



Research Article

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# Use of Traps to Monitor the Tomato Leaf Miner Moth Attack in a West Portugal Greenhouse

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## Abstract

The tomato leaf miner (*Tuta absoluta* (Meyrick)) is a devastating pest responsible for serious damages to solanaceous crops worldwide. The larvae preferentially consume the leaf tissue of the mesophyll, but also the interior of stems and fruits. The tomato leaf miner has a high reproductive potential and causes a high reduction of crops productivity under open-field and protected conditions. This study was conducted in a greenhouse for tomato fresh production. *T. absoluta* flight activity was recorded using two different traps devices during the spring 2023 growing season in the western region of Portugal. Two traditional Delta® and one smart Trapview® trap, baited with pheromones, were installed in the greenhouse and the captures in the three traps compared. During the period of study, an accumulation of 943.4-degree days was calculated, corresponding to 2.26 potential generations. Positive and significant correlations were observed in adult captures between the two conventional Delta® traps and between the smart Trapview® trap and the Delta® trap placed 1 m apart. Early detection of pests and an accurate monitoring of population dynamics are essential for taking appropriate control measures. Results showed that the Trapview® traps are reliable devices for monitoring remotely the *T. absoluta* populations in tomato greenhouses, automatically registering the daily captures avoiding the travelling to the trap site. The use of smart traps in *T. absoluta* pest monitoring allows access to data in a simple real-time way, and a more effective and accurate crop protection.

**Keywords:** Insect biology; Integrated Pest Management; Delta trap; Monitoring; *Lycopersicon esculentum* Miller; Smart-trap; *Tuta absoluta* (Meyrick)

**Abbreviations:** IPM: Integrated Pest Management; DAP: Days After Planting

## Introduction

Tomato (*Lycopersicon esculentum* Miller) is a worldwide economically important edible and nutritious vegetable crop. It is the second most important vegetable crop in the world, after the potato [1]. The largest tomato production in Europe is from Southern countries being Italy the main producer, followed by Spain and Portugal [2]. Fresh tomato is predominantly produced in greenhouses, whereas tomato for processing is grown in the open field. In Portugal, tomato for fresh consumption has increased along the last 20 years reaching an area of 1776 ha in 2021 with an average productivity of 84.5 t per hectare. Portuguese exports of fresh tomatoes reached 107.2 t, exceeding the import by 153% [3].

The tomato leaf miner moth (*Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)) [4] is one of the most important invasive pests affecting tomato production globally [5-8]. Warm

zones with low altitudes (less than 1000 m) are especially favorable to the development of miner moth, while low temperatures are limitative factor for its survival [9]. *Tuta absoluta* is native to South America (Peru) and was accidentally spread throughout Spain at the end of 2006, and then quickly dispersed into the Mediterranean basin and Northern Africa [10-12]. In Portugal, the pest was reported for the first time in 2009 [13]. During the last 10 years, *T. absoluta* has also been reported in the Middle East and Asia, including India, Iran, Israel, Syria, and Turkey [12,14]. Although tomato plant is the preferred host, leaf miner also attacks other cultivated solanaceous species, namely potato, eggplant, pepper, tobacco and other families such as broad bean, alfalfa, watermelon as well as wild hosts [15,16].

The great economic importance of damages caused by the tomato leaf miner infestation was attributed to several causes,

such as a short *T. absoluta* generation time, a great capacity to adapt to various climatic conditions, a considerable wide host range, an aptitude to develop insecticide resistance and a lack of resistant plant varieties [7,17,18,19].

Recent reviews documented different aspects of *T. absoluta* bioecology and pest status, namely its biology, hosts, yield losses, insecticide resistance, and Integrated Pest Management (IPM) strategies [7,8,20–22]. *Tuta absoluta* may attack the tomato plants at all developmental stages, from seedling to mature stage [23], with larvae feeding inside the aerial plant organs, forming mines in leaves, flowers, and immature and ripened fruits. Tomato leaves may be destroyed and the tomato fruits market value decreases. In the absence of adequate control measures, yields can be reduced more than 90% [24]. Yield losses in Africa usually range from 11-43%, but at high pest population density losses can reach 100% [12,17]. Indirect losses caused by secondary pathogens contamination are also important, as mines and galleries in leaves and fruits are entry routes for infection by different pathogens, especially bacteria [18,25].

