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Development, growth and body weight of *Hippodamia variegata* fed *Aphis fabae* in the laboratory

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Abstract

Among the predators of aphids, lady beetles are considered their most effective natural enemies. In the present study the predation rate of the variegated lady beetle *Hippodamia variegata* (Goeze) was studied, fed on the black bean aphid *Aphis fabae* Scopoli under laboratory conditions. The duration of the development of the immature stages and the prey consumption of larval instars were examined under four constant temperatures: 15, 20, 25, and 30 °C. The total larval prey consumption was 158, 164, 192 and 175 *A. fabae* apterous adults at 15, 20, 25 and 30 °C, respectively. The development of the immature stages of *H. variegata* lasted 61.7 days at 15 °C, 30.3 days at 20 °C, 19.0 days at 25 °C, and 10.2 days at 30 °C. The thermal constant for development of the immature stages of the *H. variegata* was estimated to 184 degree days and the lower thermal threshold at 13.2 °C. The body weight of emerged female adults was 11.09, 11.55, 12.90 and 11.89 (mg) at 15, 20, 25 and 30 °C, respectively. The optimum temperature for the *H. variegata* as a biological control agent was 25 °C. At that temperature, the highest total prey consumption and lowest larval mortality were recorded. These results might be useful tools for the prediction of *H. variegata* phenology and the interaction with its prey.

Key words: Coccinellidae, Aphididae, predators, black bean aphid, biological control.

Introduction

Aphids (Hemiptera Aphididae) are some of the most serious agricultural pests of economic importance throughout the world. Apart from direct damage to plants due to feeding, indirect damage from transmitting more than 100 plant viruses has been documented (Blackman and Eastop, 2000). The control of aphids is based, mostly, on chemical insecticides. However, the extensive use of certain classes of insecticides has led to resistance development in many aphid populations (Margaritopoulos *et al*., 2007). Most insecticides used for controlling aphids may cause adverse effects to people, animals and to the environment and also may cause residue problems and mortality of beneficial organisms.

Many lady beetles (Coleoptera Coccinellidae) are known to be voracious predators of aphids (Frazer, 1988; Hagen, 1974), whiteflies (Gerling, 1990), mealybugs (Hodek, 1973), scale insects (Eliopoulos *et al*., 2010) and mites (Riddick *et al*., 2014). *Hippodamia* (*Adonia*) *variegata* (Goeze) has been reported as the most important natural enemy of aphids infesting various crops in many countries (Kontodimas and Stathas, 2005). For example, in Greece, *H. variegata* is one of the dominant coccinellid predators (Kavallieratos *et al*., 2004; Kontodimas and Stathas, 2005; Karagounis *et al*., 2006) preying on *Aphis gossypii* Glover on cotton and *Myzus persicae* Sulzer on tobacco and peach trees.

To initiate a successful biological control program, it is essential to characterize growth, stage structure, fecundity and predation rate of predators (Farhadi *et al*., 2011). In *H. variegata*, a considerable geographic variation in biological traits has been reported (Dobzhansky, 1933; Kontodimas and Stathas, 2005). The main objectives of this study were to estimate: (a) the effect of temperature on development, adult body weight, sex ratio and predation rate and (b) the thermal requirements for development (Lower development threshold and Thermal constant) of immature stages of *H. variegata* fed on *Aphis fabae* Scopoli in the laboratory.

Materials and methods

Insect rearing

The *A. fabae* was established in the laboratory on *Vicia faba* L. (fava beans) from individuals collected at the Technological Educational Institute (TEI) of Peloponnese. The culture was maintained on *V. faba* plants. The plants were kept inside 40 × 45 × 50cm cages with a wooden washable flush floor; all other sides and the top were covered with fine aphid proof muslin to allow aeration and to prevent aphid escape. The cages were kept in the laboratory, under controlled conditions (20 ± 1 °C, 60 ± 5% RH and L16:D8).

