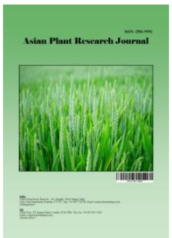

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Influence of Temperature Variability on Survival and Development of Citrus Leaf Miner, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracilariidae) in Tanzania

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Authors' contributions

This work was carried out in collaboration among all authors. Author GN did the conceptualization, software analysis, data validation and formal analysis, literature searches, data curation of the study. Author BSW wrote, reviewed and edited the manuscript did data visualization. Authors GMR worked as a part of project administration. All authors read and approved the final manuscript.

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ABSTRACT

Due to limited information on *Phyllocnistis citrella's* Biology, study was aimed to examine the effects of different temperature levels on biology of citrus leaf miner under controlled laboratory conditions at the Entomology laboratory of Sokoine University of Agriculture (SUA), Tanzania from December 2011 to September 2012. Leaf samples (1,985 leaf samples) considered to contain larvae in serpentine mines were collected and individually incubated at room temperature. The emerging adults were wet preserved in 70% ethanol while some dry preserved using special micro pins under

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microscope for future identification. A Monterey LG8920 pheromone trap was set in every $10m^2$ at SUA citrus orchard to trap adult moths to supplement sample sources. Citrus leaf miners species collected were identified and four different temperature levels (20, 25, 30 and 35°C) were monitored at each developmental stage. Results revealed longer development time (egg to adult) at 20^0C and shorter at 30^0C in 28 and 10 days while the total mortality was 26.67% at 20^0C and 6.67% at 30^0C respectively. Oviposition period and rate were negatively and positively influenced by temperature (from 9.26 days at 20^0C to 6.21 days at 30^0C and 15.2 eggs/ female/ day at 30^0C and 6.8 eggs/ female/ day at 20^0C respectively). The overall fecundity was 28.2 eggs/ female at 20^0C and 57.1 eggs/ female at 30^0C. High larval mortality rate was recorded compared to the egg stage while no mortality observed at pupa stage. Conclusively, the pest on this temperature range (20^0C, minimum to 30^0C, maximum) has high fecundity, low mortality rate and the developmental time was positively correlated with high temperature, therefore considered optimum temperature range for its survival and development. Other researches especially Integrated Pest management are imperative for sound management package as per the pest in Tanzania and beyond.

Keywords: Leafminer Biology; Citrus leafminer; Phyllocnistis citrella; Citrus leafminer fecundity and Citrus leafminer Oviposition.

1. INTRODUCTION

Tanzania is one of the countries known to have significant levels of citrus production in Africa although not among the worldwide major producers. Based on [1]; the quantity produced is 46,840 tons (from 13,312 ha) per annum which ranked Tanzania sixth after Morocco, South Africa, Egypt, Nigeria and the Democratic Republic of Congo. The crop is mainly produced in Tanga, Coast and Morogoro Regions while Dar es Salaam Region is popular for citrus seedling nurseries. In other regions, citrus is grown as door yard trees and not in plantations [2].

Citrus production in Tanzania is comparatively very low with reference to the world statistics. According to [1]; citrus production increased in quantity from about 6,000 tons in 1970 to 52,054 tons in 1999, the highest ever attained in Tanzania. Thereafter, the yield continuously declined to 35,706 tons in 2009 despite the new orchards and plantations established since early 2000. Largest area ever recorded under citrus was 42,475 ha in the year 2001. A biotic stress (drought and declined soil fertility) and biotic constraints (insect pests and diseases) are believed to have contributed to the decline in yield [3].

Diseases such as *Citrus tristeza* have been reported to exist wherever citrus is grown in Tanzania [4, 5]; reported that insect pests such as *Bactrocera invadens* and codling moths have been causing enormous damage to fruits with high economic loss. Recent observation on citrus leaf miner suspected to be micro-Lepidoptera of the species *Phyllocnistis citrella* in some orchards in Morogoro. While some efforts have been initiated to address the disease problems (*Citrus tristeza* and *Xanthomonas campestris* pv. c*itri*) and a few insect pests such as fruit flies (*Bactrocera invadens* and other species) little has been done to address the citrus leaf miner problem.

