

Biological aspects of tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae) in conditions of Northeastern Tunisia: possible implications for pest management

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Abstract

The tomato leafminer *Tuta absoluta* Meyrick is a key insect pest of tomato in Tunisia and in many other countries around the world. The present study was carried out to attempt to acquire better knowledge of the biology of this insect and to implement suitable prophylactic control tools for *T. absoluta* in Northeastern Tunisia. Under greenhouse conditions, *T. absoluta* females exhibited a stronger preference to lay eggs on leaves of the tomato plant apex, in comparison to laying eggs on leaves of either the middle or lower part of the plant. Using pheromone water traps during the period January–May, three flight peaks of *T. absoluta* males were recorded in Takelsa greenhouses, with the highest trap counts recorded in spring. The use of insect-proof screens significantly prevented *T. absoluta* infestations on host plants. The tomato cultivars Shams and Chebli were shown to be the least suitable under open-field conditions for egg-laying by *T. absoluta*, compared to the cultivar Ferrinz. The use of both insect-proof screens and tomato cultivars with lower suitability for pest's egg-laying might be a promising prophylactic control tactic against *T. absoluta* in Northeastern Tunisia.

Key words: cultivar suitability, insect-proof, pest management, population dynamics, prophylactic tool, tomato, *Tuta absoluta*.

Abbreviations: IPM, Integrated Pest Management.

Introduction

The tomato leafminer *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) is a major insect pest infesting tomato crops in countries of the Mediterranean basin (Germain et al. 2009; Desneux et al. 2010; Desneux et al. 2011; Balzan, Moonen 2012; Tropea Garzia et al. 2012) and in its area of origin, namely South America (Miranda et al. 1998; Lietti et al. 2005). This pest is able of causing major crop yield and economic losses in tomatoes (EPPO 2005; Germain et al. 2009; Hassan, Alzaidi 2009; Silva et al. 2011). For this reason, developing and implementing effective control tools for this pest is of primary importance.

Chemical control using insecticides can be considered as an effective management option for this pest (Lietti et al. 2005; Silvério et al. 2009; Lebdi-Grissa et al. 2010). Nevertheless, in several cases, insecticide treatments may not be effective against *T. absoluta* due to the occurrence of insect resistance to the active chemical ingredients used (Siqueira et al. 2001; Lietti et al. 2005; Silva et al. 2011). Furthermore, in an Integrated Pest Management (IPM) programme, pesticide treatments using either chemical or biologically-based insecticides may not be compatible with biological control of this pest using parasitoids and/or predators, since some active ingredients have been proven to be harmful to some non-target biocontrol agents

of *T. absoluta* (Arno, Gabarra 2011; Biondi et al. 2012; Biondi et al. 2013). Based on recent findings, it has been concluded that, in some circumstances, the combined use of biopesticides and natural enemies, specifically braconid wasps (for example, those known as parasitoids of *T. absoluta*) should not be considered for effective and sustainable IPM and organic programmes for tomatoes (Biondi et al. 2013). Among the alternatives to the use of conventional broad-spectrum pesticides, eco-friendly plant extracts with bioinsecticide properties (Moreno et al. 2011; Tomé et al. 2013), mass trapping using tomato leafminer's sex pheromone (Hassan, Alzaidi 2009), application of a pheromone-based mating disruption technique (Cocco et al. 2013), and biological control using *Trichogramma* parasitoids (Cabello et al. 2012; Chailleux et al. 2012) may provide environmentally safe and adequate control of this pest. Likewise, adopting effective prophylactic tools may also be another promising and eco-friendly way to control this invasive pest.

In Tunisia, *T. absoluta* was first found in October 2008 to infest open-field tomato crops of the Sahel region (Akouda delegation, Governorate of Sousse). In this country, the insect has spread rapidly and it is currently considered a key insect pest on tomato crops. For this reason, acquiring better knowledge on its bio-ecology and developing and implementing effective control tactics of this pest are key

components in IPM programmes in Tunisia. In this regard, the present study was performed and aimed at: (i) studying some important bio-ecological aspects of *T. absoluta* (distribution of eggs and larvae on different plant sections, and male flight activity), (ii) assessing the suitability of three tomato cultivars for *T. absoluta* female egg-laying, and (iii) evaluating the efficiency of insect-proof screens in preventing attacks of tomato host plants by this pest.