The *H. variegata* colony was initiated from approximately 50 adults collected in 2013 from weeds infested by aphids in Kalamata, Messinia, Greece. All predators were reared in cylindrical, 30 cm in diameter and 50 cm in length, Plexiglas cages (Iperti and Brum, 1969). Inside the cage, an overabundance of *A. fabae* aphids on *V. faba* was placed together with the predators. The plants were replaced by fresh ones when it was deemed necessary. The colonies of all predators were kept at 25 ± 0.5 °C, 60 ± 5% RH and L16:D8.

Development of immature stages, mortality and prey consumption

Egg hatchability and incubation period were studied by placing newly laid eggs (< 24-h old) of *H. variegata* in Petri dishes at four different temperatures (15 ± 0.5, 20 ± 0.5, 25 ± 0.5 and 30 ± 0.5 °C), 60 ± 5% RH and L16:D8. Egg hatching was recorded daily and each emerging larva was transferred separately inside a Blackman box (7.7 × 4.5 × 2cm) with a fine camel hair brush at the base of which a piece of water saturated moss was placed (Blackman, 1971). In each box, a detached leaf from *V. faba* plants whose petiole was inserted into the moss was placed. One hundred *A. fabae* apterous adults (1 day old) were placed on the leaf on daily basis. The larvae were examined daily for moulting. At the same time, the aphids found alive were counted and removed, and 100 new aphids were introduced into the box. The *V. faba* plant leaf was replaced with a fresh one when it was deemed necessary. Each egg and the subsequent larva were considered as replication at each temperature.

The newly emerged adults were sex ratio records and weighed using an ADAM PMB53 (Maidstone Road, Kingston, Milton Keynes, U.K.) electronic balance. Before measurement, the insects were immobilized by exposure to 0 °C for 2-3 min.

Data analysis

The Kolmogorov-Smirnov test was used to determine if data deviated from normality. The effects of temperature on preimaginal development were examined by one-way analysis of variance (ANOVA). Two-way ANOVA was also used to examine the effects of temperature and sex on adult weight. The effects of temperature and larval instar on total and daily aphid consumption of *H. variegata* were also examined. Data obtained from total and daily prey consumption at different temperatures were analyzed using one-way ANOVA, whereas data on total and daily aphid consumption during the entire larval development of *H. variegata* was analysed using one-way ANOVA with temperature as the factors. In all above cases, means were separated by Duncan's test.

The relationship between rate of development (Y, inverse of developmental period in days) and temperature of each developmental stage, as well as the total immature development, were estimated by the linear regression model: $y = a + bx$, where y is the developmental rate at temperature x (Arnold, 1959). The lower temperature threshold (T_0) for development was determined as the x-intercept of the linear equation ($-a/b$) and the summation of day-degrees (DD, $1/b$) requirements as the inverse of the slope of the above equation. Statistical analyses were conducted using SPSS ver. 18 for Windows (SPSS, 2009).

Results

Egg, larval and pupal mortality of *H. variegata* are shown in table 1. The egg to adult survival rate increased from 43.4% to 71.9% as temperature increased from 15 to 25 °C, but decreased to 50.0% at 30 °C. Egg and first instar larvae were found to have the highest mortality. Pre-imaginal mortality for *H. variegata* was not significantly different among temperatures (χ^2_3 = 7.74, $P \leq 0.052$).

The duration of larvae development was longer than the duration of the other stages, at all temperatures tested (table 2). The developmental period was temperature dependent for egg ($F_{3, 89}$ = 1022.6, $P \leq 0.001$),

Table 1. Percentage mortality of *H. variegata* feeding on *A. fabae* at four constant temperatures and long day length (L16:D8).

Life stages	Mortality %							
	15 °C		20 °C		25 °C		30 °C	
	N	%	N	%	N	%	N	%
Egg	53	26.41	42	16.67	32	9.38	50	22.00
L1	39	25.64	35	20.00	29	6.90	39	17.95
L2	29	10.35	28	10.71	27	3.70	32	6.25
L3	26	3.84	25	4.00	26	3.85	30	16.67
L4	25	4.00	24	4.16	25	4.00	25	0.00
L1-L4	39	38.46	35	34.29	29	17.24	39	35.90
Pupa	24	4.17	23	4.35	24	4.17	25	0.00

N: number of replications.