The life cycle of *T. absoluta* is completed in 30-35 days, depending on environmental conditions, in particular the temperature, with four development stages: egg, larvae, pupa, and adult [8,20]. Thermal requirements vary between 14–35 °C, which fits well with optimal tomato growth conditions [26,27].

Adult females mate immediately after emergence and during their lifetime lay up to 250-300 eggs usually on the underside of leaves, stems, and to a lesser extent on fruits [28,29]. Due to favorable climatic conditions in the greenhouse and to its vigorous reproductive potential, this pest can have up to 10-12 generations per year [17,29]. Eggs are small, oval-cylindrical (0.35 mm long), initially cream-colored and becoming orange as they approach hatching. At night, females lay eggs individually in the upper part of the plant, namely in young apical leaves, stems, and sepals [18]. Larvae take 4 to 7 days to hatch at 27 °C and immediately penetrate leaf parenchyma or plant organ [24].

Larval stage is the most damaging period completed within 12-15 days [30]. Four larval instars differ in size and color. Immediately after hatching, early-instar larvae are white or cream and start to damage the leaf by entering the mesophyll tissue [20]. During the 2<sup>nd</sup>-3<sup>rd</sup> instar, larvae become green in color, while fourth-instar-larvae have a body length of 7-10 mm and a green or purple abdomen, with a characteristic dark head [25]. Mature larvae, corresponding to the 3<sup>rd</sup>-4<sup>th</sup> instars, can cause substantial damages in plants, with total loss of the harvest. Larvae feed on the mesophyll of leaves leaving translucent areas called galleries and maintaining intact the epidermis [24]. Usually, larvae and mines are associated with expanded leaves in the medium part of the canopy. In the case of severe attacks, the leaves appear burnt and finally die [12]. Mining plant damage causes malformation [31].

When food is available and environmental conditions are favorable, larvae feed almost continuously and generally do not

enter diapause [18]. However, as shown by Campos et al. [32], moths can enter a facultative diapause under cool temperature. Matured larvae pupate mainly on the leaves or in the soil [7,20], or in packaging material [33]. Pupae have a development time of 7-10 days, with a cylindrical shape of 5-6 mm long and a green color, becoming brownish later on [17].

The adult moths are small (5-7 mm long) with a wingspan of 8-10 mm, and males are slightly smaller than females. They are brownish or silver, with narrow brown-speckled wings. The most important identifying characteristics are the filiform antennae silverfish-grey scales, and characteristic black spots in the anterior wing. Adults are nocturnal and usually remain hidden during the day with most activity occurring at sunrise or dusk. Females live for 10-15 days and males for 6-7 days [17].

*Tuta absoluta* is a pest very challenging to control, as a consequence of its endophytic habit where larvae are protected in leaves mesophyll or inside fruits [34]. Once introduced in an area, *T. absoluta* can be spread by seedlings, infested plants, fruits, and pallets or other box containers [35]. In many parts of the world, insecticides are the most common approach adopted by growers to control *T. absoluta* [12,36,37]. Chemical control leads to higher production costs and other problems, such as side effects on non-target organisms and pollinators, and the development of insecticide plant resistance [38]. However, the implementation of alternative environmentally safe methods, such as microbial insecticides and control agents have been increasingly used to manage *T. absoluta* control [17].

The use of *Bacillus thuringiensis* (Bt) is an effective biological method to control *T. absoluta* larvae [31,36,39]. Its ingestion causes the death of larvae by the toxins and a general infection due to the proliferation of bacteria through the insect's body. The use of different *B. thuringiensis* strains, which express different toxins with distinct action modes, is recommended to avoid problems of resistance to the insecticide [22].

To interrupt the biological cycle of pests and reduce the excessive use of insecticides, it is important to implement sustainable cultural practices, such as crop rotation with non-solanaceous crops [40], selective removal and destruction of infested plant material, weed control [16,41], use of insect-proof nets in lateral and upper openings and double door systems at the greenhouse entrance [11], release of parasitoids and predators [17,23,42], use of resistant tomato cultivars [43], and massive trapping with the placement of sex pheromone traps [21,44].

Pest flight prediction models often relate the sum of active temperatures (accumulation of degree-days) to certain phenological events (e.g., the start and peak of each flight). This information makes it possible to define the periods during which the risk estimates of each pest generation should be focused (damage sampling) and to carry out the phytosanitary treatments when they prove to be necessary, in particular ovicides [45]. In addition, Guimapi et al. [46] observed that when relative humidity

is included in simulations, the invasion and spread evolution of *T. absoluta* populations are closer to natural observations.

