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Original article (Orijinal araştırma)

Development of *Lysiphlebus testaceipes* (Cresson, 1880) (Hymenoptera: Braconidae) on different hosts and temperatures[1]

Lysiphlebus testaceipes (Cresson, 1880) (Hymenoptera: Braconidae)' in farklı konukçu ve sıcaklıklarda gelişimi

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Abstract

In this study, the development time, mortality, parasitization rate and sex ratio of *Lysiphlebus testaceipes* (Cresson,1880) (Hymenoptera: Braconidae: Aphidiinae) on *Aphis craccivora* Koch, 1854, *Aphis fabae* Scopoli, 1763 and *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae), were determined. The experiments were carried out at three different, temperatures (17, 22 and 27±1ºC) for each aphid species, 60±10% RH and 16:8 h L:D photoperiod. The development thresholds and thermal constants for the parasitoid were also determined for the three aphid species. The parasitization rate of *L. testaceipes* was 25.0, 53.4 and 20.5% (for 17, 22 and 27ºC, respectively) for *A. craccivora*; 62.7, 71.1 and 37.1% for *A. fabae*; and 54.2, 70.7 and 20.0% for *A. gossypii*. The development time of *L. testaceipes* was 18.5, 10.9 and 7.9 d in *A. craccivora*, 17.6, 10.2 and 7.4 d in *A. fabae*, and 19.8, 12.6 and 9.3 d in *A. gossypii* at 17, 22 and 27±1ºC. The development thresholds and thermal constants for *L. testaceipes* in *A. craccivora*, *A. fabae* and *A. gossypii* were 9.42, 9.69 and 8.12ºC, and 136.99, 128.05 and 175.44 degree days, respectively. Based on the overall results, *A. fabae* is an excellent potential host for the mass rearing of *L. testaceipes* at 20-22ºC.

Keywords: Aphid species, biological control, development threshold, parasitoid, sex ratio

Öz

Çalışmada *Lysiphlebus testaceipes* (Cresson,1880) (Hymenoptera: Braconidae: Aphidiinae)'in *Aphis craccivora* Koch, 1854, *Aphis fabae* Scopoli, 1763 ve *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) üzerinde gelişme süresi, ölüm, parazitleme ve cinsiyet oranı belirlenmiştir. Denemeler her bir yaprakbiti türü için üç farklı sıcaklık (17, 22 ve 27±1ºC), 60±10% RH ve 16:8 L:D koşullarında yürütülmüştür. Gelişme eşiği ve termal konstant üç yaprakbiti türü için hesaplanmıştır. *Lysiphlebus testaceipes*'in parazitleme oranı *A. craccivora* için %25.0, 53.4 ve 20.5, *A. fabae* için %62.7, 71.1 ve 37.1, *A. gossypii* için %54.2, 70.7 ve 20.0 olmuştur. *Lysiphlebus testaceipes*'in gelişme süresi 17, 22 ve 27±1ºC' de sırayla *A. craccivora* üzerinde 18.5, 10.9 ve 7.9 gün, *A. fabae* üzerinde 17.6, 10.2 ve 7.4 gün, *A. gossypii* üzerinde 19.8, 12.6 ve 9.3 gündür. Gelişme eşiği ve termal konstant *A. craccivora*, *A. fabae* ve *A. gossypii* üzerinde sırayla 9.42, 9.69 ve 8.12ºC; 136.99, 128.05 ve 175.44 gün derece olmuştur. Genel sonuçlara dayanarak, 20-22ºC'de A. fabae *L. testaceipes*'in kitle üretimi için çok iyi bir potansiyel konukçudur.

Anahtar sözcükler: Afit türleri, biyolojik mücadele, gelişme eşiği, parazitoit, eşeysel oran

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Introduction

Aphid damage is considered one of the major impediments to production in many cultivated crops. They not only feed on plant sap and produce honeydew but they are also responsible for the dispersal of many viruses. Chemical control is the major method used to suppress population levels among the possible control methods, including cultural and biological methods (Parrella et al., 1999). Biological control methods are being implemented for aphid management in open fields and greenhouses (Zamani et al., 2007; Uygun et al., 2010). The Aphidiinae species (Hymenoptera: Braconidae) are all parasitoids of aphids (Mackauer & Starý, 1967) and many species have been successfully used against aphid species in these areas (Ramakers et al., 1989; van Steenis & El-Khawass, 1995; Yoldaş et al., 2011).

