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ORIGINAL PAPER

Flight behavior and oviposition of *Tuta absoluta* on susceptible and resistant genotypes of *Solanum lycopersicum*

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Abstract Plants produce volatile chemical compounds that can negatively affect the preference (antixenosis) or the performance (antibiosis) of herbivorous insects. It is thought that these volatile compounds are used as cues by herbivorous insects to determine the suitability of the plant for egg deposition and, hence, offspring performance. Here, we investigated whether volatiles produced by tomato plants play a role in modulating the flight and oviposition behavior of mated *Tuta absoluta* females. We found that the behavioral steps displayed by mated females did not differ when they flew toward resistant or susceptible tomato genotypes, but they reached the susceptible genotypes faster than the resistant ones. Moreover, females landed more often and laid more eggs on the most susceptible genotype, the Santa Clara variety. Because this variety is known to be of high quality for the development of *T. absoluta* larvae, the female's decision to land and lay more eggs on this genotype seems to be mainly to maximize offspring performance. However, this is not so straightforward because the proportion of landings and eggs laid by *T. absoluta* on another susceptible genotype tested in this study was not significantly higher than on the resistant genotypes. Finally, although future studies are still needed, considering the antixenotic and antibiotic traits of the resistant genotypes studied here, they are likely to succeed if used in integrated pest management.

Keywords Tomato leafminer · Tomato plants · Behavior · Volatiles · Oviposition · Wind tunnel

Introduction

Plants are constantly exposed to herbivores, and they have evolved traits that confer resistance in order to cope with herbivore attacks. Resistance to herbivorous insects can occur through three mechanisms: antixenosis, antibiosis, and tolerance (Panda and Kush 1995). Antixenosis is characterized by deterrence of the attacker. For instance, plants are known to release volatile compounds that repel aphids (Gibson and Pickett 1983), whiteflies (Bleeker et al. 2009), thrips (Gaum et al. 1994; Koschier et al. 2000), and moths (Peterson et al. 1994; Markovic et al. 1996; Moraes et al. 2001) or to have physical traits that decrease feeding (Roux et al. 2008) and oviposition (Leckie et al. 2016) of herbivores. Antibiosis is characterized by harming the attacker, for instance, by synthesizing anti-digestive compounds that negatively affect the development of the insect once it is settled and feeds on the plant (Panda and Kush 1995). Finally, tolerance is a compensation strategy to reduce the effects of herbivory (Panda and Kush 1995; Cardoza et al. 1999; Kant et al. 2015).

Plant defenses in turn select for counter-strategies in insects, such as the ability of females to use plant-produced volatile compounds to detect suitable hosts for oviposition. Indeed, via chemical and behavioral experiments, several studies have demonstrated that female moths are attracted by certain plant volatiles. Bengtsson et al. (2001) showed that the antenna of the codling moth, *Cydia pomonella*, responded consistently to volatile compounds that are predominantly released from branches of flowers and green apples. Similarly, Tasin et al. (2005) showed that antenna

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of the grapevine moth *Lobesia botrana* responded differently when exposed to volatile compounds collected either from leaves, from flowers or from berries of grape plants. While most male and female moths are attracted by a specific blend of plant volatiles, they are not by single compounds (Ansebo et al. 2004; Cha et al. 2008). and oviposition decision is affected by them (Witzgall et al. 2005; Piñero and Dorn 2009). Avoiding to oviposit on plants that are highly defended presumably minimizes the negative effects of anti-digestive compounds on its progeny.

The tomato leafminer *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) is an oligophagous insect that is considered a major pest of tomato crops in many countries of South America, as the larvae can cause substantial damage to leaves, which causes crop losses (Haji et al. 1988). Since *Tuta absoluta* larvae have a limited dispersal capacity, the quality of their food mainly depends on which plant the female moths choose to lay their eggs (Schoonhoven et al. 2005). It has been shown that many species preferentially oviposit on host plant species on which their offspring fare best, supporting the “preference performance hypothesis” (Jaenike 1978; Thompson 1988; Mayhew 1997; Gripenberg et al. 2010). According to this hypothesis, the decision of females to oviposit or not should be driven by potential offspring performance; females would evolve to lay their eggs on plants best suitable for their offspring. Moreover, there is more evidence indicating that female preference for suitable host plants among oligophagous insects is stronger than among polyphagous insects (Gripenberg et al. 2010). Following this assumption, antixenotic signals may be used by the females as a first step to assess the quality of the plant by avoiding those with significant antibiotic effects. Hence, the ability to perceive and avoid unsuitable hosts is a crucial step for the reproductive success of many moths, including *T. absoluta*.