To support conscious decision-making in pest control and the rational use of pesticides or biological control, early detection and identification of the presence of *T. absoluta* and knowledge of its abundance is essential. The use of traps with specific pheromones has been useful for early detection in recently infested areas and for monitoring the flight activity during the growing crop season [21,22,44]. Monitoring pests can be a very laborious and expensive task. During the last decade, smart technologies for remote pest monitoring have been developed. The present study was conducted to compare the efficiency of two trap models, the traditional Delta® trap and a smart trap, to monitor the tomato leaf miner *Tuta absoluta* pest in a greenhouse.

## Materials and Methods

### Trial Location and Plant Material

The experiment was conducted in a greenhouse with 1.5 ha, located at A-dos-Cunhados (39° 08'01.2"N, 9° 20'33.8"W) in Western Portugal, around 100 km north of Lisbon. The tomato transplantation occurred on 5 January 2023, with five weeks plants of grafted Sir Elyan variety (Vilmorin-Mikado). Tomato plants were cultivated in substrate bags, with a row and inter-row spacing of 25 x 180 cm, using drip fertigation according to local cultivation practices.

Plants were grown under organic production system and only microbial insecticides were applied to control *Tuta absoluta* leaf miners. In 2023, maximum and minimum temperatures and relative humidity were hourly recorded by the Trapview® smart trap during the crop growth. The smart trap (Trapview WING SC) was produced by EFOS d.o.o. and was available as part of the EU Thematic Network H2020 Project SmartProtect (2023).

### Estimation of Degree-day Accumulation

In the greenhouse, degree-day accumulations were calculated to estimate the potential number of *T. absoluta* generations during the monitoring period using the sine wave method [47-49], downloaded from the University of California's IPM online DD model's webpage (<https://ipm.ucanr.edu/WEATHER/index.html#DEGREEDAYS>). In this simple linear method, the records of daily minimum and maximum air temperatures from the weather station were used as inputs. The thermal requirements calculated by Krechmer and Foerster [26] were used and the lower development threshold of 8.0 °C and an upper intermediate cut-off of 37.3 °C were selected.

### Monitoring the Tomato Leaf Miner

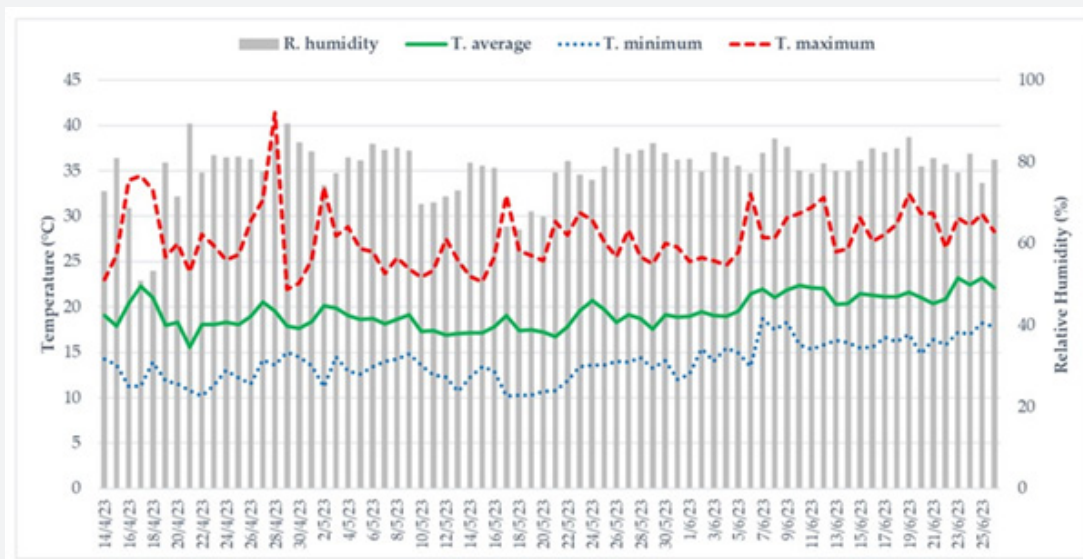
To monitor *T. absoluta* flight activity and compare captures between trap models, two traditional Delta® (Biobest) traps (Delta-37 and Delta-36), spaced 100 m apart, and one smart Trapview® located within 1 m from the Delta-36 trap were installed in the greenhouse (Figure 1a).



