Orijinal araştırma (Original article)

Temperature-dependent development of *Chilocorus bipustulatus* (L.) (Coleoptera: Coccinellidae) on *Aspidiotus nerii* Bouché (Hemiptera: Diaspididae) under laboratory conditions¹

Aspidiotus nerii Bouché (Hemiptera: Diaspididae) ile beslenen Chilocorus bipustulatus (L.) (Coleoptera: Coccinellidae)'un laboratuvar koşullarında sıcaklığa bağlı gelişmesi

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Summary

Chilocorus bipustulatus (L.) is an important predator of scale insects that is widely distributed in citrus plantations in the Mediterranean Region of Turkey. The developmental time of *C. bipustulatus*, a polyphagous predator, was studied at six different, constant temperatures (14, 18, 22, 26, 30 and 34° C) and a relative humidity (R.H. of 65±1%, with *Aspidiotus nerii* Bouché (Hemiptera: Diaspididae) as the prey. The developmental periods of immature stages were 61.67, 51.00, 41.21, 33.72, 26.76 and 27.17 days at 14, 18, 22, 26, 30 and 34 °C, respectively. The developmental threshold for *C. bipustulatus* determined with the linear model was 5.91. Linear regression was employed to determine the thermal constant of 651.1 degree-days.In this study linear and nonlinear regression models are discussed.

Key words: Temperature dependent development, linear model, nonlinear model, Chilocorus bipustulatus

Özet

Chilocorus bipustulatus (L.) (Coleoptera: Coccinellidae) Akdeniz Bölgesinde (Türkiye) turunçgil alanlarında yaygın olarak bulunan önemli bir kabuklubit avcısıdır. *Aspidiotus nerii* Bouché (Hemiptera: Diaspididae) üzerinde beslenen *C. bipustulatus*'un, nemi % 65±1'e ayarlı iklim dolaplarında 6 farklı sabit sıcaklıkta (14, 18, 22, 26, 30 ve 34°C) sıcaklığa bağlı gelişme süreleri incelenmiştir. Avcının ergin öncesi gelişme süreleri 14, 18, 22, 26, 30 ve 34°C sıcaklıklarda sırasıyla 61.67, 51.00, 41.21, 33.72, 26.76 ve 27.17 gün sürmüştür. *C. bipustulatus*'un lineer regresyon modeline göre gelişme eşiği 5.91 °C olarak hesaplanmıştır. Lineer regresyon kullanılarak hesaplanan termal konstant değeri ise 651.1 gün-derece olmuştur.

Bu çalışmada *C. bipustulatus*'un sıcaklığa bağlı gelişme oranlarının belirlenmesinde doğrusal ve doğrusal olmayan regresyon modelleri tartışılmıştır.

Anahtar sözcükler: Sıcaklığa bağlı gelişme, doğrusal model, doğrusal olmayan moddel, Chilocorus bipustulatus

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Introduction

Turkey's citrus industry is important for its economy. However, some factors like pests cause substantial losses. More than 80 species of citrus pests have been reported in Turkey (Uygun et al., California red scale (CRS), Aonidiella aurantii (Maskell), is the key pest in the Mediterranean 1992). Region of Turkey (Eronc, 1971; Uygun and Sekeroglu, 1981, 1984; Uygun et al., 1987, 1988). Different control methods have been used against it; however, chemical control is the most common method used by growers. As a result of chemical control, pest populations decrease initially, but after a short time reach high levels and continue being a problem. Pesticides also leave residues on the crops and damage the natural balance. Many studies on IPM related to keeping pest populations under economic threshold levels without eliminating the species from the agro-ecosystem and also to decrease environmental pollution, have been conducted (Uygun and Sekeroglu, 1981, 1984; Uygun et al., 1987, 1988, 1992; Anonymous, 1991). Biological control is an important part of IPM programs, and besides not causing pollution and not damaging human and wild life, prevention can be long term. Among many predators and parasitoids attacking CRS, Chilocorus bipustulatus (L.) is the most common natural enemy (Podoler and Henen, 1983; Uygun et al., 1992; Eliopoulos et al., 2010). Although it is commonly known that C. bipustulatus plays an important role in the biological control of scale insects, there have been limited laboratory and field studies that model biological control with this predator, both in Turkey and worldwide.

