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Harmonia axyridis Pallas (Coleoptera: Coccinellidae): Biological aspects and thermal requirements

Tatiana de Oliveira Ramos[1*], Terezinha Monteiro dos Santos-Cividanes[2], Francisco Jorge Cividanes[1], Laís da Conceição dos Santos[1]

[1]Department of Plant Health, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, Brazil;
[*]Corresponding Author: tatiorbio@gmail.com
[2]Agência Paulista de Tecnologia dos Agronegócios (APTA), Regional Centro Leste, Ribeirão Preto, Brazil

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ABSTRACT

The aim of this study was to determine the biological aspects and thermal requirements of *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) fed daily with *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs. Under laboratory conditions, the experiment was carried out in acclimatized chambers set to 18˚C, 21˚C, 24˚C, 27˚C, and 30˚C. Larvae of *H. axyridis* were separated, kept in a glass tube, and fed with *A. kuehniella* eggs. The Asiatic lady beetle adults were separated into 20 couples and kept in plastic cups receiving the same food as the larvae. The length of the larval, pupal, and total biological cycle (from egg to adult) stages declined significantly from 18˚C to 27˚C, but was stable between 27˚C and 30˚C. Survival was similar for larvae from the first to the fourth stadium when kept at 18˚C, 21˚C, and 24˚C with a higher total biological cycle survival at 27˚C. *H. axyridis* males presented higher longevity at 18˚C and 21˚C in comparison to females that had a longer life and a higher fecundity at 24˚C and 27˚C, respectively. The lower thermal limit for biological cycle development was 12.4˚C, and the thermal constant was 243.9 degree-days.

KEYWORDS

***Anagasta kuehniella*; Biological Control; Degree-Days**

1. INTRODUCTION

Harmonia axyridis Pallas (Coleoptera: Coccinellidae), is found in several countries, in most of which it was introduced as a biological control agent of arthropod pests [1]. In Brazil, the species was initially observed in Curitiba, Paraná, in 2002. Later, it was reported in Ribeirão Preto and Piracicaba, São Paulo in citrus crops and okra and in Viçosa, Minas Gerais, in vegetables. Its distribution is also known to include the states of Rio Grande do Sul, Mato Grosso do Sul, Santa Catarina, and the Federal District [2-5].

The Asian ladybug needs a certain amount of thermal energy to complete its life cycle; in some regions, the length of this variable can be extended for up to 90 days, depending on humidity, photoperiod, and food type [6].

The larval period of the *H. axyridis* was 10.4 days when kept at 25˚C and fed *Myzus persicae* (Sulzer) and 17.9 days when fed *Cinara atlantica* (Wilson). The average life cycle of the species was 14.1 and 12.4 days at 27˚C when fed *Anagasta kuehniella* (Zeller) or adults of *Schizaphis graminum* (Rondani), respectively [7-9]. However, it is unlikely that changes in temperature affect the biological development of *H. axyridis*, because it has successfully reproduced in different climatic conditions [10].

Studies on the thermal requirements of the species enable the forecasting of population growth through the accumulation of temperatures that occur above the minimum developmental temperature [11]. *Harmonia axyridis* showed similar values for thermal requirements when fed the aphids *Dysaphis crataegi* (Kaltenbach) and *Aphis fabae* Scopoli maintained at temperatures of 15˚C, 20˚C, 25˚C, and 30˚C [12].

Only limited information is known about the effect of temperature on *H. axyridis*. Studies related to thermal requirements should be conducted in Brazil to obtain information about the potential of the species as a pest control agent. The aim of this study was to determine the biological and thermal requirements of *H. axyridis* fed on eggs of *A. kuehniella*.