Figure 1: (a) Localization of the Delta-36 trap and smart Trapview® in the greenhouse (spaced 1 m apart); (b) Detail of Delta-36 trap; (c) Detail of smart Trapview®. Pheromone capsules were located on the top of the traps.

Delta® traps have a white triangular-shaped body open at the tops, with a removable sticky plate to retain the attracted insects, and the pheromone is positioned at the top-middle (Figure 1b). Sticky plates of the two Delta® traps were replaced weekly and captures were quantified by visual observation using a binocular magnifying glass (70x) (Zeiss Stemi 1000). The Trapview® smart trap registered the daily captures automatically by taking

daily images of sticky plate (Figure 1c). The software identifies the *T. absoluta* catches, with confirmation of identity made with the periodic observation and morphological identification of specimens. The smart trap also registers the climatic data in the greenhouse on an hourly basis (Figure 2). Collected data and different statistics are available on the web or mobile platform whenever necessary.



**Figure 2:** Daily values of minimum, average and maximum temperature (°C) and relative humidity (%) in the tomato greenhouse, from 14 April to 26 June 2023.

The three traps were placed 1.2 m above the ground. The monitoring period started on 15 April 2023, 100 days after planting (DAP), and was extended to the harvest date on 26 June 2023 (172 DAP). All traps had a pheromone lure (Biobest®) which are rubber dispensers containing the species-specific sex pheromones. The capsule slowly releases an odor similar to the sex hormone of *T. absoluta* females to attract males that are trapped in the sticky plate. The sex pheromone lures were replaced on 26 May 2023.

### Phytosanitary Control

The management practices were accordingly to those usually adopted by producers in the region. Treatments with *B. thuringiensis* (Bt) bacterium were weekly carried out in the greenhouse during the period of *Tuta absoluta* infestation, with a total of nine applications. Only *B. thuringiensis* was applied during plant growth, with different commercial strains (Turex®, Costar®, Dipel®, and Belthirul®) (Table 1).

**Table 1:** Commercial formulations of *Bacillus thuringiensis* (Bt) used in the monitoring period to control *Tuta absoluta* in a tomato greenhouse.

No.	Date	Days after plantation	Commercial product	Formulation
1	22 April	107	Turex®	<i>Bt</i> var. <i>aizawai</i>
2	29 April	114	Costar®	<i>Bt</i> var. <i>kurstaki</i>
3	6 May	121	Dipel® DF	<i>Bt</i> var. <i>kurstaki</i>
4	13 May	128	Turex®	<i>Bt</i> var. <i>aizawai</i>
5	20 May	135	Belthirul®	<i>Bt</i> var. <i>kurstaki</i>
6	27 May	142	Costar®	<i>Bt</i> var. <i>kurstaki</i>
7	3 June	149	Turex®	<i>Bt</i> var. <i>aizawai</i>
8	10 June	156	Belthirul®	<i>Bt</i> var. <i>kurstaki</i>
9	17 June	163	Dipel® DF	<i>Bt</i> var. <i>kurstaki</i>

### Statistical Analysis

Correlation coefficient and the relative significance of the p-value ( $p \leq 0.05$ ) were determined for the number of *Tuta absoluta* captured in different traps, using Statistica version 12 software.

### Results

#### Climate Data and Number of Generations

Greenhouse daily temperature and relative humidity were recorded (Figure 2). In the period from 14 April to 26 June 2023, the minimum temperature was always above 10 °C. A consistent

increase in temperature was observed from 7 June to 26 June, with minimum, average, and maximum values above 15 °C, 20 °C and 25 °C respectively.

The potential number of generations of *T. absoluta* in the monitoring period was estimated using the degree-day accumulation method. A total of 943.4 degree-days were estimated, which correspond to 2.26 moth generations according to [26], as a total of 416.7 degree-days which are necessary to complete one generation.

## Monitoring the Tomato Leaf Miner by the Two Trap Models

### Captures of *T. absoluta* in the Smart Trapview® Trap

In smart traps, it is possible to follow remotely the daily progress of the *T. absoluta* infestation. The daily catches in Trapview® from 17 April to 26 June 2023 are shown in Figure 3. A lower and upper limit of daily catches, associated with the sending of alerts to the user, can be previously defined in trap computer program, represented by the horizontal dotted color lines green (10 adult males) and red (30 adult males), respectively.

