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Temperature-dependent effects on some biological aspects of two ectoparasitoids of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae)

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Abstract

Background Temperature alters host suitability for the development of parasitoids through direct (thermal effect) and indirect (parental effect) pathways. The effects of three temperature regimes on the development and survival of two parasitoid species, *Citrostichus phyllocnistoides* (Narayanan) and *Cirrospilus ingenuus* Gahan (Eulophidae: Hymenoptera) of the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) was evaluated. The experiment was conducted at 20, 25, and 30°C temperatures with 65 ± 2% relative humidity (R.H.) and 16h: 8h (L: D) photoperiod.

Results In *C. phyllocnistoides*, the pre-ovipositional period was longer at 20°C, while non-significant difference was observed in the pre-ovipositional period of *C. ingenuus* under the effect of different temperatures ($P > 0.05$). The ovipositional period of *C. phyllocnistoides* and *C. ingenuus* was higher at 20°C and gradually decreased by increasing the temperature. Non-significant ($P > 0.05$) difference was found in post-ovipositional period of both parasitoid species. Both species exhibited the maximum fecundity at 25°C, while, the minimum fecundity was recorded at 30°C. However, the adult longevity of both parasitoid species was highest at 20°C and gradually decreased by increasing the temperature. In both parasitoids, the parasitism rate was highest at 25°C.

Conclusion This study highlighted the importance of thermal effects on some parasitoid species of insect pests to predict the future of trophic dynamics in global warming situations.

Keywords Citrus leafminer, *Citrostichus phyllocnistoides*, *Cirrospilus ingenuus*, Parasitism, Temperature

Background

The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) is a devastating pest of citrus (Arshad et al. 2018a). It is native to Southeast Asia (Clausen 1931). This micro-lepidopterous species was reported for the first time in 1856 by Calcutta (Indo-Pak subcontinent) (Stainton 1856). Since the last two decades of the twentieth century, this pest has invaded the Americas and Mediterranean basin (Vercher et al. 2005) along with citrus growing tropical and sub-tropical areas of the world (Kerns and Wright 2001).

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Adults of *P. citrella* are minute moths (2 mm long) having wings fringed with long hairs (Mustafa et al. 2014a). Females oviposit single eggs on the young, tender citrus leaves (5–45 mm long) about 24 h after mating (Amlin et al. 2002). Newly hatched larvae immediately bore a mine and enter into the tiny leaf below the epidermal layer (Mansour and Braham 2020). There are four larval instars of *P. citrella*. The first three instars feed on the sap and epidermal cells, while the fourth instar is prepupae, which is a non-feeding stage (Beattie and Hardy 2004). The prepupae spin a silken cocoon to form a pupal chamber, usually protected by folding the leaf margin (Chermi et al. 2001). *Phyllocnistis citrella* is multivoltine and has nearly continuous generations. In tropical conditions, this pest has up to 15 generations per year (Mustafa et al. 2014b).

Phyllocnistis citrella is regarded as a serious threat to citrus cultures for causing high economic losses (Uji 2000; Dahmane and Chakali 2022). Infested leaves become deformed, chlorotic and cause a reduction in photosynthetic rate (Arshad et al. 2019). Besides, mining provides an entry hole to the canker bacterium (*Xanthomonas axonopodis* pv. *citri*) by opening the leaf cuticle and facilitate its access to the plant (Gottwald et al. 2007). Previous studies have demonstrated that *P. citrella* damage has no economic impact on the mature trees under Mediterranean conditions (Diez et al. 2006), but it causes significant economic losses in citrus nurseries, young plantations, and top-grafted trees (De Prins and De Prins 2005).

Citrus growers mostly rely on the repeated applications of synthetic insecticides for the control of *P. citrella* (Amiri 2007; Elazab et al. 2021). However, the effective chemical control of *P. citrella* is difficult because the larvae and pupae are protected inside the mines or chambers, respectively (Beattie and Hardy 2004). Furthermore, the regular use of insecticides has disrupted the non-targeted natural enemies and led to resistance in the populations of *P. citrella* against insecticides (Elekcioglu 2017). The chemical control seemed a costly and short-term solution for the management of *P. citrella* (Mafi and Ohbayashi 2010). On the other hand, biological control is an effective and promising option for controlling *P. citrella* over the long term (Kalaitzaki et al. 2021). Several studies have reported a significant reduction in the *P. citrella* population governed by the indigenous natural enemies (Elekcioglu 2017).

