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# Effects of Temperature on Development and Survival of Harmonia axyridis (Coleoptera: Coccinellidae) and Chrysoperla externa (Neuroptera: Chrysopidae) Fed on Rhopalosiphum padi (Hemiptera: Aphididae)

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# EFFECTS OF TEMPERATURE ON DEVELOPMENT AND SURVIVAL OF *HARMONIA AXYRIDIS* (COLEOPTERA: COCCINELLIDAE) AND *CHRYSOPERLA EXTERNA* (NEUROPTERA: CHRYSOPIDAE) FED ON *RHOPALOSIPHUM PADI* (HEMIPTERA: APHIDIDAE)

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## ABSTRACT

The effects of various temperatures on the development and survival of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) and *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) fed on *Rhopalosiphum padi* (Linnaeus) (Hemiptera: Aphididae) were studied, and some biological aspects among these predators were compared. On average, 70 nymphs of *R. padi* were added to each experimental unit with predator larvae and maintained at 12, 16, 20, 24, 28, or 32 °C. The duration (days) and survival (%) were measured for each immature stage of the two predator species. The developmental durations of all *H. axyridis* larval instars, the total larval stage, the pupal stage and total preimaginal stage were very long at 16 °C but shortest at 24 °C for the first, second and third larval instars and at 28 ºC for the fourth larval instar, the total larval stage and the total pre-imaginal stage. Both at 24 °C and 28 °C, 92% of *H. axyridis* completed the larva to adult cycle, whereas survival was significantly reduced in the 16 to 20 °C range. At the highest temperature (32 °C), 70% of *H. axyridis* did not reach adulthood. In the case of *C. externa*, the durations of development of all larval instars and the total larval stage became progressively shorter as the temperature increased from 12 to 20 °C. At still higher temperatures the developmental duration this insect remained almost constant. The predator completed the larval stage at all temperatures from 12 to 32 °C. However, at 12 and 16 °C, mortality of the larvae was greater than 88%, and 100% of the pupae perished. Although the lower threshold temperatures estimated for both predator species were very similar, the development period from larva to adult of *H. axyridis* was only 59% as long as that of *C. externa*. By comparing certain biological parameters including durations of development and survival rates of the two predator species, it is suggested that *H. axyridis* has intrinsic advantages over *C. externa;* however, additional factors must be considered when choosing the species of predator for biological control of *R. padi* on grass pastures in Brazil.

Key Words: predators, aphids, forage, temperature

## RESUMO

O efeito de várias temperaturas sobre o desenvolvimento e sobrevivência de *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) e *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) alimentados de *Rhopalosiphum padi* (Linnaeus) (Hemiptera: Aphididae) foram estudados, assim, como alguns aspectos biológicos entre esses predadores foram comparados. Em média, 70 ninfas de *R. padi* foi adicionadas em cada unidade experimental, contendo uma larva do predador e foram mantidas à 12, 16, 20, 24, 28, 32 ºC. Avaliou-se a duração (dias) e a sobrevivência (%) de cada instar larval, estágio larval total, estágio pré-pupal, estágio pupal e estágio pré-imaginal dos predadores. A duração de *H. axyridis* foi maior a 16 ºC e reduziu significativamente a 24 ºC (do primeiro ao terceiro ínstar) e a 28 ºC (quarto ínstar larval, estágio larval total e estágio pré-imaginal). A 24 °C e 28 °C, 92% dos insetos completaram o ciclo (larva a adulto) havendo redução significativa a 16 e 20 °C. Na maior temperatura estudada (32 °C), 70% desse predador não atingiram a idade adulta. Para *C. externa*, verificou-se redução na duração com incremento da temperatura de 12 à 20°C para todos instares larvais e estágio larval total desse predador. Em temperaturas mais elevadas a velocidade de desenvolvimento do inseto estabilizou-se. O predador completou o estágio larval total em todas as temperaturas testadas. No entanto, houve mortalidade acima de 88% e de 100% das larvas e pupas, respectivamente, á 12 e 16ºC. Foi verificado que apesar da temperatura base estimada para ambas espécies de predadores terem sido próximas, o período de desenvolvimento de larva a adulto de *H. axyridis* corresponde a 59% daquele de *C. externa*. Pela comparação dos parâmetros biológicos, duração e sobrevivência, sugere-se que *H. axyridis* teve vantagens intrínsecas sobre *C. externa*, porém, outros fatores devem

ser considerados na escolha da espécie do predador para o controle biológico de *R. padi* em forrageiras no Brasil.

