

Host-Seeking Behavior of *Aphidius gifuensis* (Hymenoptera: Braconidae) Modulated by Chemical Cues Within a Tritrophic Context

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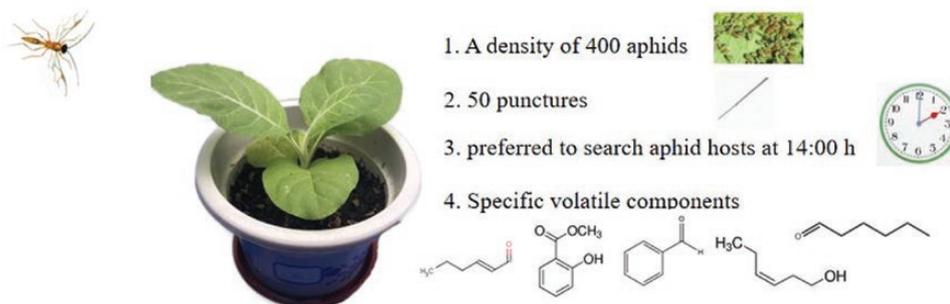
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Abstract

Aphidius gifuensis Ashmead is a generalist endoparasitoid that parasitizes a variety of aphid species. In China, it is widely used as a biological control agent to protect vegetables and tobaccos in open fields; control efficiency is largely dependent on its host-seeking ability. In this study, a six-choice olfactometer was used to investigate the olfactory responses of *A. gifuensis* to tobacco plants that had suffered damage (either varying degrees of mechanical damage or from aphid-feeding at different time intervals) and tobacco volatiles with different dosages. Furthermore, the regularity of *A. gifuensis* females' response toward an aphid/tobacco complex was monitored using a Y-tube olfactometer. Our findings suggest that tobacco plants are significantly attractive to *A. gifuensis* after they have been punctured with 50 holes, or housed with *Myzus persicae* (Sulzer) at a density of 400 aphids, except at an infestation time of 12 h. Moreover, aphid density had a more significant effect on the response than the time interval since aphid application. *Aphidius gifuensis* was found to be active during the daytime and preferred to search for their aphid hosts at 14:00 h. Five EAG-active tobacco volatiles (trans-2-hexenal, methyl salicylate, benzaldehyde, cis-3-hexen-1-ol, and 1-hexanal) were found to significantly attract *A. gifuensis* females at different concentration ranges. The practical implications of these results are discussed in the framework of the sustainable biological control of pest aphids in agricultural production systems.

Graph Abstract

Host-Seeking Behavior of *A. gifuensis*



Key words: biological control, aphid parasitoid, *Myzus persicae*, tobacco, attraction

Parasitoids have evolved multifarious tactics to locate their hosts (Turlings et al. 1993). As part of this host-seeking process, long- and short-distance orientation is guided via multisensory cues containing chemical, visual, and vibratory information, which are utilized in a joint manner. Among these, chemical cues are considered to be crucial (Vinson 1976) and emanate from either the herbivorous host or its habitat. Aside from host location, they also affect activities such as searching for food, finding copulation opportunities, and avoiding natural enemies (Schoonhoven et al. 2005). When plant species suffer from infestation by herbivorous arthropods they release specific volatile blends known as herbivore-induced plant volatiles (HIPVs) that can recruit natural enemies of the attacking pests, deter phytophagous insects, and/or elicit an enhanced defense of its own and of surrounding plants (Unsicker et al. 2009, Dicke and Baldwin 2010, Backer et al. 2015, Coppola et al. 2017).

HIPVs are considered the most important information to help parasitoids locate and recognize their hosts in a complex odorous environment. They are highly detectable and reliably imply the occurrence of herbivores due to their volatility and, as a consequence, also serve to protect the plant from infestation by pests (Vet and Dicke 1992, Takabayashi and Dicke 1996, Arimura et al. 2009). Both the herbivore species and level of infestation have an effect on the blend and relative quantities of the volatiles (Du et al. 1998, Guerrieri et al. 1999), therefore understanding their ecological functions in parasitoid host-seeking behavior is a considerable challenge. Within the context of biological control systems, understanding how parasitoid foraging behavior is affected by olfactory cues, as well as investigating how HIPVs could be used, will contribute to enhancing the control effectiveness of parasitoids in the field (Vet and Dicke 1992, Cortesero et al. 2000, Aldrich et al. 2003).

Aphidius gifuensis Ashmead (Hymenoptera: Braconidae) is a common endoparasitoid and an important natural enemy of several aphid species [e.g., *Myzus persicae* (Sulzer), *Macrosiphum avenae* (Fabricius), *Lipaphis erysimi* (Kaltenbach), *Aphis glycines* Matsumura, and *Aphidius gossypii* Glover (Hemiptera: Aphididae)] that vector over 100 plant viral pathogens and inflict reduced yields and lower the retail value of crops (Wei et al. 2003, Wu 2007). In China, *A. gifuensis* have been commercially utilized to suppress the population of aphids on tobacco plants in open fields and on vegetables in greenhouses, since this species was shown to have a high rate of parasitism against aphids (Wei et al. 2003, Yang et al. 2011). *Aphidius gifuensis* is widely employed as a biological control agent against *M. persicae*, which is also currently the main aphid host used for mass-rearing of this parasitoid in China, even though *A. gifuensis* is able to parasitize and develop from other aphid species as well (Wei et al. 2003, Zhang et al. 2014).

