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Developmental Biology of Tomato Leaf Miner, *Phthorimaea absoluta* Meyrick (Chang and Metz 2021) to varied Levels of Temperatures

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ABSTRACT: Tomato leafminer (TLM), *Phthorimaea absoluta*, an invasive and a key pest of tomato globally. Establishment of an invasive pest in an introduced environment is influenced by abiotic factors, among them temperature is one of the most important abiotic factors which determines the growth and development and number of generations per year. Temperature is one of the abiotic factors which influences the growth and development of the insect. This will be of great importance to determine the temperature ranges in which tomato leaf miner can grow best and it is essential for understanding its population dynamics and possible expansion in different agro-climatic zones. Hence, the present study was conducted to study the impact of different temperatures (10, 15, 20, 25, 30, 35 and 40 °C) at constant relative humidity (70 ± 5%) on development and survival of TLM. The results revealed that duration of incubation period, larval and pupal period decreased with increasing temperature. Fecundity and egg viability of TLM was maximum at 30°C. (141.59 eggs/female and 90.93 per cent respectively). Pre-oviposition, oviposition and position period was extended at 15°C. With respect to longevity of adults, females lived longer than males at all the temperatures tested. Egg to adult cycle was of shorter duration at 30 (30.54 days) and 35°C (23.58 days) whereas the duration was longer at 15°C (74.58 days). Thus, temperature had significant effect on various biological parameters of TLM.

Keywords: Tomato leaf miner (TLM), invasion, biology, temperature.

INTRODUCTION

Tomato crop is attacked by a variety of pests and diseases from planting to harvest. Among the various insect pests attacking tomato, tomato leafminer (TLM), *Phthorimaea absoluta* Meyrick (Chang and Metz 2021) also known as tomato pinworm, a recently introduced pest of tomato (Lepidoptera: Gelechiidae) which has become a major bottleneck and has also resulted in significant reduction in yield (Biondi *et al.,* 2018). In India, the occurrence of this pest wasinitially detected atIndian Institute of Horticultural Research (IIHR), Hesaraghatta, Bengaluru and Pune, Ahmednagar, Dhule, Jalgaon, Nashik, and Satara Districts of Maharashtra during 2014 (Sridhar *et al.,* 2014; Shashank *et al.,* 2015) and in malnad region of Karnataka (Kalleshwaraswamy *et al.,* 2015). The TLM is an extremely devastating pest that may decrease fruit quality, or even, cause 50-100% losses in open field and greenhouse crops, mainly if control methods are not applied (Nayana *et al.,* 2018). Because of its high reproductive potential, multivoltinism and potential to acclimatize to different climatic conditions, TLM is currently considered a key limiting phytosanitary factor affecting the global Solanaceous crops value chain (Desneux, 2011). Plants are damaged by direct feeding on leaves, stems, buds, calyx, young fruits and also on ripe fruits (Shashank *et al.,* 2018). Furthermore, damage is also caused by the invasion of secondary pathogens which enter through the wounds made by the pest (EPPO, 2005). For an invasive species to be established, it first has to overcome several environmental barriers. The potential for an insect species to become a pest is influenced by a variety of factors. Among the abiotic factors, temperature is a critical abiotic element because it influences insect development, survival and reproduction (Gavkare and Sharma 2017). As TLM is an invasive species, it has a strong potential to spread to larger areas in the coming years owing to atmospheric changes or other factors. As a result, understanding the environmental variables that affect the pest's life would be crucial in determining the best measures to be taken to combat this pest (Ozgocke *et al.,* 2016).

Determining the appropriate temperature conditions for the pest is essential in terms of the studies of population dynamics. The knowledge obtained will be of great value to assess the incidence in different regions and give scientific bases for its control. Predicting the seasonal occurrence and abundance of any pest is essential for the accurate scheduling of control tactics. Such predictions require an understanding of the relationship between insect development rate and temperature. Hence, understanding the impact of external factors such as temperature on the growth, survival, reproduction and rate of increase of insect populations is necessary. The present study was conducted to estimate optimum thermal requirement of tomato leafminer, *P. absoluta* in Biochemical Oxygen Demand (BOD) incubator and the effect of temperature on its development, reproduction (pre-oviposition, oviposition, post-oviposition periods and fecundity) and longevity was studied.