Temperature is the most important abiotic factor affecting the biology, ecology and populations of all living organisms (Kontodimas et al., 2004; Haghani et al., 2007). By studying the effects of this factor, the growth, ecology and future populations of organisms can be determined or estimated. Especially in biological control, knowing the responses of natural enemies to climatic conditions supplies useful knowledge on their adaptability and efficacy.

In this study, the growth of *C. bipustulatus* under different temperature regimes was investigated and its thermal thresholds and thermal constants were estimated. Thus, by using the present data, estimates of the future populations of this insect can be made for any time. In addition, data obtained from this study can be used for the development of simulation models for other natural enemies.

Materials and Methods

Rearing and experimental conditions

The stock of *A. nerii* used in this research was obtained from the cultures at the Plant Protection Department of the Süleyman Demirel University, Turkey. *A. nerii* individuals reared on potato sprouts (*Solanum tuberosum L.*) in rearing rooms adjusted to $25\pm1^{\circ}$ C, L:D 16:8 h photoperiod, and $65\pm5^{\circ}$ RH, in the Plant Protection Department of the Faculty of Agriculture, Süleyman Demirel University, were used as prey for the predator. Clean potato tubers in plastic trays were deliberately infested with crawlers of *A. nerii* to build up the population. This procedure was continued for the duration of the study.

The predator insect, *C. bipustulatus,* was originally collected from citrus orchards in Antalya in 2009. Adult individuals were transferred to culture boxes (30X40X15 cm) with potatoes infested with various stages of *A. nerii.* When the eggs of the predator were observed on *A. nerii* individuals, the adult predators were transferred to new boxes.

Climatic conditions were same as for the rooms used for the rearing of the prey. Experiments were carried out between 2009 and 2010.

Determination of the development periods of *Chilocorus bipustulatus* under different temperatures

In order to investigate the development periods of *C. bipustulatus* from egg to adult, under at five constant temperatures, newly hatched adults were used. They were transferred to culture bottles in controlled chambers at different temperatures. Adult individuals that developed from the eggs laid by these adults were used in the trial. They were transferred to bottles containing potatoes infested by *A. nerii* and cotton wool for their eggs. The cotton wool was checked for eggs everyday. The eggs were transferred to plastic bottles (20 cm in height by 10 cm diameter) containing potatoes infested with *A. nerii*. Eggs and hatched larvae were then examined every day and development times of eggs, larvae and pupae were recorded. Ten replicate bottles with 10 eggs in each were used in the trial.

All trials were performed in the laboratory under controlled conditions (14, 18, 22, 26, 30 and 34 \pm 1°C temperatures, 65 \pm 1% RH and L:D 16:8 h) in incubators (model KB 8400 F, Termax).

Statistical analysis

Data were submitted to analysis of variance (ANOVA) at P < 0.05 to examine the significance of the main effect (temperature) by using the Tukey-Kramer (HSD) test (Sokal and Rohlf, 1995). Statistical analyses were performed with the CurveExpertPro (version 1.6.3), and the SPSS (version 15.0) (SPSS 2006) statistical packages.

Models

Data were used to calculate minimum development temperature and thermal constant for the predator (Sharov, 2012; Pedigo and Zeiss, 1996). In addition, by using linear and nonlinear regression models, insect-temperature relationships were determined (Roy et al., 2002; Kontodimas et al., 2004; Herrera et al., 2005; Damos and Savopoulou-Soultani, 2008; Eliopoulos et al., 2010; Jalali et al., 2010).

Six models were applied to estimate the temperature-dependent development of *C. bipustulatus* on *A. nerii;* they are the most commonly used models for that purpose.

