Temperature-Dependent Biological and Demographic Parameters of *Coleomegilla maculata* (Coleoptera: Coccinellidae)

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Abstract

Knowledge of the most suitable environmental conditions for an organism growth and development is a prerequisite for developing mass rearing technology. The temperature requirements for development and the optimal range of temperatures for growth and reproduction of *Coleomegilla maculata* De Geer were studied. The development time of individual *C. maculata* larvae was determined at 18, 21, 24, 27, 30, 33, and 36 °C. Development times were converted to development rates and fitted to a nonlinear temperature-dependent model and to the linear day-degree model. Life and fertility table analysis was used to determine the optimal temperature for population growth within a range of favorable temperatures including 24, 25, 26, 27, and 28 °C. Nonlinear estimates of thermal maximum (*Tm*), for the postembryonic development of *C. maculata* was 35.09 ± 10.35 °C. Estimation of *Tm* based on pupal development was much lower at 27.23 ± 1.52 °C. Linear and nonlinear estimates of low temperature development threshold were 13.13 ± and 4.77 ± 3.03 °C for the whole postembryonic development and 10.95 and 9.18 ± 1.36 °C for the pupal stage alone, respectively. The most favorable temperature for population growth was 25 °C, where *C. maculata* showed significantly higher intrinsic rate of increase (*rm* = 0.066) and significantly lower doubling time (10.57 d) than the other favorable temperatures tested. A negative value of *rm* was obtained at 28 °C, indicating population decline occurring at this temperature making it unfavorable for *C. maculata*.

Key words: pink spotted lady beetle, life cycle, rearing, life table, day-degree
Riddick et al. 2014h,c; Riddick and Wu 2015) and artificial diets (Rojas et al. 2016) have been done. However, standard mass production techniques have not been established and commercial production in the United States has been inconsistent.

Several important aspects of mass rearing C. maculata remain poorly studied, like the favorable physical environment factor including temperature, humidity and photoperiod. Schneider (2009) considered environmental factors as basic and highly important for the development of optimal mass rearing techniques of an insect species. Because insects are poikilothermic, temperature is a very important factor that can significantly impact the ability to culture successfully and mass produce an insect (Schneider 2009). Temperature impacts not only insect developmental rate (Logan et al. 1976) but also immature survival (IS) and fecundity (Schneider 2009). Many studies have focused on temperature as one of the main environmental factors impacting development and reproduction in many species of Coccinellidae (Rodriguez-Saona and Miller 1999, Canhilal et al. 2001, Schüder et al. 2004, Mota et al. 2008, Jalali et al. 2009, Statas et al. 2011). Obrycki and Tauber (1978) studied the effect of temperature on development of C. maculata and concluded that the optimal temperature for development was between 24 and 26.7°C. However, for improving mass rearing technology, more detailed studies are necessary on the temperature requirements and the effects of temperature on the life cycle of C. maculata.

The objectives of this study were to determine the temperature requirements for development of C. maculata, the effects of temperature on the life cycle and biological parameters of C. maculata, and the optimal range of temperatures for maximizing the fitness of C. maculata in culture.

Materials and Methods

Colony Origin and Rearing Procedures

The stock colony of C. maculata was initiated from field collections conducted in the spring and summer of 2010 in Stoneville, MS. This study was conducted during the spring of 2014 and at the time the stock had been in culture approximately 4 yr.

Adults were maintained in a two-section modular cage as described by Rojas et al. (2016). This design provides an upper space for feeding and a lower space for oviposition. Adult beetles were fed with a combination of food sources including Entofood (Koppert Biological Systems Inc., Howell, MI), ground bee pollen granules (product 2530, NOW Foods, Bloomingdale, IL), Lygus hesperus (Knight) (Heteroptera: Miridae) eggs, ground lyophilized Lygus hesperus with a combination of food sources including Entofood (Koppert LLC) paper to provide shelter space and increase surface area. Boxes were closed and sealed on the sides using laboratory labeling tape to prevent first instars from escaping. The stock colony of C. maculata was maintained in an environmental chamber at 26°C, 75% RH, and 14 h photophase.

