Pakistan J. Zool., vol. 49(2), pp 685-691, 2017. **DOI**: http://dx.doi.org/10.17582/journal.pjz/2017.49.2.685.691

Effect of Different Temperatures on the Biology of *Citrostichus phyllocnistoides* (Narayanan) (Hymnoptera: Eulophidae) a Parasitoid of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae)

Naime Zülâl Elekcioğlu*
University of Çukurova, Karaisalı Vocational School, 01170, Adana, Turkey

Article Information
Received 20 July 2016
Revised 16 November 2016
Accepted 14 January 2017
Available online 2 April 2017

Authors' Contributions
NZE designed and carried out the study, analyzed the data and wrote the article

Key words
***Citrostichus phyllocnistoides*, Temperature, Developmental time, Parasitization.**

ABSTRACT

The effect of constant temperatures on developmental time and parasitization rate of *Citrostichus phyllocnistoides* was evaluated on *Phyllocnistis citrella* at nine constant temperatures ranging from 15ºC±1 to 35ºC±1 in 2.5ºC increments in the laboratory. Developmental periods of immature stages ranged from 34.98 days at 15ºC to 9.15 days at 35ºC. The lower developmental threshold for *C. phyllocnistoides* estimated was 8.93°C. Mortality rate of immature stages decreased as the temperature increased, ranged from 27% at 15ºC to 8% at 35°C. Longevity of both sexes were different at all temperatures studied (except 30 and 32.5°C), ranged from 22.1 days at 15°C to 8.0 days at 35°C for males and 23.8 days at 15°C to 8.3 days at 35°C for females. Parasitization rate of *C. phyllocnistoides* on *Phyllocnistis citrella* increased with the temperature up to 32.5ºC being 49%, then decreased at 35ºC.

INTRODUCTION

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) (CLM) is a pest of regular occurrence in nurseries, young plantations and tender flushes of citrus groves. It is most damaging in the nurseries considering the economic loss incurred and the havoc it plays if left unattended. It was first detected in Turkey in 1994 (Uygun *et al*., 1995) where it has been spreading through citrus growing regions. Because of the extensive mining of young shoots caused by this pest it is considered a serious threat to citriculture. The pest not only causes direct damage to the leaves of new sprouts, but also infect the twigs and fruits (Clausen, 1931; Heppner, 1995; Mustafa *et al*., 2013). Under typical Mediterranean conditions, CLM damage is of economic importance only on young and top-grafted trees, and is considered to be merely an aesthetic factor for mature trees (Gonzalez, 1997). In Turkey, the citrus leafminer is active during the summer and autumn months (Uygun *et al*., 2000). Current control of CLM by growers in Turkey as in the other countries was primarily based on repeated application of insecticides during sprouting of young leaves (Yumruktepe *et al*., 1996). Although they have effectively controlled this species, the continued use of pesticides for several decades has disrupted the biological control by natural enemies and has led to a resurgence in *P. citrella* populations. Decreasing efficacy and increasing concern over adverse environmental effects have brought the need for the development of new types of selective control alternatives or methods of crop protection with or without reduced use of synthetic insecticides (Huang *et al*., 1989). Biological control is the best option for controlling this pest (Pena and Duncan, 1993). In many areas, a reduction in the pest population has been observed because of the presence of natural enemies (Ding *et al*., 1989; Ujiye *et al*., 1996). In Turkey, a number of indigenous natural enemies attack citrus leafminer, including the Eulophid parasitoids; *Cirrospilus*, *Ratzeburgiola*, *Pnigalio*, *Chrysocharis*, *Neochrysocharis*, *Baryscapus, Diglyphus, Chrysonotomia, Sympiesis* and *Pteromalus* genus (Başpınar *et al*., 1996; Elekcioğlu, 2013). These parasitoid species were previously found on various wild hosts and started to attack *P. citrella* when it made its appearance. Several exotic species have also been introduced into Turkey (Uygun *et al*., 1997). During the last 15 years, the most common and widespread parasitoid species found on *P. citrella* has been an exotic species, *Citrostichus phyllocnistoides* Narayanan (Hymenoptera: Eulophidae), with parasitism level up to 51% (Elekcioğlu and Uygun, 2013). This species had already been reported as primary parasitoid of citrus leafminer in many countries (Argov *et al*., 1998; Liotta *et al*., 2003; Garcia-Marí *et al*.,

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* Corresponding author: nelekcioglu@cu.edu.tr
0030-9923/2017/0002-0685 $ 9.00/0

2004; Wang *et al*., 2006; Hoy *et al*., 2007; Tsagkarakis *et al*., 2013). *C. phyllocnistoides*, was successfully released in Florida, Australia, Israel and several countries of the Mediterranean Basin (Argov and Rossler, 1996; FAO, 1996). However, the success of any biological control programme depends on the critical study of the biology and ecology of the biological control agent (Pena *et al*., 1996). This study was undertaken to investigate the biology of *C. phyllocnistoides* to improve its use in biological control programs.

