



The Role of Host Plants, Alternative Food Resources and Herbivore Induced Volatiles in Choice Behavior of an Omnivorous Predator

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Macrolophus pygmaeus, Rambur (Heteroptera, Miridae) is a generalist predator found on various plant species and has also the ability to feed both on animal and plant tissue. Foraging behavior of *M. pygmaeus* and ultimately its efficacy as a biological control agent, is known to be affected by olfactory stimuli. Here, we elaborate on the response of this omnivore predator to volatiles produced by host plants by conducting olfactometric bioassays under laboratory conditions. In particular, we explored: (i) the relationship between previous experience and plant choice of *M. pygmaeus* nymphs by comparing its attractiveness to pepper and aubergine plants and (ii) how the presence of an aphid prey, *Myzus persicae* (Sulzer), or floral resources may influence choices made by *M. pygmaeus*. When the two host plants were provided, aubergine plants were found to be more attractive than pepper plants, regardless the previous experience of *M. pygmaeus*. Furthermore, the presence of *M. persicae* made aubergine plants more attractive to *M. pygmaeus* nymphs than uninfested aubergine plants. The gas chromatography-mass spectrometry analysis showed that plants infested with *M. persicae* emitted additional compounds as compared to the volatiles emitted from uninfested plants. In particular, four compounds, (*E*)- β -farnesene, (*E,E*)-TMTT, 2-methylbutanal oxime and dodecanal were found present only in the headspace of aubergine plants with aphids. However, *M. pygmaeus* did not show preference for the floral resources. Our results indicate that the response of *M. pygmaeus* is tuned toward the various stimuli in its habitat.

Keywords: *Macrolophus pygmaeus*, aubergine, pepper plant, Y-tube olfactometer, volatiles

INTRODUCTION

The generalist predator *Macrolophus pygmaeus*, Rambur (Hemiptera: Miridae), is a widely used biological control agent for several pests such as aphids, whiteflies, mites as well as the serious invasive pest *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on tomato crops (Perdikis et al., 2011; Calvo et al., 2012; Urbaneja et al., 2012; Zappala et al., 2013; Moreno-Ripoll et al., 2014). In particular, in Greece, *M. pygmaeus* is recorded to act as an efficient natural enemy (through natural colonization) in the control of aphids in field tomato crops (Lykouressis et al., 1999–2000). In addition, this mirid is also characterized for its omnivorous behavior, feeding on both plant and prey, which in some cases, depending on the suitability of the plant, allows development and

reproduction in the absence of prey (Perdikis and Lykouressis, 1997, 1999, 2000). Unlike other omnivores, *M. pygmaeus* is considered as harmless and has been widely used in biological control programs (Castañé et al., 2011). However, Moerkens et al. (2016) reported that *M. pygmaeus*, can cause economic damage in tomato crops regardless the presence or absence of prey. In addition, Sanchez et al. (2018), reported that high populations of this mirid may lead to a yield reduction due to increased plant feeding. Yet, further research is necessary regarding other species of host plants. Recently, Zhang et al. (2018), have reported that *M. pygmaeus* can induce plant defenses in pepper plants. Similar results have been presented in previous studies concerning *M. pygmaeus* and other mirid species (Pappas et al., 2015, 2016; Pérez-Hedo et al., 2015a,b). In general, further consideration is required prior of using omnivores in biological control, since their ability to feed on both plant tissue and prey may have positive (survival of omnivores) and negative (preference for plant resources rather than prey) effects (Eubanks and Denno, 1999; Perdakis and Lykouressis, 1999; Maselou et al., 2014).

In order to switch between more than one trophic level, omnivores are equipped with morphological and physiological as well as phylogenetic and behavioral traits (Coll and Guershon, 2002). The behavioral characteristics for collection of information and action by a predator, has drawn a lot of attention by researchers in the recent years especially for predator species which can be used effectively in biological control (Ingegno et al., 2011, 2013, 2016; Lins et al., 2014; De Backer et al., 2015). Predator's orientation and the ability to detect and explore available patches with prey is related to various stimuli such as chemicals, visual and acoustic signals or even variation in temperature and humidity (Greany and Hagen, 1981; Letourneau, 1998).

It is well documented that olfactory stimuli are used by natural enemies for host or prey location and enhance efficacy by reducing searching time and increasing attack rates on prey (Dicke and Sabelis, 1988; Vet and Dicke, 1992; Dicke and Vet, 1999). These odors can derive from the prey or the host plant but can also be induced in plants in response to herbivore feeding (Greany and Hagen, 1981; Dicke et al., 1990; Vet and Dicke, 1992; Dicke and Vet, 1999; Hilker and Meiners, 2002). Environmental abiotic conditions and developmental stage of the plant are known to influence the blend of emitted volatiles (Boege and Marquis, 2005; Koricheva and Barton, 2012). The induction of volatiles are known to differ among herbivore species, or in the presence of more than one pests, but it has also been reported that different plant species infested by the same herbivorous species, emit different compounds of volatiles (Dicke and Sabelis, 1988; Turlings et al., 1990; Vet and Dicke, 1992; Tumlinson et al., 1993; Dicke, 1994, 1999; Sabelis et al., 1999; Bruce and Pickett, 2007; Ingegno et al., 2011; Cai et al., 2014). Moreover, plant feeding by omnivores may also affect the reproduction and development of herbivores through induced plant defenses (Pappas et al., 2015; Zhang et al., 2018).

Omnivores have been reported to utilize volatiles from host plants and herbivore-induced plant volatiles (HIPVs) to detect their host plant and prey (Lins et al., 2014; Rim et al., 2015, 2017). This response to HIPVs may be either inherited or acquired

through experience (Vet and Dicke, 1992; Steidle and van Loon, 2003; de Boer and Dicke, 2006). Therefore, the effect of these volatiles to an omnivore's performance as biological control agent and their possible use as attractants, is of high importance during the implementation of a biological control strategy.

It has been reported that *M. pygmaeus* responds to volatiles from plants with prey, but not to volatiles emitted directly by the prey (Ingegno et al., 2011; Lins et al., 2014). Lins et al. (2014) found that *M. pygmaeus* exhibits a learning ability (experience) toward prey which may result in more efficient foraging. *M. pygmaeus* was able to discriminate a tomato plant infested by *T. absoluta* from a non-infested plant using olfactory cues (De Backer et al., 2015) as well as spider mite infested tomato plants over clean air (Pappas et al., 2018). However, little information is available regarding its response to volatiles produced from alternative plant food resources such as flowers. A previous study has shown that the presence of flowers on aubergine or pepper plants reduces *M. pygmaeus* predation on the aphid *Myzus persicae*, Sulzer (Heteroptera, Aphididae) (Maselou et al., 2014).

