Effect of temperature on biology of Citrus Leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) under lab conditions

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Abstract

Citrus leafminer, *Phyllocnistis citrella* Stainton, recently has become a serious pest in Iran, especially in citrus nurseries. This study was aimed to assess the effect of temperature on the biological parameters of the *P. citrella* at three constant temperatures: 20, 27 and 35°C, 70±10% RH, photoperiod of 14: 10 (L: D) and on seedlings of *Citrus sinensis* cultivar Valencia as host. Results showed significant reduction in incubation period from 6.22 at 20°C to 2.18 days at 35°C and adversely mortality increase from 7 to 21% at 20 and 35°C, respectively. Larval period was 8.06, 5.26 and 4.14 days and pupal period was 11.18, 6.86 and 5.03 days at 20, 27 and 35°C, respectively. The highest mortality in both larval and pupal periods was observed at 35°C (11.4 and 15.8%, respectively). Both females and males lived longer at 20°C (9.31 and 7.38 days) and shorter at 35°C (5.38 and 4.21 days, respectively). Moth emergence rate was the highest at 27°C (female: 82.2% and male: 84.5%). The sex ratio for the offspring was 1:1.2, 1:1.4 and 1:1.3 male: female at 20, 27 and 35 °C, respectively.

Key words: Citrus leaf miner, *Phyllocnistis citrella*, developmental time, mortality

اثر دما بر زیست‌شناسی مینوز برگ مرکبات، *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) در شرایط آزمایشگاهی

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چکیده

مینوز برگ مرکبات، *Phyllocnistis citrella* Stainton، در سال‌های اخیر به یک آفت جدی مرکبات به‌ویژه در نهالستان‌ها تبدیل شده‌است. در مطالعه کنونی زیست‌شناسی آفت در سه دمای ۲۰، ۲۷ و ۳۵ درجه سلسیوس، رطوبت نسبی ۷۰±۱۰ درصد و دوره روشنایی ۱۴ ساعت روی گیاهچه‌های پرتقال رقم والنسیا به عنوان میزبان بررسی شد. نتایج نشان دادند طول دوره جنینی از ۶/۲۲ روز در دمای ۲۰ درجه سلسیوس به ۲/۱۸ روز در دمای ۳۵ درجه سلسیوس کاهش یافت در حالی‌که به طور معکوس با افزایش دما میزان تلفات از ۷ به ۲۱ درصد افزایش یافت. دوره لاروی در سه دمای مذکور به ترتیب ۸/۰۶، ۵/۲۶ و ۴/۱۴ روز و دوره شفیرگی به ترتیب ۱۱/۱۸، ۶/۸۶ و ۵/۰۳ روز برآورد شد. بیشترین مرگ و میر دوره لاروی و شفیرگی در دمای ۳۵ درجه سلسیوس به ترتیب ۱۱/۴ و ۱۵/۸ درصد مشاهده شد. طول عمر حشرات کامل ماده و نر در دمای ۲۰ درجه سلسیوس طولانی‌تر (به‌ترتیب ۹/۳۱ و ۷/۳۸ روز) و در دمای ۳۵ درجه کوتاه‌تر (به ترتیب ۵/۳۸ و ۴/۳۱ روز) بود. بالاترین درصد ظهور حشرات کامل در دمای ۲۷ درجه مشاهده شد (۸۲/۲ درصد در ماده‌ها و ۸۴/۵ درصد در حشرات نر). نسبت جنسی در دمای ۲۰، ۲۷ و ۳۵ درجه سلسیوس به ترتیب ۱: ۱/۲، ۱: ۱/۴ و ۱: ۱/۳ (نر: ماده) محاسبه شد.

