



Relative importance of host and plant semiochemicals in the foraging behavior of *Trichogramma achaeae*, an egg parasitoid of *Tuta absoluta*

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Abstract

Herbivore-induced plant volatiles (HIPVs) and host sex pheromones are important semiochemicals used by natural enemies to locate prey or hosts. The egg parasitoid *Trichogramma achaeae* Nagaraja & Nagarkatti has recently shown potential for use as a biological control agent of *Tuta absoluta* (Meyrick), a key pest of tomato crops worldwide. In this study, we used olfactometer tests to examine the behavioral response of *T. achaeae* females to *T. absoluta* sex pheromone or to HIPVs produced by tomato plants infested with *T. absoluta* eggs or larvae. Our results showed that *T. achaeae* was attracted to *T. absoluta* sex pheromone. Parasitoids were also innately attracted to volatiles produced by tomato plants, whether uninfested or infested. However, parasitoids could not distinguish between volatiles from uninfested or *T. absoluta*-infested tomato plants. We characterized the headspace volatiles of tomato plants used in the olfactometer tests and found out that oviposition and larval feeding by *T. absoluta* significantly enhanced HIPV emission. This study suggests that the sex pheromone of *T. absoluta* is a potential tool to manipulate the behavior of *T. achaeae* and improve its attraction to the tomato crop. The analysis of volatiles released by tomato plants, either infested or uninfested, coupled with the response of *T. achaeae* in the olfactometer tests was consistent with what was expected in terms of the foraging behavior of a generalist parasitoid. The results and implications are further discussed in the context of sustainable *T. absoluta* management.

Keywords Tomato · South American tomato pinworm · Sex pheromone · Biocontrol · Volatile organic compounds

Key message

- The parasitoid *Trichogramma achaeae* is considered a suitable candidate for managing the invasive pest *Tuta absoluta*
- We investigated the response of female parasitoids to *T. absoluta* sex pheromone, uninfested tomato plants, and plants infested with eggs or larvae of *T. absoluta* in an olfactometer
- The results indicated that *T. absoluta* sex pheromone may be deployed by growers to attract these parasitoids to tomato crops
- The parasitoids might depend on associative learning to respond appropriately to infested plants

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Introduction

Plant- and host-derived semiochemicals play a key role in the foraging behavior of natural enemies of insect pests. Host-derived volatile compounds, such as kairomones, are the most reliable cues for orienting natural enemies to locate their prey/host at short distance; however, their exploitation is often hampered by the evolution of hosts that emit undetectable quantities of kairomone (Vet and Dicke 1992). On the other hand, host-derived volatile compounds, such as sex pheromones, are often produced in substantial amounts by females for attracting mates at short and long distances, which in turn can also be exploited secondarily by parasitoid females to locate insect hosts (Reddy et al. 2002; Noldus et al. 1991). For example, some egg parasitoids are known to rely on the host's female sex pheromone to attach onto and travel on the female's body, and then subsequently parasitize her newly oviposited eggs (Arakaki et al. 1996; Huigens et al. 2010).

Likewise, when fed upon by herbivorous arthropods, various plant species will emit specific volatile blends that could attract natural enemies (predators or parasitoids), repel conspecifics, and/or induce an increased defense of its own and of neighboring plants (Unsicker et al. 2009; Dicke and Baldwin 2010; Backer et al. 2015; Coppola et al. 2017). In general, herbivore-induced plant volatiles (HIPVs) consist of several groups of compounds including terpenoids, green leaf volatiles, and benzenoids (Mumm and Dicke 2010; Fatouros et al. 2012). HIPVs are produced through various metabolic pathways, but most are derived from the terpenoid or isoprenoid pathways (Sacchettini and Poulter 1997; Degenhardt et al. 2009). The qualitative and quantitative characteristics of HIPVs vary according to the herbivore involved and to the plant species, genotype, or age (Turlings et al. 1993; Takabayashi et al. 1994). Additionally, insect oviposition has also been reported to affect the volatile blend released by the host plant (Mumm et al. 2003; Kopke et al. 2008; Fatouros et al. 2012), which is often distinct from that induced by larval feeding (Hilker and Meiners 2011). All these aforementioned factors and their combination determine the ultimate response of herbivores and natural enemies to the HIPVs emitted, including responses of repellence or attraction.