Based on the results of Maselou et al. (2014, 2015) and considering the importance of *M. pygmaeus* as a biological control agent and the rather limited knowledge of the role of volatiles stimuli to its behavior, we set out to elucidate the behavioral response of the predator toward plant resources using olfactometric bioassays. We specifically addressed the following hypotheses: (i) if plant choice in *M. pygmaeus* is influenced by previous experience, predators reared on plant species of different suitability (aubergine plants vs. pepper plants), would show different host plant selection behavior, (ii) the presence of alternative food resources (flower) or prey (aphid-infested plants) alter the volatile blend and considering that they are a feeding source for *M. pygmaeus*, their volatiles would be more attractive for the omnivore than the volatiles emitted from uninfested and non-flowering plants. We also assessed the predator preference for flowering plants vs. non-flowering plants with prey.

MATERIALS AND METHODS

Plant Cultivation and Insect Rearing

Aubergine (cv. Bonika F1) and pepper plants (cv. Vidi) were grown from March to October in a greenhouse in the Agricultural University of Athens at temperature $22.5 \pm 2.5^\circ\text{C}$ (mean \pm SD), under natural light. The plants used in all experiments were approximately of the same age, 20–25 cm tall with 5–6 true leaves. *M. pygmaeus* rearing was initiated by releasing adults and nymphs collected from a tomato field in central Greece (Co. Boeotia). The predators were reared separately on potted sweet pepper (cv. Vidi) and aubergine (cv. Bonika F1). Each week new non-flowering aubergine or pepper plants were provided to maintain the rearings. The two colonies of *M. pygmaeus* were maintained for at least 15 generations on each plant species provided *ad libitum* with *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs as a food supply. Eggs of the *E. kuehniella* were obtained from Koppert BV (Entofood, Berkel en Rodenrijs, The Netherlands). *M. persicae* rearing was established on aubergine plants by releasing adults and nymphs collected from aubergines in the premises of Agricultural

University of Athens. All cultures were kept in wood-framed cages (80 cm length × 80 cm width × 70 cm height) in a greenhouse under the same conditions ($22.5 \pm 2.5^\circ\text{C}$, natural light).

OLFACTOMETER BIOASSAYS

The olfactory bioassays were conducted at $25 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH under constant light. For each objective we tested the volatile sources presented in **Table 1**. In total eleven comparisons were carried out, six referring to our first objective, (effects of omnivore's experience on plant choice) and five to the response of *M. pygmaeus* nymphs toward the presence of floral resources and prey. Responses of predator nymphs to volatiles were observed in a two-choice Y-tube glass olfactometer (2.5 cm and 3.0 cm inside diameter at the entry and in the two side arms, respectively) formed by an entry arm (12 cm in length) and two side arms (15 cm in length, 70° angle). The tube was positioned horizontally, and the two side arms were each connected to a glass container (3.5 L or 0.5 L in volume for the assays with plants or individual flowers, respectively). The airflow was produced by an air pump adjusted at the end of the olfactometer with a flow meter to 250 mL min^{-1} and passed through an activated charcoal filter. The glass containers with volatile sources were kept behind a white panel, preventing insects from visually detecting the plants. When plants were used for the bioassays, pots were covered with aluminum foil to restrict the emission of volatiles from soil or plastic. All procedures were carried out wearing gloves. The flowers were cut just before the experiment and maintained in contact with water until use in the experiments. Fifth instar *M. pygmaeus* predators were used in the experiments after being deprived from prey for 24 hours to exclude the influence of variable hunger levels. These were obtained from nymphs of 1st or 2nd instar that were transferred from the rearing cages to potted caged aubergine or pepper plants with eggs of *E. kuehniella* in a temperature controlled room in 25°C , $65 \pm 5\%$ R. H. and a photoperiod of 16:8 (L:D) h and left to develop to the 5th instar. Approximately 1 h before trials were initiated, the predators were placed into individual tubes. Each nymph was

individually introduced at the downwind end of the entry arm and observed until covering more than 10 cm inside each chosen arm. The experiments took place from 12:00 p.m. to 17:00 p.m. since *M. pygmaeus* has been previously reported to be more active during the afternoon (Perdikis et al., 2004). Nymphs not making a choice for a side arm within 10 min were considered as having made no choice and were excluded from data analysis. Each predator was tested only once and then discarded. The final number of *M. pygmaeus* nymphs that had made a choice for each pair of volatiles was 40. After testing a batch of five nymphs, the volatile sources were switched between left and right sides of the arms to minimize positional bias. After testing ten nymphs, the Y-tube and glass containers were washed with neutral soap and alcohol (70%) and autoclaved at 120°C for 20 min. Approximately, 8–10 insects were tested daily and plants or flowers were replaced daily, therefore 5–6 plants or 10–15 flowers were used per treatment.

Headspace Collection and Analysis of Plant Volatiles

Volatile collection was performed from uninfested, flowering and aphid infested aubergine plants as described by Anastasaki et al. (2018) with slight modifications. A glass container (3.5 L) as described above was used for volatile collection. Single-potted plants were placed in each glass container, with pot and soil wrapped with aluminum foil. Purified air, through an activated charcoal filter, passed through the glass containers. Plant volatiles were drawn by vacuum pump (Dymax 5, Charles Austen Pumps Ltd, UK) with a rate of 360 mL/min onto a Teflon made trap (5 cm length × 3 mm id) containing 30 mg Porapak Q (80/100 mesh, Supelco, Bellefonte, USA) tapped with a 2 mm glass wool and 3 mm Teflon tubes in each end. Prior to the analysis, traps were sequentially washed with 1 mL methanol, diethyl ether, and n-pentane (Fisher Chemicals, Bishop, UK) and blown dry with N_2 . Collection of headspace volatiles was performed continuously for 6 h. Three replicates per treatment were carried out. The collection of volatiles was carried out at the same experimental conditions and time period as for the olfactometer bioassays (12:00–18:00). After volatile collection,

TABLE 1 | Olfactometer bioassays involving *M. pygmaeus* nymphs originating either from pepper or aubergine plants (see details in the Materials and Methods).

Objective	<i>M. pygmaeus</i> host plant origin	Odor source 1		Odor source 2
I	Pepper	Pepper plant	vs.	Air
		Aubergine plant	vs.	Air
		Pepper plant	vs.	Aubergine plant
	Aubergine	Aubergine plant	vs.	Air
		Pepper plant	vs.	Air
II	Pepper	Aubergine plant	vs.	Pepper plant
		Flower from pepper plant	vs.	Air
		Flower from aubergine plant	vs.	Air
		Aphid-infested aubergine plant	vs.	Uninfested aubergine plant
		Flowering aubergine plant	vs.	Uninfested aubergine plant
		Flowering aubergine plant	vs.	Aphid-infested aubergine plant

traps were extracted immediately with 0.5 mL n-pentane. Sample volumes were reduced to 150 μ L and stored in a freezer (at -20°C) in a sealed GC vial with conical inserter until use.