کلمات کلیدی: مینوز برگ مرکبات، *Phyllocnistis citrella*، دوره نمو، مرگ و میر

Introduction

Iran is the fourth largest citrus producing country after China, India and Pakistan in Asia with 164 thousand hectares cultivated area and about 2600 thousand tons of citrus fruit production per year (FAO, 2014). Citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is a serious pest that is able to deposit 50 eggs during its life cycle. Its larvae have 4 instars (3 instars and a prepupal stage) before they enter to pupal stage, which occurs within the mine (Beattie and Smith, 1993; Heppner, 1995). Adults live for only about one week. Their entire life cycle requires 14 to 50 days depending on temperature. This pest is active throughout the seasons and completes 5-13 generations in a year. The larvae mine immature foliage, leading to reduction of yield, especially in nursery and newly planted trees (Ba-Angood, 1977, 1978; Heppner, 1995; Cardwell *et al.*, 2008). In addition, these mines provide an opening for the entry of pathogens such as *Xanthomonas axonopodis* pv. *citri,* a bacterium that causes citrus canker (Chagas *et al.*, 2001). During recent years, many types of insecticides have been applied against *P. citrella* leading to pest resistance and elimination of its natural enemies.

This study was intended to improve the existing knowledge about the biology and ecology of the pest in terms of its developmental rates, lower and higher thermal thresholds, thermal constants and overwintering. Ba-Angood (1977, 1978) reported the egg, larval, pupal and total life cycle as 2-6, 7-8, 8-9 and 18 days respectively at the field condition and 9.5-3.5, 18.5-10.4, 19.5-9.2 and 50-23 days respectively at 20, 25, 30 and 35°C. Huang *et al.* (1989) recorded these periods as 0.5- 10.5, 3.0-49.5 and 3.5-17.0 days and Beattie and Smith (1993) found them to be 1-10, 5-6, 6-22 days, respectively, although the temperatures are not included in these studies. Elekcioglu and Uygun (2004) studied the development and fecundity of this pest at 5 constant temperatures and calculated these periods as 7.3-1.9, 21-4.1, 23.9-4 and 51.7-10.1 respectively at 15, 20, 25, 30 and 35°C. Their results showed that at 30°C the net reproduction rate and female: male ratio was highest and mortality was lowest compared to the other temperatures.

In 1961, *P. citrella* was recorded for the first time from southern provinces (Farahbakhsh, 1961), and then in 1995 citrus leafminer discovered in north parts of Iran (Jafari, 1995). Jafari *et al.* (2000) argued that total developmental time was about 19 and 16.5 days at 25 and 30°C, respectively.

In another study, average duration of egg, larval, prepupal and pupal stages of *P. citrella* was reported as 3.65±1.37, 8.95±1.85 and 7.5±1.91 days (mean±SD), respectively, under 25°C and 70% RH conditions (Namvar and Safaralizade, 2008). We have studied the effect of three constant temperatures on developmental rate and fecundity of *P. citrella* in order to provide better understanding of its life history in Iran.

Materials and Methods

Host plant

Seeds of *Citrus sinensis* var. Valencia were planted using the method described by Smith and Hoy (1995) with little modifications. The seeds were planted in a 1:1 mixture of vermicompost and sand in a cavity seedling tray (Hummert International, Earth City, MO) containing 96 cavities (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.

Insects

Infested flushes were obtained from Dashte-Naz nurseries in Mazandaran province located in northern part of Iran (36.6° N, 52.1° E; 16 m a.s.l.) to supplement the insect colony. After formation of pupa cells at the leave edge, pupae were separated from the infested leaves with a soft brush and put in to the photographic film's canisters, containing moist cotton. The last abdominal segment was longer in female pupae which included two long hairs lacking in males (Jacas and Garrido, 1996). After the emergence of moths, one pair of female and male were introduced to a Valencia sapling covered with a small tulle cage. To provide the food, cotton wool moistened with honey solution (%10) was placed in each cage. The cages were kept in climatic rooms at 70±10% RH and photoperiod of 14 hours under different constant temperatures, 20, 27 and 35±1°C. Every other day, the laid eggs were counted. For each temperature, 100 leaves containing individual egg were transferred to a separate petri dish kept in incubator under the same conditions. The duration and the mortality at different developmental stages were recorded. Lack of a new mine, pupal chamber 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.

Climatic Data

Outdoor climatic data, including monthly average of minimum, maximum, and mean temperature, during 2006-2014 was provided by the weather station of Meteorological Organization in Dashte-Naz station.