Citrostichus phyllocnistoides (Narayan) (Hymenoptera: Eulophidae) is a primary ecto-parasitoid of *P. citrella* around the world (Tsagkarakis et al. 2013). Elekcioglu and Uygun (2013) reported *C. phyllocnistoides* causing up to 51% parasitism in Turkey. Similarly, *Cirrospilus ingenuus* Gahan (Hymenoptera: Eulophidae) is another

solitary ecto-parasitoid of *P. citrella* (Hoy and Nguyen 2003). This parasitoid is native to Asia and commonly found in China, Australia, India, Japan, Indonesia, Oman, Thailand, Malaysia and Taiwan (Zhu et al. 2002). Both parasitoids were released in several countries for the classical biological control of *P. citrella* (Elekcioglu and Uygun 2013). In Pakistan, Arshad et al. (2018b) reported both *C. phyllocnistoides* and *C. ingenuus* from the Sargodha region of Punjab province.

Knowledge of the thermal effect on natural enemies of insect pests is crucial to develop a cost-effective mass rearing programs, as it has the potential to use in augmentative biological control of insect pests. This study aimed to evaluate the effect of temperature on the ovipositional periods, fecundity, longevity, and parasitization of *C. phyllocnistoides* and *C. ingenuus* on *P. citrella* under controlled laboratory conditions.

Methods

The study was conducted at the Pak-China Joint Research Centre for Citrus Diseases and Insect Pests Management, College of Agriculture, University of Sargodha, Sargodha, Pakistan.

Rearing of *Phyllocnistis citrella*

Leaves infested with *P. citrella* were collected from the citrus plantations around the vicinity of the College of Agriculture, University of Sargodha (32°07'53.6"N, 72°41'09.3"E) for rearing in the laboratory. The collected leaves were kept under controlled climatic conditions at 26±2°C temperature, 60±5% RH. and 16h: 8h (L:D) photoperiod until the adults' emergence. After emergence, the adults were transferred into Perspex cages (50×50×100 cm, Shenzhen Yijin, Guangdong, China), provided with young citrus plants pruned to a height of 40–50 cm for easy handling. The plants were planted in 0.5-L plastic pots filled with fertile sand. Every 10 days, three plants were exposed to adult *P. citrella* moths for 2–3 days for egg-laying. Following that period, the moths were removed from the cages and infested plants were kept undisturbed in rearing chambers until adult emergence. Adults *P. citrella* moths were fed on a mixture of honey and water (1:3).

Rearing of parasitoids

Two parasitoids of *P. citrella*; *C. phyllocnistoides* and *C. ingenuus* were studied. Parasitoids were obtained from the colony reared in the laboratory. The Perspex cages (50×50×100 cm) were used for the rearing of each parasitoid. For the emergence of adult wasps, the parasitized *P. citrella* larvae or pupae were kept under the same conditions as for rearing *P. citrella*. After emergence, the adult parasitoids were released in the cages with a ratio

of 1:1 of both male and female wasps. Honey water (10% w/v) soaked filter paper strips were placed in cages to feed the adult wasps. *P. citrella* infested plants were provided in each cage for the oviposition of gravid female wasps. After oviposition, the plants were kept undisturbed under controlled conditions for the emergence

Parasitism rate

A total number of parasitoids and *P. citrella* adults emerged from the parasitized and unparasitized individuals, respectively were counted from the experiment to determine the parasitization rate at different temperatures. The percent parasitism was calculated using the formula suggested by Li et al. (2018).

$$\text{Parasitism(\%)} = \frac{\text{No. of parasitized hosts}}{\text{No. of unparasitized hosts} + \text{No. of parasitized hosts}} \times 100$$

of the next generation. A continuous supply of infested plants was provided to get a maximum population of parasitoids.