Materials and methods

Biological aspects of *Tuta absoluta*

Eight commercial greenhouses located in the tomato growing area of Takelsa (Cap-Bon region, North-East of Tunisia) were used in the present study. The climatic conditions in these greenhouses during the study period are mentioned in Table 1. Each of the tomato greenhouses had an area of 500 m² and was planted with 625 tomato plants (cv. Sankara) grown in nine rows with a 0.80 m distance between plants. These tomato plants were drip irrigated. Random sampling of 75 tomato leaves was carried out weekly in each of the greenhouses, between January 1st and May 12th 2010, to assess the population structure of *T. absoluta* on tomatoes and to determine the distribution of eggs and larvae on host plant sections. It should be pointed out that the middle part of the tomato plant contains the highest number of leaves, compared to plant apex and bottom of the plant. Leaves were selected from the upper area of each of the three plant parts, taking into account the increase in length of each plant part during the growing period. Sampled leaves were observed under a binocular microscope (Leica® Model MS5) and the different life stages of *T. absoluta* were reported and counted.

In addition to the evaluation of population structure and life stage localization on tomato leaves, the male flight activity of *T. absoluta* was also studied in three of the eight greenhouses, using sex pheromone lures (Koppert Biological Systems, the Netherlands) placed inside water traps. In each greenhouse, two pheromone water traps were hung on tomato plant canopy at height 50 cm above the ground. Minimum distance between traps was 25 m. The number of *T. absoluta* males caught inside traps was recorded weekly and sex pheromone lures used were renewed every four weeks. Whenever necessary, dirty water in traps was replaced with clean water.

Effectiveness of insect-proof screens

Two greenhouses, one with and another without insect-proof screens, were used in the study. Both greenhouses

had the same growing conditions described earlier. The evaluation procedure consisted of counting weekly the number of larvae on sampled leaves (75 leaves per greenhouse) within each of these two greenhouses in order to evaluate the role of the insect-proof screens in preventing attacks by *T. absoluta*.

Suitability of tomato cultivars for *Tuta absoluta* egg-laying

An open-field plot located in Takelsa, covering an area of 1 ha and planted with three tomato cultivars (cv. Ferrinz, 320 plants; cv. Chebli, 285 plants; and cv. Shams, 200 plants) was used in the present study to evaluate tomato cultivar suitability for *T. absoluta* infestations (egg-laying). Sampling was carried out in the study site from early May to early July 2012. Twenty leaves of each cultivar were harvested weekly and observed in the laboratory under a binocular microscope (Leica® Model MS5). Eggs of *T. absoluta* laid on all sampled leaves were counted for each of the three tomato cultivars. The purpose was to determine cultivar(s) with lower suitability for egg-laying by *T. absoluta* females.

Statistical analysis

The effect of plant cultivar on egg-laying by *T. absoluta* on leaves was tested using one-way ANOVA. Significantly different means of treatments were determined using Duncan's multiple range test at $P = 0.05$. A *t*-test was also used to compare the number of larvae present on leaves between the greenhouse equipped with insect-proof and the greenhouse without insect-proof screens. All statistical analyses were performed using the software SPSS Statistics 17.0 (SPSS Inc. 2009).

Results and discussion

Aspects of *Tuta absoluta* biology

During the 4-month study period extending from mid January to early May, immature life stages of *T. absoluta* (eggs and larvae) were found on at least one of the three parts (apex, middle or lower part) of their host plants. It clearly appeared that *T. absoluta* mated females exhibited a preference to lay eggs on leaves of the plant apex, compared to laying eggs on leaves of the two other parts of tomato host plant (Table 2). Indeed, as indicated by the data in Table 2, eggs were totally absent on leaves of either the middle or the lower part of the host plant. In a previous study, it was shown that before flowering, *T. absoluta* females chose the under-side of the leaf for oviposition in the apical part of the plant canopy (Torres et al. 2001). Additionally, in agreement with our findings, Leite et al. (2004) demonstrated that

Table 1. Average monthly temperature and relative humidity in Takelsa greenhouses (year 2010)

Parameter	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Temperature (°C)	13.6	14	15.1	17.6	20	23.7	27.6	28.1	24.3	21.3	17.3	13.8
Relative humidity (%)	78	77	82	83	76	74	73	73	80	82	81	77

Table 2. Population structure of *Tuta absoluta* within the tomato plant canopy (600 leaves per sampling date) in eight greenhouses (Takelsa, January-May 2010). E, number of eggs; L1, number of first instar larvae; L2, number of second instar larvae; L3, number of third instar larvae; L4, number of fourth instar larvae.