Palavras-Chave: predador, afídeo, forragem, temperatura

The aphid *Rhopalosiphum padi* (Linnaeus) (Hemiptera: Aphididae) is highly polyphagous (Blackman & Eastop 2000), and transmits viruses to grass (Poaceae) species (Hutchinson & Bale 1994). In recent years, it has been found associated with *Brachiaria* spp. (Poales: Poaceae) in Brazil, causing reduction in plant quality (Auad et al. 2009). Thus, management strategies for this species are needed, and a promising strategy is biological control, since there is no record of effective use of insecticides against this aphid on *Brachiaria* sp. with the Ministry of Agriculture, Livestock and Supply (MAPA), Brazil.

Research findings with predatory coccinellids and lacewings highlight the potential of these agents to control aphids in various crops (Auad et al. 2003; Maia et al. 2004; Tsaganou et al. 2004; Lee & Kang 2004; Barbosa et al. 2008; Yu et al. 2013), which suggests their use for control of *R. padi*, given the natural occurrence of predators and prey in pasture (Auad et al. 2009). Among predators, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) and *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) are used in augmentative and conservation biological control programs in several crop cultures in various regions of the world (Albuquerque et al. 1994; Koch 2003). In pasture systems there is a rich insect fauna, which promotes the conservation of these predators, resulting in reduced population density of *R. padi* in the early stages of colonization. However, several abiotic factors interfere with the actions of these natural enemies. Among these factors, temperature acts directly on the population dynamics of insects (Huffaker et al. 1999), each species has its own heat requirement, which determines how the environment affects its population growth (Salvadori & Parra 1990).

The efficiency of a predator should be evaluated based on its development and mortality at different temperatures before fieldwork is conducted to evaluate its potential against a pest in a given cropping system (Cardoso & Lázzari 2003). Besides temperature, the development of predators is affected by prey quality (Canard & Principi 1984; Frazer 1988; Venzon & Carvalho 1993; Canard 1997). Therefore research is needed on the effectiveness of *R. padi* as prey of *H. axyridis* and *C. externa* at different temperatures; and this should include the determination of the critical temperature range for development of these predators as a basis for determining whether field temperatures are optimal for development of *R. padi*; which is an important condition for success-

ful biological control. Thus, the objective was to study the effect of different temperatures on the development and survival of *H. axyridis* and *C. externa* fed on *R. padi* as well as comparing some biological aspects among these predators.

## MATERIAL AND METHODS

### Collection and Maintenance of Prey and Predators

A culture of *R. padi* was obtained from a greenhouse at Embrapa Gado de Leite, Brazil and reared following the methodology of Auad et al. (2009). Cultures were maintained in growth chambers at $25 \pm 1$ °C, $70 \pm 10\%$ RH and 12:12 h L:D.

*Chrysoperla externa* and *H. axyridis* adults were collected from the experimental field of Embrapa Gado de Leite, Brazil, and placed in PVC rearing units (10 cm diam × 10 cm height) with their ends sealed with organza and the inside coated by white paper (*C. externa*) or strips of ribbed paper (*H. axyridis*), which served as substrates for oviposition. These rearing units were kept under the same climatic conditions as their prey. The eggs of both predator species (*C. externa and H. axyridis*) were removed from the oviposition substrate, transferred to Petri dishes (2.5 cm diam and 2.5 cm height) and maintained until hatching.

### Experiment

Each experimental unit was comprised of a Petri dish (2.5 cm diam × 2.5 cm height) containing one foliar disc of *Brachiaria ruziziensis* placed on a 1.0 cm thick layer of 1% agar in order to keep the leaf disc turgid. Subsequently, on average 70 nymphs of *R. padi* were added to each plate. Thirty and sixty larvae of *C. externa* and *H. axyridis*, respectively, up to 18 h of age were placed individually on these plates and fed ad libitum on *R. padi*, which were replenished daily. The experimental units were kept in growth chambers ($70 \pm 10\%$ and 12 h photophase) at 12, 16, 20, 24, 28, 32 °C and the lowest temperature used only for *C. externa*.

To evaluate the comparative biology between the 2 predator species only data within the range of 20 to 32 °C were compared. A completely randomized design with 30 and 60 repetitions for *C. externa* and *H. axyridis,* respectively, was used.

The duration (days) and survival (%) of each larval instar, the total larval stage, pre-pupal,

pupal and pre-imaginal stages of predators were evaluated daily. The results of duration for predators at different temperatures were subjected to regression analysis. The survival data (%) were submitted to analysis of variance (ANOVA) and means were compared by the Scott Knott test ($P < 0.05$). The same analyses were performed to compare the duration and survival among predators.

Data on duration of development of different instars, the total larval stage, the total pupal stage and the total pre-imaginal stage were subjected to regression analysis. The lower threshold temperature (LTT) (°C) and thermal constant (K) in degree days (DD) were estimated based on a hyperbolic relationship, according to the methodology proposed by Bean (1961).

