# CHAPTER FOUR

**Olfactory responses of *Diglyphus isaea* (Hymenoptera: Eulophidae) to *Liriomyza* (Diptera: Agromyzdae) species-induced volatiles of *Phaseolus vulgaris*, *Pisum sativum*, *Solanum lycopersicum* and *Vicia faba***

## Abstract

The role of volatile stimuli in host finding of *Diglyphus isaea* was studied on *Liriomyza huidobrensis*, *L. sativae* and *L. trifolii* on four plant species (*Phaseolus vulgaris*, *Pisum sativum, Solanum lycopersicum* and *Vicia faba*). Two experiments were carried out in the laboratory using a Y-tube olfactometer. In the first experiment four-day-old pre-mated *D. isaea* females were exposed to undamaged and mechanically damaged plants, plants infested with third-instar larvae of one of the three *Liriomyza* species and a blank control. The second experiment compared the behaviour of *D. isaea* females on *P. vulgaris*, *P. sativum* and *S. lycopersicum* infested with third-instar larvae of *L. huidobrensis* against a *L. huidobrensis*-infested *V. faba* control. First and final choices and time spent by parasitoids in arms of the Y-tube olfactometer were recorded. Mechanically damaged and undamaged plants were not more attractive than the blank control. Compared to the blank control, preference of *D. isaea* for *L. huidobrensis*-infested plants was similar for all plant species, while *V. faba* was preferred when plants were infested with *L. sativae* and *P. vulgaris* and *S. lycopersicum* were preferred when plants were infested with *L. trifolii*. Against a *V. faba* control, parasitoids were more attracted to *V. faba* than to *P. sativum* and *P. vulgaris*. However, there were no significant differences in attraction between the *V. faba* and *S. lycopersicum*. This work shows that attraction of *D. isaea* to plant volatiles released by leafminer-infested plants is dependent to a various degree on interactions between *Liriomyza* species and host plants.

**Introduction**

The agromyzid leafminers *Liriomyza huidobrensis* (Blanchard), *L. sativae* Blanchard and *L. trifolii* (Burgess) are polyphagous and important pests of agricultural crops worldwide (Spencer, 1985; Murphy & LaSalle, 1999; EPPO, 2006; Burgio *et al*., 2007). *Diglyphus isaea* (Walker) (Hymenoptera, Eulophidae) is used as a biological control agent of *Liriomyza* species in many parts of the world (Heinz & Parrella, 1989; Murphy & LaSalle, 1999; Ode & Heinz, 2002). Although *D. isaea* parasitises several species of *Liriomyza* on different crops, the distribution of parasitoids among crops is uneven, that is, parasitoids are associated with certain host plants and habitats but not with others (Zehnder & Trumble, 1984; Johnson & Hara, 1987; Chabi-Olaye *et al*., 2008). Occurrences of these patterns indicate the huge challenge that parasitoids encounter before locating *Liriomyza* hosts. In locating their hosts, parasitoids must search for potential hosts living on different plants growing in diverse habitats (Zhao & Kang, 2002).

Host plant and leafminer-larvae related factors that are important in host finding by *D. isaea* include visual cues from the plants (colour of leaves, mine shape, mine size), size of leafminer larvae and volatiles released by the feeding larvae (Finidori-Logli *et al*., 1996). Among these factors, chemical stimuli (volatiles released due to adult and larval plant damage) act as cues that direct many parasitoids to plant habitats and their hosts in the long to short distance range (Dicke & Minkenberg, 1991). A study by Zhao & Kang, (2002) on *L. sativae* revealed that *D. isaea* was more attracted to volatiles emitted from mined bean (*Phaseolus vulgaris* L.) leaves than from other host plants infested with *L. sativae* larvae. From these studies, it was concluded that herbivore-induced volatile (HIV) components were variable among plants depending on the type of plant damage and important in guiding *D. isaea* to their hosts.

A variety of crops grown in Kenya, for example snow peas (*Pisum sativum* L., Fabaceae), French beans (*Phaseolus vulgaris* L., Fabaceae), tomatoes (*Solanum lycopersicum* L., (Solanaceae), faba bean (*Vicia faba* L., Fabaceae) and cut flowers, which are of high commercial value are prone to high levels of *Liriomyza* attack (Chabi-Olaye *et al*., 2008). Very little is known about differences in volatile composition of these host plants when attacked by different *Liriomyza* species with different feeding

habits, e.g. *L. huidobrensis* mines extend towards the base of the leaf, which tend to sieve leaf segments between the veins (Wei & Kang 2006). Furthermore, *L. huidobrensis* larvae consume mesophyll both in palisade and in spongy tissues (Parrella & Bathke, 1984, Wei *et al*., 2000), whereas *L. sativae* and *L. trifolii* larvae only feed in palisade tissue (Kang, 1996; Salvo & Valladares, 2004).

In the tritrophic interaction involving host plants, leafminers and *D. isaea*, both the host insects and the parasitoid are generalists (Zhao & Kang, 2002). Considering the wide host range of the three *Liriomyza* species and *D. isaea,* it is critical to establish which host plant-leafminer combinations are suitable for *D. isaea* in biological control programmes. Host plant association of *D. isaea* is poorly understood in Kenyan horticultural systems. As an important step in understanding the role of volatiles in guiding *D. isaea* to plant and leafminer habitats, this study was undertaken to determine the behavioural responses of *D. isaea* to herbivore-induced volatiles of different plants induced by *L. huidobrensis*, *L. sativae* and *L. trifolii*.