Temperature regimes

The experiments were performed under completely randomized design (CRD) in growth chamber (BIOBASE, BJPX-A400) at three different temperature levels, 20, 25, and 30°C ± 1°C at 65 ± 2% relative humidity (R.H.) and 16h: 8h (L:D) photoperiod.

Ovipositional period, fecundity and adults' longevity of parasitoids

Citrus leaves infested with third instar *P. citrella* larvae were placed in Petri plates (140 mm diameter) lined with water-soaked filter papers. A total of 5 pairs of each parasitoid was tested by considering each pair as one replicate. One pair of adult parasitoids was offered 10 larvae of *P. citrella* at 48 h intervals. After 48 h, a new set of the host larvae were provided. Adult parasitoids were fed on small droplets of honey water (10% w/v) solution deposited on the leaves. The hosts and parasitoids were observed three times every day under a stereomicroscope. The evidence of stinging and feeding of parasitoids on *P. citrella* larvae ranged from barely detectable markings or very small black dot left after ovipositor insertion. The data of pre-ovipositional, ovipositional, and post-ovipositional periods, fecundity, and adults' longevity were determined by daily observations (Elekcioglu 2017). The duration between the start of the experiment and the day when oviposition began was calculated as the pre-ovipositional period. The period between the first and last egg-laying of the parasitoids was considered as the ovipositional period. While the period between final egg-laying and the parasitoid's death was recorded as the post-ovipositional period.

Data analysis

Data were subjected to one-way analysis of variance (ANOVA) at $\alpha = 0.05$. Prior to the analyses, data were transformed through square-root transformation to normalize the variance after checking the normality of residuals and homogenized with the Shapiro–Wilk test. Original means were separated using the Fisher's least significant difference (LSD) multiple range test. All the data were evaluated by using Minitab 17.0 software.

Results

Ovipositional periods

The temperature had significant effects on the ovipositional period of both *C. phyllocnistoides* ($F_{2,12} = 68.5$, $P < 0.001$) and *C. ingenuus* ($F_{2,12} = 32.7$, $P < 0.001$), while non-significant effect was observed on post-ovipositional ($F_{2,12} = 2.00$, $P = 0.178$ for *C. phyllocnistoides* and ($F_{2,12} = 3.45$, $P = 0.065$) periods of both parasitoids (Fig. 1). Some of the females of both parasitoids started egg-laying on the 1st day right after being released on the *P. citrella* larvae, while some of them within 2 days. The pre-ovipositional period of *C. phyllocnistoides* was longer at 20°C (2.2 ± 0.178 days), while non-significant difference was observed in the pre-ovipositional period of *C. ingenuus* under the effect of different temperatures ($F_{2,12} = 2.92$, $P = 0.092$). In both *C. phyllocnistoides* and *C. ingenuus*, the maximum duration of ovipositional was recorded at 20°C (11.40 ± 0.357 days and 11.20 ± 0.521 days, respectively). It gradually decreased by increasing the temperature. *C. phyllocnistoides* had a short post-ovipositional period (< 1 day) as the females died soon after their final egg-laying, while *C. ingenuus* survived for more 1.5 to 3 days after laying their last eggs (Fig. 1).