The identification of the chemical compounds was performed with gas chromatography-mass spectrometry (GC-MS). One microliter of the extract was injected in a Varian CP-3800 GC, with a 1079 injector coupled with a 1200L quadrupole mass spectrometer. Separation of the analytes was performed with a Varian VF5ms capillary column (30 m, 0.25 mm i.d, 0.25 μ m film thickness). Splitless mode was set to 0.75 min. Then, the injector split ratio was set at 80:1. At 5 min, the split ratio was set to 70:1. The flow rate of the carrier gas helium was

1 mL min^{-1} . The column temperature was maintained at 40°C for 1 min, increased with a rate of $1.2^{\circ}\text{C min}^{-1}$ to 65°C and with a rate at $3^{\circ}\text{C min}^{-1}$ to 180°C . The column was heated with a rate of $15^{\circ}\text{C min}^{-1}$ to the final temperature of 250°C . Mass spectrometer was operated in Electron ionization mode (EI) with ion energy of -70 eV , filament current $50\ \mu\text{A}$ and source temperature 200°C . Data acquisition was performed in full scan (MS) with scanning range 40–300 amu. Compounds were identified by comparing their retention time and mass spectra with that of commercial standards whenever possible or tentatively by comparing their elution order, mass spectra and RI values from Adams (2007); NIST, 2005; Wiley 275 mass spectra libraries and literature data (Adams, 2007; Anastasaki

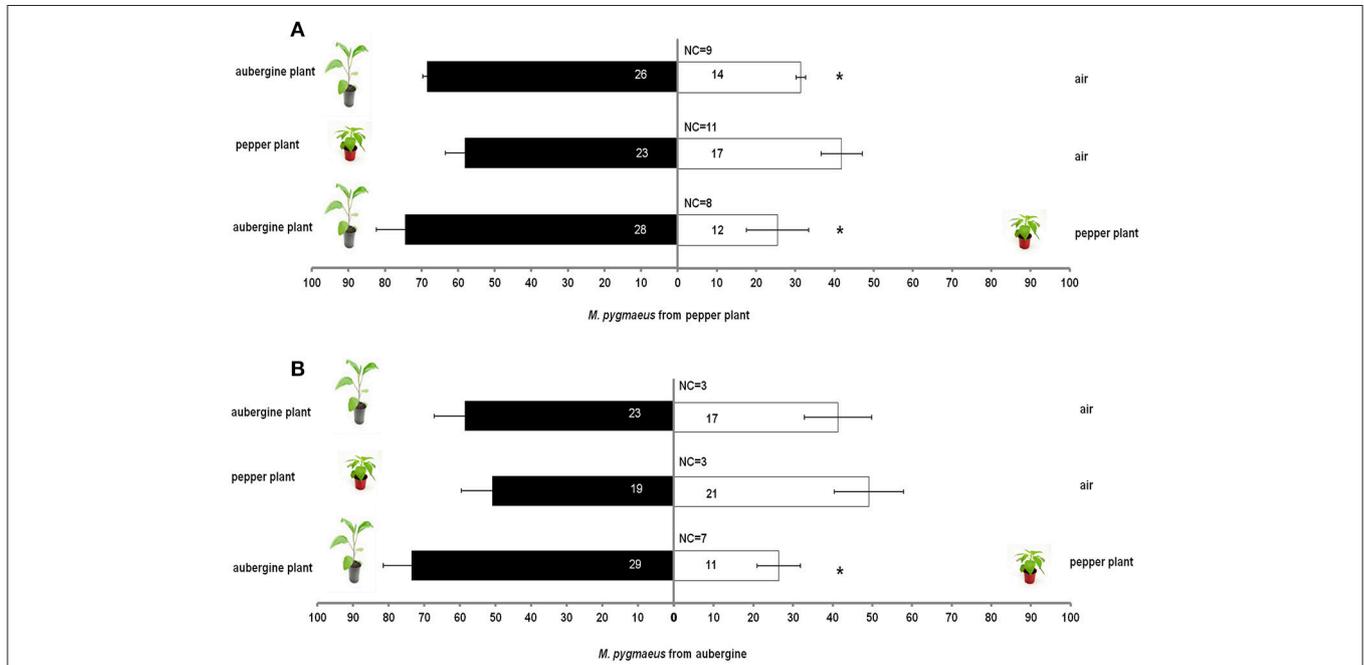


FIGURE 1 | Response of *M. pygmaeus* nymphs originating from pepper (A) or aubergine (B) plants in a Y-tube olfactometer to the odors of pepper plant, aubergine and clean air for each compared pair. Number in bars represent individuals that moved toward the odor source. *t* test ($P < 0.05$; *df*: 1) (NC, number of individuals that did not make a choice).

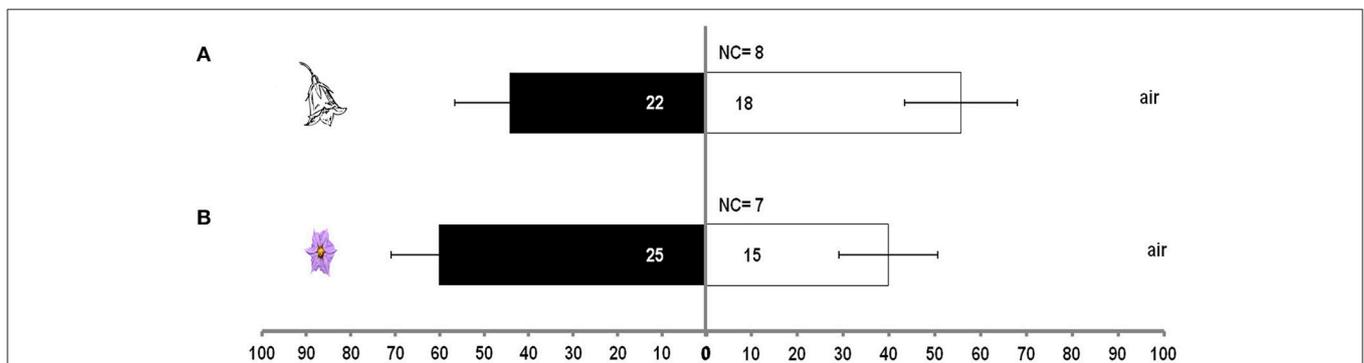


FIGURE 2 | Response of *M. pygmaeus* nymphs originating from pepper or aubergine plants in a Y-tube olfactometer to the odors of one pepper flower or one aubergine flower over clean air. Numbers in bars represent individuals that moved toward the odor source. *t* test ($P < 0.05$; *df*: 1) (NC, number of individuals that did not make a choice). (A) Flower from pepper plant. (B) Flower from aubergine plant.