**Materials and methods**

**Plants**

Four plant species, *P. vulgaris* (variety Julia), *P. sativum* (variety Oregon Sugar Pod III) *S. lycopersicum* (variety Moneymaker) and *V. faba* (a local Kenyan open pollinated variety) were used in the experiments. Plants were grown and maintained in a leafminer-free screen house at the International Centre of Insect Physiology and Ecology (*icipe*) in Nairobi, Kenya as described in Chapter 2. The average leaf areas of the plants before the experiments were: *P. vulgaris*, 111.68 cm$^2$, *P. sativum*, 76.65 cm$^2$, *S. lycopersicum* 98.43 cm$^2$ and *V. faba* 37.40 cm$^2$.

**Insect rearing**

The three *Liriomyza* species, *L. huidobrensis*, *L. sativae* and *L. trifolii*, were obtained from the International Centre of Insect Physiology and Ecology (*icipe*) leafminer rearing facility (see Chapter 2). All species were reared at $27 \pm 2$ ℃ with a photoperiod of 12L: 12D and relative humidity of approximately 30 %. *Liriomyza huidobrensis* was

reared on *V. faba* while *L. sativae* and *L. trifolii* were reared on *P. vulgaris*. All species had been reared on the respective host plants for approximately 18-20 generations prior to the experiments.

For experimental purposes, three potted plants of each of the four plant species were exposed to 50 four-day-old adult male and female leafminers (sex ratio 1:1) for a period of 24 hours. Plants were then monitored until at least 30 larvae had developed to the third-instar stage. Any damage to the exposed plants was avoided as much as possible to prevent the release of volatiles not associated with the insect damage. The mean number of mined leaves and leafminer larvae per plant species were: FrLH3 (81 larvae/10 mined leaves), SNLH3 (36 larvae/17 mined leaves), TMLH3 (36 larvae/15 mined leaves), FBLH3 (92 larvae/17 mined leaves), FrLS3 (105 larvae/10 mined leaves), TMLS3 (31 larvae/15 mined leaves), FBLS3 (60 larvae/19 mined leaves), TMLT3 (30 larvae/16 mined leaves) and FBLT3 (61 larvae/14 mined leaves).

*Diglyphus isaea* adults were supplied by Dudutech Pvt (K) (Ltd) (Kenya) where the parasitoid was mass-reared on *P. sativum* under uniform conditions. Parasitoids were received from Dudutech within 24 hours of emergence. They were then allowed to mate for a period of 48 hours in ventilated Perspex cages (40 cm × 20 cm × 20 cm) and thereafter given a pre-oviposition period of 24 hours. The parasitoids were fed on a 10 % honey solution and kept at $25 \pm 1$ $^{\circ}$C, approximately 45 % relative humidity, and a 12h: 12h L: D photoperiod during the mating and pre-oviposition period. After this period, 30 females per treatment were randomly selected and placed singly in a small cylindrical vial (3 cm long, internal diameter 1 cm). All parasitoids used in the experiments were four-day-old naïve female adults, with no previous exposure to either leafminer or plants. Each individual female was used only once.

**Y-tube olfactometer behavioural experiments**

A Y-tube olfactometer was used to determine the response of *D. isaea* females to different volatiles emitted from the four host plant species (*P. vulgaris*, *P. sativum*, *S. lycopersicum* and *V. faba*) with damage induced by three different leafminer species (*L. huidobrensis*, *L. sativae* and *L. trifolii*). The Y-tube olfactometer was similar to that described by Ngi-Song *et al*., 1996; Wei & Kang, 2006). It consisted of a 15 cm-long

stem, 15 cm-long arms joined at an angle of 60°, an internal diameter of 2.3 cm and an observation arena measuring 95 cm × 60 cm × 45 cm arena (Fig.4.1).