Experimental Design

This study consisted of two experiments designed to identify a narrow range of the most favorable temperatures for growth, development and reproduction of C. maculata, based on the method used by Rojas et al. (2013) on Phytoecusius persimilis Athias-Henriot (Acari: Phytoseiidae). The first experiment consisted of determining the effects of temperature on immature development and survival of C. maculata within a wide range of temperatures (from 18 to 36°C) at 3°C intervals. The second experiment consisted of determining the effect of a narrow range of favorable temperatures (based on results from the first experiment) at 1°C intervals on the entire life cycle of C. maculata using life and fertility table analyses.

Temperature Requirements for Development. Coleomegilla maculata eggs from the stock colony were allowed to develop in an environmental chamber at 26 ± 1°C and 65 ± 8% RH. A total of 175 newly eclosed first instars, which were able to move in response to being contacted with a fine brush, were selected from the stock eggs. Selected first instars were randomly assigned to 1 of 7 groups of 25 larvae, which were individually placed inside tight-fit dishes (9 x 50 mm) (Falcon 35-1006, Becton Dickinson Co., Franklin Lakes, NJ) modified with a screened window (9-mm diameter) located at the top. The bottoms of the dishes were partially lined with tissue paper and saturated water crystals of cross-linked potassium polyacrylate and polyacrylamide copolymer (T-400, Terawet Inc., San Diego, CA) were provided as a source of water. Food consisted of a 1:1 mix of Entofood and ground bee pollen granules and was provided in detached caps of 1.5-ml centrifuge tubes (Fisherbrand 02-682-550, Fisher Scientific Co. LLC, Horizon Ridge, CT) to minimize microbial contamination from frass particles. Each of the 7 groups of 25 isolated larvae was assigned to a different temperature treatment (18, 21, 24, 27, 30, 33, and 36 ± 0.5°C). Each treatment group was placed in a different environmental chamber (Percival I30VLC8, Percival Scientific, Perry, IW), each set at the corresponding temperature treatment and all of them at a 80 ± 5% RH and 14 h photophase. The temperature inside each chamber was calibrated using certified thermometers submerged in ethylene glycol inside sealed bottles (Exact-Temp, I-030-1BLST, Thermo Scientific, Dubuque, IW). Relative humidity was controlled by the chambers and it was monitored by certified electronic hygrometers (Traceable®, 06-662-4, Control Company, Friendswood, TX). Dishes containing C. maculata larvae were not placed directly on the chamber trays, but instead were placed inside plastic boxes (110 x 110 x 35 mm) (Product 156C, Pioneer Plastics Inc.) to buffer temperature fluctuations.

Data Analysis. Larvae were monitored daily for molting events and mortality and the day of each molt was recorded for each larva. The duration of each instar was determined for each larva for all temperature treatments by counting the number of days between eclosion and adult emergence dates. Data were analyzed using analysis of variance and mean development time for each instar and pupa, as well as total development time, were compared among temperature treatments using the Tukey–Kramer HSD (Honestly Significant Difference) test using JMP version 11 software (SAS Institute 2013).

Data for individual postembryonic development time were converted to development rate as 1 per d. Development rates were
fitted to the Hilbert and Logan (1983) model using nonlinear regression analysis. The Hilbert and Logan (1983) model is defined as

\[
f(T) = \psi \left( \frac{(T - T_m)^2}{(T - T_b)^2 + D^2} - e^{-\frac{T_m - T_b}{D}} \right)
\]

where \(T\) is temperature, \(T_m\) is the thermal maximum, \(T_b\) is the lowest temperature at which development occurs (=temperature development threshold [LDT]) and \(T_a\), \(T_m\), \(AT\), \(D\), and \(\psi\) are parameters to be estimated by least squares nonlinear analysis. The least squares estimated value of \(T_m\) was used as a limit mark for viable temperature conditions for \(C.\ maculata\).