Date	Plant apex					Middle of the plant					Bottom of the plant				
	E	L1	L2	L3	L4	E	L1	L2	L3	L4	E	L1	L2	L3	L4
January 13	26	0	0	0	0	0	0	0	2	0	0	0	0	0	0
January 20	14	1	0	0	0	0	15	13	1	0	0	4	0	3	1
January 29	8	0	0	0	0	0	13	9	9	1	0	3	2	3	2
February 4	15	0	1	0	0	0	6	7	7	3	0	3	5	3	0
February 11	12	0	0	0	0	0	2	13	6	1	0	7	5	3	0
March 5	18	1	0	0	0	0	10	2	1	2	0	11	8	2	2
March 11	6	1	0	0	0	0	5	10	10	4	0	6	10	4	1
March 19	9	0	0	0	0	0	9	11	11	4	0	0	3	9	0
March 25	18	2	0	0	0	0	5	8	8	2	0	0	2	0	3
April 1	4	18	0	0	0	0	24	7	7	2	0	0	2	1	0
April 27	3	7	6	0	0	0	31	20	17	5	0	0	0	0	0
May 5	5	4	5	0	0	0	17	9	14	7	0	0	0	0	0

there was a preferential deposition of *T. absoluta* eggs on the apical leaves of the tomato plant canopy. Furthermore, Leite et al. (1999) found that *T. absoluta* oviposited more on leaves of the apical and medium portions than in the basal parts of the tomato plant. The same authors stated that this oviposition behavior could be linked to the lower calcium content of apical leaves, which are tender, compared to middle or basal leaves of the host plant.

It was highlighted in the present study that the highest number of larvae, regardless of their respective life stages, was found on leaves of the middle part of the plant (Table 2). This finding could be attributed to the higher number of leaves (more food resources are offered to larvae) in the middle part, compared to the number of leaves present on either apical or lower parts of the plant. On the other hand, young instar larvae (L1 and L2) were found on leaves of all three parts of the plant; however third and fourth instar larvae were found only on leaves of the middle and lower parts of the plant (Table 2). This can be explained by the fact that, during their growth and development period, it might be possible that young instar larvae moved from leaves of apical parts to leaves of middle and basal parts of the plant and reached third and fourth instar larval stages, before pupating.

Regarding male flight activity of the tomato leafminer, our study revealed the occurrence of three flight peaks. The first peak was recorded on January 20th in all of the three studied greenhouses. The second flight peak was observed on March 17th in the first greenhouse and on March 24th in either the second or the third greenhouse. The third peak occurred in late April in all three study greenhouses (Fig. 1). The highest trap counts (100 and 140 males per trap per week) were recorded in pheromone traps of the second greenhouse (G2) during the spring period (Fig. 1). In a greenhouse located in Biskra (Center-east Algeria), from mid December to late May, three flight peaks of *T. absoluta*

males were recorded: the first on March 24th, the second on April 14th and the third on May 19th 2011 (Allache et al. 2012). The increase of male catches in pheromone traps during spring (March-April) could be due to temperature increases, as it was shown previously by Lacordaire and Feuvrier (2010).

These results related to population structure combined with those linked to male flight activity of *T. absoluta* are very useful for decision making regarding control of this pest since (i) they provide information about localization on plant canopy and density of eggs and young (first and second) instar larvae, which cause plant damage