2. MATERIALS AND METHODS

The study was developed at the Laboratory of Insect Ecology (LECOL), Department of Plant Health, Universidade Estadual Paulista/Faculdade de Ciências Agrárias e Veterinárias (Unesp/FCAV), Jaboticabal, São Paulo Stade. The third generation of *H. axyridis* eggs used in the experiment was obtained from our own laboratory rearing, kept in a climate-controlled room at 25˚C ± 1˚C with a 14-h photophase and relative humidity of 70% ± 5%. The coccinellid species was identified by Dr. Lúcia Massuti de Almeida from the Federal University of Paraná. The experiment began with 100 *H. axyridis* eggs kept in a 6.0 cm diameter and 2.0 cm high Petri dish placed in each of the evaluated temperatures of 18˚C, 21˚C, 24˚C, 27˚C, and 30˚C ± 1˚C with a 14-h photophase and 70% ± 5% relative humidity. In sequence, the larvae in the first stadium were separated in glass tubes (8.0 cm high × 2.5 cm diameter) and fed *A. kuehniella* eggs. Twenty-four hours after the stadium change, the larvae were weighed using a precision balance. Daily observations were made to determine the length and survival rate for each larval stadium, pupal phase, and total egg-adult period.

Adults were weighed immediately after emergence and were used to form couples that were kept in 250 mL transparent plastic cups (10.0 cm high × 7.0 cm diameter) sealed with voile cloth. Each couple was fed *A. kuehniella* eggs *ad libitum* and observed daily to evaluate the length of the pre-oviposition, oviposition, and post-oviposition periods; daily and total oviposition capability; longevity; and adult survival. The experimental design was completely randomized with 20 replicates held at constant temperatures of 18˚C, 21˚C, 24˚C, 27˚C, and 30˚C. The data were submitted to variance analysis, and the averages were compared with a Tukey test, with significance evaluated at a 5% probability by using the software AgroEstat and Assistat [13].

The results related to survival were arcsine transformed (P/100)1/2. The *H. axyridis* thermal requirements were determined by the hyperbolic method, by calculateing the lower developmental threshold (Tb) (in degrees Celsius) and the thermal constant (K) (in degree-days) by using linear regression between the development time and the corresponding temperatures of 18˚C, 21˚C, 24˚C, 27˚C, and 30˚C [14]. The time scale in degree-days was obtained as the daily sum of accumulated degree-days above the lower developmental threshold of *H. axyridis*.

3. RESULTS AND DISCUSSION

In general, the duration of the larval period of *H. axyridis* was influenced by temperature, ranging from 2.1 to 5.6 days at temperatures of 30˚C to 18˚C, respectively (**Table 1**). The average length of the egg stage at 24˚C was about 3.3 days, average of 2.8 days for the embryonic period of *H. axyridis* fed on *M. persicae* [8]. The first and second stadium lengths differed significantly among *H. axyridis* raised at 18˚C, 21˚C and 24˚C. However, larvae in the third and fourth stadiums showed a similar development period under 18˚C and 21˚C, while the development duration was the same at 27˚C and 30˚C in all stadiums. The stadium development time at 27˚C was close to that observed by [9]. They kept *H. axyridis* under the same thermal conditions and recorded 2.5, 1.7, 1.8, and 4.1 days for the length of the first, second, third, and fourth stadiums, respectively, using *A. kuehniella* eggs as a food source. On the other hand, the fourth stadium larvae had the longest development period. The longer larvae permanence in this stadium is probably related to the higher exploitation of important nutrients. These nutrients are important to transform the pupa into an emerged adult [15]. At 27˚C, *H. axyridis* completed its larval stage in 9.2 days, which differed from the results obtained by [9]. They found a length of 10.2 days for the species kept at 27˚C and receiving the same food source.

The average duration of the pupa stage at 27˚C (**Table 1**) was similar to the 3.9 days found by research using the aphid *S. graminum* as food for *H. axyridis* at 27˚C [9]. In this study, increased temperatures were found to reduce the time of development of the pupal stage. This characteristic is important because it allows one to obtain a larger number of individuals of the species in a smaller period of time under laboratory conditions to be used in

Table 1. Mean duration (days) of the developmental phases of *H. axyridis* fed eggs of *A. kuehniella*. Temp.: 18˚C, 21˚C, 24˚C, 27˚C, 30˚C ± 1˚C; RH: 70% ± 5%; and photophase: 14 h.

