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Effect of temperature, host plant, and host instar on the development and survival of the parasitoid *Semielacher petiolatus*

Argyro P. Kalaitzaki[1], Antonios E. Tsagarakis[2], Dionyssios Perdikis[2], Dionyssios P. Lykouressis[2]
[1]*Hellenic Agricultural Organization 'DEMETER', Institute of Olive Tree, Subtropical Plants and Viticulture, Chania, Greece*
[2]*Agricultural University of Athens, Laboratory of Agricultural Zoology and Entomology, Athens, Greece*

Abstract

Laboratory studies were conducted to assess the effect of temperature on the development and survival of the parasitoid *Semielacher petiolatus* (Girault) on its host, the citrus leaf miner *Phyllocnistis citrella* Stainton. Experiments were conducted at five constant temperatures (15, 20, 25, 30 and 32.5 °C), with RH 60 ± 10% and a photoperiod of 14:10 h (L:D). The relationship between the developmental rate and temperature was investigated using both linear and non-linear (Lactin's formula) models. The shortest developmental period was recorded at 30 °C and the longest at 15 °C. No adults emerged at 32.5 °C, as no pupa survived. Mortality (%) from egg to adult was lower at temperatures within the range 20-30 °C than at either 15 °C or 32.5 °C. The lower developmental thresholds estimated by the non-linear model for egg, larval and pupal development were higher than those estimated by the linear model. The estimated upper developmental thresholds for egg, larval and pupal development were 36.5, 32.9 and 30.9 °C, respectively. The optimum temperature for development ranged between 26.7-29.5 °C. Our results provide a broad insight into the biological characteristics and thermal requirements of *S. petiolatus*, factors important to further characterize its role as biological control agent under typical Mediterranean climatic conditions.

Key words: Hymenoptera, Eulophidae, ectoparasitoid, *Phyllocnistis citrella*, constant temperatures, developmental threshold, thermal constant, biological control, optimum temperature.

Introduction

Semielacher petiolatus (Girault) (Hymenoptera Eulophidae) is a solitary ectoparasitoid, indigenous to Australia (Bouĉek, 1988; Heppner, 1993; Smith and Beattie, 1996) and the Solomon Islands (Schauff *et al*., 1998). In Australia *S. petiolatus* is the major parasitoid of the citrus leafminer *Phyllocnistis citrella* Stainton (Lepidoptera Gracillariidae) (Smith *et al*., 1997).

The above parasitoid has been introduced into several Mediterranean countries for the biological control of citrus leafminer, including Greece (Kalaitzaki *et al*., 2011), Cyprus, Israel, Morocco, Oman, Syria, Tunisia (Schauff *et al*., 1998), Turkey (Elekcioglu and Uygun, 2006), and Spain (Garcia-Mari *et al*., 2004), as well as the USA (Florida) (Lim and Hoy, 2005).The parasitoid was found in Italy in 1998, although it is unknown how it arrived there (Mineo *et al*., 1998). *S. petiolatus* was introduced in Greece in 1996, during the frame of a classical biological control project for the citrus leafminer. Among the introduced species were the eulophids *Citrostichus phyllocnistoides* (Narayanan), *Quadrastichus* sp. and *Cirrospilus quadristriatus* (Subba Rao et Ramamani) (Hymenoptera Eulophidae), and the encyrtid *Ageniaspis citricola* Logvinovskaya (Hymenoptera Encyrtidae). The parasitoids were released in several areas of Greece (Kalaitzaki *et al*., 2011; Tsagarakis *et al*., 2013). Among the released parasitoids, *C. phyllocnistoides* and *S. petiolatus* became established in Greece, dispersing very rapidly over large distances from the release sites, and contributing to a reduction in the citrus leafminer population (Kalaitzaki *et al*., 2011).

S. petiolatus also spread and became an abundant parasitoid of the citrus leafminer in Italy (Sicily) (Mineo *et al*., 1998; Mineo, 1999; Massa *et al*., 2001; Zappalà, 2010), Jordan (Ateyyat, 2002), Algeria (Schauff *et al*., 1998), Israel (Argov and Rössler, 1996), Morocco (Abbassi *et al*., 1999), Turkey (Uygun *et al*., 1997), and Portugal (Zappalà, 2010), although it failed to establish in Spain (Garcia-Mari *et al*., 2004).

