# Resistance mechanism of tomato against *Tuta absoluta;* the role of trichomes in the resistance

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# Resistance mechanism of tomato against *Tuta absoluta*; the role of trichomes in the resistance

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## Abstract

*Tuta absoluta* is a devastating pest of tomato. The use of host plant resistance to control pests is an interesting, potentially useful technique, but there is no known cultivated tomato resistant to *T. absoluta.* The objective of this resistance was to screen wild tomato accession and identify resistance mechanism against *T. absoluta*. Sixteen wild tomato accessions from six species were tested in two experiments. The tested parameters were damaged leaf area, oviposition rate, emerged larvae, adult and larvae survival, larvae and adult preference. Those parameters were correlated with the density of each trichome type and with available LC-MS data. From no-choice test parameters accession LA1777, LA1718 and LA716 were the most resistant and LA1401 and LA1139 were the most susceptible, and all *S. lycopersicum* accessions were susceptible. From choice test, the accessions G1.1561, LA1718, LA716, LA1645, LA0483 and LA1408 were not preferred by the larvae, and the accessions LA1777 and G1.1561 were the least preferred by the adults. The accession LA1777 and LA716 were out of the most resistance. The resistance of this genotype was related to the presence of trichome Type I and IV.

Key words:

*Tuta absoluta,* tomato, LA1777, LA716, glandular trichomes, LC-MS.

# 1. Introduction

## 1.1. Classification and distribution of *Tuta absoluta*

*Tuta absoluta* was described in 1917 by Meyrick as *Phthorimaea absoluta* from specimens collected in Peru, and it classified under family Gelechiidae, order Lepidoptera and phylum Arthropoda (Muniappan, 2010). After its first description, this insect has spread with the tomato producing areas of South America become commonly known as a tomato leaf miner (Vargas, 1970). In the year 2006, it was detected for the first time in Europe, Spain. Since then, it has spread rapidly to all Mediterranean countries, North Europe, Middle East and Africa (Figure 1-1) (USDA–APHIS, 2011; Cocco et al., 2012).

Figure 1-1: Distribution of Tuta absoluta (Russell IPM Ltd, 2010).

## 1.2. Host

Tomato is the main host of *Tuta absoluta.* In tomato plants, the female adults lay eggs on all above ground part of the plant (leaves, shoots and flowers as well on the fruits) (Vargas, 1970). Despite the clear preference of this insect on tomato species, it also affects common bean, potato, eggplant and tobacco (EPPO, 2009; Cocco et al., 2012). It also has been using weeds as an alternative host such as; *Lycium chilense*, *Solanum nigrum* and *Datura stramonium*; *Datura ferox* and *Nicotiana glauca* (Estay, 2000; EPPO, 2005).

## 1.3. Biology of *Tuta absoluta*

*Tuta absoluta* is a complete metamorphism insect (Holometabolism) (Figure 1-2). It can produce 10 to 12 generations per year, and the average duration of its life cycle is 28.7 days at a constant temperature of 25$^{o}$C and 75% relative humidity (Garzia et al., 2012). In general, males and virgin females live longer than mated females, and the sex ratio is about 1.33 females per male (USDA–APHIS, 2011). A female adult can live up to 42 days and is capable of lay up to 260 eggs during its life (Garzia et al., 2012). Females can lay 72.3% of their eggs within the first 5 days of life and the 90% within the first 10 days after settlement (USDA–APHIS, 2011).

The eggs are laid one by one (rarely in batches) on all aboveground parts of the host plant, and it hatches within 4-6 days. Larvae I (L1) hatches from the eggs and enter the plant tissue to start feeding. The larvae growth, from larvae instar I to IV, takes around 11-15 days and mature larvae purge themselves of food and build a silken cocoon where the larva transforms into pupa. Then depending upon the conditions, pupa development will take around 5-8 days to grow up as adults (USDA–APHIS, 2011).

Figure 1-2: Life cycle of Tuta absoluta modified from Muniappan, 2010.

### 1.4. Importance and botany of tomato

Tomato (Solanum lycopersicum L.) is one of the most important food crops in the world in terms of human consumption, while total global production exceeds 3.7 million hectares worldwide with an average anual production more than 126 million metric tons (FAO, 2009). Tomatoes are consumed and used as a salad, paste, peeled tomatoes, diced products, and various forms of juice, sauces, and soups that is also a significant source of vitamin A and C as well as source of lycopene, a carotenoid pigment with antioxidant properties (Guimaraes et al., 2007).

The tomato systematics of genus describtion were under debate and reorganized several times (Darwin et al., 2003). Nowadays, tomatoes are recognised as nested inside the genus *Solanum* (Peralta et al., 2008). Together with the cultivated tomato (*S. lycopersicum*), there are 17 recognised wild species: *S. cheesmaniae, S. galapagense, S. chilense, S. chmielewskii, S. lycopersicum, S. habrochaites, S. neorickii, S. pennellii, S. arcanum, S. corneliomulleri, S. huaylasense, S. peruvianum, S. pimpinellifolium, S. juglandifolium, S. lycopersicoides, S. ochranthum, S. sitiens* (Peralta and Spooner, 2005).

Tomatoes are native to Western South America and the natural distribution goes from central Ecuador, through Peru to northern Chile and in the Galapagos Islands. Tomatoes were introduced to Europe from South America and become known to botanists in the 16th century (Peralta and Spooner, 2005; Desneux et al., 2010). All tomato species are diploid (2n=24) and their exhibits great difference in morphological characters such as matting system, habitat preference, trichome densities and types, resistant to pest and diseases and other agronomic traits important for breeding (Desneux et al., 2010).

Wild relatives of tomato have been used as sources of insect resistant (Oliveira et al., 2009). Insect resistance is generally associated with the presence of trichome types and densities (Tissier, 2012). Trichomes are specialized structures on the epidermis (glass et al., 2012) and based on the presence/absence of a glandular head trichomes can be classified as glandular (type I, IV, VI & VII) or non-glandular trichomes (types II, III, V). Glandular trichomes are the sites of synthesis and storage of secondary metabolites (Schilmiller et al., 2010; McDowell et al., 2011).

## 1.5. Statement of problem

Tomato leaf minor is one of the major devastating pests of processing and fresh tomatoes, both in greenhouse and open field (Cocco et al., 2012). *Tuta absoluta* larvae can absolutely destroy the tomato canopy by excavating the leaves, stems and buds; and burrows into fruits causing the quality decline of fresh tomato and yield loss that range from 50% to 100% (Cocco et al., 2012; USDA–APHIS, 2011). Because of the biology and behaviour of *T. absoluta* it is challenging to control it. Chemical control methods have been trusted to control this insect, but the feeding habits of the larvae, the increasing number of resistant strains of this pest, together with the negative impact of the chemical into the environment makes the chemical control method not sustainable (Moreno, 2011; Deliperi and Delrio, 2012). In addition, mating disruption methods (like sex pheromones) have been used to control *T. absoluta*, but this technique is a lot more expensive than pesticide applications (Cocco et al., 2012). The biological control methods are still under development for further investigation to find it in an operative way (Russell IPM Ltd, 2010).

Because of stated above, exploring wild accession of tomato for new sources of resistance is needed. To our knowledge, there is no longer cultivated variety resistant to *T. absoluta* (Oliveira et al., 2009). Resistant to several herbivore insects have been described in wild relatives of the tomato. Resistant to *Bemisia tabaci* and *Trialeurodes vaporariorum* was described in *S. galapagense.* Insect resistance in wild relatives of tomato was associated with the presence of glandular trichomes and secondary metabolites such as acyl sugars and methyl ketones (Kang et al., 2009; Schilmiller et al., 2010; McDowell et al., 2011).

### 1.6. Objectives and research questions

This study screened resistant accessions from 16 genotypes of tomato against *Tuta absolute* and identified the mechanism involved in the resistance. We had two main research questions:

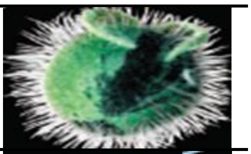
- What are the most resistance accessions of tomato against *Tuta absoluta*?
- What are the resistance mechanisms?

## 2. Materials and Methods

### 2.1. Plant Materials

Seeds of sixteen tomato accessions were ordered from the Tomato Genetics Resource Centre (TGRC) as its name listed (Table 1). To carry out the work, more than 10 seeds of each of the 16 accession were sown on January 25, 2013 and allowed to grow for three weeks in a polyethylene pot with soil. To break seed dormancy, the tomato seeds were treated with 1% sodium hypochlorite for 30 minutes, washed and sown immediately. Then, five seedlings from each of the grown accession were transplanted individually to polyethylene pot and allowed for further growth of five weeks. The seedlings were grown in a greenhouse (Unifarm, Wageningen UR) for a period of two months at $23^0$C and 77% relative humidity. After this period, plants moved into the insect proof greenhouse under $25^0$C and 75% relative humidity, where the phenotype experiments were performed for five months, until the end of the experiment. Sixteen accessions were arranged in a randomized complete design with five replications. Afterwards, choice and no-choice experiments were performed.

Table 1:accessions and species used in this study

| Accession name | *Species name* | |
|---|---|---|
| LA0521<br>LA1139 | *S. cheesmaniae* |  |
| LA1401<br>LA0483<br>LA1408 | *S. galapagense* |  |
| G1.1561<br>LA1718<br>LA1777 | *S. habrochaites* |  |
| FCN13-1-6-1<br>Cv. Uco plata INTA<br>FCN93-6-2<br>Cv. Moneymaker | *S. lycopersicum* |  |
| LA716 | *S. pennellii* |  |
| LA1645<br>LA1580<br>LA1584 | *S. pimpinellifolium* |  |

## 2.2. *Tuta absoluta* rearing

*Tuta absoluta* was reared in a climate room (Nergena, Wageningen UR) at 25$^0$C, RH 75% and 16-8h day-night. It was synchronized two times a week and maintained by feeding the clean tomato plant, S*. lycopersicum* cv. Moneymaker. To get each stage of *T. absoluta*, four insect cage boxes were prepared, which contained adults, larvae, pupas and the mixture of all, the left-side box (Figure 2-1). Adults were laying eggs in the larger box on the right side. The plant which contained eggs from larger box moves to the next box to obtain larvae instar I (one) and on the next time of synchronization, the plant that contained the larvae go to the third box to get larvae instars II-IV, as well pupa had been present. New adults have been found from the last box (left side) which can also contain other stages, and those adults trapped and released back to the larger box with new plant by using an insect trapper jar. To maintain eggs and available feed for larvae growth, new plants have been placed in the first three right-side box.