Development rates were also used to calculate the day-degree requirement for postembryonic development (K), which is also defined as the sum of day-degrees above the low LDT (Nedvěd and Honěk 2012). The day-degree model consists of fitting a linear regression model to the development rate versus temperature in using only data of “ecologically relevant temperatures” as defined by Nedvěd and Honěk (2012). The value of K was calculated as the reciprocal of the regression slope and the low LDT as the intercept divided by the slope of the regression model (Schneider 2009).

Most Favorable Temperature Range. The full life cycle of \(C.\ maculata\) was evaluated in five different temperature conditions at 1°C interval, which included 24, 25, 26, 27, and 28 ± 0.5°C. Originally, the experiment included a treatment for 23°C, but the fecundity data were lost for this temperature treatment due to an environmental chamber malfunction. RH of 65 ± 5% and a photoperiod of 14:10 (L:D) h were set for all treatments. Development time and IS were determined in groups of 24 newly eclosed first instars of the same age, which were reared in three-compartment clear boxes (170 × 84 × 42 mm) (product 182C, Pioneer Plastics Inc.), that had been modified by cutting a hole between each compartment and gluing the cover permanently. Screened windows were added to both short sides in addition to three detachable screened windows at the bottom using the same design as those on the covers of the cages for the stock colony (as described by Rojas et al. 2016). Each compartment was filled with 10 crumpled tissue paper squares (approximately 40 × 40 mm) to increase surface area and provide shelters for larvae.

Five groups were created for every temperature treatment. Groups were provided with the same food mix as described in “Temperature Requirements for Development”, and water was provided with saturated crystals of potassium polyacrylate and polyacrylamide copolymer placed in screened devices on the cover of the cage as described by Rojas et al. (2016). Groups were monitored daily, and food and water were supplied as needed until all larvae developed to the pupal stage. Pupae were monitored daily for adult emergence and the date of adult emergence, sex, and tenar adult weight were recorded. Development time was calculated as the number of days between eclosion and emergence dates for each individual because all larvae from each group had the same eclosion date at the beginning of the experiment. IS was calculated as the number of emerging adults divided by the initial number of first instars in each cage (n = 24). Teneral adults were also observed using a stereo microscope to detect malformations, especially those malformations that prevented adult beetles from walking (abnormal legs) or reproducing (genitalia). The number of adults displaying these type of malformations was recorded for each cage and temperature treatment. Adults with defective elytra, but able to walk and mate were counted as normal.

Twelve adult pairs from each temperature treatment were randomly selected and placed individually into clear plastic boxes (74 × 74 × 27 mm) (product 006c, Pioneer Plastics Inc.) modified with a screened window (22-mm diameter) on the cover. Adults were provided with food, water, and an oviposition substrate consisting of a crumpled tissue paper squares as described in “Temperature Requirements for Development” for the larvae. Pairs were monitored daily for oviposition and mortality for 100 d. Eggs were counted, cut from the tissue, placed in small petri dishes (55 diameter × 15 mm), recorded, labeled by oviposition date, and allowed to develop at the same conditions. Adults cannibalize eggs regularly, thus cannibalized eggs were identified and counted using the residues of shells glued to the oviposition substrate.

Data Analysis. Egg viability was calculated as hatched eggs per total eggs; IS was calculated as total adults emerging per first instars; fecundity as was determined as total eggs oviposited per female (including cannibalized eggs) for a period of 100 d; the rate of egg cannibalism was calculated as number of cannibalized eggs per total number of eggs oviposited. ANOVA was used to compare development time, adult weight, and fecundity. A single linear regression model was used to fit development time data versus temperature, which in this case included salvaged data from the 23°C treatment. The Z test was used to compare egg viability, rate of egg cannibalism, IS, and teneral adult mortality. Life and fertility tables from each of the five rearing temperature groups were calculated by substituting the initial values of \(x\) and \(l_x\) in the adult life table, with development time and the product of egg viability, IS, and teneral adult survival, respectively. Egg cannibalism was not taken into account to calculate the initial \(l_x\) in the fertility table analysis, because egg cannibalism may be an artifact resulting from the rearing conditions and could be reduced by a better oviposition system design.