MATERIALS AND METHODS

Insect rearing

Phyllocnistis citrella was obtained from citrus orchards in east Mediterranean region and reared on *Citrus aurantium* L. New plants, suitable for the pest to lay eggs upon, were replaced twice weekly whilst those exhibiting hatched pupae were removed. The plants with III larval stage of *P. citrella* were removed to another climate room and *Citrostichus phyllocnistoides* adults were released on the leaf miner. To ensure continuous production, saplings were obtained regularly each week and, when necessary, placed in the leafminer and parasitoid rearing room. *P. citrella* stock was maintained in a growth chamber at 30±1°C, and 80±5% relative humidity (RH) under a 12 h of artificial light (8,000 lx) 4 h of twilight (50 lx) and 8 h dark and *C. phyllocnistoides* stock at 30°C±1°C, 60±5% RH, 16 h of artificial light (8,000 lx) cycles in climatic rooms in Biological Control Research Station.

Temperature regimes

Experiments were conducted at nine constant temperatures ranging from 15±1°C to 35±1ºC in 2.5ºC increments, 60±5% relative humidity and 16:8 h light:dark (L:D) in temperature cabinets.

Determination of the effect of different temperatures on the developmental time of immature stages of C. phyllocnistoides

Five citrus plants each infested with 60 third larval instars of the host, *P. citrella,* were placed in rearing cages, were placed into climate cabinets at constant temperatures of 15, 17.5, 20, 22.5, 25, 27.5, 30, 32.5 and 35±1°C. *C. phyllocnistoides* adults were collected with an aspirator in the rearing room and released into plastic jars whose topsides were covered by tulle and then placed onto the saplings. *C. phyllocnistoides* adults were kept on the saplings for 24 h to allow them to oviposit, and then removed. Hatched larvae were allowed to develop until they reached the third larval instar, and 100 *C. phyllocnistoides* adults (male+female) newly emerged from pupae were released onto the larvae. Parasitoid adults were counted and removed after 24 h and all of the experiments were checked every 12 h.

The development of the parasitoid was observed until adult emergence. Thermal unit values were calculated at each temperature with the following formula: thermal units (degree-days) = (constant temperature-development threshold) x development time. The development thresholds were predicted from the regression equations for the development rate.

Parasitization

Leafminer larva on each leaf were examined under the stereomicroscope after emergence of all leafminer and parasitoid adults to determine the parasitization rate. *C. phyllocnistoides* adults which completed their development and *P. citrella* adults obtained from unparasitized individuals from the above experiments conducted at 15-35±1°C were counted and the parasitization rate of *C. phyllocnistoides* at different temperatures were determined. Calculations were made after correction of mortality of host due to feeding. The percentage parasitism was calculated as the ratio of the number parasitized host (number of adult parasitoid) to the total number of all adult individuals (parasitoid+*P. citrella*) (van Driesche, 1983).

Determination of the effect of different temperatures on the lifespan

To determine the longevity of *C. phyllocnistoides* adults at different temperatures, 10 newly emerged (max. 1 h old) female and male parasitoid adults were maintained under nine constant temperatures (15-35±1°C). Parasitoid adults were put into petri dishes and covered by tulle (3 cm Ø × 1 cm h). Honey water (10% w/v) absorbed into pieces of sponge was given as a nutrient. Dishes were examined every 12 h to count the surviving parasitoids.

Determination of the length of the preoviposition, oviposition and postoviposition periods

A single male and female pair of newly emerged adults were released onto saplings (one pair per sapling) bearing III larval stage of *P. citrella* to determine the length of the preoviposition period of *C. phyllocnistoides*. These adults were removed with an aspirator after 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 days, and each interval was repeated 4 times. The period between the beginning of the experiment and the day when the parasitizing first began was accepted as the preoviposition period. To determine the length of the oviposition period, newly emerged female and male parasitoids were kept in glass tubes for a length of time equivalent to the preoviposition period and then released onto saplings on which *P. citrella* of III larval stages was present. Adults of *C. phyllocnistoides* were transferred to another sapling every 12 h and this process was repeated until the adults were dead. In this way, the time points of the first and last egg laying events of the parasitoid were determined. The period between the final eggs lay and the parasitoid's death was accepted as the postoviposition period. Experiments were repeated with 10 pairs of the parasitoid. Honey water (10% w/v) absorbed into pieces of sponge was given as a nutrient. Here, only the results of the studies conducted at 30±1°C were given.