Mathematical models and data analysis

The linear models have been used widely to estimate the lower developmental threshold (T_{min}) and thermal constant (K) of arthropods (Campbell *et al*., 1974; Lactin *et al*., 1995). In this study the traditional and Ikemoto-Takai linear models were used to predict the lower developmental threshold and thermal constant of developmental stages (egg to

adult) of *P. citrella.* The traditional and Ikemoto–Takai linear formula are shown in the following equations, respectively (Campbell *et al.*, 1974; Ikemoto and Takai, 2000):

$$D = \frac{K}{(T - T_{min})}$$

$$DT = K + T_{min} \times D$$

Where D indicates the duration of development (days), T, ambient temperature, T_{min}, the lower temperature threshold and K is the thermal constant. The latter function was proposed by Ikemoto and Takai (2000). This equation is derived from the traditional linear model to obtain more reliable estimates of the parameters.

The traditional and Ikemoto-Takai linear models were analyzed using linear regression of Minitab, v 17.1. The SE of T_{min} and K in Ikemoto-Takai linear model were obtain directly from the regression analysis but in the traditional model, the standard errors were calculated from the following equation (Campbell *et al.*, 1974; Kontodimas *et al.*, 2004; Karimi-Molati *et al.*, 2014):

$$SE_{T_{min}} = \frac{r}{b} \times \sqrt{\left(\frac{S^2}{N \times r^2}\right) + \left(\frac{SE_b}{b}\right)^2}$$

$$SE_K = \frac{SE_b}{b^2}$$

Data from all tests were submitted to Shapiro Wilk test to verify for normality, as well as to Levene's test for equality of variances. If it was necessary, data was transformed to square root of arcsine (Sokal and Rohlf, 1995) to standardize means and normalize variances. But, non-transformed data were presented in the figures. Differences between treatments were done by one-way analyses of variance (ANOVAs), followed by a Tukey's test for multiple comparisons at P<0.05 (SPSS, 2007). All data were expressed as mean±SE.

Results

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 11 days at 35°C. The highest mortality during all stages was observed at 35ºC while the lowest mortality recorded at 27°C. The differences were found to be statistically significant at all temperatures studied (Table 1).

The regression equation estimates for both linear models (traditional and Ikemoto-Takai), coefficients of determination (R^2), T_{min} and K for each developmental stage are shown in Table 2. The estimated temperature threshold for all immature stages by Ikemoto–

Takai model was a little higher than those calculated by traditional model, although the estimated thermal constant was lower in Ikemoto–Takai model compared with traditional one. The curves of influence of temperature on developmental rate of total immature period fitted by two linear models are shown in Fig. 1. Based on Ikemoto–Takai and traditional models, the T_{min} values of total immature period were 8.25 and 7.06°C respectively and this pest required 293.7 DD and 312.5 DD respectively above a lower threshold temperature for development from egg to adult eclosion.

Table 1. Developmental period of immature stages (egg to pupa) and mortality of *Phyllocnistis citrella* at different temperatures.

Stage	Temperature (ºC)	n	Mortality (%)	Developmental time (days)		
				mean±SE	Min.	Max.
Egg	20	100	7	6.22±0.14 a*	3	9
	27	100	9	2.72±0.13 b	1	6
	35	100	21	2.18±0.11 c	1	6
Larva	20	93	5.4	9.56±0.15 a*	5	12
	27	91	3.3	5.26±0.13 b	3	8
	35	79	11.4	4.14±0.11 c	3	7
Pupa	20	88	1.9	11.18±0.23 a*	7	15
	27	88	2.3	6.86±0.15 b	4	10
	35	70	15.8	5.03±0.17 c	3	9
Total	20	100	18	25.51±0.34 a*	18	32
	27	100	14	14.86±0.25 b	10	22
	35	100	41	11.04±0.24 c	7	17

* Means followed by the same letter are not significantly different using Tukey's Test at $P < 0.05$.

The changes in longevity of moths during different temperatures were coinciding with immature stages. The longevity decreased with increasing temperature, from 9.31 to 5.38 days in females and 7.38 to 4.21 days in males at 20 to 35°C, respectively. Both female and male moths showed the highest emergence at 27°C (82.2 and 84.5%, respectively). However, the highest oviposition rate was observed at 35°C with average of 45.82±0.98 eggs per female (Table 3).