Lysiphlebus testaceipes (Cresson, 1880) (Hymenoptera: Braconidae: Aphidiinae) have been accepted one of the effective aphid parasitoids in this group. Originally from Cuba, it was released in France and Corsica to control *Aphis spireacola* Patch, 1914 (Hemiptera: Aphididae). However, the parasitism of *Toxoptera aurantii* (Boyer de Fonscolombe, 1841) rather than *A. spireacola* was recorded (Starý et al., 1988). After release, it spread gradually throughout the Mediterranean and to the western Atlantic with wide host range (Starý et al., 2004). The successful introduction of any parasitoid to a new environment depends on several factors. Firstly, releases in classical biological control should be done in each climatic zone that is occupied by the host, so that the parasitoid has a chance to establish in all areas where the host occurs. Secondly, the releases should be large enough to ensure rapid establishment. Often more than one release in an area is needed for successful establishment. To achieve these goals, the mass rearing of candidate parasitoids is an integral step (Debach, 1974; Uygun et al., 2010). Understanding the factors that regulate interactions between aphid parasitoids and their hosts will improve both conservation and augmentation of the parasitoids. Temperature and host are key abiotic and biotic factors, respectively, that regulate insect population dynamics, development rates and seasonal occurrence (Campbell et al., 1974; Harvey, 2000). Both the host-aphid species and temperature can affect the rate of development and longevity of aphid parasitoids (Deng & Tsai, 1998). There is considerable literature on temperature-dependent biology of *L. testaceipes*. Variation in observed developmental periods of *L. testaceipes* have been attributed to genetic variability among distinct populations and differences among the host-aphid species (Tang & Yokomi, 1995; Elliott et al., 1999; Royer et al., 2001; Rodrigues et al., 2004; Starý et al., 2004).

In the present study, the effects of three host-aphid species and three constant temperatures on the development, parasitism percentage, pupal mortality percentage and sex ratio of *L. testaceipes* were studied to evaluate their potential as hosts for its mass rearing.

Material and Methods

Insect and plant sources

The aphids used in the experiments, namely cowpea aphid, *Aphis craccivora* Koch, 1854 black bean aphid, *Aphis fabae* Scopoli, 1763 and cotton aphid *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae), were collected from horse bean and cotton fields in Balcalı, Adana, Turkey in March 2011 and the experiments were set at the same year. Laboratory colonies of the cotton aphid were established on cotton (*Gossypium hirsutum* L. cv. Çukurova 1518), and black bean and cowpea aphids were established on horse bean, *Vicia fabae* L. Each colony was separately reared in a 70 x 55 x 40 cm cage at 23±2ºC, 65±10% RH and 16 h of artificial light of 6,000 lx in an insect rearing room. The aphids were reared in the laboratory for three generations before being used in the experiments.

Lysiphlebus testaceipes population was originally collected from *A. gossypii* in a citrus orchard in Seferihisar, İzmir, in May 2008, and maintained on *A. fabae* in the laboratory for 10-12 generations before the individuals were used in the current experiments. *Lysiphlebus testaceipes* was reared in a 70 x 55 x 40 cm cage at 20±2ºC, 60±10% RH and 16 h of artificial light of 6,000 lx in an insect rearing room.

Effect of temperature and aphid species on parasitoid development and parasitization rate

The development rates of *L. testaceipes* on *A. craccivora*, *A. fabae* and *A. gossypii* were studied at three different temperatures (17, 22 and 27±1ºC), 60±10% RH and 16 h of artificial light (5,000 lx). The

apterous adults of *A. craccivora, A. fabae* and *A. gossypii* were transferred separately with a fine camel hairbrush to excised leaf disks (Ø 5 cm) of the host plant inverted on wet cotton on the Petri dishes. Offspring born within 24 h were taken from the cotton disk and transferred to potted cotton and horse bean seedlings (3-4 true leaf stage). Each plant had 80 first instar aphids on it and is referred to as a unit. The units, which were transferred to a climate chamber set at 22ºC, were tightly covered with a 5 L plastic cage (30 cm high x 14 cm lid diameter) which had three openings (10 cm Ø) covered with mesh, one on the bottom and two on the sides of the cylinder. These nymphs were used for parasitization when they reached the second or third instar.