The tomato pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a major pest of tomato crops in South and Central America, and more recently in Europe (Biondi et al., 2018; Giorgini et al. 2019). *Tuta absoluta* was first reported in eastern Spain in 2006 and since then has invaded other European countries (Desneux et al. 2010), the Middle East, Africa (Mansour et al. 2018), and parts of Asia (Campos et al. 2017; Han et al. 2019). The

main damage caused by *T. absoluta* on tomato plants is due to larval feeding on leaves, stems, or fruits, leading to losses of as much as 100% in the absence of control measures (López 1991; Picanço et al. 1998; Tropea Garzia et al. 2012). Although *T. absoluta* is a well-studied horticultural pest, most of its control strategies are still based on insecticide use (Lietti et al. 2005; Guedes and Picanço 2012), the effectiveness of which is often undermined by insecticide resistance (Siqueira et al. 2000; Lietti et al. 2005; Silva et al. 2011; Roiditakis et al. 2018; Silva et al. 2019) and/or reduced contact with the larvae hidden inside plant stems or fruits (Cocco et al. 2013).

Several indigenous Afro-Eurasian natural enemies have been reported as potential biological control agents of *T. absoluta*, from which eulophid and braconid parasitoid wasps, and especially mirid predators, stand out as potentially more effective (Zappalà et al. 2013; Giorgini et al. 2019). Nevertheless, among the various natural enemies of *T. absoluta* studied to date, the egg parasitoids of the genus *Trichogramma* have received more substantial attention as potential biological control agents (Parra and Zucchi 2004; Pratisoli et al. 2005; Cagnotti et al. 2016; Cherif et al. 2018). For example, the generalist egg parasitoid *Trichogramma achaeae* Nagaraja & Nagarkatti (Hymenoptera: Trichogrammatidae) has been recently considered as a valid candidate to help manage *T. absoluta*, which infests greenhouse tomato crops in Mediterranean countries (Cabello et al. 2012; Cascone et al. 2015; Giorgini et al. 2019 and references therein). *Trichogramma achaeae* is a generalist parasitoid known to parasitize the eggs of lepidopteran species in at least ten different families and is considered native to Asia (China, India, and Russia). However, following accidental or deliberate introductions for biological control of lepidopteran pests, *T. achaeae* is now distributed worldwide, being recorded in Europe, Africa, and the Americas (Cabello et al. 2009; Wright and Stouthamer 2011; Polaszek et al. 2012). This parasitoid is also commercially available in various countries (Cabello et al. 2009; van Lenteren et al. 2018) following the consistent increase of the egg parasitism rate and pest control after inundative releases (Cabello et al. 2012; Oliveira et al. 2017).

To further improve this parasitoid efficiency, it is important to understand the chemical ecology of trophic interactions among tomato plants, *T. absoluta*, and *T. achaeae*. While it is known that *T. absoluta* eggs and larvae can elicit the production of HIPVs by tomato plants (Backer et al. 2015; Anastasaki et al. 2018), the responses of *T. achaeae* to those HIPVs remain unexplored. Likewise, it is unknown whether *T. achaeae* females could be attracted to *T. absoluta* sex pheromone. The pursuit of such knowledge is crucial to improve the effective use of *T. achaeae* in the augmentative biological control of *T. absoluta* in tomato greenhouses. The aim of our study was to use a series of olfactometer tests to

assess the response (attraction) of *T. achaeae* female parasitoids to: (i) HIPVs induced by *T. absoluta* egg deposition on tomato plants, (ii) HIPVs induced by *T. absoluta* larvae feeding on tomato plants, or (iii) to synthetic sex pheromone of *T. absoluta*. Additionally, we characterized the HIPV chemical profile of tomato plants infested with *T. absoluta* eggs or larvae.

Materials and methods

Plants and insects

The tomato plants (cultivar ‘San Marzano nano’) used in the olfactometer tests were grown in a glasshouse under the following conditions: temperature of 24 ± 2 °C, relative humidity (RH) of $65 \pm 5\%$, and photoperiod of 16L:8D. Plants used in the olfactometer tests were 5 weeks old, with 4–6 completely expanded leaves and a height of 18 cm.