Massa *et al.* (2001) reported that *S. petiolatus* could also be found on insect hosts other than citrus leafminer, including two Lepidoptera (*Cosmopterix pulchrimella* Chambers on *Parietaria diffusa* M. et K. and *Stigmella aurella* F. on *Rubus ulmifolius* Schott) and two Diptera (*Liriomyza* sp. on *Mercurialis annua* L. and *Chromatomyia horticola* Goureau on *Sonchus* spp.) in Italy and a further Lepidoptera (*Dialectica scalariella* Zeller on *Echium* sp.) in Jordan.

However, to evaluate a natural enemy for its potential as a biological control agent, studies on its biological characteristics such as development and survival under different conditions as well characterization of its interactions with other natural enemies (Lim *et al*., 2006; Naselli *et al*., 2017) are essential. The development time, adult longevity, total fecundity, host mortality, and host-size choice have been studied at 25 °C by Lim and Hoy (2005). In a further study its host discrimination capacity, relative humidity tolerance and parasitisation of alternative hosts were also assessed (Lim *et al*., 2006). However, no experimental data concerning its thermal requirements (developmental thresholds, optimum temperature, and thermal constant) for development are currently available.

Various mathematical formulae have been used to express the relationship between temperature and the rate of development of insects. Linear degree-day models are usually used, but the non-linear models describe development of insects under variable temperatures (Stinner *et al*., 1975; Sharpe and DeMichele, 1977; Hilbert and Logan, 1983; Campolo *et al.*, 2014).

In the present study the immature development of *S. petiolatus* was studied at several temperatures, and both linear and non-linear models were used to describe the relationship between temperature and its rate of development. This study aimed to: (1) studying the development and survival of egg, larval and pupal stages of *S. petiolatus* when reared on two instars of *P. citrella* at different temperatures; (2) studying whether the host plant (on two important Greek citrus cultivars) and the sex ratio of the parasitoid affect its development; (3) describing the relationship temperature and the rate of development using both linear and non-linear models; and finally (4) estimating the thermal requirements for development of the parasitoid on each host - plant system.

Materials and methods

Insect rearing

The *P. citrella* colony was initiated using adults that emerged from infested shoots collected from citrus orchards in the Chania prefecture of Crete. The 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 lighting at the Institute of Olive Tree, Subtropical Plants and Viticulture, Chania.

Rearing of the parasitoid *S. petiolatus* was initiated from adults that had emerged from parasitized larvae of *P. citrella* collected in citrus orchards in the area of Chania, Crete. The rearing of *S. petiolatus* was maintained on sour orange plants infested with *P. citrella*. Plants were placed in transparent Plexiglas cages (50 × 50 × 100 cm) in growth chambers in the laboratory, at 25 ± 0.5 °C, 60 ± 10% RH with a 14L:10D photoperiod.

Development and mortality of immature stages

The development of *S. petiolatus* was studied using eggs less than 8 h old oviposited on citrus leafminer larvae on the leaves of either sweet orange *Citrus sinensis* (L) Osbck (cv. Washington navel) or mandarin *Citrus reticulata* Blanco (cv. Clementine - Algerian). In order to use *P. citrella* larvae for parasitization, young plants of both plant species were introduced into the rearing greenhouse of the citrus leafminer. The plants became infested with the citrus leafminer and leaves bearing the 2^{nd} and 3^{rd} instar larvae of *P. citrella*, the preferred host stages for *S. petiolatus* oviposition (Argov and Rössler, 1996; Lim *et al*., 2006), were detached from the plants and placed separately into cylindrical transparent plastic cages (9 cm diameter × 21 cm height) with young adult parasitoids.

Following their introduction into the cage, the parasitoids were left undisturbed for 8 h to oviposite. The leaves were then inspected for the presence of parasitoid eggs using a binocular stereoscopic microscope. Leaves with hosts on which one egg had been deposited were transferred and placed individually on a water soaked cotton layer in a 9 cm diameter plastic Petri dish.

The development time and mortality of the parasitoid immature stages were studied on sweet orange leaves at 15, 20, 25, 30 and 32.5 °C, and on mandarin leaves at 20, 25 and 30 °C, in a controlled growth room with RH 60 ± 10%, 14:10 (L:D) h photoperiod and 10,000 Lux light intensity. The cohort size at each temperature was 80, 62, 43, 50 and 44 at 15, 20, 25, 30 and 32.5 °C on sweet orange and 55, 44 and 54 eggs at 20, 25 and 30 °C on mandarin, respectively. Each egg was considered as a replicate. The developmental time and mortality of the immature stages of the parasitoid were recorded at 12 h intervals until adult emergence, at all temperatures except for 32.5 °C where observations were conducted at 8 h intervals. The host stage of the parasitized larvae of *P. citrella* as well as the sex of the emerged parasitoids was recorded in all cases.