Fluorescent tubes fixed at 1 m above the observation arena provided artificial light while temperatures inside the experimental room were maintained at $25 \pm 1$ °C. A pump was used to draw air through activated charcoal to purify it from any contaminating odours. The air from the activated charcoal was led to two flow meters, each leading to an arm of the Y-tube via the target (plant treatment).
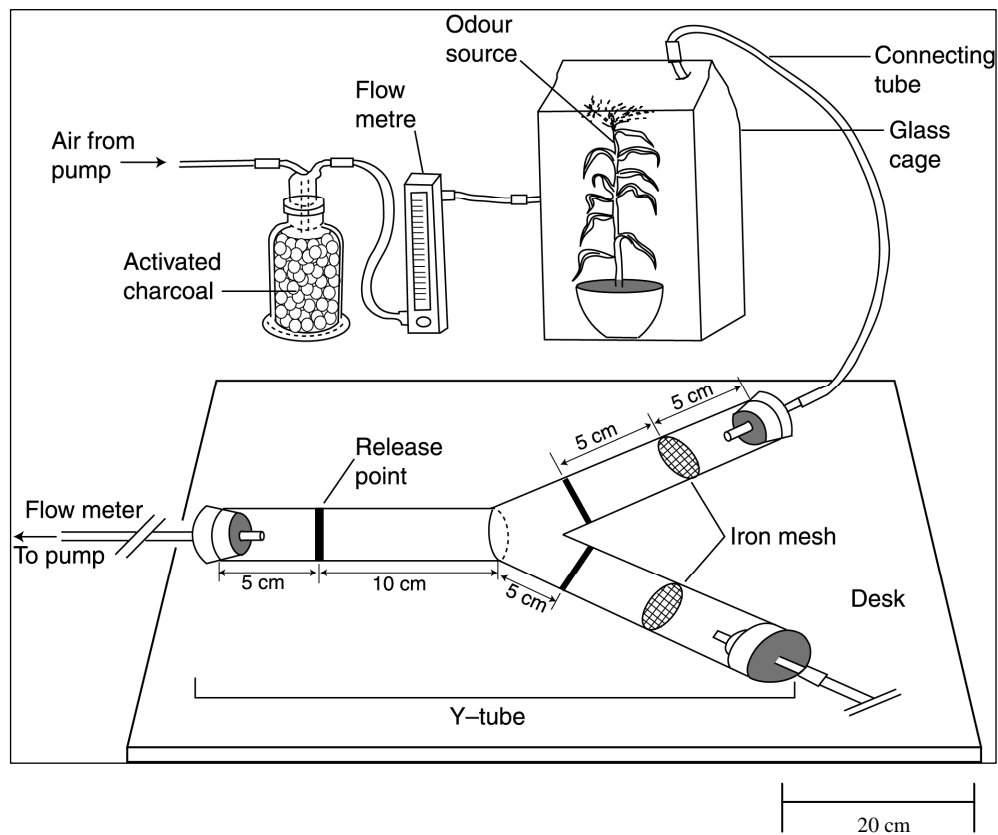


**Fig. 4.1. Schematic diagram of the Y-tube olfactometer (Ngi-Song *et al*., 1996)**

The treatments in the first experiment were as follows: (1) blank (control), (2) healthy undamaged plants, (3) mechanically damaged plants (mechanical damage was induced by making four scratch lines along the entire length of eight leaves per plant to simulate larval damage) and (4) plants infested with third-instar larvae of *Liriomyza* species. All plant treatments were compared pairwise with the blank control.

The second experiment was undertaken to determine differences in responses of *D. isaea* to third-instar *L. huidobrensis*-damaged *P. vulgaris*, *P. sativum* and *S.*

123

*lycopersicum* plants, each compared against a control consisting of *V. faba* infested with third-instar larvae. *Vicia faba* has been suggested as a good host plant for rearing *Liriomyza* species and parasitoids (Videla *et al*., 2006). Some commercial insectaries in Kenya have adopted the use of *V. faba* infested with *L. huidobrensis* for mass-rearing *D. isaea* (East Africa Growers Ltd, Kenya, pers. comm.). While *D. isaea* can directly benefit from larger-sized hosts (Videla *et al*., 2006) and abundance of host larvae, this can only be of advantage if the plants and/or plant-leafminer interactions enable the parasitoid to locate the host. The ability to provide volatile cues that can attract *D. isaea* was therefore tested for *V. faba* against other similarly treated plant species.

Each female parasitoid was introduced individually into the stem of the Y-tube at a distance of 5 cm from the opening of the stem. A parasitoid was considered to have made a choice when it traversed the 10 cm-distance of the stem and an additional 5 cm into either arm (visually assessed by a line marked on both arms) (Du *et al*., 1996). After release, each parasitoid was allocated 5 minutes in the olfactometer to make a choice between the two treatments. A "no choice" was recorded when a parasitoid failed to satisfy the conditions outlined above.

With the Y-tube olfactometer, short-range responses to volatile compounds can be detected by walking insects in the absence of visual cues (Wei & Kang, 2006). In the current study, the olfactometer was used for a small flying parasitoid. According to Du *et al*. (1996), a legitimate criticism of many Y-tube experiments has been the failure to take into account mistakes made by insects during initial exploration of the apparatus. Therefore, Du *et al*. (1996) suggested recording final choices of insects after a set time period. Wei & Kang (2006) proposed that parasitoids might spend longer time in the arm of an olfactometer containing the preferred odour. Thus, the final choices and the amount of time spent by parasitoids in each arm of the olfactometer were recorded in the current study.

The position of the arms containing plant treatments was reversed to avoid bias after every five parasitoids tested. The Y-tube was replaced with a clean one after 10 parasitoids were tested. The plant treatment was replaced with a similar set of plants after every 10 parasitoids tested. Thirty female parasitoids were tested for each plant treatment. The used-Y-tubes were rinsed with dichloromethane and water and dried for later use in an oven maintained at 100 $^{\circ}$C.

**Data Analysis**

The percentage responses for each of the treatments versus the blank control and the *V. faba* control were calculated. The number of non-responding insects in each set of the experiment was also noted but was excluded from the analyses. Chi-square tests for differences between numbers of parasitoids making choices between plant treatments and controls for both first and final choices were done using the PROC FREQ model (SAS/STAT, SAS version 9.1.3) (SAS Institute Inc., 2002-2003). The paired-sample *t*-test (two tailed) was used to analyse differences between percentages of time spent in each arm of the olfactometer for each test. Non-responding female *D. isaea* ranged between 3 % and 23 % for all the experiments.