The biology of *A. gifuensis* is well understood, and its host-seeking behavior has been recorded on *Capsicum annuum* L. var. *grossum*, *Brassica oleracea* L., *Chrysanthemum coronarium* L., *Solanum lycopersicum* L. attacked by *M. persicae* (Tan and Liu, 2014, Wang 2015), *Triticum aestivum* L. attacked by *M. avenae* (He 2019) and *Sitobion akebiae* Shinji (Takemoto 2016), *Nicotiana tabacum* and *Brassica napus* spp. attacked by *M. persicae* and *L. erysimi* (Yang et al. 2009), *Vicia faba* L. attacked by *Acyrtosiphon pisum* Harris (Takemoto and Yoshimura 2020). A previous study found that *A. gifuensis* was strongly attracted to healthy sweet peppers and crown daisies, and that aphid infestation amplified this attraction (Wang 2015). Yang et al. (2009) found that *A. gifuensis* females were significantly attracted to tobacco and oilseed rape plants with a high level of aphid infestation, but were not attracted to intact

or mechanically-damaged host plants. Dong et al. (2008) assessed the electrophysiological activities of several kinds of chemical cues, including aphid sex pheromone, alarm pheromone, and tobacco volatiles; their results suggested that the electrophysiologically active components may be involved in the host-seeking behavior of *A. gifuensis*. However, data on the olfactory responses elicited by bioactive molecules are still lacking. As such, the mechanisms of host foraging in *A. gifuensis* require further clarification.

The purpose of this study was to investigate the possible roles of cues from the host-plant complex (*M. persicae*-*N. tabacum*) in host foraging by *A. gifuensis*. Three areas were investigated: 1) olfactory response of *A. gifuensis* to tobacco plants with different levels of mechanical damage, and with different levels of aphid density at different time intervals; 2) daily olfactory behavioral characteristics of *A. gifuensis* females to aphid and tobacco complexes over time; and 3) olfactory response of *A. gifuensis* to several tobacco volatile compounds that have previously been identified as electrophysiologically active (Dong et al. 2008). Understanding the olfactory responses of parasitoids to chemical cues and characterizing the infochemicals responsible are an important foundation for exploiting parasitoid-recruiting tactics. We hope, therefore, that exploration of the above topics will lead to improvements in the effectiveness of parasitoid-based biological control programs against aphid pests.

Materials and Methods

The maintenance rearing of aphids (*M. persicae*) and parasitoids (*A. gifuensis*), the cultivation of tobacco, as well as the olfactory measurements to determine the host-seeking behavior of *A. gifuensis* on the tobacco plants, were all implemented in climate-controlled rooms of the Biological Control Research Institute, College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou, Fujian, China. All choice bioassays were performed between 08:00 and 16:00 unless otherwise stated.

Insect Colony Rearing

Aphids

Green peach aphids were collected from cabbage fields in Fuzhou, Fujian, China, and maintained on tobacco plants (cultivars K326) within a 40 × 40 × 40 cm insect rearing cage. The aphid colony was reared in a controlled insectary for more than 60 generations under conditions of 25 ± 1°C, 75 ± 5% relative humidity (RH), and a L:D photoperiod of 14:10 h.

Parasitoids

The initial colony of *A. gifuensis* was obtained from aphid mummies provided by Shaowu Tobacco Co. Ltd., Nanping, Fujian, China. The laboratory-reared strain was reared on *M. persicae* fed on tobacco for more than 60 generations in an insect rearing chamber at 25 ± 1°C, 65 ± 1% RH and a L:D photoperiod of 14:10 h, following the rearing protocols proposed by Wei et al. (2003). All experimental parasitoids were transferred from tobacco plants as mummified aphids and placed into glass tubes (200 × 25 mm) covered by cotton wool. The aphid mummies were kept in climate-controlled incubators at 22 ± 1°C, 60 ± 5% RH and a L:D photoperiod of 12:12 h. Every 24 h, newly emerged adult parasitoids were gently shifted into new tubes covered by cotton wool moistened with 50% honey solution and maintained for up to 48 h before the experiments. All parasitoids utilized in this study were naive, assumed to be mated, and used only once. Before each trial, *A. gifuensis* adults were starved for 2 h to enhance their sensitivity to odor (Yang et al. 2009).

Plant Cultures

Tobacco seeds (cultivars K326) were provided by Nanping Tobacco Co. Ltd., Fujian, China and cultivated in a controlled room in accordance with [Wei et al. \(2003\)](#). The tobacco seedlings were maintained in trays (length × width × height: 30 × 25 × 10 cm) with soil (20.17 mg/kg of available N, 10.85 mg/kg of available P and 53.98 mg/kg of available K), and after they had grown four true leaves, individual tobacco plants were transferred into plastic pots (upper diameter: 9 cm, bottom diameter: 6.5 cm, height: 7 cm) for further growth. Whole tobacco plants with a height of 10–15 cm and 4–6 true leaves were used in the experiment. Prior to the experiments, the pots were wrapped with aluminum foil to eliminate effects caused by odor from the soil or soil microorganisms ([Supp Fig. 1 \[online only\]](#)).

Chemicals

[Dong et al. \(2008\)](#) demonstrated that six tobacco volatiles compounds, namely trans-2-hexenal, linalool (racemic mixture), methyl salicylate, benzaldehyde, cis-3-hexen-1-ol, and 1-hexanal, can elicit different levels of electroantennogram (EAG) responses in *A. gifuensis* males and females. As such, we selected these chemical standards for use in olfactory assays. Information on these standards is given in [Table 1](#). Each compound was dissolved in liquid paraffin oil (purity > 99%; Beijing solabo Technology Co., Ltd, Beijing, China) and prepared into five concentrations: 1 (i.e., no dilution), 10^{-1} , 10^{-2} , 10^{-3} , and 10^{-4} (v:v) for the dose–response tests. Diluted standards were maintained in a refrigerator at 4°C until the start of the experiments.