Thermal requirements for development

The thermal requirements for the development of *S. petiolatus* were estimated using the linear model as described by Campbell *et al.* (1974). The temperature threshold (t) for development was estimated by extrapolating to zero the linear part of the relationship between temperature and development rate (the reciprocal of the average development period in days). This relationship is described by the regression equation $y = a + bT$ where y is the rate of development and T is the temperature in °C. The temperature threshold was calculated as $t = -a/b$ and the thermal constant k (i.e. the amount of heat units required for development) as $k = 1/b$ degree-days (DD) (Campbell *et al*., 1974). In the current study the data obtained at 15, 20, 25 and 30 °C were used in the calculations. The data recorded at the highest temperature (32.5 °C) were excluded because they didn't fall within the linear region of the relationship between temperature and rate of development.

The relationship between temperature and developmental rate was also investigated using the improved non-linear model of Lactin *et al.* (1995) (modification 2). This model, which is based on a version of Logan *et al.* (1976), eliminates a redundant parameter and introduces an intercept parameter to allow estimation of the lowest developmental threshold. Also, it describes the relationship between developmental rate and temperature above optimal values and allows estimations of the upper temperature threshold. This model is composed of four parameters and has the following form:

$$r(\mathrm{T}) = e^{\rho \mathrm{T}} - e^{[\rho \mathrm{T}_{max} - (\mathrm{T}_{max} - \mathrm{T})/\Delta]} + \lambda,$$

where $r(\mathrm{T})$ is the developmental rate at temperature T and ρ, T_{max}, Δ and λ are fitted parameters.

Statistical analysis

The data for the developmental period of each stage of *S. petiolatus* (egg, larva and pupa) were analyzed with a 3-way ANOVA for the factors: temperature (20, 25 and 30 °C), the host plant (sweet orange or mandarin) and the sex of the emerged adults. The data for egg and larval development on sweet orange were analyzed for two factors: the temperature (15, 20, 25, 30, and 32.5 °C) and the sex of the emerged adults. Analyses were not conducted for the pupal stage since no pupae survived at 32.5 °C. The relative duration of each stage was assessed using data collected from sweet orange with a 3-way ANOVA for: citrus leafminer stage, temperature, sex, and 15, 20, 25, 30 °C and 32.5 °C for egg and larva. Similar analysis was done at 20, 25 and 30 °C on mandarin. Means were separated using the Tukey-Kramer HSD test.

Mortality data were arcsine transformed and tested for significance using the χ^2 test. Data analyses were carried out using the statistical program JMP 7.0 (SAS, 2007). Calculation of the linear model was performed using Microsoft Office Excel 2007. Non-linear regression was carried out using the statistical program JMP 7.0 (SAS, 2007).

Results

Developmental time and mortality of immature stages

The percentage mortality of the various stages of the parasitoid are shown in table 1. More than 41% of *S. petiolatus* immature stages reached adulthood, at all temperatures examined, except for 32.5 °C at which 31% reached the pupal stage but none survived subsequently. On sweet orange the mortality for total development ranged from 30 to 100%. Significantly higher mortality occurred at 32.5 °C on sweet orange ($\chi^2 = 57.12$, df = 4, $P < 0.001$). On mandarin no significant differences were found in mortality rates from 20 to 30 °C ($\chi^2 = 3.82$, df = 2, $P > 0.57$).

Rearing *P. citrella* on different host plants did not affect the mortality rate of *S. petiolatus* at any temperature tested (20 °C: $\chi^2 = 0.16$, df = 1, $P > 0.68$; 25 °C: $\chi^2 = 0.008$, df = 1, $P > 0.92$; 30 °C: $\chi^2 = 0.05$, df = 1, $P > 0.81$) (table 1). Among the immature stages, the larval stage was the most sensitive and the pupal stage the most robust, at all temperatures tested except for 32.5 °C, at which no pupa survived (table 1).

