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Effect of temperature on life history parameters of the polyphagous parasitoid *Pnigalio pectinicornis*

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Abstract

Pnigalio pectinicornis (L.) (Hymenoptera Eulophidae) is a widespread polyphagous ectoparasitoid of leaf-mining or gall-producing insects found in subtropical and temperate regions. In an attempt to evaluate the effectiveness of this parasitoid as a biological control agent, life table parameters and the effect of temperature on its fecundity, longevity, host-instar choice, superparasitism and host feeding were studied on *Phyllocnistis citrella* Stainton (Lepidoptera Gracillariidae). Tests were conducted at 20, 25 and 30 ± 0.5 °C with 60 ± 10% RH under a 14L:10D photoperiod. *P. pectinicornis* oviposition period was significantly longer at 20 °C than at 25 °C and 30 °C. In terms of fecundity, females laid the highest mean number of eggs (110.3) at 20 °C followed by 38.6 and 35.6 at 25 °C and 30 °C, respectively. Female longevity was almost double (71.5 days) at the lowest temperature tested (20 °C). The net reproductive rate (R_o) was highest (18.8, female offspring produced per female) at 20 °C, and lowest (2.1) at 30 °C. Following the above pattern, mean generation time (T: 51.36-32.2), intrinsic rate of increase (r_m: 0.073-0.027) and finite rate of increase (λ: 1.076-1.027) were maximum at 20 °C and minimum at 30 °C. Population-doubling time (DT) peaked to 25.29 days at 30 °C and was reduced to only 9.42 days at 20 °C. *P. pectinicornis* prefers to oviposit on 4th instar larvae two and three times more than on 3rd instar larvae or pupae of *P. citrella*, respectively at all temperatures tested. Temperature significantly affected percent of self-superparasitism which was highest at 20 °C (27.3%). Temperature did not significantly affect daily host feeding rates. The above results indicate that *P. pectinicornis* is much better adapted at lower temperatures typical for the Mediterranean climatic conditions during late spring and autumn. Gaining knowledge about these parameters is essential to understand its population dynamics in the field and its role in regulating the population of this herbivorous insect compared to other parasitoid species.

Key words: biological control, fecundity, host feeding, polyphagous ectoparasitoid, r_m, temperature.

Introduction

Pnigalio Schrank (Hymenoptera Chalcidoidea Eulophidae) is mainly a Holarctic genus which includes about 100 described species of ectoparasitoids, primary parasitoids and hyperparasitoids (Schauff *et al.*, 1998; Gebiola *et al.*, 2009). Regarding the species for which biological characteristics have been studied, they are either specialist or generalist idiobionts, with a solitary or gregarious larval development on leafminers and/or gall-making insects belonging to the orders Coleoptera, Diptera, Hymenoptera and Lepidoptera (Noyes, 2019). Although the genus *Pnigalio* includes many important biological control natural enemies, relatively few data exist on their biological traits.

Specifically, several biological parameters have been documented for just five species: *Pnigalio soemius* (Walker) (Hymenoptera Eulophidae) reared on *Cosmopterix pulchrimella* Chambers (Lepidoptera Cosmopterigidae) which reared on *Parietaria diffusa* Mert. et Koch (Urticaceae) (Bernardo *et al.*, 2006); *Pnigalio agraules* (Walker) (likely to be *Pnigalio mediterraneus* Ferriere et Delucchi according to Gebiola *et al.*, 2009) (Hymenoptera Eulophidae) reared on *Cameraria ohridella* Deschka et Dimic (Lepidoptera Gracillariidae) developed on *Aesculus hippocastanum* L. (Hippocastanaceae) (Grabenweger *et al.*, 2009); *Pnigalio minio* (=*flavipes*) (Ashmead) (Hymenoptera Eulophidae) reared on *Phyllocnistis citrella* Stainton (Lepidoptera Gracillariidae) and *Phyllonorycter elmaella* Doganlar et Mutuura (Lepidoptera Gracillariidae) on *Citrus latifolia* Tanaka and on apple trees, respectively (Barrett and Brunner, 1990; Duncan and Peña, 2000); and *Pnigalio pectinicornis* (L.) (Hymenoptera Eulophidae) reared on *P. citrella* developed on *Citrus sinensis* L. Osbeck and on *Citrus reticulata* Blanco (Kalaitzaki *et al.*, 2007).

In this study we focused on *P. pectinicornis* which is a widespread parasitoid found in subtropical and temperate regions in 30 countries of the Palearctic region as well as in Australia and New Zealand indicating its ability to survive in a variety of environmental conditions (Gençer, 2012; Noyes, 2019), while its development can continue even in winter weather conditions that occur in most citrus-producing areas in Mediterranean, provided that suitable hosts are available for the parasitoid (Kalaitzaki *et al.*, 2007). It is a polyphagous ectoparasitoid of 165 leaf-mining or gall-making insect species from four insect orders (Lepidoptera, Coleoptera, Diptera, Hymenoptera) recorded on a wide range of plant (65) species (Boucek and Askew, 1968; Noyes, 2019). Several of these insect hosts are pests of agricultural and ornamental importance, such as *Prays oleae* Bernard (Lepidoptera

Hyponomeutidae) (Ramos and Panis, 1975; Arambourg and Pralavorio, 1986), *P. citrella* (Schauff *et al*., 1998; Urbaneja *et al*., 1999; Gomes de Silva *et al*., 2006; Kalaitzaki *et al*., 2007), *Leucoptera malifoliella* (Costa) (Lepidoptera Lyonetiidae) (Balázs and Jenser, 2004), *Phthorimaea operculella* (Zeller) (Lepidoptera Gelechiidae) (Pucci *et al*., 2003) and *C. ohridella* (Lupi, 2005). Despite its significant natural contribution in the control of many phytophagous insects, a limited knowledge on biological data is available for this species.

