total Ion Chromatogram (Total Ion Current [TIC]) of *H. rhamnoides volatiles* was used to obtain MS data using Mass Hunter software, and these data were used for qualitative and quantitative analyses (Nusra et al.2021).

2.3 Test volatiles and preparation

Six key volatiles were screened by volatile analysis. Table1 lists the names, purity, and sources of the six standard compounds. The compounds were dissolved in liquid paraffin, and each was prepared in 0.1, 1, 10, 50, and 100-μg/μL solutions.

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2.4 EAG response (mv) test

EAG is a biological identification instrument that is widely used in experimental entomology, it is mainly used to detect those insects that perceive the world through their antennae. Healthy and highly active *T. vishnou gigantina* imagoes aged 1–3 day were selected, and the test apparatus were sterilized before use. An antenna was removed from each imago at the base using a scalpel, and one flagellum was removed from the end of the antenna. The two ends of the antenna kept in full contact with two electrodes using conductive adhesive, the output end was connected to a host computer (Syntech UN-06), and the setup was covered with a transparent hood, to keep the experimental conditions stable. The sample compounds prepared in section 1.3 were each tested. The test gas flow rate was 400 mL/min, and the measurement was recorded after baseline

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stabilization. The stimulation time was set to 0.5 s with an interval of at least 30 s to ensure that the sensory function of the antennal sensilla was completely restored. Different compounds were tested at the same concentration level on six male and six female imagoes separately from each other. Experimental insects are not reused; separate tests are conducted for male and female adults. Each sample is stimulated an average of 5 times, with 3 sets of replicate experiments. During testing, the concentration of the standardized compounds is increased from low to high. When the same concentration gradient of the tested compounds has been completed, it is necessary to replace the clean test tubes. and liquid paraffin was used as the blank control. Data were collected and analyzed using Spike software (Syntech). The EAG measurements were directly read out by an antennal potentiometer. The relative value of EAG response was calculated as follows:

Relative EAG response(mv) = (Relative value of EAG response of test sample)(mv) – [(pre-test EAG response of control group)(mv) + (post-test EAG response of control group)(mv)] / 2.

2.5 Olfactory behavioral response bioassay by Y-tube olfactometry

The behavioral response of *T. vishnou gigantina* to the six pest-induced *H. rhamnoides* volatiles was tested using a Y-tube olfactometer with an inner diameter of 3 cm, one 15 cm main arm, two 8 cm side arms with an angle of 75° between them, and an air flow rate of 400 mL/min. To ensure that all parts were uniformly illuminated, a light source was placed 30 cm above the olfactometer. The highest concentration in the EAG response test (100 μg/μL) was selected, and 20 μL of each test sample was dropped onto a strip of filter paper using a pipette and placed at the end of one side arm. The same volume of n-hexane was dripped onto another strip of filter paper and placed at end of the other arm as the control. If the *T. vishnou gigantina* entered the position at more than two-thirds of the attractor arm or control arm and stayed for more than 1 min, it was recorded as having a smell source tendency; otherwise, it was recorded as having no response. The olfactory behavioral responses of *T. vishnou gigantina* to volatiles of the same concentration were recorded as a group. After the each test group, the test apparatus was cleaned and dried. The effects of the experimental environment were eliminated by interchanging the attractor arm with the control arm. Twenty imagoes were tested under each volatile concentration level at a male-to-female ratio of 1:1, and each behavioral response bioassay was repeated for five times. Please mention how many concentrations were tested for each volatile .

2.6 Data analysis and statistics

The relative content (percentage) of each volatile was calculated using the area normalization method. The generated data were statistically analyzed using the IBM SPSS Statistics 26. The significance of EAG differences between different concentrations of the same standard compound was calculated using the Duncan multiple comparisons method, and the results of olfactory behavioral responses were analyzed using the χ2 test.

3. Results

3.1 Collection and identification of volatiles from pest-affected *H. rhamnoides* leaves

In total, 33 volatiles were detected in the pest-affected *H. rhamnoides*, which included six alcohols, three hydrocarbons, Eleven terpenes, two ketones, four aldehydes, and seven esters (Table 2). Among these, the levels of six compounds differed significantly from those in healthy *H. rhamnoides* leaves. Relative content of the six volatiles the he ~~relative contents~~ were Leaf acetate (35.09±1.12)%, 2-ethylhexanol (22.91±3.09)%, humulene (2.42±0.76)%, Aristolochene (1.92±0.42)%, Hexyl 2-methyl butyrate (0.58±0.09)%, and longifolene (2.41±0.65)%.