Table 2. Development time (days) of *H. variegata* feeding on *A. fabae* at four constant temperatures and long day length. Numbers in brackets denote standard error of means.

Life stages	15 °C		20 °C		25 °C		30 °C		*F*
	N	Days	N	Days	N	Days	N	Days	
Egg	23	10.17 (0.11)a	22	5.91 (0.11)b	23	3.65 (0.11)c	25	2.06 (0.11)d	1022.6*
Larva	23	35.43 (0.49)a	22	16.45 (0.51)b	23	10.30 (0.49)c	25	5.26 (0.47)d	729.7*
Pupa	23	16.04 (0.21)a	22	7.95 (0.22)b	23	5.04 (0.21)c	25	2.84 (0.21)d	740.6*
Adult	23	61.65 (0.47)a	22	30.32 (0.48)b	23	19.0 (0.47)c	25	10.16 (0.45)d	2316.1*

N: number of replications. Means followed by a different small letter within a row differ significantly ($P < 0.05$) by Duncan's test.

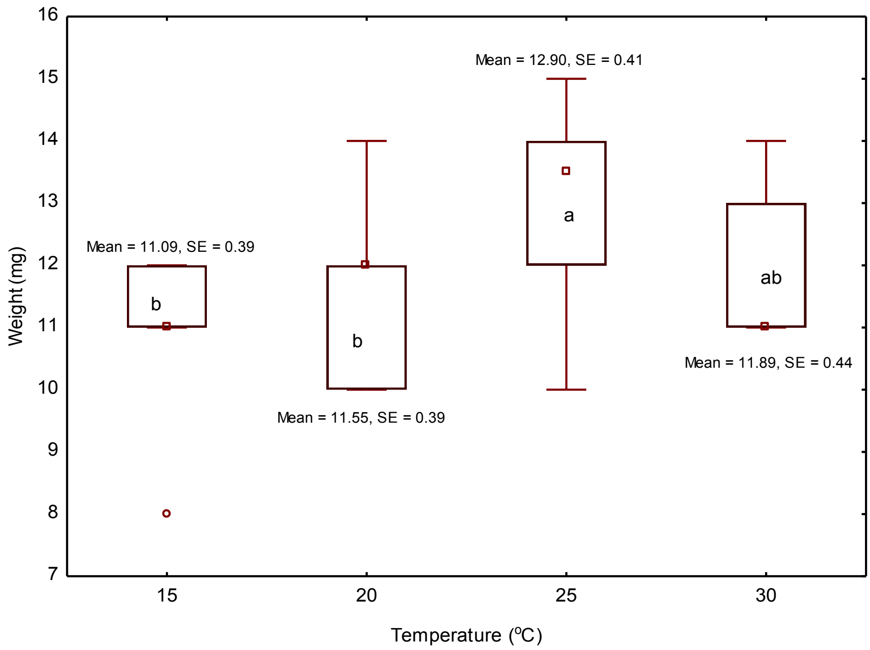

Figure 1. Box plots showing mean body weight of *H. variegata* females fed on *A. fabae* at four constant temperatures and long day length. Means followed by a different letter are significantly different from each other based on a Duncan's test at $P < 0.05$.

larval ($F_{3, 89} = 729.7$, $P \leq 0.001$), pupal ($F_{3, 89} = 740.8$, $P \leq 0.001$) and for the total preimaginal development of *H. variegata* ($F_{3, 89} = 2316.1$, $P \leq 0.001$).

The weight of emerged adults was 9.74, 9.82, 10.69 and 9.76 (mg) at 15, 20, 25, and 30 °C, respectively, but do not seem temperature dependent ($F_{3, 89} = 1.157$, $P < 0.331$). However, sex affects adult weight significantly, with females being heavier than males ($F_{1, 93} = 148.23$, $P < 0.001$). Additionally, female body weight was significantly different between temperatures ($F_{3, 37} = 3.57$, $P < 0.023$). The weight of emerged females was 11.09, 11.55, 12.90 and11.89 mg at 15, 20, 25, and 30 °C, respectively (figure 1). Sex ratio (% females) was 47.8%, 50.0%, 43.5% and 36.0% at 15, 20, 25 and 30 °C, respectively. Sex ratio was not different amongst temperatures ($\chi^2_3 = 1.11$, $P \leq 0.775$).