Even though the above-mentioned studies offer valuable information concerning distribution, insect hosts and thermal requirements for development of the parasitoid on each host plant system, knowledge of the effect of temperature on its reproduction, host choice, host feeding, longevity and self-superparasitism is still lacking. This knowledge is essential to understand the role of *P. pectinicornis*, as an indigenous polyphagous parasitoid of *P. citrella*, a citrus insect pest that invaded Greece 27 years ago, in comparison to several exotic parasitoids systematically introduced in citrus groves (Kalaitzaki *et al*., 2011; Tsagarakis *et al*., 2013). Several studies on the seasonal abundance of *P. citrella* and its natural enemies in citrus orchards showed that shortly after its invasion, *P. pectinicornis* was one of its most abundant parasitoid species in Greece (Kalaitzaki *et al*., 2007; 2011, Tsagarakis *et al*., 2013), Spain (Urbaneja *et al*., 2000) and Portugal (Gomes da Silva *et al*., 2006). Nevertheless, shortly after the introduction of the exotic parasitoid *Citrostichus phyllocnistoides* (Narayanan) (Hymenoptera Eulophidae), in several regions of the Mediterranean basin, guild composition changed entirely with the latter parasitoid displacing *P. pectinicornis* which was forced to move back to its original hosts (Garcia-Mari *et al*., 2004; Karamaouna *et al*., 2010; Kalaitzaki *et al*., 2011).

Life history parameters have been measured for other citrus leaf miner parasitoids, belonging to Eulophidae family, such as *P. minio* (Duncan and Pena, 2000), *Semielacher petiolatus* (Girault) (Kalaitzaki *et al*., 2021), *Cirrospilus* sp. near *lyncus* (Urbaneja *et al*., 2001), *Quadrastichus citrella* Reina et La Salle (Llácer *et al*., 2006) and *Cirrospilus vittatus* Walker (Urbaneja *et al*., 2002) by studies aiming to provide understanding of their importance as *P. citrella* parasitoids. This is not the case for *P. pectinicornis* since its life table and biological parameters have not been investigated and no insight has been gained on its potential to compete and exploit a habitat or the factors that forced it to move back to its original hosts.

Under this light, experiments to study the effect of temperature on adult life parameters of the parasitoid *P. pectinicornis* on *P. citrella* reared on sour orange trees under laboratory conditions were undertaken. Specifically, the objectives of the present study were to: (1) evaluate the effect of temperature on female pre-, post-, oviposition periods, fecundity, offspring sex ratio and longevity, (2) study host feeding, host instar choice and self-superparasitism of *P. pectinicornis* reared on the citrus leafminer *P. citrella*, and to (3) estimate life table population parameters of the parasitoid at different temperatures using appropriate mathematical formulae.

Materials and methods

Insect rearing

The parasitoid *P. pectinicornis* was reared using the citrus leafminer *P. citrella* as a host. Host adult insects originated from infested citrus shoots collected from orchards located in Chania prefecture of Crete. *P. citrella* rearing was maintained on 2-3 year old potted sour orange plants (*Citrus aurantium* L.) placed in transparent Plexiglas cages (50 × 50 × 100 cm) consequently kept in growth chambers at 25 ± 0.5 °C, 60 ± 10% RH and a 14L:10D photoperiod. *P. pectinicornis* adults used for the bioassays emerged from parasitized *P. citrella* larvae as described previously (Kalaitzaki *et al*., 2007).

Oviposition, fecundity, longevity, host feeding, host-instar choice and self-superparasitism

Pre-oviposition, oviposition and post-oviposition periods, fecundity, longevity, host feeding, host-instar choice and self-superparasitism of *P. pectinicornis* were determined by daily observations of females, utilizing transparent cylindrical PVC cages (9 cm in diameter and 21 cm high) as described by Kalaitzaki *et al*. (2007; 2021). Preliminary tests were conducted to determine the optimal number of hosts to expose, ensuring an excess of hosts for oviposition. In each cage, detached sour orange leaves bearing four 3rd, four 4th instar larvae (prepupa) and four pupae of *P. citrella* (12 exposed hosts) were placed in a small plastic jar (25 ml) filled with water along with one female and two male adults (less than 24 hours old) of the parasitoid. Parasitoids were fed *ad libitum* on small drops of honey deposited inside the cage. All leaves bearing the *P. citrella* hosts were removed and new ones were introduced in the cage at 24 hours intervals. Subsequently, the hosts were checked under a stereoscopic binocular microscope for evidence of either oviposition or host feeding. Exposed hosts were scored as parasitized if one (single parasitized) or more eggs (self-parasitized) were deposited onto or near the paralyzed host (idiobiont parasitoid) and as dead by host feeding if there was evidence of partial or complete desiccation of the larvae due to feeding by the adult female. Dead male parasitoids were replaced with 1-day old ones. Measurements were made every 24 hours throughout the female lifespan. Each leaf with parasitized host carrying one or more eggs was placed in a Petri dish until adult parasitoid emergence in order to examine the parasitoid's sex. Parasitoid sex rate was expressed as percent of ♀ over total number of insects (♂ + ♀). Twenty females were initially used for each temperature treatment, while females that never oviposited during their life span were excluded from analysis. The number of replicates (females) for each temperature is shown in table 1. The experiments were conducted under controlled conditions in a growth chamber (Conviron) at 20, 25 and 30 ± 0.5 °C, 60 ± 10% RH, 10,000 lux illumination and 14L:10D photoperiod.

Demographic growth parameters

In order to calculate population parameters of *P. pectinicornis*, data obtained from this study were combined with those from our previous study concerning developmental time, and survival rates of the immature stages of the parasitoid (Kalaitzaki *et al*., 2007). The age specific survival (l_x: fraction of females alive at age x) and the age specific fecundity (m_x: mean number of females born from surviving females at age x) per day were calculated for each temperature (20, 25, 30 °C). Net reproductive rate (R_o) and mean generation time (T) were calculated using the equations $R_o = \Sigma l_x m_x$, $T = \Sigma x l_x m_x / R_o$ respectively (Carey, 1993). Intrinsic rate of increase (r_m) was calculated by iterative solution of the equation $\Sigma e^{-rmx} l_x m_x d_x = 1$ and consequently used to calculate doubling time (DT = $ln2/r_m$) and finite rate of increase ($\lambda = e^{rm}$) (Birch, 1948; Carey, 1993).