The citrus leaf miner, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is an important pest of citrus and related *Rutaceae* and ornamental plants almost worldwide [6]. Adults of *Phyllocnistis spp* are minute moths of 4 mm wingspread with white and silvery iridescent scales plus a black dot on each wingtip. It has 4 larval stages characteristically living by sap feeding in the new foliage (flush) leaving meandering serpentine mines [7]. At the end of fourth (resting) instar, the pest forms a cocoon at the edge of a leaf which causes a leaf to roll [8].Temperature has been observed to have high influence on development and fecundity of citrus leaf miner [9, 10]; observed that the life cycle of citrus leaf miner from egg to adult lasted for 13 to 52 days depending on weather conditions.

The citrus leaf miner mines the leave surface, tissue of young shoots, stems, and less frequently the fruits [11]. The attack by citrus leaf miner on young trees may result in death while older trees survive attack with reduced yield. Fruit development is not directly affected [10]; however, growth of nursery citrus stock and newly planted citrus trees can be retarded and subsequently reduce yields. Nonetheless, *P. citrella* may also help in the spread of

Xanthomonas axonopodis pv. *citri* leading to the citrus canker disease [12].

Despite its apparent economic importance and distribution in Tanzania, there is limited information on *P. citrella's* Biology, specifically developmental time, mortality rate, longevity and fecundity at different temperatures under laboratory conditions [13]. Therefore, the present study was aimed to examine effects of different temperature levels on biology of citrus leaf miner under controlled laboratory conditions.

2. MATERIALS AND METHODS

2.1 Identification of *P. citrella*

A survey was conducted in major citrus growing Districts of Tanzania viz: Morogoro, Muheza and Kinondoni and a total of 12500 trees were assessed. Leaf samples considered to contain larvae in serpentine mines were collected. A total of 1985 leaf samples were collected and individually incubated at room temperature in perforated plastic containers. The emerging adults were wet preserved in 70% ethanol while some dry preserved using special micro pins under microscope for future identification. A Monterey LG8920 pheromone trap was set in every 10 m^2 at SUA citrus orchard to trap adult moths to supplement sample sources. Citrus leaf miners species collected were identified as described by [14].

2.2 Assessment of *P. citrella's* Biology

2.2.1 Developmental and mortality rate of immature stages

Insects samples collected from orchards during surveys and those emerged from collected leaf samples were reared on *Citrus sinensis* seedlings at 27±3°C, 80±5 % RH and a photoperiod of 16 hours artificial light in a Caron's (Model 6045) insect growth chamber for four weeks as per protocol adopted by [15]. New citrus plants suitable for the pest to lay eggs upon were replaced once every week whilst those exhibiting hatched pupae were removed.

The saplings with fresh leaves were covered with tulle cages and 20-30 adults leaf miner were released into the cages, suctioned and the leaves were examined by hand magnification lens every other day. The generation time and mortality at different developmental stages were daily recorded until exclusion. Four different temperatures levels (20, 25, 30 and 35°C) were used and monitored at each developmental stage.

2.2.2 Longevity and fecundity of *P. citrella*

At exclusion, the newly emerged adults were kept at controlled temperatures (20, 25, 30 and 35°C) in the same tulle cages. Adults were fed with water soaked in sponge and honey stripe. To determine the daily number of eggs laid, the adults were taken from the cages with a suction trap and released to new saplings every day. The number of eggs laid per female was calculated by as follows:

$$Nel = \frac{Nec}{Nfm}$$

Where: Nel = Number of eggs laid per female, Nec = Number of eggs per cage and Nfm = Number of females.