Linear model

The calculations for minimum development threshold and thermal constant of *C. bipustulatus* were based on the following linear model (Champbell et al., 1974; Roy et al., 2002; Kontodimas et al. 2004; Jalali et al., 2010).

y = a + bT

where \mathcal{Y} is development rate at temperature T and a and b are constants. The slope factor b is the slope of the function and b is the regression coefficient. a is the intercept and it can be calculated from the function. Estimation of constants was based on data obtained at 18, 22, 26, 30 degree temperatures only (Campbell et al., 1974).

The model was fitted using the Excel (M.S.) and SPSS (version 15.0) (SPSS 2006) softwares.

Minimum development temperature $(T_{\min} \square)$ was calculated from

$$T_{\min} \square = -\frac{a}{b}$$

The thermal constant (K) was calculated from $K = \frac{1}{b}$ (Champbell et al., 1974; Damos and Savopoulou-Soultani, 2008; Palyvos and Emmanouel., 2009)

Polynomial model

The polynomial regression model (4^{th} degree) was used to describe the nonlinear relationship between the development rates of *C. bipustulatus* from egg to adult by using all experimental data from all temperatures (14-34°C).

 $y = a + bT + cT^2 + dT^8 + eT^4$

where **a b c d** and **e** are empirical constants (Harcourt and Yee, 1982; Kuo et al., 2006; Sandhu et al., 2010).

 T_{\min} , T_{opt} and T_{\max} were also calculated by using polynomial regression.

These empirical constants are also used in nonlinear models.

Logan6 and Logan10 models

To estimate the optimum and maximum development temperatures of *C. bipustulatus,* the Logan 6 and Logan 10 models were used. A lower development threshold can not be estimated by using these two methods because they are asymptotic to the left of the temperature axis (Kontodimas et al., 2004).

Logan6 model

$$D(T) = \psi . (e^{(p.x)} - e^{(p.T_L - (T_L - x)/\Delta T)})$$

(Logan et al. 1976, Logan 1988, Gould and Elkinton 1990, Morales-Ramos and Cate 1993, Got et al. 1996, Briere and Pracros 1998, Briere et al. 1998, Hentz et al. 1998, Sigsgaard 2000, Tobin et al. 2001, Roy et al. 2002; Kontodimas et al. 2004; Palyvos and Emmanouel, 2009) where $\mathcal{D}(\mathcal{T})$ is the rate of development at temperature \mathcal{T} (°C) (days⁻¹), ψ is the maximum developmental rate, \mathcal{P} is a constant defining the rate at optimal temperature, $\mathcal{T}_{\mathcal{I}}$ is the lethal maximum temperature, and $\Delta \mathcal{T}$ is the temperature range over which physiological breakdown becomes the overriding influence (Roy et al., 2002).

Logan10 model

$$1/D = \alpha \left[1/(1+k.e^{-\rho.temp}) - e^{((t\max-temp)/\Delta)} \right]$$

(Logan et al. 1976)

where α , k and β are empirical constants, and temp is the rearing temperature (°C), the maximum temperature (°C), and ΔT is the temperature range over which physiological breakdown becomes the overriding influence (Roy et al., 2002).

The curves were fitted with nonlinear regression using the Marquardt algorithm in SPSS.

Lactin and Briere models

The Lactin and Briere models were used to estimate the minimum, optimum and maximum development temperatures of *C. bipustulatus* (Kontodimas et al., 2004).

Lactin model

$$D(T) = \mathbf{e}^{p.temp} - \mathbf{e}^{(p.t_m - \frac{t_m - temp}{\Delta})} + \lambda$$

where p, *temp*, *t_m*, Δ and λ are fitted coefficients (Lactin et al. 1995; Lactin and Johnson 1995; Brière and Pracros 1998; Royer et al. 1999; Muniz and Nombela 2001; Tobin et al. 2001; Roy et al. 2002; Kontodimas et al. 2004; Arbab et al. 2006, 2008; Jalali et al., 2010).

Briere model

$$\frac{1}{D}$$
 = a.temp.(temp-t_{min}). $\sqrt{t_{max} - temp}$

where $\lim \square$ is the lower threshold, $\lim \square$ the lethal temperature (upper threshold) and \square is an empirical constant (Brière et al. 1999; Roy et al. 2002; Kontodimas et al. 2004).