**Results**

**Plant treatments vs. blank control**

There were no significant differences in first and final choices and time spent between undamaged plants and the blank control for all plant species except for the final choice of parasitoids for undamaged *S. lycopersicum* (70 % vs. 30 % for control) (Fig. 4.2a,b,c). However, as their first choice (Fig. 4.3a), *D. isaea* females significantly preferred the arm leading to host plants infested with *L. huidobrensis* (*P. vulgaris*: 75 % vs. 25 % for control; *P. sativum*: 71 % vs. 29 % for control; *S. lycopersicum*: 79 % vs. 21 % for control; *V. faba*: 80 % vs. 23 % for control). No significant differences ($P > 0.05$) were recorded for *D. isaea* females when *P. vulgaris* (41 % vs. 59 % for control), and *S. lycopersicum* (54 % vs. 46 % for control) infested with *L. sativae* were used. However, *D. isaea* females significantly preferred *V. faba* plants infested with *L. sativae* (70 % vs. 30 % for control). Significant first choice preferences were also recorded for *D. isaea* females for *P. vulgaris* (69 % vs. 31 % for control) and *S. lycopersicum* (66 % vs. 35 % for control) infested with *L. trifolii*. However, there was no significant first choice difference for *V. faba* infested with *L. trifolii* compared to the blank control (37 % vs. 63 % for control).
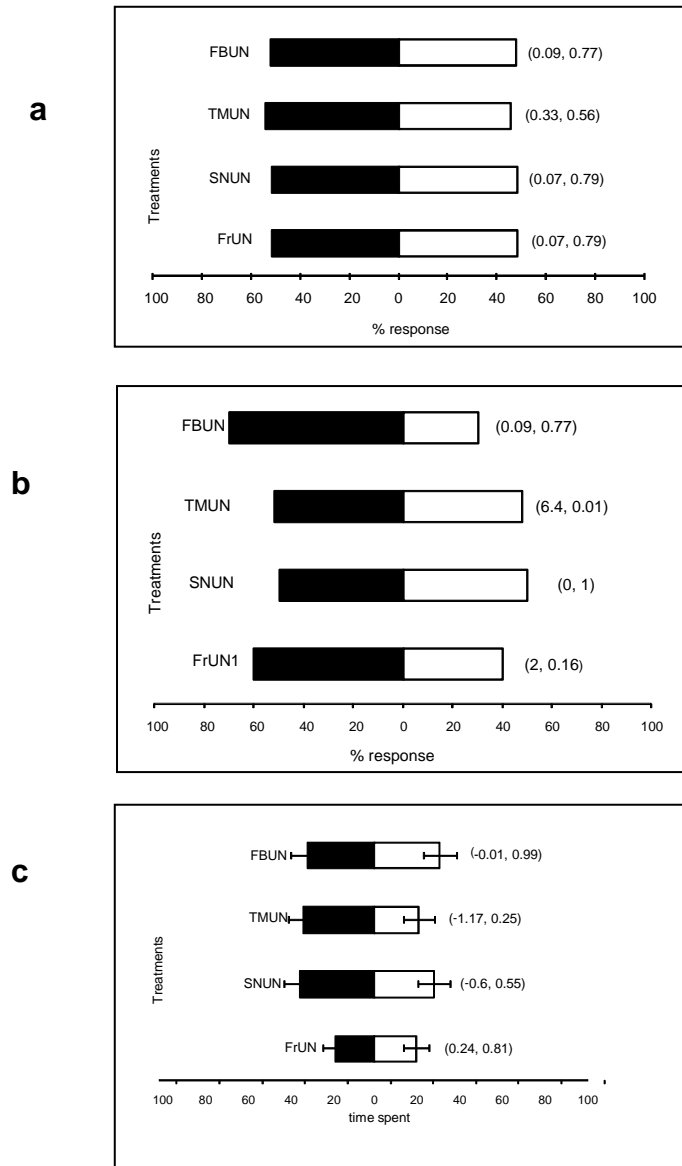
**Fig. 4.2. First (a) and final (b) choice of naïve *D. isaea* females in a Y-olfactometer with one of four undamaged host plants versus a blank control. Bars indicate mean percent response for first/final choices of 30 individual females over an observation time of 5 min. $\chi^2$ test statistic for differences between the number of parasitoids in each arm and P-values are given in parentheses. Treatments (y-axis): *P. vulgaris* (FrUN), *P. sativum* (SNUN), *S. lycopersicum* (TMUN), *V. faba* (FBUN).**
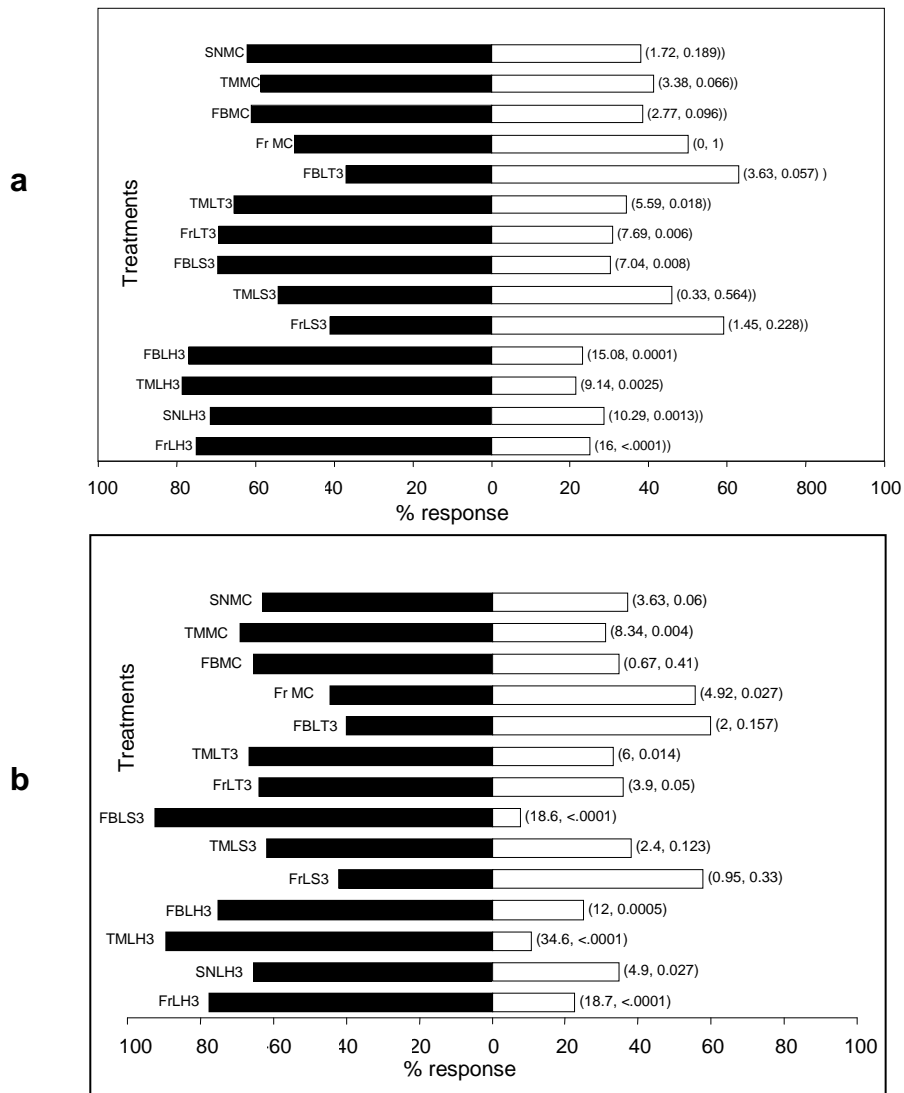
**Fig. 4.3. First (a) and final (b) choice of naïve *D. isaea* females in a Y-olfactometer with one of four host plants infested with third-instar larvae of one of the *Liriomyza* species versus a blank control. Bars indicate mean percent response (x-axis) for first/final choices of 30 individual females over an observation time of 5 min. $\chi^2$ test statistic for differences between the number of parasitoids in each arm and P-values are given in parentheses. Treatments (y-axis): plants infested with third-instar larvae of *L. huidobrensis*: *P. vulgaris* (FrLH3), *P. sativum* (SNLH3), *S. lycopersicum* (TMLH3), *V. faba* (FBLH3), third-instar larvae of *L. sativae*: *P. vulgaris* (FrLS3), *S. lycopersicum* (TMLS3), *V. faba* (FBLS3), third-instar larvae of *L. trifolii*: *P. vulgaris* (FrLT3), *S. lycopersicum* (TMLT3), *V. faba* (FBLT3), mechanically damaged plants: *P. vulgaris* (FrMC), *P. sativum* (SNMC), *S. lycopersicum* (TMMC) and *V. faba* (FBMC)**

