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Biology and external morphology of invasive tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

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ABSTRACT: The tomato leaf miner, *Tuta absoluta* (Meyrick), native to South America, has gained entry to India in the recent past. Being an exotic pest, generating information on biology under local conditions is essential. The incubation, larval and pupal periods were 3.90±0.91, 11.80±1.25 and 9.15±1.70 days, respectively. There were four larval instars based on head capsule collected. Characters useful to distinguish sex in different stages like larvae, pupa and adults were identified. Larvae were sexed based on the presence of pink coloured gonads in males, pupae based on the distance of genital aperture from the anterior segment of last segment and adults based on the abdominal size and genitalia. Description of male and female genitalia has been given. The study is helpful in designing future integrated management of this recently invaded pest in India.

Keywords: Invasive tomato leaf miner, biology, *Tuta absoluta*

INTRODUCTION

The tomato leaf miner or pin worm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) has been reported a major pest of tomato, *Lycopersicon esculentum* as well as other solanaceous crops (OEPPD EPPO, 2005). The place of origin is thought to be South America, hence the pest is commonly referred to as South American tomato leaf miner. This pest is considered as one of the key pests of tomato in South America (Garcia and Espul, 1982). *Tuta absoluta* was accidentally introduced to Spain in 2006, from where it spread north to the Netherlands and east to Iran (Desneux *et al*., 2010, 2011; Baniameri and Cheraghian, 2012). In northern Africa, it was reported in Tunisia in 2008 (Abbes *et al*., 2012), Morroco in 2008 (Ouardi *et al*., 2012), North of the Sahel in 2008 (Desneux *et al*., 2010), Western Africa in 2010 (USDA APHIS, 2011) and in Senegal 2012 (Pfeiffer *et al*., 2013). Explosive spread and dissemination of *T. absoluta* is mainly correlated with fruit import and further distribution (Potting, 2009).

Its occurrence in India was reported for the first time by different workers in different parts of India. Its occurrence in and around Bangalore was reported by Sridhar *et al.,* (2014); around Pune by Shashank *et al.,* (2015); in malnad and Hyderabad-Karntaka region by Kalleshwaraswamy *et al.,* (2015) and in Telangana by Kumari *et al.,* (2015). One of the possible pathways for a long distance dissemination of *T. absoluta* could be through packaging materials (boxes) from infested countries (EPPO, 2010). Wherever it has been introduced, this has become the most serious pest causing severe damage on tomato (Germain *et al*., 2009). Cost-benefit analysis showed that *T. aboluta* significantly increased costs of pest management, primarily as a result of increased use of insecticides (Lietti *et al*., 2005). The tomato leafminercan cause crop losses up to 100% and is considered a key pest of greenhouse and open-field tomato (Arturo *et al.,* 2012). Kalleshwaraswamy *et al.,* (2015) speculated that the introduced populations are probably resistant to various group of insecticides, the basic studies like invasion dynamics, life history, reproductive biology are need of the hour to provide the information for its management. Literature survey indicated occurrence of 3 instars in Egypt (Kaoud, 2014), 4 instars in Turkey (Erdogan and Balaroglu, 2014) and 5 instars in Serbia (Toševski *et al.,* 2011). This indicates that the number of larval instarsvaried across geographical location. Similarly, it is tough to distinguish the adults of this microlepidoptera with common gelechids like groundnut leaf miner and potato tuber moth through naked eye. Hence, efforts were made to describe the male and female genitalia. Geographical location may also influence the other aspects of biology and hence this study was focused on the biology and external morphology of this newly invaded pest.

MATERIALS AND METHODS

The study was conducted at the Department of Agricultural Entomology, University of Agriculture and Horticultural Sciences, College of Agriculture, Navile, Shivamogga at room temperature (25±2°C). A laboratory population was established with field collected larvae and pupae from tomato growing regions of Kommanalu, Shivamogga, India (14° 01^{1} 16.2^{11} N; 75° 35^{1} 34.2^{11}). This culture was kept in 50x30x30 cm rearing cages in which tomato seedlings were placed as a source of food. Newly emerged adults were transferred into another cage containing tomato plants. Eggs laid on tomato leaves were collected and used for recording following biological and morphological characters.