METHODOLOGY

Rearing of insect culture. Later instar larvae of TLM were collected from infested tomato fields near Kommanal village (13°58′25. 97″N; 075°34′43.86″E) and reared in laboratory at Department of Entomology, College of Agriculture, Shivamogga. Field collected larvae were reared on tomato leaves in insect rearing cages for one generation before using for the experiments. Adults thus emerged were transferred to insect rearing cages for mating and provided with 10 per cent honey solution in cotton swab as food and tomato leaves as an oviposition substrate. Eggs laid on a single day were used for the experiments.

Incubation period, larval period, pupal period, pre-oviposition, oviposition, post-oviposition periods (in days) and fecundity (number of eggs/ female) for TLM were determined at seven different temperatures (10, 15, 20, 25, 30, 35 and 40 °C) at constant relative humidity of 70 ± 5%. The experiments were carried out in BOD growth chamber to study the endurance and development of TLM on tomato (hybrid, JKTH 811) in the laboratory. The experiment was performed in two steps.

Assessment of development and survival of egg, larvae and pupal stages of TLM. The rate of development and survival of immature stages of TLM were evaluated at constant temperature as mentioned above. For each temperature with a constant relative humidity treatment, 20 eggs laid on a single day were transferred carefully with the help of a fine camel hair brush to tomato leaflets, the end of petiole was then inserted in moist cotton swab and wrapped with aluminum foil to delay wilting/drying of leaflets and kept in insect breeding boxes (9cm diameter). Then the whole set up was placed in BOD incubator and incubated on the respective temperature. The experiment was replicated five times with 100 eggs per trial (each temperature) in total. These eggs were observed daily for hatching, once the eggs had hatched, observations were recorded on incubation period, larval period and pupal period. Newly emerged larvae were reared individually on tomato leaflets (petiole wrapped with moist cotton) in insect breeding boxes at the respective temperatures. When larvae consumed on an average 70 to 80 per cent of the leaves or when leaves began to wilt, new leaves were provided. The leaves were replaced until the last larva pupated. Observations on the development time and survival of each larval in star were recorded and pupal sexing was done as described by Nayana and Kalleshwaraswamy (2015).

Assessment of fecundity and longevity TLM during its adult stage at respective temperature and relative humidity. Pupae thus obtained were sexed and kept separately for adult emergence. Adults obtained from larvae reared at each temperature were used to determine the fecundity and fertility of TLM. Adults developed from the pupae were kept in pairs in small cage (15 cm breadth × 21.5 cm length and 15 cm height) and was provided with 25 days old tomato seedling (oviposition substrate) for egg laying and 10 per cent honey solution in cotton swab as food. The oviposition substrate was observed for egg laying and replaced daily until the last adult died. Observations on daily survival, fecundity, adult longevity, pre-oviposition period, oviposition period and post-oviposition period were recorded. The mean incubation period larval and mean pupal duration was taken according to the treatment and statistical analysis was performed.

Data analysis. The experiment was conducted in completely randomized design with five treatments and twenty replications. The data regarding incubation period, larval and pupal duration, fecundity and fertility of females, male and female adult longevity of *P. absoluta* was recorded and expressed in days and the data was subjected to one way ANOVA with Tukeys test.

RESULT AND DISCUSSION

The influence of temperature on life cycle of the TLM from egg to adult was studied at respective temperature treatments. But population development ceased at 10 and 40°C. Though eggs hatched at these temperatures but no larvae developed through to adult moths and hence the data at these temperatures were not included for the analysis.