Adult parasitoids were obtained from aphid mummies isolated in a 50 ml falcon tube (10 cm by 1.5 cm Ø). Upon adult emergence, the gender was determined under a stereomicroscope. Two male adults and one female were introduced into a 5-L plastic cage for a minimum 4-h mating period. A small piece of fine muslin fabric containing 3% sugar solution was placed in the cage for nutrition. Thereafter, for each temperature, one mated parasitoid female was introduced into each unit covered by the plastic cage for a 24-h oviposition period and then removed. The experimental units were kept at the same temperature and monitored daily for adult parasitoid emergence. Individual development time from oviposition to the beginning of mummy formation and from oviposition to adult emergence was determined for males and females, and combined. The sex of the adult parasitoids was determined under a stereomicroscope. The data were used to calculate the effects of temperature and aphid on the female-male ratio of the parasitoid. The number of unemerged parasitoids from mummies was used to assess the mortality percentage for each cage. The parasitization rate was calculated as the proportion of transferred aphids that became mummies. At each temperature, 10 replicates of each aphid species were used.

Data analysis

The effect of temperature on the developmental periods of *L. testaceipes* on each aphid species from the oviposition to the beginning of mummy formation, and from oviposition to adult emergence, was analyzed by one-way analysis of variance (ANOVA, $\alpha = 0.05$). If a significant difference was detected, multiple comparisons were made by using Tukey's HSD multiple range test. Data from the three aphid species were also pooled to test for the possible effect of aphid species on the development of parasitoids at different temperatures, i.e., an interaction effect, by using two-way ANOVA. The effect of temperature on the parasitization rate and mortality ratio of the mummy stage for each aphid species were analyzed with one-way ANOVA ($\alpha = 0.05$). If a significant difference was detected, the Tukey's multiple range test was applied to separate the means. The data on the mortality rate and the parasitism percentage were arcsine square root transformed before the application of the tests (SPSS Inc. 2008).

Chi-square (χ^2) analysis was applied to determine if there was any effect of temperature and aphid species on the sex ratio of the parasitoid in comparison to a hypothesized sex ratio of 1:1. The analysis was applied separately to the sex ratio of *L. testaceipes* that became adults on each aphid species at the different temperatures. In addition to Chi-square analysis, a phi-Cramer's V test was applied to measure the effect of each aphid species on the sex ratio of *L. testaceipes*. The analyses were carried out with SPSS 17.0 statistical software (SPSS Inc. 2008).

Separately, a linear technique was employed to compute the lower development threshold of the egg-larval stages and total immature stages of the parasitoid by using development rate data as the dependent variable and temperature as the independent variable. The lower development threshold was determined as the intercept point of the linear equation with the x-axis and the degree-day requirements were calculated as the inverse of the linear equation slope (Campbell et al., 1974).

Results and Discussion

Effect of temperature and aphid species on parasitoid development time

The development time of *Lysiphlebus testaceipes* on the three aphid species was shortest at 27ºC, the highest temperature tested, and longest at 17ºC, the lowest temperature tested (Table 1). As the temperature increased, the developmental period shortened, with the three mean development periods significantly different from each other ($\alpha < 0.05$). The shortest development time of the egg and nymphs at both 17 and 27ºC was for *A. gossypii*; but at 22ºC it was for *A. fabae*. However, the development time

from egg to adult for females, males and females-males combined was longest in *A. gossypii* and shortest in *A. fabae* at the three temperatures.

Males of *L. testaceipes* reared on *A. fabae* took a longer time to complete their development than females at 17 and 27ºC but a shorter time at 22ºC. When all the individuals reared on the three aphids at three temperatures were considered collectively, in general, the males developed in a shorter time than females. This difference, however, was often less than half a day (Table 1).

Table1. Development times of *Lysiphlebus testaceipes* on *Aphis craccivora*, *Aphis fabae* and *Aphis gossypii* at three constant temperatures

Host species	Temperature (ºC)	n	Development Time (d, mean±SE)*			
			Egg - Nymph	Female (♀)	Male (♂)	Total (♀&♂)
Aphis craccivora	17	155	11.4±0.06 a	18.6±0.09 a	18.50±0.09 a	18.5±0.07 a
	22	328	6.5±0.03 b	10.9±0.04 b	10.8±0.04 b	10.9±0.04 b
	27	170	4.3±0.04 c	8.0±0.05 c	7.6±0.05 c	7.9±0.04 c
Aphis fabae	17	501	11.2±0.02 a	17.6±0.06 a	17.7±0.08 a	17.6±0.05 a
	22	553	6.1±0.02 b	10.3±0.04 b	10.1±0.37 b	10.2±0.03 b
	27	179	4.1±0.02 c	7.4±0.06 c	7.5±0.07 c	7.4±0.04 c
Aphis gossypii	17	560	10.8±0.04 a	19.8±0.10 a	19.7±0.07 a	19.8±0.08 a
	22	614	6.7±0.03 b	12.7±0.04 b	12.5±0.02 b	12.6±0.06 b
	27	32	4.0±0.00 c	9.6±0.04 c	9.0±0.12 c	9.3±0.24 c