3.2 EAG response of *T. vishnougigantina* to pest-induced *H. rhamnoides* volatiles at different concentrations

The EAG responses of *T. vishnou gigantina* imagoes differed not only among the six different pest-induced volatiles but also between different concentrations of the same volatile (Table 3). At a concentration of 0.1 μg/μL, the EAG responses to all volatiles were weak. When the concentration increased to 1 μg/μL, compared with a volatile concentration of 0.1 μ g / μ L the relative values of EAG responses from both female and male *T. vishnou gigantina* imagoes increased significantly (P < 0.05). When the volatile concentration reached 10 μg/μL, the relative EAG values of *T. vishnou gigantina* females for Hexyl 2-methyl butyrate and Longifolene were significantly higher (P < 0.05). At concentrations of 50 μg/μL, the relative values of EAG

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responses of imagoes were all significantly higher than those at concentrations of 10 μg/μL ($P < 0.05$), with the highest relative values observed in response to Hexyl 2-methyl butyrate. When the volatile concentration reached 100 μg/μL, the relative values of all EAG responses were maximized, except for those to Hexyl 2-methyl butyrate and Leaf acetate, which were not significantly different from values at a concentration of 50 μg/μL ($P > 0.05$).

Therefore, at a concentration of 100 μg/uL, the relative EAG response values of adult *T. vishnou gigantina* were the highest compared to those at other concentrations. The relative values of EAG responses of *T. vishnou gigantina* to different volatiles were also compared. Responses to Hexyl 2-methyl butyrate were the highest (2.53 and 2.24), followed by those to longifolene (2.44 and 2.18), and responses to Leaf acetate were the lowest (1.59 and 0.78). Overall, the relative values of EAG responses of female and male *T. vishnou gigantina* imagoes to the six pest-induced *H. rhamnoides* volatiles continued to increase with increasing concentrations, females consistently showed stronger responses than males.

3.3 Olfactory behavioral responses of *T. vishnou gigantina* to six pest-induced *H. rhamnoides* volatiles

The test results revealed significant differences in the olfactory behavioral responses of female and male imagoes to the six pest-induced volatiles ~~between~~ (Table 4). The compounds with the highest behavioral trends were Hexyl 2-methyl butyrate, with trend rates of 82.50% and 76.92% followed by longifolene (80.00% and 68.42%), and the volatile with the weakest olfactory behavioral response was Leaf acetate (43.24% and 46.15%) and humulene and aristolochene which was almost similar . The $\chi 2$ test results confirmed that the total number of *T. vishnou gigantina* imagoes attracted to Hexyl 2-methyl butyrate and longifolene was significantly different from that attracted to the blank control ($P < 0.05$), indicating that the two compounds exerted a chemotactic effect on both female and male *T. vishnou gigantina* imagoes. Only female *T. vishnou gigantina* imagoes showed a significant behavioral preference for 2-ethylhexanol, with a convergence rate of 76.31% over the blank control. In contrast, three volatiles, including humulene, Leaf acetate, and Aristolochene, showed no significant attracting effect on *T. vishnou gigantina* imagoes ($P > 0.05$). Overall, the behavioral trends of female *T. vishnou gigantina* imagoes toward the six standard compounds ~~were the following~~ in the descending order of attraction : Hexyl 2-methyl butyrate, longifolene, 2-ethylhexanol, Aristolochene, humulene, and Leaf acetate; the trends for male imagoes were Hexyl 2-methyl butyrate, longifolene, humulene, Leaf acetate, 2-ethylhexanol, and Aristolochene.

4. Discussion

EAG response and olfactory behavioral response tests are able to identify factors in plant volatiles with chemo-ecological effects on insects (Bhowmik et al.2016). Our results showed that the six standard compounds at specific concentrations could induce EAG and olfactory behavioral responses in both male and female *T. vishnou gigantina* imagoes. Hence, we can conclude that pest-induced *H. rhamnoides* volatiles play a positive role in attracting *T. vishnou gigantina* to hosts. Similarly, volatiles induced by *Phaudaflamman* larvae have a strong attracting effect on both male and female imagoes.