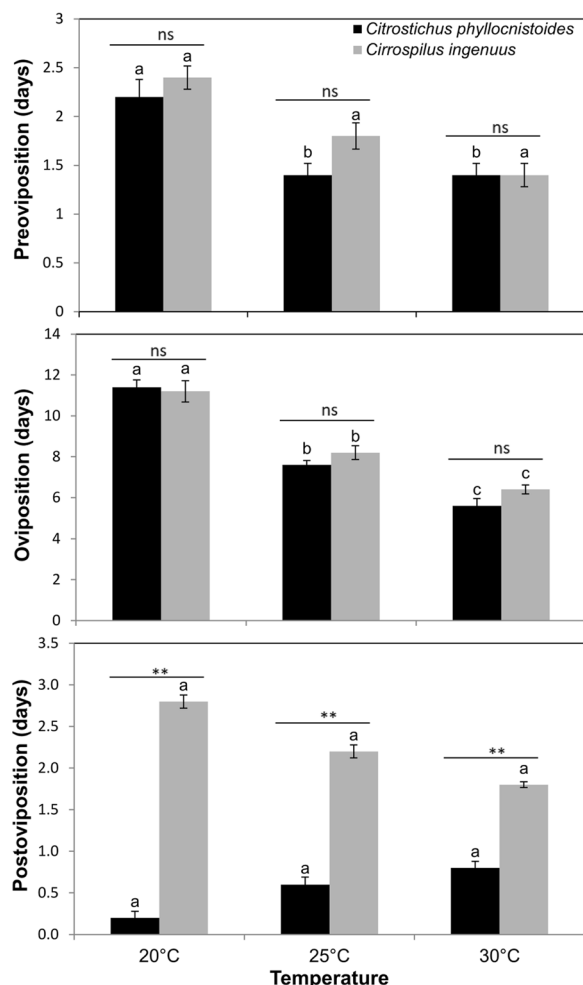


Fig. 1 Effect of temperature on the pre-oviposition, oviposition and post-oviposition period (means ± S.E.) of *Citrostichus phyllocnistoides* and *Cirrospilus ingenuus* reared on *Phyllocnistis citrella*. means within the same parasitoid sharing similar letters are not significantly different ($\alpha=0.05$; LSD test). ** shows the significance ($P < 0.05$) and ns shows the non-significance ($P > 0.05$) among two parasitoids for each temperature regime

Table 1 Effect of temperature on the fecundity (means ± S.E.) of *Citrostichus phyllocnistoides* and *Cirrospilus ingenuus* reared on *Phyllocnistis citrella*

Temperature	n	Fecundity	
		<i>C. phyllocnistoides</i>	<i>C. ingenuus</i>
20°C	5	15.8 ± 0.867 a	14.2 ± 0.438 b
25°C	5	17.0 ± 0.938 a	17.6 ± 0.606 a
30°C	5	10.6 ± 0.536 b	9.4 ± 0.456 c

Within each column, means sharing the same letters are not significantly different ($\alpha=0.05$; LSD test), n = number of female parasitoids

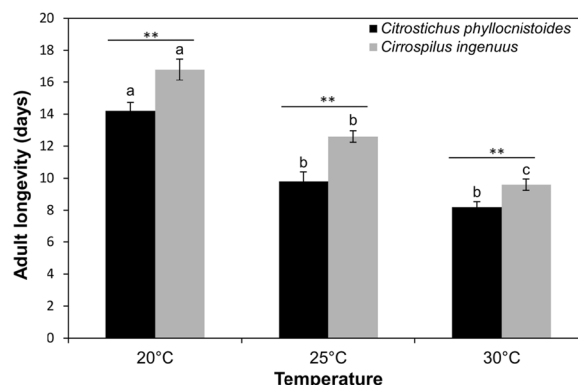


Fig. 2 Effect of temperature on adult longevity (days) (means ± S.E.) of *Citrostichus phyllocnistoides* and *Cirrospilus ingenuus* reared on *Phyllocnistis citrella*, means within the same parasitoid sharing similar letters are not significantly different ($\alpha=0.05$; LSD test), ** shows the significance ($P < 0.05$) among two parasitoids for each temperature regime

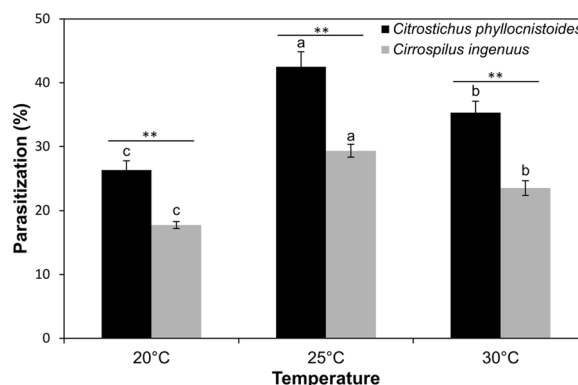


Fig. 3 Effect of temperature on the percent parasitism (means ± S.E.) of *Citrostichus phyllocnistoides* and *Cirrospilus ingenuus* reared on *Phyllocnistis citrella*, means within the same parasitoid sharing similar letters are not significantly different ($\alpha=0.05$; LSD test), ** shows the significance ($P < 0.05$) among two parasitoids for each temperature regime