* Within the columns means followed by the same letters are not significantly different ($\alpha > 0.05$, Tukey; $df_{A.craY\text{-}L} = 2, 650$, $F_{A.craY\text{-}L} = 6090$, $Sig_{A.craY\text{-}L} = 0.000$; $df_{A.craTot♀} = 2, 422$, $F_{A.craTot♀} = 6173$, $Sig_{A.craTot♀} = 0.000$; $df_{A.craTot♂} = 2, 225$, $F_{A.craTot♂} = 4296$, $Sig_{A.craTot♂} = 0.000$; $df_{A.craTot♀\text{-}♂} = 2, 649$ $F_{A.craTot♀\text{-}♂} = 10211$, $Sig_{A.craTot♀\text{-}♂} = 0.000$; $df_{A.fabY\text{-}L} = 2, 1230$, $F_{A.fabY\text{-}L} = 4926$, $Sig_{A.fabY\text{-}L} = 0.000$; $df_{A.fabTot♀} = 2, 692$ $F_{A.fabTot♀} = 7478$, $Sig_{A.fabTot♀} = 0.000$; $df_{A.fabTot♂} = 2, 535$, $F_{A.fabTot♂} = 6396$, $Sig_{A.fabTot♂} = 0.000$; $df_{A.fabTot♀\text{-}♂} = 2, 1230$, $F_{A.fabTot♀\text{-}♂} = 13921$, $Sig_{A.fabTot♀\text{-}♂} = 0.000$; $df_{A.gossY\text{-}L} = 2, 1203$, $F_{A.gossY\text{-}L} = 6090$, $Sig_{A.gossY\text{-}L} = 0.000$; $df_{A.gossTot♀} = 2, 10$, $F_{A.gossTot♀} = 6173$, $Sig_{A.gossTot♀} = 0.000$; $df_{A.gossTot♂} = 2, 2$ $F_{A.gossTot♂} = 4296$, $Sig_{A.gossTot♂} = 0.000$; $df_{A.gossTot♀\text{-}♂} = 2, 13$, $F_{A.gossTot♀\text{-}♂} = 10211$, $Sig_{A.gossTot♀\text{-}♂} = 0.000$).

Two way analysis of variance of the total development times of *L. testaceipes* in the three aphids at the three temperatures revealed significant differences attributable to temperature, parasitoid, and temperature by host-aphid species interaction ($\alpha = 0.05$; $df_{species} = 2, 3027$ $F_{species} = 193.759$, $Sig_{species} = 0.000$; $df_{temp} = 2, 3027$ $F_{temp} = 11754$, $Sig_{temp} = 0.000$; $df_{speciesXtemp} = 4, 3027$ $F_{speciesXtemp} = 3.687$, $Sig_{speciesXtemp} = 0.005$). Tukey's multiple range tests ($\alpha = 0.05$) applied after univariate analysis of variance (ANOVA) showed that host species was significant for the development time of *L. testaceipes* ($\alpha < 0.05$) but temperature was not significant ($\alpha \geq 0.05$).

Tang & Yokomi (1995) reported that the development times of *L. testaceipes* in *A. gossypii* on the host plant, *Hibiscus rosa-sinensis* L., 1753, at 15, 18, 21, 24, 27 and 30ºC were 25.0, 23.4, 15.3, 13.7, 10.5 and 9.5 d, respectively. Elliott et al. (1999) reported that the development times of *L. testaceipes* in the wheat aphid *Schizaphis graminum* (Rondani, 1852) on the host plant, *Hordeum vulgare* L., 1753, at 10, 14, 18, 22 and 26ºC were 49.1, 24.1, 15.2, 10.6 and 9.3 d, respectively. A major difference between these two studies is the 8.2 d difference between the developmental times of the parasitoid at 18ºC. However, it may not be correct to attribute this difference in development time only to the aphid host species. Weathersbee et al. (2004) reported that the composition and concentration of secondary plant metabolites can influence insect herbivore fitness, and that these effects are reflected in the parasitoid's development time. The host plant is an important factor in the development of aphids. The mean development times of *A. gossypii* to maturity on cotton at 15, 20, 25 and 30ºC were 12.0, 8.1, 5.7, and 4.5 d (Kersting et al., 1999), and on grapefruit at 20, 25, and 30ºC, they were 7.4, 6.4, and 5.9 d (Satar et al., 1998), respectively. During the development of *L. testaceipes* from egg-laying to egg hatching, the host is not killed by the parasitoid during or soon after egg-laying, i.e., the parasitoid uses the living host as a