Statistical analysis

Data either on oviposition periods, fecundity, longevity, sex ratio host feeding, self-superparasism and parasitism at various temperatures or on host-instar choice were analysed using analysis of variance (ANOVA). Within each temperature, to compare differences between single parasitized and self-superparasitized hosts (with 2, 3, 4, 5, 6, 7, 8, 9 eggs/host), percentage data were subjected to analysis of variance (ANOVA). Data on the percentage of single-parasitism and self-superparasitism were arcsine - transformed before analysis. Analyses were followed by post hoc Tukey - Kramer test (HSD) to compare the significance between the means ($p < 0.05$). Data analysis was carried out using the statistical program JMP 16.1 (SAS Institute, 2021).

Results

Oviposition

A percentage of 5, 10 and 20% of females of *P. pectinicornis* failed to oviposit at 20, 25 and 30 °C, respectively. *P. pectinicornis* was found to be synovigenic with a relatively long pre-oviposition period which was significantly affected by temperature ($F_{2,52} = 6.957$, $P = 0.0022$). The pre-oviposition period was longest at 20 °C (8.73 ± 0.64 days) but did not significantly differ from that recorded at 30 °C (7.68 ± 0.63 days) (table 1). Oviposition period was significantly longer at 20 °C (55.63 ± 6.38 days) than that observed at 25 °C (33.50 ± 3.33 days) and 30 °C (25.87 ± 2.87 days) ($F_{2,52} = 11.065$, $P < 0.001$) (table 1). No significant effect of temperature was found on the length of post-oviposition period ($F_{2,52} = 0.496$, $P = 0.612$) (table 1).

Fecundity

The fecundity of *P. pectinicornis* was significantly affected by temperature ($F_{2,52} = 22.72$, $P < 0.001$) with the highest number of eggs laid at 20 °C (110.26 ± 13.21 eggs / female) compared to 25 °C (38.61 ± 4.10 eggs / female) and 30 °C (35.63 ± 5.33 eggs / female). The same pattern was observed between the average daily oviposition rate of *P. pectinicornis* and temperature ($F_{2,52} = 12.184$, $P < 0.001$); maximum values were recorded at 20 °C (1.57 ± 0.14 eggs / day) while a significant reduction was observed at 25 and 30 °C (0.91 ± 1.11 and 0.83± 0.08 eggs / day, respectively) (figure 1 and table 1). A quadratic relationship was found between daily egg-oviposition and female age at all tested temperatures (20 °C: $R^2 = 0.558$, $a = 2.636 \pm 0.131$, $b = -0.014 \pm 0.001$, $c = -0.0004 \pm 0.00005$ and $P < 0.0001$; 25 °C: $R^2 = 0.422$, $a = 1.118 \pm 0.118$, $b = -0.011 \pm 0.002$, $c = -0.0008 \pm 0.0001$ and $P < 0.0001$; 30 °C: $R^2 = 0.531$, $a = 1.512 \pm 0.112$, $b = -0.015 \pm 0.002$, $c = -0.00069 \pm 0.0001$ and $P < 0.0001$). Females laid up to 10 eggs/day at 20 °C, and 8 at 25 °C and 30 °C, (table 1). Within 24 hours, a single female parasitized in average 1.10 ± 0.04, 0.76± 0.05 and 0.68 ± 0.06 hosts (either by depositing a single egg per host or by self-superparasitizing) (table 1), and a total of 78.52 ± 9.12, 32.66 ± 3.37 and 29.47 ± 4.39 hosts at 20, 25 and 30 °C, respectively, during its overall life. The percentage of parasitism was significantly affected by temperature ($F_{2,52} = 8.071$, $P < 0.001$). The highest percentage was observed at 20 °C compared to 25 and 30 °C. The same pattern was observed between the average daily parasitism rate of *P. pectinicornis* and temperature ($F_{2,52} = 8.028$, $P < 0.001$).

Table 1. Life history parameters (mean ± SE) of *P. pectinicornis* feeding on *P. citrella* at three temperatures. (Numbers in parenthesis show the min and max values).

Biological characteristic	Temperature 20 °C	 25 °C	 30 °C
Fecundity (laid eggs)	110.26 ± 13.21 a (22-201)	38.61 ± 4.10 b (7-70)	35.63 ± 5.33 b (6-73)
Longevity (days)	71.52 ± 6.78 a (18-118)	44.33 ± 3.52 b (20-63)	40.81 ± 3.83 b (19-74)
Oviposition rate (laid eggs/female/day)	1.57 ± 0.14 a (0-10)	0.91 ± 1.11 b (0-8)	0.83 ± 0.08 b (0-8)
Pre-oviposition period (days)	8.73 ± 0.64 a (4-13)	5.61 ± 0.56 b (3-11)	7.68 ± 0.63 ab (3-12)
Oviposition period (days)	55.63 ± 6.38 a (6-101)	33.5 ± 3.33 b (9-55)	25.87 ± 2.87 b (8-44)
Post-oviposition period (days)	7.15 ± 1.87 a (1-29)	5.22 ± 1.09 a (2-19)	7.25 ± 1.79 a (2-31)
Host-feeding (larvae/female)	26.53 ± 3.18 b (8-48)	19.78 ± 2.08 a (10-31)	15.51 ± 1.98 a (8-26)
Host-feeding rate (larvae/day)	0.38 ± 0.03 a (0-4)	0.44 ± 0.03 a (0-3)	0.38 ± 0.04 a (0-3)
Parasitism rate (hosts/female/day)	1.10 ± 0.08 b (0-6)	0.76 ± 0.08 a (0-5)	0.68 ± 0.064 a (0-5)
Parasitism (%)	9.17 ± 0.71 b (3.53-14.14)	6.35 ± 0.68 a (2.78-16.25)	5.69 ± 0.54 a (1.82-8.97)
Sex ratio (females/ total adults)	0.29 ± 0.05 a (0-1)	0.17 ± 0.04 ab (0-0.66)	0.10 ± 0.03 b (0-0.38)
N (number of replicates)[1]	19	18	16

Within rows, values followed by the same letter do not differ significantly according to Tukey - Kramer test (P = 0.05).

