Behavioral responses of the three Trichogramma species to different odor sources

Ghazwan Alsaedi, Ahmad Ashouri and Reza Talaei-Hassanloui

Abstract
Tuta absoluta Meyrick (Lepidoptera: Gelechiidae), commonly known as the tomato borer, represents one of the tomato main pests since it had been explored. Eggs of the T. absoluta moth on tomato plants can be parasitized by variety species of Trichogramma as T. brassicae, T. evanescens and T. embryophagum. This study was conducted to test the behavioral responses of parasitoids to infested plants, herbivore induced plant odors from plants infested with T. absoluta and Bacillus thuringiensis (Bt treated plants) in an airflow Y-tube olfactometer. Odors emitted by tomato plant elicited a behavioral response from females of Trichogramma species. The results showed that parasitoid T. embryophagum females have been positively attracted to the healthy tomato plants (60%) comparing with clean air jar, T. brassicae, T. evanescens and T. embryophagum females have been positively attracted to the tomato plants infested with T. absoluta eggs/larvae (65%, 60% and 60%) comparing with non-infested plants female parasitoids were not attracted, and had a negative response, experiments preference ability to use Trichogramma spp. to control T. absoluta eggs. Trichogramma spp. females were consistently weaker toward Bt spray on tomato plants, that refer there are no effect from using Bt on control T. absoluta eggs.

Keywords: Trichogramma spp., Tomato, Tuta absoluta, Bacillus thuringiensis, olfactometric bioassays

1. Introduction
Plant chemicals may function as an indirect defence trait responsible for attracting natural enemies of herbivores and important chemical cues for parasitoids and predators to locate their host and direct defence may not only have a major effect on the herbivore’s survival, but also on the survival of the natural enemies of the herbivore, leading to a possible conflict between the plant and the natural enemy that is attracted to the herbivore on that plant (Dicke et al 1990, Turlings et al 1999, Agrawal et al 2002, Gols et al 2008, Dicke and Baldwin 2010, Kessler and Heil 2011) [12, 40, 1, 20, 16, 26]. When volatiles are used to recruit natural enemies, they have been termed host-induced synomones, and they are regarded as an indirect chemical defence reaction by the plants (Dicke 1999, Dicke and van Loon 2000) [13, 15]. Deposition of insect eggs also can induce the production of volatiles or change leaf chemistry in a way that the plants attract and arrest certain egg parasitoids (Meiners and Hilker 1997, De Moraes et al 1998; Hilker et al 2002, Mumm et al 2003, Fatourou et al 2008) [29, 10, 23, 31, 18]. Chemical cues play a major role in the process of host selection by parasitoids, a process that has been discriminated into several steps such as habitat location, host location and host acceptance (Milonas et al 2009) [28]. The egg parasitoids are expected to frequently exploit chemical cues from other sources for host location as well. In the case of lepidopteran hosts, this includes, for example, pheromones and compounds released from scales of the wings of ovispositing females (Shu and Jones 1989, Colazza et al 1997) [38, 8]. Egg parasitoids belonging to the genus Trichogramma (Hymenoptera: Trichogrammatidae) are important natural enemies of a wide range of pests and are successfully used in biological control programmes worldwide (van Lenteren 2000) [42]. Trichogramma wasps were attracted to volatiles of egg-induced plants, and this attraction depended on the Trichogramma strain used (Nina et al 2014) [32]. Since then, a number of studies have investigated the response of Trichogramma spp. to volatiles derived from fresh plant material and plant extracts using airflow olfactometers. In most studies, parasitoids were found to be responsive (Boo and Yang 1998) [6]. Female T. brassicae wasps were shown to spy on an anti-aphrodisiac pheromone innately, and mated P. brassicae females marked with this pheromone, and parasitize the freshly laid eggs of butterflies (Fatourou et al 2005a) [17]. This espionage-and-ride strategy was confirmed in T. evanescens after learning (Huigens et al 2009) [24]. Furthermore B. thuringiensis and the parasite T. achaeae can provide...
good control of *T. absoluta* in tomato greenhouses (Desneux et al 2010, Molla et al 2011) [11, 30]. *B. thuringiensis* and *T. achaea* have been shown to be effective against *T. absoluta* and could be a supplement to the mirid predator *N. tenuis* (Calvo et al 2012a) [7]. We conducted a laboratory experiments in Y-tube olfactometer to investigate whether the parasitoids responded to the natural odors of tomato plants and *Bt*. We tested responses of three species of *Trichogramma* females to infested tomato plants of *T. absoluta* (Lepidoptera: Gelechiidae), and to plants infested spray with/without *Bt*.

