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Life table and biological characteristics of the parasitoid *Semielacher petiolatus* reared on *Phyllocnistis citrella*

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Abstract

Semielacher petiolatus (Girault) (Hymenoptera Eulophidae) is an ectoparasitoid of the citrus leaf miner *Phyllocnistis citrella* Stainton (Lepidoptera Gracillariidae) that has been widely released in several Mediterranean countries. In an attempt to evaluate the insects temperature adaptations that play a significant role in its establishment potential, life table parameters and the effect of temperature on its fecundity, longevity, host-instar choice, superparasitism and host feeding were studied. Tests were conducted at 20, 25 and 30 ± 0.5 °C with 60 ± 10% RH and a 14L:10D photoperiod. *S. petiolatus* oviposition period was 21.05 ± 2.54 days at 30 °C but as short as 2.25 ± 0.23 days at 20 °C. Fecundity had a maximum value (173.3 ± 27.09) at 30 °C followed by 143.57 ± 22.67 and 5.13 ± 0.81 at 25 and 20 °C, respectively while, 28.57% of the females failed to oviposit at 20 °C. Female longevity averaged between 25.20 and 20.57 days at 30 and 25 °C, respectively but dropped to just 5.77 days at 20 °C. The net reproductive rate (R_0) was highest (50.8) at 30 °C, and highly reduced (1.1) at 20 °C. Following the above pattern, mean generation time (T: 15.45 - 23.39), intrinsic rate of increase (r_m: 0.006 - 0.216) and finite rate of increase (λ: 1.006 - 1.24) were maximum at 30 °C and minimum at 20 °C. Population-doubling time (DT) peaked to 106.94 days at 20 °C and was reduced to only 3.21 days at 30 °C. The host-choice tests showed that second and third instar larvae of *P. citrella* were equally selected at all temperatures. Superparasitism occurred at a rate of 5.79, 15.39 and 14.52% at 20, 25 and 30 °C, respectively and up to 6 eggs had been oviposited per host. Daily host feeding rates averaged 0.24, 0.41 and 0.31 hosts at 20, 25 and 30 °C, respectively. The above results indicate that *S. petiolatus* is much better adapted at higher temperatures typical for the Mediterranean climatic conditions during summer while its population increase is expected to be marginal at temperatures close to 20 °C. Overall, conservation tactics by maintaining a rich biodiversity in citrus groves for providing alternative food and shelter to the parasitoid, especially during spring before populations of *P. citrella* increase, should enhance its activity against early summer population of *P. citrella* in citrus orchards in Mediterranean countries.

Key words: Hymenoptera, Eulophidae, ectoparasitoid, temperature, fecundity, intrinsic rate of increase, biological control.

Introduction

Semielacher petiolatus (Girault) (Hymenoptera Eulophidae) is an indigenous to Australia and Solomon Islands, solitary idiobiont ectoparasitoid, predominantly parasitizing the citrus leaf miner *Phyllocnistis citrella* Stainton (Lepidoptera Gracillariidae) (Bouček, 1988; Smith *et al*., 1997; Schauff *et al*., 1998). Following serious problems caused by the invasion of *P. citrella* particularly in young trees, nurseries, and overgraftings (Caleca *et al*., 1996; 2000; 2003; Caleca, 2000; Peña *et al*, 2000; Garcia-Mari *et al*., 2002), a number of parasitoids including *S. petiolatus* were introduced into the Mediterranean region (Cyprus, Israel, Morocco, Syria, Tunisia, Turkey, Egypt, Spain and Greece) against this pest (Schauff *et al*., 1998; Garcia-Mari *et al*., 2004; Elekcioglu and Uygun, 2006; Zappalà, 2010; Kalaitzaki *et al*., 2011). In Greece, between 1996 and 1999, *S. petiolatus* along with the other eulophids *Citrostichus phyllocnistoides* (Narayanan), *Quadrastichus* sp. and *Cirrospilus quadristriatus* (Subba Rao et Ramamani) (Hymenoptera Eulophidae), and the encyrtid *Ageniaspis citricola* Logvinovskaya (Hymenoptera Encyrtidae) reared and massively released in several regions, as a part of a biological control program against the citrus leafminer (Kalaitzaki *et al*., 2011; Tsagkarakis *et al*., 2013a). Studies on the seasonal abundance of the citrus leafminer and its parasitoids in Greece during 1999 and 2013 showed that *S. petiolatus* and *C. phyllocnistoides* were very effective against *P. citrella* and readily became predominately established in Greece with a wide geographical distribution in citrus growing areas (Kalaitzaki *et al*., 2011; Tsagkarakis *et al*., 2013a; 2013b). The successful establishment of *S. petiolatus* has also been demonstrated in studies from other citrus growing countries including Italy, Jordan, Algeria, Israel, Morocco, Turkey, Portugal, and Spain in Andalucía and Balear Islands (Argov and Rössler 1996; Uygun *et al*., 1997; Mineo *et al*., 1998; Schauff *et al*., 1998; Abbassi *et al*., 1999; Mineo 1999; Massa *et al*., 2001; Ateyyat, 2002; Siscaro *et al*., 2003; Garcia-Mari *et al*., 2004; Rizzo *et al*., 2006; Zappalà, 2010), however, it failed to establish in Valencia of Spain (Garcia-Mari *et al*., 2004).