Observations on the development of immature stages and their morphology

Ten female and male pupae were collected from larvae reared on the tomato leaves (JKDTH811). When adults emerged, moths were provided with 10% sucrose solution and allowed to mate and lay eggs in small insect cages (50x30x30 cm). Fifty eggs laid on the same day were collected and each egg was placed into a seperate petri dishes (3.5 cm diameter) containing a tomato leaf with their petiole covered with wet cotton. Eggs were examined daily for hatching and the change in their colour was recorded. Fresh foliage was provided for newly emerged larvae and observed daily till they pupate and emerge as adults. Individual insects were checked daily for development and survival. Egg period (days from egg laying to hatching neonate larvae), first larval instar period (days from hatching of egg to first moulting), second larval instar period (days from first head capsule to second moulting), third larval instar period (days from second head capsule to third moulting), fourth larval instar period (days from third head capsule to pupa stage, total larval period (days from first instar to pupae), pupal period (days from pupae to adult emergence) were recorded. Data was recorded on individuals that survived to adult stage were included in the analysis.

To establish the larval instar period, the width of the head capsule was measured using an ocular micrometer under a stereo binocular microscope. For measurements, at each stage, average of 20 insects were used. The sex of individual was distinguished as male or female both at larval stage and pupal stage. Male and female larvae were identified based on the characteristics as indicated by Shanower *et al.*, (1993) for *Aproarema modicella* (Deventer) (Lepidoptera: Gelechiidae) and were photographed under a stereo binocular microscope.

Biological parameters of adults

When emerged, adults (two males and a female) were paired in transparent glass tube (5 cm height x 3 cm diameter) for mating covered with muslin cloth on the top. Tomato leaves were placed with their petiole covered with wet cotton to facilitate egg laying and 10% sugar solution to feed the moths. The experiment was repeated 20 times. Experiments were observed daily and fresh foliage was provided when required. The pre-oviposition period, oviposition period, post-oviposition period, fecundity and longevity were calculated. Egg length and width, Pupal length, width and weight of (adult) *T. absoluta* was also recorded.

Dissection and preparation of slides of male and female genitalia

Male and female genitalia were dissected under stereo binocular microscope. Genitalia of adults were dissected using the technique described by Clark (1941)with little modification wherever required.Before dissection of genitalia, adults were photographed to distinguish male and female. Thenthe abdomen was detached from thorax with the help ofa fine needle. The abdomen was then transferred to a test tube containing a few ml of 10 per cent potassium hydroxide (KOH). This was heated slowly in a water bath till the convection currents were observed in the solution and then kept for cooling. After cooling, the abdomen was transferred to a glass cavity dish containing water and the macerated soft tissues were pressed out with the help of a pair of fine pointed forceps. After repeated washings in water, the genitalia was immersed in absolute alcohol to dehydrate for 5 minutes (for 2 minutes in case of female genitalia) and parts of genitalia were stretched for clear visualance. Then male genitalia was mounted on glass slide using Euparol mountant and slides were kept for drying in hot air oven at 45 degree celcius for 8 – 10 days. In case of female, genitalia was stained using Chlorazole black before mounting on slides. The mounted slides were photographed using *Leica M 205 C* trinocular microscope and described.

RESULTS

Developmental biology of immature stages and their morphology

The eggs took an average of 3.90±0.91 (Table 1) for hatching. The duration of larval development from egg hatching to pupationlasted from 9.5-14.0 days with an average 11.80±1.25 days. The duration of pupal period ranged between 6.0-12.0 days with an average of 9.15±1.70days (Table 1). The whole life cycle of the

Table 1. Pre-adult biological parameters of *Tuta absoluta*

Stage*	Range of Development period (days)	Mean±SD
Egg	3 .0 – 5.5	3.90±0.91
Larval	9.5 – 14.0	11.80±1.25
Pupal	6.0 – 12.0	9.15±1.70
Total Pre-adult period	21.0 – 27.50	24.50 ± 2.15

*N=20

Table 3. Egg length and width, Pupal length, width and weight of *Tuta absoluta*

Parameter	Range	AV. ± SD
Egg length(mm)	0.65 – 1.00	0.78 ± 0.10
Egg width (mm)	0.10 – 0.15	0.14 ± 0.02
Pupal length (mm)	3.55 – 4.05	4.28 ± 0.43
Pupal width (mm)	0.63 – 1.03	0.85 ± 0.11
Pupal weight (mg)	2.25 – 4.70	3.19 ± 0.69

N=20

T. absoluta from egg to adult emergence under laboratory conditions ranged from 21.0-27.50 days with an average of 24.5±2.15 days.