### 2. Material and methods

#### 2.1 Plants and Insects

Tomato plants (*Solanum lycopersicum* L.) were grown in a greenhouse (25±2°C, 60–70% RH, 16L: 8D). Seeds originated from the University of Tehran, Department of Plant Protection, Ecology and Behavior Lab (Karaj, Iran) during 2015. Five weeks old Plants were used in the experiments. The plants were grown in small pots (8 cm height, 9 cm diameter) in mixture of soil and perlite (1:1). Tomato plants were introduced into cages with approximately 30 *T. absoluta* females and removed 24 h later, after oviposition was used for the volatiles odors, tomato plants with eggs were kept at the same conditions in the growth chambers until used for volatile odors. All used insects were collected from University of Tehran. Mated females of *T. absoluta* were obtained by pairing a virgin male and a virgin female moth, one day after mating, *T. absoluta* females were used in the experiments. *T. brassicae* *T. evanescens* and *T. embryophagum* (Hymenoptera: Trichogrammatidae), were reared on eggs of the moth *Ephestia kuehniella* in the University of Tehran, in a climate chamber (25±2°C, 60–70% RH, L16:D8). Only mated, 2–5 days old, wasps were used in the experiments. None of the wasps used in the experiments have previous contact with any plant material or host residues.

Y-tube olfactometer and general bioassay Y-tube olfactometer (stem 14cm; arms 19cm at 130° angle; internal section 22×22mm) sandwiched between two glass sheaths. The odour sources were reversed after testing parasitoid females. The responses of 20 parasitoid females (12h mated) will be tested for the treatments one adult females of *T. brassicae*, *T. evanescens* and *T. embryophagum* was released simultaneously and their preference for one of the two odour sources was recorded. After 5 minutes, the wasp collected in each of the end arm was counted; insects then were positioned individually at the beginning of the central arm of the Y-tube and observed for 5 minutes. When the wasps crossed the threshold line (located in the middle of each arm) and stayed in the arm end for 20 sec, this was considered as “choice”, and when a wasp did not make a choice within 5 minutes, it was recorded as a “no response” and excluded from the statistical analysis. In total, 140 wasps were tested with seven different experiments and each wasp was used only once, there were seven experiments: The first experiment were designed to investigate female wasps responses to healthy tomato plants comparing with clean air Jar in a Y-tube olfactometer, leaves tomato plants being used in this experiment were from 5 to 6 week age tomato seedling. That plant was in a healthy status and growing from plastic pot that including natural soil mixed with local peat moss. Twenty females of *Trichogramma* sp. had been bioassayed in these experiments. The second experiment was designed to investigate female wasp's responses to infested tomato plants with *T. absoluta* larvae/egg comparing with clean air Jar in a Y-tube olfactometer, tomato plants reared under the same conditions described in the first experiment. The third was performed to investigate female wasps responses to healthy tomato plants comparing with infested tomato plants with *T. absoluta* larvae/egg tomato plants reared under the same conditions described in the first experiment. The fourth experiment was done to investigate female wasps responses to healthy plants spryed with *B. thuringiensis* (*Bt*) comparing with infested tomato plants infested with *T. absoluta* larvae/egg and sprayed with *B. thuringiensis* (*Bt*). Three suspension concentrations (10^4, 10^5, 10^6 cells/ml) and water (control) sprayed on tomato plants reared under the same conditions described for the first experiment. The fifth experiment was designed to indicate female wasps responses to infested tomato plants with *T. absoluta* larvae/egg comparing with infested tomato plants by *T. absoluta* larvae/egg and sprayed with *B. thuringiensis* (*Bt*). Three suspension concentrations (10^4, 10^5, 10^6 cells/ml) and water (control) sprayed on tomato plants reared under the same conditions described for the first experiment. The sixth experiment was planned to investigate female wasps' responses to *T. absoluta* egg comparing with clean air Jar in a Y-tube olfactometer. Tomato plants reared under the same conditions described for the first experiment. The seventh experiment was planned to investigate female wasps' responses to *T. absoluta* egg comparing with clean air Jar in a Y-tube olfactometer. Tomato plants reared under the same conditions described for the first experiment.

#### 2.2 Data analysis

Differences between behavioral responses of parasitoids under pairs of treatments were analyzed by chi-square tests. Individuals that did not make a choice were excluded from the statistical analysis. Statistical analysis was performed using the software IBM SPSS Statistics 21.

### 3. Results

The results showed the Parasitoids were able to detect the odor and were attracted toward the odor-source arm (Fig. 1, 2, 3), but intensity was different among the Parasitoid females species.