[1] Includes only ovipositing females.

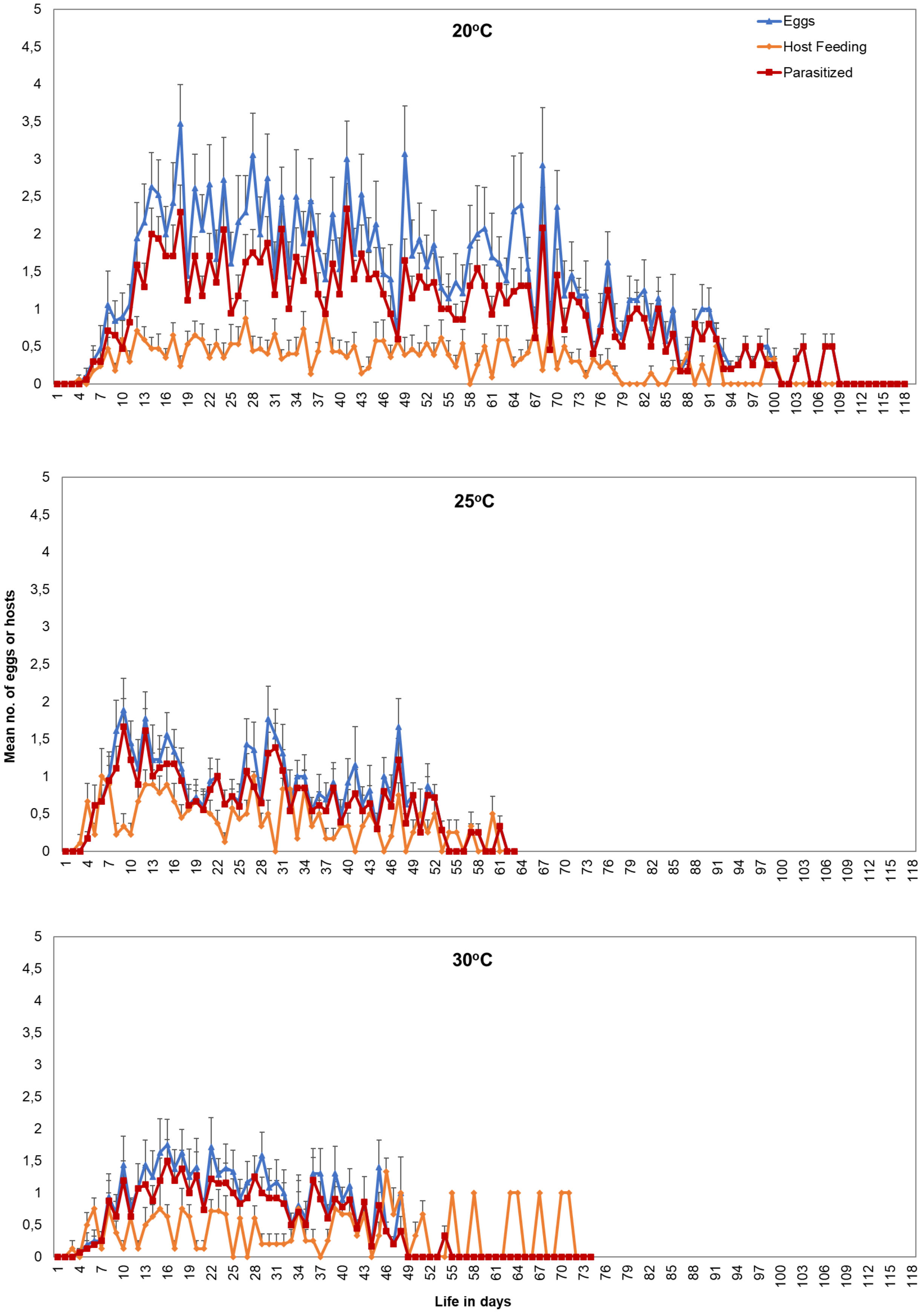

Figure 1. Daily distribution of *P. pectinicornis* fecundity, parasitization and host-feeding (mean + SE) at 20, 25, 30 °C.

Table 2. Mean number (± SE) of third, fourth (prepupa) instar larva and pupa of *P. citrella* parasitized by *P. pectinicornis* at 20, 25 and 30 °C.

	20 °C	25 °C	30 °C
3rd instar larva	19.42 ± 3.62 b	11.16 ± 1.38 b	9.37 ± 1.96 b
4th instar larva (prepupa)	54.73 ± 6.39 a	19.16 ± 2.36 a	17.43 ± 2.75 a
Pupa	4.37 ± 0.65 c	2.33 ± 0.31 c	2.63 ± 0.71 b

Within columns means followed by the same letter do not differ significantly different Tukey - Kramer test (P = 0.05).

Table 3. Single parasitism and self-superparasitism (% ± SE) of *P. pectinicornis* at 20, 25 and 30 °C.

No. of parasitoid eggs oviposited per host	20 °C	25 °C	30 °C
1	72.65 ± 7.15 a	86.08 ± 2.91 a	82.80 ± 3.68 a
2	18.83 ± 1.67 b	12.87 ± 0.69 b	13.80 ± 0.82 b
3	5.63 ± 0.95 c	2.20 ± 0.21 c	3.18 ± 0.26 c
4	2.01 ± 0.53 d	0.43 ± 0.07 cd	0.21 ± 0.06 d
5	0.60 ± 0.27 de	0.21 ± 0.05 cd	0 d
6	0.06 ±0.04 e	0 d	0 d
7	0.06 ±0.04 e	0 d	0 d
8	0 e	0 d	0 d
9	0.13 ± 0.12 e	0 d	0 d

Means followed by different small letters on each respective temperature are significantly different (P < 0.05; HSD test).