Tuta absoluta was continuously reared inside bug dorms at the Istituto per la Protezione Sostenibile delle Piante (IPSP) under the same conditions described above. The original strain of *T. absoluta* was collected in 2015 in tomato greenhouses located in Battipaglia (Salerno, Italy). *Trichogramma achaeae* parasitoids used in the olfactometer tests were purchased biweekly from a commercial supplier (Agrobio, Almeria, Spain) in the form of pupae developed in the eggs of *Ephesia kuehniella* Zeller. Upon their arrival, the parasitoids were transferred to a climatic chamber at the same conditions described above until their use. The identity of the *Trichogramma* species was confirmed by COI and ITS2 gene amplification and sequencing as described by Cascone et al. (2015). Adult parasitoids were placed individually into micro-glass vials and sexed under a stereomicroscope 1–2 h prior to olfactometer tests. All female parasitoids used in olfactometer tests were 48–72 h old, mated, fed (1:1 water/honey solution), and naïve, i.e., with no previous oviposition experience or contact with plants.

Olfactometer tests

The behavioral response of *T. achaeae* females toward *T. absoluta* sex pheromone and HIPVs from infested tomato plants was assessed in a Y-tube olfactometer system (described in detail by Fatouros et al. 2012). Commercial pheromone dispensers or tomato plants were placed individually inside a 20-L glass jar, which was subsequently closed tightly for 15–20 min prior to the beginning of each choice test to allow the consistent diffusion of odor plumes and to reach a pressure balance inside the system. Each of the two glass jars was independently connected to an arm of the Y-tube, and the air flow was adjusted to 100 ml/minute for each arm. Female parasitoids were allowed 30 min to

make a choice between the two odor sources. All tests were conducted between 10:00 and 14:00 h in a laboratory evenly illuminated by fluorescent lights and with an ambient temperature of 24 ± 2 °C.

During each run, we subjected 48–72-h-old female parasitoids to the following choice tests: (i) tomato plant infested with *T. absoluta* eggs versus uninfested tomato plant, (ii) tomato plant infested with *T. absoluta* eggs versus clean air, (iii) tomato plant infested with *T. absoluta* larvae versus uninfested tomato plant, (iv) clean air versus *T. absoluta* sex pheromone, (v) uninfested tomato plant versus clean air, and (vi) tomato plant infested with *T. absoluta* larvae versus clean air. The first–second instar larvae of *T. absoluta* used in tests (iii and vi) were engendered from eggs in tests (i and ii), and the plants were also maintained the same for these two tests (iii and vi). The reason for the larvae-infested treatment is that eggs and larvae of *T. absoluta* can co-occur on the same plants, and by associative learning, the adult parasitoids could increase their chance of encountering its specific host (the egg) on plants infested with multiple stages of the pest. In treatments involving egg infestation (i and ii), the tomato plants were subjected to *T. absoluta* oviposition 48–72 h prior to olfactometer tests. This exposure time resulted in plants with approximately 28.60 ± 2.00 (SE) *T. absoluta* eggs each (min. 20, max. 58 eggs). We allowed the eggs about 7–10 days after the last oviposition exposure date to hatch into larvae and then carried out the olfactometer test (iii and vi). Thereafter, each tomato plant had an average of 12.76 ± 1.10 (SE) *T. absoluta* larvae (min. 7, max. 24 larvae). During test iv, the *T. absoluta* sex pheromone was released by a rubber septum dispenser (Biogard, CBC Europe S.r.l) containing (E,Z,Z)-3,8,11-tetradecatrien-1-yl acetate and (E,Z)-3,8-tetradecadien-1-yl acetate.

Each of the six choice tests was replicated 20–26 times, where a replicate was represented by a group of ten female parasitoids placed inside a glass tube that was inserted at the end of the Y-tube and allowed 30 min to make a choice. Groups of replicates (2–4) were conducted on 6–8 different dates, where a new set of pair of odors was always provided. A choice was scored only when a female parasitoid touched the very end of one of the Y-tube arms, or when it was collected in the trapping bulb connected to the Y-tube near the end of each arm. Females that did not make a choice were referred to as ‘unresponsive’ and were excluded from the statistical analysis (see below). In order to avoid any spatial bias, the position of the Y-tube arms was also reversed after running each choice test replicate.

Chemical analysis and HIPV profiling

After each olfactometer choice test, the tomato plants (uninfested, egg- and larva-infested) were individually placed inside a 20-L glass jar for a 3-h volatile extraction from

the headspace of those plants. There were 6–8 replicates for the chemical analysis/HIPV profiling of infested plants (with eggs or larvae) and 14 for uninfested plants (each plant considered as one replicate). The volatiles were collected in Tenax traps and stored at $-20\text{ }^{\circ}\text{C}$ for later volatile profiling by means of gas chromatography. Tomato plants infested with *T. absoluta* eggs from test (ii) were subjected to volatile extraction only after larvae emergence and being used in the olfactometer test (iii), whereas tomato plants infested with eggs from test (i) and uninfested plants were subjected to volatile extraction immediately after being used in the olfactometer. Aboveground parts of all plants (stem and leaves) were weighed out using a precision scale (Mettler Toledo) after volatile collection (destructive sample).