First experiment showed the *T. embryophagum* strong attractiveness toward the jar that contained a healthy tomato seedling (Fig. 3), 12 *T. embryophagum* females of 20 females (almost 60%) were attracted (*X^2=6.700, P=0.035*) (Table 1), while 5 females showed either no responding toward the clean air jar comparing. Also there was no attractiveness between *T. brassicae* and *T. evanescens* toward the jar that contained a healthy tomato seedling (Fig. 1, 2) were 8 and 9 females of 20 females (almost 40% and 45%) respectively (*X^2=1.600, P=0.449; X^2=1.900, P=0.387*) (Table 1). The results of the Parasitoid females Trichogramma spp. showed the greatest attraction movements toward *T. absoluta* eggs/larvae infested tomato seedlings, without and with *Bt* (experiment 2, 3, 4) comparing with the clean air, healthy tomato seedling and tomato seedling with *Bt* were similarly positive. The responding *T. brassicae*, *T. evanescens* and *T. embryophagum* females in these experiments were 13, 12 and 20 females (almost 65%, 60% and 60%) respectively for second experiment (*X^2=9.100, P=0.011; X^2=6.700, P=0.035; X^2=9.100, P=0.011*); 13, 12 and 13 of 20 females (almost 65%, 60% and 65%) respectively for third experiment (*X^2=9.700, P=0.008; X^2=6.400, P=0.041; X^2=9.700, P=0.008*) and for fourth experiment was 12, 11 and 12 of 20 females (almost 60%, 55% and 60%) respectively (*X^2=6.400, P=0.041; X^2=6.100, P=0.047; X^2=6.700, P=0.035*) (Table 1). The
The results of the fourth, fifth and sixth experiment showed the females *T. brassicae*, *T. evanescens* and *T. embryophagum* females’ no attractiveness toward *T. absoluta* eggs/larvae infested tomato seedling with Bt comparing with the *T. absoluta* eggs/larvae infested tomato seedling (experiment 5) since they were 5, 9 and 9 of 20 female (almost 25%, 45% and 45%) respectively ($X^2=1.300$, $P=0.522$; $X^2=1.300$, $P=0.522$; $X^2=1.300$, $P=0.522$) (Table 1); Bt comparing with the clean air (experiment 6) since they were 6, 5 and 7 of 20 female (almost 30%, 25% and 35%) respectively ($X^2=2.800$, $P=0.247$; $X^2=2.500$, $P=0.287$; $X^2=0.700$, $P=0.705$) (Table 1).

### Table 1: Responses of *Trichogramma* spp. females to the odors of *Tuta absoluta* egg and larvae infested tomato plants in a Y-tube olfactometer and number of non-responding individuals recorded for each odor pair.

<table>
<thead>
<tr>
<th>Odor-source pairs</th>
<th><em>Trichogramma</em> sp.</th>
<th>No. of responses to stimulus test</th>
<th>No. of responses to control stimulus</th>
<th>No. of Non responses</th>
<th>$X^2$</th>
<th>$P$</th>
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</thead>
<tbody>
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<td><strong>Healthy Plant and Clean Air</strong></td>
<td></td>
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<tr>
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<td>8</td>
<td>8</td>
<td>4</td>
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<td>0.449</td>
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<td>7</td>
<td>4</td>
<td>1.900</td>
<td>0.387</td>
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<td><em>T. embryophagum</em></td>
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<td>5</td>
<td>3</td>
<td>6.700</td>
<td>0.035</td>
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<td><strong>Infested Plant and Clean Air</strong></td>
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<tr>
<td><em>T. brassicae</em></td>
<td>13</td>
<td>4</td>
<td>3</td>
<td>9.100</td>
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<tr>
<td><em>T. evanescens</em></td>
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<td><strong>Infested Plant and Healthy Plant</strong></td>
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<td><em>T. brassicae</em></td>
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<td>5</td>
<td>2</td>
<td>9.700</td>
<td>0.008</td>
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<td><em>T. evanescens</em></td>
<td>12</td>
<td>4</td>
<td>4</td>
<td>6.400</td>
<td>0.041</td>
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<td>0.008</td>
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<td><strong>Infested Plant+ Bt and Healthy Plant+ Bt</strong></td>
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<td>1.300</td>
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<td>4</td>
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<td>5</td>
<td>0.700</td>
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<td><em>T. embryophagum</em></td>
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<td>2</td>
<td>4</td>
<td>12.400</td>
<td>0.002</td>
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</table>

**Fig 1:** shows responses of *T. brassicae* females to the odors of *T. absoluta* infested tomato seedlings with/without Bt in a Y-tube olfactometer and the non-responding of *T. brassicae* females.
Seventh experiment showed the higher significantly number of individual *T. brassicae*, *T. evanescens* and *T. embryophagum* females in attractiveness toward *T. absoluta* eggs comparing with clean air since they were 12, 12 and 14 of 20 female (almost 60%, 60% and 70%) respectively ($X^2=6.400$, $P=0.041$; $X^2=6.700$, $P=0.035$; $X^2=12.400$, $P=0.002$) (Table 1).