The demographic parameters net reproductive rate (\(R_n\)), intrinsic rate of increase (\(r_m\)), generation time (\(G\)), and doubling time (\(DT\)) were calculated according to Carey (1993) using the modified spreadsheet of Portilla et al. (2014) as

\[
R_n = \sum_{x=0}^{w} l_x m_x
\]

\[
1 = \sum_{x=0}^{w} e^{-r_m x} l_x m_x
\]

\[
G = \sum_{x=0}^{w} x l_x m_x
\]

\[
DT = \frac{\ln(2)}{r_m}
\]

where \(x\) is the age class in days, \(w\) is the oldest surviving age, \(l_x\) is the female survival rate from egg to age \(x\), and \(m_x\) is mean female progeny per female occurring during age \(x\).

The jackknife method (Manly 1997, Efron and Tibshirani 1993) was used on demographic parameters to obtain 12 estimates by deletion and replacement of a given number of observed data points, as described by Maia et al. (2000) for statistical comparisons. The total number of females (NF) including those that did not complete development was calculated based on the number of live adult females (AF) used in the fertility table as AF/IS and the resulting number was rounded to 0 decimals. Surviving adult females were numbered from 1 to 12 and the rest of the nonsurviving individuals from 13 to the resulting number of AF/IS. During each deleting series, dead females that did not complete development were deleted from the life table, which impacted the survival to adult and surviving females
(1–12) were deleted from the fertility table, which impacted reproductive output estimates. The number of deletions per estimate was calculated as NF/12 and deletions were non-repetitive, meaning that a given female number was deleted only once and replaced in the rest of the estimates. Deletions were selected randomly using a random number generator. ANOVA was used to analyze the 12 estimates of demographic parameters per treatment and means were compared among treatments using the Tukey–Kramer HSD test.

**Results**

**Temperature Requirements for Development**

Development times of the postembryonic developmental stages of *C. maculata* at 7 different temperatures are presented in Table 1. Survival from first instar to adult was not significantly different among the assayed temperature treatments for 18–30°C and 0.64, 0.84, 0.84, 0.84, and 0.6 for 18, 21, 24, 27, and 30°C. Immature mortality was significantly higher (survival = 0.32) at 33°C compared to the cooler temperature treatments (|Z| > 1.96, P < 0.0488). Larvae of *C. maculata* did not complete development at 36°C, most of them died during the first stadium and the rest dying during the second stadium. No third instars were observed at 36°C.

Mortality of teneral adults due to defective molting was observed at 27°C; however, no significant difference in survival from first instar to reproductive adult was observed among temperature treatments 18–27°C. The incidence of adults molting defectively (miss-mols) increased with temperature resulting in a significant reduction of adult survival to the reproductive age at 30°C as compared to cooler temperatures (|Z| > 4.24, P < 0.0001). No reproductive adults were obtained at 33°C, where all emerging adults molted defectively, most of them dying during the teneral period. Based on immature and teneral adult survival, it seems *C. maculata* started showing signs of high-temperature stress at 27°C. However, development time started to show signs of impairment at 30°C, where development time of first, second, and third instars did not decrease significantly as compared to the next cooler temperature (27°C) (Table 1).

Hilbert and Logan (1983) model fit the developmental rates (1/d) versus temperature data (Table 2; Fig. 1). The estimate of a thermal maximum (*Tm*), differed substantially between total postembryonic development (35.09 ± 10.35°C) and the pupal stage (27.23 ± 1.52°C) of *C. maculata* (Table 2). The estimated value of the lowest temperature at which development occurs (parameter *Tb*) by the Hilbert and Logan model was 4.7°C for the total postembryonic development and 9.18°C for the pupal stage development (Table 2).