source of nutrition for its development. This makes the development period of the parasitoid dependent to some extent on the species of aphid. In this regard, the present study corroborated the assertion of Tang & Yokomi (1995) and Elliott et al. (1999) that the development time of *L. testaceipes* is affected by host-aphid species. Separately, Rodrigues et al. (2004) reported that the development times of *L. testaceipes* in *A. gossypii* fed on chrysanthemum were 26.9, 14.8, 11.3, and 12.2 d at 15, 20, 25, and 30ºC, respectively. The development times obtained from the present study were similar to those determined in these studies, in spite of the host plant difference.

Effect of temperature and aphid species on the parasitization and pupal mortality rate of *Lysiphlebus testaceipes*

The parasitization rate of aphids by *L. testaceipes* at the three temperatures, was highest at 22ºC for the three aphid species, and the rate for *A. fabae* stands out as significantly higher ($\alpha < 0.05$). The lowest rate for the three aphid species was at 27ºC and the highest rate at this temperature was again for *A. fabae*, while the parasitization rates of *A. craccivora* and *A. gossypii* were close to each other. The lowest rates at the three temperatures were for *A. craccivora* (except at 27ºC for *A. gossypii*) (Table 2). When compared to the other two species, *A. craccivora* exhibited the fastest response to antennal or ovipositor contact by the parasitoid, moving its body violently and erratically, especially the abdomen, and throwing themselves to the soil.

When the effects of temperature, aphid species and temperature by aphid species interaction on parasitization rate were examined with two-way analysis of variance, the effect of temperature on the parasitization rate was significant ($\alpha = 0.05$, df = 2, 60, F = 5.925, P = 0.005) but the effect of species was not significant ($\alpha = 0.05$, df = 2, 60, F = 3.178, P = 0.050). The multiple comparison test (Tukey, $\alpha = 0.05$) for parasitization rate and temperature placed the parasitization rate on the three aphid species at 17ºC in one group and the rate for 22 and 27ºC in a different group. Rodrigues et al. (2004) reported that the parasitization rates of *L. testaceipes* for *A. gossypii* fed on chrysanthemum were 76, 68, 65 and 40% at 15, 20, 25 and 30ºC, respectively. In the present study, the lowest parasitization rate was also obtained at the highest temperature. Separately, the parasitization rates for the three aphid species suggest that *L. testaceipes* most successfully parasitized *A. fabae* to the other aphid species at all temperatures.

Table 2. Parasitization rate, pupal mortality rate and female-male ratio of *Lysiphlebus testaceipes* on *Aphis craccivora, Aphis fabae* and *Aphis gossypii* at different temperatures (Mean±SE)*

Host species	Temperature (ºC)	Number of Exposed Unit	Parasitization Rate (%)**		Pupal Mortality Rate (%)***		Female/Male Ratio
	17	6	25.0±4.79	b	0.0±0.00		1:0.79
Aphis craccivora	22	9	53.9±11.00	a	0.0±0.00		1:0.46
	27	6	20.5±6.54	b	0.0±0.00		1:0.40
	17	10	62.7±8.92	ab	0.0±0.00	b	1:0.57
Aphis fabae	22	10	71.1±4.12	a	3.1±2.18	b	1:0.97
	27	7	37.1±8.66	b	20.4±5.63	a	1:0.84
	17	5	54.2±10.02	a	5.9±1.81	b	1:0.80
Aphis gossypii	22	5	70.7±15.49	a	4.7±2.59	b	1:0.63
	27	4	20.0±7.50	a	56.8±6.80	a	1:0.27

* Within the columns means followed by the same letters are not significantly different ($\alpha \geq 0.05$, Tukey).

** Each unit has 80 second or third instars *aphid* nymphs.

*** Parasitization rate and pupal death ratio were arcsine-square root transformed before one-way ANOVA and Tukey; untransformed data are presented ($df_{A.cracPar} = 2,20$, $F_{A.cracPar} = 1.08$ $Sig_{A.cracPar} = 0.36$; $df_{A.fabePar} = 2, 24$, $F_{A.fabePar} = 4.07$ $Sig_{A.fabePar} = 0.030$; $df_{A.gossPar} = 2,10$, $F_{A.gossPar} = 1.37$ $Sig_{A.gossPar} = 0.30$; $df_{A.fabeMortality} = 2,24$, $F_{A.fabeMortality} = 16.02$ $Sig_{A.fabeMortality} = 0.000$; $df_{A.gossMortality} = 2,10$, $F_{A.gossMortality} = 7.45$, $Sig_{A.gossMortality} = 0.01$).