Massa and Rizzo (2000) and Massa *et al*. (2001) reported that *S. petiolatus* was found on six insect hosts other than citrus leafminer in Italy and Jordan, including *Agromyza hiemalis* Becker (Diptera Agromyzidae) on *Urtica* spp., *Chromatomyia horticola* (Goureau) (Diptera Agromyzidae) on *Sonchus* spp., *Liriomyza* sp. (Diptera Agromyzidae) on *Merculiaris annua* L., *Cosmopterix pulchrimella* Chambers (Lepidoptera Cosmopterigidae) on *Parietaria diffusa* M. et K., *Stigmella aurella* (F.)

(Lepidoptera Nepticulidae) on *Rubus ulmifolius* Schott and *Dialectica scalariella* Zeller (Lepidoptera Gracillariidae) on *Echium* sp. These alternative hosts could be a positive establishment factor since they allow the parasitoid to maintain its populations during periods of very low citrus leafminer populations without any risk both for native leafminers and native parasitoids, as demonstrated by Rizzo *et al*. (2006).

To evaluate the potential of this parasitoid to establish and effectively contribute in the control of the citrus leafminer, studies have been conducted on its biological characteristics and behaviour including its development under various temperatures (Argov and Rössler, 1996; Lim and Hoy, 2005; Kalaitzaki *et al*., 2018), adult longevity, total fecundity, host mortality, host-size choice at 25 °C (Lim and Hoy, 2005) and host discrimination capacity, relative humidity tolerance and parasitization of alternative hosts (Massa *et al*., 2001; Rizzo *et al*., 2006; Lim *et al*., 2006). Even though the above-mentioned studies offer valuable information, knowledge of the effect of temperature on its reproduction and longevity is still lacking. This knowledge is essential in order to evaluate the potential of this parasitoid to become established in new areas, to interpret the variability in the results of its releases or to plan eventual new releases where it may be required given that *P. citrella* is present in all citrus-producing regions in the world (CABI, 2019). The effect of temperature on life table parameters have been estimated for other citrus leaf miner parasitoids such as *Cirrospilus* sp. near *lyncus*, *Quadrastichus citrella* Reina et La Salle and *Cirrospilus vittatus* Walker aimed to understanding their importance as parasitoids of the *P. citrella* (Urbaneja *et al*., 2001; 2002; Llácer *et al*., 2006). This is not the case for *S. petiolatus* since its life table parameters have not been estimated and consequently its successful establishment or biological control efficiency against the citrus leaf miner cannot be directly explained and compared to that of other parasitoids. Additionally, the effect of temperature on host choice, host feeding and self-superparasitism of *S. petiolatus* has not been examined even though they constitute essential parameters in the assessment of the potential of a parasitoid to compete and exploit a habitat.

In order to complement the knowledge acquired in a previous study concerning developmental time and survival rates of the immature stages of the parasitoid (Kalaitzaki *et al*., 2018), additional aspects of the adult population parameters of *S. petiolatus* were studied. 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 *S. petiolatus* reared on *P. citrella*, (3) estimate life table population parameters of the parasitoid at different temperatures using appropriate mathematical formulae.

Materials and methods

Insect rearing

The *P. citrella* colony was initiated using adults emerged from infested shoots collected from citrus orchards located in Chania prefecture of Crete. Rearing was maintained on two to three-year old potted sour orange plants (*Citrus aurantium* L.) in a glasshouse at 22.5 ± 2.5 °C under natural illumination at the Institute of Olive Tree, Subtropical Plants and Viticulture, Chania. Adults of the parasitoid *S. petiolatus* used for the bioassays originated from parasitized larvae of *P. citrella*, collected in citrus orchards in the area of Chania, Crete and maintained in transparent Plexiglass cages as described by Kalaitzaki *et al*. (2018).

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

Preoviposition, oviposition and postoviposition periods, fecundity, longevity, host feeding, host-instar choice and self-superparasitism of *S. petiolatus* 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). Optimal number of hosts to be exposed was established in preliminary tests in order to determine the necessary number ensuring an excess of hosts for oviposition: in each cage, detached sour orange leaves bearing ten 2nd and ten 3rd instar larvae of *P. citrella* (20 in total) 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 on the inner side of the cage. Leaves were left for 24 hours and then were replaced with fresh ones. At 24 hours intervals, all leaves bearing the *P. citrella* larvae were removed and new ones were introduced in the cage. Subsequently, the larvae were checked under a stereoscopic binocular microscope for evidence of either oviposition or host feeding. Exposed larvae were defined as parasitized if one or more eggs were deposited onto or near the paralyzed host (idiobiont parasitoid) and as dead by host feeding if there was evidence by partial or complete desiccation of the larvae due to feeding by the adult female. Dead male parasitoids were replaced with a 1 day old ones. Measurements were made every 24 hours throughout the female lifespan. Each leaf with parasitized larva by one or more eggs was placed in a Petri dish till adult parasitoid emergence in order to examine the sex. Parasitoid sex rate was expressed as percent of ♀ over total number of insects (♂ + ♀). Twenty-one replicates (females) were conducted for each temperature treatment while non-ovipositing females were excluded from analysis. The potential of the virgin females to oviposit was also evaluated at each temperature by using 1 day old virgin females without males (10 females). 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 *S. petiolatus*, data obtained from this study were combined with these from our previous study concerning developmental time, and survival rates of the immature stages of the parasitoid (Kalaitzaki *et al*., 2018). The age specific survival (l_x: fraction of females alive at age x) and the age specific fecundity (m_x: 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_0) and mean generation time (T) were calculated using the equations $R_0 = \Sigma l_x m_x$, $T = \Sigma \times l_x m_x / R_0$ 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 ($\lambda = e^{rm}$) respectively (Birch, 1948, Carey, 1993). Calculations were performed using the QBASIC program developed by Jervis and Copland (1996).

