

# CHEMICAL CUES MEDIATING BEHAVIORAL AND ELECTROPHYSIOLOGICAL RESPONSES OF *FOPIUS ARISANUS* (HYMENOPTERA: BRACONIDAE): THE ROLE OF HERBIVORE-INDUCED PLANT VOLATILES

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**Abstract.** The aim of the present study was to establish how a selection of herbivore-induced plant volatiles (HIPVs) regulate the behavioral response of *Fopius arisanus*. A total of ten HIPVs were selected which are major bioactive components produced by fruits immediately after being infested with eggs of *Bactrocera dorsalis*, a common pest parasitized by *F. arisanus*. The behavioral and electroantennogram (EAG) responses of both sexes of *F. arisanus* to these chemicals were investigated. Our results suggest that all tested HIPVs can elicit different levels of antennal and behavioral response for both sexes at a variety of concentrations. There were no significant differences in response levels between sexes for all compounds, except trans farnesol at a concentration of 10<sup>-2</sup> ml/ml (v/v), indicating that it may be of value in biological control programs using *F. arisanus*. Furthermore, we observed that the behavioral and EAG dose-dependent curves of both sexes of *F. arisanus* could be largely divided into four types, namely steady decrease (ethyl octanoate, methyl octanoate, linalool, tetradecane, pentadecane), steady increase (trans farnesol), inverted V (cis-3-hexenyl acetate,  $\beta$ -myrcene,  $\beta$ -ocimene) and fluctuating (benzaldehyde). The ecological role of these HIPV components in the host location process of *F. arisanus* is discussed.

**Keywords:** HIPVs, biological control, EAG response, behavioral responses, infochemicals

## Introduction

Within the context of biological control programs, the effectiveness of a parasitoid relies heavily on its capacity to search for suitable hosts (Godfray, 1994). Insects have developed multisensory systems for perceiving various cues from their habitats, including visual, chemical and vibrational cues, which are utilized in an interactive manner to make foraging choices (Schellhorn et al., 2014). Of these, chemical cues play the most important role and influence activities such as food finding, mate searching and escaping natural enemies and competition. These, in turn, affect the large-scale

geographical distribution of insect populations (Schoonhoven et al., 2005; Vinatier et al., 2011). Chemical cues from either the phytophagous host or its habitat may be used by parasitoids at long- and short-range. Generally, cues derived from the host are less detectable but a more reliable indication of the presence of herbivorous hosts. Cues from the host plant, on the other hand, are less reliable but more detectable, which presents the parasitoid with a reliability-detectability dilemma (Vet and Dicke, 1992).

Parasitoids have developed diverse tactics to overcome this predicament, one of which is the utilization of herbivore-induced plant volatiles (HIPVs) as reliable cues to find their herbivorous hosts in a complex odorous environment. This also has the indirect effect of defending host habitats from infestation (Vet and Dicke, 1992; Ngi-Song et al., 2000). These volatiles usually comprise hundreds of chemical components distributed within the green leaf volatiles, and include esters, aromatic compounds, monoterpenes, sesquiterpenes and homoterpenes (Degenhardt et al., 2009); however, not all components of HIPVs trigger behavioral responses in parasitoids. In some tritrophic contexts, single critical compounds only or a blend of compounds with specific ratios have been found to affect the recruitment and recognition of insects (Bruce and Pickett, 2011). This indicates that it is necessary to investigate how parasitoids respond to volatile infochemicals for each parasitoid-host system and to characterize which infochemicals are involved.

The subject of the present research is *Fopius arisanus* Sonan (Hymenoptera: Braconidae), an egg-pupal endoparasitoid originating in the Indo-Pacific region and known to parasitize the eggs and first instars of approximately 40 frugivorous tephritid fruit fly species (Bautista and Harris, 1996; Rouse et al., 2007a). This parasitoid was introduced into Hawaii at the end of the 1940s and into French Polynesia later in 2002; in both cases it rapidly became the dominant parasitoid of tephritid pests, greatly suppressing the populations of *Bactrocera dorsalis* Hendel and *Ceratitis capitata* Wiedemann (Diptera: Tephritidae), respectively (Vargas et al., 2007, 2012, 2013). These cases of successful application demonstrated that *F. arisanus* possessed a huge potential for augmentative biological control, alone or in combination with other management measures to control fruit flies (Harris et al., 2000).

The biology of *F. arisanus* is well-known, and its host-searching behavior has been reported on coffee fruits infested by *C. capitata* eggs (Wang and Messing, 2003). Furthermore, many studies have investigated the relationships between fly-infested plants and the ovipositional behavior or parasitism performance of parasitoids (Liquidó, 1991; Harris and Bautista, 1996; Bautista et al., 2004; Ayelo et al., 2017). Additionally, *F. arisanus* was fascinated with fresh guava and orange fruits (Altuzar et al., 2004), and infestation by *Anastrepha* eggs enhanced this attraction (Rouse et al., 2007b; Pérez et al., 2013). In recent years, our research team has analyzed the volatile components of four host crops, namely guava (*Psidium guajava* L. [Myrtales: Myrtaceae], banana (*Musa paradisiaca* L. [Zingiberales: Musaceae]), citrus (*Citrus sinensis* L. [Sapindales: Rubiaceae]) and tomato *Solanum lycopersicum* Mill. [Tubiflorae: Solanaceae], by gas chromatography-mass spectrometry (GC-MS). We found that after egg deposition by *B. dorsalis*, host fruits can emit ten new chemical components in comparison with non-infested fruits (Gu et al., 2017). When we compared these volatile compounds with the volatile profile of the egg surface of *B. dorsalis* (Ji et al., 2016), we found that the emitted compounds derived from the fruits, rather than from the host pest. Until now, the ecological role of HIPVs emitted from fruits after *B. dorsalis* infestation in the host-location behavior of *F. arisanus* is poorly understood.