## RESULTS

Development Stages of *Harmonia axyridis* at Various Temperatures

The regression curves for the durations of development stages of *H. axyridis* follow a quadratic trend with high correlation of determination ($R^2$); that is, as the temperature increases, the duration of developmental stages declines according to the adjusted model (Fig. 1).

From the first to third larval instar, the duration was extended at 16 °C and reduced on average 3.5 times at 24 °C. However, the durations of development of the first and third larval instars were very similar at 24, 28 and 32 °C. Also the durations of development of the second larval instar at 28 and 32 °C were similar. In the 4th larval instar, the total larval stage and the total pre-imaginal stage, the durations of development gradually decreased as the temperature increase up to 28 °C, but then increased as the temperature rose to 32 °C (Fig. 1).

Pre-pupal duration was about 1 day, irrespective of temperature (data not shown). However, the temperature was inversely proportional to the duration of the pupal stage (Fig. 1).

The first-instar larvae showed sensitivity to high temperatures with somewhat reduced survival at 32 °C that was statistically significant ($F = 3.11, P = 0.032$). The temperatures studied (16 to 32 °C) did not significantly influence the survival of the second ($F = 2.396, P = 0.07$), nor the third ($F = 1.66, P = 0.18$) and fourth larval instars ($F = 0.88, P = 0.48$) (Table 1).

There were significant reductions in survival of the total larval stage ($F = 6.008, P = 0.001$), and the pre-pupal ($F = 2.59, P = 0.06$) and pupal ($F = 4.74, P = 0.005$) stages at 32 °C compared to lower temperatures, at which the survival of *H. axyridis* was above 75% to 93% (Table 1). At 24 °C and 28 °C, 92% of the insects completed the larva to adult cycle, percent survival was significant reduced ($F = 18.35, P = 0.0001$) in the 16 to 20 °C range. At the highest temperature (32 °C) 70% of predators failed to reach adulthood (Table 1).

The lower threshold temperatures (LTT) (Table 2) of *H. axyridis* were 13.51 and 10.22 °C for the second and fourth instars, respectively, which implies that the fourth instar can continue developing when low temperatures prevent development of the second instars. The LTT and thermal constant (K) (i.e., number of degree days required to complete the total larval stage) are greater (LTT = 11.58 °C, k = 135.13 DD) than those of the total pupal stage (LTT = 5.30 °C, K = 80.64 DD). The number of degree days required to complete the biological cycle (larva to adult) was 227.27 for *H. axyridis*.

Developmental Stages of *Chrysoperla externa* at Various Temperatures

The regression curves for the durations of development of the various larval instars and other developmental stages of *C. externa* each followed a quadratic trend with a high coefficient of determination ($R^2$) (Fig. 2). There were strong reductions in the durations of development of all larval instars as the temperature increased from 12 to 20 °C. Above this temperature range the trend line reached a point of inflection. At 32 °C the durations of the first, second, third larval instars and total larval stage of *C. externa* were reduced by 15.3, 19.4, 24.6 and 54.4 days, respectively, compared to those at 12 °C (Fig. 2).

Only larvae kept at temperatures between 20-32 °C reached the pupal stage. The duration of the pre-imaginal stage at 32 °C was only 15.26 days compared to 31.97 days at 20 °C (Fig. 2).

Survival rates of first instar larvae of *C. externa* were reduced significantly ($F = 3.885, P = 0.005$) at 12 °C and 28 °C compared to other temperatures. The survival rates of the second ($F = 13.276, P = 0.001$) and third larval instars ($F = 15.235, P = 0.001$) were significantly reduced at 12 and 16 °C compared to highest temperatures (Table 1). *Chrysoperla externa* completed its larval stages at all temperatures tested. However, there was greater than 88 % mortality of the larvae subjected to 12 and 16 °C. Furthermore, the duration of the total larval stage was significantly reduced ($F = 47.39, P = 0.001$) at 20, 28 and 32 °C compared to 24 °C (Table 1). At the lowest temperatures (12 and 16 °C) the insects failed to grow to the adult stage, because there was 100% pupal mortality. At the other temperatures, survival rates of the pupal stage were not significantly different ($F = 1.85, P = 0.164$) (Table 1). The life cycle from larva to adult of *C. externa* was completed only in the interval 20-32 °C, and with the greatest survival rates of the total pre-imaginal stage at 20 °C and 24 °C ($F = 6.44, P = 0.0004$) (Table 1).