The developmental time in days for egg, larval, and pupal stages of the parasitoid *S. petiolatus* reared on *P. citrella* on sweet orange and mandarin leaves at various temperatures are shown in tables 2 and 3. Three-way ANOVA showed that temperature and sex had a significant effect on the total developmental time of *S. petiolatus* ($F_{2, 181} = 866.71$, $P < 0.0001$ and $F_{1, 181} = 42.43$, $P < 0.0001$, respectively). However, significant differences between the two host plants were not found ($F_{1, 181} = 2.52$, $P = 0.114$), indicating that the rearing of *P. citrella* on different citrus host plants did not affect the development time of *S. petiolatus* at any temperature tested. The interaction between temperature and host plant was significant only for the pupal developmental period ($F_{2, 181} = 8.84$, $P < 0.0002$) and for the total developmental period ($F_{2, 181} = 3.48$, $P < 0.032$). The interaction among the three factors was not significant. Mean total developmental time from oviposition to adult emergence varied from 26.33 days at 15 °C to 7.04 days at 30 °C on sweet orange (table 3) and from 11.47 at 20 °C to 7.17 days at 30 °C on mandarin (table 2). Within the range 15-30 °C the developmental period decreased as the temperature increased. The shortest total developmental time of the immature stages was recorded at 30 °C on both host plants. At 32.5 °C the developmental time of egg and larva increased significantly as compared to those at 30 °C, while no pupa survived to adulthood on sweet orange (table 3).

The developmental time of the pupal stage was the longest, at all temperatures, followed by that of larval stage. The egg stage had the shortest developmental period.

On sweet orange, three-way ANOVA showed that temperature and sex had a significant effect on the total developmental time of *S. petiolatus* ($F_{3, 114} = 3900.64$, $P < 0.0001$ and $F_{1, 114} = 32.26$, $P < 0.0001$, respectively) (table 4). However, significant differences for development on the two instars of the citrus leafminer were not found, showing that the host instar did not affect the development time of *S. petiolatus* at all tested temperatures ($F_{1, 114} = 2.23$, $P = 0.137$). The interaction between

Table 1. Percentage mortality (%) of egg, larval and pupal stages of *S. petiolatus* reared on *P. citrella* in leaves of sweet orange (cv. Washington navel) and mandarin (cv. Clementine) at various constant temperatures.

Temp. °C	Egg (n[c]/n[d])		Larva (n[c]/n[d])		Pupa (n[c]/n[d])		Total[a] (n[d])	
	Sweet orange	Mandarin	Sweet orange	Mandarin	Sweet orange	Mandarin	Sweet orange	Mandarin
15	10.0 (80/72)	-	41.6 (72/42)	-	21.4 (42/33)	-	58.8 (33)	-
20	11.3 (62/55)	10.9 (55/49)	30.9 (55/38)	32.6 (49/33)	7.8 (38/35)	12.1 (33/29)	43.5 (35)	47.3 (29)
25	2.3 (43/42)	2.3 (44/43)	23.8 (42/32)	23.2 (45/33)	6.3 (32/30)	6.1 (33/31)	30.2 (30)	29.5 (31)
30	10.0 (50/45)	9.4 (54/49)	13.3 (45/39)	18.3 (49/40)	17.9 (39/32)	10.0 (40/36)	36.0 (32)	34.0 (36)
32.5	4.5 (44/42)	-	69.0 (42/29)	-	100 (29/0)	-	100 (0)	-

[c] Number of insects at the beginning of each stage. [d] Number of insects completing development. [a] From oviposition to adult emergence.

Table 2. Mean development time ± SE in days for egg, larva and pupa of *S. petiolatus* reared on *P. citrella* in leaves of sweet orange (cv. Washington navel) and mandarin (cv. Clementine) at various constant temperatures.

Temp. °C	Egg (n^c)		Larva (n^c)		Pupa (n^c)		Total^a (n^d)	
	Sweet orange	Mandarin	Sweet orange	Mandarin	Sweet orange	Mandarin	Sweet orange	Mandarin
20	1.64 ± 0.03a (62)	1.67 ± 0.03a (55)	3.94 ± 0.15a (55)	3.71 ± 0.12a (49)	5.85 ± 0.08a (38)	6.08 ± 0.15b (29)	11.43 ± 0.14a (35)	11.47 ± 0.16a (29)
25	0.88 ± 0.01b (43)	0.87 ± 0.01b (44)	2.24 ± 0.08b (42)	2.05 ± 0.06b (43)	4.46 ± 0.08c (32)	4.28 ± 0.08cd (48)	7.58 ± 0.09b (30)	7.21 ± 0.09c (31)
30	0.83 ± 0.01b (50)	0.83 ± 0.00b (54)	2.17 ± 0.42b (45)	2.40 ± 0.12b (49)	4.03 ± 0.07d (39)	3.76 ± 0.08d (77)	7.04 ± 0.11c (32)	7.17 ± 0.22c (36)

[c] Number of insects at the beginning of each stage. [d] Number of insects completing development. [a] From oviposition to adult emergence. Means followed by different small letters on each respective immature stage of parasitoid are significantly different ($P < 0.05$; HSD test).