Longevity

A significant effect of temperature on female longevity was found ($F_{2,52} = 11.10$, $P < 0.0001$). Maximum female longevity was recorded at 20 °C (71.52 ± 6.78 days) but decreased by about half at 25 (44.33 ± 3.52 days) and 30 °C (40.81 ± 3.83 days) (table 1).

Sex ratio

Temperature significantly affected the sex ratio of the emerging females (female incidence) ($F_{2,51} = 4.17$, $P < 0.02$). The proportion of females was significantly higher at 20 °C (0.29) compared to 30 °C (0.10), but not significantly different from that recorded at 25 °C (0.17) (table 1). At 20 °C, 94.7% of the females studied produced both female and male offspring while the remaining 5.3% produced only male offspring. The proportion of females giving only male offspring were 38.9 and 50% at 25 and 30 °C respectively, indicating that these females did not mate: males derive from arrhenotokous parthenogenesis, a common characteristic of Hymenoptera.

Host feeding

Females preferred to feed on 3rd instar larvae. Temperature did not significantly affect daily host feeding rates (evidence of partial or complete desiccation of the larvae) ($F_{2,33} = 0.811$, $P = 0.453$). Feeding rates on *P. citrella* larvae were 0.38, 0.44 and 0.38 larvae/day at 20, 25 and 30 °C, respectively (table 1). The first host feeding incidence by females was recorded 4, 3 and 3 days after the adult emergence while in total, each female parasitoid fed on 26.53, 19.78 and 15.51 larvae at 20, 25 and 30 °C, respectively during her life (figure 1, table 1). A quadratic relationship was found between daily host feeding and female age at 20 and 25 °C (20 °C: $R^2 = 0.446$, $a = 0.621 \pm 0.039$, $b = -0.003 \pm 0.0004$, $c = -0.0009 \pm 0.0001$ and $P < 0.0001$; 25 °C: $R^2 = 0.255$, $a = 0.7019 \pm 0.071$, $b = -0.006 \pm 0.001$, $c = -0.0002 \pm 0.00001$ and $P < 0.0001$) while no impact of the age of the female was observed on her host feeding rate at 30 °C (figure 1).

Host-instar choice

P. pectinicornis prefers to oviposit on 4th instar larvae two or three-fold more than on 3rd instar larvae and pupal stage of *P. citrella*, respectively at all temperatures tested (20 °C: $F_{2,56} = 36.83$, $P < 0.0001$; 25 °C: $F_{2,53} = 27.99$, $P < 0.0001$; 30 °C: $F_{2,47} = 13.79$, $P < 0.0001$) (table 2).

Self-superparasitism

The maximum number of eggs laid on a single host in one day was 9, 5 and 4 at 20, 25, 30 °C, respectively (table 3). *P. pectinicornis* females ovipositing a single egg per host were recorded at a percentage of 72.65, 86.08 and 82.80% at 20, 25 and 30 °C, respectively during their lives (table 3). Temperature significantly affected percent of self-superparasitism which was highest at 20 °C (27.3%) ($F_{2,50} = 11.544$, $P < 0.0001$). The percentage of host larvae with a single egg was significantly higher compared to host larvae with 2, 3, 4, 5, 6, 7 and 9 eggs at any temperature tested (20 °C: $F_{8,162} = 415.99$, $P < 0.0001$; 25 °C: $F_{8,150} = 471.43.62$, $P < 0.0001$; 30 °C: $F_{8,130} = 254.26$, $P < 0.0001$). Self-superparasitism was observed on the 5th, 7th and 5th days after adult emergence at 20, 25 and 30 °C, respectively (figure 2). Pearson correlation coefficient (r) analysis revealed a moderate positive correlation between female age and self-superparasitism at 20 °C and a weak positive correlation at 25 and 30 °C (20 °C: $r = 0.414$, $n = 118$, $P < 0.0001$; 25 °C: $r = 0.111$, $n = 62$, $P = 0.007$; 30 °C: $r = 0.210$, $n = 74$, $P < 0.0001$).