The LTT in the different instars of *C. externa* were highest in the second instar (LTT = 10.20 °C)
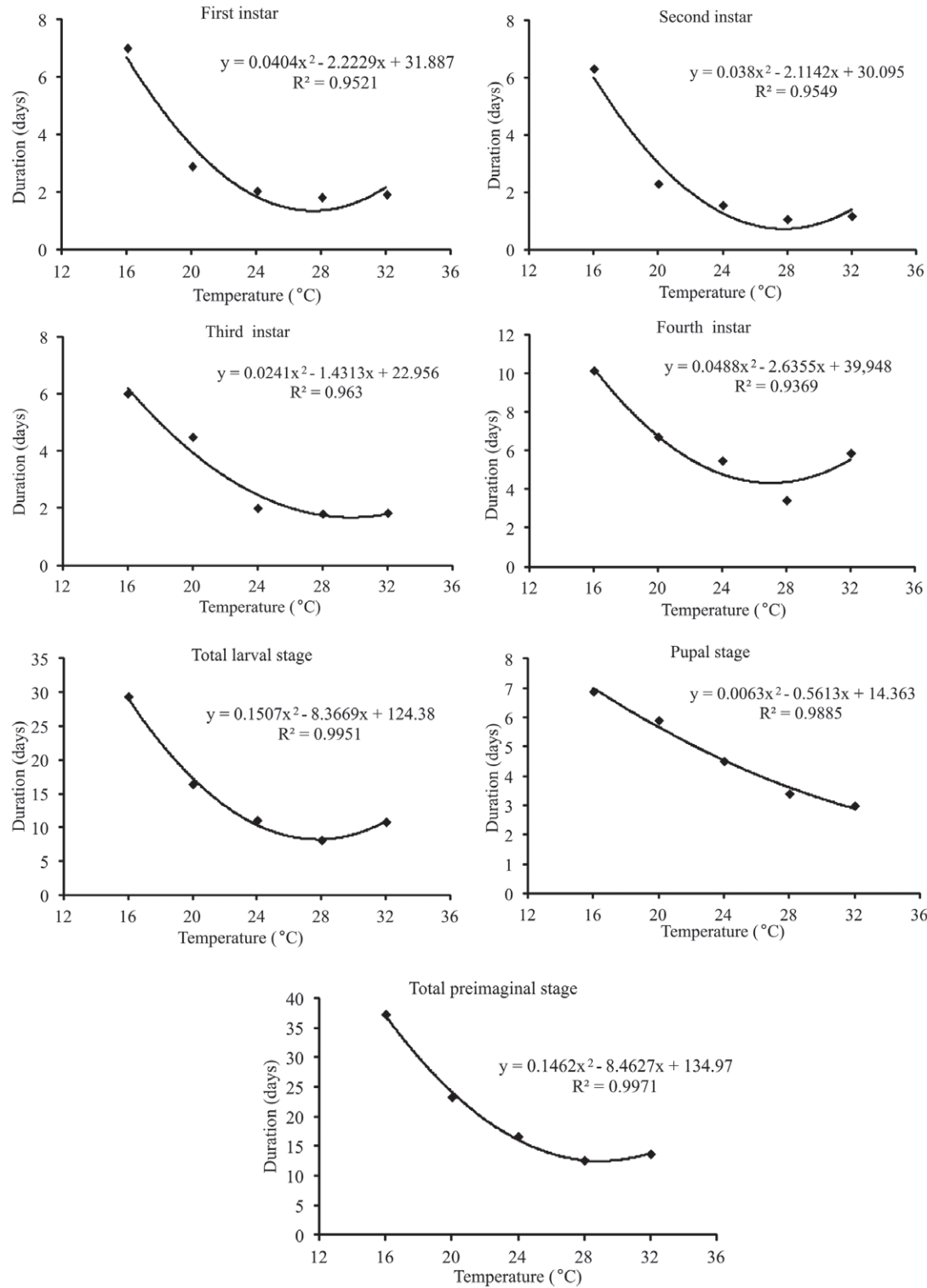
Fig. 1. Effect of temperature on duration of development of *Harmonia axyridis* life stages fed on *Rhopalosiphum padi* revealed by fitted regression curves for the durations of the first, second, third, fourth larval instars, the total larval stage, the pupal stage and the total preimaginal stage.