Table 3. Mean development time ± SE in days of egg, larva and pupa of *S. petiolatus* reared on *P. citrella* in leaves of sweet orange (cv. Washington navel) at various constant temperatures.

Temp. °C	Egg (n^c)	Larva (n^c)	Pupa (n^c)	Total^a (n^d)
15	2.98 ± 0.01a (80)	8.45 ± 0.28a (72)	14.90 ± 0.13a (42)	26.33 ± 0.51a (33)
20	1.64 ± 0.03b (62)	3.94 ± 0.15c (55)	5.85 ± 0.08Ab (38)	11.43 ± 0.14b (35)
25	0.88 ± 0.01c (43)	2.24 ± 0.08d (42)	4.46 ± 0.08c (32)	7.58 ± 0.09c (30)
30	0.83 ± 0.01c (50)	2.17 ± 0.42d (45)	4.03 ± 0.07d (39)	7.04 ± 0.11c (32)
32.5	0.88 ± 0.01c (44)	6.27 ± 0.81b (42)	- (0)	- (0)

[c] Number of insects at the beginning of each stage. [d] Number of insects completing development. [a] From oviposition to adult emergence. Means followed by different small letters within a column are significantly different ($P < 0.05$; HSD test).

Table 4. Mean development time ± SE in days of egg, larva and pupa of male and female of *S. petiolatus* reared on *P. citrella* in leaves of sweet orange (cv. Washington navel) and mandarin (cv. Clementine) at various constant temperatures.

		Sweet orange							
°C	(n^c)	Egg		Larva		Pupa		Total^a (n^d)	
		Male	Female	Male	Female	Male	Female	Male	Female
15	33	3.00 ± 0.01a	2.98 ± 0.02a	7.72 ± 0.19a	9.15 ± 0.46b	14.54 ± 0.11a	15.23 ± 0.21b	26.2 ± 0.97a (16)	27.37 ± 0.35b (17)
20	35	1.62 ± 0.03b	1.73 ± 0.05b	3.82 ± 0.18c	4.29 ± 0.26c	5.88 ± 0.08c	5.77 ± 0.24c	11.32 ± 0.16c (26)	11.80 ± 0.23c (7)
25	30	0.87 ± 0.12c	0.88 ± 0.01c	2.23 ± 0.11d	2.24 ± 0.11d	4.32 ± 0.13de	4.55 ± 0.11de	7.44 ± 0.15d (12)	7.68 ± 0.11de (18)
30	32	0.83 ± 0.01c	0.83 ± 0.00c	2.03 ± 0.08d	2.31 ± 0.12d	3.86 ± 0.09e	3.97 ± 0.28de	6.73 ± 0.07de (16)	7.34 ± 0.18e (16)

		Mandarin							
°C	(n^c)	Egg		Larva		Pupa		Total^a (n^d)	
		Male	Female	Male	Female	Male	Female	Male	Female
20	29	1.69 ± 0.02a	1.62 ± 0.08a	3.55 ± 0.15a	3.99 ± 0.17a	5.76 ± 0.16a	6.69 ± 0.22b	11.02 ± 0.11a (19)	12.32 ± 0.25b (10)
25	31	0.87 ± 0.00b	0.87 ± 0.00b	1.99 ± 0.12b	2.07 ± 0.08b	3.78 ± 0.08d	4.45 ± 0.09c	6.65 ± 0.04cd (8)	7.40 ± 0.10c (46)
30	36	0.83 ± 0.00b	0.83 ± 0.00b	2.27 ± 0.13b	2.51 ± 0.19b	3.61 ± 0.13d	3.90 ± 0.10d	6.72 ± 0.15d (17)	7.56 ± 0.36cd (19)

[c] Initial number of replicates. [d] Number of insects completing development. [a] From oviposition to adult emergence. Means followed by different small letters on each respective immature stage of parasitoid are significantly different ($P < 0.05$; HSD test).

Table 5. Regression equations that describe the relationship between the rate of development (Y) and temperature (X) of the each immature stage of *S. petiolatus* reared on second and third larva stage of *P. citrella* and on two host plants, and the respective temperature threshold for development (*t*) in °C and the thermal constant (*k*) in degree-days.