Statistical analysis

Data either on oviposition periods, fecundity, longevity, sex ratio and host feeding at various temperatures or on host-instar choice and clutch size (1, 2, 3, 4, 5, 6) were analysed using analysis of variance (ANOVA). Data on the percentage of self-superparasitism were arcsine - transformed before analysis. Tukey - Kramer test (HSD) was used to compare the means among temperatures while differences between the means of the two host instars at each temperature were compared using t-test ($P = 0.05$). All statistical analyses were conducted using the Statistical Analysis System JMP 7.0 (SAS, 2007).

Results

Oviposition

All females of *S. petiolatus* laid eggs at 25 and 30 °C but 28.57 % of them failed to oviposit at 20 °C. Overall, temperature did not significantly affect the length of the pre-oviposition period ($F_{2,53} = 2.12$, $P > 0.130$). On the contrary, oviposition period was significantly longer at 30 °C (21.05 ± 2.54 days) and 25 °C (16.38 ± 1.62 days) than that observed at 20 °C (2.25 ± 0.23 days) ($F_{2,53} = 26.30$, $P < 0.001$) (table 1). No significant effect of temperature was found on the length of post-oviposition period ($F_{2,53} = 0.03$, $P > 0.968$) (table 1).

Fecundity

The fecundity of *S. petiolatus* was significantly affected by temperature ($F_{2,87} = 21.25$, $P < 0.001$) with the highest number of eggs laid at 30 °C (173.30 ± 27.09 eggs / female) and 25 °C (143.57 ± 22.67 eggs / female). Significantly lower number of eggs (5.13 ± 0.81 eggs / female) were laid by *S. petiolatus* females at 20 °C (table 1). The average daily oviposition rate of *S. petiolatus* was similar between 25 and 30 °C (6.14 and 6.07 eggs / day) but significantly reduced at 20 °C (0.68 eggs / day) ($F_{2,87} = 5.33$, $P < 0.006$) (table 1 and figure 1). A quadratic relationship was found between daily eggs oviposition and female age at all tested temperatures (20 °C: $R^2 = 0.764$, $a = 1.126 \pm 0.236$, $b = 0.013 \pm 0.004$, $c = -0.0838 \pm 0.020$ and $p < 0.026$; 25 °C: $R^2 = 0.891$, $a = 11.632 \pm 0.44$, $b = 0.108 \pm 0.017$, $c = -0.028 \pm 0.001$ and $p < 0.0001$; 30 °C: $R^2 = 0.736$, $a = 10.485 \pm 0.57$, $b = 0.074 \pm 0.0193$, $c = -0.017 \pm 0.0017$ and $p < 0.0001$). Females laid up to 26 eggs per day at 30 °C, 22 at 25 °C and only 7 eggs/day at 20 °C (data not shown). Within 24 hours a single female parasitized in average 0.69 ± 0.25, 5.11 ± 0.61 and 5.02 ± 0.57 hosts (either by depositing a single egg per host or superparasitizing), and during her overall life 4.73 ± 0.62, 119.62 ± 16.73 and 145.70 ± 20.39 hosts at 20, 25 and 30 °C, respectively.

Sex ratio

Temperature did not significantly affect the sex ratio of the emerging females (female incidence) ($F_{2,36} = 1.42$, $P > 0.254$). The proportion of females at 20, 25 and 30 °C was 0.46, 0.45 and 0.46 respectively (table 1). At 20 °C, 86.6% of the females studied produced both female and male offspring while the remaining 14.4% produced only male offspring, demonstrating that these last females did not mate; as common in all Hymenoptera also in this species males derive from arrhenotokous parthenogenesis. Interestingly, all female parasitoids produced offsprings from both sexes at 25 and 30 °C.

Longevity

A significant effect of temperature on female longevity was found ($F_{2,87} = 39.84$, $P < 0.0001$). Mean longevity increased with temperature starting from 5.77± 0.40 days at 20 °C, rising to 20.57 ± 1.71 days at 25 °C and 25.44 ± 2.37 days at 30 °C (table 1).