Ecologically relevant temperatures as defined by Nedvěd and Honěk (2012) included 18, 21, 24, 27, and 30°C. At 33°C, development time of *C. maculata* did not decrease significantly by showing signs of high temperature stress. The K value for the full postembryonic development of *C. maculata* was 217.13 day-degrees and the lower LDT was 13.1°C. Values of K were 160.93 and 55.01 day-degrees for larval and pupal development, respectively, and LDT values were 13.7 and 10.95°C for larval and pupal development, respectively. Values of LDT of full postembryonic development obtained by the day-degree linear model were higher than those estimated by the nonlinear model (TD = LDT) of Hilbert and Logan (1983) (13.1 and 4.77°C, respectively). However values were more closely similar when only pupal development was analyzed (10.95 and 9.18°C for the linear and nonlinear models, respectively).

**Most Favorable Temperature Range**

Egg viability was surprisingly low (below 12%) in all the treatments; however, IS was above 82% in all the temperatures (Table 3). Most of the temperature treatments did not significantly affect IS. Only at 26°C was IS significantly lower than at 24 and 27°C (Z > 1.96, N = 240, P < 0.001) (Table 3). Egg viability significantly decreased with the increase of temperature at 26, 27, and 28°C, and it was significantly lower at these temperatures than at 25°C (Z > 2.14, N = 4580, P < 0.001). Egg viability at 24°C did not differ significantly from that observed at 25 and 26°C (Table 3). Combined egg viability and IS ratios (egg viability × IS) was higher at 25°C (10.33%), followed by 24°C (9.62%), 26°C (8.17%), 27°C (3.7%), and 28°C (0.7%). Mortality of teneral adults due to defective molting increased with temperature and was significantly higher at 27 and 28°C as compared to that observed at 24 and 25°C (Z > 1.96, N = 210, P < 0.001) (Table 3). Egg cannibalism significantly increased with temperature (Z > 6.69, N = 9160, P < 0.001), except between the temperature treatments of 25 and 26°C, where there was no significant difference (Table 3).

Development time was linear and inversely correlated with temperature (*R² = 0.738; F = 1806; df1 = 1, df2 = 640; P < 0.0001) (parameters: α = 47.65 ± 0.72, β = −1.19 ± 0.28) as expected for a favorable range of temperatures. Fecundity as total eggs oviposited per female was not significantly affected by temperature and no significant differences were observed among temperature treatments (Table 4). There was significant difference in female weight among temperature treatments (F = 4.47, df1 = 4, df2 = 292, P = 0.0016), but the difference did not occur among all temperature treatments. Female weight was significantly higher at 25°C than at 27 and 28°C and was significantly lower at 27°C as compared with rearing temperatures of 24 and 25°C. No significant difference in female weight was observed among the other rearing temperatures (Table 4).

Although differences in IS and fecundity were small and not significant, respectively, among the narrower range of rearing temperatures tested in the second experiment (Tables 3 and 4), demographic parameters showed significant differences among temperature treatments (Table 5). Particularly the net reproductive rate (R₀), which was significantly different among all temperature treatments

**Table 1.** Mean duration of the postembryonic developmental stages of *C. maculata* at six different temperatures

| (°C) | N  | First instar | Second instar | Third instar | Fourth instar | Pupa | Total | Adult weight (mg) |
|------|----|--------------|---------------|--------------|---------------|------|-------|------------------|
| 18   | 16 | 9.63 ± 0.49a | 6.25 ± 0.42a  | 6.69 ± 0.2a  | 11.0 ± 0.2a   | 8.06 ± 0.06a | 41.63 ± 0.88a | 12.61 ± 0.34b   |
| 21   | 21 | 6.48 ± 0.22b | 4.29 ± 0.16b  | 4.57 ± 0.11b | 7.43 ± 0.13b  | 5.71 ± 0.1b  | 28.48 ± 0.3b  | 13.29 ± 0.25ab  |
| 24   | 21 | 5.0 ± 0.17c  | 3.24 ± 0.33c  | 3.19 ± 0.09c | 5.24 ± 0.1c   | 4.0 ± 0c     | 20.67 ± 0.38c | 14.41 ± 0.28a   |
| 27   | 21 | 3.24 ± 0.14d | 2.1 ± 0.12d   | 2.1 ± 0.07d  | 4.52 ± 0.12d  | 3.43 ± 0.11d | 15.38 ± 0.11d | 13.32 ± 0.39ab  |
| 30   | 15 | 3.07 ± 0.18d | 1.73 ± 0.12d  | 1.87 ± 0.09d | 3.2 ± 0.11e   | 3.0 ± 0e     | 12.87 ± 0.24e | 11.14 ± 0.36c   |
| 33   | 8  | 3.0 ± 0.5d   | 1.75 ± 0.25d  | 1.75 ± 0.16d | 3.23 ± 0.16e  | 2.88 ± 0.13e | 12.63 ± 0.71e | 9.61 ± 0.3c     |