Our study results showed that after *H. rhamnoides* leaves were damaged by *T. vishnou gigantina*, the relative content of terpenoids and esters in their volatiles increased significantly, and these pest-induced volatiles exerted a notable attracting effect on both male and female imagoes. In particular, Hexyl 2-methyl butyrate showed a stronger attracting effect on female *T. vishnou gigantina* imagoes than on males, which suggests that *T. vishnou gigantina* is specific in its response to pest-induced *H. rhamnoides* volatiles, This result is similar to findings on numerous phytophagous insects such as Hemiptera (Badra et al. 2021), Coleoptera (Ballhorn et al. 2013), and Diptera (Hern and Dorn 2004).Therefore, pest-induced volatiles are beneficial for controlling insect oviposition and mating.

In the EAG response test, the highest concentration of the six volatiles was 100 μg/μL. Compared to other concentrations in both EAG and behavioral tests, Hexyl 2-methyl butyrate produced the strongest attracting (i.e., chemotactic) effect on both female and male imagoes, followed by 2-ethylhexanol and longifolene. 2-ethylhexanol produced a significant attracting effect on female imagoes, although there was no significant attraction to males. Among the *H. rhamnoides* volatiles induced by *T. vishnou gigantina*, the semiochemicals Hexyl 2-methyl butyrate and longifolene elicited particularly notable stimulus responses. Pest-inducing volatiles are able to target and lure adults of different sexes and play an important role in adult host localization. The stimulatory responses of these single informative compounds are conducive to the

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Commented [APPK11]: Table 3 shows that attraction was at par control of insect egg-laying and mating, as well as luring or avoiding insects, which provides a scientific basis for better use of plant volatiles to monitor and control *T. vishnou gigantina* in the future. However, the olfactory mechanism of phytophagous insects is very complex, and the EAG response to a single plant volatile is significantly different from that to a full set of plant volatiles (Hare 2011). Future studies should investigate the physiological and behavioral responses of *T. vishnougigantina* based on a combination of different pest-induced plant volatiles.

The responses of female and male *T. vishnougigantina* imagoes to the same *H. rhamnoides* volatiles were basically the same. Under the specified concentration gradient, EAG and olfactory behavioral responses both significantly increased with the increase in volatile concentration. However, the EAG response to pest-induced volatiles at the same concentration level showed certain differences between female and male *T. vishnou gigantina* imagoes. For example, in a study on volatiles from *Eucalyptus* leaves, limonene at a dose of 100 µg had a significant oviposition repellent effect on *Helicoverpaarmigera* egg-holding female moths (Yuan et al. 2021). This might be related to the number and functional categories of sensors on the antennae of imagoes and need to be examined at greater depth in future studies.

Insects are sensitive to different odours, and the volatiles of different plants are unique compounds with specific components and ratios, which are very important for insects to locate their hosts accurately (Ingrao et al. 2019). According to the study, due to the relatively homogeneous vegetation in Wuqi, Shaanxi, the volatiles of *T. vishnou rhamnoides* have a strong luring effect on the *T. vishnou gigantina*, and the rate of infestation in the *H.rhamnoides* forests of Wuqi, Shaanxi, is very high (Liuet al. 2013). To study the behavioural responses of *T. vishnou gigantina* to pest-inducing plant volatiles and to identify the relationship between pest-inducing plant volatiles and *T. vishnou gigantina* behaviours, either by using the pest-inducing key volatiles as attractants to improve the efficacy of insecticides or by interfering with the pest's behaviour in terms of host recognition and host localisation, mating and searching for oviposition sites, which can be used as a contraindicator. This will provide a new way to control *T. vishnou gigantina*, as well as a theoretical basis for the effective control of other pests and the development of efficient lure agents.

5. Conclusion

This study investigated that the electroantennogram and olfactory behavioral of *Trabala vishnou gigantina* is obviously impacted by pest-induced *Hippophae rhamnoides* volatiles. This information will be extremely valuable to the development of pest control methods by revealing the chemical compounds released by *H. rhamnoides* upon initial infestation contribute to further attracting *T. vishnou gigantina* in the future.