After trapping on Tenax (30 mg) and carboxen (30 mg) packed tubes, the samples were analyzed by CIS4–TDU–GC/MS. Gerstel TDU was heated at $300\text{ }^{\circ}\text{C}$ for 7 min under a helium stripping flow of 30 ml min^{-1} . The TDU unit was directly assembled over the PTV injector (CIS4 Gerstel, Germany) with a liner-in-liner coupling, which eliminates the carryover effect and analyte loss. During this stage, the CIS4 was cooled to $-20\text{ }^{\circ}\text{C}$ by computer-controlled liquid CO_2 pulsed flow. After cryo-trapping on a Tenax packing liner, the PTV was quickly ramped to $260\text{ }^{\circ}\text{C}$ for desorption and the analyte was transferred to CIS4. An Agilent 7890 GC equipped with a 5975 MSD was used for the analysis, all from Agilent Tech. (Palo Alto, CA, USA). Helium was used as the carrier gas, and the flow was kept constant at 1.2 ml/min . The chromatographic settings were as follows: injector in splitless mode set at $260\text{ }^{\circ}\text{C}$, J&W Innowax column (50 m, 0.20 mm i.d. , $0.4\text{ }\mu\text{m df}$); oven temperature program: initial temperature $40\text{ }^{\circ}\text{C}$ for 1 min, then $10\text{ }^{\circ}\text{C min}^{-1}$ increase until $130\text{ }^{\circ}\text{C}$, then $5\text{ }^{\circ}\text{C min}^{-1}$ increase until $210\text{ }^{\circ}\text{C}$, then $20\text{ }^{\circ}\text{C min}^{-1}$ increase until $260\text{ }^{\circ}\text{C}$, hold time 3 min. The mass spectrometer was operating with an electron ionization of 70 eV , in scan mode in the m/z range 29–330, at three scans sec^{-1} .

The deconvoluted peak spectra obtained by Agilent MassHunter software were matched against the NIST 11 spectral library for tentative identification. Kovats' retention indices were calculated for further compound confirmation and compared with those reported in the literature for the chromatographic column used. Authentic standards were also injected to confirm compound identity.

Data analysis

A two-sided binomial proportion test was carried out on the olfactometer data to test for differences in odor choice made by *T. achaeae* females. Only data on female parasitoids that made a choice between odor sources within 30 min were included in the analysis. The unresponsive females were excluded from the statistical analysis. Therefore, a total of

194, 158, 146, 156, 119, and 192 female parasitoids were included in the analyses for choice tests (i), (ii), (iii), (iv), (v), and (vi), respectively. Differences in the fresh weight of tomato plants (uninfested, egg- or larva-infested) after volatile extraction were also tested using ANOVA. The analysis was run using R software (R Core Team 2018).

A partial least squares-discriminant analysis (PLS-DA) was used to determine whether HIPV profiles classify the treatments assessed (uninfested plant, and plant infested by eggs or larvae) in different groups. PLS-DA was performed using the R packages mixOmics (Rohart et al. 2017) and RVAideMemoire (Hervé 2017). Additionally, MANOVA and ANOVA were run to test for differences in HIPV concentration among uninfested or egg- and larva-infested tomato plants, which was followed by a pairwise comparison using the post hoc Tukey test at $P < 0.05$ (R Core Team 2018).

Results

In the olfactometer tests, *T. achaeae* females preferred the odor from synthetic *Tuta absoluta* sex pheromone as opposed to clean air ($\chi^2 = 4.00$, $P = 0.04$) (Fig. 1). Likewise, a greater proportion of female parasitoids preferred the odor of uninfested tomato plants ($\chi^2 = 4.07$, $P = 0.04$) and of tomato plants infested with *T. absoluta* eggs ($\chi^2 = 3.97$, $P = 0.04$) or larvae ($\chi^2 = 5.05$, $P = 0.02$), when contrasted with clean air (Fig. 1). However, there was no significant difference regarding parasitoid choice when contrasting either larvae-infested ($\chi^2 = 1.98$, $P = 0.15$) or egg-infested tomato plants ($\chi^2 = 0.05$, $P = 0.94$) against uninfested tomato plants. There was no significant difference in the fresh weight of the tomato plants used among the different infestation treatments (uninfested, egg- or larva-infested) ($F = 2.83$, $P = 0.07$).