The purpose of the present research was to construct dose-response curves of key bioactive HIPVs that modulate the tritrophic system using electrophysiological and behavioral assays. Understanding how parasitoids respond to such chemicals will significantly contribute to the current knowledge on insect chemical communication and hopefully improve their effectiveness as biological control agents against destructive fruit pests like *B. dorsalis*.

## Materials and methods

### Parasitoid rearing

The initial colony of parasitoid *F. arisanus* was obtained from a field culture, reared on *B. dorsalis* eggs as described by Manoukis et al. (2011). Experimental parasitoids were kept at the insect mass rearing chamber of the Beneficial Insects Institute, Fujian Agriculture and Forestry University (BII, FAFU), Fujian Province, China under conditions of  $25 \pm 1$  °C,  $65 \pm 5\%$  relative humidity (RH), and a L:D photoperiod of 10:14 h. The laboratory-reared strains were periodically introduced with a parasitoid population collected from the field to prevent genetic decline and to preserve the original behavioral properties of the species. Naïve mated parasitoid females and males aged 7–12 days (without previous exposure to host or plant odors) acquired from the fifth to sixth generation were used in the assays. All experiments were conducted between 08:00 and 16:00.

### Chemical components

Ten chemical standards, namely ethyl octanoate, benzaldehyde, methyl octanoate, linalool, tetradecane, pentadecane, cis-3-hexenyl acetate,  $\beta$ -myrcene, trans farnesol and  $\beta$ -ocimene, were used in electroantennogram (EAG) and behavioral assays. Information on these standards is listed in *Table 1*. Each compound was dissolved into n-hexane (purity > 99%; Beijing solabo Technology Co., Ltd, Beijing, China) and diluted to concentrations of  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$  ml/ml (v:v) for the dose-response tests. Diluted standards were maintained in a refrigerator at 4 °C until required.

**Table 1.** Details of the ten chemical standards

| Chemicals             | Formula  | CAS no.    | Purity (%) | Storage condition             | Source   |
|-----------------------|--|------------|------------|-------------------------------|--|
| Benzaldehyde          | C <sub>7</sub> H <sub>6</sub> O                | 100-52-7   | 98.5       | Room temperature              | Shanghai Macklin Biochemical Technology Co., Ltd |
| Ethyl octanoate       | C <sub>10</sub> H <sub>20</sub> O <sub>2</sub> | 106-32-1   | 99         | Room temperature              | Shanghai Macklin Biochemical Technology Co., Ltd |
| Methyl octanoate      | C <sub>9</sub> H <sub>18</sub> O <sub>2</sub>  | 111-11-5   | 99         | Room temperature, dry, sealed | Shanghai Macklin Biochemical Technology Co., Ltd |
| Linalool              | C <sub>10</sub> H <sub>18</sub> O              | 78-70-6    | 98         | Room temperature              | Shanghai Macklin Biochemical Technology Co., Ltd |
| Tetradecane           | C <sub>14</sub> H <sub>30</sub>                | 629-59-4   | 98         | Room temperature              | Shanghai Macklin Biochemical Technology Co., Ltd |
| Pentadecane           | C <sub>15</sub> H <sub>32</sub>                | 629-62-9   | 98         | Room temperature              | Shanghai Macklin Biochemical Technology Co., Ltd |
| cis-3-hexenyl acetate | C <sub>8</sub> H <sub>14</sub> O <sub>2</sub>  | 3681-71-8  | 98         | Room temperature              | Shanghai Macklin Biochemical Technology Co., Ltd |
| $\beta$ -myrcene      | C <sub>10</sub> H <sub>16</sub>                | 123-35-3   | 90         | 2–8 °C                        | Aladdin reagent (Shanghai) Co., Ltd              |
| Trans farnesol        | C <sub>15</sub> H <sub>26</sub> O              | 106-28-5   | 97         | 2–8 °C                        | Shanghai Macklin Biochemical Technology Co., Ltd |
| $\beta$ -ocimene      | C <sub>10</sub> H <sub>16</sub>                | 13877-91-3 | 90         | Room temperature              | Shanghai Macklin Biochemical Technology Co., Ltd |

