The Effect of Temperature on Development and Fecundity of Citrus Leafminer, *Phyllocnistis citrella*

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Abstract

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Citrus leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillaridae), recently has become a serious pest in Syria, especially in citrus nurseries. Development and fecundity of *P. citrella* were recorded at four constant temperatures ranging from 20, 25, 30, 35±1°C, 70±10% relative humidity and photoperiod of 14:10 hours (L:D) and on seedlings of *Citrus sinensis* cultivar Valencia as host. This study was carried out in the laboratories of Latakia Center for Breeding and Applications of Biological Enemies during 2022. Results obtained indicated that the developmental time (egg to adult) of *P. citrella* decreased with increasing temperatures, ranging from 20.82 days at 20°C to 10.25 days at 35°C. Results also showed significant reduction in the incubation period from 7.58 days at 20°C to 2.66 days at 35°C, and mortality rate increase from 13 to 22% at 20 and 35°C. respectively. The larval period was 9.44, 5.24, 4.48 and 4.0 days and pupal period was 9.22, 6.84, 5.76 and 4.78 days at 20, 25, 30 and 35°C, respectively. The highest mortality rate in egg, larval and pupal periods was observed at 35°C (22.00, 11.50 and 15.94%, respectively). At all temperatures studied the females lived significantly longer than the males. Both females and the males lived longer at a temperature of 20°C (the female 13.3 and the male 11.5 days) and shorter at 35°C (5.8 days for females and 4.5 days for males). The study showed that the preoviposition period was very short. Results showed significant reduction in Oviposition period, ranging from 8.43 to 5.52 days at 20 and 35°C, respectively. The fecundity was found to be 27.74, 46.413, 57.8 and 49.5 eggs/female at 20, 25, 30 and 35°C, respectively. The sex ratio for the offspring was 1:1.2, 1:1.4, 1.0: 1.5 and 1:1 male: female at 20, 25, 30 and 35°C, respectively. It can be concluded from this study that the temperature of 30°C was optimal for *P. citrella* development.
Keywords: Citrus leaf miner, *Phyllocnistis citrella*, development, fecundity, sex ratio.

Introduction

Many insect pests were recorded to attack citrus trees, some of them cause serious damage and crop economic loss (Ghanim, 2009; Moustafa, 2004). The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) is a key pest that limits the production of citrus in Syria and worldwide (Abo-Kaf *et al*., 2006), especially to young trees (Ghanim, 2009; Heppner & Dixon, 1995; Moustafa, 2004; Smith & Hoy,1995). It causes damage to citrus through mining and feeding on the parenchymatous tissues of the youngest tender leaves. *P. citrella* produces silvery mines on the surface of fruits, leaf and stem and reduces the quality of fruits, and photosynthetic area of the leaf which eventually reduces the quantity of production (Abdalla & Mohammed., 2004; Belasque *et al*., 2005). Moreover, CLM has also been associated with transmission of the citrus canker disease caused by *Xanthomonas axonopodis* pv. *citri* (Atiq *et al*., 2007; Jesus *et al*., 2006).

In spite of the wide distribution and apparent economic importance of *P. citrella*, there is limited information on its biology and ecology. This knowledge would prove useful in improvement of control programs directed against the pest in citrus orchards. The present study was designed to study the effect of different temperatures on development and fecundity of *P. citrella* under controlled laboratory conditions.

Materials and Methods

Host plants

The laboratory work was carried out at the Latakia Center for the Breeding and Applications of Biological Enemies. The center is located on the semi-humid bioclimatic floor, 12 km from the city of Latakia. Citrus (Valencia) seeds were grown using the method of Smith & Hoy (1995). The seeds were sown in a mixture of vermicompost and sand at a ratio of 1:1. In a seedling tray containing 100 holes (2.5×2.5×6 cm). Each seedling with 3-4 leaves was transferred to a little plastic pot (8 cm in diameter). Seedlings with 30 to 50 cm tall were ready to be used as hosts for the citrus leafminer.

Insect colony

Infested flushes were obtained from citrus trees of the Valencia variety grafted on sour orange rootstock from a citrus orchard in the village of Fedeo (Latakia Governorate) to supplement the insect colony. After formation of pupae at the leaf edge, they were separated from the infested leaves with a soft brush and placed into a photographic film's canister, containing moist cotton. The last abdominal segment was longer in female pupae which included two long hairs that are lacking in males (Jacas & Garrido, 1996). After moths emergence, one female and male pair were introduced to a Valencia young twig covered with a small fine mesh cage. Cotton wool moistened with honey solution (10%) was placed in each cage to provide food. The cages were kept in growth rooms at 70±10% RH and photoperiod

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of 14 hours under different constant temperatures of 20, 25, 30 and 35±1°C. Every other day, the laid eggs were counted. For each temperature, 100 leaves containing individual eggs were transferred to a separate petri dish kept in the incubator under the same conditions. The duration and the mortality of different developmental stages were recorded. Lack of new mines, pupal chambers or moth emergence were used as indicators of egg, larval or pupal mortality, respectively. The sex ratio of pupae was determined before adult emergence and then the mortality and longevity of female and male moths were recorded.