Tomato plants (*Solanum lycopersicum* L.; Solanaceae) are one of the most preferred and suitable hosts known for *T. absoluta*. It is known that the females use volatile compounds to distinguish susceptible from resistant tomato plants (Proffit et al. 2011). However, there is a lack of knowledge about how volatile signals are perceived by *T. absoluta* females and which behavioral steps are displayed by them when they search for tomato plants. Furthermore, since those females use volatile signals to decide where to oviposit, it is well possible that they discriminate susceptible from resistant tomato plants to avoid exposing their offspring to the stronger defenses of the latter. Antibiotic effects conferring resistance of wild tomato plants to *T. absoluta* have been attributed to 2-tridecanone, 2-undecanone, and zingiberene, which are compounds not found in susceptible cultivars (Giustolin and Vendramim 1996; Leite et al. 1999; Azevedo et al. 2003). Indeed, in this paper, we will show that *T. absoluta* uses such signals to distinguish susceptible from resistant tomato plants and to decide to land and oviposit on the most susceptible plant on which their offspring could, potentially, fare best. However, this is not so straightforward because the proportion of landings and eggs laid by *T. absoluta* on another susceptible genotype tested in this study was not significantly higher than on the resistant genotypes tested.

In order to inspect the role of antixenotic signals in modulating the flight behavior of *T. absoluta* mated females and their final decision to land and oviposit on suitable host plants, we carried out two distinct assays. The first assay involved a no-choice test where we evaluated five behaviors displayed by mated females during their flight toward susceptible or resistant tomato plants, which includes the proportion of females displaying each behavior, relative to the number of females released in the wind tunnel. Moreover, if such a decision is based mostly on volatiles, females would take longer to land on unsuitable plants than they do on suitable ones in no-choice tests. Conversely, if moth females need to evaluate the plant surface in order to make their choice on ovipositing, then time to land would not differ according to plant suitability but oviposition would. Thus, we also measured the average time spent by *T. absoluta* mated females as they flew toward susceptible and resistant tomato genotypes in a wind tunnel. The second assay involved a no-choice test where we assessed the number of eggs laid by one mated female on each tomato genotype, by placing one female and one tomato genotype in a cage. In doing so, we investigate whether the female would retain its urge to oviposit even if unable to choose a better plant.

Materials and methods

Plants

As resistant plants we used three *Solanum lycopersicum* accessions that are registered at the Horticultural Germoplasm Bank (HGB) of the University of Viçosa as HGB-1708, HGB-674, and HGB-1497. As susceptible plants we used two *S. lycopersicum* genotypes: the accession HGB-489 and the commercial variety Santa Clara. These accessions were previously classified as resistant or susceptible to *T. absoluta* by Castro Antônio (2006) and Oliveira et al. (2009). The Santa Clara variety was included as a benchmark for susceptibility as it is considered the preferred host of *T. absoluta* (Oliveira et al. 2009; Giustolin et al. 2002; Leite et al. 2001; Thomazini et al. 2001).

Tomato plants were grown in a greenhouse (25 ± 2 °C, 60–70% relative humidity [RH]). Seedlings were transplanted to plastic pots (5L) with a soil substrate for vegetables (Plantmax, Eucatex Agro). Plants were watered daily, fertilized weekly (15–15–20 N–P–K), and used for experiments when they were approximately 40 days old. The use of the fungicide Cercobin 700 PM was necessary during the dry season, between June and July, for powdery mildew control on the leaves, and the insecticide Cartap BR 500 was applied to control whiteflies. Plants were not used for experiments until 20 days after the application of chemicals, which was necessary due to the residual period of these chemicals on the plants (Ministério da Agricultura 2009).

Insects

Eggs, larvae, and pupae of *T. absoluta* were collected from tomato leaves (*S. lycopersicum* var. Santa Clara) in a commercial plantation in Viçosa, Minas Gerais, Brazil. Since 2008, the rearing has been maintained in insect-proof cages (30 × 20 × 30 cm^3) within a climate room (25 ± 2 °C, 60–70% RH, 12:12h [light:dark] photoperiod). After egg hatching, new detached tomato leaves (Santa Clara variety) with their petioles immersed in water were periodically provided for larvae feeding (*ad libitum*). After 7–10 days, the leaves were removed from the cage, and the pupae were carefully collected from the leaves. Subsequently, the pupae were sexed according to Coelho and França (1987) and transferred to another climate room [25 ± 2 °C, 60–70% RH, 12:12 h (light:dark) photoperiod] without tomato plants to avoid female exposure to tomato volatiles. Males and females were kept in separate insect-proof cages and were fed with a sugar solution (10% w/v). One day prior to the experiments, one virgin male and one virgin female were confined in a small plastic cage (10 × 3 cm^2). We left them there for 12 h and observed whether the mating occurred, checking the cages during the last hour of the scotophase and the first 2–3 h of the photophase (Hickel 1989). Once mated, we removed the males from the cages and returned them to the rearing. Only mated females (1–2 days old) were used for the experiments.