### **Choice measurements**

This bioassay was designed to determine whether different concentrations of HIPVs imposed differing impacts on the behavioral responses of *F. arisanus*. This was done using a six-choice olfactometer as described by Gu et al. (2018). The test area comprised a release area, an adapting area, a selection area and six 100-ml odor bottles. Humidified and charcoal-filtered air was passed through the test area uniformly at a rate of 200 ml/min. A piece of cotton wool was placed in each odor bottle which was moistened with 200 µl of the test component; one concentration was used for each bottle and pure n-hexane served as the control group. Each trial was tested with only one chemical type. Prior to the experiment, *F. arisanus* adults were starved for 1 h to improve their sensitivity to odor. One group of 30 parasitoids of the same sex were then concomitantly introduced into the release area. Based on pre-observations, most parasitoids need about 30 min to adapt to this experimental apparatus and then make a “choice”. Therefore, after 30 min the number of parasitoids that stayed at each area for at least 10 s or trapped in each odor bottle was documented. The response rate was then calculated using the following formula:

$$R_{response} = \frac{N_{response}}{N_{release}} \times 100$$

$N_{response}$  denotes the number of selected males or females and  $N_{release}$  denotes the total number of released males or females. After each trial, the parasitoids were removed and not used again in the experiment. The apparatus was then cleaned using an abluent and then 75% ethanol, rinsed using distilled water, and thereafter dried for several hours with an air-blowing drier. After each individual trial, different chemicals of various concentrations were randomly deposited into odor bottles to eliminate any positional effects. Six replicates were performed for each six-choice experiment of each chemical type.

### **Antennal preparation and EAG measurements**

In order to evaluate the dose-dependent effects of each volatile component, the EAG technique was used to determine the electrophysiological responses of the antennae of male and female *F. arisanus* to volatile components at different concentrations. The antennae of *F. arisanus* adults were completely excised with the aid of a stereomicroscope and a few top flagellar segments of the antennae were cut off.

Thereafter, the cut antennal tips were inserted into Ag-AgCl glass electrodes filled with saline solution, which was used to maintain an electrical connection between the electrodes. The EAG response signals were passed through a high impedance amplifier (IDIC-2, Syntech, Hilversum, Netherlands) and further processed with EAG software (Syntech).

Different concentrations of the tested components diluted with n-hexane (control) were exposed in ascending order to abate the effect of olfactory adaptation possibly caused by strong stimulation. Stimulus solutions were prepared by first pipetting 20 µl of each solution onto individual filter papers (6 × 15 mm), and thereafter allowed to adequately diffuse for 2 min before the assay. The stimuli were pipetted onto filter paper strips and were immediately deposited in disposable Pasteur pipettes that in turn were connected to an air flow control device (Syntech CS-05, Netherlands Syntech Co., Ltd.) which continuously flowed purified and humidified air over the prepared antennae

at a rate of 100 ml/min. Each air tube was placed 10 mm from the antenna. The control stimulus containing n-hexane was tested both at the beginning and end of each trial to ensure that the preparations were functional throughout. Five antennae were used for each chemical, and each assay was replicated five times. The electrophysiological response of *F. arisanus* to each chemical was expressed by the EAG relative value ( $RV_{EAG}$ ), which was calculated using the following formula:

$$RV_{EAG} = \frac{V_1 + V_2}{V_{c1} + V_{c2}}$$

$V_1$  and  $V_2$  denote the two EAG values of the test compounds, and  $V_{c1}$  and  $V_{c2}$  denote the two EAG values of the control treatments at the start and the end of each replicate, respectively.

### **Statistical analysis**

Statistical differences between concentrations for both the behavioral responses and EAG relative values were evaluated using Tukey's honestly significant differences (HSD) test for multiple mean comparisons after ANOVA. A comparison of the differences between male and female parasitoids was conducted using an independent t-test. In both cases  $P < 0.05$  was considered statistically significant. All statistical analyses were performed using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

## **Results**

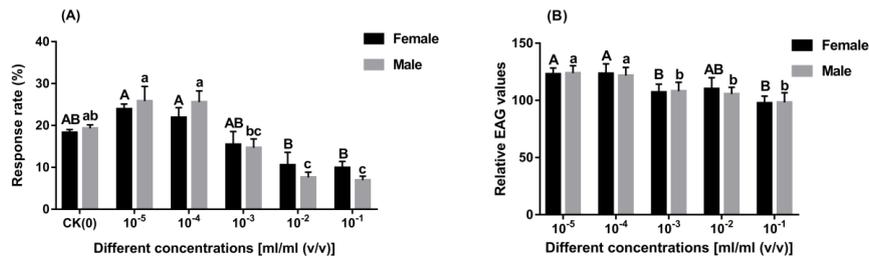
### ***The tendencies of response rates and EAG relative values of *F. arisanus* to ten synthetic HIPVs at different concentrations***

Our results show that all dosages of the tested components can elicit different levels of antennal and behavioral response in *F. arisanus* (both male and female). The response rates and EAG relative values of both sexes of *F. arisanus* to the same component showed some significant differences between concentrations (*Figs. 1-10*;  $F$  and  $P$  values see *Table 2*).

