

The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae): pupal key characters for sexing individuals

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Abstract: The present study was conducted to determine the crucial characters for sexing individuals of female and male pupae of the tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). The experiments were performed on 50 live pupae, which were examined under a stereozoom microscope and photographed. Two small tubercles present on the eighth abdominal segment of female pupae are helpful to distinguish the tomato leafminer from male pupae. The genital opening in females is found between the tubercles and is indicated by a longitudinal suture in the middle of the eighth abdominal segment. The male genital opening is also found on the ninth abdominal segment. Typically, female pupae and adults are heavier and bigger than males. This key information and pictures will be useful for laboratory studies that perform single pair matings, sex ratios, and other biological experiments.

Key words: Tomato leafminer, *Tuta absoluta*, pupa sex discrimination

The tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is an invasive pest in Turkey and was first reported in İzmir in 2009. It is originally from Peru, South America, but it is now reported in many countries, and constitutes a serious problem for tomato production around the world (Duarte et al., 2015; EPPO, 2005). It was recorded in 2006 in Spain and then was rapidly distributed to the Mediterranean region (Urbeneja et al., 2012). It is widely established in many countries, and is currently found in Europe (such as Portugal, France, Bulgaria, Hungary, Kosovo, Switzerland, Greece, the Netherlands, Germany, Italy, and the United Kingdom), Russia, Turkey, Albania, Iraq, Lebanon, Israel, Tunisia, Morocco, Algeria, Saudi Arabia, and Libya (Garzia et al., 2012; Cocco et al., 2013). The tomato leafminer is a multivoltine species that is present all-year-round depending on the environmental conditions, especially temperature (Garzia et al., 2012).

The tomato leafminer is a serious pest of tomato production in open field and under greenhouse conditions (Lietti et al., 2005). It is an oligophagous pest of Solanaceae and attacks certain nonsolanaceous cultivars. The female usually deposits her eggs on the underside of tomato leaves and stems, and sometimes on fruits. The damage is caused by larvae preferably feeding by mining the tomato leaves, flowers, stems, and fruits. Mature larvae pupate in the soil, decaying plant material, or any sheltered sites. The damage to the leaf mesophyll is caused by expanding mines, which

reduce the photosynthesis and yield. Without any control, the damage can reach 100% of the yield production (Desneux et al., 2010).

Given the importance of the pest, many studies (Stol et al., 2009) have been conducted to relate its biology (Pereyra and Sanchez, 2006; Doğanlar and Yiğit, 2011; Torres et al., 2011; Öztemiz, 2012), ecology, management (Giustolin et al., 2001; Silva et al., 2011; Dağlı et al., 2012), effects of X-rays (Cagnotti et al., 2012), and reproduction as parthenogenesis (Megido et al., 2012). In some of these studies it is important to have knowledge of *T. absoluta* pupal characters. The adults can be sexed by examining their genitalia through dissection, but this takes time and requires experience. On the other hand, if the individuals need to be alive for further experiments, that will not be possible. In certain other experiments, virgins are needed. These need to be identified before the adults emerge. Various morphological differences have been reported for determining the sex of lepidopterous pupae (Anton and Garrido, 1996), and, although the morphology is similar, sexual differences are not exactly the same in all studied species. The sexual differences between female and male pupae of *Scrobipalpula absoluta* have been reported previously by drawing (Coelho and Franca, 1987), now known as *T. absoluta*. However, the pupa of *T. absoluta* is tiny, and ventral segmentations are hard to identify in individuals. The practical and visualized external key

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characters of pupae will be useful for distinguishing them easily in the laboratory.

The objective of this study is to show sex-specific morphological details in live tomato leafminer pupae, photographed under a microscope that enables sexing of pupae for laboratory studies.

The colony of tomato leafminer was established on tomato leaves in the laboratory at 24 ± 1 °C, $60 \pm 5\%$ RH, and a 16:8 (light:dark) photoperiod. Tomato plants were grown in pots with fertilized soil and were irrigated every other day. The larvae were reared in a plastic container ($30 \times 21 \times 7$ cm) with a piece of paper towel at the bottom and a chiffon lid. Larvae were developed through four instars in tomato leaves and then pupated. Pupae were collected daily by carefully examining the rearing containers and excising tomato leaves where they usually pupated in the laboratory. The pupal length (mm), width (mm), and weight (mg) were measured (Yilmaz and Genç, 2012; Genç, 2015). Adults were held in screen cages, fed with a honey solution (10%), and then sexed (Yilmaz and Genç, 2012).

Individuals from the laboratory colony were used to conduct the experiments. The experiments were carried out with 5 replications, with 10 live pupae for each replication. In order to sex the pupae, ventral parts of the 7th, 8th, and 9th abdominal segments were examined externally under an Olympus SZX9 stereozoom microscope and photographed with an attached Olympus digital camera (Genc, 2005; Genç, 2015).

Mature larvae were pupated at the bottom of larval-rearing containers or in larval mines of tomato leaves in the laboratory. Dorsal, ventral, and lateral views of the live pupae were examined, as shown in Figure 1. Pupae were initially soft, cylindrical, and green, later becoming dark brown before adult emergence (Figure 1). Male pupae weighed 2.33 ± 0.5 mg, and were 0.578 ± 0.06 mm in width and 1.963 ± 0.064 mm in length. Female pupae weighed 3.13 ± 0.6 mg, and were 0.679 ± 0.05 mm in width and 2.135 ± 0.131 mm in length.