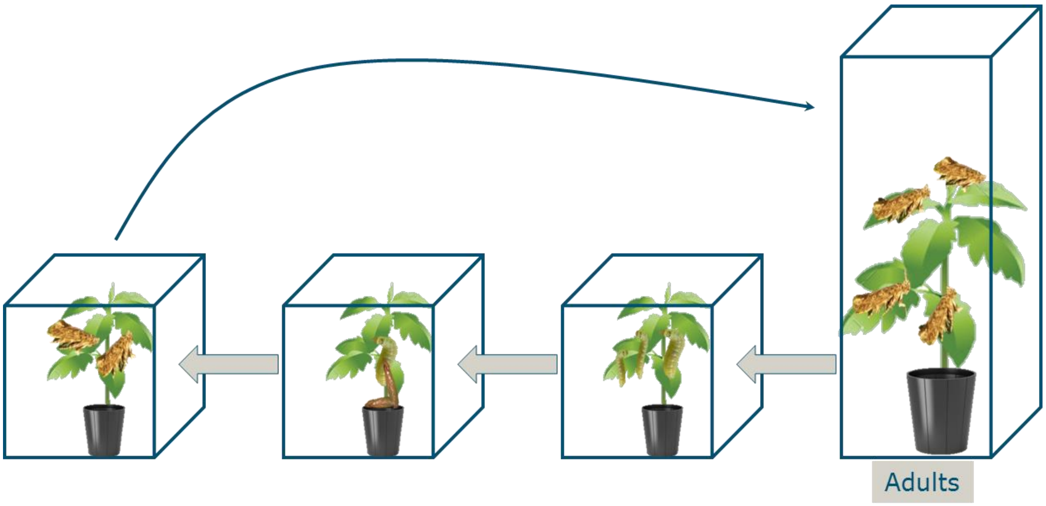


Figure 2-1: Tuta absoluta Synchronization.

## 2.3. Experiments

### 2.3.1. No-choice experiments

### 2.3.1.1. Clip-on cage test

Three months after sowing, a clip-on experiment was performed. Which contained one insect per clip-on cage (2.5cm diameter and 1. 0cm height); one clip-on per plant and five plants per accession were prepared for this test (Figure 2-2). The cages were placed on the first to third fully expanded leaf from the top of the plant. Three parameters were considered; adult survival (AS), oviposition rate (OR) and a ratio of emerged larvae from eggs (EL); following the formula described (Bas et al., 1992).

*Adult survival* (AS): one non-sexed *T. absoluta* adult was placed into a clip-on cage. The clip-on cage was placed on the second to third fully expanded leaf on the adaxial part of the leaflet. The cage was left in a period to seven days, and the survival of the insects was counted daily. Because in the seven days, there were not adults alive and then AS was calculated out only at the first two days, after inoculation.

*Oviposition rate* (OR): *T. absolute* adults were anaesthetized using $CO_2$ and one female were placed per clip-on cage. One clip-on cage per plant and five plants per accession were used. Seven days after infestation, the number of death, live *T. absolute* and laid eggs were recorded on the last day.

Emerged larvae (EL)*:* five days after counting eggs, the number of larvae per cage was counted and the ratio larva/eggs were calculated.

After all, one-way ANOVA was analysed for each parameter followed by the least significant difference.

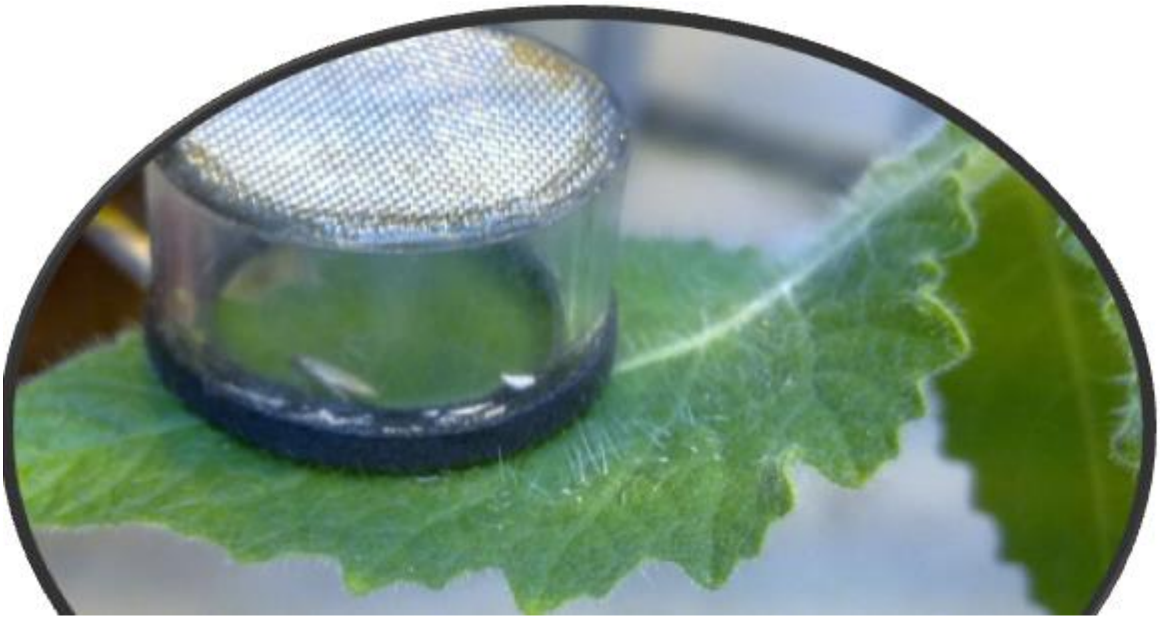


Figure 2-2: Clip-on cage in no-choice experiment.

#### 2.3.1.2. Detached leaflet test

Five leaflets from the upper first and second leaf branches from each of the 16 accession were detached. Individual leaflets were put per petri-dish, which were prepared by dropping a little of agar water (1.5%) at the one end (Figure 2-3). Then two larvae per petri-dish were applied. The larvae were starved for a period of two hours before inoculation. One leaflet per petri-dish and five petri-dishes per accession were used. All 85 petri-dishes were wrapped with para film and set in the growing conditions. In this experiment, we have considered two variables, damaged leaf area and larvae survival.

*Damaged leaf area***:** the larvae were left to feed over a period of 24 hours. After that period, the cages were moved to a dark area for 15 minutes, and pictures were taken to measure damaged leaf area through chlorophyll fluorescence. The pictures were performed using mobile camera version 3 with an exposure of 800 microseconds and an LED pulse 2.5 (DC17). Scoring was performed using the CFII analysis software single pulse option. First, the data were retrieved in the software, then the projected leaf area was determined by a mask, thirdly the photosynthetic activity was calculated at each pixel after a 3x3 smoothing, and finally, the affected area was determined that marked and token out in blue colour (Figure 2-4). Then the damaged leaf area of the infected pixels transformed to $\text{Log}_2$ (x + 1) and general analysis of variance done using the total leaf area (mask pixel) as a covariate (Table 5, appendix).

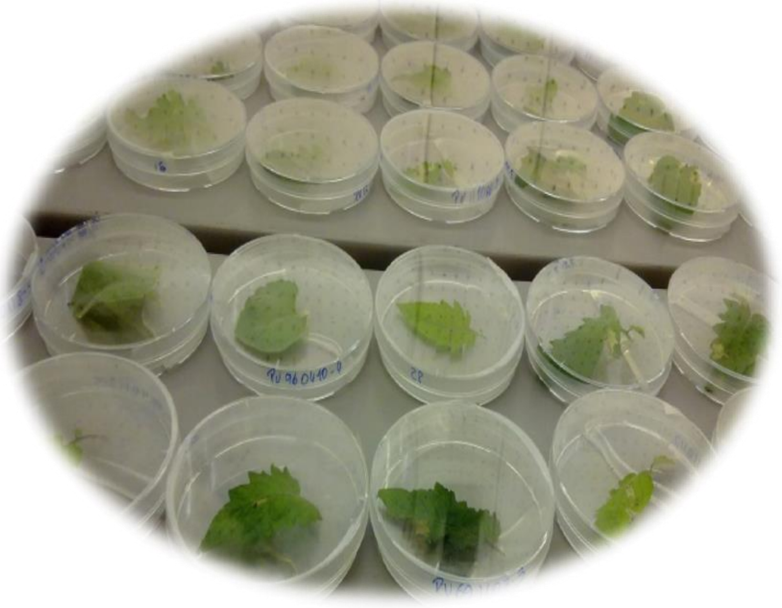


Figure 2-3: Detached leaf experiment set-up.

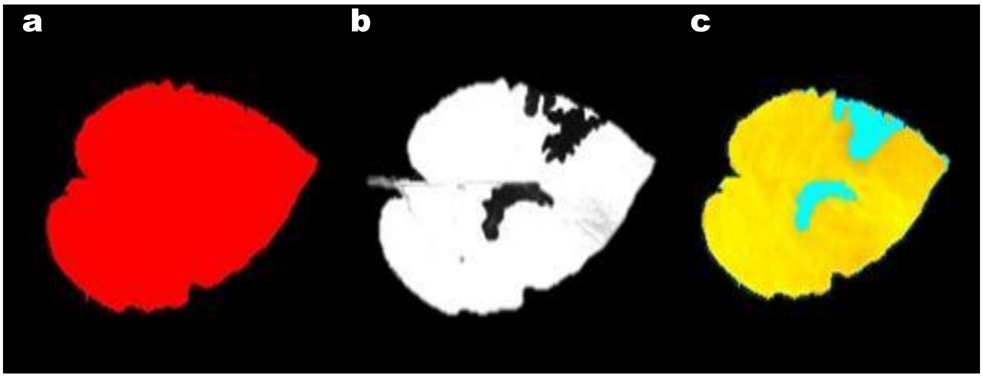


Figure 2-4: Calculation of the damage leaf area. a) Projected leaf area determined by a mask; b) photosynthetic activity calculated for each pixel after a 3x3 smoothing and c) in the affected area was determined and marked in blue.