As their final choice *D. isaea* females significantly preferred *P. vulgaris* (77 % vs. 23 % for control), *P. sativum* (65 % vs. 35 % for control), *S. lycopersicum* (89 % vs. 11 % for control) and *V. faba* (75 % vs. 35 % for control) plants infested with *L. huidobrensis* (Fig. 4.3b). There were no significant difference in final choice compared with blank controls for *P. vulgaris* (42 % vs. 58 % for the control) and *S. lycopersicum* (62 % vs. 38 % for the control) infested with *L. sativae*. However, there was a significant difference in final choice for *V. faba* (92 % vs. 8 % for the control) infested with *L. sativae*. For plants infested with *L. trifolii*, there were no significant preferences for final choices by *D. isaea* female for *V. faba* (40 % vs. 60 % for the control), in contrast to *P. vulgaris* (64 % vs. 36 % for the control) and *S. lycopersicum* (67 % vs. 33 % for the control).

*Diglyphus isaea* females significantly spent more time in the arms leading to plants infested with *L. huidobrensis* compared to blank controls (*P. vulgaris*: 55 % vs. 16 % for the control; *P. sativum*: 45 % vs. 21 % for the control; *S. lycopersicum*: 55 % vs. 4 % for the control; *V. faba*: 45 % vs. 15 % for the control) (Fig. 4.4). For plants infested with *L. sativae*, there were no significant differences in the amount of time that *D. isaea* females spent in arms leading to either of *P. vulgaris* (17 % vs. 29 % for the control) and *S. lycopersicum* (34 % vs. 22 % for the control) compared to the blank control. However, *D. isaea* females significantly spent more time in the arm leading to *V. faba* (43 % vs. 13 % for the control) infested with *L. sativae* compared to blank controls. For plants infested with *L. trifolii*, *D. isaea* females significantly spent more time in the arm leading to *S. lycopersicum* (44 % vs. 15 % for the control). However, there was no significant difference in the amount of time spent in the arms leading to *P. vulgaris* (23 % vs. 22 % for the control) and *V. faba* (34 % vs. 34 % for the control).