Experimental Design

Experiment 1: Olfactory responses to tobacco plants with different levels of mechanical damage

This assay was conducted to evaluate the olfactory responses of *A. gifuensis* females and males to tobacco plants exhibiting six levels of mechanical damage. Specifically, their olfactory preference was measured (in terms of the number of respondents) using an improved six-arm olfactometer ([Gu et al. 2018](#)) when supplied concomitantly with the differentially damaged tobacco plants. The test area in the olfactometer comprised a release area, a pre-choice area, and a chosen area ([Supp Fig. 2 \[online only\]](#)). Each connection was sealed with polytetrafluoroethylene tape. Humidified air was uniformly passed through activated charcoal and introduced to six odor bottles (diameter: 20 cm, height: 10 cm) that contained samples of one tobacco plant with respective damage levels of 0 (control), 25, 50, 100, 200, and 400 punctures. The mechanical damage was inflicted using a sterilized insect needle (size #1; Jiangxi Sunrise Plant Protection Tech. Co. Ltd, Nanchang, Jiangxi, China) to mimic the damage caused by aphid-feeding. Puncture-treated plants were randomly deposited into their respective odor bottles and immediately used for the experiment. A group of 30 parasitoids (either all females or all males) were then simultaneously introduced into the center of

the release area; they were therefore isolated and given equal opportunity to perceive the odors coming from six directions. The number of parasitoids that entered each odor bottle or stayed at each chosen area were counted after 10 min. At the end of the trial, all parasitoids were removed from the olfactometer using an aspirator. After every replication, the olfactometer was thoroughly cleaned with 75% ethanol, flushed with distilled water and thereafter naturally dried. Measurements were taken separately for females and males and the experiment was replicated five times (in total 150 parasitoid females and 150 males were determined), and each replicate was performed on a different day.

Experiment 2: Olfactory responses to tobacco plants with varying aphid densities for different durations

This assay was performed using the same six-arm olfactometer to test the olfactory responses of *A. gifuensis* females to tobacco plants exhibiting six levels of aphid density at different time intervals. In order to prepare the plants, apterous adults of mixed-instar *M. persicae* were respectively diverted to tobacco leaves using a moist fine brush. Aphid densities of 25, 50, 100, 200, and 400 aphids per plant were used; a tobacco plant without aphids served as the control. Aphid-tobacco complexes were then placed into cylindrical plastic containers (diameter: 25 cm, height: 15 cm) with tiny pores; this allowed air exchange but prevented the aphids from escaping and ensured the absence of other insects. These were then randomly positioned into their respective odor bottles. After the aphids had been present on the tobacco leaves for the specific durations of 3, 6, 12, 24, and 48 h, a group of 30 female parasitoids were released into the release area of the olfactometer. The number of parasitoids that chose each odor bottle or stayed at each chosen area within a 10-min period was recorded. This experiment was replicated five times for each duration (i.e., 150 parasitoid females per duration, 750 in total), and replicates were also performed on different days. After each replication, the tested parasitoids were removed from the equipment, and the apparatus was cleaned as previously described.

Experiment 3: Daily olfactory behavior regularity of *A. gifuensis* females to aphid-tobacco complexes

The evaluation was undertaken using a Y-tube olfactometer (glass, 5 cm internal diameter, 15 cm long main stem, 15 cm long test arms, 60° angle) and tested the olfactory behavioral characteristics of *A. gifuensis* to *M. persicae* and *N. tabacum* complexes as time passed over the period of 1 d. This two-arm olfactometer system was similar to that used in our previous study ([Cai et al. 2020a](#)), consisting of an air pump (to generate an air stream), activated charcoal filter, humidifying device, glass stems, and two odor bottles ([Supp Fig. 3 \[online only\]](#)). The air stream was passed through the activated charcoal filter to clean the air and thereafter through the humidifying device at a rate of 200 ml/min. Finally, the air was divided and passed through the two odor bottles (diameter: 20 cm, height: 10 cm) which each contained one of

Table 1. Details of the six chemical standards

Chemical	CAS no.	Purity (%)	Storage conditions	Source
trans-2-hexenal	6728-26-3	97	0–6°C	Shanghai Macklin Biochemical Technology Co., Ltd
linalool	78-70-6	99	4°C	Shanghai Macklin Biochemical Technology Co., Ltd
methyl salicylate	119-36-8	99	Room temperature, cool and ventilated	Shanghai Macklin Biochemical Technology Co., Ltd
benzaldehyde	100-52-7	99	Room temperature, cool and ventilated	Shanghai Macklin Biochemical Technology Co., Ltd
cis-3-Hexen-1-ol	928-96-1	99	Room temperature, cool and ventilated	Aladdin reagent (Shanghai) Co., Ltd
hexanal	66-25-1	99	Room temperature, cool and ventilated	Shanghai Macklin Biochemical Technology Co., Ltd