Behavioral response in a wind tunnel

The flight behavior of *T. absoluta* was observed in a wind tunnel, following the procedure of earlier studies (Kennedy and Marsh 1974; Tasin et al. 2005; Proffit et al. 2011). In short, it consists of releasing one female at one end of the tunnel and observing its flight toward one plant genotype (source) located at the other end. The wind tunnel was equipped with a horizontal fan that pushed air through it, and outcoming air was aspirated by another fan on the opposite site. Illumination was provided by lamps at 10 lx located outside and on top of the wind tunnel.

Before testing the flight behavior of *T. absoluta* toward the susceptible or resistant tomato genotypes, we carried out two control behavioral assays with mated females in the wind tunnel (size 63 × 63 × 200 cm^3; wind speed 0.25 m/s, 23 ± 2 °C, and 70 ± 5% RH). The first one was to investigate at which time of the day mated females prefer to fly and to land on the plants in the wind tunnel, and the second one was to investigate whether plant volatiles play the main role in the female's decision to fly and land on the plant. In the first experiment, we observed the flight response of *T. absoluta* toward tomato leaves (ca. 30 cm high; three detached Santa Clara leaves) during four 1-h time windows: (1) the penultimate hour of the photophase ('On2'; $n = 151$); (2) the last hour of the photophase ('On1'; $n = 163$); (3) the first hour of the scotophase ('Off1'; $n = 70$); and (4) the second hour of the scotophase ('Off2'; $n = 73$). In the second experiment, we assessed the proportion of *T. absoluta* landing on tomato leaves (ca. 30 cm high; three detached Santa Clara leaves) versus fake leaves ('plastic plants'; green plastic-shaped-like tomato leaves). Both tomato and plastic plants were put into glass vials with water and were tested simultaneously in the wind tunnel in the first hour of the scotophase (i.e., 'Off1') ($n = 138$).

The flight behavior of *T. absoluta* toward the susceptible and resistant tomato genotypes was assessed in another wind tunnel (size 3 × 1 × 1 m^3; wind speed 0.35 m/s, 23 ± 2 °C, and 70 ± 5% RH).To assure the best performance of the females in the wind tunnel we, therefore, performed our experiments in the first hour of the scotophase (see Fig. 1). Since the goal was to observe the female behaviors toward each tomato genotype, they were offered individually to one mated female. *T. absoluta* females were placed individually in a glass tube (15 × 3 cm^2) and acclimated to the wind tunnel 1 h prior to the experiments. Three leaves from each genotype (for number of replicates see Table 1) were detached from tomato plants and placed with their petiole into glass vials (100 mL) containing water; these vials were then placed at the end of the wind tunnel 1.70 m away from where the females were released. Each female was used only once, and flight behaviors were observed from the moment it was released until it reached the plant, but never for more than 2 min. Observed behaviors were as follows: (1) antennation (when the female remained at the tip of the glass tube moving the antennae); (2) take-off (when the female left the release platform); (3) oriented flight (when the female flew zigzagging upwind toward the plant); (4) flight near the source (when the female flew within 0.10 m of the plant); and finally (5) landing on the plant. We also measured the total time spent by these females as they flew toward each tomato genotype in the wind tunnel.