The developmental rate of immature stages was estimated. Number of individuals that failed to complete their development were excluded from the analysis. Developmental times for each stage and total stages (egg – adult) were determined.

Data analysis

Differences between treatments were analysed by one-way analyses of variance (ANOVAs), followed by a Tukeyís test for multiple comparisons at P<0.05 (SPSS, 2007). All data were expressed as mean±SE. The traditional linear model was used to predict the lower developmental threshold and thermal constant of developmental stages (egg to adult) of *P. citrella*.

Results and Discussion

Development and mortality rate of immature stages

Temperature increase significantly shortened the developmental period of immature stages of *P. citrella*. The development period from egg to adult insect ranged from 20.82 days at 20°C to 10.25 days at 35°C. The mortality rate was lower at 30°C then at 25°C, and the difference was statistically significant at P=0.01. The mean duration of embryonic development was 7.58±0.18, 3.50±1.77, 2.33±1.54 and 2.26±0.18 days at temperatures of 20, 25, 30 and 35°C, respectively. The average duration of larval development was 9.44±1.21, 5.24±0.614, 4.48±0.55 and 4.0±0.28 days at temperatures of 20, 25, 30 and 35°C, respectively. The average pupal duration was 9.22±0.787, 6.84±0.898, 5.76±0.89 and 4.789±0.149 days at temperatures of 20, 25, 30 and 35°C, respectively. Average duration of development from egg to adult was 20.823±1.75, 14.4±1.515, 11.8±2.117 and 10.25±1.242 days at temperatures of 20, 25, 30 and 35°C, respectively.

A study by Jafari *et al*. (2000) on the biology of citrus leaf miner on Valencia seedlings in the northern regions of Iran, showed that the total growth period of the insect (from egg to egg) is 19 and 16.57 days at temperatures of 25 and 30°C. Safaralizade (2008) showed that the average development period for the egg, larva and pupa of the citrus leaf miner was 3.65, 8.95 and 7.5 days, respectively, at a temperature of 25°C, a relative humidity of 70% and a photoperiod of 10:14 hours (light:dark). The total development period for the immature stages of the insect was 20.1 days. Pinto & Fucarin (2000) showed that the incubation period was 2.1 days, the duration of larval development was 4.5 days, the prepupal period 1.7 days, and the pupal period 5.7 days at a temperature of 27°C, 60-70% relative humidity, and 14 hours of light. Chagas & Parra (2000) studied the effect of seven temperatures (18, 20, 22, 25, 28, 30 and 35±1°C), and the shortest developmental periods for larvae and pupae were at 32°C (3.6 and 6.4 days, respectively). Elekçioğlu & Uygun (2004) study of the biology of the citrus miner insect in Turkey at temperatures 15, 20, 25, 30 and 35°C showed that the incubation period for the egg decreased from 7.3 days at a temperature of 15°C to 1.9 days at a temperature of 35°C, and the developmental period of the larvae decreased from 21.0-23.9 days at a temperature of 15°C to 4.0-4.1 days at a temperature of 35°C. The highest mortality rate for the immature stages of the insect was at a temperature of 15°C, and the lowest at a temperature of 30°C.

Results obtained at all tested temperatures showed that the mortality rate was low in the larva and pupal stages, and was higher during the egg stage. The mortality rate was higher at 35°C for all stages of the insect (Table 2). The slightly higher mortality rate at 35°C may be due to the fast development of the leaves to become harder and consequently unsuitable for the larvae that could not feed on them any more and died (Elekçioğlu & Uygun, 2004).

The results obtained in this study were not in agreement with the results of Elekçioğlu & Uygun (2004), who reported at five constant temperatures (15, 20, 25, 30, 35±1°C) and 25-35°C varying temperatures, that the mortality rate at all studied temperatures was low during the egg period and higher in the larval period, whereas no deaths were detected during the pupal period.

Table 1. Mean duration and range (days) of immature stages of *Phyllocnlstls citrella* at different temperatures.