Mean ± SEM. Means with the same letter are not significantly different after the Tukey–Kramer HSD test at α = 0.05.
$F = 355.71, \text{df}_1 = 4, \text{df}_2 = 55, P < 0.0001$) (Table 5). The highest $R_o$ value was observed at 25°C (23.11 ± 0.66) and the lowest at 28°C (0.94 ± 0.03) (Fig. 2). Because $R_o$ measures the mean net reproductive output per female, as reproducing female progeny per female, values of $R_o$ lower than 1 indicate a decline rather than growth of the population. In addition, at 28°C life and fertility table analysis yielded a negative value of intrinsic rate of increase ($r_m$) (Table 5), which also signals a population decline. At a temperature of 25°C, the $r_m$ value was significantly higher than that observed at 24, 27, and 28°C ($F = 1121.12, \text{df}_1 = 4, \text{df}_2 = 55, P < 0.0001$), which produced a DT value that was significantly shorter than that obtained at 24, 27, and 28°C ($F = 67.25, \text{df}_1 = 3, \text{df}_2 = 44, P < 0.0001$) (Table 5). The reproductive output ($R_o$) was significantly lower at 26°C than at 25°C; however, $r_m$ and DT values did not differ significantly between these two temperature conditions ($10.94 ± 0.17$ and $10.57 ± 0.11$ d, respectively) (Table 5). This may be the result of a significantly shorter generation time ($G$) occurring at 26°C ($52.32 ± 0.47$ d) as compared with that occurring at 25°C ($58.08 ± 0.37$ d) ($F = 278.3, \text{df}_1 = 4, \text{df}_2 = 55, P < 0.0001$).

### Discussion

Egg viability observed in this study was surprisingly low as compared to that observed in other coccinellids (Nedvéd and Honěk 2012). However, the levels of egg viability observed in this study at...
Table 3. Egg viability, egg cannibalism, immature survival, and teneral adult mortality of *C. maculata* at five different temperatures

| Temperature (°C) | Egg viability | Egg cannibalism | Immature survival | Teneral adult mortality |
|------------------|---------------|-----------------|-------------------|------------------------|
| 24               | 0.101 ± 0.006bc | 0.316 ± 0.007d | 0.925 ± 0.028a | 0.009 ± 0.009d |
| 25               | 0.117 ± 0.007ab | 0.457 ± 0.007c | 0.883 ± 0.029ab | 0.038 ± 0.019cd |
| 26               | 0.097 ± 0.006c  | 0.452 ± 0.007c | 0.842 ± 0.033b  | 0.059 ± 0.024bc |
| 27               | 0.077 ± 0.005d  | 0.563 ± 0.007b | 0.925 ± 0.024a  | 0.108 ± 0.030b |
| 28               | 0.008 ± 0.003e  | 0.634 ± 0.008a | 0.875 ± 0.03ab  | 0.210 ± 0.04a |

Proportion ± SE. Proportions with the same letter are not significantly different after the Z test at z/2 = 0.025.