T (˚C)	Egg	1st	2nd	3rd	4th	Larval	Pupal	Egg-adult
18	[a]5.6 ± 0.10a	6.5 ± 0.23a	4.7 ± 0.32a	4.4 ± 0.23a	8.2 ± 0.31a	23.8 ± 0.69a	10.3 ± 0.18a	39.3 ± 3.7a
21	4.5 ± 0.10b	4.5 ± 0.14b	3.7 ± 0.11b	4.1 ± 0.12a	8.4 ± 0.17a	20.7 ± 0.37b	7.2 ± 0.07b	31.1 ± 1.5a
24	3.3 ± 0.07c	3.5 ± 0.09c	2.8 ± 0.09c	3.0 ± 0.10b	5.3 ± 0.07b	14.6 ± 0.24c	5.3 ± 0.06c	23.1 ± 1.2b
27	2.5 ± 0.07d	2.2 ± 0.07d	1.4 ± 0.09d	1.9 ± 0.10c	3.7 ± 0.11c	9.2 ± 0.12d	3.9 ± 0.06d	15.9 ± 0.8c
30	2.1 ± 0.07e	2.0 ± 0.03d	1.3 ± 0.08d	1.8 ± 0.09c	4.2 ± 0.10c	9.3 ± 0.16d	4.0 ± 0.00d	15.2 ± 0.8c

[a]Averages (±deviation) followed by the same letters in columns did not differ by the Tukey test at 5% probability ($p > 0.05$).

biological control programs [16].

The biological cycle (egg-adult length) of *H. axyridis* was inversely proportional in the temperatures evaluated. The periods at 18˚C and 21˚C were significantly longer than at 24˚C, 27˚C, and 30˚C. Research keeping *H. axyridis* at 25˚C and using *M. persicae* as food, observed that the species completed its development in 19.8 days, a value greater than the obtained in this research, probably due to nutritional differences in the foods used in the studies [8].

The *H. axyridis* egg viability at 18˚C and 27˚C was higher than that at 30˚C, it was lower than that at 21˚C and 24˚C, but not significantly (**Table 2**). The survivals of the first and second stadium larvae were similar when they were submitted to different temperatures, while first and fourth stadium larvae presented lower surviving at 30˚C. Value did not differ from the 18˚C to 24˚C temperature range but it was higher at 27˚C. This shows that first stadium larvae, which are newly hatched, and fourth stadium larvae, which will become pupae, were more sensitive to temperature than second and third stadium larvae. This may indicate that second and third stadium larvae have a stronger potential to colonize habitats under different climatic conditions.

There were no significant differences in larval survival at different temperatures; however, pupae that had been exposed to 30˚C had lower adult emergence. The observed survival for the complete biological cycle (egg to adult) of *H. axyridis* was similar at 18˚C and 24˚C, higher at 27˚C, and lower at 30˚C. Research using the sorghum aphid *S. graminum* as food had a survival rate of 86.7% at 24˚C, superior to what was found in this study [9].

Harmonia axyridis had a shorter biological cycle length (**Table 1**) and females had a shorter pre-oviposition period at 27˚C and 30˚C; however, temperatures of 30˚C had the undesirable effect of reducing the oviposition period (**Table 3**). An important observation was that the oviposition period was longer at 18˚C and 24˚C. However, females were more fertile at 27˚C. The reproductive investment in insects is associated with the biologic cycle survival. In this study, the opposite was observed. At 27˚C, *H. axyridis* laid a higher number of eggs and had a higher biological cycle survival rate (**Table 2**) [17].

Even though temperatures of 27˚C and 30˚C reduced the development period in the biological cycle (**Table 1**), under the same thermal conditions, males and females had a shorter lifespan. *H. axyridis* males had higher longevity at 18˚C and 21˚C, while females had higher longevity at 18˚C and 24˚C, surviving for an average of 248.3 days. From 18˚C to 24˚C, female longevity was 3 times higher than that observed to *H. axyridis* females fed *C. atlantica* at 24˚C (85.6 days) [9]. The authors highlighted that coccinellidae longevity is highly variable, ranging from a few months up to 3 years.