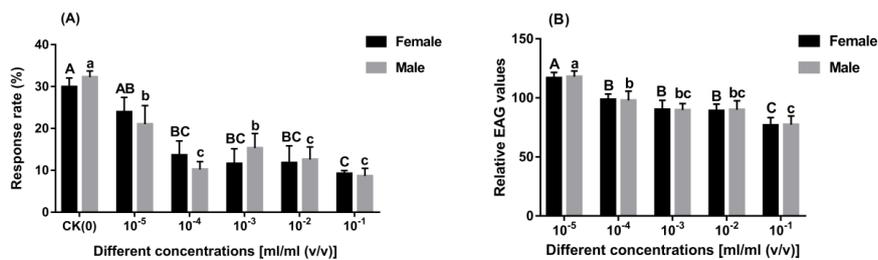
The dose threshold that can trigger the statistically highest behavioral and antennal response for each compound is shown in *Table 2*. It can be seen that both parasitoid females and males have a diverse dose threshold range for different chemicals. Furthermore, for each chemical, there was no difference in trend between males and females for both the response rate and EAG relative values. Benzaldehyde is an exception to this rule, for which the EAG relative values steadily decreased as the dose increased whereas the behavioral response rates of parasitoid males fluctuated with concentration (*Fig. 2*). The tendencies of the response rates and EAG relative values of *F. arisanus* males and females could be divided into the 4 types described below.

#### ***Steady decrease***

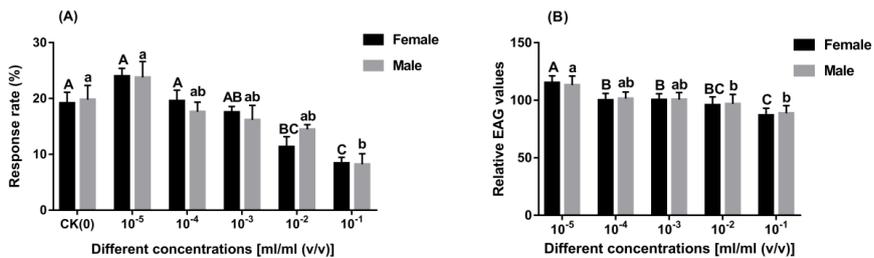
Response rates and EAG relative values of both male and female *F. arisanus* declined as the dose increased for ethyl octanoate, methyl octanoate, linalool, tetradecane and pentadecane (*Figs. 1 and 3-6*). For benzaldehyde, both male and female EAG relative values and female response rates belonged to this type (*Fig. 2B*).



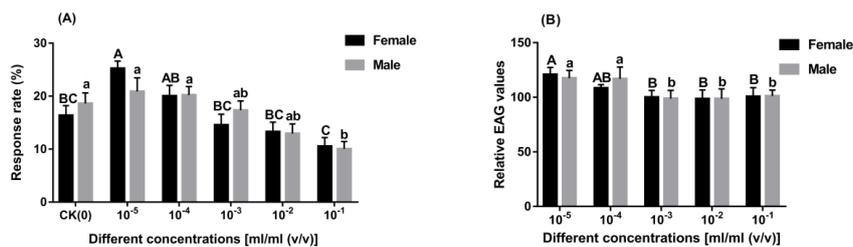
**Figure 1.** Behavioral (A) and EAG (B) dose-responses of *F. arisanus* to ethyl octanoate. The data are expressed as mean  $\pm$  SE. Different uppercase (female) or lowercase (male) letters indicate significant differences between concentrations (Tukey's HSD test after ANOVA,  $P < 0.05$ )



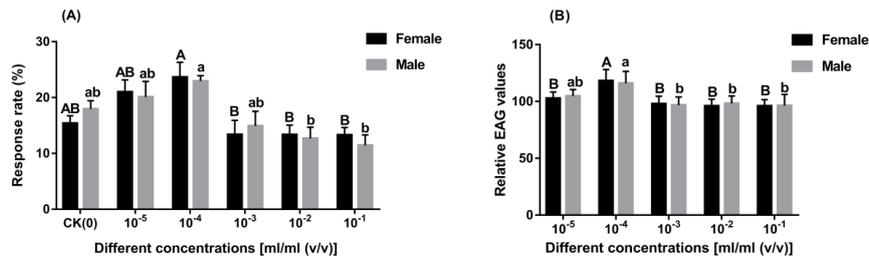
**Figure 2.** Behavioral (A) and EAG (B) dose-responses of *F. arisanus* to benzaldehyde. The data are expressed as mean  $\pm$  SE. Different uppercase (female) or lowercase (male) letters indicate significant differences between concentrations (Tukey's HSD test after ANOVA,  $P < 0.05$ )



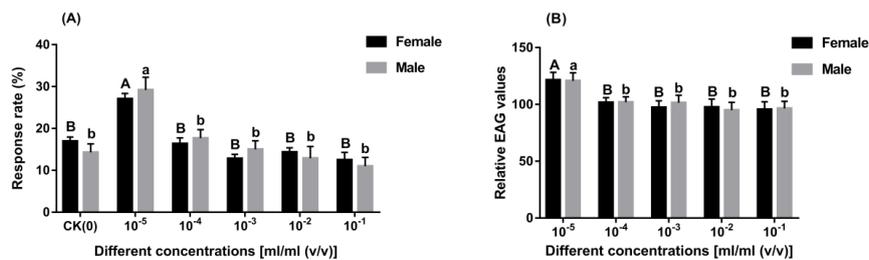
**Figure 3.** Behavioral (A) and EAG (B) dose-responses of *F. arisanus* to methyl octanoate. The data are expressed as mean  $\pm$  SE. Different uppercase (female) or lowercase (male) letters indicate significant differences between concentrations (Tukey's HSD test after ANOVA,  $P < 0.05$ )