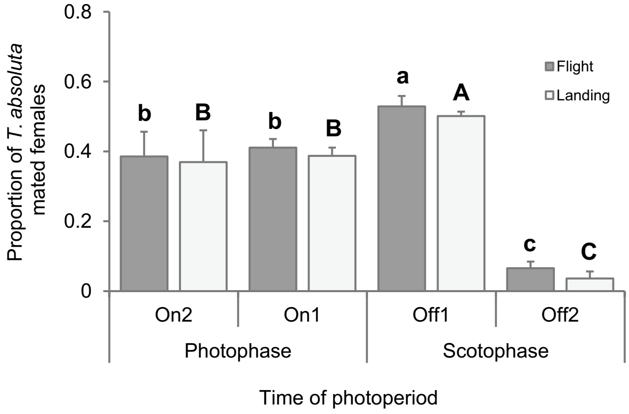

Fig. 1 Flight response of *T. absoluta* in a wind tunnel during photophase and scotophase (size 63×63×200 cm^3, wind speed 0.25 m/s, 23±2 °C, and 70±5% RH). Leaves from the tomato genotype Santa Clara variety were placed at the end of wind tunnel and one *T. absoluta* mated female was placed in a platform on the opposite site. Dark gray bars indicate mean proportion (+SE) of moths that flew toward the tomato leaves, and the *light gray bars* indicate mean proportion (+SE) of moths that landed on them in four different time points: On1 and On2 which correspond to the last 2 h of the photophase and Off1 and Off2 which correspond to the first 2 h of the scotophase. *Different uppercase letters* denote significant differences (GLM with contrast, $P \leq 0.05$) in landing and *lowercase letters* denote significant differences (GLM with contrast, $P \leq 0.05$) in flight over the treatments

Oviposition response

For the oviposition test, one tomato leaflet was offered to one mated *T. absoluta* female ($n = 35$). All leaflets from the third leaf of each tomato plant genotype were detached and placed with their petiole into glass vials (2 mL) filled with water. Each leaflet with a *T. absoluta* female was kept in a plastic cage (10×3 cm^2) within a climate room [25±2 °C, 60–70% RH, 12:12 h (light:dark) photoperiod]. In addition to the leaflet, each plastic cage also contained a piece of cotton wool that was soaked with a sugar solution (10% w/v). After 24h, the number of eggs laid on each leaflet was assessed.

Statistical analyses

Analyses were carried out using Generalized Linear Modeling (GLM) followed by residual analyses to confirm the model suitability and the choice of error distribution. Initially, full models comprised all levels within the variable under scrutiny. Subsequent model simplification was achieved amalgamating levels, as long as this did not produce a significant ($P \leq 0.05$) change in deviance (Crawley 2007). Analyses were performed using the software R, version 2.15.1 (R Development Core Team 2012).

The flight response of *T. absoluta* in the four time windows tested (On2, On1, Off1, and Off2) were compared under quasibinomial distribution with time windows as levels of the explanatory categorical variable (x) and the proportion of mated females that flew or landed on the plant in the wind tunnel as dependent variable (y).

The proportion of females landing on plastic and tomato leaves were compared under quasibinomial distribution. The two source types were included in the model as explanatory categorical variable (x) and the proportion of mated females that landed on each source in the wind tunnel as dependent variable (y).

Differences among the flight behaviors toward resistant and susceptible genotypes were analyzed under quasibinomial distribution with genotype identities as levels of the explanatory categorical variable and the proportion of successes for each behavior (antennation, take-off, oriented flight, flight near the source and landing on the source) as the dependent variable. Each behavioral response in the wind tunnel was analyzed separately. The choice of

Table 1 List of behaviors displayed by *T. absoluta* toward five tomato genotypes in a wind tunnel

Tomato genotype	Females tested	Antennation[a]	Take-off[a]	Oriented flight[b]	Flight near the source	Landing on the source[b]
Santa Clara	74 (100%)	54 (73%)	54 (73%)	32 (59%)	24 (75%)	11 (45%) **A**
HGB-489	65 (100%)	51 (78%)	51 (78%)	23 (45%)	20 (87%)	6 (30%) **B**
HGB-1497	82 (100%)	53 (65%)	50 (60%)	24 (48%)	12 (50%)	1 (8%) **B**
HGB-1708	87 (100%)	53 (61%)	52 (59%)	28 (54%)	22 (79%)	3 (13%) **B**
HGB-674	76 (100%)	50 (66%)	52 (68%)	26 (50%)	12 (46%)	3 (25%) **B**
F value	–	1.1	1.3	0.4	1.6	2.9
P value	–	0.35	0.29	0.82	0.18	0.02*

*Denotes significant difference ($P \leq 0.05$)

[a] The number of females displaying the behaviors **antennation** and **take-off** depends on the total number of females tested

[b] The number of females **landing on the source** depends on the number of females that **flight near The source**, which itself depends on the number of females that have an **oriented flight**

quasibinomial distribution is justified to correct for overdispersion previously detected under binomial errors (Crawley 2007).

The time spent by the females flying toward each tomato genotype in the wind tunnel was analyzed under normal distribution with genotypes as levels of the explanatory variable (x) and time as dependent variable (y).