In order to determine the specific pupal characters of tomato leafminers, the ventral sides of the 8th, 9th, and 10th abdominal segments were examined for external morphological differences, and a total of 50 alive pupae were studied. The tomato leafminer had 10 visible abdominal segments, with the 10th segment including the cremaster and having an anal opening (Figure 2). The female pupae had a longitudinal suture or slit in the middle of the 8th abdominal segment in between the two small tubercles. The male pupae had a suture in the middle of the 9th abdominal segment. The case containing wings and legs ends at the 5th abdominal segment in females, but extends to the 6th abdominal segment in the male pupae (Figure 2).

Male pupae were lighter and smaller than female pupae (Figure 3A). Pupal stage of the leafminer required 8.15 ± 2.11 days under controlled laboratory conditions. Adults were gray-brown, and males were darker than females (Figure 3B). The adult life span was short at about 9.52 ± 2.74 days in the laboratory.



Figure 1. Pupal stage of the tomato leafminer *Tuta absoluta*: A) dorsal, B) ventral, and C) lateral views.

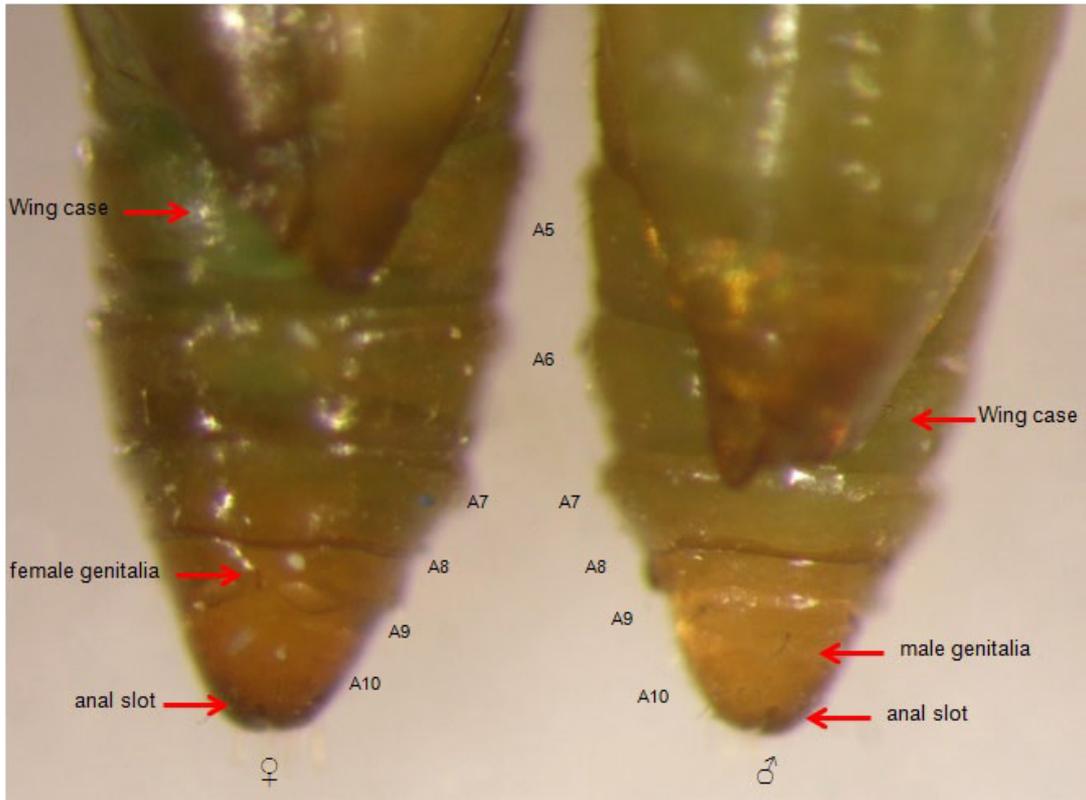


Figure 2. Ventral views of female and male pupae of the tomato leafminer. Abdominal segments indicated as 5th (A5), 6th (A6), 7th (A7), 8th (A8), 9th (A9), and 10th (A10).



Figure 3. Ventral view of the tomato leafminer, pupae and adults.

It is known that the key characters of the sex should be present in the posterior part of the abdomen in pupae and adults (Anton and Garrido, 1996; Posado et al., 2011). In general, the sex of pupae can be identified by the sutures on the ventral side of the 8th and 9th abdominal segments in Lepidoptera (Anton and Garrido, 1996; Genc, 2005, 2015; Posada et al., 2011; Genç, 2015). Studies indicated that examinations of these segments showed some similarities and differences based on the presence and/or absence of tubercles, combs, and spiracles, along with known specific sutures. Their shape, position, and appearance are different (Anton and Garrido, 1996; Posado et al., 2011) and may be specific to the family or species. The previous study indicated by drawing that the genital opening was in the 8th abdominal segment in females and in the 9th for males in the tomato leafminer (Coelho and Franca, 1987). The results here also confirmed the previous study; however, tomato leaf miner pupae are quite small, and the last abdominal segments in alive pupae are curved inwards in some individuals. The division between the segments is not very clear, so it is quite difficult to distinguish the

segments in the male and female pupae during laboratory studies. In this case, it is crucial and useful to have good pictures from living specimens that have reliable sex-specific features.

In conclusion, external morphological characters in tomato leafminer pupae were shown with actual pictures in this study. In total, 50 living pupae of the tomato leafminer were examined. Pupal sex determination was 100% accurate by using the appearance of a specific slit located in the middle of 8th or 9th abdominal segment. Observing pupae for external characters with a 10× or 20× microscope is helpful.

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