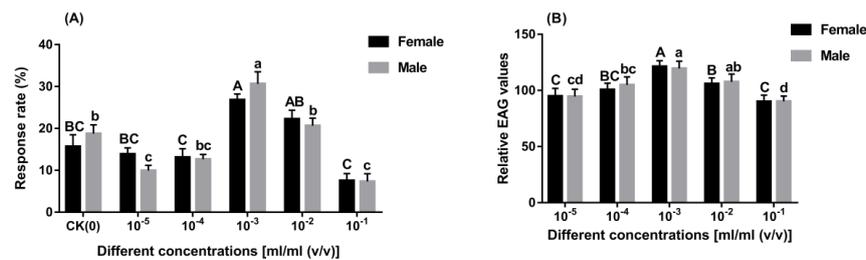
**Figure 4.** Behavioral (A) and EAG (B) dose-responses of *F. arisanus* to linalool. The data are expressed as mean  $\pm$  SE. Different uppercase (female) or lowercase (male) letters indicate significant differences between concentrations (Tukey's HSD test after ANOVA,  $P < 0.05$ )



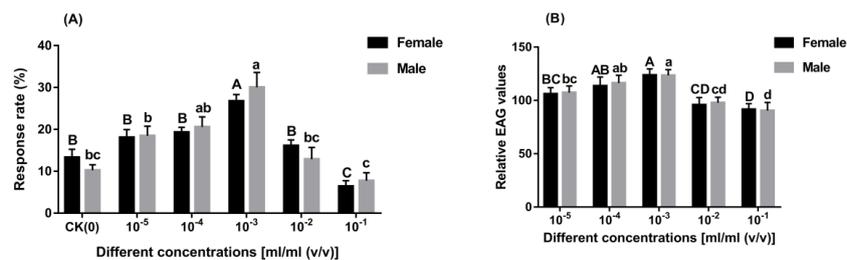
**Figure 5.** Behavioral (A) and EAG (B) dose-responses of *F. arisanus* to tetradecane. The data are expressed as mean  $\pm$  SE. Different uppercase (female) or lowercase (male) letters indicate significant differences between concentrations (Tukey's HSD test after ANOVA,  $P < 0.05$ )



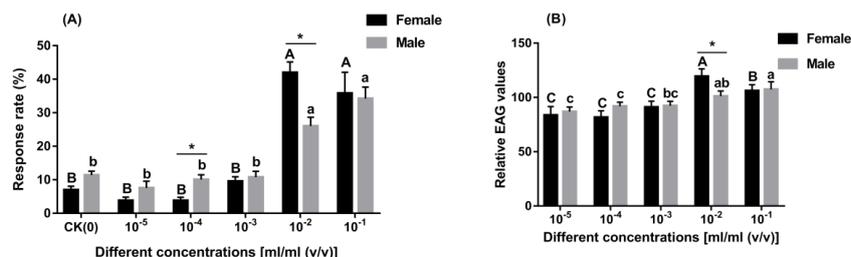
**Figure 6.** Behavioral (A) and EAG (B) dose-responses of *F. arisanus* to pentadecane. The data are expressed as mean  $\pm$  SE. Different uppercase (female) or lowercase (male) letters indicate significant differences between concentrations (Tukey's HSD test after ANOVA,  $P < 0.05$ )



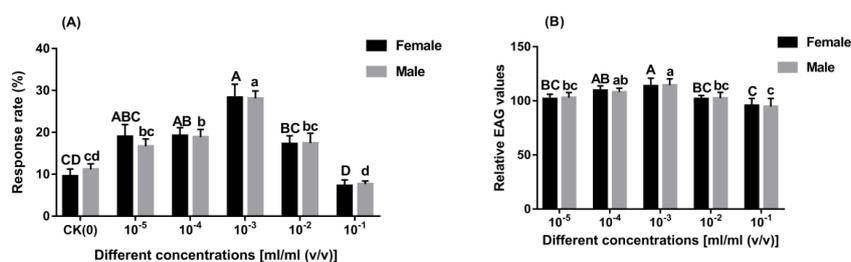
**Figure 7.** Behavioral (A) and EAG (B) dose-responses of *F. arisanus* to *cis*-3-hexenyl acetate. The data are expressed as mean  $\pm$  SE. Different uppercase (female) or lowercase (male) letters indicate significant differences between concentrations (Tukey's HSD test after ANOVA,  $P < 0.05$ )



**Figure 8.** Behavioral (A) and EAG (B) dose-responses of *F. arisanus* females to  $\beta$ -myrcene. The data are expressed as mean  $\pm$  SE. Different letters indicate significant differences between concentrations (Tukey's HSD test after ANOVA,  $P < 0.05$ )



**Figure 9.** Behavioral (A) and EAG (B) dose-responses of *F. arisanus* to *trans* farnesol. The data are expressed as mean  $\pm$  SE. Different uppercase (female) or lowercase (male) letters indicate significant differences between concentrations (Tukey's HSD test after ANOVA,  $P < 0.05$ ). Asterisks indicate significant differences between females and males using an independent *t*-test at  $P < 0.05$



**Figure 10.** Behavioral (A) and EAG (B) dose-responses of *F. arisanus* to  $\beta$ -ocimene. The data are expressed as mean  $\pm$  SE. Different uppercase (female) or lowercase (male) letters indicate significant differences between concentrations (Tukey's HSD test after ANOVA,  $P < 0.05$ )

#### Steady increase

Response rates and EAG relative values increased as the dose increased and peaked at the highest concentration. This applied only to *trans* farnesol (Fig. 9).