Assessment of development and survival of egg, larvae and pupal stages of TLM. Significant variation was recorded with respect to incubation period, larval and pupal period at different temperature treatments and constant relative humidity. The shortest incubation period was of 2.05 days at 35 °C and longest incubation period was observed at 15 °C (8.88 days). The mean larval development (time taken from egg hatching to pupation) was extended in the population maintained at 15°C (33.99 days), while mean larval development period of 11.39 days at 25 °C was significantly at par with at 30 °C (10.93 days). Duration of pupal period was inversely proportional to temperature. Mean pupal duration was15.52 days at 20 °C and 15.06± 1.12 days at 15 °C as compared to 9.65, 5.70 and 4.19 days for the pupae incubated at 25, 30 and 35 ˚C, respectively and the difference was highly significant at all tested temperatures (Table 1). The present results are in line with the findings of Mahdi and Doumandji (2013), who reported that maximum and minimum duration of incubation period of 12.1 days at 15 °C and 4.1 days at 30 °C was observed likewise larval (23 days at 30 °C and 7.9 at 15 °C) and pupal developmental period (6.5 days at 30 °C and 36.4 days at 15 °C) was decreased with increase in temperature. Similarly, in another study Salama *et al.* (2014), recorded maximum duration of incubation period of 14.65 days at 15 ˚C and then decreased to 3.7 days 30 °C and also larval and pupal period decreased with increased temperature. Egg, larval and pupal duration were significantly shortened as the temperature increased in the above studies. Same trend with respect to development period was observed in the present study but longer duration with respect to each stage was observed in the above results than those recorded in the present study which may be due to different populations of the same species may have different development parameters (Lee and Elliott, 1998; Gomi *et al.,* 2003). The above-mentioned studies were conducted with the populations of TLM from Algeria and Egypt and this may be one of the factors responsible for the reported differences in the effect of temperature on the development of TLM. The differences in the incubation period, larval and pupal development time reported in different studies could be also due to the differences in rearing temperature, tomato cultivars and the geographical populations of the insect used in different studies (Bernays and Chapman, 1994). In addition, variation in duration of different stages of the pest may also depend on for how many generations the insect was reared in the laboratory before using in the experiment, although the effect of this trait on TLM has not been determined.

Table 1: Developmental time (days) (Mean±SE) of the different life stages of *Phthorimaea absoluta* recorded at five constant temperatures.

Temperature	Incubation period	Larva period	Pupa period	Pre-oviposition period	Oviposition period	Post-oviposition period
15°C	$8.88^{a} \pm 0.33$	$33.99^{a} \pm 0.99$	$15.06^{b} \pm 1.12$	$3.41^{a} \pm 0.50$	$7.31^{ab} \pm 0.79$	$8.21^{a} \pm 0.74$
20°C	$6.06^{b} \pm 0.50$	$27.57^{b} \pm 0.64$	$15.52^{a} \pm 0.80$	$3.16^{a} \pm 1.01$	$8.07^{a} \pm 0.77$	$7.05^{b} \pm 1.04$
25°C	$5.26^{c} \pm 0.85$	$11.39^{c} \pm 0.68$	$9.65^{c} \pm 2.05$	$2.53^{b} \pm 0.51$	$6.59^{b} \pm 0.94$	$4.88^{c} \pm 0.94$
30°C	$2.91^{d} \pm 0.25$	$10.93^{c} \pm 0.23$	$5.70^{d} \pm 0.44$	$2.52^{b} \pm 0.50$	$5.02^{c} \pm 0.75$	$3.46^{d} \pm 0.51$
35°C	$2.05^{e} \pm 0.16$	$8.09^{d} \pm 0.61$	$4.19^{d} \pm 0.91$	$2.22^{c} \pm 0.45$	$4.56^{d} \pm 0.51$	$2.47^{e} \pm 0.51$

Means sharing similar letters in the same column are not significantly different by DMRT (P=0.01)

Reproduction of TLM at different temperatures. Temperature significantly affected the pre-oviposition period, oviposition period, post-oviposition period of TLM (Table 1). The pre-oviposition period at 15 °C was 3.41 days and was at par with 20 °C (3.16 days) which was significantly longer than other temperatures. At 25 °C the pre-oviposition period was 2.53 days which was at par with 30 °C (2.52 days) and the shortest pre-oviposition period was recorded at 35 °C (2.22 days). Duration of oviposition period was 4.56 days at 35 °C, 5.02 days at 30 °C. 6.59 days at 25 °C, 8.07 days at 20 °C, 7.31 days at 15 °C. There was significant difference in oviposition period at 30 and 35 °C and the variation was on par at 15 °C, 20 °C and 25 °C. The post-oviposition period was shorter at 35 °C (2.47 days) and there was significant variation with respect to post-oviposition period at different temperature *viz.,* 3.46, 4.88, 7.05 and 8.21 days at 30, 25, 20 and 15 °C, respectively (Table 1). The above results are in accordance with the findings of recent study conducted by Negi *et al.* (2020), that pre-oviposition period, oviposition period, post-oviposition period of TLM decreased with increase in temperature and the oviposition period was longest at 20°C (12.3 days). Thus, the environmental conditions such as temperature and relative humidity are factors influencing insect physiology and behaviour. Temperature has a direct influence on the insect activity and rate of development.