the odor sources to be tested. One hour prior to each trial, aphid-tobacco complexes containing 400 mixed-instar aphids were prepared as mentioned previously; a non-aphid tobacco plant served as a control. The experiment lasted from 8:00 to 23:00 and a trial was performed at the start and thereafter every 3 h; thus, a total of 6 time points were assessed within one day. At the start of each trial, a group of 20 *A. gifuensis* females were introduced into the main stem of the olfactometer. Ten minutes later, a parasitoid that had crawled across at least 1/3 the length of a test arm and stayed for at least 10 s was judged to have chosen that arm. The number of responding parasitoids for each test arm was counted and the relative response percentage of *A. gifuensis* females to the aphid-tobacco complex was calculated using the following formula: (number of parasitoids that chose aphid-tobacco complex – number of parasitoids that chose healthy tobacco)/(total number of responding parasitoids) × 100. After each trial, all parasitoids were extracted from the arms, the glass stems were cleaned as previously mentioned, and the arms and odor sources were alternated to reduce spatial effects. This experiment was replicated five times (in total, 600 parasitoids were tested) and replications were conducted on different days. Over the entire course of all five replicates, the room and equipment were fully illuminated.

Experiment 4: Olfactory responses to tobacco volatiles

This assay was performed to estimate whether different concentrations of tobacco volatiles, known to elicit EAG responses in *A. gifuensis*, could impose differing impacts on their olfactory responses. The same six-choice olfactometer as experiments 1 and 2 was used. Each trial assessed only one chemical standard at a time, which were diluted as described earlier. First, a piece of cotton ball was moistened with 25 µl of each concentration of the test component and then deposited in its respective pear-shaped odor bottle (200 ml). Pure paraffin oil was used as the control. One group of 30 *A. gifuensis* females was then concomitantly transferred into the release area. Ten minutes later, the number of parasitoids that stayed in each chosen area or became trapped in each odor bottle was documented. After each trial, the tested parasitoids and the apparatus were treated as previously described, and different chemicals with varying concentrations were randomly positioned in the odor bottles to remove any positional effects. For each chemical, five replicates were performed on different days (in total, 150 parasitoids were tested for each chemical).

Statistical Analysis

All data analyses were performed using SPSS v.17.0 (SPSS, Inc., Chicago, IL) and figures were constructed using GraphPad Prism v.7.0 (GraphPad Software, San Diego, CA). Statistical differences between olfactory responses to the different levels of mechanical damage, aphid-density, duration, and concentrations were estimated using Tukey's honestly significant differences (HSD) test for multiple mean comparisons after one-way analysis of variance. The olfactory response of parasitoids to the tobacco plants with different levels of aphid density for different durations was analyzed by a general linear model (GLM). Evaluation factors were time interval and aphid density. A comparison of the differences between male and female parasitoids in response to mechanically damaged tobacco was performed using an independent *t*-test. In all cases, $P < 0.05$ was considered statistically significant. To improve normality and homoscedasticity (Zar 1984), all percentage data were square root transformed and numerical data were log+1 transformed. However, untransformed values are shown in the figures.

Results

Experiment 1: Olfactory Response to Tobacco Plants with Different Levels of Mechanical Damage

As shown in Fig. 1, the olfactory responses of both *A. gifuensis* females and males to tobacco plants were significantly affected by mechanical damage (female: $F_{5,24} = 5.459$, $P < 0.001$; male: $F_{5,24} = 9.905$, $P < 0.001$); however, the extent of the attraction did not increase linearly with increasing damage levels. Tobacco plants with 50 punctures attracted the highest number of parasitoids for both males and females; for males this was significantly more compared to all other levels of mechanical damage and the control, for females this was significantly more than those with 0, 25 and 400 punctures. Furthermore, there were no significant differences between males and females at each damage level (all $P > 0.05$).

Experiment 2: Olfactory Responses to Tobacco with Varying Aphid Densities for Different Durations

According to the analysis from the GLM, the time interval from aphid application did not significantly influence the olfactory responses of *A. gifuensis* females to aphid-tobacco complexes ($F_4 = 0.046$, $P = 0.996$). However, a significant impact was observed for aphid density ($F_5 = 16.039$, $P < 0.001$). For each time interval, tobacco plants with a high density of aphids attracted significantly more parasitoid females compared to the aphid-free tobacco: 400 aphids for 3 and 24 h (3 h: $F_{4,25} = 4.115$, $P < 0.01$; 24 h: $F_{4,25} = 6.841$, $P < 0.01$); 200 and 400 aphids for 6 h ($F_{4,25} = 6.756$, $P < 0.01$); 100–200 aphids for 12 h ($F_{4,25} = 10.868$, $P < 0.01$); and 100, 200, and 400 aphids for 48 h ($F_{4,25} = 6.916$, $P < 0.01$). With the exception of 12 h, the number of *A. gifuensis* females responding to the aphid-tobacco complexes generally increased as the aphid density increased, peaking at the highest density of aphids (Fig. 2).