There were no significant differences in the first choices made by the female *D. isaea* parasitoids between the mechanically damaged plants and blank controls ($P > 0.05$) (Fig. 4.3a). As their final choices, *D. isaea* females did not show any significant preferences for *P. sativum* and *V. faba* that were mechanically damaged while the only significant preference for the final choice of mechanically damaged plants was for *S. lycopersicum* (70 % vs. 30 % for the control). The parasitoids preferably chose the blank control (56 % vs. 44 %) to mechanically damaged *P. vulgaris* plants. The *D. isaea*

females, however, did not significantly spent more time in any of the arms leading to the mechanically damaged plants (Fig. 4.4).
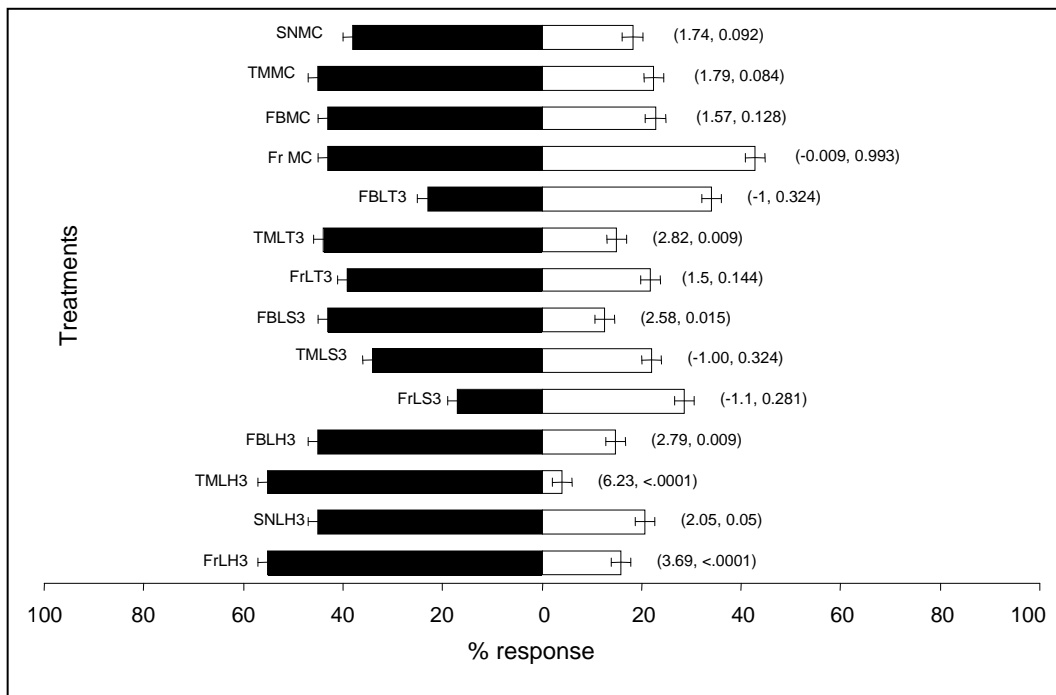


**Fig. 4.4. Time spent in each arm by naïve *D. isaea* females in a Y-olfactometer with *P. vulgaris*, *P. sativum*, *S. lycopersicum* or *V. faba* plants infested with third-instar larvae of either *L. huidobrensis*, *L. sativae* and *L. trifolii* (black bars) versus a blank control (white bars) or mechanically damaged plants (black bars) versus a blank control (white bars). Numbers in parentheses represent t-values, P-values (t-value, P-value) for paired-sample t-tests. Bars indicate mean values (± SE) of the percent time spent in each arm by 30 females over an observation time of 5 min. Treatments: plants infested with third-instar larvae of *L. huidobrensis*: *P. vulgaris* (FrLH3), *P. sativum* (SNLH3), *S. lycopersicum* (TMLH3), *V. faba* (FBLH3), third-instar larvae of *L. sativae*: *P. vulgaris* (FrLS3), *S. lycopersicum* (TMLS3), *V. faba* (FBLS3), third-instar larvae of *L. trifolii*: *P. vulgaris* (FrLT3), *S. lycopersicum* (TMLT3), *V. faba* (FBLT3); mechanically damaged plants: *P. vulgaris* (FrMC), *P. sativum* (SNMC), *S. lycopersicum* (TMMC) and *V. faba* (FBMC).**