Moreover, number of dead adults was recorded daily and the survival rate was established as the proportion of adults survived pre-established time intervals. A life table for each specific temperature was generated using formulae adopted by [16].

2.3 Data Analysis

The data obtained from daily observations were used to construct life tables according to [16]. Differences in developmental/generation time, mortality and fecundity were tested by analysis of variance (ANOVA) and Fisher's Least Significant Difference (LSD) test was used at 5 % level of probability.

3. RESULTS

3.1 Characteristic Features of *Phyllocnistis citrella*

The current findings revealed that the pest was typically identified as *Phyllocnistis spp*. The body was found to be slender of about 2 (+/-0.4) mm long in resting position and a wing span of about 4 (+/-0.2) mm. The adult had tarnished silver forewings with broadened longitudinal yellow fascia and wing expanse of about 5-7 mm. The colour was faint white with silvery scales on forewings and each wingtip had a black spot.

(a) (b) (c)

Plate 1. (a,b) Sepertine mines caused by *P. citrella* (c) Adult *P. citrella*
Sources: (a) [14] (b) Field survey, 2011 (c) [17]

3.2 Leaf Damage by *Phyllocnistis citrella*

Based on the observable symptoms on the leaf samples collected, the leaf damage corresponds to that of *P. citrella* (plates 1a and b). The adult *Phyllocnistis spp* (plate 1c) is most easily detected by its meandering serpentine larval mine, usually on the ventral side of the leaf. Larvae are minute (3 mm), translucent greenish-yellow, and located inside the leaf mine.

3.3 Biological features of *Phyllocnistis citrella*

3.3.1 Mean duration of oviposition, longevity and fecundity of *P. citrella*

The overall fecundity at all temperature levels was not significantly different. However, it was lower at 20°C with an average of 28.2 eggs per female and higher at 30°C with an average of 57.1 eggs per female. The oviposition period had been shortened with an increase in temperature. The highest *P. citrella* oviposition rate was recorded at 30°C with an average of 15.2 eggs per female per day, while the lowest was at 20°C with an average of 6.8 eggs per female per day. The females' longevity supersedes the males across the temperature regimes. It was observed to be 9.26 days at 20°C and 6.21 days at 30°C (Table 1). Both adult female and male *P. citrella* survived longer at 25°C although, the females lived for an average of 15.2 days while the males lived for 12.3 days. At 30°C adult female survived for a period of 6.3 days compared to the males that lived for only 4.9 days.

3.3.2 Developmental time and mortality rate of immature stages

The developmental time of egg, larval and pupal periods of *P. citrella* decreased with increasing temperatures (Table 2). The total developmental time from egg to adult stage ranged from 28.0 days at 20°C to 10.0 days at 35°C and the differed significantly (*P* = .05) at all temperature levels studied. Therefore, by increasing temperature from 20^0C to 35^0C, the egg, larval and pupal time to develop had been reduced from 5.2±0.10, 6.8±1.56 and 13.7±0.16 to 1.8±0.03, 3.9±0.95 and 3.7±0.18 respectively (Table 3).
The eggs' mortality rate ranged from 10% at 20°C to 3.33% at 30°C (Table 3). The insect mortality was higher at the larval stage compared to the egg stage while no mortality was recorded at the pupal stage of the pest.

4. DISCUSSION

The current findings had revealed that the pest was typically identified as *Phyllocnistis spp*. The observed descriptions were similar to [18-20]; who studied the pest in different ecological zones. The larva of *Phyllocnistis citrella* is known to be restricted to the plant family *Rutaceae*. Therefore, these had confirmed that the sample pest collected was morphologically *Phyllocnistis citrella*.

Moreover, [14]; suggested the specimen belong to genus Phyllocnistis. *Phyllocnistis* Zeller includes 87 described species, many of which are very small, with silvery vestiture, and similar in appearance [14, 21].The precise taxonomic placement of the genus has also remained questionable because of a lack of shared adult morphological characters with other micro-Lepidoptera [22]. So far, it's only one specie that is known to attack citrus; *Phyllocnistis citrella* Stainton.