Host plant		df	Regression equation	S.E. of *b*	R^2	*P*	*t*	*k*
Sweet orange	Egg	128	Y = −0.5847 + 0.0623X	0.0016	0.919	0.0001	9.38	17
Sweet orange	Larva	128	Y = −0.2350 + 0.0251X	0.0012	0.765	0.0001	9.36	40
Sweet orange	Pupa	128	Y = −0.0923 + 0.0120X	0.0004	0.851	0.0001	7.69	83
Sweet orange	Total	128	Y = −0.0613 + 0.0071X	0.0002	0.906	0.0001	8.71	143
Mandarin	Egg	94	Y = −0.5997 + 0.0631X	0.1277	0.924	0.0001	9.50	16
Mandarin	Larva	94	Y = −0.2044 + 0.0239X	0.0083	0.805	0.0001	8.55	42
Mandarin	Pupa	94	Y = −0.1201 + 0.0135X	0.0020	0.954	0.0001	8.89	74
Mandarin	Total	94	Y = −0.0645 + 0.0074X	0.0015	0.918	0.0001	8.71	135

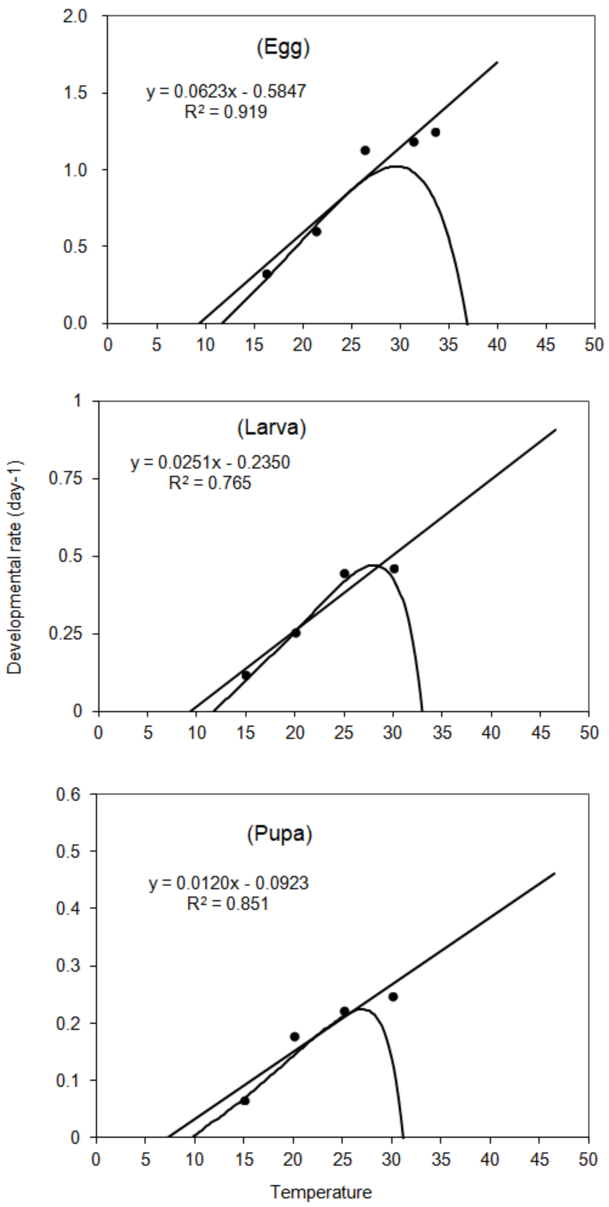

Figure 1. Developmental rate (days^{-1}) of eggs, larvae and pupae of *S. petiolatus* at constant (●) temperatures. Fitted curves according to the Lactin *et al.* (1995) model. Fitted lines according to the Campbell *et al.* (1974) model.

temperature and sex was significant ($F_{3,\ 114}$ = 8.800, P < 0.0001), but the interaction among these three factors was not significant.

On mandarin, three-way ANOVA showed that temperature and sex had also significant effect on the total developmental time of *S. petiolatus* ($F_{2,\ 84}$ = 420.25, P < 0.0001 and $F_{1,\ 84}$ = 31.48, P < 0.0001, respectively) (table 4), however, significant differences between development on the two instars of the citrus leafminer were again not found ($F_{1,\ 84}$ = 1.68, P = 0.198).

Lower temperature threshold and thermal constant

The linear regression equations that describe the relationship between temperature and the rate of egg, larval, pupal and total development on sweet orange and mandarin were estimated (table 5). The estimated lower developmental threshold for egg development was higher than those of larva, pupa and for total development on both host plants. The lower temperature threshold for the immature stages of S. *petiolatus* was similar for both host plants (table 5).

The number of degree-days required for the completion of each immature stage, and for the total development of the parasitoid are shown in table 5. The degree-days required for the completion of *S. petiolatus* development were 143 and 135 DD on sweet orange and mandarin, respectively. Among the immature stages the pupal stage had the largest thermal constant, followed by that of the larval stage, on both host plants.