Evaluation of data

Data were analyzed by using the SPSS 16.0 (SPSS, 2007) package program and following Steel and Torrie (1960) and Karman (1971), analysis of variance (ANOVA) was applied to means. Differences between means were evaluated with Duncan's test ($P = 5\%$).

The effect of temperature on developmental rate (1/days) was calculated by linear regression. The minimum developmental temperature threshold for the parasitoid was found by extrapolating the regression line (To = a/b). The degree-day requirements were determined as the inverse of the linear equation slope (DD = 1/b) after Sharov (2004).

RESULTS

Development/Mortality

The egg, larval and pupal stages of *C. phyllocnistoides* could not be examined clearly because of these stages are under the epidermis. Therefore, the length of the egg to adult period was used to determine the developmental time of the immature stages of *C. phyllocnistoides*. Temperature strongly effected the developmental time of *C. phyllocnistoides* so that the developmental period of the parasitoid got shorter with increasing temperature ($P<0.01$) (Table I). Mean developmental time varied from 9.15 days at 35°C to 34.98 days at 15ºC.

Temperature effected the mortality rate of *C. phyllocnistoides*. From egg to adult it ranged from 27% at 15ºC to 8% at 35°C. Mortality rate decreased as the temperature increased. 'Thermal units' conducted according to developmental times of *C. phyllocnistoides* differed at different temperature conditions being the lowest at 15°C (212.13 degree-days) and the highest at 25°C (264.68 °C) (Table I).

Equation of the rate of development against temperature was calculated assuming that the mean developmental rates, *i.e.* the reciprocals of developmental times, were linearly related to temperature between 15ºC and 35°C. The resulting line indicted that development rate was highly correlated with temperature ($y = .0041x - .0371$; $R^2 = 0.9911$; $P = 0.011$). The estimated lower developmental threshold for *C. phyllocnistoides* was 8.93ºC (Fig. 1).

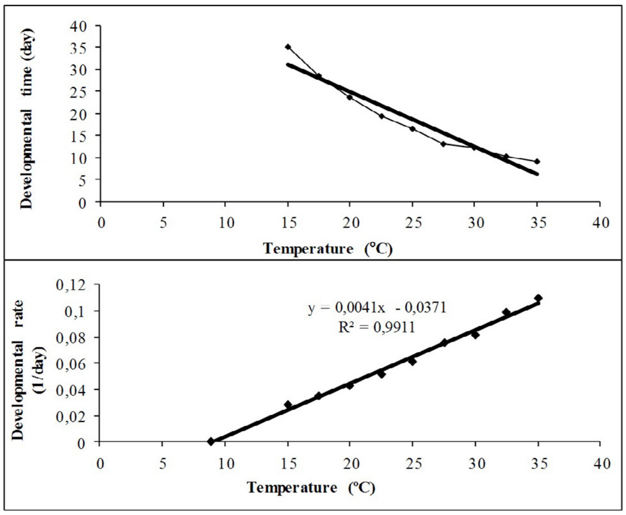

Fig. 1. Rate of total development (egg to adult) of *Citrostichus phyllocnistoides*. Line represents the linear regression of development rate on temperature within the range of 15-35ºC.

Table I.- Developmental time, thermal units in degree-days and mortality rate for *Citrostichus phyllocnistoides* on *Phyllocnistis citrella* at nine constant temperatures.

Temperature (±1ºC)	n	Developmental time* (days; mean±SEM) (min.-max.)	Mortality (%) (egg to adult)	Degree-days
15	44	34.98±1.58 a (32-38)	27	212.33
17.5	46	28.63 ±1.87 b (26-32)	23	235.06
20	47	23.53±1.21 c (19-25)	22	260.48
22.5	49	19.39± 1.75 d (15-23)	18	212.13
25	50	16.46±0.91 e (14-18)	17	264.68
27.5	52	13.08 ±0.62 f (12-14)	13	240.54
30	54	12.28±0.98 g (9-14)	10	258.74
32.5	54	10.13 ±0.44 h (9-11)	10	236.05
35	55	9.15±0.95 i (8-11)	8	238.54

*Means in the same column followed by a common letter are not significantly different at 5% level by Duncan test.