Table 1. Life history parameters (mean ± SE) of *S. petiolatus* 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)	5.13 ± 0.81 a (1-13)	143.57 ± 22.67 b (24-379)	173.30 ± 27.09 b (30-450)
Longevity (days)	5.77 ± 0.40 a (3-8)	20.57 ± 1.71 b (9-38)	25.20 ± 2.37 b (9-44)
Oviposition rate (laid eggs/female/day)	0.68 ± 0.16 a (0-1.35)	6.14±0.56 b (0-9.8)	6.07 ± 0.46 b (0-10.85)
Pre-oviposition period (days)	1.86 ± 0.22 a (1-4)	1.90 ± 0.22 a (1-4)	2.38 ± 0.30 a (1-5)
Oviposition period (days)	2.25 ± 0.23 a (1-4)	16.38 ± 1.62 b (6-31)	21.05 ± 2.54 b (5-37)
Post-oviposition period (days)	2.33 ± 0.27a (1-5)	2.28 ± 0.39 a (0-5)	2.22 ± 0.33 a (1-7)
Host feeding rate (Larvae/day)	0.24 ± 0.10 a (0-0.0.85)	0.41 ± 0.07 a (0-1.25)	0.31 ± 0.04 a (0-0.66)
Sex ratio (females/ total adults)	0.46 ± 0.06 a (0-0.75)	0.45 ± 0.09 a (0.01-0.64)	0.46 ± 0.05 a (0.02-0.69)
N (number of replicates)[1]	15	21	21

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.

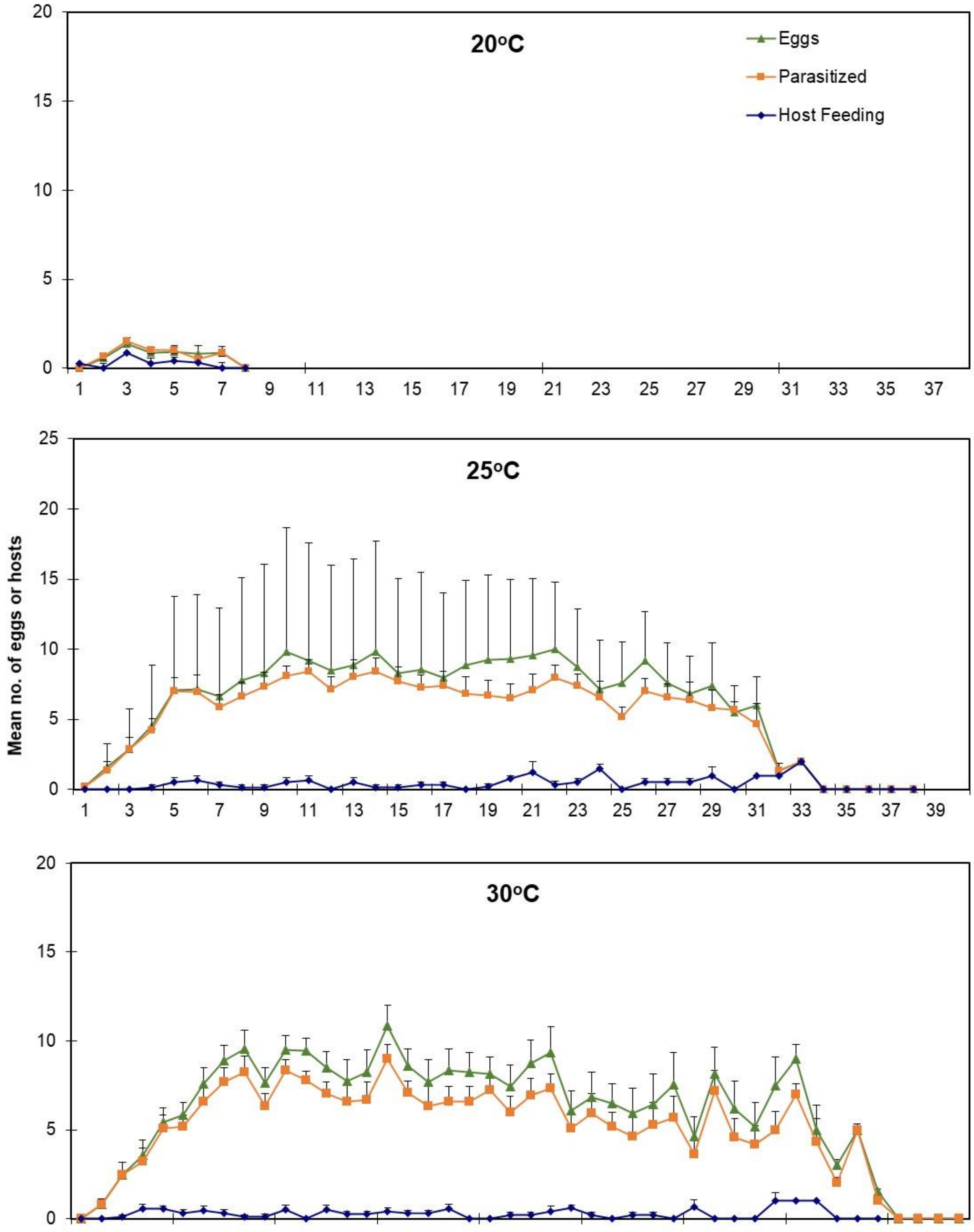

Figure 1. Daily distribution of *S. petiolatus* fecundity, parasitization and host feeding at 20, 25, 30 °C. Error bars represent standard error of the means.