Temperature (°C)	Duration of immature stages (days)							
	Number	Egg	Number	Larva	Number	Pupa	Number	Total
20	100	7.58 a (4-11)	87	9.44 a (7-13)	82	9.22 a (8-14)	100	20.82 a (17-28)
25	100	3.50 b (2-7)	91	5.24 b (3-8)	86	6.84 b (8-11)	100	14.40 b (12-24)
30	100	2.33 c (1-6)	90	4.48 c (3-7)	84	5.76 b (4-10)	100	11.80 c (8-20)
35	100	2.26 c (1-5)	78	4.00 c (3-7)	69	4.78 c (3-9)	100	10.25 d (8-18)

Means followed by the same letter in the same colomn are not significantly different at P=0.01, according to Duncan's multiple range test.
Values in paranthesis are minimum and maximum values

Some larvae developed fast and consumed the leaf before it was fully developed. In both the field and laboratory observations it was concluded that death was also caused by not crossing the existing mines and not feeding when meeting with one's own or another larvaes' mines, which is in agreement with the findings of other workers (Heppner, 1993; Siu-King & Ren-Guang, 1980; Stansly & Rouse, 1993). These results could probably be due to the protection of the pupae by the pupal cell.

At all temperatures studied, the mortality rate was higher during the egg period than in the larval and pupal periods. The mortality rate of eggs ranged from 22% at 35°C to 13% at 20°C (Table 2), and at the larval stage ranged between 5.494% at 25°C to 11.5% at 35°C. The mortality rate of pupa ranged from 2.235% at 25°C to 15.94 % at 35°C. These results are not in agreement with previous finding (Elekçioğlu & Uygun, 2004).

Longevity and fecundity

The life span of the full insect at different temperatures differed according to the sex of the insect (female or male). Both the female and the male lived longer at a temperature of 20°C (the female 13.3 and the male 11.5) days, and at the temperature of 25°C (11.5 days for females and 9.5 days for males) days and at a temperature of 30°C (7.5 for a female and 5.7 for a male) a day, and the lowest period was at a temperature of 35°C (5.8 days for females and 4.5 days for males). The life span of females was longer than that of males, which is in agreement with previous workers (Elekçioğlu & Uygun, 2004; Rathod *et al.,* 2020). Knapp (1995) showed that adults live from 2-12 days, which can be increased to 20 days. Abo-Kaf *et al.* (2006) reported that adult longevity without any feeding was 1.7±0.5 days for males, 2.3±0.59 days for females, whereas Jadhav (2015) reported that longevity for males and females was 5.6±0.99 and 7.0±0.77 days, respectively.

Results obtained in this study indicated that the preoviposition period was very short, and the highest value was reached at 20°C with an average of 1.1 days, which is in agreement with a previous report (Elekçioğlu & Uygun, 2004). Rathod *et al*. (2020) indicated that the pre-laying period ranged from 1.0 to 1.5 days, the laying period ranged from 3.0 to 3.5 days, and the post-laying period ranged from 1.0 to 1.5 days.

Ba-Angood (1977) reported earlier that mating of adult insects 9-11 hours after emergence from the pupal stage, occured at night. They live a short period (24-36 hours) which is in agreement with this study as well as that of Elekçioğlu & Uygun (2004). The egg-laying period differed according to the temperature, and decreased with the increase in temperature (Table 3).

The fertility of the adult insect differed according to the temperature used, and was lowest at 20°C (27.74 eggs/female), and reached 46.41 eggs/female at 25°C, and increased to 57.8 female eggs/female at 30°C, then decreased to 49.5 eggs/female at 35°C, and these results were more or less in agreement to what has been reported earlier by several workers (Atapour & Osouli, 2017; Beattie & Smith, 1993; Elekçioğlu & Uygun, 2004; Jadhav, 2015; Knapp, 1995; Radke & Kandalkar, 1987; Rathod *et al.,* 2020).

The sex ratio (male:female) differed according to temperature (Table 4), and was lowest at 30°C (1.0:1.5), which was in agreement with the results obtained earlier by several workers (Atapour & Osouli, 2017; Ba-Angood, 1977; Elekçioğlu & Uygun, 2004; Jadhav, 2015; Rathod *et al.,* 2020; Maryam & Shiva, 2017).

Table 2. Mortality rate (%) of eggs and immature stages of *Phyllocnistis citrella* at different temperatures.

Temperature (°C)	Mortality rate of eggs and immature stages (%)							
	Number	Eggs	Number	Larvae	Number	Pupae	Number	(Egg to adult)
20	100	13.00 b	87.00	5.74 b	82.00	2.32 b	100	20.0 b
25	100	9.00 c	91.00	5.49 b	86.00	2.23 b	100	16.0 c
30	100	10.00 c	90.00	6.66 b	84.00	4.76 b	100	20.0 b
35	100	22.00 a	78.00	11.50 a	69.00	15.94 a	100	42.0 a

Means followed by the same letter in the same Column are not significantly different according to Duncan's multiple range test at P=0.01.

Table 3. Pre-oviposition, oviposition, post-oviposition period and fecundity of *Phyllocnistis citrella* along the Syrian coast.