Effect of different temperatures on the lifespan

The lifespan of male and female adults of *C. phyllocnistoides* were longest under the constant temperature of 15°C (23.8 and 22.1 days for females and males, respectively) and were shortest under the temperature of 35°C (8.3 and 8.0 days), except the indivuduals at 30°C and 32.5°C. The lifespan of adult males and females were not significantly different at any temperature. Females survived longer than males at all the temperatures tested but not statistically different except the individuals at 30°C and 32.5°C (P<0.01) (Table II).

Table II.- Mean developmental times (days) of male and female of *Citrostichus phyllocnistoides* at different temperatures when reared on *Phyllocnistis citrella* on detached citrus leaves.

Temperature (±1ºC)	Adult life (day)	
	Male (♂)*	Female (♀)*
15	22.1 a^x A^y	23.8 a A
17.5	19.8 b B	21.7 b B
20	15.9 c C	16.5 c C
22.5	11.1 d D	11.3 d D
25	10.2 e E	10.6 e E
27.5	9.4 f F	9.8 f F
30	8.7 g G	9.1 g G
32.5	8.4 g G	8.7 g G
35	8.0 h H	8.3 h H

**x* Means within a column followed by the same letter are not significantly different by Duncan's test (P = 0.05).
y Means within a line followed by the same letter are not significantly different by Duncan's test (P= 0.05).

Lengths of the preoviposition, oviposition and postoviposition periods

Some of the parasitoid females began to lay eggs right after being released onto the leaf miner larvae and some of them in 2.0 days. So the preoviposition period of the parasitoid was detected as 0-2 days at 30°C. The oviposition period of *C. phyllocnistoides* was found to be as an average of 8.4 days at 30°C and they lay eggs until they die. The parasitoid adults died soon after they laid their last eggs so it was reported that the parasitoid had no postoviposition period.

Parasitization

The highest parasitization was recorded at 32.5°C with 49% followed by 30 ve 35°C temperatures (47% and 46%, respectively) being at the same statistical group. As it is seen from the figure the parasitization rate increased from 15°C to 32.5°C by increasing temperatures and decreased after 32.5°C (Fig. 2).

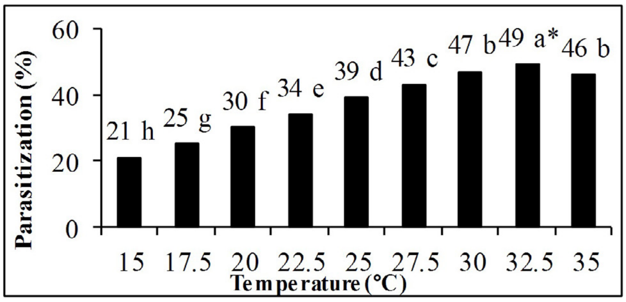

Fig. 2. The parasitization rate of *Citrostichus phyllocnistoides* at different temperatures. *Means over the columns followed by the same letter are not significantly different at 5% level by Duncan test.

DISCUSSION

Until 1986 the existent literature references cite *P. citrella* in many countries of Africa, Asia, Australia and Pacific islands, remaining confined in these areas, however, an expansion occurred in 1993 when *P. citrella* was detected in Florida (Heppner, 1993). Following the invasion in Florida, the studies concentrated primarily on the control of the pest. For an integrated management of a pest biological and ecological aspects must be known. There are many parasitoid species of CLM in citrus orchards at our study site; however, *C. phyllocnistoides* is the most abundant parasitoid of the pest. Investigation on the biology is one of the most important steps in conservation and assessment of the potential of a biological control agent. There is limited information available in the literature on developmental time, longevity and parasitization rate of *C. phyllocnistoides* at different temperatures under laboratory conditions.