The temperature threshold fluctuated between 12.3 °C for the egg stage and 13.5 °C for the larvae stage. The linear regression parameters (intercept and slopes) that describe the relationship between developmental rate (y) and temperature (x) are shown in table 3. The lower slope *i.e.* 0.1454 was recorded at the larvae instar which had the longest duration of all temperatures tested. For the rest of the stages, the slope increased as the duration of development decreased.

Daily aphid consumption during the whole larval development, as well as the total number of aphids consumed in each larval instar of *H. variegata* are shown in tables 4 and 5. The total larval prey consumption was 157.5, 163.5, 192.2 and 174.8 aphids at 15, 20, 25, and 30 °C, respectively. One-way ANOVA indicated that total aphid consumption was significantly different among temperatures ($F_{3, 89} = 12.36$, $P < 0.001$).

During the fourth larval instar of *H. variegata* consumed the highest number of aphids per day under all temperatures. The mean daily larval prey consumption was 4.5, 10.1, 19.0 and 33.9 aphids at 15, 20, 25 and 30 °C, respectively. Significant differences were observed among temperatures and mean daily aphid consumption ($F_{3, 89} = 311.75$, $P < 0.001$).

Discussion

Among the developmental stages of *H. variegata*, the egg and the first instar larvae suffered the highest mortality between 15 and 20 °C. Relatively high mortality of the first instars as compared to other stages has been reported for other Coccinellidae (Omkar and Pervez, 2004), which was possibly due to their small size and the thin and soft cuticle which makes them more vulnerable to physical stressors (Omkar and Pervez, 2004). In the present study, the eggs suffered maximum mortality at minimum and maximum temperatures of 15 and 30 °C, respectively. Many authors (e.g., Orr and Obrycki, 1990; Miller, 1992; Rodriguez-Saona and Miller, 1999; Benelli *et al.*, 2015) have reported that severe mortality during immature stages of predatory Coccinellidae occurs at temperatures near the developmental threshold.

It has been reported that exposure of different immature stages of aphidophagous coccinellids to higher temperatures results in an increase in the development rate (Stathas 2000; Omkar and Pervez 2004). The significant differences in the developmental period of each of the life stages of *H. variegata* suggest that all stages were sensitive to temperature fluctuations. Pre-imaginal development (egg to adults) in *H. variegata* varied from 61.6 to 10.2 days for temperatures between 15 to 30 °C respectively. The total developmental period (egg to adult emergence) of *H. variegata* found in the present study was higher than the study by Jalali *et al.*, (2014)

Table 3. Developmental threshold (T_0) and degree day (DD) requirements in *H. variegata* feeding on *A. fabae* at four constant temperatures and long day length.

Life stage	T_0	DD	Equation	R^2 (df)	*P*
Egg	12.3	38.8	$y = -0.31834x + 0.025744$	0.91 (1, 93)	< 0.05
Larvae	13.5	93.1	$y = -0.1454x + 0.010739$	0.87 (1, 93)	< 0.05
Pupae	12.9	50.3	$y = -0.25618x + 0.019863$	0.83 (1, 93)	< 0.05
Egg-adult	13.2	184.4	$y = -0.07137x + 0.005422$	0.90 (1, 93)	< 0.05

Table 4. Total prey consumption of *H. variegata* larvae feeding on *A. fabae* at four constant temperatures and long day length. Numbers in brackets denote standard error of means.