The temperature also influences the development time of insects, which affects the weight of larvae [1]. In general, *H. axyridis* larvae in the second, third, and fourth instars had higher weights at 27˚C, while adults had similar weights at all temperatures **(Table 4)**. The weights of *H. axyridis* larvae and adults at 30˚C were not included because there was low survival in this treatment. At all temperatures, the weight increased with each stadium change up to the adult phase [18].

Table 2. Mean survival (%) for larvae, pupae, and the total biological cycle and egg viability of *H. axyridis* fed *A. kuehniella*. Temp.: 18˚C, 21˚C, 24˚C, 27˚C, 30˚C ± 27˚C, 30˚C ± 1˚C; RH: 70% ± 5%; and photophase: 14 h.

T (˚C)	Egg	1st	2nd	3rd	4th	Larval	Pupal	Egg-Adult
18	[a]82.0 ± 3.8a	89.0 ± 3.4bc	98.0 ± 2.0a	99.0 ±1.0a	71.0 ± 3.9bc	72.6 ± 18.9b	70.0 ± 3.6bc	61.2 ± 4.1ab
21	68.7 ± 6.8ab	86.9 ± 5.8bc	100.0 ± 0.0a	100.0 ± 0.0a	58.7 ± 7.1bc	76.2 ± 32.0b	58.7 ± 7.1bc	43.0 ± 6.6bc
24	74.7 ± 6.8ab	90.5 ± 4.4bc	100.0 ± 0.0a	98.6 ± 1.3a	64.2 ± 6.2bc	86.5 ± 22.2a	64.2 ± 6.2bc	52.3 ± 3.4ab
27	83.0 ± 4.1a	94.5 ± 2.2a	98.7 ± 1.2a	97.3 ± 1.9a	77.0 ± 3.9a	76.7 ± 20.7b	76.0 ± 4.0a	63.7 ± 3.4a
30	65.0 ± 8.2b	72.7 ± 7.8c	100.0 ± 0.0a	100.0 ± 0.0a	46.6 ± 7.1c	58.2 ± 27.4c	46.6 ± 7.1c	28.1 ± 7.0c

[a]Averages (±deviation) followed by the same letters in columns did not differ by the Tukey test at 5% probability ($p > 0.01$). At each temperature (treatment), there were 100 eggs. Survival data were transformed by arcsine $(P/100)^{1/2}$.

Table 3. Mean duration (days) of the pre-oviposition, oviposition, and post-oviposition periods and longevity (days) of *H. axyridis* fed *A. kuehniella*. Temp.: 18˚C, 21˚C, 24˚C, 27˚C, 30˚C ± 1˚C; RH: 70% ± 5%; and photophase: 14 h.

T (˚C)	Pre-oviposition	Oviposition period	Post-oviposition	Total eggs	Daily eggs	Longevity (Male)	Longevity (Female)
18	[a]88.7 ± 15.6ab	158.4 ± 18.4a	13.8 ± 2.2ab	806.0 ± 78.4c	8.0 ± 1.2b	221.7 ± 11.7a	242.2 ± 16.9a
21	116.7 ± 7.9a	117.1 ± 7.4ab	24.9 ± 3.6a	967.9 ± 102.9c	8.8 ± 0.9b	185.0 ± 10.6ab	244.9 ± 13.2a
24	74.0 ± 7.5b	157.9 ± 11.6a	14.2 ± 3.5ab	1.430.2 ± 194.1bc	8.5 ± 0.9b	200.7 ± 9.8a	259.4 ± 10.1a
27	11.7 ± 1.0c	143.4 ± 12.3ab	11.5 ± 1.0 6b	2.642.4 ± 210.7a	21.2 ± 2.0a	103.1 ± 10.1b	154.7 ± 10.1b
30	14.3 ± 1.3c	96.4 ± 6.8b	14.3 ± 2.8ab	1.685.2 ± 209.6b	18.0 ± 1.9a	115.4 ± 8.3b	140.6 ± 7.01b

[a]Averages (±deviation) followed by the same letters in columns did not differ by the Tukey test at 5% probability.