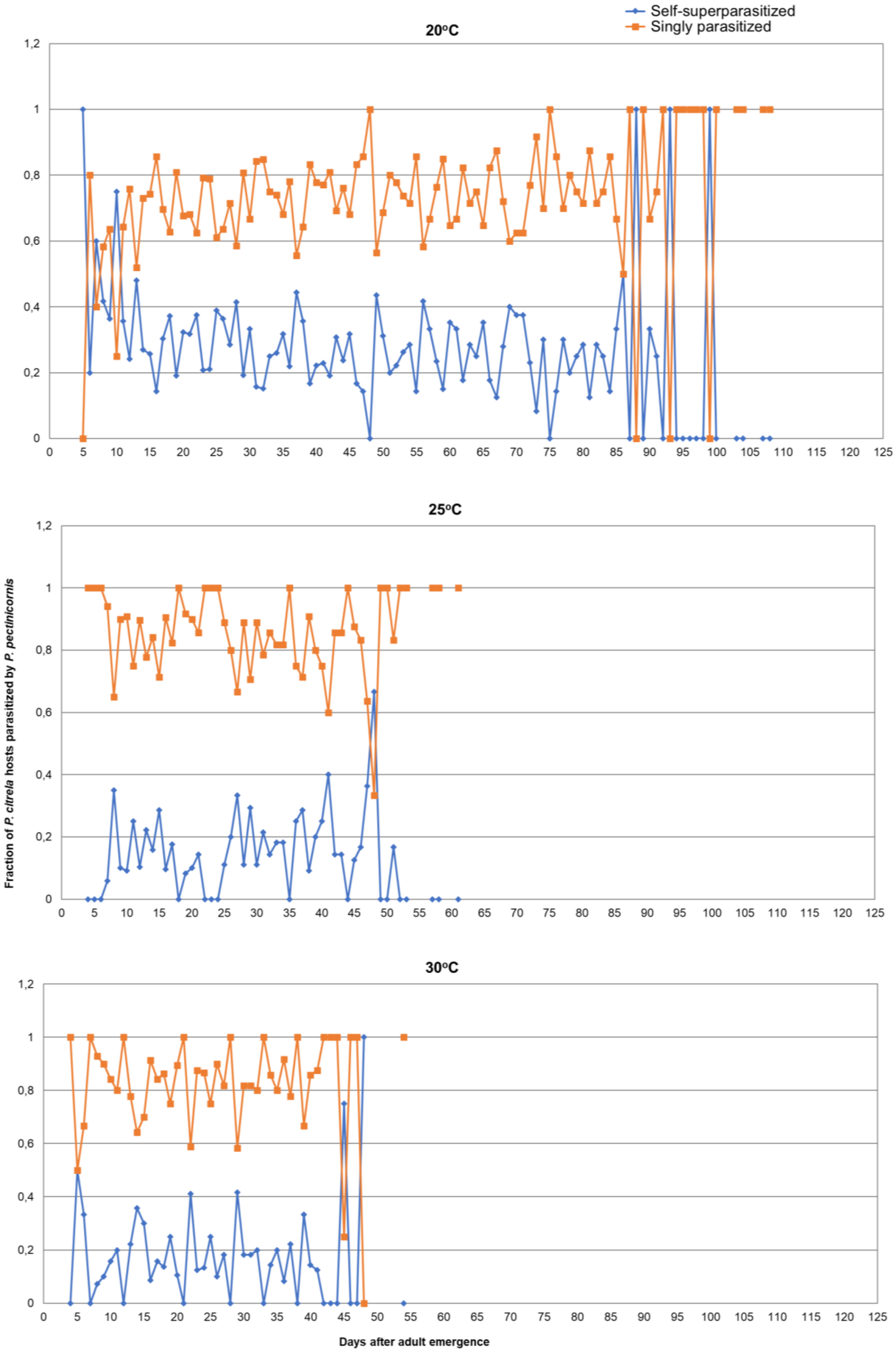

Figure 2. Fraction of *P. citrella* hosts singly parasitized and self-superparasitized by *P. pectinicornis*.

Table 4. Life table characteristics of *P. pectinicornis* feeding on *P. citrella* at three temperatures. *Ro*, net reproductive rate; *T*, mean generation time (days); r_m, intrinsic rate of increase (days^{-1}); λ, finite rate of increase; DT, doubling time (days).

	20 °C	25 °C	30 °C
R_o	18.82	4.69	2.167
r_m	0.073	0.054	0.027
T	51.36	32.22	32.83
λ	1.076	1.056	1.027
DT	9.42	12.643	25.29

Life table characteristics

Demographic growth parameters of the parasitoid *P. pectinicornis* at different temperatures are shown in table 4. The net reproductive rate (R_o) of the parasitoid was highest at 20 °C (18.82 female eggs / female / generation), and lowest at 25 and 30 °C (4.69 and 2.17, respectively) (table 4). The highest value of intrinsic rate of natural increase (r_m) of *P. pectinicornis* was also observed at 20 °C (0.073) and decreased approximately 1.5-fold at 25 °C (0.054) and 2.7-fold at 30 °C (0.027) (table 4). The mean generation time (T) of *P. pectinicornis* also decreased with temperature from 51.36 days at 20 °C to a minimum of 32.22 and 32.83 days at 25 and 30 °C, respectively (table 4). The values of the finite rate of increase (λ) of *P. pectinicornis* were maximum at 20 °C (1.076) and minimum at 30 °C (1.027). Population-doubling time (DT) peaked to 25.29 days at 30 °C and was reduced to only 9.42 days at 20 °C (table 4).

Discussion and conclusions

Oviposition

P. pectinicornis has a pre-oviposition period of a few days during which it needs to feed on hosts and other non-host nutrient sources, such as nectar, hemipteran honeydew and pollen, before achieving its full reproductive potential, similar to other synovigenic species of the genus *Pnigalio*, such as *P. minio*, *P. soemius*, and of other citrus leafminer parasitoid species (*Cirrospilus* sp. near *lyncus*, *C. vittatus* and *S. petiolatus*) (Duncan and Peňa, 2000; Urbaneja *et al.*, 2001; 2002; Bernardo *et al.*, 2006; Kalaitzaki *et al.*, 2021). Temperature significantly affected the length of both pre-oviposition and oviposition periods of *P. pectinicornis*, but not of the post-oviposition period. Maximum values of both pre-oviposition and oviposition periods were observed at the lowest (20 °C) temperature tested. A similar response to temperature was reported for *Cirrospilus* sp. near *lyncus* regarding all three periods (pre-, post- and oviposition), that were longer at lower temperatures with maximum values obtained at 15-20 °C (Urbaneja *et al.*, 2001). Contrary to *P. pectinicornis*, the pre-oviposition period of the citrus leafminer parasitoids *S. petiolatus*, *Q. citrella* as well as of the *Liriomyza* spp. synovigenic ectoparasitoid *Diglyphus isaea* (Walker) was not found to be significantly affected by temperature (Minkenberg, 1989; Llácer *et al.*, 2006; Kalaitzaki *et al.*, 2021).