*Larvae survival:* forty-eight hours after inoculation, survived larvae were counted. The method of calculating larva survival was as proposed (Bas et al., 1992) and data were analysed in one-way ANOVA.

### 2.3.2. Choice experiment

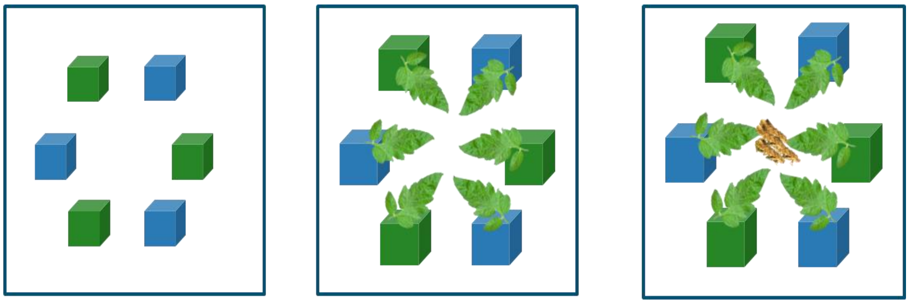
#### 2.3.2.1. Detached leaflet test

In choice test, each tomato accession was compared to the reference cultivar Moneymaker. This experiment was done to examine two parameters, larvae preference and adult settlement preference.

*Larvae preference:* One leaflet from the testing accession and one leaflet from the cv. Moneymakers were interleaved on a rectangular petri-dish, which was prepared with little agar water (1.5%) in two opposite corners (Figure 2-5). Afterwards one larva was put in the middle of the petri-dish and five petri-dishes per accession were used. Data was collected 24 hours after inoculation.The proportion of larvae between the two categories was calculated. Data were analysed by binomial distribution test. The larvae that did not make a choice or have escaped were excluded from the analysis.

Figure 2-5: Larvae choice test in petri-dish.

*Adult settlement preference:* In this choice test, three leaves from each of the 15 accession compared to three leaflets of the cv. Moneymaker (six leaves per tray) (Figure 2-6). Leaflets were put in wet Oasis floral foam, on a tray (5 cm height and 35 cm width) and arranged in a circle. Eighteen *T. absoluta* adults were placed in the centre and repeated two times in the 45-minute interval. The proportion of insects that made a choice was calculated, and data were analysed by a binomial distribution test. Insects that did not make a choice were not included into the analysis.



Randomly labelling the Oasis foam

Inserting the leaflets

Setting insects in the middle

Figure 2-6: Set up of the *T. absoluta* settlement preference test.

### 2.3.3. Trichome morphology

The type and density of trichomes were analysed per accession. Trichomes were counted by using a binocular microscope on adaxial and abaxial leaflets of each of the 16 accessions. Ten sample leaflets were detached from the second to third fully expanded leaves of each accession. Based on the identified morphology of trichome types (Channarappa et al., 1992), the densities of each trichome type were recorded from an area of 0.785 $mm^2$ on the abaxial and adaxial sampled leaflets of each accession. The density of each trichome types was analysed by one-way ANOVA. Finally, densities of each trichome type were correlated to the choice and no-choice test parameters.

### 2.3.4. Correlation of acyl-sugar with density of trichome type, choice and no-choice test

The metabolite data of Liquid chromatography–mass spectrometry (LC-MS) inquiries were received from Dr. Ben Vosman (Plant Breeding International), and the method used for LC-MS analysis was to find acyl sugars as discussed in Lucatti et al. (2013). Eight accessions were used for this correlation analysis. Those were *S. lycopersicum* accessions (FCN 13-1-6-1, FCN 93-6-2, cv. Uco Plata INTA and cv. Moneymaker), LA1584 (*S. pimpinellifolium)*, LA716 (*S. pennellii*), LA1401 (*S. galapagense*) and LA1777 (*S. habrochaites*) (Table 7, appendix). The relative concentration of metabolites was transformed to $\text{Log}_2$ (x +1), and auto scaled to $\frac{\text{Xi} - \bar{\text{X}}}{\text{SD}}$ ($\bar{\text{X}}$ and SD are the means and standard deviations of a memetabolite across accessions, respectively and Xi is the amount of a metabolite of a given accession). Auto scaled data were correlated with the transformed data of choice, no-choice tests and trichome density.

### 2.4. Data analysis

All statistical analysis was performed using GenStat 15th edition (Service Pack 1) (VSN International Ltd, 2013) and the method of analysis was as described on each test. Normality of variance was checked before analysis, and the mean was compared using a Fisher LSD test and the Pearson correlation was used for correlated parameters.

## 3. Result

### 3.1. No-choice experiments

### 3.1.1. Resistance to larvae feeding

Significant differences were found among accessions in a damaged leaf area (F=4. 28; df=15, 64; $p<0.01$) (Figure 3-1). The accession LA1777 (*S. habrochaites*) was the least affected (0.24 ± 0.19mm$^2$) (Table 4, appendix). The highest value of damage was observed for the accession LA1401 of *S. galapagense* (17.94 ± 7.34 mm$^2$) (Table 4, appendix).

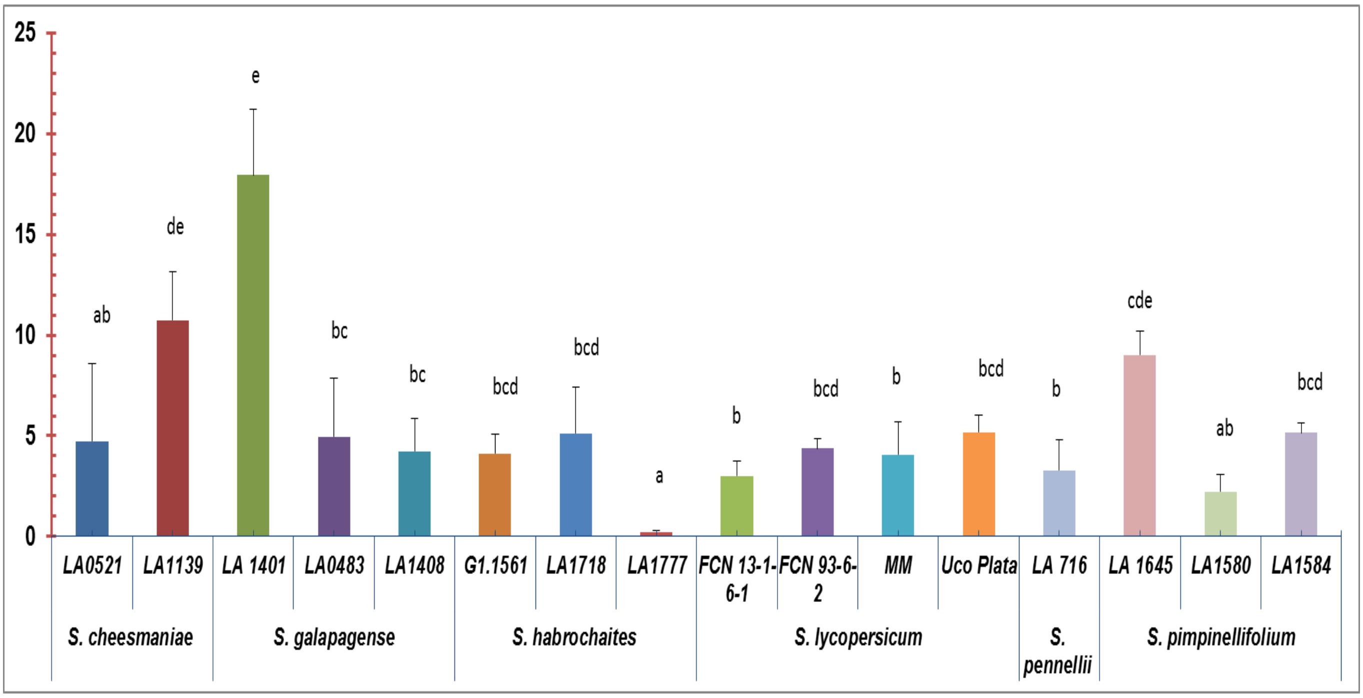


Figure 3-1: Damaged leaf area on tomato accessions caused by larvae of *T. absoluta* (mean ± SE). Different letters indicate significant differences at LSD, $p<0.05$.

### 3.1.2. Survival test

To examine the survival ability of the insect two parameters, larvae and adult survival were tested. Significant difference was detected in larvae survival among tomato accession (F=4. 12; df=15, 64, p<0.01) (Figure 3-2). Accessions LA716 (*S. pennellii*), LA1718 and LA1777 (*S. habrochaites*) has the lowest number of surviving larvae (0.28 ± 0.39, 0.42 ± 0.39 and 0.14 ± 0.32, respectively) (Table 4, appendix). No statistically significant differences were observed in the adult survival test (F=0. 68; df=15, 64; p = 0.79).