**Plant treatments vs. *V. faba* control**

As their first choice, *D. isaea* females significantly preferred the arm leading to *V. faba* plants infested with *L. huidobrensis* compared to *P. vulgaris* (68 % vs. 32 %), and *P. sativum* (68 % vs. 32 %), while there was no significant first choice preference between *S. lycopersicum* and the *V. faba* control (Fig. 4.5a). As their final choice, *D. isaea* females preferably chose the *V. faba* control compared to *P. vulgaris* (78 % vs. 22 %) and *P. sativum* (69 % vs. 31 %). However, there was no significant difference for the final choice between *S. lycopersicum* and *V. faba* infested with *L. huidobrensis* (Fig. 4.5b). *Diglyphus isaea* females spent more time in the arms leading to *V. faba* plants infested with *L. huidobrensis* compared to *P. vulgaris* and *P. sativum* (32 % vs. 12 %) and (43 % vs. 15 %) respectively. However, there were no significant differences in the time spent in arms between *S. lycopersicum* and *V. faba* control (Fig. 4.5c).
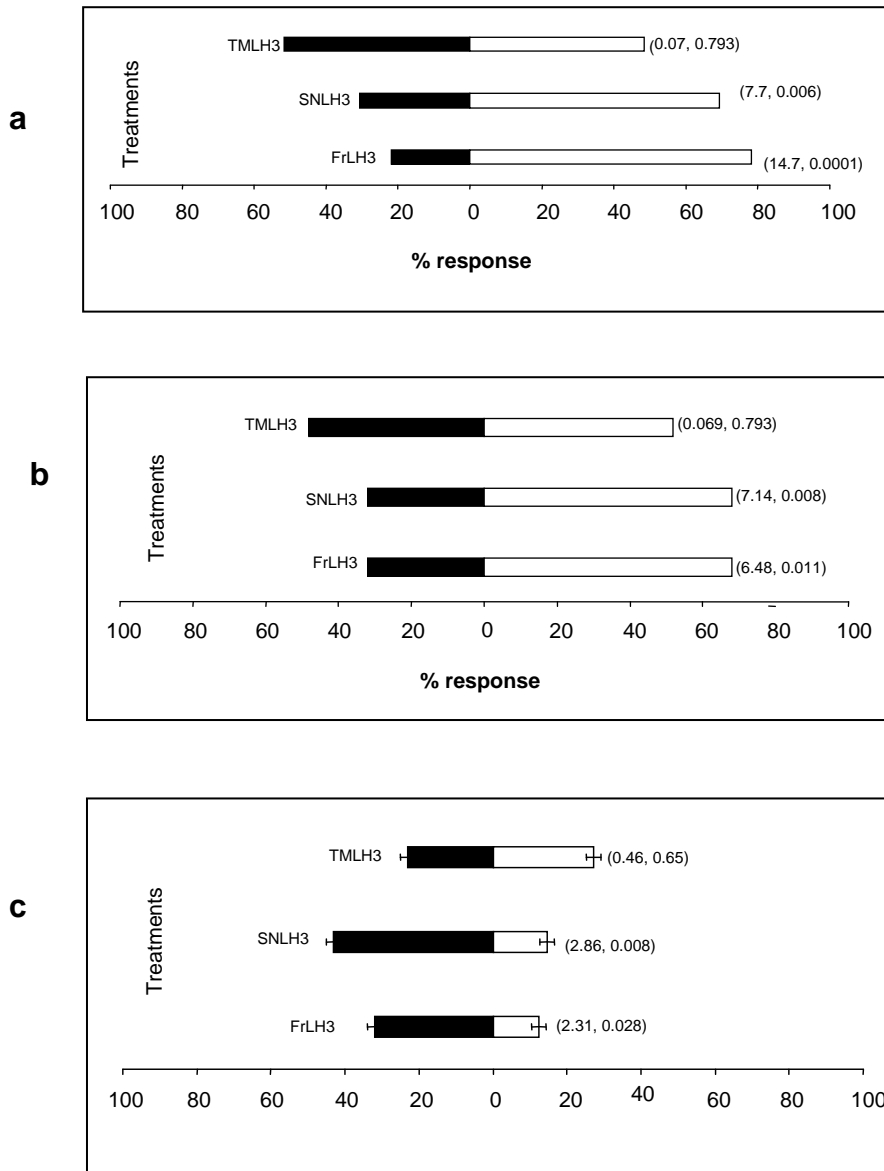
**Fig. 4.5. Responses of naïve female** *D. isaea* **in a Y-olfactometer to whole plants of** *P. vulgaris*, *P. sativum* **and** *S. lycopersicum* **infested with third-instar larvae of** *L. huidobrensis* **(black bars) versus a similarly infested** *V. faba* **control (white bars). First choice (a), final choice (b) and time spent in each arm (c) were measured.** $\chi^2$ **test for differences between numbers of parasitoids in each arm and a paired-sample t-test for differences in percent time spent in each arm were calculated. Numbers in parentheses represent t- followed by P-values. Bars indicate mean values (± SE) of the percent time spent in each arm by 30 females over an observation time of 5 min. Treatments: plants infested with third-instar larvae of** *L. huidobrensis*: *P. vulgaris* **(FrLH3),** *P. sativum* **(SNLH3),** *S. lycopersicum.* **(TMLH3),** *V. faba* **(FBLH3).**