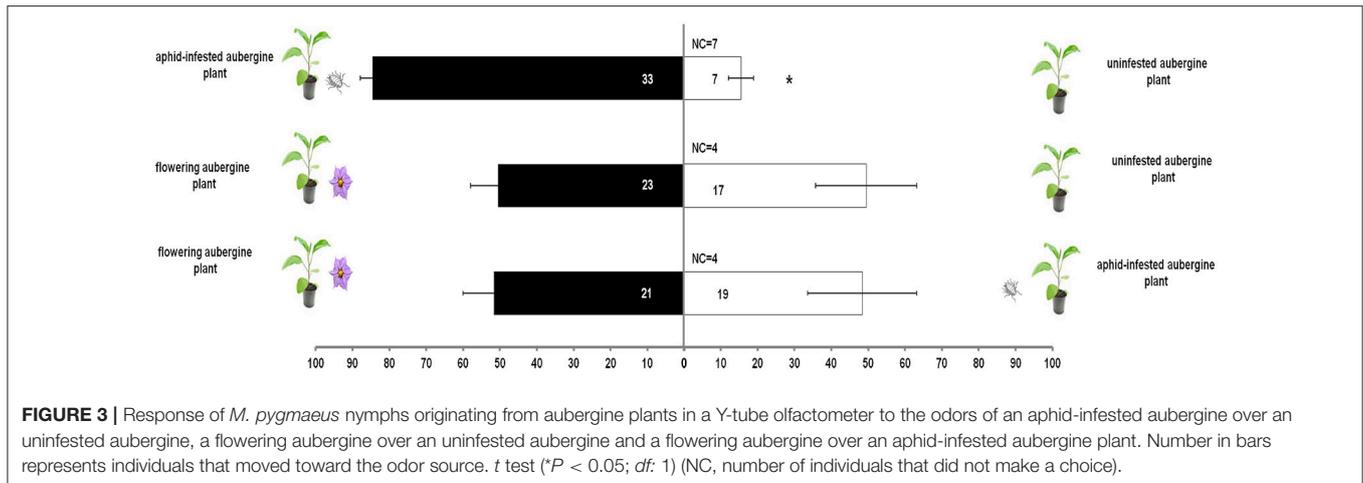


FIGURE 3 | Response of *M. pygmaeus* nymphs originating from aubergine plants in a Y-tube olfactometer to the odors of an aphid-infested aubergine over an uninfested aubergine, a flowering aubergine over an uninfested aubergine and a flowering aubergine over an aphid-infested aubergine plant. Number in bars represents individuals that moved toward the odor source. *t* test (* $P < 0.05$; *df*: 1) (NC, number of individuals that did not make a choice).

et al., 2018). We also used retention indices (RI) of a series of n-alkane (C_8 - C_{20}).

Statistical Analysis

In the olfactometer bioassays, the responses of *M. pygmaeus* nymphs were analyzed by a logistic regression to investigate the influence of predator host plant origin. Each bioassay with one pair of plants (or plant vs. air) served as a replicate. Predator host plant origin and plant treatment (pepper or aubergine) were used as fixed factors. Non-significant interactions were removed from the final model (Agresti, 2013). In comparisons of plants vs. air the number of *M. pygmaeus* choosing plants (aubergine or pepper) out of the total individuals responding was used as the response variable. In comparison of aubergine plants vs. pepper plants, the number of individuals choosing aubergine plants out of total individuals responding was used as the response variable. Logistic regression was also used to investigate the influence of flowers on *M. pygmaeus* behavioral choices. In this case, the number of individuals moving toward the flower odor chamber out of total responding was the response variable. The effect of plant treatment (i.e., flowering plant or aphid infested plant) was analyzed by logistic regression and the number of individuals choosing flowering or aphid infested plants out of total responding was used as the response variable. To determine whether there was a preference for an odor source within a treatment combination, we used one sample *t*-test on the proportion of *M. pygmaeus* preferring the response variable in each replicate. Data were arcsine-transformed and tested against arcsine (0.5), i.e., no preference for either odor source. Nymphs that did not make a choice were excluded from the statistical analysis. All analyses were performed using SPSS version 19.0 (SPSS, Chicago, IL)¹.

The total ion chromatogram peak areas of identified compounds were calculated by Varian MS Workstation software (version 6.9). The effect of treatment on the relative peak area of each volatile identified was determined with analysis of variance

and comparisons among means were performed using the LSD test. Volatile data were also log-transformed and subjected to multivariate analysis with SIMCA 14.1 software (Umetrics, Umeå, Sweden). Specifically, projections to latent structures-discriminant analysis (PLS-DA) were performed with Pareto scaling.

RESULTS

Olfactometer Bioassays

Host plant origin (aubergine or pepper) of *M. pygmaeus* nymphs had a significant effect on the response of nymphs toward aubergine or pepper plants in comparison with air ($\chi^2 = 34.9$, *df* = 15, $P = 0.002$). When *M. pygmaeus* originated from pepper plants, the nymphs were attracted to aubergine plants in comparison with air ($t = 14.4$, *df* = 4, $P < 0.001$). No other significant differences were observed between aubergine or pepper plants in comparison with air (Pepper plant vs. clean air $t_{\text{pepper}} = 1.6$, *df* = 4, $P = 0.185$; $t_{\text{aubergine}} = 0.2$, *df* = 4, $P = 0.835$; Aubergine plant vs. clean air: $t_{\text{aubergine}} = 1.1$, *df* = 4, $P = 0.332$, **Figure 1**).

There was no significant effect of *M. pygmaeus* nymph origin in comparisons of aubergines over pepper plants ($\chi^2 = 11.1$, *df* = 8, $P = 0.197$). *M. pygmaeus* nymphs were more attracted to aubergine than to pepper plants ($t_{\text{pepper}} = 2.55$, *df* = 5, $P = 0.05$; $t_{\text{aubergine}} = 4.03$, *df* = 5, $P = 0.01$, **Figure 1**).

Treatment had no significant effect in the case of single aubergine or pepper flower over clean air ($\chi^2 = 13.8$, *df* = 8, $P = 0.08$). *M. pygmaeus* nymphs did not discriminate between clean air and volatiles from an aubergine ($t = 1.1$, *df* = 4, $P = 0.337$) or a pepper flower ($t = 0.36$, *df* = 4, $P = 0.738$) (**Figure 2**).

M. pygmaeus nymphs did not discriminate between flowering and uninfested or aphid-infested aubergine plants ($\chi^2 = 26.4$, *df* = 18, $P = 0.092$, **Figure 3**). Predator nymphs were more attracted to aphid-infested aubergine plants than to uninfested ones ($t = 7.9$, *df* = 3, $P = 0.004$, **Figure 3**).