According to a report by [23]; the larvae usually have three or more sap-feeding and one non-

Table 1. Mean duration of oviposition, longevity and fecundity of *P. citrella*

Temp.	Oviposition		Longevity (days)				No. of eggs per female	
^{0}C	N		N	Female	n	Male	per day	total
20	30	9.26±1.48 (5-14)	30	14.8±0.16 (12-16)	30	11.81±0.41 (9-12)	6.8±1.24 (0-10)	28.2±4.22 (18-38)
25	30	7.62±1.82 (3-11)	30	15.2±0.18 (16-18)	30	12.3±0.29 (11-15)	12.4±2.18 (0-16)	49.62±6.88 (20-96)
30	30	6.21±1.61 (2-9)	30	12.32±0.09 (11-17)	30	9.22±0.06 (7-13)	15.2±2.98 (0-22)	57.1±12.76 (28-89)
35	30	5.86±1.12 (2-7)	30	6.3±0.18 (5-8)	30	4.9±0.08 (3-7)	13.6±2.4 (0-20)	47.2±10.85 (0-98)

Table 2. Development of immature stages of *Phyllocnistis citrella*

Temp.	Duration of immature stages (days)							
^{0}C	N	Egg	N	Larva	n	Pupa	n	Total
20	60	5.2±0.10 (4-7)	50	6.8 ±1.56 (5-9)	46	13.7±0.16 (12-18)	46	28.0±0.21 (22-28)
25	78	2.9±0.08 (2-4)	72	5.5±1.45 (3-6)	68	7.5±0.08 (7-9)	68	15.4±0.29 (13-15)
30	93	2.7±0.03 (2-3)	90	4.6±1.04 (3-6)	86	4.4±0.09 (3-7)	86	12.0±0.06 (11-13)
35	125	1.8±0.03 (1-7)	117	3.9±0.95 (4-7)	113	3.7±0.18 (4-8)	113	10.0±0.08 (10-13)

Table 3. Mortality of eggs and immature stages of *P. citrella*

Temp.	Mortality of eggs and immature stages					Total mortality	Mortality(%)
	Egg		Larva		Pupa	(egg to adult)	(egg to adult)
	N	(%)	N	(%)	n		
20	30	10	27	18.52	22	8	26.67
25	30	6.67	28	7.14	26	4	13.33
30	30	3.33	29	3.45	28	2	6.67
35	30	3.33	29	6.9	27	3	10

feeding instars with highly specialized cocoon-spinning instar. During its larval stages, *P. citrella* mines the adaxial and abaxial surfaces of new leaves [24]. A newly hatched larva immediately chews into the leaf from its egg and start feeding on the internal parts of the leaf by shearing the plant tissue [10]. The larva creates a long, slender, sub-epidermal serpentine mine with a characteristic median frass line at the terminus of which a pupal chamber (pupal cocoon fold) is constructed, usually from the curled edge of the leaf [23]. All the above characteristics had corroborates the current study and therefore the damage symptoms were the same and thus the pest is said to be confirmed *Phyllocnistis citrella* Stainton (1856) that belongs to the order Lepidoptera and the family Gracileriidae.

The present study recorded that developmental time of the egg, larval and pupal periods of *P. citrella* decreased with increasing temperatures (Table 2). The total developmental time from egg to adult stage had decreased from 28.0 days at 20°C to 10.0 days at 35°C. The current findings corroborate the research by [25]; that increasing temperature significantly shortened the developmental period of immature stages of *P. citrella*. Mean developmental duration varied from 25 days at 20°C to about 11days at 35ºC. Moreover, [26]; on the biology of *P. citrella* in northern Iran reported that the total developmental duration (egg to egg) of *P. citrella* as 19 and 16.57 days at 25 and 30^{0}C respectively. Similar trends were also reported by several researchers [7, 27–30]; that egg, larval and pupal periods and the total life cycle as 2-6, 6-7, 6-7 and 14-18 days all at 33^{0}C respectively. Nonetheless, in a field study in India, [31]; recorded an incubation period of 2-10 days, a larval period of 5-10 days, a pupal period of 6-20 days and a total life cycle of 13-52 days in the field during November-January with no indication of the temperature.