Table 4. Mean weights (mg) of the second, third, and fourth stadium larvae and adults of *H. axyridis* fed *A. kuehniella*. Temp.: 18˚C 21˚C, 24˚C, 27˚C ± 1˚C; RH: 70% ± 5%; and photophase: 14 h.

T (˚C)	2nd stadium	3rd stadium	4th stadium	Adult
18	[a]1.5 ± 0.10c	5.3 ± 0.44b	16.2 ± 2.3bc	23.8 ± 1.5a
21	1.8 ± 0.13bc	5.0 ± 0.37b	12.0 ± 0.71c	24.8 ± 1.2a
24	2.0 ± 0.14b	7.2 ± 0.46a	20.1 ± 1.6b	27.6 ± 1.2a
27	2.8 ± 0.08a	8.4 ± 0.15a	26.1 ± 1.6a	29.6 ± 2.5a

[a]Averages (± deviation) followed by the same letters in columns did not differ by the Tukey test at 5% probability. Data represent the average of 15 replications ± deviation. The weights of coccinellidae kept at 30˚C were not included in the calculations

The *H. axyridis* fourth stadium, pupal period, and biologic cycle development length values at 30˚C were not included in calculating the thermal demand because, under this temperature, the development time was longer, differing from the other observations of the relationship between the development rate and other temperatures. It has been recommended that development velocity values be rejected when there is not a linear relationship between values [19].

The values of eggs and the total biological cycle (**Table 5**) determined in our study are close to those determined for the coccinellidae *Coleomegilla maculata* (DeGeer) and *Chilocorus bipustulatus* Linnaeus with values for Tb and K for the egg stage at 11.0˚C and 11.3˚C and from 40.2 to 123.6 degree-days and for the biological cycle values varying from 11.3˚C to 11.4˚C and 101.0 to 235.8 degree-days [20,21]. However, the Tb and K obtained for *H. axyridis* in this study differed from other values for *H. axyridis* in France (Tb = 10.5˚C and K = 231.3 degree-days), in the United States (Tb = 11.2˚C and K = 267.3 degree-days), and in Denmark (Tb = 10.5˚C and K = 231.0 degree-days) [1,5,6,8-10,12,17,18,21,22]. As explained before, this difference among Tb and K values might be explained by the foods used and by the different biotypes existing in the regions where the studies were carried out, considering that the climatic conditions of a region are reflected in the insects thermal requirement values [1,4-10,12,14,17-22]. The results of this study show that the temperature of 27˚C is more favorable to *H. axyridis*, providing a faster development and a higher fecundity. *H. axyridis* has favorable development at different temperatures when fed on *A. kuehniella* eggs. The temperature of 30˚C lengthens the development time for the larval and pupal stages and reduces survival by up to 54%. The low development thermal limit values as well as the biologic cycle thermal constant suggest that *H. axyridis* should be able to develop and establish itself in different regions of Brazil.

Table 5. Lower developmental thermal limit (Tb), thermal constant (K), and determination coefficient (R^2) for the different developmental stages and the total biological cycle of *H. axyridis* fed *A. kuehniella*. Temp.: 18˚C, 21˚C, 24˚C, 27˚C, 30˚C ± 1˚C; RH: 70% ± 5%; and photophase: 14 h.

Phase	Tb (˚C)	[a]K (GD)	[b]Equation (1/D)	R^2
Egg	11.3	40.7	Y = −0.279756 + 0.02455x	99.3
1st instar	13.2	33.6	Y = −0.395167 + 0.02972x	97.9
2nd instar	14.5	19.9	Y = −0.728590 + 0.05006x	93.9
3rd instar	10.9	34.8	Y = −0.314516 + 0.02828x	90.5
4th instar	11.4	63.8	Y = −0.179831 + 0.01565x	90.0
Larva	12.0	156.4	Y = −0.077115 + 0.00639x	93.2
Pupa	12.8	55.9	Y = −0.230340 + 0.01788x	98.0
Egg-adult	12.4	243.9	Y = −0.000511 + 0.00410x	93.0

[a]DD: degree-days; [b]1/D: development speed equation; D: development length (days).

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