¹SPSS v. 19.0.0., 2010. SPSS Inc., Chicago

TABLE 2 | Volatile composition of headspace of uninfested (CON), flowering (FLO), and aphid infested aubergine plants (APH) expressed as total peak area (102 ± SE, *n* = 3).

ID	RI ^a	Compounds	CON	FLO	APH	F ^b	P ^c
1	800	Octane	3.08 ± 1.02a ^d	0.00b	3.48 ± 1.28a	14.48	0.005*
2	802	Hexanal	0.00b	3.85 ± 0.92a	0.00b	17.77	0.003*
3	811	Butyl acetate	0.00b	0.06 ± 0.04b	0.61 ± 0.16a	13.08	0.006*
4	835	2-methylbutanal oxime	0.00b	0.00b	1.00 ± 0.45a	4.92	0.054*
5	856	m-xylene	4.39 ± 1.52a	0.00b	4.29 ± 1.55ab	4.00	0.079
6	864	o-xylene	3.91 ± 0.42a	6.02 ± 1.87a	8.29 ± 1.47a	2.47	0.165
7	887	p-xylene	1.79 ± 0.21a	2.42 ± 0.11a	3.54 ± 1.15a	1.71	0.259
8	900	Nonane	1.04 ± 0.15b	2.92 ± 0.79a	0.84 ± 0.19b	5.79	0.040*
9	906	Heptanal	0.26 ± 0.26b	0.76 ± 0.16ab	1.87 ± 0.75a	3.22	0.112
10	927	α-pinene	5.02 ± 0.29a	5.28 ± 0.01a	3.99 ± 0.88a	1.62	0.273
11	938	β-citronellene	0.00b	3.78 ± 0.95a	0.00b	15.90	0.004*
12	978	m-menth-1-ene	1.60 ± 0.23a	1.90 ± 0.17a	0.84 ± 0.16b	8.47	0.018*
13	972	β-pinene	1.33 ± 0.27a	1.02 ± 0.08a	2.02 ± 0.47a	2.58	0.156
14	976	p-menthane	1.15 ± 0.46a	1.75 ± 0.48a	1.27 ± 0.66a	0.34	0.726
15	985	Hydrocarbon 1	3.81 ± 1.12a	3.90 ± 0.19a	5.17 ± 1.10a	0.69	0.540
16	993	Butyl butanoate	0.97 ± 0.35ab	0.00b	1.81 ± 0.28a	8.31	0.026*
17	1000	Decane	7.89 ± 1.34a	7.78 ± 2.23a	1.59 ± 0.61b	5.45	0.045*
18	1005	3-δ-carene	3.81 ± 0.65a	2.54 ± 0.29a	3.06 ± 0.25a	2.13	0.201
19	1016	Hydrocarbon 2	3.91 ± 0.88a	1.35 ± 0.78a	1.67 ± 0.96a	2.55	0.158
20	1021	p-cymene	1.54 ± 0.42a	0.56 ± 0.32a	1.20 ± 0.07a	2.62	0.152
21	1025	Limonene	3.89 ± 0.21a	4.54 ± 0.23a	6.16 ± 1.20a	2.63	0.152
22	1028	Eucalyptol	1.12 ± 0.27b	2.29 ± 0.08a	0.56 ± 0.14b	23.76	0.001*
23	1030	Ethylhexanol	3.40 ± 1.25a	2.53 ± 0.01a	3.08 ± 0.60a	0.30	0.753
24	1032	Benzyl alcohol	0.13 ± 0.13a	0.00a	0.00a	1.00	0.422
25	1034	Unknown 1	0.36 ± 0.14a	0.43 ± 0.25a	0.00a	1.95	0.222
26	1044	Hydrocarbon 3	0.54 ± 0.26a	0.62 ± 0.21a	0.69 ± 0.53a	0.05	0.956
27	1055	Hydrocarbon 4	1.87 ± 0.51a	1.52 ± 0.59a	0.38 ± 0.31a	2.59	0.155
28	1061	Hydrocarbon 5	1.82 ± 0.23a	1.38 ± 0.35a	1.17 ± 0.15a	1.69	0.262
29	1063	Hydrocarbon 6	1.23 ± 0.49a	0.99 ± 0.24ab	0.00b	4.35	0.068
30	1076	Dihydromyrcenol	2.78 ± 0.14a	3.44 ± 0.67a	2.30 ± 0.33a	1.73	0.255
31	1100	Undecane	5.90 ± 0.52a	0.31 ± 0.12b	1.21 ± 0.35b	66.89	0.000*
32	1101	Linalool	0.90 ± 0.19b	4.81 ± 0.32a	3.03 ± 0.97a	10.76	0.010*
33	1109	Nonanal	5.11 ± 0.46a	3.72 ± 0.53a	5.30 ± 0.41a	3.16	0.116
34	1114	(E)4,8-dimethyl-1,3,7-nonatriene	0.14 ± 0.03b	0.21 ± 0.03b	0.61 ± 0.12a	16.69	0.004*
35	1115	Hydrocarbon 7	0.43 ± 0.01a	0.42 ± 0.07a	0.17 ± 0.01b	11.20	0.009*
36	1146	Camphor	0.69 ± 0.19a	1.38 ± 0.38a	0.89 ± 0.22a	1.65	0.269
37	1148	Menthone	0.44 ± 0.07ab	0.76 ± 0.09a	0.25 ± 0.13b	6.62	0.030*
38	1165	2-(Z)-nonenal	0.83 ± 0.15a	0.63 ± 0.15a	0.66 ± 0.06a	0.75	0.511
39	1166	Benzyl acetate	0.54 ± 0.27a	0.80 ± 0.17a	0.79 ± 0.17a	0.49	0.638
40	1168	Isoborneol	0.00b	0.45 ± 0.07a	0.36 ± 0.06a	18.89	0.003*
41	1191	Phenyl ethyl acetate	0.29 ± 0.08a	0.53 ± 0.11a	0.52 ± 0.02a	2.88	0.133
42	1194	α-terpineol	0.56 ± 0.09a	0.64 ± 0.15a	0.66 ± 0.04a	0.28	0.769
43	1200	Dodecane	1.83 ± 0.12a	2.43 ± 0.06a	1.97 ± 0.33a	2.32	0.180
44	1204	Verbenone	0.13 ± 0.04a	0.11 ± 0.07a	0.19 ± 0.10a	0.32	0.737
45	1207	Decanal	3.05 ± 0.19a	2.61 ± 0.51a	3.00 ± 0.45a	0.31	0.748
46	1232	Citronellol	0.00b	0.23 ± 0.03a	0.22 ± 0.07a	8.63	0.017*
47	1253	Linalyl acetate	0.36 ± 0.08a	0.57 ± 0.01a	0.41 ± 0.15a	1.37	0.323
48	1274	Unknown 2	0.56 ± 0.17a	0.00a	0.55 ± 0.26a	3.19	0.114
49	1281	Unknown 3	0.14 ± 0.07a	0.38 ± 0.22a	0.21 ± 0.08a	0.76	0.510
50	1286	Isobornyl acetate	0.57 ± 0.13a	1.01 ± 0.07a	0.58 ± 0.22a	2.65	0.150