When the aphid species in their immature stage were examined for the mortality of *L. testaceipes* (Table 2), the highest rate was 56.8% in *A. gossypii* at 27ºC (it should be noted that no mortality was observed for *L. testaceipes* in *A. craccivora*.) In addition, *A. craccivora* was the species least parasitized by *L. testaceipes* at any temperature. The reason for this was probably the behavior of *A. craccivora,* which threw themselves from the plant to the soil in the experimental pots when they were disturbed by the parasitoid; this appears to have been a major factor in reducing its parasitization rate below that of the other two species.

The mortality of *L. testaceipes* pupae in *A. fabae* at 27ºC was 20.4%, while there was only low mortality at 17 and 22ºC. Similarly, *L. testaceipes* showed lower mortality in *A. gossypii* at 17 and 22ºC than at 27ºC (Table 2). A study by Takanashi (1990) on the development time of *Lysiphlebia japonica* (Ashmead) between 12 and 30ºC reported that no individuals were able to develop at 30ºC. Also, Deng & Tsai (1998) stated that the mortality rates of *L. japonica* in *Toxoptera citricida* (Kirkaldy) (Hemiptera: Aphididae) were 2.8, 16.2, 27.5 and 73.3% at 15, 20, 25 and 30ºC, respectively. Likewise, Rodrigues et al. (2004) demonstrated that the mortality rates of *L. testaceipes* in *A. gossypii* fed on chrysanthemum were 20, 39, 38 and 86% at 15, 20, 25 and 30ºC, respectively. The highest temperature used in the present study and others studies (Takanashi, 1990; Deng & Tsai, 1998; Rodrigues et al., 2004) caused similar mortality rates.

In the present study, the two-way analysis of variance applied to the mortality rates of *L. testaceipes* individuals separately reared on the three aphid species demonstrated a significant statistical difference between the means and also that this difference was due to temperature, host-aphid species and temperature by host-aphid species interaction. In the multiple comparison tests, both temperature and aphid species formed a separate group (Tukey, $\alpha = 0.05$; $df_{species} = 2, 60$, $F_{species} = 28.34$, $P_{species} = 0.000$; $df_{temp} = 2, 60$, $F_{temp} = 26.22$, $P_{temp} = 0.000$; $df_{speciesXtemp} = 4, 60$, $F_{speciesXtemp} = 7.861$, $P_{speciesXtemp} = 0.000$).

For the three aphids at the three temperatures, the highest female ratio of *L. testaceipes* was obtained on *A. fabae*, followed by *A. gossypii* (Table 2). Temperature affected the sex ratio of the parasitoid in *A. craccivora* and it was statistically significant ($\chi^2_{A.craccivora} = 7.694$, $df_{A.craccivora} = 2$, $Sig_{A.craccivora} = 0.021$). Chi-square tests applied separately to the data on sex ratios for the parasitoids reared on *A. fabae* and *A. gossypii* gave similar results ($\chi^2_{A.fabae} = 18.02$, $df_{A.fabae} = 2$, $Sig_{A.fabae} = 0.000$; $\chi^2_{A.gossypi} = 17.056$, $df_{A.gossypi} = 2$, $Sig_{A.gossypi} = 0.000$). Given that temperature affected the sex ratio of *L.* testaceipes reared on each aphid species, the phi-Cramer's V test was applied to measure the magnitude of this effect. The effect was strong for A. fabae (0.174) and A. gossypii (0.174), but not for A. craccivora (0.109). Furthermore, the Chi-square test revealed that the host-aphid species affected the sex ratio of the parasitoid as strongly as temperature ($\chi^2_{species} = 36.136$, $df_{species} = 2$, $Sig_{species} = 0.000$, phi-Cramer's V value = 0.121).

The sex ratio is one of the most important contributors to the success of released parasitoids. The reproduction of hymenopteran parasitoids begins with the mating of females and males shortly after their emergence from mummies. Mated females store sperm in their spermatheca. The females facultatively alter the gender of their progeny in response to changes in the environmental conditions which can affect the sex ratio by stimulating the release of sperm for fertilization of the eggs, with only females produced (haplodiploid genetic system). In contrast, if the females do not release sperm for the fertilization of eggs, only males hatch from the unfertilized eggs (DeBach, 1974; Godfray, 1994).