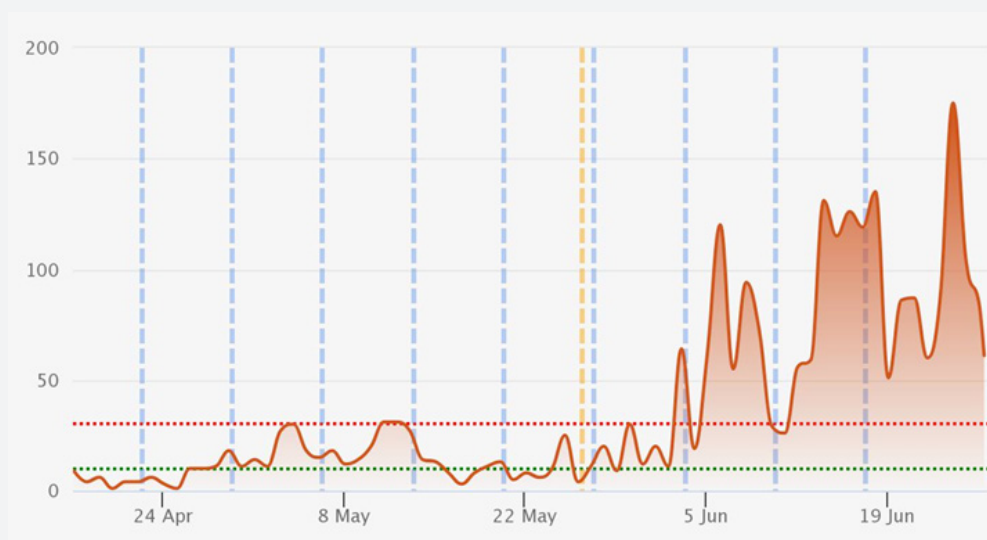


Figure 3: Daily catches of tomato leaf miner *Tuta absoluta* recorded by the smart Trapview® trap installed in the tomato greenhouse, from 17 April to 26 June 2023.

Legend: Number of new *T. absoluta* captures (red area); Spraying with *B. thuringiensis* biocide (vertical blue line); Change of pheromone lure (vertical yellow line). Horizontal green and red dotted lines represent the low and the high daily pest limit of 10 and 30 captures.day<sup>-1</sup>, respectively.

During the period from 17 April to 2 June, the captures remained low, with less than 30 males.day<sup>-1</sup>; the high limit of pest daily captures was reached only on 11 and 12 May with 31 captures per day. The daily captures increased thereafter from June, reaching a maximum of 175 captures in 24 June.

### Comparison of Captures of *T. absoluta* in Delta® and Smart Trapview® Traps

The weekly *T. absoluta* captures in the three traps (Delta-37, Delta-36, and Trapview) during the period from 21 April to 26 June are represented in Figure 4.

During the monitoring period, the Delta-37 trap recorded the highest number of captures (3083 moths), followed by Trapview® (2686 moths) and Delta-36 traps (2175 moths). Differences in weekly *T. absoluta* infestation were observed in the two traditional Delta® traps (Delta-36 and Delta-37), separated by 100 m distance in the greenhouse, however with a significant correlation

coefficient ( $r=0.760$ ,  $p=0.007$ ,  $n=11$ ). In the initial period, the captures were higher in Delta-37 trap, located near the entrance of the greenhouse, but after 9 June the captures were similar in both Delta® traps, except the last date when the tomato plants in the greenhouse had already been harvested.