(Continued)

TABLE 2 | Continued

ID	Ri ^a	Compounds	CON	FLO	APH	F ^b	P ^c
51	1300	Tridecane	0.83 ± 0.16a	0.84 ± 0.07a	1.02 ± 0.20a	0.48	0.639
52	1311	Undecanal	0.33 ± 0.07a	0.30 ± 0.01a	0.27 ± 0.05a	0.32	0.736
53	1322	Hydrocarbon 8	1.02 ± 0.53a	0.55 ± 0.32a	0.55 ± 0.36a	0.44	0.662
54	1344	Unknown 4	0.83 ± 0.15a	0.00a	0.82 ± 0.82a	0.61	0.578
55	1371	Unknown 5	3.88 ± 0.37b	5.52 ± 0.17a	3.25 ± 0.60b	7.73	0.022*
56	1400	Tetradecane	1.39 ± 0.32a	1.30 ± 0.01a	2.07 ± 0.69a	0.93	0.444
57	1405	Longifolene	0.30 ± 0.05a	0.31 ± 0.03a	0.32 ± 0.06a	0.02	0.977
58	1412	Dodecanal	0.00a	0.00a	0.16 ± 0.08a	4.00	0.079
59	1431	(Z)- α -bermamotoene	0.38 ± 0.4b	0.52 ± 0.10b	1.79 ± 0.06a	54.87	0.000*
60	1452	Geranyl acetone	0.83 ± 0.29a	0.73 ± 0.30a	0.00a	3.53	0.097
61	1453	(E)- β -farnesene	0.00b	0.00b	1.23 ± 0.10a	15.79	0.004*
62	1500	Pentadecane	1.00 ± 0.30a	0.91 ± 0.11a	1.35 ± 0.60a	0.36	0.709
63	1523	Lilal	0.43 ± 0.08a	0.37 ± 0.06a	0.35 ± 0.18a	0.14	0.869
64	1529	Unknown 6	0.80 ± 0.31a	0.44 ± 0.26a	0.46 ± 0.18a	0.64	0.558
65	1570	Unknown 7	0.25 ± 0.07a	0.90 ± 0.52a	0.00a	2.361	0.175
66	1575	(E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene	0.00a	0.00a	0.20 ± 0.05b	15.79	0.004
67	1583	Unknown 8	1.68 ± 0.09b	0.84 ± 0.26c	3.34 ± 0.29a	29.92	0.001*
68	1600	Hexadecane	0.91 ± 0.22a	0.82 ± 0.09a	0.54 ± 0.16a	1.62	0.274

^aRetention Index relative to C8-20 n-alkanes on a VF5ms column. Calculated Retention Index relative to C8-C20 n-alkanes. Identification achieved by comparing mass spectra and RI with authentic standard (s) or tentative (t) with mass spectra libraries and literature data (Adams, 2007; Anastasaki et al., 2018).

^bF values, df = 2.

^cProbability values for each F-test. Significant values ($P < 0.05$) are denoted in bold and marked with an asterisk for each compound.

^dMean values followed by different letters are significantly different ($P < 0.05$).