TABLE 1. SURVIVAL (%) AT VARIOUS TEMPERATURES OF THE DIFFERENT INSTARS, TOTAL LARVAL STAGE, PREPUPAE, PUPAE AND TOTAL PRE-IMAGINAL STAGE OF *HARMONIA AXYRIDIS* AND *CHRYSOPERLA EXTERNA* FED ON *RHOPALOSIPHUM PADI*.

| | Survival (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Harmonia axyridis* | | | | | | | |
| Temperature (°C) | 1° instar | 2° instar | 3° instar | 4° instar | Total Larval | Pre-pupal | Pupal | Pre-imaginal |
| 16 | 91.66 a | 96.66 ns | 90.33 ns | 92.33 ns | 75.00 a | 98.16 a | 98.00 a | 74.66 b |
| | $n = 60$ | $n = 55$ | $n = 53$ | $n = 48$ | $n = 45$ | $n = 45$ | $n = 44$ | $n = 43$ |
| 20 | 93.33 a | 92.83 ns | 92.50 ns | 100.00 ns | 80.00 a | 98.00 a | 100.00 a | 78.33 b |
| | $n = 60$ | $n = 56$ | $n = 52$ | $n = 48$ | $n = 48$ | $n = 48$ | $n = 47$ | $n = 47$ |
| 24 | 96.66 a | 96.50 ns | 98.33 ns | 100.00 ns | 91.66 a | 100.00 a | 100.00 a | 91.66 a |
| | $n = 60$ | $n = 58$ | $n = 56$ | $n = 55$ | $n = 55$ | $n = 55$ | $n = 55$ | $n = 55$ |
| 28 | 98.33 a | 100.00 ns | 100.00 ns | 95.00 ns | 93.33 a | 100.00 a | 100.00 a | 93.33 a |
| | $n = 60$ | $n = 59$ | $n = 59$ | $n = 59$ | $n = 56$ | $n = 56$ | $n = 56$ | $n = 56$ |
| 32 | 76.66 b | 86.83 ns | 92.16 ns | 93.33 ns | 56.66 b | 91.83 b | 69.50 b | 30.00 c |
| | $n = 60$ | $n = 46$ | $n = 40$ | $n = 38$ | $n = 34$ | $n = 34$ | $n = 31$ | $n = 18$ |

| | *Chrysoperla externa* | | | | | |
|---|---|---|---|---|---|---|
| Temperature (°C) | 1° instar | 2° instar | 3° instar | Total Larval | Pupal | Pre-imaginal |
| 12 | 70.00 b | 44.28 b | 31.81 b | 10.00 c | — | — |
| | $n = 68$ | $n = 47$ | $n = 22$ | $n = 7$ | | |
| 16 | 91.11 a | 62.77 b | 20.74 b | 12.01 c | — | — |
| | $n = 45$ | $n = 41$ | $n = 26$ | $n = 5$ | | |
| 20 | 95.00 a | 95.00 a | 91.25 a | 82.55 b | 86.25 a | 93.33 a |
| | $n = 40$ | $n = 38$ | $n = 36$ | $n = 33$ | $n = 33$ | $n = 33$ |
| 24 | 96.66 a | 100.00 a | 100.00 a | 96.66 a | 100.00 a | 96.66 a |
| | $n = 30$ | $n = 29$ | $n = 29$ | $n = 29$ | $n = 29$ | $n = 29$ |
| 28 | 80.00 b | 97.50 a | 86.04 a | 65.00 b | 87.50 a | 76.66 b |
| | $n = 40$ | $n = 32$ | $n = 31$ | $n = 27$ | $n = 27$ | $n = 27$ |
| 32 | 90.00 a | 100.00 a | 83.61 a | 76.66 b | 78.61 a | 69.00 b |
| | $n = 30$ | $n = 27$ | $n = 27$ | $n = 23$ | $n = 23$ | $n = 23$ |

Means followed by the same letter in the column did not differ by the Scott Knott test (P < 0.05).

( — ) indicates absence of data due mortality.

and lowest in the third instar (LTT = 8.7 °C). However, the third instar requires more degree-days (83.3 DD) (Table 2) than either the first larval instar (61 DD) or the second larval instar (49.0 DD). The LTTs of the total larval, total pupal and total pre-imaginal stages were 8.8, 10.69 and 8.46 °C, and 200, 153.84 and 384.62 DD, respectively (Table 2).

Comparative Biology of *Harmonia axyridis* and *Chrysoperla externa* Developmental Stages at Various Temperatures

At 24 °C the duration of the total larval stage of *H. axyridis* (11.1 days) was shorter than that of *C. externa* (12.7 days) ($F = 26.45$, $P = 0.0001$). This difference was greater at 28 °C where the duration of the total larval stage of *H. axyridis* was 8.1 days and that of *C. externa* was 12.2 days

($F = 149.20$, $P = 0.0001$). However, at 20 °C the duration of the total larval stage of *H. axyridis* (16.4 days) was longer than that of *C. externa* (14.8 days) ($F = 27.79$, $P = 0.0001$). Likewise at 32 °C the duration of the total larval stage of *H. axyridis* (10.9 days) was longer than that of *C. externa* (8.3 days) ($F = 48.146$, $P = 0.0001$).