The developmental time of *C. phyllocnistoides* decreased with increasing temperatures. The longest developmental time was at 15, and the shortest was at 35ºC, respectively and the differences were found to be statistically significant at all temperatures studied. Data from the present study are in agreement with the previous reports on the subject. Singh *et al.* (2004), indicated that *C. phyllocnistoides* completed its development in 10.5 and 9.2 days at 25±1°C and 30±1°C, 60-70% RH, respectively and they also indicated that the parasitoids lived longer when fed with fructose (60%)+honey (40%) as food. The estimated lower threshold temperature for development of *C. phyllocnistoides* was 9.8°C and thermal constant as 212.0 degree-days. It was indicated that the overwintering of this exotic biocontrol agent would be possible under typical Mediterranean temperatures (Urbaneja *et al*., 2003). There are some findings of other *P. citrella* parasitoids on the topic. Mafi and Ohbayashi (2010a), determined that developmental time of *Chrysocharis pentheus* (Walker) (Hymenoptera: Eulophidaedae), an endoparasitoid of *P. citrella* decreased in all stages as temperature increased and days from egg to adult was 14.0 days for males and 13.9 days for females at 25°C. The developmental threshold of male and female of *C. pentheus* was 8.9°C and 11.9°C, respectively. The effective accumulative temperature (thermal constant) for males and females from egg to adult was 181.8 and 238.1 degree-days, respectively. Developmental time of *Pnigalio minio* (Walker) (Hymenoptera: Eulophidaedae) decreased in all stages as temperature increased and days from egg to adult was the longest with 24.9 days at 18°C and shortest with 8.6 days at 30°C (Duncan and Pena, 2000). They determined that mortality of *P. minio* at temperatures between 18-30°C was not significantly different and suggest that development could successfully occur at higher and lower temperatures than those tested. The differences at the results can be related to the host insects and experimental conditions. Several other researchers have noted that the developmental time of other *P. citrella* parasitoids shortens with increasing temperature (Lo Pinto *et al*., 2005; Mafi and Ohbayashi, 2010b). The longevity of both males and females of *C. phyllocnistoides* was inversely related to the temperature. It could be said that the longevity of *C. phyllocnistoides* adults was affected by the increasing temperatures because as the temperature increased the longevity shortened significantly. No significant differences were found between the sexes, even though females had, on average, a higher longevity than males. However, differential longevity between sexes has been recorded in a number of species of parasitoid wasps, with females in most cases suffering greater mortality (Godfray, 1993). Duncan and Pena (2000) determined that male *P. minio* lived an average of 7.3 days after emergence. The longevity of; female *Sympiesis striatipes* Ashmead (Hym.: Eulophidae) reached to 33.8±1.5 days, male *Cirrospilus diallus* Walker (Hym.: Eulophidae) 37.65±4.25 days, female *C. diallus* 32.96±4.08 days; male *C. pictus* (Nees) (Hym.: Eulophidae) 47.82±3.80 days, female *C. pictus* 36.56±2.72 days. There are many studies that have reported that the longevity of parasitoids shortens with increasing temperature.

It was observed that most females of *C. phyllocnistoides*, like some other eulophid species, began to deposit eggs shortly after emerging and prior to mating. In a similar way, it was reported that mated *C. pentheus* females began oviposition 1-2 days after emergence and continued up to day 40 and the post-oviposition period was longer than *C. phyllocnistoides* with 5.0±0.74 days (Mafi and Ohbayashi, 2010a). These differences can be related to the host insects and experimental conditions.

It was concluded that parasitism incressed during the high temperatures up to 32.5ºC and decreased afterwards. Similarly, as in the field conditions, parasitization is higher during summer and autum than the spring in Turkey (Elekcioğlu and Uygun, 2013). Chen and Lou (1990) reported that *Elachertus* sp. (synonym of *C. phyllocnistoides*) parasitized 54.38% second to third instar larvae of CLM in the orchards of the Fuzhou suburb. Ding *et al*. (1989) recorded 67.6% parasitism of *Tetrastichus phyllocnistoides* (synonym of *C. phyllocnistoides*) in citrus orchards in Guangzhou. The difference of the parasitization rate might be due to the difference between the laboratory conditions and warrants further study. These aspects are quite common to most insect parasitoids, and similar results have been obtained from different Eulophid leafminer parasitoids (Lla´ Cer *et al*., 1998; Urbaneja *et al*., 1999).

CONCLUSION

C. phyllocnistoides is a fairly efficient parasitoid of *P. citrella*. It can be easily reared in the laboratory and released to the field as needed. All the data obtained from the study provide useful new information about the biology of *P. citrella*. However, further studies are required to better understand the parasitoid and to give it greater recognition for use in pest control.

ACKNOWLEDGEMENT

This research was supported by the Scientific and Technical Research Council of Turkey (TUBİTAK/TOVAG-104 O 526).

Statement of conflict of interest

Authors have declared no conflict of interest.

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