Host feeding

Number of dead *P. citrella* larvae by host feeding per day (evidence of partial or complete desiccation of the larvae) were equal to 0.24, 0.41 and 0.31 larvae/day at 20, 25 and 30 °C, respectively (table 1). No significant difference was found among host feeding rates at different temperatures ($F_{2,87} = 0.94$, $P > 0.39$). The first host feeding incidence by females was recorded 3, 4 and 4 days after the adult emergence while overall, each female parasitoid fed on 0.93, 8.5 and 6.9 hosts at 20, 25 and 30 °C respectively during her life. No impact of the age of the female parasitoid was observed on her host feeding rate at all temperatures studied (figure 1).

Table 2. Mean number (± SE) of second and third-instar larva of *P. citrella* parasitized by *S. petiolatus* at 20, 25 and 30 °C.

	20 °C	25 °C	30 °C
2nd instar	1.7 ± 0.3 a	57.3 ± 9.7 a	69.9 ± 10.5 a
3rd instar	3.0 ± 0.45 a	62.3 ± 8.1 a	75.8 ± 10.7 a

Within columns means followed by the same letter do not differ significantly different (P < 0.05; t' test).

Table 3. Self-superparasitism (% ± SE) of *S. petiolatus* at 20, 25 and 30 °C for different clutch sizes.

Clutch size	20 °C	25 °C	30 °C
1	94.21 ± 4.85 a	84.61 ± 1.93 a	85.48 ± 1.71 a
2	4.34 ± 0.82 b	12.01 ± 0.61 b	10.95 ± 0.71 b
3	1.45 ± 0.36 b	2.78 ± 0.27 c	2.92 ± 0.25 c
4	0 b	0.52 ± 0.12 d	0.58 ± 0.08 cd
5	0 b	0.04 ± 0.02 d	0.07 ± 0.03 d
6	0 b	0.04 ± 0.02 d	0 d

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

Table 4. Life table characteristics of *S. petiolatus* feeding on *P. citrella* at three temperatures. R_0, 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_0	1.10	38.91	50.84
r_m	0.006	0.209	0.216
T	15.45	21.01	23.39
λ	1.006	1.23	1.24
DT	106.94	3.30	3.21

Host-instar choice

S. petiolatus oviposited on both 2nd and 3rd instar larvae of *P. citrella* without preference at all temperatures tested (20 °C: $F_{1,29}$ = 4.16, P > 0.06; 25 °C: $F_{1,41}$ = 0.15, P > 0.69; 30 °C: $F_{1,41}$ = 0.16, P > 0.68) (table 2).

Self-superparasitism

Maximum number of eggs laid on a single host per day was 3, 6 and 5 at 20, 25, 30 °C, respectively. *S. petiolatus* females oviposited a single egg per host on a percentage of 94.21, 84.61 and 85.48% at 20, 25 and 30 °C, respectively during their lives (table 3). The percentage of host larvae with a single egg was significantly higher compared to host larvae with 2, 3, 4, 5 and 6 eggs at any temperature tested (20 °C: $F_{5,545}$ = 37.62, P < 0.0001; 25 °C: $F_{5,2081}$ = 1803.62, P < 0.0001; 30 °C: $F_{5,3095}$ = 1091.57, P < 0.0001). Self-superparasitism incidents occurred not sooner than the 3rd, 2nd and 4th d after adult emergence at 20, 25 and 30 °C, respectively (figure 2). Pearson correlation coefficient (r) analysis revealed no significant correlations between female age and superparasitism at all tested temperatures (20 °C: r = 0.0983, n = 8, p = 0.449; 25 °C: r = 0.011, n = 38, p = 0.529; 30 °C: r = 0.0864, n = 44, p = 0.052).

Life table characteristics

Demographic growth parameters of the parasitoid *S. petiolatus* at different temperatures are shown in table 4. *S. petiolatus* population parameters were estimated based on the total daily number of eggs deposited per female. The net reproductive rate (R_0) of the parasitoid was highest at 30 °C (50.84 female eggs / female / generation), it was reduced to 38.91 at 25 °C while at 20 °C it showed a dramatic decrease (1.10) (table 4). The highest value of intrinsic rate of natural increase (r_m) of *S. petiolatus* was also observed at 30 °C (0.216) followed by 0.209 at 25 °C while a 35-fold decrease in r_m was observed at 20 °C (table 4). The mean generation time (*T*) of *S. petiolatus* also increased with temperature from 15.45 days at 20 °C to a maximum of 23.39 days at 30 °C (table 4). The values of the finite rate of increase (λ) of *S. petiolatus* were not significantly affected by temperature with the highest values observed at 30 and 25 °C (1.24, 1.23) and the lowest at 20 °C (1.006). The doubling time (DT) of *S. petiolatus* population was significantly shorter at 25 and 30 °C (3.30 and 3.21 days) in comparison with the value observed at 20 °C (106.34 days) (table 4).