The present study overall obtained more male individuals at 17 and 22ºC than at 27ºC, with *L. testaceipes* having a higher mortality rate and male-female ratio for *A. craccivora* than for *A. fabae* and *A. gossypii*. However, the mean for *A. fabae* was similar to the mean for *A. craccivora*, especially at 17 and 22ºC. Rodrigues et al. (2004) reported that the male-female ratios of *L. testaceipes* on *A. gossypii* fed on chrysanthemum were 0.35, 0.43, 0.45, and 0.54 at 15, 20, 25, and 30ºC, respectively. These male-female ratios were quite different from the results obtained in the present study. Environmental conditions and the density of aphid, leaf texture and plant allomones are indicators for the sex ratio of *Diaeretiella rapae* (McIntosh) in *A. gossypii* on chrysanthemum (Shukla & Triphathi, 1993). Our test unit consisted of 80 individuals in 5-L cages. In contrast, Rodrigues et al. (2004) used Petri dishes that contained several aphid nymphs. The test unit differences may have caused a higher number of male *L. testaceipes* in *A. gossypii*. Moreover, *A. gossypii* has clones based on host plant (Satar et al., 2013) that probably have different clones to those on chrysanthemum (Guldemond et al., 1994).

Development thresholds and thermal constants for *Lysiphlebus testaceipes* reared on three aphid species

The thermal constants for *L. testaceipes* reared on *Aphis craccivora*, *A. fabae* and *A. gossypii* in the period from egg to pupa were 68.96 DD (degree days), 64.52 DD and 63.69 DD, and the development thresholds for that period were 11.07ºC, 11.25ºC and 11.61ºC, respectively. Furthermore, the thermal constants required for the period of egg to adult development of the parasitoids in *A. craccivora*, *A. fabae* and *A. gossypii* were 136.99 DD, 128.05 DD and 175.44 DD, the development thresholds were 9.42ºC, 9.69ºC and 8.12ºC, respectively, for the same development period (Table 3 and Figure 1).

Table 3. Development thresholds (ºC) and thermal constants (degree days, DD) of *Lysiphlebus testaceipes* reared on *Aphis craccivora, Aphis fabae* and *Aphis gossypii* in the egg and nymph period, and total adult development period

Host-aphid species	Egg and Nymph Period		Total Development Period	
	Development Threshold (ºC)	Thermal Constant (DD)	Development Threshold (ºC)	Thermal Constant (DD)
Aphis craccivora	11.07	68.96	9.42	136.99
Aphis fabae	11.25	64.52	9.69	128.05
Aphis gossypii	11.61	63.69	8.12	175.44