Tuta absoluta oviposition and larval feeding enhanced significantly the emission of HIPVs by the tomato plants (MANOVA, Pillai's trace = 1.68, $F_{20,30} = 3.563$, $P = 0.002$) (Table 1). In total, 15 plant-related volatile compounds were identified from the headspace of uninfested, egg- and larvae-infested tomato plants (Table 1). Although the uninfested and larvae-infested tomato plants emitted similar HIPV amounts for the majority of identified compounds, a significant difference in the amounts of sabinene and limonene was recorded (Table 1). Additionally, tomato plants infested with *T. absoluta* eggs released a significantly higher quantity of α -terpinene and p-cymene than that released by uninfested plants (Table 1). Lastly, the most abundant compounds found in the HIPV blends of all treatments were methyl salicylate, β -phellandrene, limonene, α -pinene, 2-carene, and α -phellandrene (Table 1).

Projection to latent structures discriminant analysis (PLS-DA) of all treatments together presented three major clusters

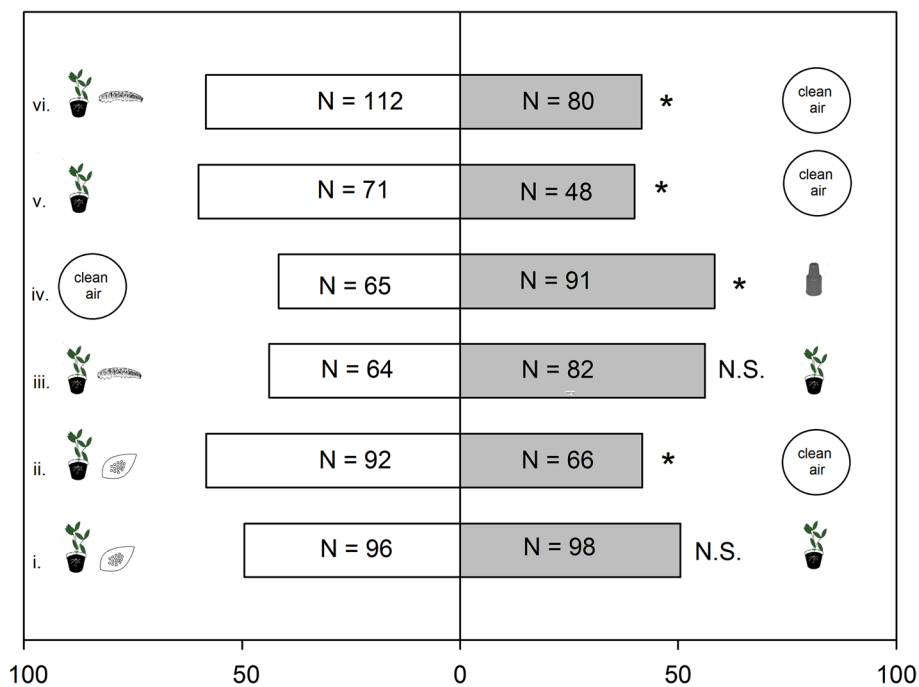


Fig. 1 Proportion (%) of *Trichogramma achaeae* adult females choosing tomato plant volatiles induced by eggs or larvae of *Tuta absoluta*, or the synthetic sex pheromone of this pest. Female parasitoids had a choice between: (i) tomato plant infested with *T. absoluta* eggs versus uninfested tomato plant, (ii) tomato plant infested with *T. absoluta* eggs versus clean air, (iii) tomato plant infested with *T. absoluta* larvae versus uninfested tomato plant, (iv) clean air versus synthetic

sex pheromone of *T. absoluta*, (v) uninfested tomato plant versus clean air, and (vi) tomato plant infested with *T. absoluta* larvae versus clean air. N=number of female parasitoids that chose one of the ‘odor’ options within 30 min (‘responsive females’). *Statistical difference between the proportions of parasitoids choosing one of the odor choices (two-sided Z-test, $P < 0.05$). N.S. no significant statistical difference

Table 1 Volatile organic compound (VOC) emission (nmol) by tomato plants in response to eggs and larvae of *Tuta absoluta*