Fecundity and longevity

Fecundity rate of *C. phyllocnistoides* ($F_{2,12}=14.5, P < 0.001$) and *C. ingenuus* ($F_{2,12}=53.0, P < 0.001$) was significantly affected with different temperatures (Table 1). Females of both parasitoids deposited a maximum number of viable eggs at 25°C. Fecundity rate of *C. phyllocnistoides* and *C. ingenuus* was 17.0 ± 0.938 and 17.6 ± 0.606 eggs, respectively at 25°C, reduced by the increasing temperature to 30°C. On the other hand, change in temperature also significantly affected the longevity of both parasitoids ($F_{2,12}=31.5, P < 0.001$ for *C. phyllocnistoides* and $F_{2,12}=45.7, P < 0.001$ for *C. ingenuus*). In both parasitoids, longevity showed a

contrary trend to fecundity as it gradually decreased by increasing the temperature (Fig. 2). Maximum longevity of *C. phyllocnistoides* adults (14.2 ± 0.521 days) was recorded at 20°C (20 > 25 > 30°C). Similarly, the longevity of *C. ingenuus* at 20°C was higher than the rest (16.80 ± 0.657 days, 20 > 25 > 30°C) (Fig. 2).

Parasitism rate

The highest parasitization of *C. phyllocnistoides* ($F_{2,12}=14.6$, $P<0.001$) and *C. ingenuus* ($F_{2,12}=30.7$, $P<0.001$) was observed at 25°C which was significantly different from the other temperatures. The parasitization rate of both parasitoids showed a similar trend by changing the temperature i.e. 25 > 30 > 20°C (Fig. 3). Temperature showed a negative and strong relation with longevity ($R^2=0.78$ for *C. phyllocnistoides* and $R^2=0.88$ for *C. ingenuus*) and oviposition ($R^2=0.89$ for *C. phyllocnistoides* and $R^2=0.83$ for *C. ingenuus*) of parasitoids (Fig. 4).

Discussion

Knowledge of biological and ecological aspects of a pest and its natural enemies are essentially important for integrated pest management (IPM) (Deguine et al. 2021). It is necessary to conduct biological investigations for the conservation and assessment of the potential of any biocontrol agent. *C. phyllocnistoides* and *C. ingenuus* had already been reported from Sargodha (Pakistan)

attacking the *P. citrella* (Arshad et al. 2018b). However, limited literature is available on the effect of temperature on the ovipositional periods, fecundity, longevity and parasitization of both parasitoids.

In this study, temperature showed non-significant effect on the pre- and post-ovipositional periods of *C. phyllocnistoides* and *C. ingenuus*. Like some other eulophids (Mafi and Ohbayashi 2010), some of the females of both parasitoids began to lay eggs shortly after being exposed to *P. citrella* larvae, while others do it in two days. Our findings are also similar to Elekcioglu (2017) who reported that the females of *C. phyllocnistoides* started egg-laying immediately after releasing on *P. citrella* larvae. Similarly, Mafi and Ohbayashi (2010) observed that mated females of *Chrysocharis pentheus* started oviposition after 1–2 days of emergence and continued egg-laying up to 40 days. The post-ovipositional period of *C. phyllocnistoides* was noticeably shorter than *C. ingenuus*, as the females died soon after depositing their last eggs. Mafi and Ohbayashi (2010) reported a shorter post-ovipositional period (5.0 ± 0.74 days) of *C. phyllocnistoides* than *C. pentheus*.