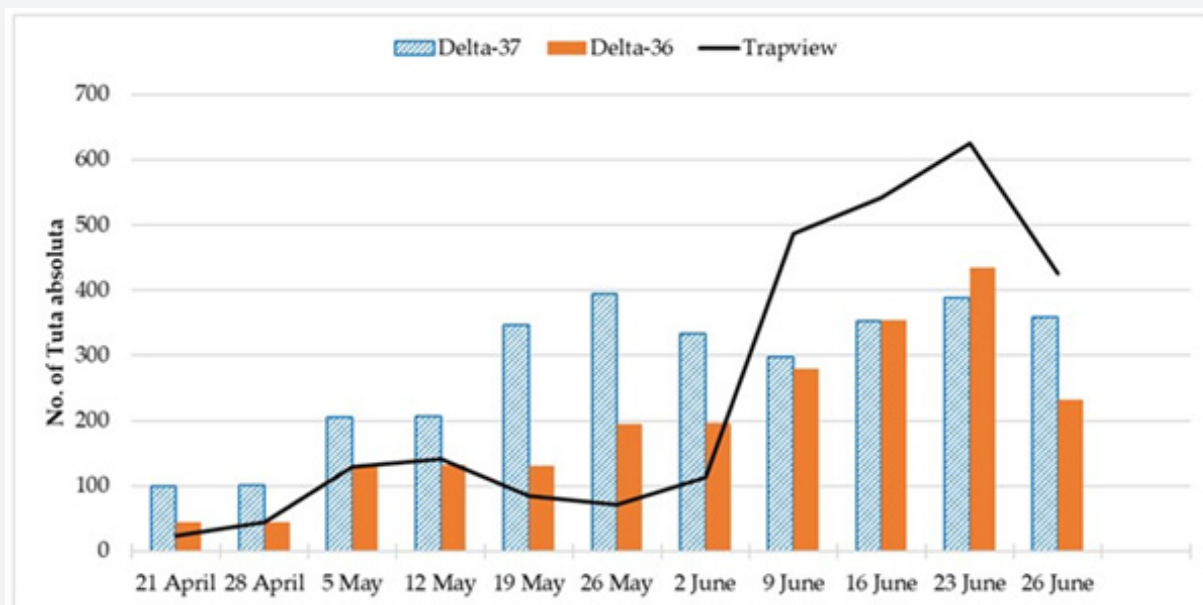
The weekly catches registered in Delta-36 and in smart Trapview®, 1 m apart (Figure 1a), were compared and a highly significant correlation coefficient ( $r=0.918$ ,  $p<0.000$ ,  $n=11$ ) was observed. A low number of catches was observed in Delta-36 and Trapview® traps from April to early June, with less than 200 captures per week. In both traps, the *T. absoluta* catches increased after 9 June (>200 males per week). Comparing the Delta-37 trap and the Trapview® (100 m apart), a non-significant correlation coefficient ( $r=0.549$ ,  $p=0.080$ ,  $n=11$ ) was observed.

### Effects of *Bacillus thuringiensis* Treatments

The weekly applications of the biocide *B. thuringiensis* (Figure

3) affected the dynamics and abundance of the pest population development in the greenhouse. *Bacillus thuringiensis* applications had a negative highly significant effect ( $r=-0.903$ ,  $p\leq 0.000$ ,  $n=9$ ) on

*T. absoluta* population, registering a lower number of captures at day 1 after each the application.



**Figure 4:** Greenhouse weekly catches of tomato leaf miner *Tuta absoluta* registered in the Delta-37, Delta-36, and Trapview traps, during the period from 21 April to 26 June 2023.

## Discussion

Monitoring and forecasting methods to estimate pest attack can be directly obtained by visual observation of plants or using indirect methods, such as the use of pheromone sex traps. However, depending on the level of infestation, counting the captures in manual sticky traps can be an exhaustive and a time-consuming task, which requires taxonomic expertise.

During the winter-spring period of 2023, a higher level of *T. absoluta* infestation was observed in the tomato greenhouse located in the Western region of Portugal compared to the previous year (data not shown). The infestation caused damage in leaves in the med-canopy, which can reduce the photosynthetic capacity and the plant's growth and yield [12]. In the period under study, a minor fruit infestation was observed indicating an effective pest control [50] by the weekly application of the biological insecticide *B. thuringiensis*.

In the initial monitoring period (from 21 April to 2 June), the Delta-37 trap located closer to the greenhouse entrance door recorded a consistently higher number of male moths captured than the Delta-36 or Trapview traps. A similar result was observed in a trial conducted in the previous year at the same location (data not shown). This can be explained by an initial *T. absoluta* infestation originated from outside the greenhouse. After 9 June, the captures in the two Delta® traps were similar indicating that the leaf miner population was more homogeneous and began to

multiply inside the greenhouse. An initial damage in border plants in the greenhouse was also observed in other studies, then spread towards the central plants, with the highest plant infestation and fruit damage generally observed in lateral rows in greenhouse trials [34]. Double-door should be used to prevent pest entering into greenhouses [8] which was not verified in the present study. The relevance of improving greenhouse structures to ensure good ventilation and climatic regulation for implementing Integrated Pest Management (IPM) in the Mediterranean tomato greenhouses was referred by Giorgini et al. [22]. *Tuta absoluta* can fly along several kilometers, dispersed by the wind, what favors the spread of the pest over new areas [1,51,52].