VOC (nmol)	Tomato plant + eggs (N=8)	Tomato plant + larvae (N=5)	Uninfested tomato plant (N=13)
α-Pinene	36.93 ± 4.48 ^b	24.25 ± 3.97 ^{ab}	21.68 ± 2.7 ^a
Camphene	6.15 ± 20 ^b	1.81 ± 0.87 ^{ab}	0.66 ± 0.4 ^a
β-Pinene	8.92 ± 3.05 ^a	4.32 ± 2.01 ^a	3.43 ± 0.93 ^a
Sabinene	1.6 ± 0.13 ^b	1.8 ± 0.07 ^b	0.99 ± 0.09 ^a
2-Carene	21.86 ± 7.34 ^b	5.84 ± 1.46 ^{ab}	4.81 ± 1.32 ^a
3-Carene	6.11 ± 0.71 ^b	6.04 ± 0.48 ^{ab}	3.96 ± 0.48 ^a
Myrcene	0.68 ± 0.48 ^a	0.35 ± 0.32 ^a	0.00 ± 0.00 ^a
α-Phellandrene	22.34 ± 8.53 ^b	9.07 ± 2.33 ^{ab}	6.07 ± 1.01 ^a
α-Terpinene	8.04 ± 2.19 ^b	3.28 ± 0.35 ^a	2.86 ± 0.22 ^a
Limonene	65.31 ± 12.08 ^b	46.36 ± 6.23 ^b	23.7 ± 2.93 ^a
β-Phellandrene	128.99 ± 39.71 ^b	56.53 ± 28.5 ^{ab}	27.65 ± 10.35 ^a
Cineolo	3.18 ± 2.02 ^a	0.12 ± 0.12 ^b	0.04 ± 0.03 ^b
γ-Terpinene	9.59 ± 1.82 ^b	8.06 ± 0.99 ^{ab}	7.08 ± 0.11 ^a
p-Cymene	5.58 ± 0.94 ^b	2.55 ± 0.19 ^a	2.10 ± 0.13 ^a
Methyl salicylate	625.83 ± 201.76 ^b	504.99 ± 210.68 ^{ab}	145.82 ± 31.89 ^a
Total	951.13 ± 287.24	675.37 ± 258.6	250.87 ± 52.59

Mean values followed by the same letters within rows do not differ statistically at Tukey post hoc ($P < 0.05$)

of samples, where the two *T. absoluta* treatments (plants infested with eggs or larvae) separated from the control (uninfested plants) and from each other (Fig. 2). The first two significant PLS components explained 44% and 13% of the total variance, respectively. Among all VOCs emitted by tomato plants, sabinene, p-symene, 3-carene, methyl salicylate, and limonene most contributed to the separation between the experimental treatments, with variable importance for the projection (VIP) values > 1.