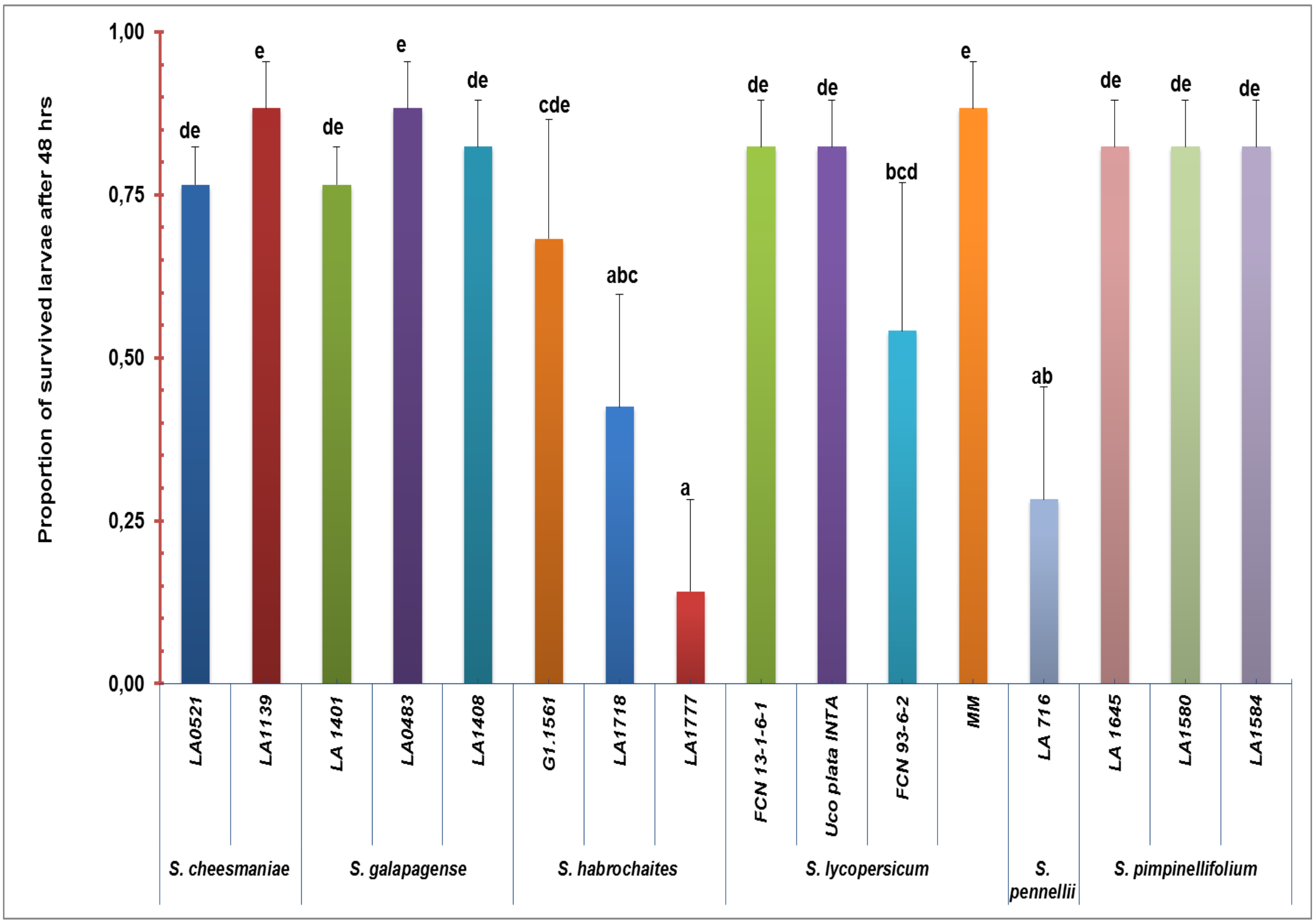


Figure 3-2: Proportion of larvae survival on tomato species (mean ± SE). Different letters indicate significant differences at LSD, p<0.05.

### 3.1.3. Oviposition rate and emerged larvae

The oviposition rate of *T. absolute* were significantly different among accessions (F=2. 57; df=15, 64; p < 0.01) (Figure 3-3). The lowest oviposition rate was found in accessions LA1718 (0.06 ± 0.13) and LA1777 (0.23 ± 0.08) and in the accession LA716 (0.26 ± 0.21). On the other hand, accession LA1139 of *S. cheesmaniae* (3.6 ± 2.45) and all *S. lycopersicum* accessions had a high number of eggs per female per day (8.26 ± 8.99 average in total) (Table 4, appendix).

There were no statistically significant differences among tomato accessions for emerging larvae (F=0. 87; df=15, 64; p = 0.60).

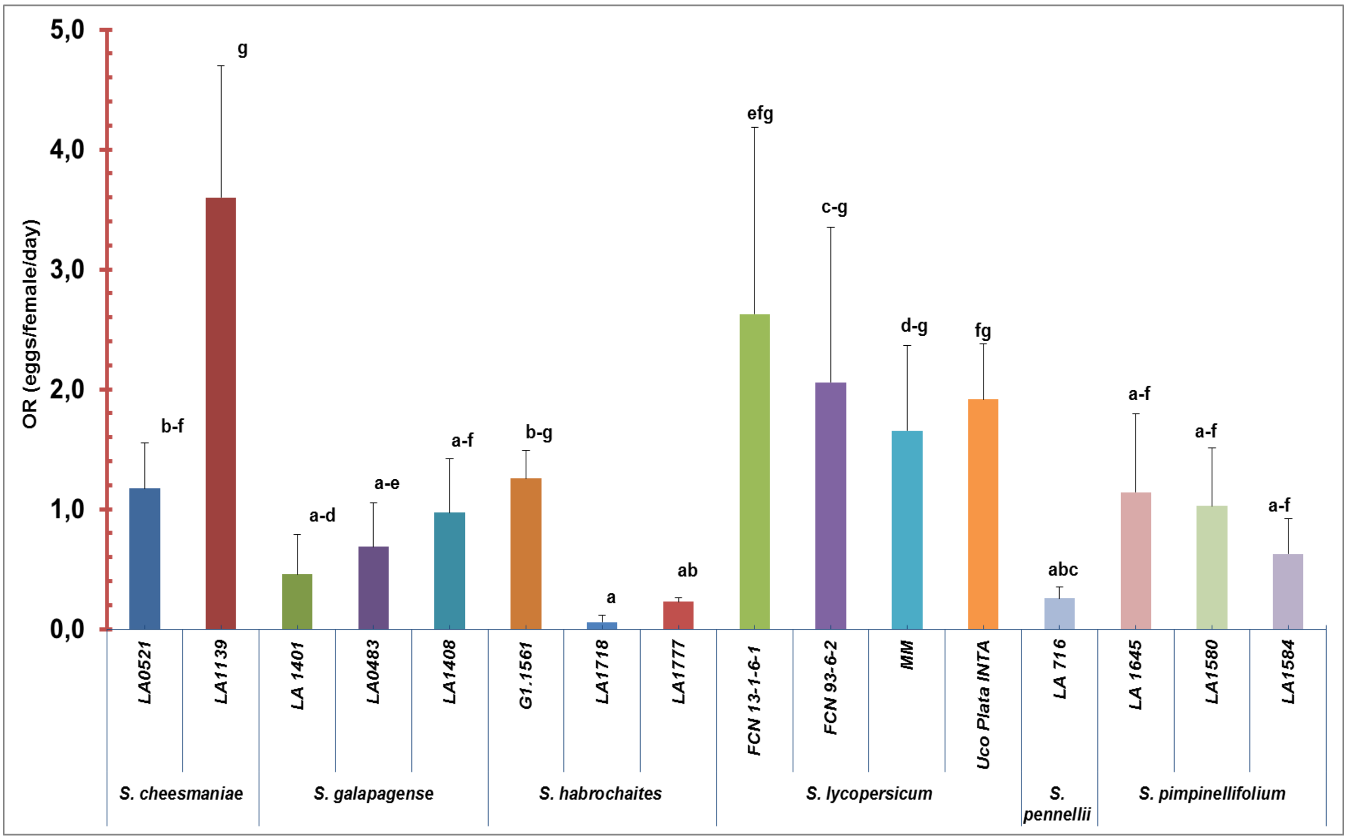


Figure 3-3: Oviposition rate of *T. absoluta* on tomato. Different letters indicate significant difference at LSD, P< 0.05.

### 3.2. Choice experiments

#### 3.2.1. Larvae preference

In this experiment we tested each accession to the reference cultivar Moneymaker (Figure 3-4) and significant difference of binomial test detected (Table 6, appendix). We saw that the larvae did not choose the accession LA 1645 (*S. pimpinellifolium*), LA716 (*S. pennellii*), LA0483 and LA1408 (*S. galapagense*), and accession G1.1561 and LA1718 (*S. habrochaites* when compared to cv. Moneymaker.