Results and Discussion

Data on temperature-dependent development of C. bipustulatus fed on A. nerii are provided in Table 1.

Table 1. Temperature-dependent development duration (days) of Chilocorus bipustulatus fed on Aspidiotus nerii

Biological	Temperature (°C)					
Stage	14	18	22	26	30	34
Egg	13.93 a	11.76 b	9.32 c	7.56 d	6.22 e	6.26 e
	(11-16)	(11-15)	(8-13)	(6-10)	(5-7)	(4-8)
L1	11.10 a	8.24 b	7.49 c	5.15 d	3.50 f	4.52 e
	(7-17)	(5-14)	(4-11)	(3-8)	(3-5)	(2-7)
L2	7.94 a	7.00 b	5.24 c	5.08 c	4.28 d	4.68 cd
	(3-13)	(4-13)	((3-10)	(3-7)	(2-9)	(2-8)
L3	9.25 a	8.42 b	6.05 c	5.41 d	3.71 e	4.06 e
	(4-15)	(6-13)	(3-11)	(2-11)	(2-6)	(2-7)
L4	15.80 a	8.42 b	7.81 b	5.12 c	3.56 e	4.40 d
	(11-29)	(5-13)	(4-13)	(2-8)	(2-9)	(2-7)
Pupa	13.93 a	12.70 b	12.55 b	8.07 c	6.92 d	7.90 c
	(9-17)	(10-20)	(7-17)	(5-10)	(5-10)	(4-16)
Total	61.67 a	51.00 b	41.21 c	33.72 d	26.76 e	27.17 e
	(56-65)	(46-63)	(35-46)	(31-36)	(22-35)	(25-31)

Means in the same row followed by a different letter are significantly different (Tukey-Kramer HSD test, P < 0.05).

The developmental time for all development stages of *C. bipustulatus* decreased with increasing temperature from 14 to 30°C (P<0.05) (Table 1). However, development time of larva 1st, larva 4th and pupal stages were longer at 34 °C than 30 °C (P<0.05). Significant differences in development time were observed among temperatures <34 °C for total immature development. *C. bipustulatus* successfully

completed development at all temperatures. Its longest development time was 61.67 days at 14 °C and shortest development time was 26.76 days at 30 °C (Table 1).

Podoler and Henen (1983) reported that the beetle completed its development from egg to adult in 80.5 days at 18 °C, 53.9 days at 22 °C, 38.2 days at 24 °C, 27.8 days at 26 °C, 27.8 days at 28 °C, 31.2 days at 30 °C and 27.1 days at 32 °C. In the present study, the predator completed its development in a shorter time.

The values of parameters of the models for the development rates of total immature stages are given in Table 2 and the curves for the development rate of *C. bipustulatus* from the models are presented in Fig. 1.

Parameters	Calculated values		
a	-0.00748 ± 0.003033		
b	0.00147 ± 0.000124		
С	5.91		
К	681.7		
R ²	0.986		
а	-0.15280 ± 0.107431		
b	0.03240 ± 0.019706		
С	-0.00230 ± 0.001309		
d	0.00007 ± 0.000037		
e	-0.00000 ± 0.00000		
t _{min}	10.00		
t _{opt}	32.00		
t _{max}	39.00		
R^2	0.997		
RSS	0.0001		
Ψ	0.502 ± 0.167		
р	0.110 ± 0.224		
t _{opt}	30.00 ± 0.003		
tmax	39.82 ± 0.606		
R^2	0.999		
RSS	0.0001		
α	-0.049 ± 0.002		
k	1.900 ± 0.000		
ρ	0.184 ± 0.000		
d 24.35 ± 1.067			
topt 38.00 ±			
t _{max}	39.00 ± 0.193		
R^2	0.934		
RSS	0.066		
α	0.00 ± 0.000		
tont	30.00 ± 0.000		
t _{max}	39.00 ± 0.000		
C	0.00		
R ²	0.999		
RSS	0.0001		
ρ	0.0013 ± 0.000		
Δ 0.9160 + 0.088			
λ	-1.003 ± 0.005		
topt	33.00		
tmay	39 01 + 0 016		
C	3 00		
~			
R^2	0 984		
	Parameters a b C K R ² a b c d e tmin topt tmax R ² RSS Ψ p topt tmax R ² RSS α k p d topt tmax R ² RSS α topt tmax R ² RSS α topt d topt tmax R ² RSS α topt Δ λ topt Δ λ topt Δ λ topt Δ		