Fecundity and longevity

The results of this study showed that 5, 10 and 20% of females of *P. pectinicornis* died without laying eggs at 20, 25 and 30 °C respectively, suggesting that temperature affects adversely the number of females that oviposited and consequently its population increase. Unfavourable temperatures have been shown also to reduce the number of females of other natural enemies that oviposited (Braman and Yeargan, 1988; Alauzet *et al.*, 1994; Kalaitzaki *et al.*, 2021). Of the three temperatures tested, the optimal temperature for oviposition was 20 °C as well as both reproductive capacity and life span of the females decreased at the higher tested temperatures. A similar effect of temperature on fecundity was reported in the case of *Cirrospilus* sp. near *lyncus* with the maximum values observed at 20 °C and minimum at 30 °C respectively, although the later parasitoid had greater maximum fecundity values than *P. pectinicornis* at all temperatures tested (Urbaneja *et al.*, 2001). However, in the case of *Q. citrella* and *S. petiolatus* maximum fecundity was recorded at 25 and 30 °C and minimum at 15 and 20 °C, respectively (Llácer *et al.*, 2006, Kalaitzaki *et al.*, 2021). Also, reproductive biology of *P. pectinicornis* appeared quite poor when compared with the *P. citrella* parasitoids *P. minio* at 27 °C (Duncan and Peňa, 2000), *Q. citrella* at the three temperatures tested (Llácer *et al.*, 2006) and *S. petiolatus* at 25 and 30 °C (Kalaitzaki *et al.*, 2021) as well as of *P. soemius* feeding on *C. pulchrimella* at 25 °C (Bernardo *et al.*, 2006). However, in the study of Grabenweger *et al.* (2009) fecundity of *P. agraules* (likely to be *P. mediterraneus* according to Gebiola *et al.*, 2009) feeding on *C. ohridella* was much lower than that found in the present study for *P. pectinicornis* at 20 °C. Daily oviposition rates of *P. pectinicornis* were affected by the female's age during oviposition, a fact similar to that observed on *Cirrospilus* sp. near *lyncus*, *Q. citrella* and *P. minio* where the number of eggs laid /day gradually decreased as age of the females progressed (Duncan and Peňa, 2000; Urbaneja *et al.*, 2001; Llácer *et al.*, 2006). While lower temperature appeared to have positive effects in terms of reproductive and life history parameters, it slightly prolongs the insect's preoviposition period. This extension may increase the risk of exposure to mortality factors, such as predation, prior to egg laying, potentially adversely affecting the overall successful reproduction of the parasitoid.

Sex ratio

Sex ratio of *P. pectinicornis* was significantly affected by temperature. The percentage of the parasitoid's female offspring was maximum at 20 °C and decreased significantly at 30 °C. Similarly to other *P. citrella* parasitoids such as *Cirrospilus* sp. near *lyncus* (Urbaneja *et al.*, 2001) and *Q. citrella* (Llácer *et al.*, 2006), the sex ratio of the *P. pectinicornis* was extremely male biased, likely in part due to absence of mating. However, the probability of mating and the resulting sex ratio may also be affected by rearing conditions and possibly in different arenas or experimental conditions or in a field setting, mating may occur more or less frequently. In any case, the proportion of *P. pectinicornis* female offspring was significantly lower compared to *P. minio* feeding also on *P. citrella*, to

P. soemius feeding on *C. pulchrimella*, to *P. agraules* (likely to be *P. mediterraneus*) feeding on *C. ohridella* at the common temperatures tested under laboratory and to *P. mediterraneus* feeding on *Bactrocera oleae* (Rossi) (Diptera Tephritidae) under field conditions in Crete (Neunschwander *et al.*, 1983; Duncan and Peňa, 2000; Bernardo *et al.*, 2006; Gebiola *et al.*, 2009; Grabenweger *et al.*, 2009). From a biological control point of view, arrhenotokous parasitoids may have lower rates of population increase compared to thelytokous. Also, where the population of an arrhenotokous parasitoid is very low, male and female individuals may have problems encountering each other (Kidd and Jervis, 1996). This distinction in reproductive strategy can influence their effectiveness in controlling pest populations.

Host feeding

Host-feeding behaviour of parasitoids, besides to providing nutrients for the adult parasitoids, extends to increasing pest control effectiveness since they additionally act as predators (Jervis and Kidd, 1986). *P. pectinicornis* elimination of its leafminer host by host-feeding is typical to other species of the genus *Pnigalio* such as *P. minio*, *P. soemius*, *P. agraules* (likely to be *P. mediterraneus*), (Barrett and Brunner, 1990; Bernardo *et al.,* 2006, Gebiola *et al.*, 2009; Grabenweger *et al.*, 2009). During her life span, each female of *P. pectinicornis* fed on an average of 26.5 hosts at 20 °C, a value which was much higher compared to that reported in the studies of Kalaitzaki *et al.* (2021) and Llácer *et al.* (2006) for *S. petiolatus*, and *Q. citrella*, respectively. However, significantly higher host-feeding rates compared to *P. pectinicornis* were found in the cases of the parasitoids *P. minio* (92.3 larvae of *P. citrella*/female), *P. soemius* (33.3 larvae of *C. pulchrimella*/female) and *P. mediterraneus* (12.3% of *C. ohridella*), as well as *Cirrospilus* sp. near *lyncus* (0.30-0.91 larvae of *P. citrella*/day), at the respective temperatures (Duncan and Peňa, 2000; Urbaneja *et al.*, 2001; Bernardo *et al.*, 2006; Gebiola *et al.*, 2009; Grabenweger *et al.*, 2009). Parasitoids tend to feed on younger and oviposit on older host stages, especially in cases of destructive host-feeding (Jervis *et al.*, 1996). Hence, stage preference is probably a reason for the low host feeding rates observed in the present study since the available *P. citrela* larval stages (3rd and 4th) are preferred by the parasitoid for egg deposition (Kalaitzaki *et al.*, 2007). Moreover, alternative food source (honey) was provided to the females and this could be another reason of the lower host feeding recorded in *P. pectinicornis*. According to Jervis *et al.* (1996) the females of destructive host-feeding parasitoids rely less upon hosts as a food source when alternative non-host food is plentiful. This was also confirmed in cases of *Neochrysocharis formosa* (Westwood) (Hymenoptera Eulophidae) and *Eupelmus vuilleti* (Crawford) (Hymenoptera Eupelmidae), as females fed with honey killed significant less hosts by host feeding compared to the non-honey-fed (Casas *et al.*, 2005, Liu *et al.*, 2015). Probably, under field conditions where 1st and 2nd instar larvae will be also available, the mortality inflicted by host feeding could be higher, since younger instars provide less resources to female parasitoids (Bernardo *et al.*, 2006).