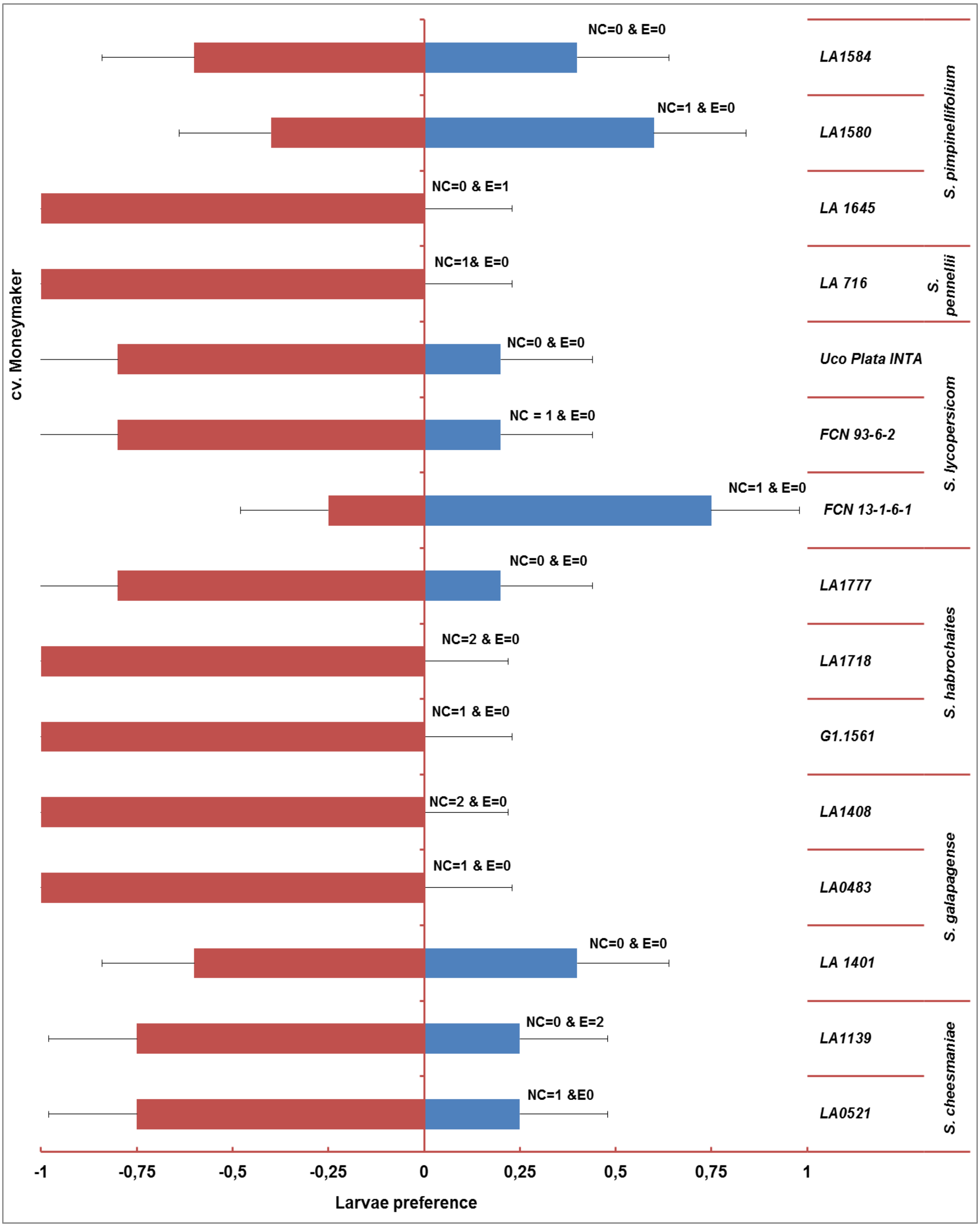


Figure 3-4: proportion of larvae feeding preference in 15 tomato accessions, each compared with cv. Moneymaker (MM). The number of non-choice insects (NC) and the number of larvae that escape (E) were not included in the analysis.

### 3.2.2. Adult settlement preference

Though most of the adults did not make a choice, we observed statistically significant difference from the binomial test (Table 6, appendix). Most of the adults set on other than accessions, on the experimental tray. Adult preference of each wild tomato accession was compared with the reference CV. Moneymaker *Tuta absoluta* did not settle on accession LA1777 and G1.1561 of (*S. habrochaites*) (Figure 3-5).

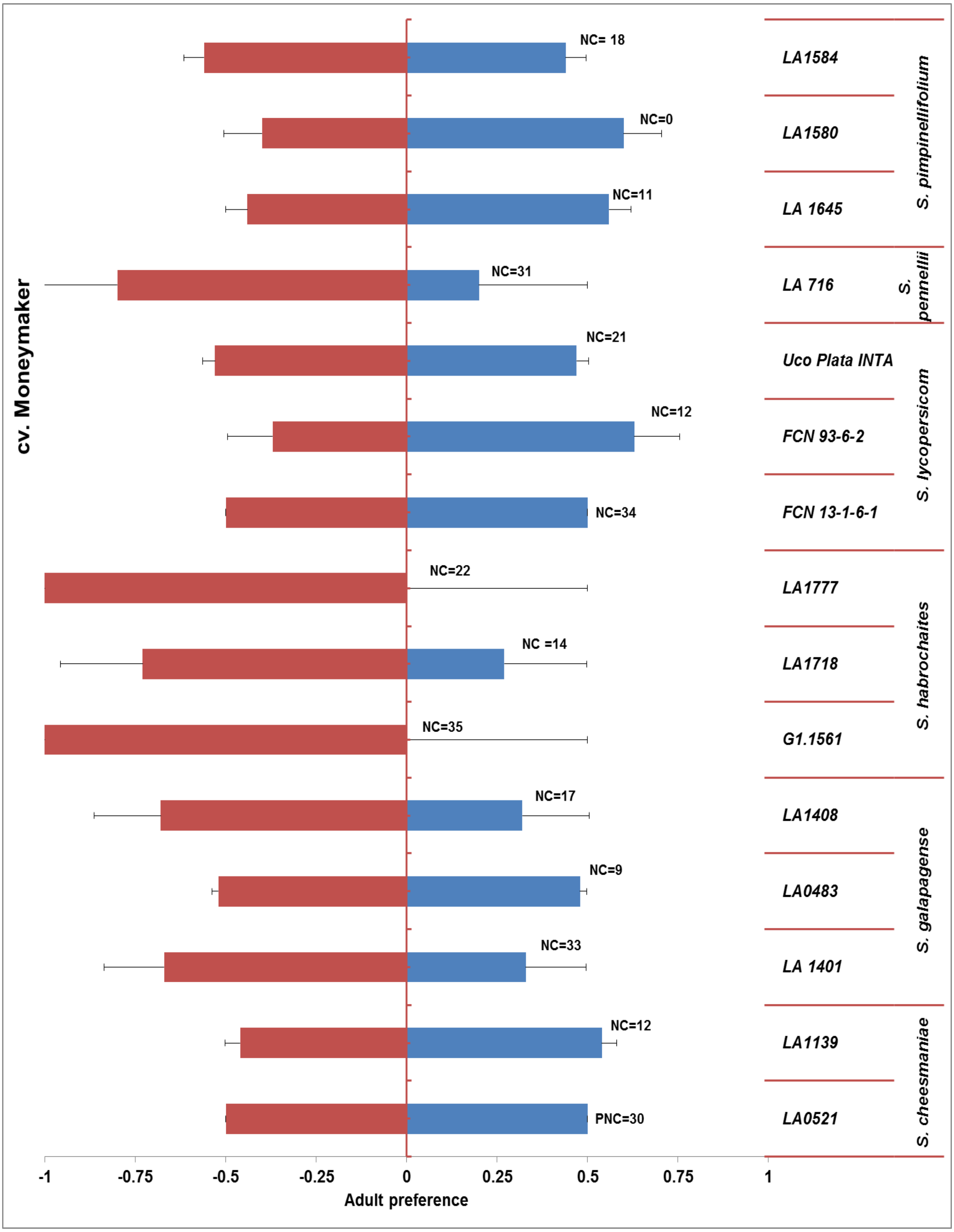


Figure 3-5: Proportion of *T. absoluta* settlement preference in 15 tomato accessions compared to cv. Moneymaker (MM). The number of non-choice insects (NC) excluded from the analysis.

### 3.3. Correlation of trichomes with choice and no-choice tests

Significant differences were detected among accessions in density of each trichome type at 0.785mm2 (ANOVA, P<0.01 (Table 2). From the glandular trichomes, Type IV was absent in all accessions of *S. lycopersicum* and *S. cheesmaniae*, and from LA1645 of *S. pimpinellifolium*. Trichome Type I was absent in accessions LA1139 of *S. cheesmaniae*, LA1645 of *S. pimpinellifolium* and in all of *S. lycopersicum* accession except cv. Uco plata INTA. Trichome Type VI presented in all the studied accessions.

On the other hand, from the non-glandular trichomes; Type III is not present in all of *S. cheesmaniae* accessions, in accession LA1645 of *S. pimpinellifolium* and in all *S. galapagense* accession except LA 1401. Trichome Type V was absent in LA 716 (*S. pennellii*), LA1408 (*S. galapagense*) and from all accessions of *S. habrochaites* except G1.1561.

Table 2: Mean number (± SD) of trichome types on leaflets of wild and cultivated cv. Moneymaker in 0.785 mm$^2$ area. Different letters indicates significant differences at LSD, $p<0.01$

| Species Name | Mean ± SD | | | | | |
|---|---|---|---|---|---|---|
| | Accession Name | Type I | Type III | Type IV | Type V | Type VI |
| *S. cheesmaniae* | LA0521 | 0.1 ± 0.32$^{ab}$ | 0 ± 0.00$^{a}$ | 0 ± 0.00$^{a}$ | 7.5 ± 2.32$^{c}$ | 10.5±5.32$^{cdef}$ |
| | LA1139 | 0 ± 0.00$^{a}$ | 0 ± 0.00$^{a}$ | 0 ± 0.00$^{a}$ | 32.1 ± 5.59$^{e}$ | 2.1 ± 1.20$^{a}$ |
| *S. galapagense* | LA 1401 | 0.6 ± 1.07$^{abc}$ | 0.6 ± 1.35$^{abcd}$ | 18.2 ± 4.49$^{d}$ | 10.5 ± 3.92$^{c}$ | 10.4 ± 4.74$^{cdef}$ |
| | LA0483 | 0.6 ± 1.07$^{abc}$ | 0 ± 0.00$^{a}$ | 10.9 ± 2.85$^{c}$ | 4.7 ± 1.89$^{b}$ | 7.6 ± 2.12$^{cd}$ |
| | LA1408 | 0.6 ± 1.35$^{abc}$ | 0 ± 0.00$^{a}$ | 27.1 ± 4.31$^{e}$ | 0 ± 0.00$^{a}$ | 3.6 ± 1.90$^{b}$ |
| *S. habrochaites* | G1.1561 | 1 ± 1.41$^{c}$ | 1.6 ± 1.35$^{e}$ | 16 ± 3.92$^{d}$ | 0.6 ± 1.58$^{a}$ | 6.6 ± 1.84$^{c}$ |
| | LA1718 | 0.5 ± 0.71$^{abc}$ | 0.9 ± 1.10$^{cde}$ | 25.7 ± 7.04$^{e}$ | 0 ± 0.00$^{a}$ | 8.7 ± 3.37$^{cde}$ |
| | LA1777 | 3.7 ± 1.34$^{d}$ | 1.5 ± 1.58$^{e}$ | 19.7 ± 4.55$^{d}$ | 0 ± 0.00$^{a}$ | 9.4 ± 2.63$^{cdef}$ |
| *S. lycopersicum* | FCN13-1-6-1 | 0 ± 00$^{a}$ | 0.1 ± 0.32$^{e}$ | 0 ± 0.00$^{a}$ | 20.2 ± 4.49$^{d}$ | 12.8 ± 3.08$^{f}$ |
| | FCN93-6-2 | 0 ± 0.00$^{a}$ | 1.6 ± 1.26$^{e}$ | 0 ± 0.00$^{a}$ | 21.2 ± 4.26$^{d}$ | 4.4 ± 2.01$^{b}$ |
| | MM | 0 ± 0.00$^{a}$ | 1 ± 1.70$^{bcde}$ | 0 ± 0.00$^{a}$ | 32.9 ± 6.12$^{e}$ | 10.4 ± 2.80$^{def}$ |
| | Uco Plata INTA | 0.6 ± 0.70$^{abc}$ | 0.8 ± 0.92$^{cde}$ | 0 ± 0.00$^{a}$ | 19.2 ± 5.51$^{d}$ | 9.4 ± 2.37$^{cdef}$ |
| *S. pennellii* | LA 716 | 0.3 ± 0.67$^{abc}$ | 0.2 ± 0.42$^{abc}$ | 18.6 ± 3.44$^{d}$ | 0 ± 0.00$^{a}$ | 4.4 ± 1.43$^{b}$ |
| *S. pimpinellifolium* | LA 1645 | 0 ± 0.00$^{a}$ | 0 ± 0.00$^{a}$ | 0 ± 0.00$^{a}$ | 40.5 ± 4.12$^{e}$ | 3.1 ± 1.29$^{ab}$ |
| | LA1580 | 0.2 ± 0.42$^{ab}$ | 1.1 ± 1.45$^{de}$ | 2.8 ± 2.90$^{b}$ | 17.6 ± 8.18$^{d}$ | 22.4 ± 9.29$^{g}$ |
| | LA1584 | 0.7 ± 0.95$^{bc}$ | 1 ± 0.94$^{de}$ | 16.7 ± 2.83$^{d}$ | 9.7 ± 6.17$^{c}$ | 11.3 ± 3.30$^{ef}$ |