Headspace Collection and Analysis of Plant Volatiles

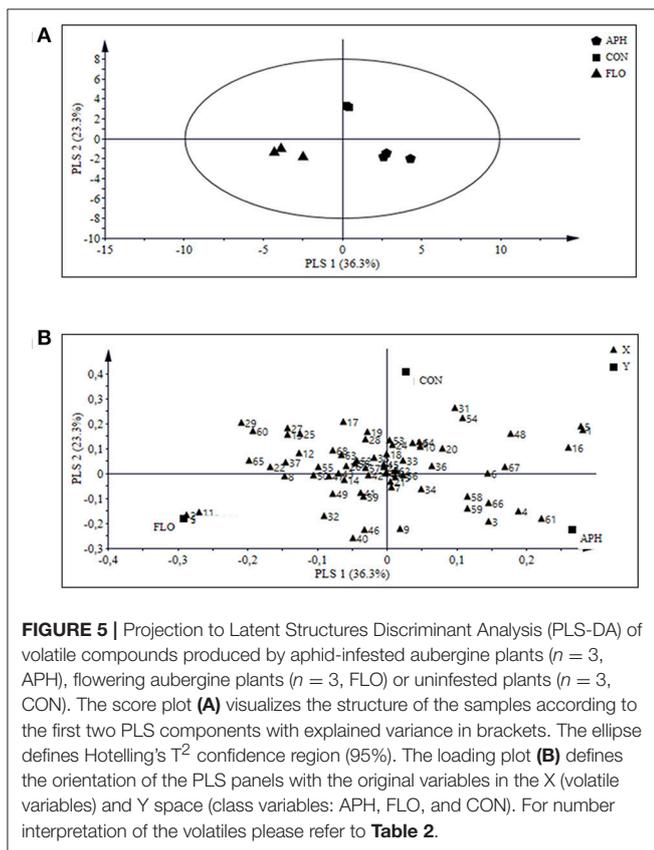
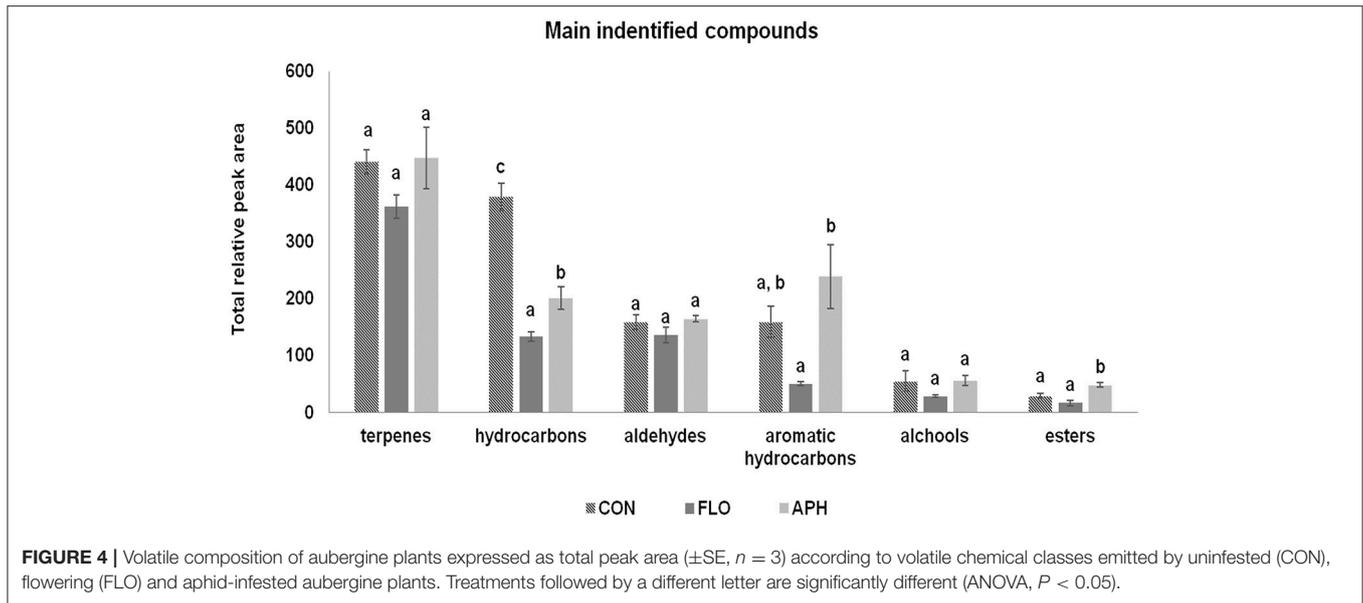
In total 68 compounds were isolated from the headspace of aubergine plants (Table 2). Among them, 49 were found in all three plant categories. We confirmed that differences exist in volatile emissions of uninfested, flowering and aphid-infested plants. Dodecanal, (E)- β -farnesene, 2-methylbutanal oxime and (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene –[(E,E)-TMTT] were found only in the volatile blend of aphid-infested aubergines. Furthermore, hexanal and β -citronellene were found only in the headspace of flowering aubergines, while benzyl alcohol was isolated only in the headspace of non-infested ones. Additionally, unknown compounds 1 and 7, hydrocarbon 6 and geranyl acetone were emitted from uninfested and flowering aubergines but not from aphid-infested plants. Terpenoids, including monoterpenes and sesquiterpenes, was the most abundant class of compounds. The main monoterpenes identified on all plant treatments were α - and β -pinene, limonene and 3- δ -carene. Significant emissions of esters, aromatic hydrocarbons and hydrocarbons ($F_{\text{est}} = 14.260$, $df = 2$, $P = 0.005$; $F_{\text{ahyd}} = 6.940$, $df = 2$, $P = 0.027$; $F_{\text{hyd}} = 46.538$, $df = 2$, $P < 0.001$) were observed in the volatile blend of all plants. The total relative peak area of terpenoids aldehydes and alcohols did not differ significantly between treatments ($F_{\text{terp}} = 1.752$, $df = 2$, $P = 0.252$; $F_{\text{ald}} = 1.741$, $df = 2$, $P = 0.253$; $F_{\text{alc}} = 1.919$, $df = 2$, $P = 0.227$) (Figure 4). Plants infested with *M. persicae* had significantly higher emissions of butyl acetate, 2-methylbutanal oxime, heptanal, (E)-4,

8-dimethyl-1,3,7-nonatriene [(E)-DMNT], citronellol, (Z)- α -bermamotoene, (E)- β -farnesene, (E,E)-TMTT and unknown 8 compared to uninfested plants (Table 2).

Projection to latent structures discriminant analysis (PLS-DA) of all treatments together presented three major clusters of samples, where the aphid-infested and the flowering plants were separated from the uninfested aubergine plants and from each other (Figure 5A). The PLS-DA analysis identified 24 compounds with a VIP value higher than 1 (Table 3). Figure 5B shows the contribution of emitted volatile compounds to the two principal components, which explained 36.3 and 23.3% of the variance, respectively.

DISCUSSION

In the present study, the response of *M. pygmaeus* nymphs toward plant resources was evaluated. Our first hypothesis that predator's plant experience affects the preference of nymphs was partly supported by our findings. Aubergine plants were more attractive to *M. pygmaeus* nymphs over air originating from pepper plants. However, our olfactory trials revealed that regardless the host plant origin of the predator pepper or aubergine plants, *M. pygmaeus* was more attracted to aubergine than to pepper plants. Previous studies have focused on adult behavior. Ingegno et al. (2011) have studied the effect of different host plants species in comparison with tomato plants on the response of *M. pygmaeus* adults. However, the influence of rearing plant on the choice behavior of *M. pygmaeus* nymphs has



not been studied previously. In the present study we tested two host-plant species, pepper and aubergine plants, which according to Perdakis and Lykouressis (2000, 2004a,b) have shown different suitability for the development and survival of *M. pygmaeus* and have been characterized among a wide range of host plants as the

least and most suitable plant, respectively. Other mirid species, such as *Dicyphus hesperus* Knight (Hemiptera:Miridae), both adults and nymphs, exhibit a similar behavior by choosing host plants on which nymph survival without prey was substantial (Sanchez et al., 2004).

Our second hypothesis that the volatiles emitted by a flower or prey would affect the response of *M. pygmaeus* nymphs was supported in the case of prey only. Predator nymphs were attracted to aphid-infested aubergines over uninfested ones. Previous studies have shown that *M. pygmaeus* adults do not respond in the presence of prey without the plant (Ingegno et al., 2011; Lins et al., 2014). Following the outcomes of Maselou et al. (2014), we carried out olfactometer bioassays in a plant scale using aubergine plants since they were found to be more attractive to *M. pygmaeus* nymphs. Likewise, there was a tendency for attraction toward flowering aubergine over non-flowering plants though not statistically significant. Given that *M. pygmaeus* nymphs can also feed on plant pollen (Perdakis and Lykouressis, 2000; Vandekerckhove and De Clercq, 2010), we expected that flowering plants would be more attractive than non-flowering plants. The fact that *M. pygmaeus* nymphs did not show a clear preference for the flowering plants suggests that these floral resources may be exploited after the establishment of the species on the plant. On the contrary, *M. pygmaeus* nymphs showed a clear preference for aphid-infested over uninfested aubergine plants. Similar outcomes on adults of *M. pygmaeus* (Ingegno et al., 2011; Lins et al., 2014), *D. errans* (Ingegno et al., 2013) and *N. tenuis* (Rim et al., 2015) were reported in Y-tube olfactometer studies. However, when we compared a flowering aubergine with an aphid-infested one, we recorded equal attraction, suggesting that floral resources may affect *M. pygmaeus* nymphs behavior. Previous studies showed that *M. pygmaeus* nymphs consumed less prey on flowering aubergine or pepper plants. This reduction was higher in aubergine than pepper plants (Maselou et al., 2014). Landis et al. (2000) showed

TABLE 3 | Variable importance to the projection (VIP) of volatiles for the corresponding PLS-DA plots, with value more than 1.0, which can be chosen as the characteristic volatile compounds for the differentiation between treatments.