The results obtained in the traditional Delta-36 trap confirmed the information in Trapview® trap 1 m away, and a significant correlation between insect captures was observed. A different result was observed when comparing the Delta-37 trap and the Trapview®, separated by 100 m. The correlation coefficient between the number of captures in these two traps was not significant, what highlights the importance of trap location in the number of captures.

The duration of the life cycle was greatly influenced by temperature [8,20,26,32]. Temperature inside the greenhouse, especially after June, was favorable to leaf miner development. In all traps, an increase in *T. absoluta* infestation was observed after 2 June when the average temperature was 21 °C, and minimum and maximum temperatures fluctuated between 13 °C and 33

°C respectively. Deutsch et al. [53] observed a life cycle of about 26 days at an optimum temperature of 30 °C, with more than 10 generations per year. Martins et al. [27] observed a reduction of *T. absoluta* life cycle duration, from 75 to 26 days when the temperature varied from 17 °C to 33 °C. In addition, Cuthbertson et al. [54] registered 92% of egg hatching at 13 °C, higher than 80% with a mean daily temperature of 21 °C, and a significant decline of temperatures above 23 °C.

Although the number of traps tested in this study was limited and the data only covered one production season, the results obtained showed that the use of conventional and tech-traps is a reliable method to monitor the population levels of *T. absoluta*, providing early warnings of the infestation and helping to prevent high population levels.

Different authors showed that pheromone traps can give early warning of infestation and will accurately capture *T. absoluta* in low to medium population infestations [12,22]. The number of males caught in pheromone traps is linearly and negatively correlated with tomato production [50]. Mating disruption was an efficient strategy to control tomato leaf miner in greenhouses and can be included in the overall tomato IPM programs [34].

In the present study, two formulations of the microbial insecticide *Bacillus thuringiensis* were weekly applied to control *T. absoluta*, in a total of 9 treatments, and no relevant damage was observed on tomato fruits. Since *T. absoluta* has multiple overlapping generations, multiple applications are required to control the vulnerable stages [12]. Several studies also demonstrated the effectiveness of *B. thuringiensis* treatment against the *T. absoluta* larvae in protected and open-field crops [31,55]. González-Cabrera et al. [36] verified that *B. thuringiensis* was highly efficient in reducing the damage produced by newly hatched, 2nd–3rd larval instars, with the great advantage of not interfering with the establishment of predator and parasitoid. Also, Alsaedi et al. [39] studied the effect of the *B. thuringiensis* var. *kurstaki* biopesticide on larval mortality in *T. absoluta* and observed a greater percentage of mortality occurred 3 days after the larvae feeding the treated leaves with *B. thuringiensis*. Treatments must be applied when the pest is more vulnerable, i.e., before the larvae penetration in the epidermis of the leaves or in the fruits [24].

Conventional traps need to be visited regularly personally to obtain information about catches, which can be expensive in terms of time and travelling [56]. The smart trap automatically registered the daily pest captures with the great advantage of not being necessary to go to the trap site. The uses of smart traps, provided that internet access is available and other sensors data are accessed in a simple and practical mode at real-time, is very important for timely crop protection against pests and diseases.

In this study, a high correlation was observed between the two-model devices placed side by side. Smart Trapview® proved to be a reliable trap for monitoring the *T. absoluta* populations in the

tomato greenhouse. The use of smart technology by agronomists and other consultants is an important tool to support pest risk assessment, facilitating decision-making and advising growers. More models for the automatic identification of different pests and algorithms to predict the biological development of pests for the optimal timing of different control measures will be implemented in the future to help producers.

### Conclusion

Smart traps use artificial intelligence algorithms to identify and automatically count insects (automatic insect detection and data acquisition) and include weather sensors. Data are sent via mobile network and can be consulted in a platform installed in a computer or mobile device. It is a fast and accurate method for estimating pest risks and allows an effective remote monitoring of pest status in real-time, with reduction of time and travelling costs.

Results showed that the Trapview® traps are reliable devices for monitoring remotely *T. absoluta* populations in tomato greenhouses. A positive and significant correlation was observed between the number of captures recorded automatically in the smart Trapview® trap and the adjacent traditional Delta® trap installed 1 m away. The present study should continue with a higher number of traps to account flight activity and be tested in other seasons in protected and open-air crops.

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