The durations of the total pupal stage of *H. axyridis* was much shorter than those of *C. externa* at 20 °C ($F = 325.99$, $P = 0.0001$), 24 °C ($F = 103.24$, $P = 0.0001$), 28 °C ($F = 107.80$, $P = 0.0001$) and 32 °C ($F = 21.08$, $P = 0.0001$). At these 4 temperatures the durations of the total pupal stage of *H. axyridis* were shorter than those of *C. externa* by 11, 6, 7 and 4 days, respectively (Table 3).

The survival rates of the total larval stage of *C. externa* and *H. axyridis* did not differ significantly at 20 °C ($F = 3.13$, $P = 0.08$) and 24 °C ($F =$

TABLE 2. REGRESSION EQUATIONS, DETERMINATION COEFFICIENTS (R$^2$), LOWER THRESHOLD TEMPERATURES (LTT) AND THERMAL CONSTANTS (K) (DEGREE-DAYS) OF THE VARIOUS LARVAL INSTARS AND OTHER IMMATURE STAGES OF *HARMONIA AXYRIDIS* AND *CHRYSOPERLA EXTERNA* FED ON *RHOPALOSIPHUM PADI*.

| Stage | Equation | R$^2$ | LTT (°C) | K (DD) |
|---|---|---|---|---|
| *Harmonia axyridis* | | | | |
| First instar | 0.0344x - 0.3730 | 0.9481 | 10.843 | 29.070 |
| Second instar | 0.0641x - 0.8658 | 0.9961 | 13.507 | 15.601 |
| Third instar | 0.0359x - 0.4293 | 0.9142 | 11.958 | 27.855 |
| Fourth instar | 0.0152x - 0.1554 | 0.9359 | 10.224 | 65.789 |
| Larval stage | 0.0074x - 0.0857 | 0.9981 | 11.581 | 135.135 |
| Pupal stage | 0.0124x - 0.0658 | 0.9552 | 5.306 | 80.645 |
| Preimaginal stage | 0.0044x - 0.0437 | 0.9983 | 9.932 | 227.273 |
| *Chrysoperla externa* | | | | |
| First instar | 0.0164x - 0.1516 | 0.9664 | 9.243 | 60.975 |
| Second instar | 0.0204x - 0.2082 | 0.9343 | 10.205 | 49.019 |
| Third instar | 0.012x - 0.1041 | 0.9447 | 8.675 | 83.333 |
| Larval stage | 0.005x - 0.0442 | 0.9439 | 8.840 | 200.00 |
| Pupal stage | 0.0065x - 0.0695 | 0.9256 | 10.692 | 153.84 |
| Preimaginal stage | 0.0026x - 0.0220 | 0.9012 | 8.462 | 384.62 |

0.305, $P$ = 0.583) (Table 3) However, at 28 °C survival of *C. externa* larvae (65%) was significantly less than survival of *H. aryxidis* larvae (93%) ($F$ = 11.19, $P$ = 0.001). In contrast at 32 °C, survival of *H. axyridis* larvae (57%) was significantly less than survival of *C. externa* larvae (77%) ($F$ = 4.88, $P$ = 0.03) (Table 3). The percentages of survival of the total pupal stages of *H. axyridis* and *C. externa* did not differ significantly at any of the temperatures, i.e., at 20 °C ($F$ = 2.44, $P$ = 0.12), at 24 °C ($F$ = 0.00, $P$ = 1.00), at 28 °C ($F$ = 2.01, $P$ = 0.16) and at 32 °C ($F$ = 0.95, $P$ = 0.33) (Table 3).

## DISCUSSION

In ectothermic animals metabolic rate increases with increasing temperature according to the $Q_{10}$ principle (Dillon et al. 2010), and biochemical processes under such conditions are accelerated (Neven 2000). We found that the development time of *H axyridis* fed on *R. padi* was progressively shortened when the rearing temperature was increased from 16 to 28 °C. However, no further reduction occurred when the rearing temperature was increased to 32 °C. This indicated that at 32 °C the metabolic rate was not increased sufficiently to further shorten the predator's development period. In turn this suggested that at 32 °C is too high for efficient functioning of the insect's metabolic processes. Helmuth et al. (2005) and Chow & Terblanche (2007) believe that physiological responses are one of the main strategies used to compensate for the change in climate.

Besides the alterations caused by temperature, one should consider the type and nutritional quality of food (Gilbert & Raworth 1998; Castro et al.