The data from the oviposition test were analyzed under quasipoisson distribution with genotypes as levels of the explanatory variable (x) and the number of eggs laid as the dependent variable (y).

Results

Tuta absoluta behavioral response in the wind tunnel

First, we demonstrated that the response of mated *T. absoluta* females toward tomato leaves in the wind tunnel differed significantly over the four time windows that were tested (flying: GLM,$F_{[3,26]} = 12.3$, $P \leq 0.001$; landing: GLM, $F_{[3,26]} = 12.6$, $P \leq 0.001$, Fig. 1). We observed that most of the females responded (i.e., flying or landing) during the first hour of the scotophase (Off1). There was a clear difference among the first (Off1) and the second (Off2) hour of the scotophase with less than 10% of the females responding during Off2 (flying: GLM with contrast, $F_{[1,23]} = 31.4$, $P \leq 0.001$; landing: GLM with contrast, $F_{[1,23]} = 33.1$, $P \leq 0.001$). The proportion of females flying or landing during the single to last (On2) and last (On1) hour of the photophase did not differ (flying: GLM with contrast, $F_{[1,23]} = 2.7$, $P = 0.11$; landing: GLM with contrast, $F_{[1,23]} = 2.2$, $P = 0.15$). Next, we verified that, during the first hour of the scotophase (Off1), *T. absoluta* females indeed responded to olfactory cues in the wind tunnel, i.e., the odors of the tomato leaves, and not to visual cues, as nearly all the tested individuals landed on real tomato leaves instead of on plastic mimics (GLM with $F_{[1,19]} = 644.4$, $P \leq 0.001$, Fig. 2).

Finally, we tested the behavioral responses of *T. absoluta* females toward different tomato genotypes during the first hour of the scotophase (Off1). Among the five flight behaviors exhibited by *T. absoluta* females in the wind tunnel, only landing was significantly affected by tomato genotypes (GLM with $F_{[4,60]} = 2.9$, $P = 0.02$, Table 1). Compared with the other genotypes, mated females preferred to land on the Santa Clara variety (GLM with contrast, $F_{[1,63]} = 6.9$, $P \leq 0.01$, Fig. 3). Additionally, the time spent by females to reach each genotype was also affected by tomato genotypes (GLM with $F_{[4,19]} = 9.1$, $P \leq 0.001$, Fig. 4). The time spent by females to reach the susceptible genotypes Santa Clara variety and HGB-489 did not differ (GLM with contrast, $F_{[1,19]} = 1.2$, $P = 0.29$), but they differed from the time to reach the resistant genotypes HGB-674 (GLM with contrast, $F_{[1,20]} = 17.2$, $P \leq 0.001$), HGB-1708 (GLM with contrast, $F_{[1,20]} = 8.1$, $P \leq 0.01$), and HGB-1497 (GLM with contrast, $F_{[1,20]} = 16.9$,

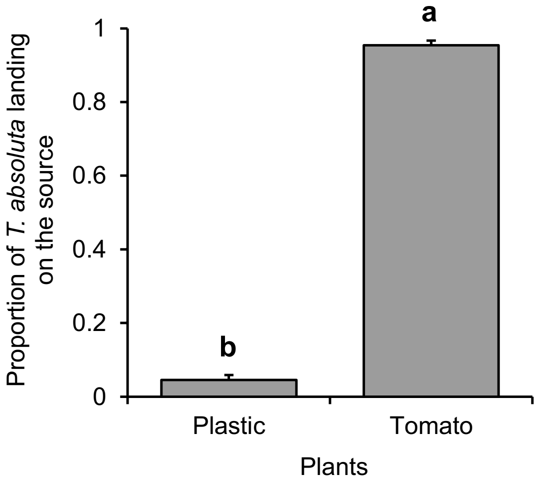

Fig. 2 Flight response of *T. absoluta* toward tomato and plastic plants in a wind tunnel on the first hour of the scotophase (size $63 \times 63 \times 200$ cm^3, wind speed 0.25 m/s, 23 ± 2 °C, and 70 ± 5% RH). Leaves from the tomato genotype Santa Clara variety were placed simultaneously with green plastic leaves at one end of wind tunnel, and one *T. absoluta* mated female was placed in a platform on the other end. *Bars* show mean proportion (+SE) of *T. absoluta* mated females that landed on each of the two plants in a choice test. *Different letters* denote significant differences (GLM $P \leq 0.05$) among plants