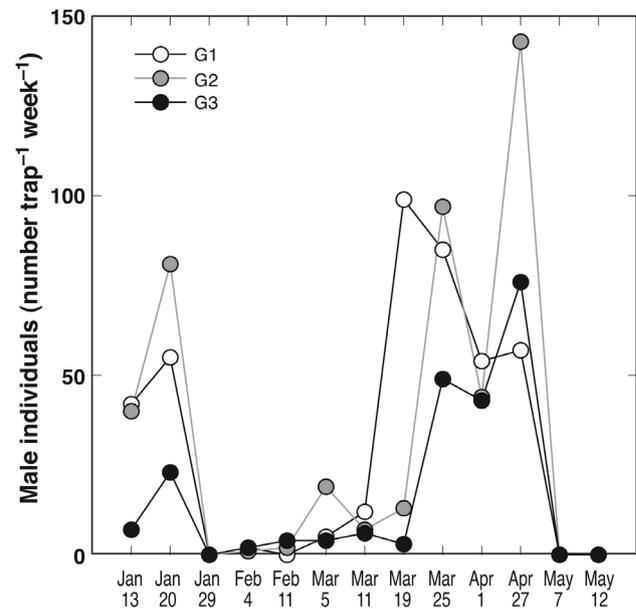


Fig. 1. Male flight activity of *Tuta absoluta* in Takelsa greenhouses (January-May 2010). G1, greenhouse 1; G2, greenhouse 2; G3, greenhouse 3.

and which are the most vulnerable stages to insecticides and (ii) they enable us to acquire knowledge about the periods with intense insect activity (male flight peaks) for which control intervention(s) is (are) highly required to avoid crop damages. Hassan, Alzaidi (2009) suggested that monitoring using pheromone traps can provide early warning of infestation and estimate the density of the insect population. Desneux et al. (2010) indicated that rigorous sampling protocols combining pheromone trapping to monitor adult abundance and direct observation to record direct plant damage need to be adopted in pest management programmes against this pest. In addition to monitoring, pheromone lures for *T. absoluta* can also be exploited for pest control purposes. Indeed, the application of mass trapping technique by placing a high number of sex pheromone traps in greenhouses is a potential option for the management of *T. absoluta* populations (Hassan, Alzaidi 2009). Sex pheromone lure can also be successfully used in a mating disruption program against *T. absoluta* in greenhouse tomato crops (Cocco et al. 2013).

Effect of insect-proof screens on *Tuta absoluta* infestation

The mean number of *T. absoluta* larvae found on leaves during the 4-month study period (from mid January to early May) was significantly different ($t = -2.3$; $df = 20$; $P = 0.032$) between the greenhouse equipped with insect proof and the greenhouse without insect-proof screens (Table 3, Fig. 2). During the study period, the weekly number of larvae found on 75 leaves did not exceed 20 in the greenhouse equipped with insect-proof screens, whereas this number exceeded 40 larvae in the greenhouse without insect-proof screens (Fig. 2). These findings show that the use of insect-proof screens significantly reduced density of *T. absoluta* larval populations on tomato leaves. Accordingly, this control tool could be successfully incorporated in an integrated pest management programme against the tomato leafminer occurring in Tunisian greenhouses. In such a context, the combination “pheromone water traps + insect-proof screens” could be useful in limiting tomato infestations by *T. absoluta*. According to Harbi et al. (2012), the use of one sex pheromone water trap combined with insect-proof covering of doors and aeration openings is sufficient to guarantee good crop protection level against *T. absoluta* infesting greenhouse tomato crops in Teboulba (Sahel region, Central-East Tunisia).

Table 3. Effect of insect-proof screens on infestation level by *Tuta absoluta* larvae. Means followed by the same letter do not differ significantly at the 5% level of significance (Duncan’s multiple range test)

Treatment	Mean number of larvae per 75 leaves
Greenhouse with insect-proof	6.09 a
Greenhouse without insect-proof	17.54 b

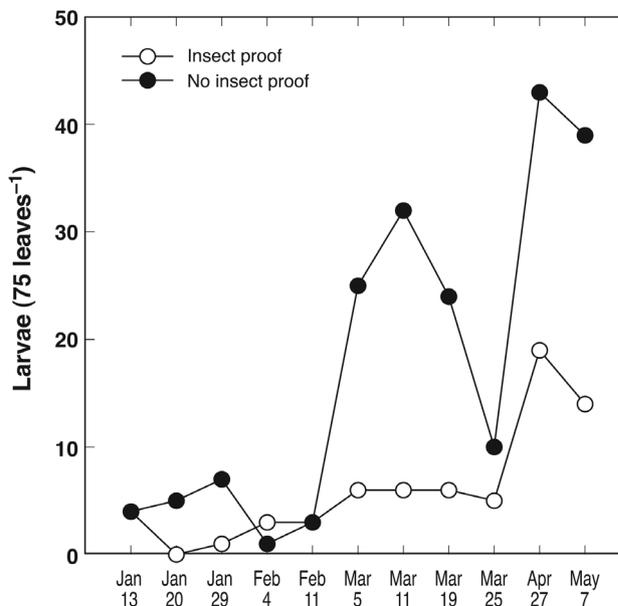


Fig. 2. Density of *Tuta absoluta* larvae on leaves of tomato plants in two different greenhouses with or without insect proof.