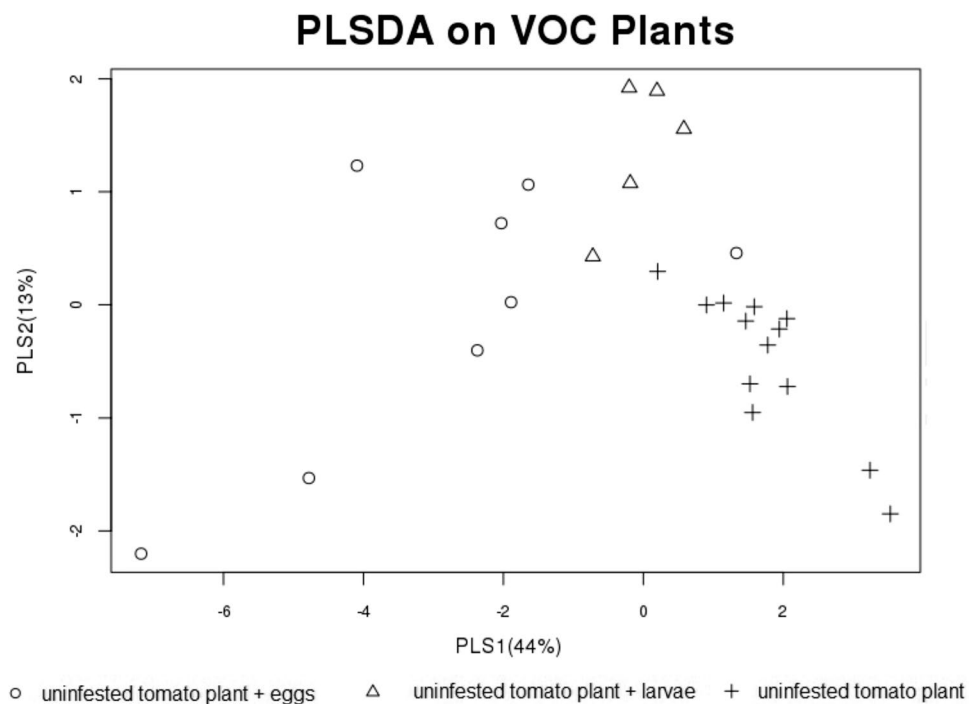
Discussion

The deployment of semiochemicals that could enhance the performance of biological control agents represents a profitable and modern strategy for plant protection. This is particularly important for invasive pests whose control still relies mostly on the repeated application of synthetic insecticides (Giorgini et al. 2019). Our results show that both host- and plant-derived cues influence the foraging behavior of *T. achaeae*. Specifically, olfactometer tests showed that female parasitoids were significantly attracted toward the sex pheromone of *T. absoluta*. In fact, the exploitation of sex pheromone to locate host eggs is relatively common among egg parasitoids, including *Trichogramma* species. For example, Reddy et al. (2002) demonstrated that the sex pheromone components of *Plutella xylostella* (L.), individually or as synthetic blends, attracted both the egg parasitoid *Trichogramma chilonis* Ishii and the larval parasitoid *Cotesia plutellae* (Kurdjumov). Likewise, Lewis et al. (1982) also

documented that a synthetic blend of *Heliothis zea* (Boddie) sex pheromone increased significantly egg parasitism by *Trichogramma pretiosum* Riley in greenhouse and in cotton fields. A similar result could be expected for *T. achaeae* in European greenhouses, or in tomato fields where mating disruption is deployed to manage *T. absoluta* (Cocco et al. 2013; Perez-Hedo et al. 2017; Biondi et al., 2018; Giorgini et al. 2019). However, field and greenhouse experiments are necessary to confirm this hypothesis regarding *T. achaeae*. For example, there have been some discrepancies between laboratory and greenhouse results regarding the attraction of other *Trichogramma* species to *T. absoluta* sex pheromone (Ahamdi and Poorjavad 2018), which worked better under laboratory conditions. Additionally, applying synthetic pheromone at the right timing and dose is crucial to guarantee the attraction of specific parasitoids, and thus these parameters should be also investigated.

Although the infestation by *T. absoluta*, especially oviposition, increased the emission of HIPVs (VOCs) by tomato plants (Table 1), *T. achaeae* females did not show a preference for egg-infested or larva-infested plants over uninfested tomato plants (Fig. 1). This pattern has also been observed for other natural enemies of *T. absoluta* such as the mirid predator *Nesidiocoris tenuis* (Heuter) (Naselli et al. 2017). In contrast, in our study, female parasitoids preferred uninfested, egg-infested, or larvae-infested tomato plants when they were tested against clean air. These results indicate that *T. achaeae* females are innately attracted to volatiles produced by tomato plants, regardless of the infestation status. In fact, it has been documented that certain plant species

Fig. 2 Projection to Latent Structures Discriminant Analysis (PLS-DA) on the volatile organic compounds (VOCs) emitted by tomato plants infested with eggs or larvae of *Tuta absoluta*



(e.g., tomato) naturally release specific compounds, such as sesquiterpenes, that significantly influence the attraction of *Trichogramma* species (Nordlund et al. 1985). The absence of *T. achaeae*'s preference for *T. absoluta*-infested plants, despite the higher release of volatiles in respect to control plants, suggests that naïve parasitoids may not be able to distinguish between infested and uninfested plants. If that is the case, the associative learning ability of *T. achaeae* females could become crucial for exploiting chemical cues released by infested tomato plants. In fact, some *Trichogramma* species have been reported to be able to learn the different components of host plant volatiles and to adapt their behavior accordingly (Pashalidou et al. 2010; Wilson and Woods 2016). Moreover, Vet and Dicke (1992) also theorized that generalist parasitoids, compared to specialists, should rely more strongly on learning infested-plant cues to adjust their foraging behavior since they have not coevolved with a specific host.