Acknowledgments

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Authors' Contributions

Yonghua Liu designed the study and performed experimental work. Shuo Tang analyzed the data. Yonghua Liu, Shuo Tang, Jiangshuai Feng, Kexu An and Xiongfei Yan wrote the article.

Conflicts of interest

The authors declare no conflict of interest.

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Table Caption

Table 1 The names, purity, and sources of six volatile substances tested

Table 2 Relative content of volatile species and components of *H.rhamnoides*

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Table 3 Relative EAG response values of *T. vishnou gigantina* to different concentrations of six compounds from damaged *H. rhamnoides*

Table 4 Behavioral responses of *Trabala vishnou gigantic Yang* to six compounds from damaged *H.rhamnoides*

Table 1 The names, purity, and sources of six volatile substances tested

Standard compounds	Purity	Source of supply
Hexyl 2-methyl butyrate	99%	Aladdin
2-ethylhexanol	99%	Aladdin
Humulene	98%	Sigma
Leaf acetate	99%	Sigma
Aristolochene	98%	Sigma
Longifolene	98%	Sigma

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Table 2 Relative content of volatile ~~species and~~ compounds in *H.rhamnoides leaves*

Volatile Group	Plant volatile	CAS	Log2FC	Relative content (%) in leaves of H. rhamnoides	
				Healthy leaves ~~*H.rhamnoides*~~	infested leaves~~*H.rhamnoides*~~
Alcohol	2-Heptanol	6033-23-4	-1.8714	1.21±0.17	0.31±0.17
	cis-5-Octen-1-ol	64275-73-6	1.2858	1.79±0.73	4.11±0.53
	1-Octanol	111-87-5	-0.5346	16.41±2.75	10.51±5.01
	1-Octen-3-ol	3391-86-4	-0.4058	1.47±0.04	1.06±0.32
	Benzeneethanol, beta -methyl-	1123-85-9	0.3858	15.07±0.37	17.71±0.77
	2-Ethyl-1-hexanol	104-76-7	3.6386	2.03±0.05	22.91±3.09
Aldehydes	1H-Pyrrole-2-carboxaldehyde	1003-29-8	-3.4465	2.39±0.27	0.20±0.01
	1-Cyclohexene-1-acetaldehyde, 2,6,6-trimethyl-	472-66-2	3.3180	0.68±0.02	5.96±1.26
	10-Undecenal	112-45-8	1.9747	2.97±0.66	10.94±4.28
	Octanal	124-13-0	-1.1304	1.97±0.23	0.84±0.54
Terpenoids	Aristolochene	26620-71-3	5.0862	—	1.92±0.42
	10,10-Dimethyl-2,6-dimethylene bicyclo[7.2.0] undecane	357414-37-0	1.2215	3.16±0.22	6.81±1.89
	Sabinyl acetate	3536-54-7	1.7666	0.79±0.26	2.53±0.93
	(-)-α-cedrene	469-61-4	3.3932	0.25±0.02	2.36±0.18
	β-sesquiphellandrene	20307-83-9	-0.0937	2.93±0.43	2.47±0.44
	γ-cadinene	39029-41-9	2.8218	0.79±0.27	5.23±2.19
	Linalool	78-70-6	0.9038	1.02±0.11	1.71±0.47
	Humulene	6753-98-6	3.9679	—	2.42±0.76
	Cedrene	11028-42-5	1.9997	1.11±0.13	4.14±1.58
	Longifolene	475-20-7	3.5186	0.23±0.08	2.41±0.65
	Perillyl alcohol	536-59-4	1.5948	0.57±0.16	1.62±0.64
Hydrocarbons	1-Tridecene	2437-56-1	1.5494	1.7±0.11	4.58±1.48
	Nonane	111-84-2	-1.3873	4.36±0.07	1.59±0.91
	p-cymene	99-87-6	1.8511	1.21±0.75	3.54±1.37
Ketone	2-Hexanone,3-methyl-	2550-21-2	-0.3902	17.18±1.31	12.71±7.13
	5-Nonanone	502-56-7	-0.0018	5.64±0.31	5.47±3.34
Ester	Acetic acid,non-3-enyl ester, cis-	13049-88-2	1.8795	0.68±0.19	2.35±0.91
	Hexyl 2-methyl butyrate	10032-15-2	4.5583	—	0.58±0.09
	Dihydro actinidiolide	15356-74-8	-2.0320	11.68±3.01	2.52±0.72
	Benzene aceticacid, ethyl ester	101-97-3	0.2201	4.02±0.34	4.28±0.61
	Butanoicacid, butyl ester	109-21-7	2.0078	17.51±6.21	59.80±14.33
	Leaf acetate	3681-71-8	3.6593	3.09±0.42	35.09±1.12
	cis-3-Hexenyl pyruvate	68133-76-6	2.0806	17.14±7.26	62.42±13.24