2011). According to Stathas et al. (2001), the preimaginal period of *H. axyridis* was shorter when fed on *Dysaphis crataegi* (Kaltenbach) compared to *Aphis fabae* Scopoli at 20 °C. Shorter duration of this period was found in this work for this predator fed on *R. padi* on the same thermal condition. This shortened development period was also observed at 24 °C and 28 °C, compared with those recorded by Lanzoni et al. (2004), who found duration of 19.8 days at 25 °C when *H. axyridis* fed on *Myzus persicae* (Sulzer). Shortened development periods of *H. axyridis* of 18.9 and 22.5 days were also observed at 27 °C by Abdel-Salam & Abdel-Baky (2001) when fed this predator was fed fresh or frozen eggs of *Sitotroga cerealella* Olivier, respectively. Thus, even in close thermal conditions, the rate of development of *H. axyridis* may be affected depending on the prey offered. These data demonstrate that *R. padi* showed favorable nutritional quality, and that this prey species can be considered to be favorable for activity of *H. axyridis* in the field.

*Harmonia axyridis* completed its immature stages at all temperatures tested (16 °C to 32 °C); however, larval survival was reduced at extreme temperatures primarily because of little survival of the first instar. This greater sensitivity of the first instar under extreme temperatures can be explained by it having thin and soft cuticle making it more vulnerable to dehydration. The increased cuticle thickness in other stages possibly confers protection at extreme temperatures (Omkar & Pervez 2004). Acar et al. (2004) showed that adults and second-instar larvae of *H. axyridis* suffered stress at 35 °C and high mortality at 40 °C, but that the third and fourth larval instars and pupae tolerated 35 °C. According to Campbell et al. (1974), high
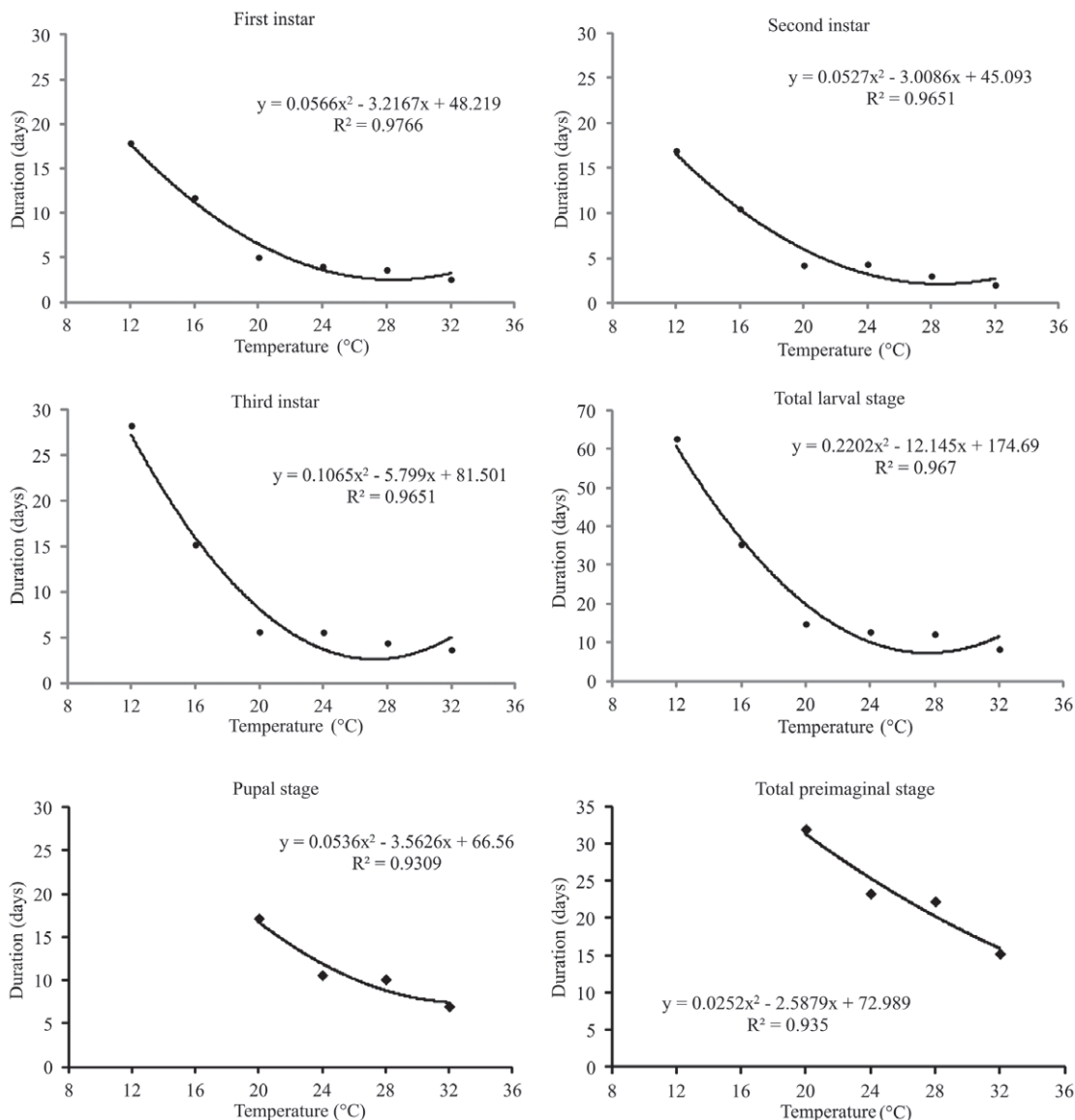
Fig. 2. Effect of temperature on duration of development of *Chrysoperla externa* life stages fed on *Rhopalosiphum padi* revealed by fitted regression curves for the durations of the first, second, and third larval instars, the total larval stage, the pupal stage and the total preimaginal stage.