Experiment 3: Daily Olfactory Behavior Regularity of *A. gifuensis* Females to Aphid-Tobacco Complexes

In the Y-tube olfactometer test, the host-foraging activity of *A. gifuensis* females was concentrated between 14:00 and 20:00 h, and the relative response rate of parasitoids choosing aphid-tobacco complexes at 14:00 h was significantly higher than in the morning (8:00 and 11:00 h) ($F_{5,24} = 2.491$, $P < 0.01$; Fig. 3). During the daytime (8:00–17:00 h), the number of *A. gifuensis* females that made a choice (i.e., regardless of whether this was control or treatment)

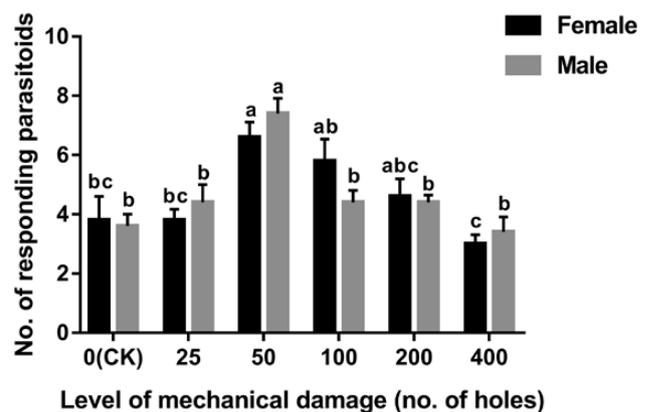


Fig. 1. Olfactory responses (mean ± SE) of *A. gifuensis* females and males to tobacco plants with different levels of mechanical damage. Different letters on the tops of the bars indicate a significant difference between concentrations by Tukey's HSD, $\alpha = 0.05$ ($n = 5$).

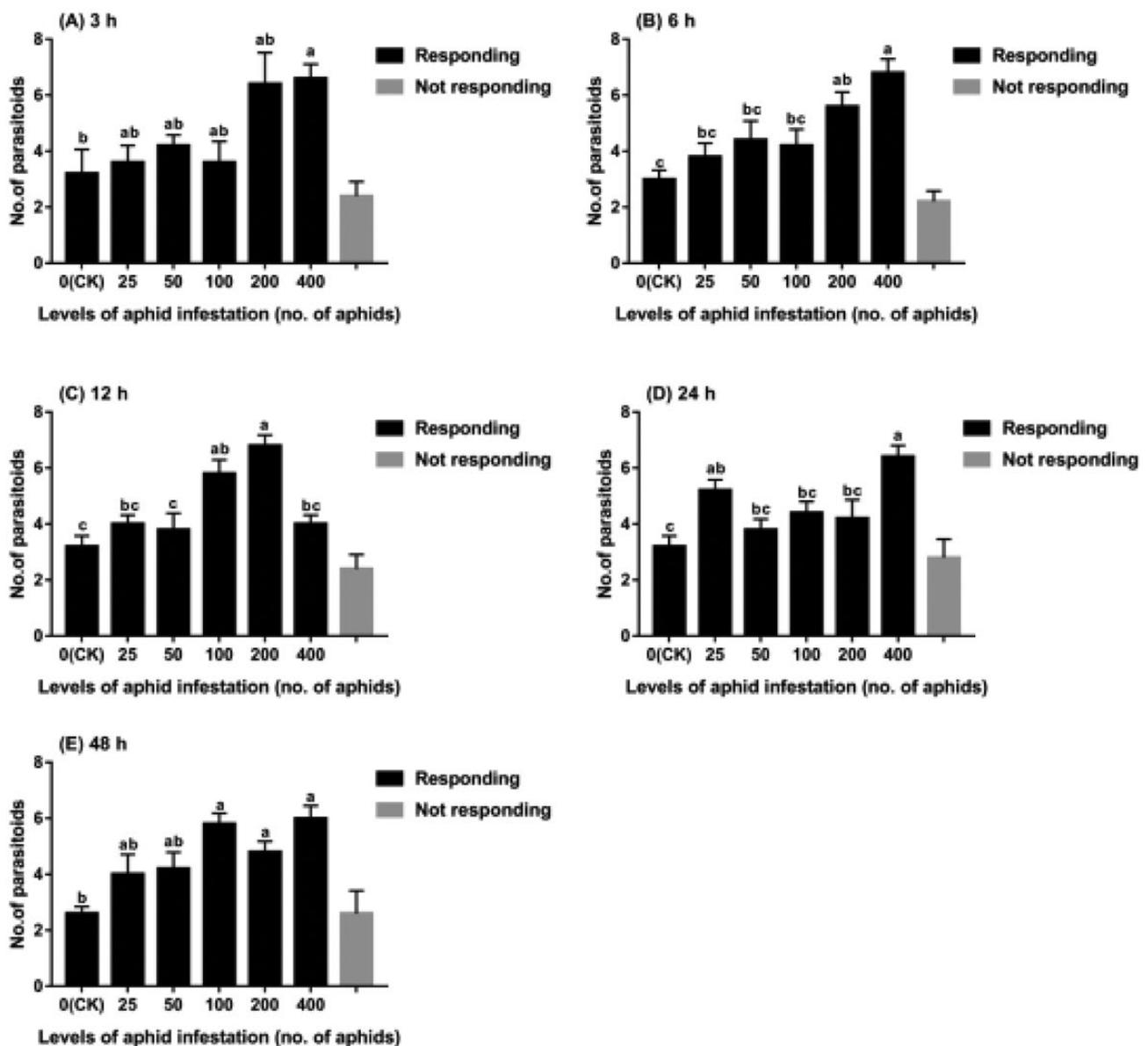


Fig. 2. Olfactory responses (mean \pm SE) of *A. gifuensis* females to tobacco plants with different levels of aphids density after different time intervals. Different letters on the tops of the bars indicate a significant difference by Tukey's HSD, $\alpha = 0.05$ ($n = 5$).

was significantly higher than that at night (20:00–23:00 h), with 83–99% making a choice in the daytime ($F_{5,24} = 8.413$, $P < 0.01$). Furthermore, the activity of parasitoids decreased as time elapsed over the day.