Host-instar choice

Contrary to *P. minio* in which females preferred to oviposit on 3rd instar larva of citrus leafminer (72.3%) under field conditions, *P. pectinicornis* preferred to oviposit on the prepupa instar compared to 3rd instar larva while it rarely oviposited on the pupa of *P. citrella* regardless the temperature regime. This preference of *P. pectinicornis* for mature *P. citrella* instars may partly explain its competitive displacement by *C. phyllocnistoides*, currently predominant in most Mediterranean countries, which prefers to parasitize second and early 3rd instar larvae making the mature *P. citrella* instars less abundant (Urbaneja et al., 2000, Garcia-Marí *et al.*, 2004; Karamaouna *et al.*, 2010; Kalaitzaki *et al.*, 2011).

Self-superparasitism

Self-superparasitism of *P. pectinicornis* occurred more frequent (27 and 16% at 20 and 25 °C, respectively) than that observed by *P. soemius* under laboratory conditions (4.1% on *C. pulchrimella* at 25 °C) (Bernardo *et al.*, 2006). Moreover, self-superparasitism of *P. pectinicornis* was also more frequent than that observed by *P. minio* under field conditions (6.25% on *P. citrella*) (Duncan and Peňa, 2000). On the contrary, self-superparasitism of *P. pectinicornis* was less frequent than that observed by *Cirrospilus* sp. near *lyncus* (34.3%) and *C. vittatus* (42.8% at 25 °C) under laboratory conditions (Urbaneja *et al.*, 2002). Temperature significantly affected percent of self-superparasitism which was highest at 20 °C (27%). Similar to other species such *S. petiolatus* and *Q. citrella* this behaviour was more frequent at the temperature where fecundity was enhanced for *P. pectinicornis* and this may be related to the higher egg potential of these parasitoids at this temperature (Keasar *et al.*, 2006; Llácer *et al.*, 2006; Kalaitzaki *et al.*, 2021). Despite the fact that a sufficient number of hosts was available to the parasitoid (12 hosts provided per day), occasional occurrences of two to nine eggs on a single host left the remaining leafminer larvae unparasitized. However, only one of the multiple eggs laid in each host successfully completed development, while the rest were wasted. The occurrence of self-superparasitism increased on the 5th day after female emergence at 20 and 30 °C and on the 7th day at 25 °C and remained at similar levels throughout the female's life. Similarly, to *P. pectinicornis*, the rate of superparastism of *S. petiolatus* on *P. citrella* was not affected by the parasitoid age (Kalaitzaki *et al.*, 2021).

Life table characteristics

The values of demographic parameters of *P. pectinicornis* including the net reproductive rate (R_o), the intrinsic rate of natural increase (r_m) and the mean generation time (T) clearly indicate that *P. pectinicornis* underperformed in comparison with the parasitoids of *P. citrella*, *Cirrospilus* sp. near *lyncus* and *Q. citrella* at the three tested temperatures, *S. petiolatus* at 25 and 30 °C, and *C. vittatus* at 25 °C, while performed better than *S. petiolatus* at 20 °C (Urbaneja *et al.*, 2001; 2002; Llácer *et al.*, 2006; Kalaitzaki *et al.*, 2021).

Results of the current study showed that of the temperatures tested, reproductive and life table parameters of *P. pectinicornis* are favoured at 20 °C in contrast to the temperatures within the range of 25-30 °C. In addition, according to Kalaitzaki *et al*., (2007) the parasitoid can develop within a broad range of temperatures (5.27-32.5 °C), with the optimum temperature for its preimaginal development ranging between 20-30 °C. These results agree with field monitoring in Western Crete and Argolida citrus groves (Greece), where even though *P. pectinicornis* was less abundant than *C. phyllocnistoides* in *P. citrella* infested leaves throughout the sampling period, its higher populations appeared from May to June and autumn (Kalaitzaki *et al*., 2011, Tsagkarakis *et al*., 2013). Similarly, in Portugal citrus groves, *P. pectinicornis* was more active in early and mid of autumn (Gomes de Silva *et al*., 2006).

Although values of key parameters of the *P. pectinicornis* such as fecundity, longevity, preimaginal development and lower thermal threshold for development exceed these of the citrus leafminer indicating its suitability as a biological control agent for this pest at all temperatures tested (Yamamoto, 1971; Minsheng *et al*., 1995), other important biological parameters such as polyphagy and lower intrinsic rate of increase compared to *P. citrella* as well as to other exotic parasitoids probably are the reasons for its lower impact on *P. citrella* population.

This is consistent with the fact that the importance of *P. pectinicornis* as parasitoid of *P. citrella* declined after the establishment of the more host-specific *P. citrella* parasitoid *C. phyllocnistoides* which forced it to return to its original niches (Garcia-Marí *et al*., 2004; Karamaouna *et al*., 2010; Kalaitzaki *et al*., 2011, Tsagkarakis *et al*., 2013, Mansour *et al*., 2021). However, other important biological parameters, such as the host-searching ability of *P. pectinicornis*, were not assessed in this study. Evaluating these factors could provide valuable insights into its suitability for biological control of insect pests, particularly in comparison to other related parasitoid species that share similar ecological niches.

The fact that the polyphagous parasitoid *P. pectinicornis* was the first one to attack the invading citrus leaf miner soon after its establishment, becoming one of its major parasitoids, demonstrates the significance of natural enemies on controlling invading herbivore pests and highlights the benefits of preserving biodiversity. Overall, conservation tactics by maintaining a rich biodiversity in citrus and olive groves by providing alternative food and shelter to the parasitoid, is of great importance.

In conclusion, the results of this study have provided valuable information on the effect of temperature on the reproductive and population parameters of the widespread polyphagous parasitoid, *P. pectinicornis*. The biological parameters associated with reproduction and longevity of this species were observed to be more favourable under lower temperatures (20 °C). Despite its low population levels after the establishment of the more specific and intrinsically superior *P. citrella* parasitoid *C. phyllocnistoides*, limiting its impact on *P. citrella* population, *P. pectinicornis* is a widespread biological control agent against several important insect pests. To enhance our understanding of the parasitoid's effectiveness across different hosts, future studies should delve into its performance on other important insect species, thereby refining our insights into the parasitoid's host range and preferences.

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