#### Inverted V

Response rates and EAG relative values increased as the doses increased and peaked at a specific dose, then reduced as the dose further increased. The group comprised the results for *cis*-3-hexenyl acetate,  $\beta$ -myrcene and  $\beta$ -ocimene (Figs. 7, 8 and 10).

#### Fluctuating

Only one trend was recorded as having this type: the response rates of *F. arisanus* males to benzaldehyde which first decreased, then increased, then decreased as the concentration increased (Fig. 2A).

#### Differences between sexes in their behavioral and antennal responses

In most cases, there were no significant differences in either the EAG relative values or the response rates of parasitoids between males and females at each concentration. However, for *trans* farnesol significant differences in response rates were observed at concentrations of  $10^{-4}$  ( $F = 0.007$ ,  $t = 3.892$ ,  $P = 0.004$ ) and  $10^{-2}$  ml/ml (v/v) ( $F = 2.230$ ,

$t = -3.683$ ,  $P = 0.004$ ) (Fig. 9A) and for the EAG relative values at  $10^{-2}$  ml/ml ( $v/v$ ) ( $F = 0.005$ ,  $t = -2.749$ ,  $P = 0.021$ ) (Fig. 9B).

**Table 2.** Concentration threshold (ml/ml) of different components that trigger the statistically highest response rate and EAG relative values of *F. arisanus*

| Chemicals             | Behavioral response |                           |                   |                           | EAG relative values           |                           |                   |                           |
|-----------------------|---------------------|---------------------------|-------------------|---------------------------|-------------------------------|---------------------------|-------------------|---------------------------|
|                       | Female              |                           | Male              |                           | Female                        |                           | Male              |                           |
|                       | Threshold           | F and P                   | Threshold         | F and P                   | Threshold                     | F and P                   | Threshold         | F and P                   |
| Benzaldehyde          | $0-10^{-5}$         | $F=7.061$ ,<br>$P<0.001$  | 0                 | $F=9.272$ ,<br>$P<0.001$  | $10^{-5}$                     | $F=30.163$ ,<br>$P<0.001$ | $10^{-5}$         | $F=24.399$ ,<br>$P<0.001$ |
| Ethyl octanoate       | $0-10^{-3}$         | $F=6.992$ ,<br>$P<0.001$  | $0-10^{-4}$       | $F=15.802$ ,<br>$P<0.001$ | $10^{-5}-10^{-4}$ , $10^{-2}$ | $F=10.801$ ,<br>$P<0.001$ | $10^{-5}-10^{-4}$ | $F=11.532$ ,<br>$P<0.001$ |
| Methyl octanoate      | $0-10^{-3}$         | $F=13.277$ ,<br>$P<0.001$ | $0-10^{-2}$       | $F=5.746$ ,<br>$P=0.001$  | $10^{-5}$                     | $F=13.794$ ,<br>$P<0.001$ | $10^{-5}-10^{-3}$ | $F=7.913$ ,<br>$P=0.001$  |
| Linalool              | $10^{-5}-10^{-4}$   | $F=8.506$ ,<br>$P<0.001$  | $0-10^{-2}$       | $F=5.120$ ,<br>$P=0.002$  | $10^{-5}-10^{-4}$             | $F=9.645$ ,<br>$P<0.001$  | $10^{-5}-10^{-4}$ | $F=7.142$ ,<br>$P=0.001$  |
| Tetradecane           | $0-10^{-4}$         | $F=4.891$ ,<br>$P=0.002$  | $0-10^{-3}$       | $F=4.708$ ,<br>$P=0.003$  | $10^{-4}$                     | $F=8.905$ ,<br>$P<0.001$  | $10^{-5}-10^{-4}$ | $F=5.093$ ,<br>$P<0.001$  |
| Pentadecane           | $10^{-5}$           | $F=17.590$ ,<br>$P<0.001$ | $10^{-5}$         | $F=7.483$ ,<br>$P<0.001$  | $10^{-5}$                     | $F=14.349$ ,<br>$P<0.001$ | $10^{-5}$         | $F=13.000$ ,<br>$P<0.001$ |
| cis-3-hexenyl acetate | $10^{-3}-10^{-2}$   | $F=12.120$ ,<br>$P<0.001$ | $10^{-3}$         | $F=19.917$ ,<br>$P<0.001$ | $10^{-3}$                     | $F=21.630$ ,<br>$P<0.001$ | $10^{-3}-10^{-2}$ | $F=16.451$ ,<br>$P<0.001$ |
| $\beta$ -myrcene      | $10^{-3}$           | $F=18.641$ ,<br>$P<0.001$ | $10^{-4}-10^{-3}$ | $F=10.917$ ,<br>$P<0.001$ | $10^{-4}-10^{-3}$             | $F=19.490$ ,<br>$P<0.001$ | $10^{-4}-10^{-3}$ | $F=21.823$ ,<br>$P<0.001$ |
| Trans-farnesol        | $10^{-2}-10^{-1}$   | $F=32.970$ ,<br>$P<0.001$ | $10^{-2}-10^{-1}$ | $F=24.057$ ,<br>$P<0.001$ | $10^{-2}$                     | $F=31.240$ ,<br>$P<0.001$ | $10^{-2}-10^{-1}$ | $F=14.070$ ,<br>$P<0.001$ |