Discussion and conclusions

S. petiolatus was found to be arrhenotokous since offspring from virgin females resulted in production of exclusively male progeny, as widely common in Hymenoptera (Heimpel and de Boer, 2008). Among the parasitoids of the citrus leaf miner, this phenomenon has been confirmed in the case of *Pnigalio minio* (Walker) (Duncan and Peña, 2000), *Cirrospilus diallus* (Walker) and *Cirrospilus pictus* (Nees) (Hymenoptera Eulophidae) (Lo Pinto *et al.*, 2005).

Similarly, to other synovigenic species such as *P. minio*, *Pnigalio soemius* (Walker), *Cirrospilus* sp. near *lyncus* and *Cirrospilus vittatus* Walker, *S. petiolatus* has a typical preoviposition period of a few days during which it needs to feed on honeydew, hosts, etc. before achieving its full reproductive potential. This was not the case for the citrus leafminer parasitoid *Quadrastichus* sp. which was reported by Llácer *et al.* (1998) to be pro-ovigenic i.e. females are able to oviposit from their eclosion. Preoviposition period of *S. petiolatus* was not significantly influenced by temperature. Similarly, to *S. petiolatus*, the *Q. citrella* as well as the *Liriomyza* spp. synovigenic ectoparasitoid *Diglyphus isaea* (Walker) were shown to have a preoviposition period of 0.3-1.8 days and 1-3 days, respectively that was not significantly affected by temperature (Minkenberg, 1989; Llácer *et al.*, 2006). Data on both length of oviposition period and fecundity of *S. petiolatus* shows a clear preference of the insect for higher temperatures in comparison with *Cirrospilus* near *lyncus*. Specifically, oviposition period of *S. petiolatus* increased in length as temperature increased up to 30 °C while in *Cirrospilus* sp. near *lyncus* oviposition period was longer under lower temperatures at a range of 15-20 °C