Suitability of tomato cultivars for *Tuta absoluta* egg-laying

Statistical analyses revealed that tomato cultivar had a significant effect on oviposition by *T. absoluta* females ($F_{2,24} = 6.37$; $P = 0.006$). As shown in Table 4, *T. absoluta* females laid weekly significantly more eggs on leaves of cv. Ferrinz relative to either cv. Chebli or cv. Shams. The two latter cultivars had statistically similar numbers of eggs laid by females of the tomato leafminer.

This difference in the *T. absoluta* egg-laying preference between the three tomato cultivars tested most likely can be attributed to differences in leaf volatile compounds. The latter represent direct cues of host plant suitability (Proffit et al. 2011). Indeed, based on oviposition bioassays, Proffit et al. (2011) demonstrated that *T. absoluta* females laid more eggs in response to cvs. Santa Clara and Carmen as compared to cv. Aromata. The same authors found that overall leaf volatile composition of cv. Aromata differed significantly from cvs. Santa Clara and Carmen, due to differences in proportions of minor compounds and due to the absence of several compounds, mostly terpenes, in cv. Aromata. De Oliveira et al. (2012) showed that oviposition rate and damage on plants were significantly

Table 4. Effect of tomato plant cultivar on number of eggs laid by *Tuta absoluta* females. Means followed by the same letter do not differ significantly at the 5% level of significance (Duncan’s multiple range test)

Cultivar	Mean number of eggs 20 leaves ⁻¹ week ⁻¹
Shams	16.66 a
Chebli	21.44 a
Ferrinz	34.77 b

lower on tomato strains rich in one of the three following allelochemicals: 2-tridecanone, zingiberene or acyl sugars. Furthermore, previous studies pointed out that tomato cultivars showed differences in susceptibility to *T. absoluta* infestation, after quantifying symptoms and damages caused by this pest on leaves of three different tomato cultivars (Bogorini et al. 2003; Oliveira et al. 2009).

Our results might provide a basis for setting-up prophylactic management tactics for this pest by selecting tomato cultivars with lower suitability for egg-laying by *T. absoluta*. However, further studies on the chemical composition of the three cultivars tested are required to better understand the direct causes of differences in egg-laying preference exhibited by mated females of the tomato leafminer.

In conclusion, overall, the results of the present study provided evidence that *T. absoluta* has a preference to lay eggs on apical leaves, compared to medium or basal leaves of tomato host plants, and revealed the presence of three flight peaks of *T. absoluta* males from January to May in greenhouses, with an increase in male density during the spring period. Additionally, our study showed that insect-proof screens were effective in reducing infestations by this insect on its host plant, and demonstrated that both tomato cvs. Chebli and Shams were characterized by lower suitability for *T. absoluta* female egg-laying relative to the cv. Ferrinz. All these results would certainly provide a basis for development and implementation of effective prophylactic and environmentally-sound pest management tools against *T. absoluta* in Northeastern Tunisia. Several future studies dealing with this topic involving the use of eco-friendly control methods, such as insect behavior-modifying (manipulating) strategies, are strongly needed to strengthen overall management programmes against this invasive pest, which currently infests both Tunisian greenhouse and open-field tomato crops. Indeed, manipulating insect behavior through the appropriate use of semiochemicals, mainly female pest sex pheromone and host-plant volatiles, could be a potential, effective alternative to the application of insecticide treatments. Sex pheromones can be used to disrupt insect mating or for mass-trapping of *T. absoluta* males, inducing large decreases in levels of pest populations. On the other hand, host-plant volatiles, whose components are known to be the source of attractants and/or repellants for insect pests, are able of negatively or positively influencing insect behavioral responses. Accordingly, more research studies on pest control practices that might be successful in enhancing the role of plant volatiles in disrupting insect pest host finding behavior are strongly required. Finally, a more powerful pest control programme reducing pesticide input could be based on combined use of insect behavior-modifying strategies and biocontrol agents (natural enemies) that are known to be sufficiently efficient on their host insect *T. absoluta*.

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