A gradual reduction in the ovipositional period of parasitoids by increasing the temperature level was noticed. Thus, temperature could be the main reason influencing the fecundity of insects (Kobori and Hanboosong 2017). The present study also showed a significant effect of temperature on the ovipositional and fecundity of both parasitoids. The temperature on which the oviposition rate of parasitoids was higher, termed as the optimal temperature for oviposition and it was 20°C for both *C. phyllocnistoides* and *C. ingenuus*. Whereas, the fecundity of both parasitoids was maximum at 25°C, which significantly reduced when moving away from this temperature. Another study conducted by Urbaneja et al. (2002) reported similar results having maximum oviposition of *Cirrospilus* sp. near lyncus at 25°C and fecundity at 20°C. The fecundity of *Hemiptarsenus varicornis* (Eulophidae: Eulophinae), a parasitoid species of *Liriomyza sativae* significantly affected by a shift in temperature (Ridland et al. 2020). On the other hand, the fecundity of another Palearctic parasitoid *Diglyphus isaea* (Hymenoptera: Eulophidae) had a higher net reproductive rate on *L. trifolii* at 15–30°C (Hondo et al. 2006).

Longevity of both *C. phyllocnistoides* and *C. ingenuus* had an inverse relationship with the temperature. The life duration of adults of both parasitoids significantly decreased as the temperature increased from 20 to 30°C. *C. phyllocnistoides* had a higher longevity than *C. ingenuus*. Several previous studies have supported our

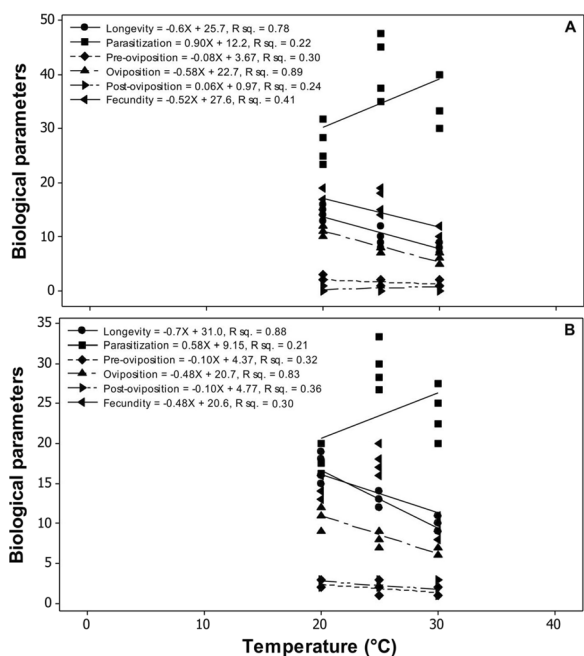


Fig. 4 Relationship between temperature and biological parameters of *Citrostichus phyllocnistoides* (A) and *Cirrospilus ingenuus* (B)

results that the longevity of adult parasitoids decreased by the increase in temperature (Elekcioglu 2017).

It was found that the parasitism rate of both parasitoids was maximum at 25°C, followed by 30°C. Elekcioglu (2017) reported increased parasitism of *C. phyllocnistoides* at high temperatures up to 32.5°C that decreased afterward. In Turkey, similar results were recorded in the field conditions where parasitism was higher during summer and autumn than in spring (Elekcioglu and Uygun 2013). The parasitism of *Tetrastichus phyllocnistoides* (synonym of *C. phyllocnistoides*) was recorded 67.6% in the citrus orchards of China (Guangzhou) (Ding et al. 1989).

Conclusions

The findings suggest that parasitoids of *P. citrella* are better adapted at 20–25°C; both parasitoids can be expected to be an important mortality factor for *P. citrella* populations at this temperature regime. Data on parasitism rate, host-feeding, and longevity can be used to determine the suitability of the parasitoids and to design an effective strategy for the introduction of parasitoids in citrus orchards. Parasitoid releases may be best suited to the spring flushes to improve overall *P. citrella* suppression.

Abbreviations

RH	Relative humidity
ANOVA	Analysis of variance
LSD	Least significant difference

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Author contributions

MIU and MA planned and designed the project and experimental layout. SMAZ and NA performed the experiment. SA performed the statistical analysis. The manuscript was prepared by MA and HMA and reviewed by MIU. All authors read and approved the final manuscript.

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Availability of data and materials

Data will not be shared.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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