Fecundity of TLM at different temperatures. Mean number of eggs laid by TLM females varied significantly from one temperature to the other. Fecundity was highest at 30 °C (141.59 eggs/female), followed by 25 °C (136.73 eggs/female), 35 °C (128.66eggs/female) and 20 °C (112.91 eggs/female) and the lowest mean number of eggs/female recorded was 86.61 at 15 °C. Percent egg hatchability varied significantly among different rearing temperature and significantly lower percent of egg viability was at 15 °C (56.50 %) and maximum of 90.93 per cent egg viability was recorded at 30 °C followed by 78.33 per cent egg viability, 71.90 per cent egg viability, 61.92 per cent egg viability at 35, 25 and 20 °C respectively (Table 2). Similarly, Krechemer and Foerster (2015) reported highest fecundity of the pest at 25°C. Fecundity of TLM on tomato leaves at 25°C and 30 °C (136.73 and 141.59 eggs/female) obtained in the present study falls within the fecundity range (47.54-260 eggs/female) reported in previous studies at same temperature (Silva *et al*., 2015) but the mean production of egg was reduced at 35 ˚C.

Effects of temperature on the longevity of TLM adults. Temperature inversely affected the longevity of males and females of TLM. The longevity of females was longer than males at each temperature selected. Female longevity was significantly less at 35 °C (9.25 days) and was longer at 15 °C (18.93 days). The longevity of females kept at 30, 25 and 20 °C was 11.00, 13.98 and 18.15 days, respectively. With respect to male longevity, duration varied significantly at different temperature. Male longevity was recorded to be on par at 30 (7.12 days) and 25 °C (7.28 days). At 15, 20 and 35 °C, male longevity was recorded to be 14.27 days, 9.84 days and 5.99 days, respectively (Table 2). In the present study with respect to adults' longevity, females lived longer than males at all the tested temperature range, which was similar to the findings of other authors wherein females' longevity were much longer than males (Haji *et al.,* 1988; Coelho and Franca, 1987; Salama *et al.,* 2014; Abo *et al.,* 2021). Mahdi and Doumandji (2013) recorded average fecundity of maximum at 30 °C (71.4 eggs/female) and females' longevity (6.47 days) was greater than males (2.6 days) at 30 °C as female body is rich in yolk substances

Effects of temperature on the survival TLM. Mean per cent survival of TLM ranged from 7 to 90.8 per cent from egg to adult at varied temperatures, with maximum per cent survival was recorded at 25 °C (90.80 %) followed by 30 °C (85.00 %). The lowest per cent survival was found to be at 15 °C (7.00 %), 20 °C (23.80 %) and 35 °C (32.6 %) (Fig. 1). These results are in line with findings of Ozgocke *et al.* (2016), who have reported that the survival rates were higher at temperatures ranged 23-27.5°C than those of other temperatures tested and it was the lowest at 15 and 34°C.

Table 2: Mean (±SE) fecundity, egg viability, longevity of females and males and duration of egg to adult cycle of *Phthorimaea absoluta* recorded at five constant temperature and relative humidity.

Temperature	Fecundity (eggs/female)	Egg viability (%)	Longevity (days)		Egg to adult cycle (days)	
			Female	Male	Female	Male
15°C	86.61^{e}±2.72	56.50^{e}±2.90	18.93^{a}±1.54	14.27^{d}±0.47	74.58^{e}±4.58	69.88^{e}±2.69
20°C	112.91^{d}±5.13	61.92^{d}±2.30	18.15^{b}±1.81	9.84^{c}±0.47	70.22^{d}±2.83	61.86^{d}±1.82
25°C	136.73^{b}±7.62	71.90^{c}±.6.16	13.98^{c}±0.85	7.28^{b}±0.51	40.28^{c}±4.58	33.80^{c}±3.85
30°C	141.59^{a}±4.85	90.93^{a}±1.29	11.00^{c}±0.71	7.12^{b}±0.53	30.54^{b}±2.83	26.55^{b}±3.15
35°C	128.66^{c}±1.97	78.33^{b}±2.49	9.25^{d}±0.27	5.99^{a}±0.52	23.58^{a}±1.84	20.32^{a}±1.41

Means sharing similar letters in the same column are not significantly different by DMRT (P=0.01)