Life stages		15 °C		20 °C		25 °C		30 °C	*F*
	N	TAC	N	TAC	N	TAC	N	TAC	
L1	23	6.7 (0.45)a	22	7.4 (0.46)ab	23	9.4 (0.45)c	25	8.2 (0.43)bc	6.7*
L2	23	10.8 (0.58)a	22	13.4 (0.59)b	23	12.6(0.58)b	25	12.3(0.55)ab	3.6*
L3	23	21.8 (1.00)a	22	24.7 (1.03)ab	23	27.5 (1.00)b	25	23.6 (0.96)a	5.6*
L4	23	118.2 (4.34)a	22	118.0 (4.43)a	23	142.7 (4.33)b	25	130.7 (5.25)ab	7.4*
L1-L4	23	157.5 (4.33)a	22	163.5 (4.43)ab	23	192.2 (4.33)c	25	174.8 (4.16)b	12.3*

N: individuals examined; TAC: Total Aphid Consumption. Means followed by a different small letter within a row differ significantly ($P < 0.05$) by Duncan's test.

Table 5. Mean daily prey consumption of *H. variegata* larvae fed on adult *A. fabae* at four constant temperatures and long day length. Numbers in brackets denote standard error of means.

Life stages		15 °C		20 °C		25 °C		30 °C	*F*
	N	MDAC	N	MDAC	N	MDAC	N	MDAC	
L1	23	0.7 (0.30)a	22	1.6 (0.31)b	23	3.2 (0.30)c	25	6.0 (0.29)d	62.4*
L2	23	1.8 (0.37)a	22	4.7 (0.38)b	23	6.2 (0.37)c	25	12.7 (0.36)d	161.6*
L3	23	3.0 (0.90)a	22	7.7 (0.92)b	23	18.2 (0.90)c	25	23.1 (0.86)d	107.8*
L4	23	10.7 (3.66)a	22	22.8 (3.74)b	23	42.9 (3.66)c	25	83.8 (3.51)d	80.2*
L1-L4	23	4.5 (0.74)a	22	10.1 (0.76)b	23	19.0 (0.74)c	25	33.9 (0.71)d	311.8*

N: individuals examined; MDAC: Mean Daily Aphid Consumption. Means followed by a different letter within a row differ significantly ($P < 0.05$) by Duncan's test.

[15.47 days at 25 °C, fed on *Agonoscena pistaciae* Burckhardt et Lauterer (Hemiptera Psyllidae)] and Wu *et al*. (2010) [12.6 to 14.5 at 25 °C depending on the host plant and fed on *Brevicoryne brassicae* (L.) and *Rhopalosiphum padi* L.], and smaller than El Hag and Zaitoon (1996) (20.1 days at 25 °C fed on *A. gossypii*). Michels and Flanders (1992) also, report a smaller developmental time (depending on the *H. variegata* population origin) between 24.4 to 30.3 days fed on *Schizaphis graminum* (Rondani) (Hemiptera Aphididae) and 23.9 to 28.1 days fed on *Diuraphis noxia* (Mordvilko). Farhadi *et al*. (2011) report a developmental time of 16.33 days at 23 °C, while El Habi *et al*. (2000) found that the total developmental time ranged from 27.58 to 7 days at 18 to 34 °C (excluding egg duration) and Obrycki and Orr (1990) found 19.4 and 19.3 days at 23 °C fed on *Rhopalosiphum maidis* (Fitch) and *Acyrthosiphon pisum* (Harris) (Hemiptera Aphididae) respectively. Lanzoni *et al*. (2004) report a developmental time of 18.1 days at 25 °C for a population origin Bologna (Italy), and reared on *M. persicae* (Hemiptera Aphididae). There are differences between the above-mentioned data and those reported in the present study. This variation may be attributed to the different prey or food quality used and the geographic variability that produces differences in populations of *H. variegata* (Dobzhansky, 1933). Also, discrepancies between studies could also be associated with different experimental conditions, the rearing system (*i.e.* relative humidity) and population origin. Butcher *et al*. (1971) and Jalali *et al*. (2014) suggested that the thermal optimum was the temperature that minimized both development and mortality time. According to Butcher *et al*. (1971) the optimum temperature for *H. variegata* development is 25 °C.