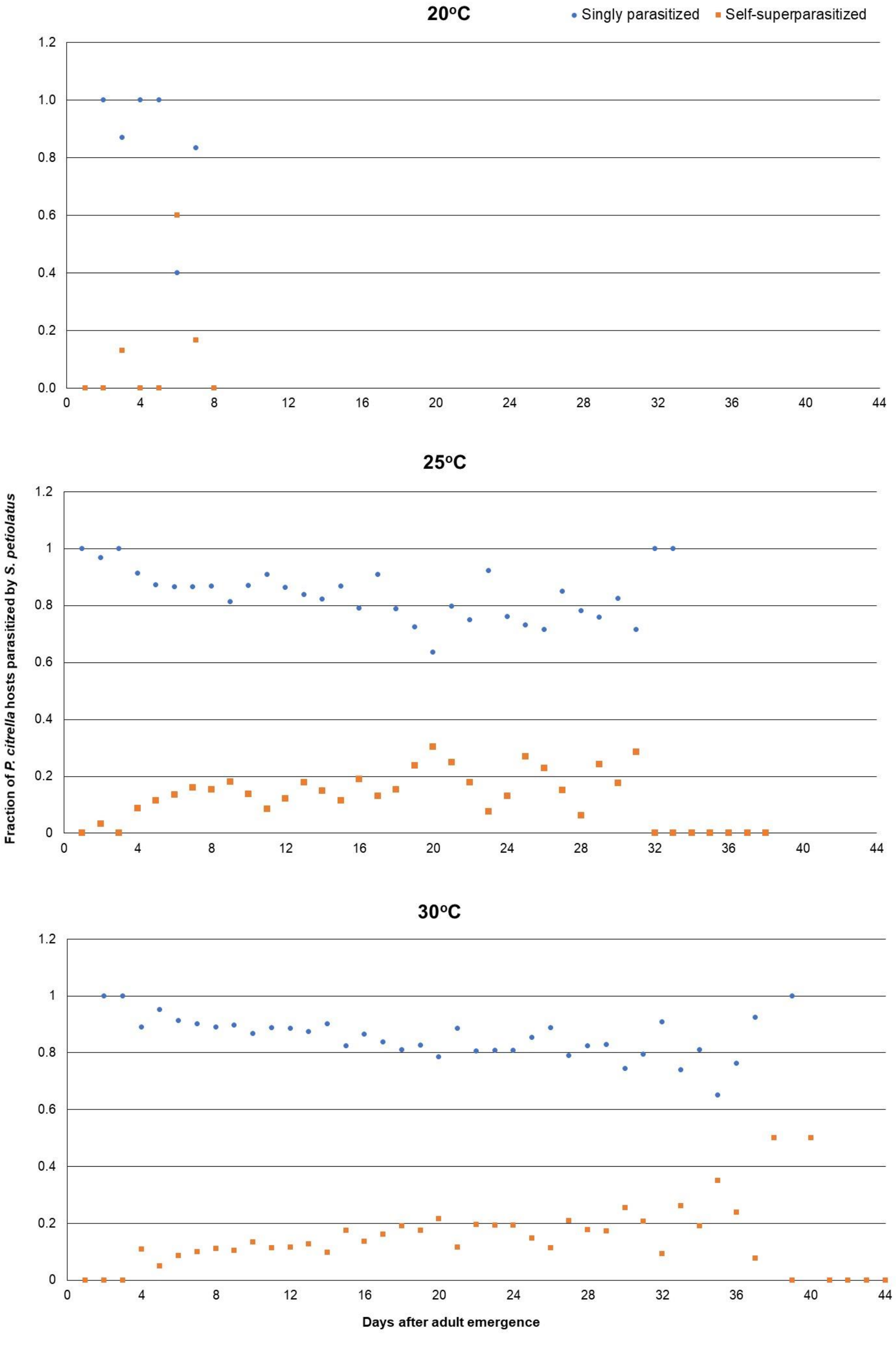

Figure 2. Fraction of *P. citrella* hosts singly parasitized and superparasitized by *S. petiolatus*.

(Urbaneja *et al*., 2001). This was also the case for the fecundity of *S. petiolatus* which was adversely affected at 20 °C where its lowest value was recorded (5.13 eggs/female), in contrast to *Cirrospilus* sp. near *lyncus* where maximum fecundity was recorded at 20 °C and minimum at 30 °C (Urbaneja *et al*., 2001). However, in the case of *Q. citrella* maximum fecundity was recorded at 25 °C and minimum at 15 °C (Llácer *et al*., 2006).

Daily oviposition rates of *S. petiolatus* were not adversely affected by the female's age during oviposition, a fact contrary 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 Peña, 2000; Urbaneja *et al*., 2001; Llácer *et al*., 2006).

No differences in *S. petiolatus* oviposition rate was found between 2nd and 3rd instar larvae of *P. citrella* regardless the temperature regime. These experimental results are not in agreement with the statement of Lim and Hoy (2005) reporting that in their preliminary observations *S. petiolatus* females preferred to attack 3rd instar larvae three times as often as other instars.

Self-superparasitism of *S. petiolatus* were less frequent (15.39% and 14.52% at 25 and 30 °C, respectively) than that observed on *Cirrospilus* sp. near *lyncus* (34.3%), *C. vittatus* (42.8% at 25°C) (Urbaneja *et al*., 2002) and *Q. citrella* (8.1-28.8%, from 15 to 25 °C) (Llácer *et al*., 2006). Less frequency of superparastism may indicate more successful parasitism since hosts that are already parasitized (self or conspecific superparasitism) are generally of lower quality, as embryos developing within them have to compete for food resources (Godfray, 1994). A slightly higher percentage of superparasitism (22%) was reported by Lim and Hoy (2005) for *S. petiolatus* at 25 °C even though more hosts were provided in the later (30 larvae/day) compared to the present study (20 larvae/day). Temperature significantly affected percent of self-superparasitism which was lowest at 20 °C (5.79%). Lower incidence of this phenomenon at 15 °C in comparison to higher temperatures has been reported in *Q. citrella* (Llácer *et al*., 2006) and this may be related to the lower egg load of both parasitoids at lower temperatures (Keasar *et al*., 2006). Generally, this behaviour was more frequent at the temperature where fecundity was enhanced for *S. petiolatus*.

The occurrence of self-superparasitism increased on the 4th day after female emergence at 25 and 30 °C, and remained at similar levels throughout the female's life. Lim and Hoy (2005) recorded that self-superparasitism of *S. petiolatus* increased on the 2nd day after the first oviposition at 25 °C and was not significantly affected by the female's age. Similarly, to *S. petiolatus*, the rate of superparastism of *Fopius arisanus* (Sonan) (Hymenoptera Braconidae) on *Bactrocera dorsalis* Hendel (Diptera Tephritidae) was not affected by the parasitoid age (Gonçalves *et al*., 2017). These experimental results are not supported by Tunca *et al*. (2016) who found that the tendency to superparasitism of the parasitoid *Ooencyrtus pityocampae* (Mercet) (Hymenoptera Encyrtidae) on *Philosamia ricini* (Danovan) (Lepidoptera Saturniidae) increased with the age of the parasitoid females. In any case, the occurrence of self-superparasitim in *S. petiolatus* seems to be low and probably results from the inability of the parasitoid to distinguish hosts attacked by self and conspecifics as indicated by Godfray (1994).

Female life span of *S. petiolatus* was found to be 5-fold longer at 30 °C compared to the minimum observed at 20 °C. The female life span of *S. petiolatus* recorded in this study was similar to that reported by Lim and Hoy (2005) at 25 °C but longer than that of *Cirrospilus* sp. near *lyncus* and *Quadrastichus* sp. at 25 and 30 °C and shorter at 20 °C (Llácer *et al*., 1998; Urbaneja *et al*., 2001).

Concerning the host feeding rate of *S. petiolatus*, no relationship between temperature and the age of the female was found. Higher host feeding rates were found in the cases of the parasitoids *Cirrospilus* sp. near *lyncus*, and *C. vittatus*, however, all three citrus leafminer parasitoids had lower host feeding rates than those of the *Liriomyza* ecto-parasite *Diglyphus isaea* (Walker) (Hymenoptera Eulophidae) at the respective temperatures (Minkenberg, 1989; Urbaneja *et al*., 2001; Urbaneja *et al*., 2002).