Temperature (°C)	Traits investigated				
	Pre-oviposition period (days)	Oviposition period (days)	post-oviposition period (days)	Fecundity	
				Eggs per day	Total
20	1.10 (2-1)	8.43±1.50 (5-12)	1.50 (0-4)	6.29±0.80 c* (0-9)	27.74±13.90 c (19-45)
25	Less than 1.0	7.65±1.82 (2-8)	<1 (0-2)	14.84±2.75 b (0-20)	46.41±13.80 b (12-83)
30	Less than 1.0	6.35±1.65 (1-9)	<1 (0-2)	17.60±2.93 a (0-25)	57.80±25.08 a (15-81)
35	Less than 1.0	5.52±1.13 (2-7)	<1 (0-2)	14.40±2.43 b (0-22)	49.5±17.54 b (15-74)

Means followed by the same letter in the same column are not significantly different according to Duncans multiple range test at P=0.01. Values in parentheses are minimum and maximum values.

Table 4. Sex ratio of *Phyllocnistis citrella* observed at different temperatures.

Temperature °C	Total number of adults observed	Number of Male	Number of Female	Sex ratio Male: female
20	50	22	28	1.0:1.2
25	50	18	32	1.0:1.4
30	50	15	35	1.0: 1.5
35	50	25	25	1.0:1.0

الملخص

ميهوب، سماري، عبد النبي بشير وهيفاء السيدة. 2025. تأثير درجة الحرارة في تطور وخصوبة حافرة أوراق الحمضيات (*Phyllocnistis citrella*). مجلة وقاية النبات العربية، 43(1):33-37. https://doi.org/10.22268/AJPP-001293

تعدّ حشرة حافرة أوراق الحمضيات (*Phyllocnistis citrella*) (Lepidoptera: Gracillaridae) آفةً بالغة الخطورة في سورية، وبخاصةٍ في مشاتل الحمضيات. تمّ في هذا البحث دراسة تطور وخصوبة حشرة *P. citrella* عند أربع درجات حرارة ثابتة، وهي: 20، 25، 30 و 35±1°س، ورطوبة نسبية 70±10%، وفترة ضوئية 10:14 ساعة (ضوء: ظلام). تمّت الدراسة على شتلات *Citrus sinensis* صنف "فالنسيا" كعائل. أجريت هذه الدراسة في مختبرات مركز اللاذقية لتربية وتطبيقات الأعداء الحيوية خلال عام 2022. بيّنت الدراسة انخفاض مدة تطور الحشرة (من بيضة إلى حشرة كاملة) لـ *P. citrella* مع ارتفاع درجات الحرارة، حيث تراوحت ما بين 20.82 يوماً عند درجة حرارة 20°س إلى 10.25 يوماً عند درجة حرارة 35°س. أظهرت النتائج انخفاضاً معنوياً في مدة حضانة البيض من 7.583 عند درجة حرارة 20°س إلى 2.66 يوماً عند درجة حرارة 35°س، وزيادة الوفيات من 13 إلى 22% عند 20 و 35°س، على التوالي. بلغ متوسط طول عمر اليرقات 9.44، 5.24، 4.48 و 4.0 يوماً، ومتوسط طول عمر العذراء 9.22، 6.84، 5.76 و 4.78 يوماً عند متوسط درجات حرارة 20، 25، 30 و 35°س، على التوالي. سُجّل أعلى معدل وفيات في البيض واليرقات والعذارى عند درجة حرارة 35°س (22.0، 11.5 و 15.94%، على التوالي). عاشت الإناث تحت جميع درجات الحرارة التي تمت دراستها مدّةً أطول معنوياً من الذكور. عاش كلّ من الأنثى والذكر لمدّةٍ أطول عند درجة حرارة 20°س (الأنثى 13.3 والذكر 11.5 يوماً) ومدّةً أقصر عند درجة حرارة 35°س (5.8 يوماً للإناث و 4.5 يوماً للذكور). أوضحت الدراسة أن فترة ما قبل وضع البيض وما بعد وضع البيض كانت قصيرة للغاية. أظهرت النتائج انخفاضاً كبيراً في فترة وضع البيض (تراوحت بين 8.43 إلى 5.52 يوماً) عند درجة حرارة 20 و 35°س، على التوالي. بلغت الخصوبة 27.74، 46.41، 57.80 و 49.50 بيضة/أنثى عند درجة حرارة 20، 25، 30 و 35°س، على التوالي. بلغت النسبة الجنسية 1: 1.2، 1: 1.4، 1.0: 1.5 و 1: 1 ذكر: أنثى عند درجة حرارة 20، 25، 30 و 35°س، على التوالي. خلصت النتائج إلى أن درجة الحرارة المثلى لنمو وتطور حشرة *P. citrella* هي 30°س.

كلمات مفتاحية: حافرة أوراق الحمضيات، *Phyllocnistis citrella*، التطور، الخصوبة، النسبة الجنسية.

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