Experiment 4: Olfactory Responses to Tobacco Volatiles

As shown in Fig. 4, most *A. gifuensis* females chose one of the six options during each trial, with the response rate ranging from 92.00% to 96.67%. With the exception of linalool (racemic mixture), the olfactory responses of *A. gifuensis* females to single tobacco volatiles were significantly affected by concentration (benzaldehyde: $F_{5,24} = 21.639$, $P < 0.01$; linalool: $F_{5,24} = 2.144$, $P = 0.095$; cis-3-hexen-1-ol: $F_{5,24} = 12.181$, $P < 0.01$; 1-hexanal: $F_{5,24} = 12.018$, $P < 0.01$; methyl salicylate: $F_{5,24} = 22.606$, $P < 0.01$; trans-2-hexenal: $F_{5,24} = 19.567$, $P < 0.01$). The concentration ranges (ml/ml) of a single tobacco volatile that induced the statistically highest olfactory

response rate of *A. gifuensis* females varied by chemical: benzaldehyde, 10^{-2} ; cis-3-hexen-1-ol, 10^{-1} to 1; 1-hexanal, 10^{-4} to 10^{-2} ; methyl salicylate, 10^{-3} to 10^{-1} ; and trans-2-hexenal, 10^{-3} to 10^{-2} .

Discussion

In the six-choice test of experiment 1, both *A. gifuensis* females and males significantly responded to the odors of tobacco plants with 50 punctures. Yang et al. (2009) performed a similar experiment with *A. gifuensis* females but used a Y-tube assay and compared only mechanically damaged tobacco plants with 400 punctures versus clean air. In their experiment, they did not observe a significant difference and thus concluded that *A. gifuensis* females did not respond to mechanically damaged tobacco plants. While our results also show no significant difference between the control and 400 punctures, our more elaborate experimental design disagrees with their conclusion. Our results suggest that both *A. gifuensis* females

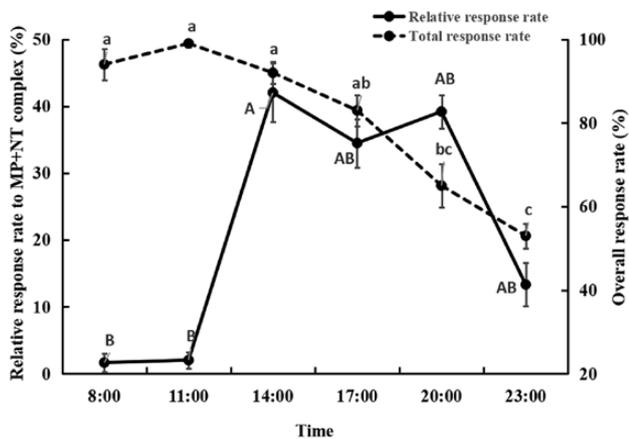


Fig. 3. Daily olfactory response (mean \pm SE) characteristics of *A. gifuensis* to *M. persicae* (MP) and *N. tabacum* (NT) complexes in a Y-tube olfactometer. Different capital and lowercase letters at the data points denote a significant difference by Tukey's HSD, $\alpha = 0.05$ ($n = 5$).

and males do in fact significantly respond to mechanically damaged tobacco plants, but only at low levels of mechanical damage. We suspect that the similar response trend of males and females facilitated the copulating males to finding females or mating sites thought plant-derived cues.

Mechanically damaged tobacco plants have also been shown to attract other braconid parasitoids such as *Cardiochiles nigriceps* Viereck (Morales et al. 1998) and *Microplitis mediator* (Haliday) (Dong et al. 2004) (Hymenoptera: Braconidae). We therefore deduced that mechanical damage qualitatively and quantitatively alters the emission and composition of the tobacco volatiles, resulting in a distinct olfactory response in *A. gifuensis*. Generally, the volatile organic compounds (VOCs) induced by mechanical damage are passively produced, and may originate from three components: 1) green leaf volatiles (GLVs) that form by a series of enzymatic reactions; 2) volatile components originally stored in the cells, tissues or organs of the plant; and 3) volatile components generated from the hydrolytic reaction of precursors (Paré and Tumlinson 1997, 1998). Thus, we suppose that in the case of highly extensive mechanical damage, the relevant enzymes, volatile precursors, cells, or tissues of the tobacco plants were seriously damaged and therefore the VOCs to potentially attract *A. gifuensis* could not be produced, formed, or released.

From our second experiment, we found that the time interval from aphid application did not have a significant impact on the behavioral choices of *A. gifuensis* females. However, aphid density was significant, whereby attraction increased as the density increased (except for the time interval of 12 h), in line with the research of Yang et al. (2009). This phenomenon has also been observed for other complexes such as *B. napus* with *M. persicae* or *L. erysimi* (Wang 2015). This enhanced attraction may result from aphid-induced volatile components released by both the plant and the host pest. Quantitative and qualitative comparisons of the composition of the VOCs emitted from aphid-tobacco complexes with varying densities of aphids are needed, since they drive the distinctive behavior of *A. gifuensis*. In our previous research, the headspace volatile profiles of tobacco plants with or without aphid-infestation at different time intervals were compared by GC-MS. The results indicated that total content and types of tobacco volatiles increased after aphid infestation, and several specific components such as tridecane, 1h-3a,7-methanoazulene, tetradecane, pentadecane, hentriacontane, nonane,

1,8-nonadien-3-ol, heneicosane, sulfuric acid, limonene, cedrene, and dichloro acetaldehyde were newly emitted from aphid-infested tobacco plants (Song et al. 2021). This will help to expound the ecological role of key components in how parasitoids locate their hosts. It deserves to be mentioned that the significant olfactory response of *A. gifuensis* females to tobacco plants with high aphid densities in the laboratory may partly explain how this parasitoid is adequately controlled in the main tobacco producing areas of China (Wei et al. 2005, Yang et al. 2009). Because *M. persicae* always have a clustered distribution in tobacco fields (Chen et al. 1990), sufficient volatiles to evoke parasitoid responses could be produced, thus enabling *A. gifuensis* females to easily locate their host in the field.