The non-linear model produced a good fit to the data for sweet orange within the range of 15-30 °C (figure 1) as indicated by the coefficients of determination (R^2 = 0.988 – 0.994) (table 6). The temperature threshold for the egg, larval and pupae development estimated by this model was higher than that obtained from the linear model (tables 5 and 6). The optimal development temperature estimated for all stages of *S. petiolatus* ranged between 26.7 and 29.5 °C, whereas the upper developmental threshold ranged between 30.9 and 39.5 °C.

Discussion and conclusions

The parasitoid *S. petiolatus* reached adulthood from immaturity with a success rate >41%, at all temperatures tested. Parasitoid development was favoured at

Table 6. Parameter estimates using the Lactin *et al.* (1995) model and R^2 for temperature-dependent development rates of immature stages of *S. petiolatus* reared on 2nd and 3rd larva stages of *P. citrella* in leaves of sweet orange.

Stage	ρ	T_{max}	Δ	λ	R^2	T_o	t
Egg	0.043	39.501	5.046	−1.563	0.988	29.501	11.50
Larva	0.022	32.919	2.269	−1.294	0.998	28.238	11.65
Pupa	0.012	30.967	1.753	−1.125	0.994	26.750	9.85

ρ - rate of increase to optimum temperature; T_{max} - maximum temperature threshold (°C); Δ - difference between the lethal temperature and the optimum temperature of development; λ - parameter that makes the curve intercept the x-axis; T_o - optimum temperature for development; t - lower development threshold (°C).

temperatures ranging from 20-30 °C but not at the highest temperature tested (32.5 °C). The failure of *S. petiolatus* to complete its development at a constant temperature of 32.5 °C, with corresponding increases in the duration of the egg and larval stages in comparison to those at 30 °C, shows that *S. petiolatus* development is prohibited at this temperature. The adverse effects of extreme temperatures, within the range 32.5 to 35 °C, have not been observed in previous studies on other eulophid citrus leafminer parasitoids such as *Pnigalio pectinicornis* (L.) (Kalaitzaki *et al.*, 2007), *Cirrospilus vittatus* Walker (Urbaneja *et al.*, 2002) *Cirropsilus* sp. near *lyncus* (Urbaneja *et al.*, 1999), *Quadrastichus citrella* Reina et La Salle (Llácer *et al.*, 2006) and *C. phyllocnistoides* (Elekcioğlu, 2017), reared on *P. citrella*. Specifically, the survival of *P. pectinicornis* did not differ significantly at 30 or 32.5 °C (Kalaitzaki *et al.*, 2007) and Urbaneja *et al.* (1999; 2002) and Llácer *et al.* (2006) reported that at constant temperature as high as 35 °C, 48.7%, 22.2% and 23.3% of the immature stages of *Cirrospilus* sp. near *lyncus*, *C. vittatus* and *Q. citrella* completed their development, respectively.

Among the introduced and the native species *S. petiolatus* is at present one of the most frequent parasitoids of *P. citrella* (Siscaro *et al.*, 2003; Kalaitzaki *et al.*, 2011; Tsagkarakis *et al.*, 2013) and this study indicates that climatic conditions might be the reason for this. *S. petiolatus* has spread throughout Mediterranean regions such in the island of Crete (Greece) and Sicily (Italy) (Mineo *et al.*, 1998; Mineo, 1999; Massa *et al.*, 2001; Zappalà, 2010, Kalaitzaki *et al.*, 2011), where the summer temperatures closely resemble those tested. This theory is further supported by the fact that *S. petiolatus* parasitization is highest during the first months of *P. citrella* infestation, in early summer (Kalaitzaki *et al.*, 2011; Zappalà, 2010), when ambient temperatures are most likely to fall within the permissive range.

In general, the males of *S. petiolatus* developed faster than the females. Although similar results have been previously recorded for *S. petiolatus* at 25 °C (Lim and Hoy, 2005), as well as for other parasitoids of *P. citrella* such as *Quadrastichus* sp. (Llácer *et al.*, 1998), *P. pectinicornis* (Kalaitzaki *et al.*, 2007), *C.* sp. near *lyncus* (Urbaneja *et al.*, 1999) and *C. vittatus* (Urbaneja *et al.*, 2002), not differences between the development of the sexes were observed for *Q. citrella* (Llácer *et al.*, 2006).