Discussion

Jafari *et al.* (2000) studied the biology of *P. citrella* in northern parts of Iran and reported the total developmental duration (egg to egg) of *P. citrella* as 19 and 16.57 days at 25 and 30°C, respectively. Using Valencia orange saplings as the host, Namvar and Safaralizade (2008) reported the average period of egg, larval and pupal stage of the citrus

leafminer 3.65, 8.95 and 7.5 days under 25°C, 70% RH and photoperiod of 14 (l): 10 (D), respectively. Therefore, the total immature stages lasted 20.1 days.

Table 2. The estimated lower temperature threshold (T_{min}), thermal constant (K), coefficients of determination (R^2) and regression equations for various immature stages (egg to pupa) of *Phyllocnistis citrella* by traditional and Ikemoto-Takai linear models.

Linear model	Stage	Regression equation	T_{min} °C	K (DD)	R^2	P-value
Traditional	Egg	DR= -0.208 + 0.0196 T	10.57±4.74	50.76±13.5	93.43	0.016
	Larva	DR= -0.069 + 0.0090 T	7.71±3.83	109.89±20.4	96.69	0.011
	Pupa	DR= -0.054 + 0.0073 T	7.42±1.16	136.98 7.79	99.69	0.03
Ikemoto-Takai	Egg	DT= 43.72 + 12.86 D	12.86±2.25	43.72±9.27	97.02	0.011
	Larva	DT= 100.30 + 9.35 D	9.35±2.38	100.3±16	93.92	0.015
	Pupa	DT= 133.91 + 7.93 D	7.93±0.88	133.91±7.1	98.77	0.07

We studied the biology of this pest at three constant temperatures for the first time in Iran. Developmental duration of immature stages was shorter as the temperature increased from 20 to 35°C. However, at 27°C the female: male ratio and moth emergence was at highest level as mortality of immature stages was lowest. The optimum temperature for the pest development appears to be 27°C. Previous studies on the developmental duration of various stages of *P. citrella*, under lab-condition, correspond to our results. Pinto and Fucarino (2000) recorded the incubation period of 2.1 days, larval period of 4.5 days, prepupal and pupal period of 1.7 and 5.7, days respectively at 27°C, 60-70 % RH and photoperiod of 14 hours. Chagas and Parra (2000) evaluated the effect of seven temperatures (18, 20, 22, 25, 28, 30 and 32±1ºC) on the developmental times of *P. citrella* and stated that the incubation period was the shortest at 30ºC (1.3 day), however, the shortest larval and pupal periods were observed at 32ºC (3.6 and 6.4 days, respectively). Elekcciogul and Uygun (2004) studied the biology of citrus leafminer in Turkey, with similar climatic conditions to the north of Iran. In their study, the developmental duration of *P. citrella* was investigated at 15, 20, 25, 30 and 35ºC. In addition, incubation period decreased from 7.3 days at 15ºC to 1.9 day at 35ºC and larval and pupal periods decreased from 21 and 23.9 days at 15ºC to 4.1 and 4 days at 35ºC, respectively. They found the highest and the lowest immature mortality 15°C and lowest at 30°C respectively. Santos *et al*. (2011) studied the ecology of the citrus leafminer in six citrus genotypes, at 25± 1ºC, 70 ± 10% RH, and 14 h photophase, and recorded the incubation period 4.2-4.5 days, larval period 6.9-8.3 days and pupal period 8.1-9.6 days. The developmental durations in these studies agree with ours, although Ba-Angood (1978) studied the biology of *P. citrella* under four constant temperatures (20, 25, 30 and 35 °C) and a fluctuating temperature (25-35.9°C) that were higher than ours. For example, incubation, larval and pupal period at 20-35 °C lasted 9.5-3.5, 18.5-10.4 and 19.5-2 days, respectively, however, the type of citrus

host was not mentioned. It seems that the longer period of development in all different temperatures is related to the host plant.