**Discussion**

*Diglyphus isaea* females preferred plants infested with third-instar *L. huidobrensis* larvae compared to the blank control in both first and final choices. In addition, female parasitoids spent more time in the arms with plants compared to blank controls. In contrast to plants infested with larvae of *L. huidobrensis*, only *P. vulgaris* and *S. lycopersicum* infested with *L. sativae* and *V. faba* infested with *L. trifolii* were preferred to blank controls. The results suggest that, depending on plant and leafminer combination, plants infested with larvae of *Liriomyza* emitted volatile compounds that were detected by *D. isaea*. For example, Dicke & Minkenberg (1991) showed that *D. isaea* uses a plant volatile emitted from tomato leaves infested by *Liriomyza byoniae* (Kaltenbach) as long-range host location cues. In general, herbivore-inflicted injury has been shown to induce plants to release volatile terpenoids, and natural enemies including parasitoids have been reported to use terpenoids as major cues to locate hosts (Dicke *et al*., 1990; Turlings *et al*., 1990; Turlings & Tumlinson, 1992; Ngi-Song *et al*., 1996; De Moraes *et al*., 1998; Kessler & Baldwin, 2001; Birkett *et al*., 2003; Colazza *et al*., 2004; De Boer *et al*., 2004; Mumm & Hilker, 2005; Wei & Kang, 2006).

The results of this study show some variation in the response of *D. isaea* females related to leafminer and host plant species. This variation in the response of *D. isaea* females to different host plants attacked by *Liriomyza* species has potentially important implications for biological control programmes. If olfactory cues are important in habitat location, then results from this study can partially explain the variation in spatial distribution patterns and efficacy of parasitoids, including *D. isaea,* among crops in the field (Zehnder & Trumble, 1984; Johnson & Hara, 1987; Schuster *et al*., 1991; Chabi-Olaye *et al*., 2008). Results of the current study further emphasize that successful biological control can be achieved by using the right combinations of *D. isaea*, leafminer and host plant (Johnson & Hara, 1987).

Mechanically damaged bean plants released a higher proportion of green leaf volatiles than *L. huidobrensis* and *L. sativae* damaged plants (Wei & Kang, 2006). Zhao & Kang (2002) demonstrated that volatiles from physically damaged bean plants elicited strong electronantennogram (EAG) responses by *D. isaea*. However, in the current study, mechanically damaged plants, including bean plants, did not attract this

parasitoid, suggesting that volatile compounds released by mechanically damaged plants do not play a role in attracting *D. isaea*. This would be beneficial because parasitoids can avoid false alarms. However, differences between the current study and Zhao & Kang (2002) and Wei & Kang (2006) may be attributed to the different cultivars used and further studies are needed to determine the role of volatiles from mechanically damaged plants in attracting *D. isaea*.

The current study showed that undamaged plants did not attract *D. isaea*. This result is consistent with findings of Zhao & Kang (2002), where neither undamaged hosts nor non-host plants of *L. sativae* elicited distinctive EAG responses from the parasitoid. Although some parasitoids use volatiles emitted by undamaged plants (Ngi-Song *et al*., 1996) to locate habitats and possibly the microhabitats of their hosts (Turlings *et al*., 1990, 1995; De Moraes *et al*., 1998; Kessler & Baldwin, 2001), it is likely that for *D. isaea* herbivore induced volatiles (HIVs) are more important in host finding than volatiles emitted by undamaged plants.

Overall, the results of the current study indicate that the amount of time spent by *D. isaea* in arms of the Y-olfactometer was consistent with the first and final choices it made. While recording either the first and final choice or time spent may be sufficient based on the results of the current study and would greatly reduce experimental time, all three parameters should be recorded because in one incidence among the experimental treatments (*P. vulgaris* infested with *L. trifolii)* first and final choice and time spent in arms were not in agreement.

*Diglyphus isaea* used in this study were reared on *P. sativum*. Thus, the results from *P. sativum* could have been influenced by the odour experience the adults were receiving when emerging from dried *P. sativum* plant debris. However, the adults showed strong responses to other host plant species and overcame the bias associated with host plants where they were reared. This therefore makes this parasitoid a good candidate for augmentative biological control.

In the pair wise comparisons between *L. huidobrensis* infested host plants and a *V. faba* control, parasitoids significantly preferred *V. faba* plants compared to *P. vulgaris* and *P. sativum* for first, final choices and the time spent in oflactometer arms. This result emphasizes that volatiles emitted from *V. faba* infested with *L. huidobrensis* were preferred to olfactory cues from *P. sativum* and *P. vulgaris*. However further

studies are needed to test if this olfactory preference can be translated into improved parasitism and/or parasitoid fitness of *D. isaea*.

Although *D. isaea* responded actively to whole plant treatments in the current study, the results were obtained under experimental conditions. Each plant was presented separately in the olfactometer, whereas in natural surroundings olfactory stimuli are more complex. To obtain more information on host plant-host-parasitoid interactions, EAG assays are needed to assess the behaviour of *D. isaea* to different herbivore induced plant volatiles. Further, herbivore induced volatiles from the different whole plants (undamaged, mechanically damaged and infested with third-instar larvae of *Liriomyza* species) should be collected, analyzed, identified and quantified and be used in the Y-tube olfactometer assays.

Olfaction is one of the many important factors involved in the search for a host by *D. isaea*. The results of the current study showed that interactions between host plant, *Liriomyza* and *D. isaea* are more complex than previously recognized. To improve biological control of *Liriomyza* species with *D. isaea* the results of the current study should be combined with findings on visual cues, plant chemistry and plant-*Liriomyza* species interactions.

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