Eggs were oval, creamy white coloured when laid and yellowish at maturity. The length of egg ranged from 0.65 – 1.00mm with an average of 0.78±0.10mm and width ranged from 0.10 – 0.15mm with an average of 0.14±0.02. The larvae were greyish-white up to second instar and later, their colour changed to green to greenish-pink. During the present study, four larval instars were observed and three moults took place through the shedding of larval skin and casting head capsule. The pupae were greenish-yellow initially and later turned to brown. The pupal length ranged from 3.55 – 4.05 mm with an average of 4.28±0.43 mm and the width ranged from 0.63 –1.03 mm with an average of 0.85±0.11mm. Pupal weight varied from 2.25 – 4.70 mg with an average of 3.19±0.69 mm (Table 3). The different stages of *T. absoluta* larvae along with head capsule are shown (Fig.1).

The duration of first instar was 2.0 – 5.5 days with an average of 3.25±1.18 days. Similarly, the duration of second, third and fourth instar was 1.5 – 3.0 days, 2.0 – 3.5 days and 2.5 – 5.5 days, respectively with an average of 2.35±0.53, 2.45±0.64 and 3.89±0.95 days, respectively. The head capsule width at first, second and third moult was 0.14 – 0.19, 0.20 – 0.26 and 0.30 – 0.44 mm, respectively with an average of 0.16±0.03, 0.23±0.01 and 0.35±0.04 mm, respectively. The width of head capsule left before pupation was 0.48 – 0.61 mm with an average of 0.56± 0.08 mm. The total larval period was 9.5 – 14.0 days with an average of 11.80±1.25 days (Table 2). Pupae took 6.0 – 12.0 days to emerge as adults with an average of 9.15±1.70 days.

The sex of the larvae was identified based on the presence of gonads. Presence of pink coloured gonads in the region of the sixth and seventh abdominal segments was a distinguishing characteristic of male larvae. The gonads are visible through the cuticle and can be recognised under stereo binocular microscope (Fig. 1) during second and third instar stage and can be distinguished with female larvae not possessing any pink colored structures (Fig. 1).

Male and female pupae were distinguished under a stereo binocular microscope. The position of the genital aperture was a distinguishing character to separate male and female pupae. In case of male, the genital aperture is present away from the anterior margin of the last abdominal segment. However, in case of female, the aperture of the oviduct and bursa copulatrix is present adjacent to anterior margin of the last abdominal segment. The genital aperture of the male pupa is approximately

Table 2. The duration and width of the head capsule for each instar larva of Tuta absoluta

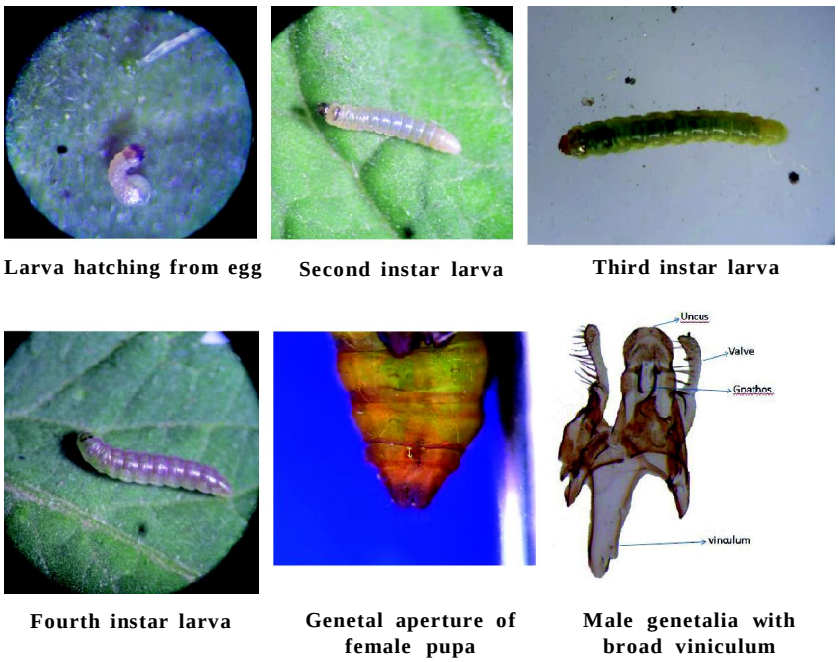
Larval stage	Range of Larval developmental period (days)	AV. ± SD	Range of head capsule width (mm)	Mean ± SD
L_1	2.0 – 5.5	3.25±1.18	0.14 – 0.19	0.16±0.03
L_2	1.5 – 3.0	2.35±0.53	0.20 – 0.26	0.23±0.01
L_3	2.0 – 3.5	2.45±0.64	0.30 – 0.44	0.35±0.04
L_4	2.5 – 5.5	3.89±0.95	0.48 – 0.61	0.56±0.08
Total	9.5 – 14.0	11.80±1.25		