Table 3. Sex ratio (Male/Female), fecundity, percentage of emergence and longevity of *Phyllocnistis citrella* at different temperatures.

Temperature (°C)	Sex Ratio (M:F)	n	Total No of eggs per female (mean±SE)	Female			Male		
				n	Moth emergence (%)	Longevity (mean±SE)	n	Moth emergence (%)	Longevity (mean±SE)
20	1:1.2	45	26.11±1.17 b* (12-45)**	45	80	9.31±0.28 a (6-12)	45	77.8	7.38±0.22 a (4-10)
27	1:1.4	45	44.02±1.24 a (20-54)	45	82.2	6.92±0.23 b (4-10)	45	84.5	5.79±0.24 b (2-9)
35	1:1.3	45	45.82±0.98 a (33-62)	45	73.3	5.38±0.21 c (3-9)	45	66.6	4.21±0.16 c (2-9)

* Means followed by the same letter are not significantly different using Tukey's Test at $P < 0.05$.
** Values in parenthesis are minimum and maximum values.

The traditional model gave lower T_{min} and higher K value than the Ikemoto–Takai model. This model had a higher value of R^2 than the Ikemoto–Takai model (Fig. 1), indicating a slightly higher degree of confidence in parameter estimates. Based on this model, *P. citrella* required 312.5 DD above a lower threshold temperature of 7.06 °C for immature developmental period. The T_{min} values of 7.06 °C and 8.25°C (using the traditional and Ikemoto–Takaimodels, respectively) estimated in this study for total stages were different from 10.4 °C reported by Elekcioglu and Uygun (2004) or 12.1°C estimated by Yamamoto (1971) for this pest. Elekcioglu and Uygun (2004) studied the developmental rate of this pest within range of five constant temperatures (15-35°C). One of the reasons for the difference in T_{min} values in these studies is the number of experimental temperatures. Although it is possible to fit a regression line with just three points (Dent and Walton, 1997), increasing the points (temperatures in current study) will led to higher degree of confidence in parameter estimates. Therefore, this test should be performed at different temperatures to obtain an accurate developmental threshold or thermal constants for *P. citrella*.

Considering the monthly average of minimum temperature (Figure 2), the minimum of ambient temperature decreased below 10°C from October and during winter it was reduced below 7°C. The population density of citrus leafminers from mid October reached to the minimum level in the middle of February in field condition (Jafari *et al*., 2000). Therefore, the temperatures, near the T_{min} range, inhibit this pest development and together with short photoperiod induce the overwintering phases.
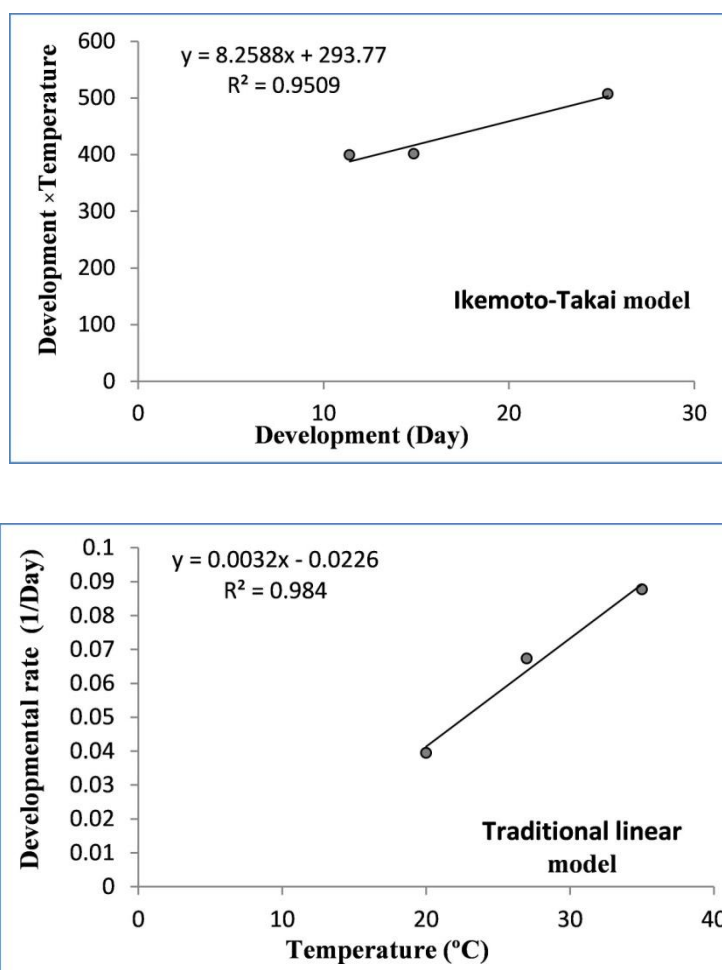
Fig. 1. Fitting the traditional and Ikemoto−Takai linear models (line) to observed values (●) of developmental rate of overall immature stages of *Phyllocnistis citrella* at different temperatures.