In our third experiment using the Y-tube olfactometer, the activity of host-seeking in *A. gifuensis* females was mainly concentrated at 14:00 h. This is highly consistent with field observations which have indicated that *A. gifuensis* prefers to search and parasitize host aphids colonized on tobacco plants in open tobacco fields (cultivars K326) between 13:00 and 14:00 h, regardless of the aphid density (Wu et al. 2000). If our laboratory findings accurately reflect performance in the field, this feature could be a valuable reference for developing parasitoid-based biological control strategies in open tobacco fields; it could help optimize the application timing of parasitoid release, insecticides or parasitoid attractants. In addition, the number of *A. gifuensis* which responded (irrespective of their choice) during daytime (8:00–17:00 h) was significantly higher than during nighttime (20:00–23:00 h), and their activity levels reduced as time passed. Since this assay was conducted in a climate-controlled room, and illumination was uniformly and continuously supplied during the whole assay duration, we hypothesize that the parasitoids' circadian rhythm imposed these effects, which is worthy of further investigation. The prominent impact of circadian rhythm on chemical communication interaction within a tritrophic context has been noted (Joo et al. 2019), but associated information on the tritrophic levels of aphids-aphid parasitoids-plants is still lacking.

Incorporating infochemicals from pest habitats as part of biological control systems will help to improve the control effectiveness of parasitoids. As such, it is necessary to investigate which volatile compositions are identified by the parasitoid olfactory system as well as the minimal concentration that is sufficient to stimulate an olfactory response (James 2005). It is possible to distinguish potentially bioactive volatile components and confirm their ecological roles in parasitoids through electrophysiological and olfactory behavioral experiments (Webster et al. 2010). Previous research established that six synthetic volatiles from tobacco were biologically active to *A. gifuensis* by EAG examinations (Dong et al. 2008). We tested all six components at different dosages and found that all except linalool (racemic mixture) could induce a significant olfactory response in *A. gifuensis* females. This is in line with the results of Dong et al. (2008) who found that the EAG response elicited by linalool was the lowest among the tested compounds. Whilst both our results and that of Dong et al. (2008) demonstrated that linalool was not attractive to *A. gifuensis*, it has been documented as triggering positive olfactory behavioral or/and EAG responses in other braconid parasitoids such as *Cotesia marginiventris* Cresson (Ngumbi and Fadamiro 2012), *Cotesia sesamiae* (Cameron) (Tamiru et al. 2015) and *Fopius arisanus* (Sonan) (Cai et al. 2020b) (Hymenoptera: Braconidae).

The concentration ranges of single tobacco volatiles that induce a significantly high response in *A. gifuensis* females varied with chemical type. Therefore, we speculate that *A. gifuensis* has diverse concentration thresholds for perceiving different types of component which can elicit corresponding levels of olfactory behavior.