In the present study, consumption rates increased from the first through the fourth instar. Fourth instars consumed more aphids than the total number consumed by other instars. The higher voracity of the 4th instar larvae is also frequently observed in other coccinellid species such as *Coccinella septempunctata* L. and *Hippodamia convergens* Guerin-Meneville fed on *Myzus persicae nicotianae* Blackman (Katsarou *et al*., 2005), *Harmonia axyridis* Pallas fed on *A. gossypii* (Lee and Kang, 2004), and *Coccinella undecimpunctata* L. fed on *M. persicae* (Cabral *et al*., 2009). This is possibly a result of the higher energy intake requirements for growth and for retaining critical body weight for pupation (Hodek and Honek, 1996). Michels and Flanders (1992) report higher total aphid consumption (depending on the *H. variegata* population origin) between 290.0 to 414.4

aphids and a mean daily aphid consumption of 21.9 to 31.9 aphids fed on *S. graminum* and 192.7 to 505.1 and 13.6 to 37.2 aphids fed on *D. noxia* at 20 °C, respectively. Farhadi *et al*. (2011) found total aphid consumption for the 1st, 2nd, 3rd and 4th instar larvae 4.93, 9.14, 20.36 and 102.48 aphids for females larvae and 4.56, 8.82, 20.38 and 81.21 aphids for males larvae, respectively, fed on *A. fabae* at 23 °C. The variation among values reported by us and other authors may be attributed to the differences in the prey used, population origin, different experimental conditions, rearing system (*i.e.* relative humidity, temperatures), etc. The variation might be attributed to differences in the size (body mass) of the different aphid species (Bhadauria *et al*., 2001). This variation (Farhadi *et al*., 2011) may be attributed to the difference in origin of the population used (*i.e.* Karaj region, Tehran Province, Iran *vs* Kalamata, Greece). Considerable geographic variation in biological traits within populations of *H. variegata* has been reported (Dobzhansky, 1933; Kontodimas and Stathas, 2005).

Also, comparing our results with those from other studies (e.g. Farhadi *et al*., 2011) shows that factors such as the aphid species on which the predator Coccinellidae feed and rearing conditions can influence the developmental period until adult emergence, general survival and adult longevity. Moreover, as suggested by Francis *et al*. (2000; 2001) in *Adalia bipunctata* (L.), and Giles *et al*. (2002) in *C. septempunctata,* biological characters must be studied as a tritrophic system, predator - aphids - plant. Kalushkov (1998) found that developmental time, mortality and adult weight of *A. fabae* as suitable food for *A. bipunctata* depended on the host plant. Also, the preimaginal developmental time, mortality and size of adult *C. septempunctata* appears to be altered by the biochemical reaction between aphid prey and host plant (Giles *et al*., 2002). As referred by Francis *et al*. (2000; 2001), the allelochemical substances the aphid host plants contain may not only affect the aphid but also the predator.

The variation in adult body weight had a direct effect on various reproductive parameters, most notably on fecundity and the oviposition period of females (Shah and Khan, 2014). Also, Omkar and Srivastava (2003) reported for *C. septempunctata* that increased food intake of suitable prey results in increased weight. In the present study, *H. variegata* consumed the most prey at 25 °C and in the same temperature they developed into larger adults.

The lowest developmental threshold and day-degree found in the present study were 13.2 °C and 184.4 DD. In comparison with our results, pre-imaginal development of a Russian population of *H. variegata* was shown to require 90.2 DD above a lower developmental threshold of 19.7 °C (Michels and Bateman, 1986). For egg development, Honek and Kocourek (1988) reported for the same parameters 8.2 °C and 61.9 DD. Differences in thermal requirements for the development of *H. variegata* could be related with prey species, food quality, whereas discrepancies between studies could also be associated with different experimental conditions (*i.e.* relative humidity).

Conclusion

The results of the present study show that fourth larvae instar are the stages where *H. variegata* are most effective as predators. The optimum temperature as a biological control agent for *H. variegata* was 25 °C. At that temperature, the highest total prey consumption and lowest larval mortality were recorded. For further study, fecundity and life table parameters of *H. variegata* will be worthy of pursuit.

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