Alternatively, the amount of volatiles produced by *T. absoluta* (eggs or larvae)-infested plants in our experiment may not have been sufficient to allow *T. achaeae* females to distinguish between infested and uninfested tomato plants. For example, while limonene and sabinene have been shown to attract egg parasitoids (e.g., Trichogrammatidae) (Rani and Sandhyarani 2012; Dicke 1994), their 'higher' concentrations (limonene and sabinene) on *T. absoluta*-infested plants in our experiment (Table 1) were not enough to allow *T. achaeae* to clearly distinguish between uninfested and infested tomato plants. Following the same line of reasoning, other studies have documented that a higher density of *T. absoluta* infestation will result in greater amounts of HIPVs (Silva et al. 2017), which attract more *Trichogramma* parasitoids (Alsaedi et al. 2016). Additionally, it appears that egg parasitoids such as *T. pretiosum* released in greenhouses are also more attracted to tomato plants containing a higher number of *T. absoluta* eggs (Faria et al. 2008). Thus, testing the response of *T. achaeae* parasitoids to HIPVs from tomato plants sustaining higher densities of *T. absoluta* eggs and larvae should be considered in future studies.

The most abundant compounds found in the headspace of *T. absoluta* egg- and larva-infested and uninfested tomato plants were methyl salicylate, β -phellandrene, limonene, α -pinene, 2-carene, and α -phellandrene, which is in line with previous studies (Silva et al. 2017). Nonetheless, it is not uncommon to find some variation in the results of current and previous studies regarding the amount of headspace compounds found in tomato plants (Frag and Paré 2002; Degenhardt et al. 2010; Proffit et al. 2011; Backer et al. 2015). Such differences in the emitted blends may be explained by variations in the size and structure of the volatile collection chambers, plant age and cultivar, herbivore density, and the environmental conditions (Dudareva et al. 2006). In our study, volatile extraction and olfactometer tests

were conducted on plants of similar age and morphological structure (5.5 ± 1.0 leaves per plant) as well as similar egg (28.60 ± 2.00) and larvae (12.76 ± 1.10) densities per plant. In any case, the PLS-DA analysis showed that uninfested and infested tomato plants had significantly different volatile profiles, with these differences appearing 72 h after *T. absoluta* oviposition.

In Mediterranean countries, *T. achaeae* has been considered a potential candidate for the biological control of *T. absoluta* by inundative releases in tomato greenhouses (Urbaneja et al. 2012; El-Arnaouty et al. 2014; Kortam et al. 2017). Nevertheless, the necessity of multiple parasitoid releases in each season has been considered a drawback by several researchers (Cabello et al. 2009; Chailleux et al. 2012, 2013). Regardless, *T. achaeae* has been a key component of *T. absoluta* IPM programs in some Mediterranean greenhouse districts by complementing the activity of mirid predators, which may not be very effective if used alone. In this context, the specific timing that biocontrol agent is released results in better control of *T. absoluta*. For example, *T. achaeae* and mirid predators could work in unison, especially if released at different times: the former targeting early egg infestation and the latter subsequently targeting remaining eggs and larvae of *T. absoluta* (Urbaneja et al., 2012; Chailleux et al. 2013; Zappalà et al. 2013). This approach is also potentially favorable to reduce the damage linked to the phytophagous behavior of the mirid predators, which occurs frequently when they are released at the beginning of the season (Giorgini et al. 2019). Field tests in different conditions (protected, open field) are needed to investigate this assertion.

Semiochemicals are of paramount importance to help natural enemies locate and recognize their hosts or prey. Understanding the nature of these chemicals and their ecological role in trophic interactions is essential to design more sustainable strategies for pest management, including biological control. Our data indicate that female parasitoids of *T. achaeae* respond to both host sex pheromone and volatiles released by tomato plants, regardless of their infestation status. Nonetheless, further studies will be necessary to investigate how the host sex pheromone could be deployed to manipulate *T. achaeae* and thus improve the parasitism of *T. absoluta* in greenhouses and open fields. Additionally, it is crucial to assess the optimal releasing rate of these semiochemicals to enhance the performance of *T. achaeae*. Lastly, further studies should investigate the potential for *T. achaeae* to learn the cues from *T. absoluta*-infested tomato plants to adjust their foraging behavior accordingly, as well as the influence of host density on the *T. achaeae* response to HIPVs. The information obtained through such studies would complement our current results and would generate mechanistic knowledge that will promote a more efficient and sustainable management of *T. absoluta*.

Author contribution

PC, MG, and EG conceived and designed the experiments. MM worked on HIPV profiling and data analysis. LG, PC, HR, GS, and LI worked on olfactometer bioassays. LG analyzed olfactometer data. LG, PC, MG, and EG wrote and edited the manuscript. All authors reviewed and approved the final version of the manuscript.

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