Total mortality was high at 20°C (26.67%), but relatively low at the other temperatures indicating that the lower the temperature the higher the mortality rate. This means that *P. citrella* can optimally survive better in warm compared to the cool climates. In a field study as reported by [7]; insect mortality was higher when temperature was also high. Similarly, [27]; reported that 19 % of the eggs laid by the pest in the field died while [30]; recorded only 5.2 % of the larvae developed into pupa while the total *P. citrella* mortality during development amounted to 96 %. The highest mortality during all stages was observed at 35^0C and the lowest recorded at 27^0C [25].

According to [32]; 60-80 % mortality of the larval instars was recorded however; these figures are much higher than the values recorded in this study. Therefore, the direct impact of temperature seems not only the mortality factor, but rather other factors especially relative humidity and the insect species (especially natural enemies that predates and/or feed on the larvae) which might be responsible for the high mortality in the field. Moreover, exposure to pesticides and other agrochemicals in the field might negatively affect the pest's mortality in the field compared to those managed in the laboratory.

According to [27]; the longevity of 2.37 and 3.75 days for males and females *P. citrella* respectively and 24.87 eggs per female were recorded while both sexes and the number of eggs were lower in longevity. This is at per with the current study. Nonetheless, [28]; reported *P. citrella's* longevity of 1.0-22.5 days and 1.0-7.5 days for females and males respectively and 7-108 eggs per female without temperature. More so, [29]; observed that females could live 5-10 days and lay 20 eggs par night and more than 50 eggs per its life. This correspond the present study especially at the constant temperatures of 25°C and 30°C. [33]; observed longevity of 2-12 days in adult which could increase to 20 days and a fecundity of 48 (36-76) eggs during its life. At all temperatures tested females predominated in number over the males and the highest ratio was at 30°C with 1.5:1.0 (female: male). Similarly, [7]; reported that the females slightly surpassed the males in number and these are in conformity with the current study at all temperatures studied. The results of the life tables showed that at 30°C the female: male ratio was highest and mortality was lowest compared to the other temperatures studied and therefore concluded that 30°C was the optimal temperature for the pest to grow.

5. CONCLUSIONS

The current study revealed that the pest has the optimum survival temperature range from 20 (minimum) to 30^0C (maximum). Therefore, the pest at this temperature range has high fecundity, low mortality rate and the developmental time was positively correlated with high temperature, thus considered the optimum temperature range for its survival, development and perpetuation. Field studies on aspects of the biology in the surveyed regions and beyond would be imperative. Moreover, other researches especially Bio-control, Integrated Pest management (IPM) and genetic characterization are of paramount importance in order to come up with a sound management package as per Citrus leaf miner pest in Tanzania and beyond.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDICES

Life tables for *P. citrella* at different temperatures:

Appendix A: Life tables for *P. citrella* at temperature of 20^{0}C

Age (day)	Total No. of individuals (Nx)	Survivorship lx=Nx/No	Mortality rate dx=lx-lx+1	Probability of dying qx=dx/lx	Mean No. of moths alive Lx=(Nx+Nx+1)/2	Life expectancy Ex=ΣLx/Nx
1	15	1.00	0.13	0.13	14	4.50
2	13	0.87	0.07	0.08	12.5	
3	12	0.80	0.13	0.17	11	
4	10	0.67	0.07	0.10	9.5	
5	9	0.60	0.13	0.22	8	
6	7	0.47	0.13	0.29	6	
7	5	0.33	0.13	0.40	4	
8	3	0.20	0.13	0.67	2	
9	1	0.07	0.07	1.00	0.5	