The monthly average of mean temperatures of Dashte-Naz region indicate that the mean temperature in July and August (27°C), was higher than the months before and after (Fig. 2). Jafari *et al*. (2000), based on their field studies in the northern regions, stated that the highest population density of *P. citrella* occurred during late summer and early autumn, especially in July and August. There is a coincidence between the optimum temperature for pest development and its population outbreak in the field. Thermal constant, the amount of thermal energy required for the completion of a stage, estimated as 312.5 DD for total immature stages of *P. citrella* by traditional linear model in this study. This value was reported as 238 DD by Yamamoto (1971). The threshold of 12.1°C (Yamamoto, 1971) thermal constant was determined as 206 DD (Ujiye, 1990, 2000) or 142-121 DD (different population collected between October to November, Lim and Hoy, 2006). Condition of the host plant could significantly affect the thermal requirements of *P. citrella* (Vargas *et al*., 2001). More studies on the temperature requirements of this pest during development, lead to more accurate prediction of the pest population.
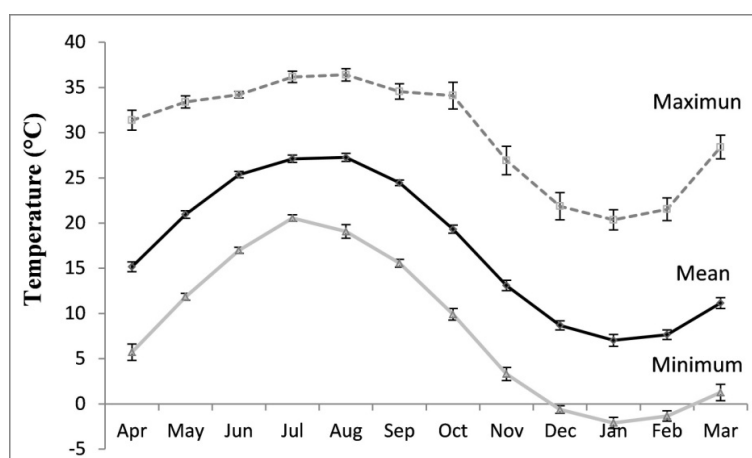
Fig. 2. The monthly average of minimum, maximum and mean temperature during 2006-2014 in Dashte-Naz region.

According to the present study, at all temperatures the female lived longer than the males and total number of laid eggs weren't significantly different at 27 and 35°C (about 45 eggs per female). However, the fecundity significantly decreased at 20°C (26.1 eggs per female). During the similar study, Elekcioglu and Uygun (2004) observed that the fecundity were lower and higher at 20°C (29.36 eggs per female) and 30°C (57.1 eggs per female) respectively, and no eggs deposited at 15°C. Abo-Kaf *et al*. (2006) recorded the longevity of non-feeding female and male moths as 2.3 and 1.7 days, respectively at 28°C. In fact, it should be considered that feeding conditions, as well as temperature, could affect longevity of the pest. Moreover, the field biology studies of citrus leaf miner on Kinnow and rough lemon during different seasons showed that at higher temperature, the growth and development of citrus leafminer was faster than at lower temperature and the average female longevity was more than male on the respective host plants during different seasons (Singh, 2014).

Acknowledgements

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