The significant pearson correlation was detected between larvae survival and Type I trichomes (r = -0.61), and oviposition rate with Type IV and V (r = -0.72, and +0.66, respectively) (Table 3). No significant correlation was observed in the damaged leaf area, adult survival, emerged larvae, larvae and adult preference.

Table 3: Correlation of oviposition rate (OR) and larvae survival (LS) with trichome types at Pearson correlation coefficient (r), p<0.05

|  | Parameters of no-choice test |  |
|---|---|---|
| Trichome Type | OR | LS |
| Type_I | -0,44 | -0,61* |
| Type_III | -0,18 | -0,33 |
| Type_IV | -0,72* | -0,48 |
| Type_V | 0,66* | 0,43 |
| Type_VI | -0,17 | 0,05 |

* : indicates parameters which have a significant difference at the 5 % significance level of Pearson correlation

- No significant Pearson correlation detected in the correlation analysis of Liquid chromatography-mass spectrometry (LC-MS) metabolites with trichome density, no-choice and choice tests (Table 9, appendix).

# 4. Discussion

## 4.1. Resistance accessions to *Tuta absoluta*

The results of non-significant differences among tomato accessions such as adult survival, emerged larvae, and non-significant correlated parameters did not present in a graph or a table. To develop germplasms which could be a multi-factor and stable resistance, understanding the different plant defence mechanisms against herbivory damage are important. In the persude of this aim, the use of an accurate high-throughput phenotyping methods (i.e. Image analysis) couple to omics analysis (RNAseg, SNPs, LC-MS) are an ideal approach (Sobreira et al., 2009).

Our results show that the accessions LA1777 (*S. habrochaites*) and LA716 (*S. pennellii*) were overall the most resistant. Moreover, we have observed very small amount of larvae survival and oviposition rate in accession LA716 (*S. pennellii*), LA1777 and LA1718 (*S. habrochaites*) showing that those accessions were able to reduce damage caused by *T. absoluta. S. habrochites* and LA716 (*S. pennellii*) were already reported to be resistant to *T. absoluta* as well to other pest herbivores, i.e., spider mites *Tetranychus urticae* and Silverleaf whitefly *Bemisia tabaci* (Maluf et al., 2010). These two accessions possess high densities of glandular and non glandular trichomes that cover the total adaxial and abaxial leaves (Figure 4-1).

Figure 4-1: Photo of the most resistant accessions. On the left the accession LA1777 of *S. habrochaites* and on the right the accession LA716 of *S. pennellii*.

It was reported that accession of *S. galapagense* is resistant to a broad range of pest, and that resistance is associated with the co-occurrence of high densities of trichomes type IV and acyl-sugars (Firdaus et al., 2013). However, in our study, the accession LA1401 (*S. galapagense*) reported as highly resistantce to *B. tabaci* was fully susceptible to *T. absoluta* (Firdaus et al., 2012). This accession, together with the accessions LA1139 (*S. cheesmaniae*) and LA1645 (*S. pimpinellifolium*) showed the highest damage leaf area. The resistance could be related with the presence of insect feeding deterrents, present in some wild tomato species that are not present in the other tomatoes assessed.

Tomato leaf volatiles are essential cue's finding and oviposition in tomato leafminor and mated females discriminated between cultivated and wild tomato accessions; they laid more eggs in *S. lycopersicum* (Proffit et al., 2011). We have shown that *T. absoluta* adults did not settle on the accession LA1777 and G1.1561 of *S. habrochaites*. However, ability of host plant selection was not restricted to *T. absoluta* adults. Larvae can make choices on wild tomato accession. We found that accessions LA 1645, LA716, LA 1408, LA0483, G1.1561 and LA1718 were not preferred by *T. absoluta* larvae. The fact that there was no correlation between damage leaf area and larva preference, allowed us to assume that different resistance mechanisms are acting at this level.

## 4.2. Importance of tomato trichomes for *T. absoluta* resistance

We have detected a correlation between trichomes I, IV and V with resistance (oviposition rate and larva survival). Trichomes are epidermal structures, that originate from the epidermal cells of above ground plant tissue, have been implicated in protection against various biotic and biotic attacks, extreme temperature and excessive light (Kang et al., 2009). As well, the glandular trichomes are an important source of essential oils, i.e., natural fragrance or products that can be used by the pharmaceutical industry (Glas et al., 2012) and most of these substances involved in plant defence to herbivorous (McDowell, 2011). We observed that trichome composition shown (Figure 4-2) significantly differ among tomato accessions (Table 2).

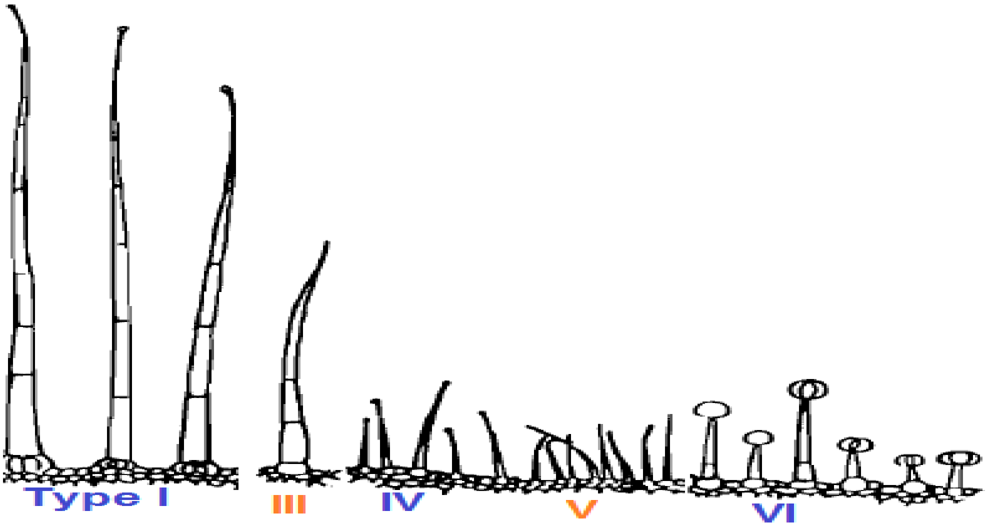


Figure 4-2: morphological feature of studying trichome types (Channarappa et al., 1992).

The diversity of trichome types and chemical composition among tomato species made different species of tomato respond in a different way against herbivore attack (Valverde et al., 2001). For instance, it was reported that wild tomato accessions with a high density of trichome type IV (i.e. *S. habrochaites*, *S. pennellii*, *S. galapagense*) are resistant to silverleaf whitefly (Maluf et al., 2010; Firdaus et al., 2012; Firdaus et al., 2013, Lucatti et al., 2013, more). Glandular trichomes are the major sites of different phytochemical production that prevents herbivore attack than non-glandular trichromes (Schilmiller et al., 2010). Although we have observed a negative correlation between glandular trichome Type, I and IV with the oviposition rate and the larva survival, not all the accessions with trichomes type IV, or I was resistant (Table 2). In addition, no correlation was found between the presence of a trichome type IV and the damaged leaf area or the adult survival. These findings reinforce the hypothesis that the presence of specific types of trichomes is not the only way, but would be the combination of trichomes and metabolites. Similar results were found during the screening of several accessions of the Lycopersicon group where the presence of trichomes type IV without high accumulation of acylsugars and vice-versa did not confer resistance to whiteflies (Lucatti et al, 2013).

We have performed an LC-MS analysis that allowed us to identify 48 acyl sugars. However, the broad coverage of the different acyl sugar, and the previous relation of these metabolites with insect resistance, no correlation was found for this work.

Previous studies, have related *T. absoluta* resistance of tomato to the presence of principal compounds, as cuticular lipids such as tricosane presented a negative correlation with the number of mines unlike tacosane and hexacosane presented a positive correlation (Oliveira et al., 2009). However, we only had acyl sugar LC-MS data and no detected correlation to *T. absoluta* resistance. This leads a suggestion, there may be other methylketones and sesquiterpanes involved for resistance mechanism. High level of allelochemicals, sesquiterpenesb (Zingiberene) and acylsugars found in *S. habrochaites* and *S. pennellii,* respectively are responsible for arthropod resistance, such as spider mites (*Tetranychus utricae*) and silverleaf whitefly (*Bemisia argentifolii)* including *T. absoluta* (Maluf et al. 2010).

## 5. Conclusions and Recommendations

In this study, we have performed a detailed study of *T. absoluta* resistance in tomato. Overall, the accession LA1777 of *S. habrochaites* was the most resistant accession. In addition, the accessions LA1718 (*S. habrochaites*) and LA716 (*S. pennellii*) reduced 55% of larvae survival when compared to the other accessions.