Appendix B: Life tables for *P. citrella* at temperature of 25^{0}C

Age (day)	Total No. of individuals (Nx)	Survivorship lx=Nx/No	Mortality rate dx=lx-lx+1	Probability of dying qx=dx/lx	Mean No. of moths alive Lx=(Nx+Nx+1)/2	Life expectancy Ex=ΣLx/Nx
1	15	1.00	0.00	0.00	15	14.4
2	15	1.00	0.00	0.00	15	
3	15	1.00	0.00	0.00	15	
4	15	1.00	0.07	0.07	14.5	
5	14	0.93	0.00	0.00	14	
6	14	0.93	0.07	0.07	13.5	
7	13	0.87	0.00	0.00	13	
8	13	0.87	0.00	0.00	13	
9	13	0.87	0.00	0.00	13	
10	13	0.87	0.07	0.08	12.5	
11	12	0.80	0.00	0.00	12	
12	12	0.80	0.07	0.08	11.5	
13	11	0.73	0.07	0.09	10.5	
14	10	0.67	0.00	0.00	10	
15	10	0.67	0.07	0.10	9.5	
16	9	0.60	0.07	0.11	8.5	
17	8	0.53	0.20	0.38	6.5	
18	5	0.33	0.07	0.20	4.5	
19	4	0.27	0.13	0.50	3	
20	2	0.13	0.07	0.50	1.5	
21	1	0.07	0.07	1.00	0.5	

Appendix C. Life tables for *P. citrella* at temperature of 30^{0}C

Age (day)	Total No. of individuals (Nx)	Survivorship lx=Nx/No	Mortality rate dx=lx-lx+1	Probability of dying qx=dx/lx	Mean No. of moths alive Lx=(Nx+Nx+1)/2	Life expectancy Ex=ΣLx/Nx
1	15	1.00	0.00	0.00	15	16.83
2	15	1.00	0.00	0.00	15	
3	15	1.00	0.00	0.00	15	
4	15	1.00	0.00	0.00	15	
5	15	1.00	0.00	0.00	15	
6	15	1.00	0.07	0.07	14.5	
7	14	0.93	0.00	0.00	14	
8	14	0.93	0.00	0.00	14	
9	14	0.93	0.00	0.00	14	
10	14	0.93	0.07	0.07	13.5	
11	13	0.87	0.00	0.00	13	
12	13	0.87	0.00	0.00	13	
13	13	0.87	0.07	0.08	12.5	
14	12	0.80	0.00	0.00	12	
15	12	0.80	0.07	0.08	11.5	
16	11	0.73	0.00	0.00	11	
17	11	0.73	0.00	0.00	11	
18	11	0.73	0.20	0.27	9.5	
19	8	0.53	0.27	0.50	6	
20	4	0.27	0.07	0.25	3.5	
21	3	0.20	0.07	0.33	2.5	
22	2	0.13	0.07	0.50	1.5	
23	1	0.07	0.07	1.00	0.5	

Appendix D. Life tables for *P. citrella* at temperature of 35^{0}C

Age (day)	Total No. of individuals (Nx)	Survivorship lx=Nx/No	Mortality rate dx=lx-lx+1	Probability of dying qx=dx/lx	Mean No. of moths alive Lx=(Nx+Nx+1)/2	Life expectancy Ex=ΣLx/Nx
1	15	1.00	0.20	0.20	13.5	3.77
2	12	0.80	0.20	0.25	10.5	
3	9	0.60	0.07	0.11	8.5	
4	8	0.53	0.07	0.13	7.5	
5	7	0.47	0.13	0.29	6	
6	5	0.33	0.07	0.20	4.5	
7	4	0.27	0.07	0.25	3.5	
8	3	0.20	0.13	0.67	2	
9	1	0.07	0.07	1.00	0.5	

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