Table 2. The parameters of development rate models (mean ± SE) of Cihilocorus bipustulatus fed on Aspidiotus nerii



Fig. 1. Relationship between development rate (1/d) of immature stages and temperature (°C) described by the linear, polynomial, Logan 6, Logan 10, Lactin and Briere models for *C. bipustulatus* (the minimum and maximum data values were omitted in Linear model).

For all models, R^2 was higher than 0.93 and RSSs were low. Temperature-dependent development models vary for different insect species (Sandhu et al., 2010) and higher values for R^2 and lower values for RSS reveal better fit (Aghdam et al. 2009; Jalali et al., 2010; Kontodimas, 2012).

Minimum temperature thresholds (C) were 5.91, 0.00 and 3.00 °C for the Linear, Briere and Lactin models, respectively. The Logan 6 and Logan 10 models were unable to estimate the minimum temperature threshold. The thermal constant was 651.1 degree-days for the beetle when using the linear model (Table 2, Fig. 1).

For the development thresholds and thermal constants of *C. bipustulatus*, there is limited information. Podoler and Henen (1983) and Eliopoulos et al. (2010) reported that the minimum temperature threshold of *C. bipustulatus* was 10.6 and 12.4 degrees, respectively. The thermal constant was reported as 558.92 and 474.7 degree-days by Podoler and Henen (1983) and Eliopoulos et al., (2010), respectively.

Our results indicate that the Antalya biotype of *C. bipustulatus* is much more tolerant of cool temperatures than those from Attiki in Greece (Eliopoulos et al. 2010) and Israel (Podoler and Henen, 1983).

Optimum and maximum temperatures (T_{max}) were calculated with the Polynomial, Logan6, Logan10, Briere and Lactin models as 32.0, 39.0; 30.0 39.0; 38.0, 39.0; 30.0, 39.0 and 33.0, 39.0 °C, respectively (Table 2, Fig. 1). Optimum and maximum temperatures for *C. bipustulatus* were 34.6 and 35.2 °C (Eliopoulos et al., 2010).

Differences in values between the current study and in the literature may have been due to the use of different biotypes of *C. bipustulatus*.

The Linear, Polynomial (4th), Lactin and Briere models are recommended for the description of temperature-dependent development of *C. bipustulatus*. Roy et al. (2002) reported that the Lactin model (*Tetranychus mcdanieli*) and the Briere model (*Stethorus punctillum*) were superior for estimating the low temperature threshold. The Linear and Lactin models are highly recommended for the description of temperature dependent development of the predators *Nephus includes* and *N. bisignatus* (Kontodimas et al., 2004). Furthermore, the linear model fitted well to the experimental data and should be capable of describing temperature dependent development of the coccinellid, *Adalia bipunctata* (Jalali et al., 2010). The Bieri-1 equation for the temperature-dependent development of *Nephus bisignatus* and *N. includes* was determined the most appropriate because it could estimate correctly all the requested parameters (T_{min}, T_{opt}, T_{max}) (Kontodimas, 2012).

According to Kontodimas et al. (2004), the linear equation not only fitted the experimental data very well, it was the easiest to calculate. Moreover, it is the only equation that allows the calculation of the thermal constant.

In conclusion, it was determined that not only the lowest but the highest temperatures were needed in the evaluations on insect development. The data also provide indirect evidence for the times when the predator is active. The results also indicate that this predator is active during the whole year in Antalya and surroundings. *Chilcorus bipustulatus* is an important predator of scale insects in Turkey (Uygun et. Al., 1992). The current study shows that this predator is able to survive at both low and high temperatures. Therefore, more detailed studies should be conducted on the influence of temperature and other ecological factors on this predator.

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