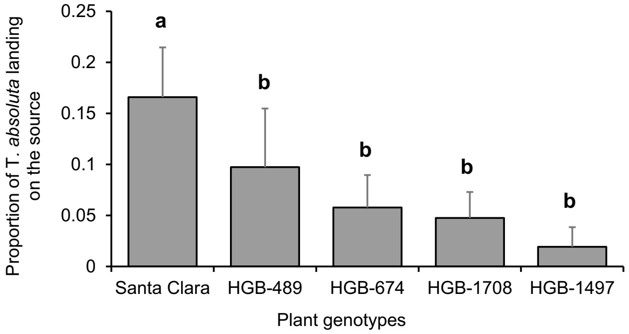

Fig. 3 Behavioral response of *T. absoluta* toward tomato plants in a wind tunnel on the first hour of the scotophase (size $3 \times 1 \times 1$ m^3, wind speed 0.35 m/s, 23 ± 2 °C, and 70 ± 5% RH). *Bars* show mean proportion (+SE) of *T. absoluta* mated females that landed on each of the five tomato genotypes in a no-choice test. Leaves from each tomato genotype were placed at the end of the wind tunnel, and one female was placed in a platform on the opposite site. The susceptible tomato genotypes are Santa Clara variety and HGB-489 and the resistant genotypes are HGB-674, HGB-1708, and HGB-1497. *Different letters* denote significant differences (GLM with contrast, $P \leq 0.05$) among plant genotypes

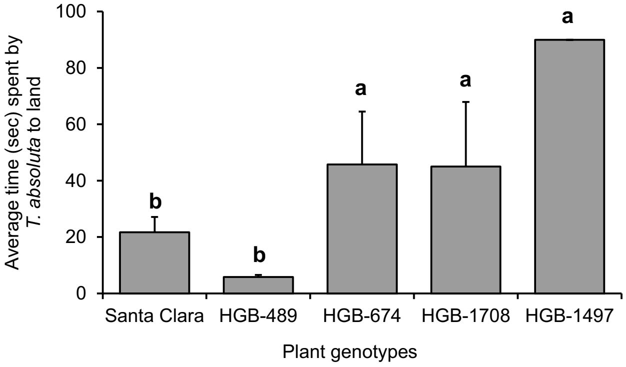

Fig. 4 Time spent by *T. absoluta* to reach the tomato plants in a wind tunnel on the first hour of the scotophase ($3\times1\times1$ m^3, wind speed 0.35 m/s, 23 ± 2 °C, and 70 ± 5% RH). *Bars* show the average time in seconds spent by *T. absoluta* mated females as they flew toward each one of the five tomato genotypes. SE is indicated for the plant genotypes Santa Clara, BGH-489, BGH-674, and BGH-1708, but not for BGH-1497. Leaves from each tomato genotype were placed at the end of the wind tunnel, and one female was placed in a platform on the opposite site. The susceptible tomato genotypes are Santa Clara variety and HGB-489, and the resistant genotypes are HGB-674, HGB-1708, and HGB-1497. *Different letters* denote significant differences (GLM with contrast, $P\leq0.05$) among plant genotypes

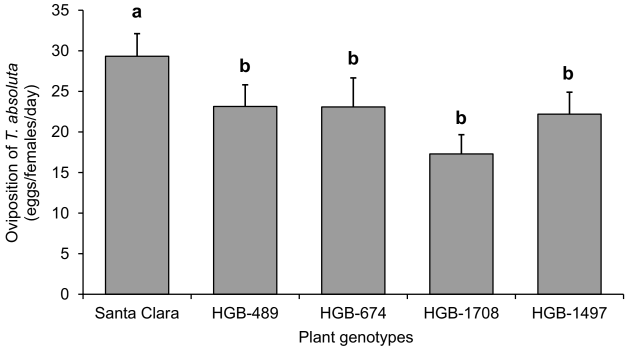

Fig. 5 Oviposition of *T. absoluta* on five tomato genotypes. *Bars* show the average number of eggs laid (+SE) by *T. absoluta* mated females on single leaflets from five different tomato genotypes in a no-choice test. The susceptible genotypes are Santa Clara variety and HGB-489, and the resistant genotypes are HGB-674, HGB-1708, and HGB-1497. *Different letters* denote significant differences (GLM with contrast, $P\leq0.05$) among plant genotypes

$P\leq0.001$). In other words, females spent less time to land on the susceptible genotypes than on the resistant ones.