## Discussion

Plants can alter the emission of their volatile profile in response to herbivore foraging or pest oviposition (Hilker and Meiners, 2011). The selective exploration of HIPVs by parasitoids when searching their phytophagous hosts has been recorded on different parasitoids species (Dicke and van Loon, 2000), concentrated on those that parasitize folivorous larvae. Recently, various methods of parasitoid-based pest control have been developed that incorporate the use of volatile infochemicals as part of non-toxic, low-cost and effective strategies (Pickett et al., 2014). Infochemicals are disseminated through plants as a defense mechanism to directly and/or indirectly protect themselves from infestation by pests (Khan et al., 2010). For example, foraging parasitoids use HIPVs (a type of infochemicals) as reliable cues at both long- and short-range to locate their hosts in nature; this effectively protects the infested plant from their pests (Ngi-Song et al., 2000). Numerous studies have demonstrated that infestation by tephritid fruit flies, including *B. dorsalis*, enhances the attraction of *F. arisanus* adults to diverse crops species (Rousse et al., 2007b; Pérez et al., 2013; Ayelo et al., 2017; Gu et al., 2017). Thus, using infochemicals from the host habitats of pests will help design and enhance parasitoid-based biological control programs. It is essential to evaluate which volatile composition are identified at the olfactory level along with the minimum dosages required to evoke behavioral responses in a particular parasitoid (James, 2005).

Our research group has previously qualitatively and quantitatively compared the volatile profiles of four kinds of host crops before and after oviposition by *B. dorsalis*.

The results suggested that ten compounds were exclusive to infested crops compared to healthy ones, and are generated by the host crop rather than the pest (Gu et al., 2017; Ji et al., 2016). Indeed, we concluded that guava fruits release benzaldehyde, cis-3-hexenyl acetate, tetradecane, pentadecane and trans farnesol, and that citrus fruit release ethyl octanoate, methyl octanoate, pentadecane,  $\beta$ -ocimene, linalool and  $\beta$ -myrcene in response to infestation by *B. dorsalis* (Gu et al., 2017). The emissions of most of these HIPVs is possibly attributed to the infested crop's active defense response because they are only detected after herbivore infestation and they are not emitted from healthy fruit, which is important in the host location process of parasitoids. Our present study revealed that these ten HIPV components, at different concentrations, can elicit different levels of electrophysiological and behavioral response in both sexes of *F. arisanus*. This provides yet more evidence that *F. arisanus* uses chemical cues derived from host crops, in concordance with speculations by other authors (Altuzar et al., 2004; Ayelo et al., 2017)

Insects are equipped with a highly sensitive olfactory system that enable them to probe and distinguish related volatile infochemicals with a high level of specificity and selectivity (Bruce and Pickett, 2011). By using electrophysiological and behavioral studies, it is possible to identify potentially bioactive volatile compounds and establish their biological function in parasitoids (Webster et al., 2010). In the present research, behavioral choice and EAG assays in *F. arisanus* confirmed 10 synthetic HIPVs to be biologically active. It is worth noting that among these volatile components, many single components have been demonstrated as eliciting positive behavioral or/and EAG responses in other braconids parasitoids [e.g. linalool for *Cotesia marginiventris* Cresson (Ngumbi and Fadamiro, 2012), ethyl octanoate for *Psytalia concolor* Szépligeti (Benelli et al., 2013), benzaldehyde for *Lysiphlebia japonica* Ashmead (Hou et al., 2008), tetradecane and cis-3-hexenyl acetate for *Microplitis croceipes* Cresson (Morawo et al., 2016; Chen and Fadamiro, 2007),  $\beta$ -myrcene and  $\beta$ -ocimene for *Aphidius ervi* Haliday (Corrado et al., 2007; Takemoto and Takabayashi, 2015) (Hymenoptera: Braconidae)] or in other hymenopteran parasitoids [e.g. pentadecane for *Trichogramma exiguum* Pinto and Platner (Paul et al., 2002) (Hymenoptera: Trichogrammatidae) and trans farnesol for *Diaeretiella rapae* M'Intosh (Hymenoptera: Aphidiidae)] (Reed et al., 1995).