We have observed different resistance mechanisms affecting different stages of the development of the insect. In a breeding programme, these independent mechanisms could be incorporated to get a fully resistant genotype.

Oviposition rate and larvae survival were negatively correlated with the presence of tricomes type IV and I, respectively indicating a role of those trichome types in resistance. Although, I was shown that trichomes type IV are the place of synthesis and storage of acyl sugars, and the known role of acyls sugars in insect resistance, no correlation was found with resistance. This indicates that other metabolites are involved in *T. absoluta* resistance.

This result suggests that commercial varieties of tomato having LA1777 (*S. habrochaites*) and LA716 (*S. pennellii*) as a source of resistance to *T. absolute* will be successful in the future breeding program. As well, it is essential to study the genetic basis of resistance by combining resistance factors from the glandular trichomes type, I and IV in combination with methylketons and sesqueterpenes that could be identified using GC-MS analysis, RNAseg and SNPs study.

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## Appendix

Table 4: Dla, LS & OR of the different accession of wild tomato including the *S. lycopersicum* cv.MM as a reference. Different letters indicate statistical differences according to LSD test ($p<0.05$)

| | | Damaged leaf area* | | | Larvae survival* | | | Oviposition rate* (eggs/days) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| species name | Accession name | n= no_ of sampled leaflets | mean ± SD | SE | n=no_ of larvae | mean ± SD | SE | n=no_ of female *T. absoluta* | Mean ± SD | SE |
| *S. cheesmaniae* | LA0521 | 5 | 4.74 ± 8.57$^{b}$ | 3.833614 | 10 | 0.77 ± 0.13$^{de}$ | 0.058579 | 5 | 1.17±0.85$^{b-f}$ | 0.379043 |
| | LA1139 | 5 | 10.74 ± 5.33$^{cd}$ | 2.38424 | 10 | 0.88 ± 0.16$^{e}$ | 0.071744 | 5 | 3.6±2.45 $^{g}$ | 1.097678 |
| *S. galapagense* | LA 1401 | 5 | 17.94 ± 7.34$^{d}$ | 3.283687 | 10 | 0.77 ± 0.13$^{de}$ | 0.058579 | 5 | 0.46±0.75$^{a-d}$ | 0.333197 |
| | LA0483 | 5 | 4.96 ± 6.54$^{b}$ | 2.923628 | 10 | 0.88 ± 0.16$^{e}$ | 0.071744 | 5 | 0.69±0.82$^{a-e}$ | 0.368117 |
| | LA1408 | 5 | 4.24 ± 3.59$^{b}$ | 1.608913 | 10 | 0.82 ± 0.16$^{de}$ | 0.071744 | 5 | 0.97±1.00$^{a-f}$ | 0.448125 |
| *S. habrochaites* | G1.1561 | 5 | 4.12 ± 2.12 $^{b}$ | 0.947312 | 10 | 0.68 ± 0.41$^{cde}$ | 0.182843 | 5 | 1.26±0.52 $^{b-g}$ | 0.232115 |
| | LA1718 | 5 | 5.12 ± 5.14$^{bc}$ | 2.298565 | 10 | 0.42 ± 0.39$^{abc}$ | 0.173205 | 5 | 0.06 ±0.13$^{a}$ | 0.057143 |
| | LA1777 | 5 | 0.24 ± 0.19$^{a}$ | 0.087178 | 10 | 0.14 ± 0.32$^{a}$ | 0.141421 | 5 | 0.23±0.08$^{ab}$ | 0.034993 |
| *S. lycopersicum* | FCN13-1-6-1 | 5 | 3.02 ± 1.64$^{bc}$ | 0.737157 | 10 | 0.82 ±0.16 $^{de}$ | 0.071744 | 5 | 2.63±3.48$^{efg}$ | 1.558126 |
| | FCN93-6-2 | 5 | 4.38 ± 1.13$^{bc}$ | 0.504381 | 10 | 0.82 ± 0.16$^{de}$ | 0.071744 | 5 | 2.06±2.89$^{c-g}$ | 1.291732 |
| | MM | 5 | 4.06 ± 3.61$^{b}$ | 1.612638 | 10 | 0.54 ± 0.51$^{bcd}$ | 0.227411 | 5 | 1.66±1.58$^{d-g}$ | 0.707972 |
| | cv. Uco plata INTA | 5 | 5.18 ± 1.93$^{bc}$ | 0.863366 | 10 | 0.88 ± 0.16$^{e}$ | 0.071744 | 5 | 1.91±1.04$^{fg}$ | 0.464231 |
| *S. pennellii* | LA 0716 | 5 | 3.28 ± 3.47$^{b}$ | 1.550935 | 10 | 0.28 ± 0.39$^{ab}$ | 0.173205 | 5 | 0.26±0.21$^{abc}$ | 0.094761 |
| *S. pimpinellifolium* | LA 1645 | 5 | 9.02 ± 2.58$^{cd}$ | 1.154729 | 10 | 0.82 ± 0.16$^{de}$ | 0.071744 | 5 | 1.14±1.46$^{b-f}$ | 0.651529 |
| | LA1580 | 5 | 2.22 ± 1.89$^{ab}$ | 0.84463 | 10 | 0.82 ± 0.16$^{de}$ | 0.071744 | 5 | 1.03±1.08$^{a-f}$ | 0.483187 |
| | LA1584 | 5 | 5.14 ± 1.06$^{bc}$ | 0.476025 | 10 | 0.82 ± 0.16$^{de}$ | 0.071744 | 5 | 0.63±0.65$^{a-f}$ | 0.291373 |

* = significant difference at $P<0.05$: Dla = Damaged leaf area, LS = Larvae survival & OR = Oviposition rate

Table 5: General analysis of variance using the total area of the leaf (Mask pixil) as a covariate

| Analysis of variance (adjusted for covariate) | | | | | | | Covariate regressions | | |
|---|---|---|---|---|---|---|---|---|---|
| Variate: Damaged leaf area | | | | | | | Variate: Damaged leaf area | | |
| | Covariate: Mask (pix) | | | | | | Covariate | coefficient | s.e. |
| Source of variation | d.f. | s.s. | m.s. | F-value | cov.ef. | P value | | | |
| LA_number | 15 | 42.5675 | 2.8378 | 3.03 | 0.92 | 0.01 | | | |
| Covariate | 1 | 0.0009 | 0.0009 | 0 | | 0.976 | | | |
| Residual | 63 | 59.0282 | 0.937 | | 0.98 | | Mask (pixel) | -1E-06 | 3.48E-5 |

Table 6: Probability of binomial distribution on the number of insects that made choice for adult settlement and larvae preference. 'P' value approximated to '0' when it couldn't proximate to the next higher number after three digits

1. adult settlement preference

| species name | accession name | n = number of insects | proportions of insects that chosen a given accession | proportion insects that chosen MM | SD | SE | probability of binomial distribution of the given accession | probability of binomial distribution of MM |
|---|---|---|---|---|---|---|---|---|
| | LA0521 | 6 | 0.50 | 0.50 | 0 | 0 | ~0 | 0 |
| *S. cheesmaniae* | LA1139 | 24 | 0.54 | 0.46 | 0.06 | 0.04 | 0.034 | 0.009 |
| | LA 1401 | 3 | 0.33 | 0.67 | 0.24 | 0.17 | 0 | 0 |
| | LA0483 | 27 | 0.48 | 0.52 | 0.026 | 0.02 | 0.034 | 0.055 |
| *S. galapagense* | LA1408 | 19 | 0.32 | 0.68 | 0.26 | 0.18 | ~0 | 0.034 |
| | G1.1561 | 1 | 0 | 1 | 0.71 | 0.5 | 0 | 0 |
| | LA1718 | 22 | 0.27 | 0.73 | 0.32 | 0.23 | ~0 | 0.106 |
| *S. habrochaites* | LA1777 | 14 | 0 | 1 | 0.71 | 0.5 | 0 | 0.055 |
| | FCN 13-1-6-1 | 2 | 0.50 | 0.50 | 0 | 0 | 0 | 0 |
| | FCN 93-6-2 | 24 | 0.63 | 0.37 | 0.18 | 0.13 | 0.081 | 0.001 |
| *S. lycopersicum* | Uco Plata INTA | 15 | 0.47 | 0.53 | 0.05 | 0.03 | ~0 | ~0 |
| *S. pennellii* | LA 716 | 5 | 0.20 | 0.80 | 0.42 | 0.3 | 0 | ~0 |
| | LA 1645 | 25 | 0.56 | 0.44 | 0.08 | 0.06 | 0.055 | 0.009 |
| | LA1580 | 30 | 0.60 | 0.40 | 0.15 | 0.11 | 0.034 | 0.081 |
| *S. pimpinellifolium* | LA1584 | 18 | 0.44 | 0.56 | 0.08 | 0.06 | ~0 | 0.004 |

2. *Larvae feeding preference*

| | accession name | n = number of insects | # of insects that chosen given accession | # of insects that chosen MM | SD | SE | probability of binomial distribution of insects that choice a given | probability of binomial distribution of insects that choice MM |
|---|---|---|---|---|---|---|---|---|

|  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|
|  |  |  |  |  |  |  | accession |  |
| *S. cheesmaniae* | LA0521 | 4 | 0.25 | -0.75 | 0.52 | 0.23 | 0.16 | 0.31 |
|  | LA1139 | 3 | 0.25 | -0.75 | 0.52 | 0.23 | 0.16 | 0.31 |
| *S. galapagense* | LA 1401 | 5 | 0.4 | -0.6 | 0.53 | 0.24 | 0.31 | 0.31 |
|  | LA0483 | 4 | 0 | -1 | 0.52 | 0.23 | 0.03 | 0.16 |
|  | LA1408 | 3 | 0 | -1 | 0.48 | 0.22 | 0.03 | 0.31 |
| *S. habrochaites* | G1.1561 | 4 | 0 | -1 | 0.52 | 0.23 | 0.03 | 0.16 |
|  | LA1718 | 3 | 0 | -1 | 0.48 | 0.22 | 0.03 | 0.31 |
|  | LA1777 | 5 | 0.2 | -0.8 | 0.53 | 0.24 | 0.16 | 0.16 |
| *S. lycopersicum* | FCN 13-1-6-1 | 4 | 0.75 | -0.25 | 0.52 | 0.23 | 0.31 | 0.16 |
|  | FCN 93-6-2 | 4 | 0.25 | -0.75 | 0.53 | 0.24 | 0.16 | 0.31 |
|  | Uco Plata INTA | 5 | 0.2 | -0.8 | 0.53 | 0.24 | 0.16 | 0.16 |
| *S. pennellii* | LA 716 | 4 | 0 | -1 | 0.52 | 0.23 | 0.03 | 0.16 |
| *S. pimpinellifolium* | LA 1645 | 4 | 0 | -1 | 0.52 | 0.23 | 0.03 | 0.16 |
|  | LA1580 | 4 | 0.6 | -0.4 | 0.53 | 0.24 | 0.31 | 0.16 |
|  | LA1584 | 5 | 0.4 | -0.6 | 0.53 | 0.24 | 0.31 | 0.31 |