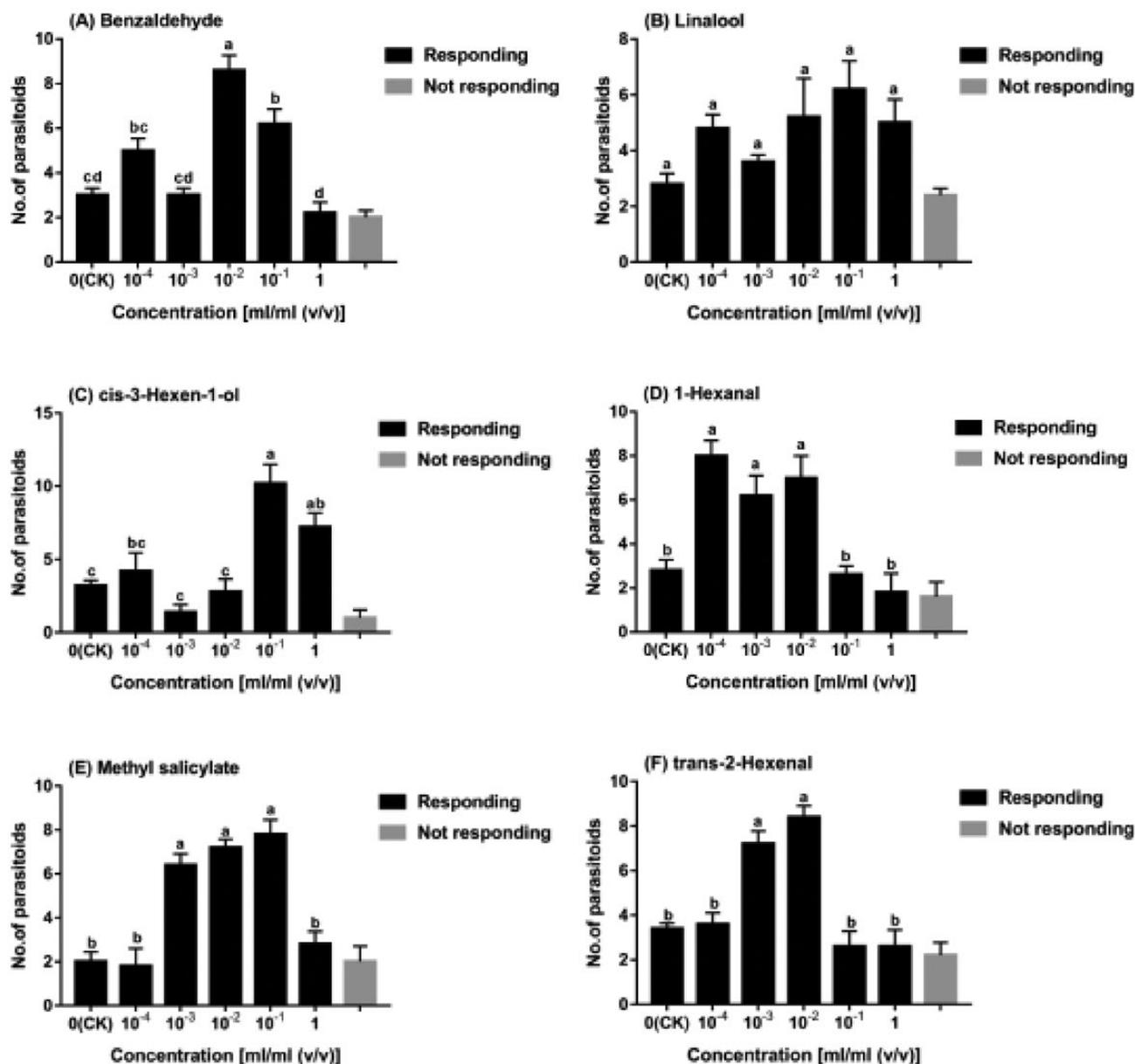


Fig. 4. Olfactory responses (mean \pm SE) of *A. gifuensis* females to six tobacco volatiles with various concentrations. Different letters on the tops of the bars indicate a significant difference by Tukey's HSD, $\alpha = 0.05$ ($n = 5$).

From another perspective, this also demonstrates that the relative content of plant volatiles could have an important role in the behavior of parasitoids. Previous research has suggested that three kinds of GLVs, including trans-2-hexenal, cis-3-hexen-1-ol, and 1-hexanal, are common constituents of HIPVs (Moraes et al. 1998, Powell et al. 1998) and, moreover, GLVs have been characterized as an important cue for parasitoid host location at long distance (Baehrecke et al. 1989).

A previous study using a four-choice olfactometer stated that methyl salicylate at a concentration of 1/9 (v:v) significantly repelled *M. persicae* and inhibited the colonization of this aphid on tobacco plants, with a repellent rate of 18.49% and inhibition rate of 67.01% (Liu et al. 2013). Additionally, 1-hexanal at a concentration of 10⁻¹ (v:v) also had a significant repellent effect on *M. persicae* in a Y-tube test (Wu 2011). Alongside the primary functions of attracting beneficial insects and deterring pests, we assert that the perfect synthetic

infochemical candidate for field application should also be equipped with extra valuable characteristics: 1) it should possess other positive ecological roles to different trophic levels in the agroecosystem, such as exerting positive influences on targeted natural enemies or the crop of economic importance, etc.; and 2) it should not have any detrimental effects on non-target organisms or the environment. For instance, methyl salicylate (MeSA) is an exemplary substance that possesses multifunctional roles: it can not only attract *A. gifuensis* as exhibited in this research, but also deter and inhibit *M. persicae* as suggested in other research (Liu et al. 2013). Furthermore, this substance can attract predators, serve as signaling hormone involved in tomato plant pathogen defense (Rowen et al. 2017), and enhance the parasitism performance of parasitoids against pests such as *Anagrus nilaparvatae* Pang et Wang (Hymenoptera: Mymaridae) (Wang and Lou 2013). As such, plant volatile components with multifunctional roles could achieve many purposes simultaneously. Accurately

characterizing these infochemicals, as well as exploring their potential in various field applications, is thus of great value in integrated pest management. However, more work is needed before they can be applied in the field.

Conclusion

This study determined how *A. gifuensis* responds to tobacco plants affected by the presence of *M. persicae* under various treatment scenarios. We also investigated the effect of isolated tobacco volatiles with various dosages, and the regularity of the parasitoids' response to tobacco/aphid complexes within a day. Our main results indicate that the tobacco plant can recruit *A. gifuensis* as a result of low-level mechanical damage or the presence of high numbers of aphids, and at different time intervals. According to our laboratory examination, *A. gifuensis* females preferred to search for their host at 14:00 h and were active during the daytime. Furthermore, five of the six tested EAG-active tobacco synthetic volatiles were found to attract significantly more parasitoids (at different dosage ranges) compared to the control group. The results of this research provide meaningful information on the host-seeking behavior of *A. gifuensis*. Furthermore, we expect that this research will assist in enhancing the control effectiveness of parasitoids in biological control programs, helping to depress aphid populations in tobacco fields.

Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

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Author Contributions

Conceptualization, P.C. and C.L.; methodology, P.C. and Y.S.; performed the experiments, Y.S., W.C., Y.G. and J.L.; analysed the data and prepared the figures, P.C. and Y.S.; writing—original draft preparation, P.C.; writing—review and editing, P.C., Y.S., and C.L. All authors have read and agreed to the published version of the manuscript.

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