Table 4. Development time, adult weight and fecundity of *C. maculata* at five different temperatures

| Temperature (°C) | Development time (d) | Adult weight (mg) | Fecundity (eggs/female) |
|------------------|----------------------|-------------------|-------------------------|
|                  | Female | Male | Female | Male |                  |                     |
| 24               | 18.86 ± 0.14a | 18.83 ± 0.22a | 15.56 ± 0.56ab | 13.02 ± 0.21ab | 434.3 ± 90.3a |
| 25               | 17.82 ± 0.15b | 17.68 ± 0.15b | 15.67 ± 0.25a  | 13.4 ± 0.17a  | 466.1 ± 96.3a |
| 26               | 17.3 ± 0.17b   | 17.3 ± 0.19b    | 14.72 ± 0.27abc | 13.59 ± 0.2a  | 459.3 ± 122.2a |
| 27               | 15.82 ± 0.11c  | 15.82 ± 0.14c   | 14.1 ± 0.25c   | 12.78 ± 0.24ab| 433.0 ± 110.4a|
| 28               | 13.72 ± 0.13d  | 13.57 ± 0.11d   | 14.3 ± 0.27bc  | 12.34 ± 0.2b  | 325.2 ± 83.6a |

Mean ± SEM. Means with the same letter are not significantly different after the Tukey–Kramer HSD test at α = 0.05.

Table 5. Means of demographic parameters obtained by the Jackknife method from life and fertility tables of *C. maculata* at five different temperatures

| Temperature (°C) | Demographic parameter | Ro | GD | T | r | m |
|------------------|------------------------|----|----|--|---|---|
| 24               |                        | 20.2 ± 0.56b | 57.96 ± 0.3a | 11.77 ± 0.13b | 0.059 ± 0.0006b |
| 25               |                        | 23.11 ± 0.66a| 58.08 ± 0.37a| 10.57 ± 0.11c | 0.0657 ± 0.0007a|
| 26               |                        | 17.67 ± 0.6c | 52.32 ± 0.47b| 10.95 ± 0.17bc| 0.0635 ± 0.0009a|
| 27               |                        | 7.73 ± 0.31d | 49.88 ± 0.27c| 14.92 ± 0.42a | 0.0468 ± 0.0011c|
| 28               |                        | 0.94 ± 0.03c | 44.6 ± 0.25d | –             | –0.0015 ± 0.0006d|

Mean ± SEM. Means with the same letter are not significantly different after the Tukey–Kramer HSD test at α = 0.05.

**Fig. 2.** Cumulative L*mx* values of *C. maculata* at five different temperatures. The total sums represent the values of net reproductive rate (Ro).
a temperature of 25 and 26°C were not different from those observed by Rojas et al. (2016) on a colony of the same origin and fed the same diet formulation as in our study designated as their control. Rojas et al. (2016) reports a threefold increase of egg viability of C. maculata when fed with an artificial diet containing 7% of Tenebrio molitor L powder, suggesting that nutrition may have play a role. It is possible that the diet mix used in this study is suboptimal causing embryos to die prematurely. However, microscope inspections of the unhatched eggs did not show any evidence of embryonic development and the great majority of the unhatched eggs retained their yellow coloration suggesting that they were not fertilized. Fertilized eggs change color within 2–3 d depending of temperature, but embryos can be observed inside earlier. It may also be possible that nutritional deficiencies affected males more than females impacting sperm production and viability. Also, large numbers of unfertilized eggs may suggest an adaptive response to high levels of egg cannibalism, which is common among Coccinellidae (Riddick and Chen 2014), or as a way to increase first instar survival by providing unfertilized eggs as food. Some coccinellids like Adalia bipunctata L, can complete larval development feeding exclusively on conspecific eggs (Dimetry 1974). Egg cannibalism allowed Harmonia axyridis (Pallas) larvae to complete development under low quality host availability (Snyder et al. 2000). An alternative explanation for the low egg viability observed is the presence of sublethal levels of phytochemicals such as flavonoids, alkaloids, tannins, and polyphenolics in diets using bee pollen (Roulston and Logan 1983, Lundgren and Wiedenmann 2004). There is also the possibility that the stock colony used in this study may have been too inbred causing low levels of egg viability; however, at the time of this study, the C. maculata colony had been in culture for <4 yr or approximately 11 generations. Colonies of C. maculata of different origins used in other studies have been in culture for similar number of generations showing much higher egg viability (Riddick et al. 2014b,c). In any case, the conclusions from this study were not impacted by this unusual level of egg viability because, even at the low levels observed, egg viability was significantly higher at 25°C than that at observed at the other temperatures (except at 24°C).