The behavioral and EAG dose–response curves of the ten compounds studied in the present research can be divided into four types: steady decrease, steady increase, inverted V and fluctuating. Regarding the group “steady decrease”, the strongest response was obtained at the lowest dose, whereas for “steady increase”, this was obtained at the highest dose. We therefore speculated that *F. arisanus* may have a dosage threshold for perceiving different components which can trigger corresponding levels of behavioral responses and physiological activities. For example, if the dosage is lower than the threshold value, the olfactory sensitivity of the parasitoids would elevate as the dose increased; however, if the dosage is higher than the threshold, sensitivity would descend as the dose increased. According to this, the concentration threshold of the volatile components that belonged to the group “inverted V” could be easily determined, i.e. cis-3-hexenyl acetate,  $\beta$ -ocimene and  $\beta$ -myrcene at  $10^{-3}$  ml/ml (v/v). The step-like ability of benzaldehyde to lure *F. arisanus* was observed at our tested concentrations. Hiroyuki and Junji (2015) also reported a similar response in a braconid parasitoid to a volatile substance: using a Y-tube olfactometer, they found that *A. ervi* females, an effective natural enemy of several aphid species, were significantly attracted

to  $\alpha$ - phellandrene at doses of 30 and 0.1 ng, but not at 10- and 1- ng. Both our findings and those of Hiroyuki and Junji (2015) indicate that HIPVs can carry information at a variety of dose ranges, instead of being restricted to a single optimal dose range. The reason why *F. arisanus* has evolved to respond to two dose ranges in their host location behavior warrants further investigation.

The behavioral dose-response curves of volatile substances belonging to the “steady decrease” group are of particular interest. Of these compounds, only benzaldehyde at a concentration of  $10^{-5}$  ml/ml (v/v) attracted significantly fewer parasitoids compared to the control group, whereas in contrary to other compounds in this group. This suggested that benzaldehyde at our tested concentrations may impose a dose-dependent repellent effect on *F. arisanus*. The ecological role of benzaldehyde on parasitoids deserves further investigation. Regarding the differences between sexes, both the behavioral and EAG responses exhibited similar trends. Only one compound showed a significant difference, i.e. trans farnesol at  $10^{-4}$  and  $10^{-2}$  ml/ml (v/v) for behavioral responses and  $10^{-2}$  ml/ml (v/v) for EAG responses. We hypothesize that both female and male *F. arisanus* may utilize the same olfactory receptors to perceive the other nine infochemicals (although with varying sensitivity), whilst having distinct sensing mechanisms for trans farnesol. In parasitoid-based biological control programs, the female has a vital role in pest suppression, directly resulting in the death of pests (Mills and Getz, 1996); thus, trans farnesol may be a more valuable volatile component.

In summary, HIPVs are considered as kairomones for several parasitoid species (Carrasco et al., 2005; Dweck et al., 2010; Benelli et al., 2013) and have already been successfully applied in the field (Uefune et al., 2012). However, detailed knowledge concerning the effect of HIPVs on the host-seeking behavior of biological control agents in the field requires further investigation prior to any possible commercial utilization (Kaplan, 2012). Besides recruiting beneficial arthropods, we believe that suitable HIPV candidates for field application should also possess additional useful features: (1) improving the defensive activities of intrinsic or imported biological control agents; (2) repelling pests or impose negative effects on the colonization and development of damaging pests; (3) exerting positive effects on the crop of economic importance; (4) without any adverse effects on non-target organisms or environment. For example, methyl salicylate at a certain concentration can not only attract *Aphidius gifuensis* Ashmead females (Hymenoptera: Braconidae) (Song, 2019), but can also inhibit the colonization and development of *Myzus persicae* Sulzer (Hemiptera: Aphididae) on tobacco leaves and provide a repellent effect (Liu et al., 2013). Furthermore, this substance can improve the parasitism performance of *Anagrus nilaparvatae* Pang et Wang (Hymenoptera: Mymaridae) towards eggs of *Nilaparvata lugens* Stal (Homoptera: Delphacidae) eggs (Wang and Lou, 2013).

Outside of direct field application, HIPVs also could be applied to enhance the effectiveness of mass-rearing beneficial insects since these compounds can improve the parasitization performance on alternative hosts. In another use, the host-seeking ability of *F. arisanus* females could be augmented by associative learning using the bioactive volatile compounds: the compounds could be incorporated into the diet of parasitoids at different phases and/or through a “reward” strategy before field release. As such, HIPVs with multiple positive ecological roles could achieve many things simultaneously, thus enhancing the efficiency of a series of plant protection strategies. Accurately characterizing these chemicals, along with investigating their potential in a variety of applications, is therefore of great value in integrated pest management.

## Conclusion

Infochemicals are produced from either the herbivorous host or its habitat and are used by parasitoids to search for their hosts. A greater understanding of the tritrophic context of fruit – fruit fly – parasitoid systems will help improve the effectiveness of biocontrol programs that use parasitoids against destructive pests. In the present study, our results demonstrated that individually applied HIPVs can elicit different levels of antennal and behavioral responses from both sexes of *F. arisanus* when used at a variety of concentrations. This indicates that the tested components could be utilized to modulate parasitoid behavior, at least under laboratory conditions. However, thus far, the field application of a specific infochemical has only been reported for the pest sex pheromone, where it was used to improve parasitoid foraging activity. The use of infochemicals in pest control therefore appears to be a desirable tactic and thus we strongly recommend that field investigations are performed to both evaluate their practical effects in nature and comprehensively understand their ecological roles. We hope that the findings of the present study will help to screen for appropriate HIPV components, and enhance biocontrol efforts in sustainably suppressing fruit fly populations.

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