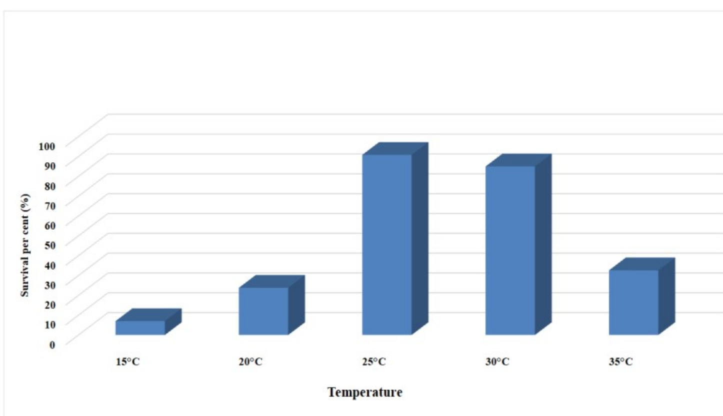

Fig. 1. Survival rate from egg to adult of tomato leaf miner, *Phthorimaea absoluta* at different temperatures.

Effects of temperature on the egg to adult cycle in TLM. Total developmental period of egg to adult cycle of females was found to be shortest at 35 °C which accounted to be 23.58 days followed by 30.54 days at 30°C, 40.28 days at 25°C, 70.22 days at 20 °C and longest period was recorded at 15 °C (74.58 days). Similarly in males, duration of egg to adult was observed to be longest at 15°C (69.88 days) and shortest at 35°C (20.32 days) (Table 2). The above results are in line with the findings of Barrientos *et al.*

(1998) that the duration of the developmental cycle of TLM was highly dependent on climatic conditions with an average development period of 76.3 days at 14 °C, 39.8 days at 19.7 °C and 23.8 days at 27.1°C.

Cuthbertson *et al.* (2013) reported that temperatures between 19 and 23 °C were the most conducive for moth development. Temperatures of 10 °C and lower were shown to be fatal for moth development. Similar results were recorded in the present study, wherein only few eggs of TLM though hatched at 10 °C, but failed to complete the cycle as larvae at this temperature were unable to burrow into the leaf epidermis which in turn suggest that 10 °C may be the lower end of the temperature range for the pest's development. Machekano *et al.* (2018) reported that, the highest temperature where TLM could not survive was 43.0°C and the highest temperature for 100% survival was 37°C. In the present study also development and survival TLM was not observed at 40 °C. Therefore, *P. absoluta's* successful invasion, quick dissemination and establishment in the introduced area may have been aided by host plant availability, climate adaptability and high thermal tolerance to a greater extent. The rate of development was arrested at higher temperatures (45 ˚C), signifying the adverse effects of extreme temperatures on growth of insect, resulting in early mortality of larval population (Jaba *et al.,* 2020). Also, extreme temperatures have an impact on its survival, especially while it is in its early growth stage.

With the increase in temperature, there was decrease in duration of respective stages of a pest at respective temperature. Like other lepidopterans, the developmental time of TLM increased with decrease in temperature (Park *et al*., 2014). This phenomenon can be explained by the ectothermic nature of insects. At high temperatures the metabolism is faster, hence developmental time becomes shorter and vice-versa (Benkova and Volf, 2007; Sgolastra *et al*., 2011; Damos and Savopoulou-Soultani 2012). In order to promote greater growth and development, the metabolic activity of the insect body is initiated between 26 °C and 34 °C. Also, as a direct effect of elevated temperature, increased temperature may increase the food demands of insects as a result of increased metabolic rates, perhaps leading to compensatory feeding (Levesque *et al.,* 2002). Temperature is the most significant factor influencing the growth and development of insects (Bale *et al.,* 2002). The effects of temperature on insects are species specific.

Temperatures between 15 and 25°C are cited as highly favourable for TLM reproduction. Larvae develop well between 20 and 30°C (Martins *et al.,* 2016). The highest temperature threshold that causes TLM mortality is 35°C. Temperatures below 20 °C increase the development period of the larval stage, that may expose this stage to abiotic and biotic mortality factors.

CONCLUSION

Results of the present study indicates that, *P. absoluta*is able to complete lifecycle at wide range of temperature. However, development was ceased at temperature 10 °C and 40 °C and also duration was extended at the lower temperature. From the present study it is clearly showed that the temperature ranged between 25 to 30°C found to be optimum for growth, development and reproduction of TLM. As the temperature was increased, developmental period decreased. These observations will be helpful in predicting population development and the regions where the pest can spread. The pest's minimum and maximum temperature requirement would help us to forecast the pest's likely distribution as well as the time when it would begin inflicting damage.

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Conflict of interest. None.

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