Table 7: LC-MS data of six tomato accessions correlated to the choice and no-choice test parameters, and with the trichome Types

| **accession name** | | FCN13-1-6-1 | FCN93-6-2 | Uco plata INTA | MM | LA 1401 | LA1584 | LA1777 | LA 0716 |
|---|---|---|---|---|---|---|---|---|---|
| choice and no-choice tests | Dla | 1.89 | 2.39 | 2.56 | 1.90 | 4.12 | 2.60 | 0.30 | 1.66 |
| | AS | 0.40 | 0.60 | 0.40 | 0.75 | 0.60 | 0.40 | 0.80 | 0.60 |
| | LS | 0.86 | 0.55 | 0.86 | 0.91 | 0.82 | 0.86 | 0.15 | 0.31 |
| | OR | 1.38 | 1.22 | 1.46 | 1.23 | 0.42 | 0.62 | 0.29 | 0.31 |
| | EL | 0.31 | 0.27 | 0.55 | 0.18 | 0.20 | 0.46 | 0.60 | 0.20 |
| | larvae preference | 0.10 | 1.20 | 0.75 | 0.00 | 0.15 | 0.90 | 0.70 | 0.25 |
| | adult preference | 0.50 | 6.00 | 3.75 | 0.00 | 0.75 | 4.50 | 3.50 | 1.25 |
| trichome Types | I | 0.00 | 0.00 | 0.60 | 0.00 | 0.60 | 0.70 | 3.70 | 0.30 |
| | III | 0.10 | 1.60 | 0.80 | 1.00 | 0.60 | 1.00 | 1.50 | 0.20 |
| | IV | 0.00 | 0.00 | 0.00 | 0.00 | 18.20 | 16.70 | 19.70 | 18.60 |
| | V | 20.20 | 21.20 | 19.20 | 32.90 | 10.50 | 9.70 | 0.00 | 0.00 |
| | VI | 12.80 | 4.40 | 9.40 | 10.40 | 10.40 | 11.30 | 9.40 | 4.40 |
| autoscaled LC-MS data | G3:19 | -0.73 | -0.58 | -0.14 | 1.45 | 0.00 | 0.00 | 0.00 | 0.00 |
| | S3:14 I | -0.51 | -0.90 | 0.01 | 1.39 | 0.46 | 0.57 | 0.46 | -1.50 |
| | S3:14 II | 0.00 | 0.00 | 0.00 | 0.00 | -0.18 | 1.38 | -0.19 | -1.01 |
| | S3:14 III | -0.68 | -0.63 | -0.14 | 1.45 | 0.61 | 0.29 | 0.58 | -1.48 |
| | S3:15 I | -0.50 | -0.52 | -0.48 | 1.50 | 0.38 | 1.01 | -0.03 | -1.36 |
| | S3:15 II | -0.51 | -0.51 | -0.48 | 1.50 | 0.80 | 0.83 | -0.39 | -1.24 |
| | S3:15 III | 0.04 | 0.68 | 0.70 | -1.43 | 0.00 | 0.00 | 0.00 | 0.00 |
| | S3:16 I | -0.57 | -1.12 | 0.88 | 0.81 | 0.47 | 0.33 | 0.68 | -1.48 |
| | S3:16 II | -0.61 | -0.61 | -0.27 | 1.48 | 0.53 | 0.42 | 0.55 | -1.50 |
| | S3:18 I | -0.60 | -0.99 | 0.35 | 1.24 | -0.14 | -0.03 | 1.30 | -1.13 |
| | S3:18 II | -0.49 | -0.27 | -0.71 | 1.48 | 0.56 | 0.39 | 0.55 | -1.50 |
| | S3:18 III | 0.00 | 0.00 | 0.00 | 0.00 | 0.65 | 0.22 | 0.60 | -1.47 |
| | S3:19 | 0.00 | 0.00 | 0.00 | 0.00 | 0.14 | -0.22 | 1.25 | -1.17 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| S3:20 | -1.00 | -0.54 | 0.27 | 1.27 | 1.27 | -0.19 | 0.09 | -1.16 |
| S3:20 I | -0.59 | -0.57 | -0.32 | 1.49 | 0.00 | 0.00 | 0.00 | 0.00 |
| S3:21 I | 0.00 | 0.00 | 0.00 | 0.00 | 0.70 | -0.07 | 0.75 | -1.39 |
| S3:21 II | 0.00 | 0.00 | 0.00 | 0.00 | -0.01 | 0.22 | 1.10 | -1.32 |
| S3:21 III | 0.00 | 0.00 | 0.00 | 0.00 | 0.67 | 0.19 | 0.60 | -1.47 |
| S3:21 IV | -0.54 | -0.50 | -0.46 | 1.50 | 0.80 | 0.90 | -0.59 | -1.10 |
| S3:21 V | -0.67 | -0.62 | -0.17 | 1.46 | 0.00 | 0.00 | 0.00 | 0.00 |
| S3:22 I | -0.78 | -0.66 | 1.40 | 0.03 | 0.42 | 0.45 | 0.62 | -1.49 |
| S3:22 II | 0.00 | 0.00 | 0.00 | 0.00 | 0.48 | 0.32 | 0.68 | -1.48 |
| S3:22 III | 0.00 | 0.00 | 0.00 | 0.00 | 0.70 | 0.70 | 0.03 | -1.42 |
| S3:22 IV | -0.78 | -0.91 | 0.59 | 1.10 | 0.23 | 0.00 | 1.09 | -1.33 |
| S3:22 V | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | -0.14 | 1.28 | -1.16 |
| S3:22 VI | -0.52 | -0.44 | -0.54 | 1.50 | 0.00 | 0.00 | 0.00 | 0.00 |
| S3:23 | -0.74 | -0.48 | -0.25 | 1.47 | 0.00 | 0.00 | 0.00 | 0.00 |
| S3:23 I | 0.00 | 0.00 | 0.00 | 0.00 | -0.11 | 0.54 | 0.93 | -1.36 |
| S3:23 II | 0.00 | 0.00 | 0.00 | 0.00 | 0.71 | 0.88 | -0.31 | -1.28 |
| S3:23 III | 0.07 | -0.93 | -0.50 | 1.37 | 0.83 | 0.65 | -0.11 | -1.37 |
| S3:23 IV | -0.78 | -0.91 | 0.59 | 1.10 | 0.00 | 0.00 | 0.00 | 0.00 |
| S4:15 | -0.76 | -0.94 | 1.05 | 0.65 | 0.39 | -0.46 | 1.18 | -1.11 |
| S4:16 I | -0.45 | -0.55 | -0.50 | 1.50 | 0.72 | -0.05 | 0.73 | -1.39 |
| S4:16 II | -0.45 | -0.55 | -0.50 | 1.50 | 0.00 | 0.00 | 0.00 | 0.00 |
| S4:17 I | -0.89 | -0.84 | 0.95 | 0.78 | -0.40 | 0.58 | 1.02 | -1.21 |
| S4:17 II | 0.00 | 0.00 | 0.00 | 0.00 | 0.62 | 0.45 | 0.42 | -1.49 |
| S4:17 III | -1.12 | -0.46 | 0.40 | 1.17 | 0.00 | 0.00 | 0.00 | 0.00 |
| S4:18 | -0.81 | -0.92 | 0.93 | 0.80 | 1.09 | 0.32 | -0.10 | -1.31 |
| S4:20 I | 0.00 | 0.00 | 0.00 | 0.00 | 0.86 | 0.16 | 0.42 | -1.44 |
| S4:20 II | 0.00 | 0.00 | 0.00 | 0.00 | 0.27 | -0.30 | 1.21 | -1.18 |
| S4:21 I | 0.00 | 0.00 | 0.00 | 0.00 | -0.10 | -0.37 | 1.40 | -0.94 |
| S4:22 I | 0.00 | 0.00 | 0.00 | 0.00 | -0.21 | -0.30 | 1.43 | -0.91 |
| S4:22 II | 0.00 | 0.00 | 0.00 | 0.00 | 0.38 | -0.44 | 1.18 | -1.12 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| S4:22 III | 0.00 | 0.00 | 0.00 | 0.00 | 0.83 | 0.42 | 0.20 | -1.45 |
| S4:23 I | 0.00 | 0.00 | 0.00 | 0.00 | 0.30 | -0.21 | 1.15 | -1.24 |
| S4:23 II | 0.00 | 0.00 | 0.00 | 0.00 | 1.17 | 0.39 | -0.40 | -1.15 |
| S4:24 I | 0.00 | 0.00 | 0.00 | 0.00 | 0.26 | -0.27 | 1.20 | -1.19 |
| S4:24 II | -0.92 | 1.09 | -0.78 | 0.61 | 0.92 | 0.76 | -0.58 | -1.11 |

AS= Adult survival, LS = Larvae survival, EL = Emerged larvae, OR = oviposition rate

S = Sugar, G = glucose, the numbers next to S or G are the number of lateral branches, the number after two dots is the number of carbons in the lateral branch & the roman numbers on acyl sugars (I - VI) represents Isomers.