N=20

Table 4. Adult longevity (days) and fecundity (eggs/female) of *Tuta absoluta* on tomato

Parameter	Stage	Range	Mean±SD
Adult longevity (days)	Male	5.5 – 10	7.55±1.60
	Female	9.5–15.5	12.65±1.97
Adult pre-oviposition period (days)	Female	1 – 2.5	1.75±0.49
Oviposition period (days)	Female	5 – 8	6.95±1.04
Fecundity (eggs/female)	Female	112 – 156	137.2±13.86
Adult post-oviposition period (days)	Female	2 – 6.5	4.35±1.43

N=20



Larva hatching from egg | Second instar larva | Third instar larva

Fourth instar larva | Genetal aperture of female pupa | Male genetalia with broad viniculum

Fig 1. Immature life stages of *T. absoluta*

in the middle third of the last abdominal segment (Fig. 4a) while the genital aperture of female is situated in the one-third of the last abdominal segment, closer to the anterior side of the segment (Fig. 1).

Biological parameters of adults

Adults are about 1 cm long, with a wing span of about 1 cm. The abdomen in female moths is wider compared to narrower and pointed abdomen of males. The male and female longevity varied from 5.5 – 10.0 and 9.5 – 15.5 days, respectively with an average of 7.55±1.60 and 12.65±1.97 days, respectively. The pre-oviposition period, Oviposition period and post-oviposition period varied from 1.0 – 2.5, 5.0 – 8.0 and 2.0 – 6.5 days, respectively with an average of 1.75±0.49, 6.95±1.04 and 4.35±1.43 days, respectively (Table 4).

Description of male and female genitalia

The aedegus with a prominent bulge at one end, uncus hood-shaped and broad, valvae digitate with a medial hump and setose apically, inner margin convex medially, tegumen broadened basally, gnathos broad, posteriorly rounded. The vinculum is broad and well extended medially (Fig.1). In female genitalia, the antrum is long and broadly funnel shaped and corpus bursa have a spine like signum.

DISCUSSION

The study was mainly aimed at recording biological and morphological parameters of *T. absoluta* in its new habitat, as it was recently introduced to India. There is a possibility of change in the growth and developmental parameters when an insect occupies a new area. It

appears that the duration of egg development of *T. absoluta* was similar to those reported by Silva *et al.,* (2015) and Duarte *et al.,* (2015) from Brazil. The duration of larval period was comparatively longer as compared to the studies of Silva *et al.,* (2015) and Duarte *et al.,* (2015). However, the width of head capsule was more or less same as that Silva *et al.,* (2015). The development of pupae took longer time as compared to results of above said workers.

The longevity of both females and males varied to greater extent as compared to Silva *et al.,* (2015) and Duarte *et al.,* (2015). The longevity of female (12.65±1.97) was longer compared to males (7.55±1.60 days). However, Silva *et al.,* (2015) reported the average longevity of male and female was 16.47±0.77 and 15.53±0.66 days, respectively, at 25°C days. The fecundity also varied to great extent. It was less as compared to reports of Silva *et al.,* (2015), but is more or less same as that of reports of Duarte *et al.,* (2015).

It is not surprising that variation or difference we recorded in different developmental periods compared to Silva *et al.,* (2015) and Duarte *et al.,* (2015) in a population acquainted to different ecological condition. Identification of sex larvae and pupae will facilitate behavioural studies with moths of known sex. This may also help in development of large scale laboratory mass multiplication technique for various biological studies aiming at its management. Taxonomic notes on the male genitalia is helpful for easy and quick identification and comparing with other gelechids attacking on the same crop. Kalleshwaraswamy *et al.,* (2015) reported occurrence of *T. absoluta* on potato. In potato, *T. absoluta* shares its ecological niche with *Phthorimaea operculella* (Zeller). Both these species cause similar type of damage (Roditakis *et al.,* 2010). Cross attraction of *P. operculella* to *T. absoluta* pheromone lures has also been reported (Roditakis *et al.,* 2010). As pheromone traps have already been exploited for monitoring and management of *T. absoluta* (unpublished data), this study is helpful in designing and evaluating various management strategies against the recently invaded pest to India.

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