The developmental period of the egg stage was shorter compared to the other stages, whereas the duration of the pupal stage was the longest of all three developmental stages. Earlier studies of the development of *P. citrella* parasitoids such as *Quadrastichus* sp. (Llácer *et al.*, 1998), *C.* sp. near *lyncus* (Urbaneja *et al.* 1999), *C. vittatus* (Urbaneja *et al.*, 2002), *C. phyllocnistoides* (Urbaneja *et al.*, 2003), *Q. citrella* (Llácer *et al.*, 2006) and *P. pectinicornis* (Kalaitzaki *et al.*, 2007) also showed a similar pattern.

The development time of the immature stages did not differ significantly between the orange and mandarin cultivars. This indicates that the parasitoid can be effective on both citrus cultivars. Similar results were also seen in earlier laboratory studies on the development of the *P. citrella* parasitoid *P. pectinicornis* (Kalaitzaki *et al.*, 2007). These results are also in agreement with earlier field studies conducted in Chania which showed that the percent of parasitism of *P. citrella* by *S. petiolatus* did not differ significantly between sweet orange and mandarin (Kalaitzaki *et al.*, 2011).

The lower temperature threshold for the egg to adult development was estimated at 8.71 °C and was the same on the sweet orange and mandarin. By the latest finding, we might conclude that different citrus varieties do not have an effect on the lower survival threshold of *S. petiolatus* as was also the case for *P. pectinicornis* (Kalaitzaki *et al.*, 2007). Also, the findings of Urbaneja *et al.* (1999) for *C.* sp. near *lyncus* were almost identical with those of the present study, with 8.8 °C being the lower threshold temperature for complete development. However, the estimated lower threshold temperature for the total development of *P. pectinicornis* was found to be lower (5.27 °C) (Kalaitzaki *et al.*, 2007). In general, the lower temperature threshold for the egg was consistently higher than for the two other developmental stages, indicating that it is more sensitive to low temperatures, findings identical to those for *P. pectinicornis* (Kalaitzaki *et al.*, 2007).

The mean temperatures recorded for Chania, Crete, (Greece) ranged between 10.5 °C in the coldest months (January-February) and 26 °C in the hottest months (July-August) (1997-2008). According to the findings of this study, *S. petiolatus* can withstand prevalent local winter temperatures and its development could be continuous in summer under the typical climatic conditions. Thus the development of *S. petiolatus* will be most affected by the availability of its host. The *P. citrella* population shows an increase during late spring and summer and declines during autumn (Kalaitzaki *et al.*, 2011). Minkenberg (1989) stated that the lower thermal threshold for parasitoids is generally higher than the one of their hosts. In the case reported by Ujiye (2000) the lower thermal threshold for *P. citrella* was found to be 12.1 °C. Because the lower threshold of *S. petiolatus* is lower than that of its host *P. citrella*, during the winter its survival could be assisted by alternative hosts. As Rizzo (2003) indicated, *S. petiolatus* has been collected from several different hosts, thereby assuring development of the parasitoid during seasons of low or no availability of *P. citrella*. Possible alternative hosts for *S. petiolatus* are *Liriomyza* sp. on *Mercurialis annua* L., and *Chromatomyia horticola* (Goureau) on *Sonchus* sp. (Massa *et al.*, 2001) which are very common found on weeds in citrus groves in the Chania area during winter and spring.

S. petiolatus can complete its development from egg to adult in a significantly shorter period than *P. citrella*, as their life cycles require about 7.5 and 16 days to complete at 25-26 °C, respectively. This finding indicates that this parasitoid would act as an effective biological control agent of *P. citrella*.

In conclusion, the results of this study have revealed valuable information on the thermal requirements of the parasitoid *S. petiolatus*. As *S. petiolatus* is well adapted to the local Mediterranean climatic conditions it represents an efficient agent for the effective biological control for *P. citrella*. Its suitability is further confirmed by the fact that this exotic parasitoid has spread rapidly through the citrus growing areas and has become widely established in several Mediterranean countries including Greece, Italy, Turkey, Tunisia and Israel where it has been shown to play an important role in the regulation of the *P. citrella* population.

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Authors' addresses: Argyro P. Kalaitzaki (corresponding author, e-mail: kalaitzaki@nagref-cha.gr), Hellenic Agricultural Organization 'DEMETER', Institute of Olive Tree, Subtropical Plants and Viticulture, Agrokipio, 73100 Chania, Greece; Antonios E. Tsagarakis, Dionyssios Perdikis, Dionyssios P. Lykouressis, Agricultural University of Athens, Laboratory of Agricultural Zoology and Entomology, Iera Odos 75, 118 55 Athens, Greece.

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