5. Discussion

Our results reveal that Parasitoid females have been shown to respond to volatiles emanating from both undamaged plants (Li et al 1992, Reddy et al 2002, Romeis et al 2005) $^{[27, 35, 36]}$ and damaged plants (Turlings et al 1990, Dicke et al 1999) $^{[40, 44]}$, tomato plants are known to respond to herbivore and activate both direct and indirect defense mechanisms (Kant et al 2004, Angeles Lopez et al 2012) $^{[25, 33]}$. Insect parasitoids and predators are known to utilize the different volatile profile of infested plants vs. non-infested plants to detect their hosts and prey (Wei et al 2007) $^{[44]}$. These volatile compounds can be induced either by feeding (herbivore induced plant volatiles) or by egg laying (oviposition-induced plant volatiles) activities of insect herbivores and may play multiple roles as communication signals and defence agents, mediating interactions with other plants, microorganisms and animals (Kessler and Heil 2011, Pangesti et al 2013, Hilker and Fatouros 2015) $^{[26, 33, 21]}$. Elucidating the chemical ecology of natural enemies, herbivores and host plants is important in the development of effective and successful integrated pest management strategies where abundance and distribution of natural enemies could be manipulated by semi-chemicals (Hilker and Fatouros 2015) $^{[21]}$. According to the theory proposed by Vet and Dicke (1992) $^{[43]}$, specialist parasitoids should respond innately to volatiles released by host-damaged plants, our results are in agreement with this for *T. embryophagum* females and exact opposite for *T. brassicae*, *T. evanescens*, Wei et al (2007) $^{[44]}$ found Insect parasitoids
and predators are known to utilize the different volatile profile of infested plants vs. non-infested plants to detect their hosts and prey (Vet and Dicke 1992, Tumlinson et al 1993, Anastasaki et al 2015) [43, 39, 2] who that tomato plants bearing T. absoluta eggs are activated to produce herbivore-induced plant volatiles that may mediate indirect defense against herbivores. These compounds (plant volatiles) are reported to play a role in tomato resistance against other insects (Bleeker et al 2009) [3]. Volatiles emitted from herbivore-infested tomato plants had quantitative and qualitative differences from undamaged plants. Even below-ground infestation by a microbe has been found to cause changes in the headspace volatile profile of tomato plants (Battaglia et al 2013) [4].

The composition of the Herbivore-induced plant volatiles plays an important role in the attraction of the parasitoid, and some later induced volatiles like aromatic compounds and sesquiterpenes may distort and/or mask the attractiveness of the active volatiles (Penafior et al 2011) [34]. The results of our Bt experiments showed the responses of parasitoids Trichogramma spp. females were consistently weaker or there were no effect from using Bt on T. absoluta toward Bt because the larvae feed far less, which results in a strong reduction in volatile emissions (Schuler et al 1999) [33], and Because of the direct effect of the Bt also on the feeding rate of S. littorals, Turlings et al (2005) [41] opted for a standardized artificial induction of the plants by scratching leaves and applying regurgitant. Our results reveal that Trichogramma spp. generalist egg parasitoids may use plant volatiles to locate plants with potential hosts and In agreement with Penafior et al (2011) [39], oviposition by herbivorous insects can induce indirect plant defense responses whereby volatiles are emitted that attract egg parasitoids (Hilker and Meiners 2006) [22]. Plants that are able to produce herbivore-induced plant volatiles in response to egg deposition have the advantage of defending themselves early on, before hatching larvae can damage the plant (Anastasaki et al 2015) [2].

Insect oviposition induced volatiles in several plant species has been associated with some kind of physical damage during oviposition, adult feeding or an excretion that is used for eggs adherence (Colazza et al 2004, Fatouros et al 2012) [9, 19]. The egg induced volatile emission effect in tomato, an economically important crop plant. It has been proved that generalist egg parasitoids such as Trichogramma species respond to oviposition-induced volatiles released by the plants after oviposition by an herbivore (Fatouros et al 2012, Anastasaki et al 2015, Hilker and Fatouros 2015) [19, 2, 21].

6. Conclusion
This study showed that parasitoids Trichogramma species have very good preference to tomato plants and to the presence of T. absoluta as one of herbivores, since tomato volatiles induced by T. absoluta feeding are more attracting natural enemies of the eggs herbivore. That suggests, there will be good opportunities and new options for putting these parasitoids within the sort of valuable assets of any of the IPM strategies against T. absoluta for generations to come in order to reduce the use of chemicals and, consequently, improve food safety and environment quality.

7. Acknowledgements
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8. References