The data in the table are mean ± SE. * and ** indicate significant differences in the relative contents of insect-infested *H.rhamnoides* leaves compared with ~~in~~ healthy leaves at the p=<0.01 and 0.05 levels, respectively.CAS-????

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Table 3 Relative EAG response values of *T. vishnou gigantina* to different concentrations of ~~six~~ volatile compounds from damaged *H. rhamnoides*

Compounds	Sex	Relative EAG response （μg/μL）				
		0.1	1	10	50	100
Hexyl 2-methyl butyrate	Female	0.33±0.16d	0.78±0.23c	1.55±0.46b	2.45±0.54a	2.53±0.38a
	Male	0.21±0.09c	0.48±0.13b	0.95±0.24b	1.86±0.48a	2.24±0.53a
2-ethylhexanol	Female	0.09±0.01e	0.34±0.02d	0.68±0.23c	1.43±0.33b	2.33±0.46a
	Male	0.08±0.01e	0.22±0.03d	0.52±0.12c	0.95±0.24b	1.46±0.32a
Humulene	Female	0.11±0.01d	0.45±0.08c	0.88±0.12b	1.56±0.28a	1.64±0.33a
	Male	0.10±0.01d	0.29±0.04c	0.68±0.11b	0.92±0.21a	0.98±0.18a
Leaf acetate	Female	0.09±0.01e	0.33±0.04d	0.59±0.15c	0.87±0.21b	1.59±0.26a
	Male	0.11±0.02d	0.31±0.03c	0.38±0.09c	0.56±0.15b	0.78±0.16a
Aristolochene	Female	0.15±0.03e	0.41±0.14d	0.75±0.20c	1.18±0.29b	1.62±0.35a
	Male	0.12±0.02d	0.33±0.05c	0.35±0.10c	0.66±0.12b	1.11±0.23a
Longifolene	Female	0.23±0.04e	0.85±0.20d	1.55±0.35c	2.05±0.38b	2.44±0.41a
	Male	0.18±0.02e	0.44±0.16d	0.96±0.22c	1.86±0.28b	2.18±0.35a

Data in the table are means ± SE; peer data followed by different lower-case letters indicate significant differences ($P<0.05$).

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Table 4 Behavioral responses of *Trabala vishnou gigantic Yang* to volatiles from damaged *H.rhamnoides*

Compounds	Sex	Total numbers			Luring rate(%)	χ^2 test (*P*)
		Odor arm	Control arm	No response		
Hexyl 2-methyl butyrate	Female	33	6	1	82.50	4.6213^* (*P*=0.0256)
	Male	30	7	2	76.92	4.4453^* (*P*=0.0375)
2-ethylhexanol	Female	29	7	2	76.31	4.4322^*(*P* =0.0366)
	Male	18	17	5	45.00	0.1543^{NS}(*P* =0.7015)
Humulene	Female	17	18	4	43.59	0.1325^{NS} (*P* =0.7256)
	Male	18	15	5	47.36	0.2463^{NS} (*P* =0.6954)
Leaf acetate	Female	16	19	2	43.24	0.1312^{NS}(*P* =0.7421)
	Male	18	18	3	46.15	0.2135^{NS}(*P* =0.6854)
Aristolochene	Female	16	18	2	44.44	0.1468^{NS}(*P* =0.6895)
	Male	15	19	2	41.67	0.1025^{NS}(*P* =0.7652)
Longifolene	Female	32	7	1	80.00	4.4547^*(*P* =0.0242)
	Male	26	8	4	68.42	3.8856^*(*P* =0.0415)

Significance levels of χ^2 test are indicated by NS (*P*>0.05) and *(*P*<0.05)

Any missing insects