The remaining flight behaviors including antennation (GLM with $F_{[4,59]}=1.1$, $P=0.35$), take-off (GLM with $F_{[4,59]}=1.3$, $P=0.29$), oriented flight (GLM with $F_{[4,59]}=0.4$, $P=0.82$), and flight near the source (GLM with $F_{[4,59]}=1.6$, $P=0.18$) were not significantly affected by the tomato genotypes. Table 1 shows, in more detail, the number (and percentage) of females displaying each one of the behaviors observed in the wind tunnel.

Oviposition response in a no-choice test

When one female was confined in a cage with only one leaflet, the average number of eggs laid by the female differed per genotype (GLM with $F_{[4,174]}=2.5$, $P=0.04$). The females laid more eggs on Santa Clara leaves than on leaves from the other genotypes (GLM with contrast, $F_{[1,173]}=6.7$, $P=0.01$, Fig. 5).

Discussion

Our results show that, in a wind tunnel, the behavioral steps exhibited by *T. absoluta* mated females before landing on a tomato plant are independent of the host plant's genotype. Nevertheless, females landed (Fig. 3) and oviposited (Fig. 5) more on the susceptible genotype Santa Clara variety than on the other genotypes. In addition, females spent less time to reach the susceptible genotypes than the resistant ones (Fig. 4). Hence, our results are in agreement with the previous finding that the Santa Clara variety is the most preferred host plant for oviposition by *T. absoluta* (Thomazini et al. 2001; Oliveira et al. 2009; Proffit et al. 2011). Since it has also been found that this host plant is also one of the most suitable for larval performance (Ecole et al. 1999; Leite et al. 2001; Giustolin et al. 2002), it seems reasonable to argue that *T. absoluta* females choose hosts that are suitable for their offspring, supporting the preference–performance hypothesis. Although our study partially supports this hypothesis, this was not so straightforward, because females landed and oviposited as often on the susceptible accession HGB-489 as on the resistant ones. In other words, because females were not able to identify this susceptible genotype as such, this finding weakens the above evidence *pro* preference–performance hypothesis.

An insect can employ two strategies to find a source: the first involves insect excitation, in which the insect prepares itself to take off in response to a stimulus perceived by its chemoreceptors (Bernays and Chapman 1994). This behavior was easily recognized among *T. absoluta* females tested in the wind tunnel. While still inside the glass tube, the female ceased moving, put its antenna in a "V" form and, upon excitation, immediately shook its wings and took off upwind. The second strategy used to find a source is the orientation that occurs after take-off. Females are guided by the odor carried by the wind and when stimulated, they fly upwind in a zigzag motion (Kennedy and Marsh 1974), exhibiting what we refer to as "oriented flight." If the female loses the odor plume and is unable to reorient, it will fly in any direction. Displaying wider lateral zigzags across the windline can help to re-contact a lost plume (Cardé and Willis 2008). Although during the observations in the wind tunnel, *T. absoluta* females exhibited an optomotor response to host location making zigzags in the wind tunnel toward the plant, once females lost the odor plume they were usually unable to reorient and re-contact the lost plume. As a result, we could not detect any effect of tomato genotypes on "oriented flight" behavior of *T. absoluta* in the wind tunnel. Since flight attraction is mainly mediated by plant volatiles (Fig. 2) and that wind itself is not enough to stimulate *T. absoluta* females to initiate flight (E. Lima, personal observation), we hypothesize that possible differences in the odors from susceptible/ resistant genotypes were not sufficient to inhibit pre-flight excitation, antennation behavior, and take-off in *T. absoluta* females. The observation that moths do not fly upwind at all in the wind tunnel in the absence of plant odors has also been raised by Tasin et al. (2005) when testing *Lobesia botrana* females and by Bengtsson et al. (2001) when testing *Cydia pomonella*.

Tuta absoluta females and other nocturnal moths (e.g., another Gelechiidae, *Phthorimaea operculella*) do not initiate their search behavior before sunset (Fenemore 1988), suggesting that visual components play a less significant role than volatiles in host location by nocturnal moths. In line with this, we show that females responded best during the first hour of darkness (Fig. 1) and that attraction is mainly mediated by plant volatiles (Fig. 2). Since during oriented flight, they reached the susceptible plants faster than the resistant ones (≈20–30 cm/s; Fig. 4), such behavior seemed to be guided primarily by short distance cues. Because the moth's flight behaviors discussed above were not affected by genotypes, it seems that *T. absoluta* moths use short-range cues to decide whether or not to land and oviposit on their host. Importantly, both physical and chemical cues affect oviposition decisions (Pontes et al. 2010). Moth females are known to evaluate certain physical and chemical properties of the plant, before accepting it as a suitable host (e.g., appearance, color, shape: Renwick and Chew 1994; Rojas and Wyatt 1999, leaf texture: Honda 1995, leaf surface chemistry: Bernays and Chapman 1994). Our results, however, suggest that the decision of *T. absoluta* to land on susceptible plants was based mostly on volatiles. The fact that the time to land on a plant differed among genotypes seems to indicate that females made their decision before landing on a plant.