Temperature-dependent development rates obtained in this study fit the Hilbert and Logan (1983) model; however, this model provided different estimates of the thermal maximum (Tmax) and LDT (Tb) when the whole postembryonic development versus only pupal development were analyzed. These discrepancies may have been due to other factors impacting the larval stage, such as food and water, which may interact with temperature. The pupal stage shows a markedly lower variability in developmental time within each of the temperatures as compared with the total postembryonic development time. This is also reflected in the parameter estimates, which showed markedly lower standard errors when estimated from the pupal stage versus the whole postembryonic development. However, because the highest nonlethal temperature tested was 33°C, we must conclude that the Tmax for the total postembryonic development (at 35.09°C) is the most accurate to describe the high temperature limit of C. maculata. On the other hand, the linear and nonlinear estimates of LDT for pupal development had the closest values, suggesting that these estimates were closer to the real low temperature limit requirements for development of C. maculata.

Lactin et al. (1995) observed limitations of the original Logan model and made some modifications to correct it by reducing the number of parameters. However, Lactin et al. (1995) models did not provide a low-temperature threshold estimate as the Hilbert and Logan (1983) model did with the parameter Tb, which provides a nonlinear estimate of LDT. Zahiri et al. (2010) considered that variants of the Logan et al. (1976) model fit insect developmental rates more realistically than other models in the upper temperature ranges, and the Hilbert and Logan (1983) model ranked high (no. 2 fitting total development data) on the least square comparison of 23 temperature-dependent models, including two models from Lactin et al. (1995), fitting developmental rate data from Hypera postica (Gyllenhal).

The nonlinear estimate of LDT for C. maculata postembryonic development was 4.82°C, which is substantially lower than the linear estimate by the day degree model of 13.1°C. These differences were narrower in the pupal development estimates of LDT between linear (10.95°C) and non-linear (9.18°C) models. Although the nonlinear estimate of LDT is probably more realistic, the linear estimate provides a better base for comparison of C. maculata temperature requirements with other coccinellids species because of its common use (Nedvěd and Honěk 2012).

Temperatures of 24°, 25°, and 26° were favorable for C. maculata, but overall the most favorable temperature for reproduction was 25°C. This is consistent with the results obtained by Obrycki and Tauber (1978) indicating that the optimal temperature for development and survival of C. maculata was between 24 and 26.7°C. Although the C. maculata population experienced growth at 27°C, the net reproductive output at this temperature was notably diminished (Fig. 2). Demographic parameter values at 28°C indicated a slow population decline in C. maculata despite that IS and fecundity at this temperature were not significantly diminished respect to other, more favorable, temperatures. Life and fertility table analysis results of the narrow favorable temperatures tested, which shows that 28°C was unfavorable for C. maculata, seem to agree with the estimate of Tm obtained from the pupal developmental rate data fitted to the Hilbert and Logan (1983) model (27.23°C). Although larval development was not interrupted at 28°C (non-lethal), approximately 21% of adults showed crippling malformations and egg viability was only 0.8% at this temperature. A temperature of 28°C was not lethal for individuals, but it was lethal for the population as shown by the negative value of rm at this temperature.

This study is a good example of the value of life and fertility table analysis, providing a clear and conclusive demographic answer for the optimal temperature conditions for C. maculata. Results from the first experiment alone would have led to the inaccurate conclusion that a temperature of 27°C was favorable to C. maculata, based on IS and adult weight. Adding fecundity data would have not changed this erroneous conclusion, because fecundity at 27°C, as determined in the second experiment, was not significantly different than that observed at lower temperatures. The life and fertility table analysis provided a whole life cycle perspective showing that 27°C is in fact unfavorable for C. maculata and this may have been the result of combined impacts of temperature on all biological parameters (statistically significant or not). The most favorable temperatures for C. maculata were 25 and 26°C because the combined effect of higher egg viability and fecundity, and lower egg cannibalism and teneral adult mortality, resulted in a significantly higher intrinsic rate of increase and shorter DT at these two temperatures.

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