During her life span, each female of *S. petiolatus* fed on up to 8.5 hosts at 25 °C, a value which was much lower compared to that reported in the study of Lim and Hoy (2005) (i.e. 28 hosts at 25 °C). However, Lim and Hoy (2005) reported that in their study host feeding rates could have been overestimated since some females can puncture the host without actually feeding on it. In our study, host feeding by *S. petiolatus* was validated by the partial or complete host desiccation. Moreover, alternative food source (honey) was provided to the females which was not used by Lim and Hoy (2005), and this absence of alternative food could be a reason of the higher host feeding recorded by Lim and Hoy (2005). Finally, in our study 2nd and 3rd instar host larvae were used which are preferred by *S. petiolatus* for oviposition (Kalaitzaki *et al*., 2018). Probably, under field conditions where 1st instar larvae will be also available, the mortality inflicted by host feeding could be higher, since younger instars provide less resource to female parasitoids (Bernardo *et al*., 2006).

The values of demographic parameters of *S. petiolatus* including the net reproductive rate (R_0), the intrinsic rate of natural increase (r_m) and the mean generation time (T) clearly indicate that *S. petiolatus* performed better than *Cirrospilus* sp. near *lyncus* and *C. vittatus* at 25 °C, similar with *Cirrospilus* sp. near *lyncus* and *Q. citrella* at 30 °C and underperformed in comparison to *Cirrospilus* sp. near *lyncus*, *C. vittatus* and *Q. citrella* at 20 °C (Urbaneja *et al*., 2001; 2002; Llácer *et al*., 2006).

Data on reproductive and life table characteristics of *S. petiolatus* showed that this parasitoid is favoured within the temperature range of 25 - 30 °C in contrast to temperatures close to 20 °C that seem rather limiting for its population increase. Additionally, it can develop within a broad range of temperatures (8.7-30 °C), with the optimum temperature for its development ranging between 26.7-29.5 °C while failing to develop at temperatures over 32.5 °C (Kalaitzaki *et al*., 2018). At this temperature range, which is typical for summer and early autumn periods in Greece and other Mediterranean countries, *S. petiolatus* is effective in controlling *P. citrella* populations since r_m values of the parasitoid are much higher than those of the citrus leafminer (Minsheng *et al*., 1995; Gomes de Silva *et al*., 2006; Zappalà, 2010; Kalaitzaki *et al*., 2011). These results agree with field monitoring in Western Crete citrus groves (Greece), where, even though *S. petiolatus* was less abundant than *C. phyllocnistoides* in *P. citrella* infested leaves throughout the sampling period, its higher populations appeared from mid-June to late August while the activity of the most dominant and more specific *P. citrella* parasitoid *C. phyllocnistoides* was more intense from September to November (Kalaitzaki *et al*., 2011). Similarly, in Sicilian citrus groves (Italy) *S. petiolatus* is more active in early summer (June-August) while *C. phyllocnistoides* from September to October (Siscaro *et al*., 2003; Rizzo *et al*., 2006; Zappalà, 2010). These results indicate that *S. petiolatus* has a narrower temperature adaptation window than the most dominant *C. phyllocnistoides* which exhibited a superior performance between 15-35 °C existing everywhere due to its climatic adaptations enabling it to withstand both cold and hot conditions (Urbaneja *et al*., 2003; Elekcioglu, 2017). Another possible explanation of the seasonal fluctuation of *S. petiolatus* on citrus leafminer could be partly explained by its migration on hosts of native flora (*A. hiemalis* on *Urtica* spp., *C. horticola* on *Sonchus* spp., *Liriomyza* sp. on *M. annua*, *C. pulchrimella* on *P. diffusa*, *S. aurella* on *R. ulmifolius* and *D. scalariella* on *Echium* sp.) (Massa and Rizzo, 2000; Massa *et al*., 2001; Siscaro *et al*., 2003; Rizzo *et al*., 2006) which are available almost everywhere in citrus groves of Mediterranean regions from autumn to spring, providing alternative food and shelter to this parasitoid.

In conclusion, the results of this study have revealed valuable information on the effect of temperature on the reproductive and population parameters of *S. petiolatus*. The results showed that biological parameters concerning reproduction and immature stages development of this species is favoured within the temperature range of 25-30 °C (Kalaitzaki *et al*., 2018). Future efforts to be established in new areas or reintroduced it in areas that had difficulties to be established should be considered as it represents an efficient agent for the effective biological control for *P. citrella*, which has been spread and established rapidly in several Mediterranean countries. Its establishment in citrus orchards in Mediterranean countries should be facilitated by focusing on promoting biodiversity in citrus groves in order to provide alternative food and shelter to the parasitoid especially during spring before populations of *P. citrella* increase. Moreover, it is important to evaluate the role of other hosts as alternative hosts of the parasitoid improving our knowledge of host range and preference.

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