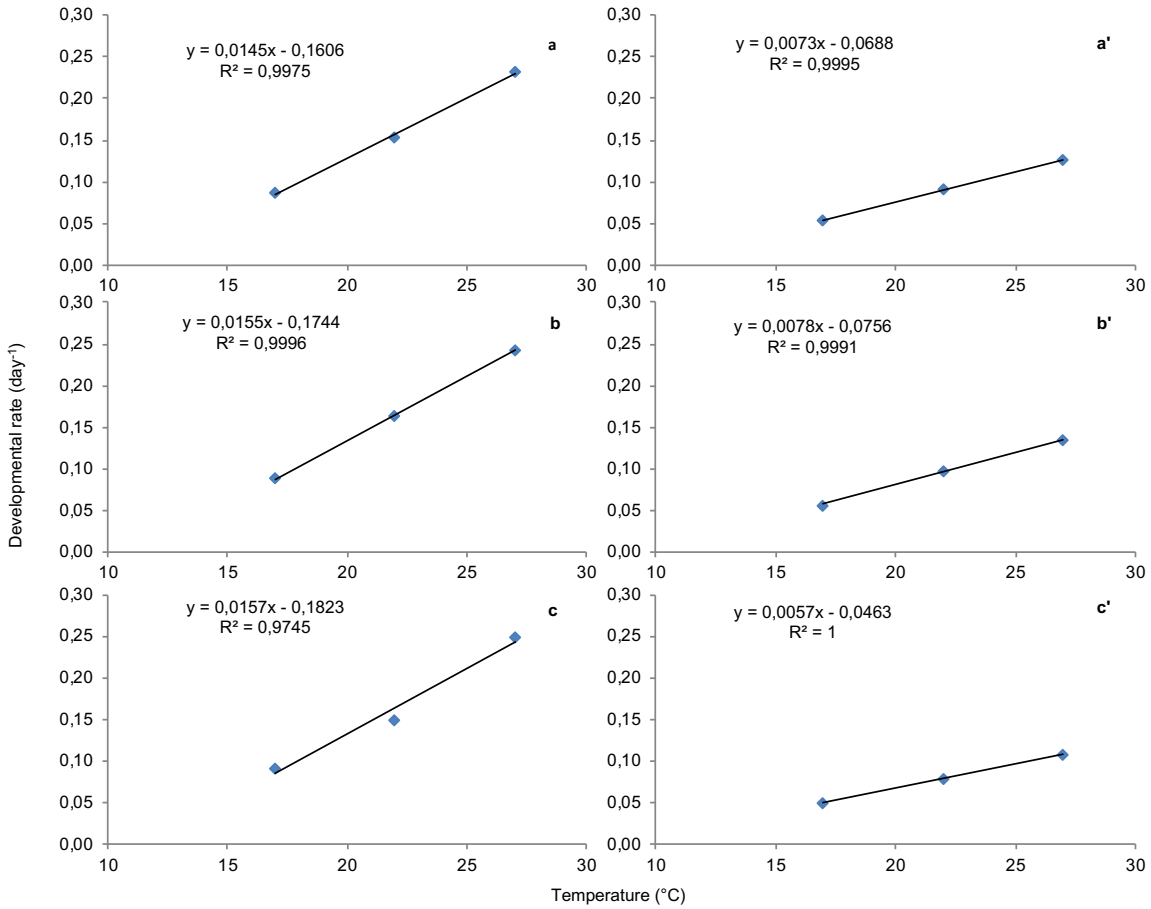

Figure 1. Regression lines and equations for development rates for the egg-larval and adult stages of *Lysiphlebus testaceipes* in (a and a') *Aphis craccivora*, (b and b') *Aphis fabae* and (c and c') *Aphis gossypii*.

The lowest development threshold in the egg-larval period was for the parasitoids reared on *A. craccivora* but the lowest thermal constant was calculated for the parasitoids in *A. gossypii*. As for the development threshold for the total development period, while the lowest threshold for the parasitoid was for *A. gossypii*, the lowest thermal constant was for *A. fabae*. In a study conducted by Tang & Yokomi (1995) on the development time of *L. testaceipes* in *T. aurantii,* the development threshold and the effective temperature to achieve maturity were 7.5ºC and 212.8 DD, respectively. The development threshold calculated by Tang & Yokomi (1995) was lower than for the aphid species obtained in the present study but the thermal constant was higher. *Lysiphlebus testaceipes* has geographical isolates, even for same host plant, and aphids can have different developmental thresholds (Royer et al., 2001).

Rodrigues et al. (2004) stated that the development times of *L. testaceipes* in *A. gossypii* fed on chrysanthemum were 26.9, 14.8, 11.3 and 12.2 d, respectively, the parasitization rates were 76, 68, 65 and 40%, respectively, and the emergence rates were 80, 61, 62 and 14%, at 15, 20, 25 and 30ºC, respectively. On the basis of these results, Rodrigues et al. (2004) recommended 25ºC as the best temperature for both the reproduction and establishment of *L. testaceipes*. Zamani et al. (2007) reported development thresholds and thermal constants for the egg to adult period for *Aphidius colemani* Viereck 1912 (Hymenoptera: Braconidae) in *A. gossypii* and *Myzus persicae* (Sulzer, 1776) of 2.97 and 2.65ºC, respectively, and 256.41 and 270.27 DD, respectively. As in the present study, Zamani et al. (2007) found different development thresholds for *A. colemani* on different host aphids.

In the light of the findings of this study, the three temperatures and the three aphid species could be used for the production of *L. testaceipes*. However, for the reasons stated earlier, *A. fabae* is a better overall host for *L. testaceipes* than both *A. craccivora* and *A. gossypii*. More specifically, *A. fabae* feeding on *V. faba* at 20-22ºC is potentially the most suitable combination of host-aphid host-plant and temperature for the mass production of *L. testaceipes*. However, the high mortality rate of the parasitoid observed in mummies at 27ºC may be a factor limiting its performance in hot areas such as the Çukurova Basin of Turkey. In addition, a study on the interactions between *L. testaceipes* and others parasitoids such as *Lysiphlebus fabarum* (Marshall, 1896) and *Lysiphlebus confusus*, Tremblay & Eady, 1978 in citrus plantations would be beneficial to understanding the prospects of *L. testaceipes* being successful in the same ecological niche as the other parasitoid species.

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