No	Compound	VIP value
1	Hexanal	1.92
2	m-xylene	1.92
3	Octane	1.90
4	β -citronellene	1.81
5	Hydrocarbon 6	1.70
6	(<i>E</i>)- β -farnesene	1.69
7	Undecane	1.63
8	Butyl butanoate	1.60
9	Isoborneol	1.56
10	Geranyl acetone	1.50
11	2-methylbutanal oxime	1.44
12	Butyl acetate	1.43
13	Unknown 4	1.43
14	Citronellol	1.37
15	Unknown 2	1.37
16	Hydrocarbon 3	1.35
17	Heptanal	1.34
18	Decane	1.26
19	β -pinene	1.22
20	Unknown 8	1.18
21	Unknown 1	1.18
22	Linalool	1.14
23	(<i>E,E</i>)-TMTT	1.11
24	(<i>Z</i>)- α -bermamotoene	1.08

that the presence of flowering plants in agroecosystems is a conceptually simple mean to increase densities of predators and parasitoids, since several natural enemies use pollen and/or nectar as alternative food. However, increased abundance of predators and parasitoids to be of benefit for biological control, should translate into increased predation (Stephan et al., 2016). Studies on mirids have shown that habitat management could lead to higher predator abundance and as a result to higher prey consumption (Perdikis et al., 2011; Ingegno et al., 2017).

In our study, a higher number of volatile compounds were detected in the headspace of aubergine plants compared to other studies (MacLeod and Gonzales de Treconis, 1983; Van Den Boom et al., 2004; Rim et al., 2015; Darshane et al., 2017). Terpenoids were the most abundant volatiles in all plant treatments in agreement with previous studies for different host plants (Ingegno et al., 2016; Anastasaki et al., 2018). *M. pygmaeus* females responded positively to compounds emitted by *T. absoluta* infested tomato plants and also to spider mite infested plants (De Backer et al., 2017; Pappas et al., 2018). In both of the above studies a different profile of induced volatiles was identified compared to our study.

The volatile blend emitted by uninfested, flowering and aphid-infested aubergine plants differed both qualitative and quantitatively. The PLS-DA analysis revealed a separation between different treatments, indicating an alteration of volatile

blend on aubergine plants after aphid infestation as well as in flowering aubergine compared to uninfested aubergine plants. The loading plot shows that hexanal and β -citronellene were responsible for the differentiation of flowering aubergine plants, while the terpenoids (*E*)-(β)-farnesene and (*E,E*)-TMTT as well as the nitrogen compound 2-methylbutanal oxime for the differentiation of aphid-infested aubergines plants. These compounds isolated only in aphid-infested aubergine plants, had also a VIP value greater than 1. The terpenoid (*E*)- β -farnesene isolated only from aphid-infested aubergine plants, is known to play important role as a foraging cue for aphid natural enemies (Du et al., 1998; Verheggen et al., 2007; Hegde et al., 2011). (*E*)- β -farnesene is a well-known alarm pheromone released from aphids to warn individuals of the same species (Pickett and Griffiths, 1980), and has also been found to act as a kairomone for several aphid predators (Verheggen et al., 2008). Plants may also emit (*E*)- β -farnesene either constitutively (Gibson and Pickett, 1983) or inductively (Schnee et al., 2006). In our study, aphids were not exposed to any predator, which may suggest that the emission of (*E*)- β -farnesene was emitted by the plant itself and not by aphids, though, we cannot exclude the possibility that other kind of irritation might have occurred. In addition, we did not isolate (*E*)- β -farnesene from uninfested aubergine plants which further suggests that its emission was induced by aphid feeding. (*E,E*)-TMTT is related to herbivory attack (Paré and Tumlinson, 1999; Tholl et al., 2011). It has been found to be emitted by cotton (Hegde et al., 2011) and pepper plants (Moayeri et al., 2007) after aphids' infestation. In a recent study, (*E,E*)-TMTT emitted from *T. absoluta*-infested tomato plants was shown to provoke antennal responses of *M. pygmaeus* in a gas chromatography coupled with mass spectrometer and electroantennographic detectors (GC-MS-EAD) (De Backer et al., 2017). Another compound found only in aphid-infested aubergine plants was 2-methylbutanal oxime. This nitrogen containing compound was recorded from aubergine plants infested by *Spodoptera litura* larvae (Rim et al., 2015) and *Tetranychus urticae* (Van Den Boom et al., 2004; Rim et al., 2015). Also it, was found to elicit an olfactory response and attraction of natural enemies of *Lymantria dispar* (McCormick et al., 2014).

In this study we demonstrated that aphid infestation induces a change in the volatile emissions of aubergine plants that influences the foraging behavior of *M. pygmaeus* nymphs. Although aubergines at their flowering stage had a distinct volatile profile both from the uninfested and aphid-infested aubergine plants, *M. pygmaeus* nymphs did not discriminate between them. These observations are in agreement with the hypothesis that insects respond to a ratio of volatiles for host recognition rather than to individual compounds (Bruce, 2015). In addition, small qualitative differences are usually more important than obvious quantitative differences in volatiles that affect insect behavior (Bruce et al., 2010). Studies on electroantennogram (EAGs) responses of *M. pygmaeus* adults exposed to volatile compounds emitted either by host or non-host plants showed significant EAGs records in plants with very different volatile profiles and the maximum deflection values in the EAGs correlated with the concentrations of

sesquiterpenes and alcohols (Ingegno et al., 2016). In other experiments, *M. pygmaeus* adults perceived better the entire volatile blend of *Tuta absoluta*-infested tomato plants than individual compounds and synthetic blends (De Backer et al., 2017).

In conclusion, our results show that *M. pygmaeus* nymphs prefer aubergine over pepper irrespectively of their host plant origin. They were also shown to prefer aphid-infested over uninfested aubergine plants. Flowering plants were shown to be equally attractive to *M. pygmaeus* nymphs compared to aphid-infested plants. The results of our experiments may have practical implications for the application of *M. pygmaeus* in biological control programs. For example, in order to determine the appropriate time for the introduction of this mirid in the crop as a biological control agent. However, a more detailed knowledge of the role of HIPVs is desirable to better understand the behavior of *M. pygmaeus*.

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AUTHOR CONTRIBUTIONS

DM, PM: Conceived and designed the experiments; DM, EA: Performed the experiments; DM, EA, PM: Analyzed the data and wrote the paper; PM: Contributed reagents, materials, analysis tools; DM, EA, PM: contributed to revisions.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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