Female preference for Santa Clara variety is consistent with the preference–performance hypothesis that states that females will evolve to lay more eggs on host plants on which their offspring fare best (Gripenberg et al. 2010). Being highly susceptible to this insect, Santa Clara variety is indeed safe enough to feed *T. absoluta*' s offspring. This preference–performance correlation is expected to be particularly strong in cases where the insect is oligophagous and the larva is sessile, such those of the leaf miner studied here (Gripenberg et al. 2010). In such cases, the larvae are confined to a resource unit chosen by their mother and thus females such as *T. absoluta* are expected to find a place of good quality for their offspring. It is thus surprising that the accession HGB-489 has been only partially susceptible to *T. absoluta* females. Some reports have in fact shown cases were the female's oviposition preference did not correlate with larval performance (Craig et al. 2000; Fritz et al. 2000; Cronin et al. 2001). What is striking in our case is the fact that, according to Castro Antônio (2006), Santa Clara variety and HGB-489 do not differ in their effect on the weight and survival rates of larvae and pupae of *T. absoluta*. Therefore, based on the preference–performance hypothesis, we did not expect to find differences between those plant genotypes either for landing or oviposition of this moth. It is tempting to claim that such differences could arise from environmental conditions on which the plant was grown (Cronin et al. 2001), plant growth rate (Craig et al. 2000), and time limitation for oviposition (Doak et al. 2006) but no data are available to sustain such a claim. However, because we found resistance for the other three accessions and susceptibility for Santa Clara variety, this possibility is remote. It seems more reasonable to hypothesize that on the accession HGB-489, the strength of selection appears still to be weak among female preference and larvae performance. It should not come as a surprise that plant genotypes that have been in existence only for decades as the result of plant breeding are not necessarily recognized by an insect as a potential host plant.

Because the *T. absoluta* larvae used here were reared on Santa Clara variety, one might argue that female preference for this variety could be a result of learning. However, it was recently demonstrated that *T. absoluta* females reared on susceptible (including Santa Clara variety) or resistant genotypes did not show a preference for these genotypes after one, two, or even fifteen generations, indicating that learning did not occur (Curtinhas 2011). Proffit et al. (2011) also indirectly showed that *T. absoluta* does not seem to learn. They reared this insect on tomato plants from the cultivar Aromata and still the females landed and laid more eggs on Santa Clara plants than on Aromata ones. Additionally, adults assayed here were kept in a separate room to prevent contact with plant odors. Hence, it seems unlikely that the female preference was a result of learning and that the larvae feeding affected the results of our experiments. There is evidence showing that experience-based modulation of preference is a complex process mediated by a combination of factors such as mating experience, plant odors, and sex pheromones (Thöming et al. 2013; Proffit et al. 2015). Furthermore, female preference as a consequence of learning was also not found for other lepidopterans, such as

Euphydryas editha (Parmesan et al. 1995), *Papilio polyxenes* (Heinz and Feeny 2005), and *Heliconius erato* (Kerpel and Moreira 2005).

Recently, the importance of electrophysiological techniques for the elucidation of the role of semiochemicals in insect behavior has been emphasized, particularly for insects in which chemoreceptors are accessible (Bruce et al. 2005). With this in mind, it would be interesting to use behavioral and electrophysiological techniques to confirm the importance of volatile compounds in female attraction or repellency. Some studies exploring the compounds potentially involved in tomato plant resistance to *T. absoluta* have been developed. However, most of these studies were performed using leaf extracts (Ecole et al. 1999; Suinaga et al. 1999; Oliveira et al. 2009) and to the best of our knowledge, only Proffit et al. (2011) evaluated the tomato leaf volatiles of the Santa Clara variety and their potential role in plant resistance against *T. absoluta*. For the other genotypes reported here (HGB-489, HGB-674, HGB-1708, and HGB-1497), the volatile compounds considered to be important in tomato leaf aroma (Andersson et al. 1980; Buttery et al. 1987; Smith et al. 1996) and their role in plant resistance were yet not reported. Finally, we suggest the use of the Accessions HGB-1497 and HGB-674 for future studies, and we suggest that these accessions have a promising role as tools in host plant resistance for integrated pest management.

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