temperatures lead to increased mortality due to the denaturation of proteins by toxic accumulations of metabolites and this harmful effect occurs mainly when the temperature remains constant.

The meager survival in the total pre-imaginal stage of *H. axyridis* at 32 °C was due to the moderately harmful effect of temperature on the larval, pupal and pre-pupal stages; however, survival in these stages were not so drastically affected in the 16-28 °C range. Survival of the total pre-imaginal stage of *H. axyridis* fed on *R. padi* ranged from 74% at 16 °C to more than 91% at 24

and 28 °C. Thus survival on *R. padi* was greater than recorded by Adbel-Salaam & Adbel-Baky (2001) on fresh (84%) or frozen eggs (80 %) of *S. cerealella* at 27 °C. Whereas *S. cerealella* eggs are is widely used for rearing coccinellids, the superior nutritional quality of *R. padi* is evident from our data. Our results corroborate those of Castro et al. (2011), who found 100% larval, pre-pupal and pupal survival when *H. axyridis* was fed on the aphid, *Cinara atlantica* (Wilson) at 25 °C. However, Lanzoni et al. (2004) found only 49% pre-imaginal survival when *H. axyridis* was fed

TABLE 3. EFFECT OF TEMPERATURE ON THE DURATIONS (DAYS) AND SURVIVAL RATES (%) OF THE TOTAL LARVAL AND TOTAL PUPAL STAGES OF *HARMONIA AXYRIDIS* AND *CHRYSOPERLA EXTERNA* FED ON *RHOPALOSIPHUM PADI*.

| Temperature (°C) | Total larval stage | | Total pupal stage | |
|---|---|---|---|---|
| | *H. axyridis* | *C. externa* | *H. axyridis* | *C. externa* |
| | Duration (Days) | | | |
| 20 | 16.43 a | 14.78 b | 5.91 b | 17.17 a |
| 24 | 11.09 b | 12.72 a | 4.52 b | 10.62 a |
| 28 | 8.14 b | 12.15 a | 3.41 b | 10.13 a |
| 32 | 10.85 a | 8.26 b | 3.00 b | 7.00 a |
| | Survival (%) | | | |
| 20 | 80.00 a | 65.00 a | 100.00 a | 81.00 a |
| 24 | 91.00 a | 96.66 a | 100.00 a | 100.00 a |
| 28 | 93.33 a | 65.00 b | 100.00 a | 87.00 a |
| 32 | 56.66 b | 76.66 a | 69.00 a | 78.61 a |

Means followed by the same letter in the rows did not differ by Scott Knott ($P < 0.05$).

on *M. persicae* at 25 °C. Some aphid species can be toxic because they sequester allelochemicals present in host plants, or for other reasons they may be inadequate as food of *H. axyridis*, such as *Brevicoryne brassicae* (Linnaeus) and *Megoura viciae* Buckton (Tsaganou et al. 2004).

The durations of each of the *C. externa* instars and the total larval stage were on average 4 × greater at 12 °C compared to those of larvae maintained at 20 °C. Fonseca et al. (2001) and Oliveira et al. (2009) also found reductions in the durations of development of this predator between 15 and 21 °C when *C. externa* was fed on *Schizaphis graminum* (Rondani), and between 12 and 20 °C when *C. externa* was fed on *Sipha flava* (Forbes). Under higher temperature conditions (24-32 °C) used our studies, the durations of development tended to stabilize, denoting that temperature sets the maximum biological activity of the insect, and may also act as a stressor when the temperature is below or above the optimum range.

Survival rates of larval instars, the total larval stage and the pupal stage were greater than 90% when *C. externa* was maintained at 20 to 32 °C. Nearly total survival of immature stages was obtained at 15-30 °C when *C. externa* was fed either on *S. graminum* (Fonseca et al. 2001), or on *Alabama argillacea* (Hübner) (Figueira et al. 2000). However, Aun (1986 ) found that the survival rates of the total larval stage of *C. externa* fed on *Anagasta kuehniella* (Zeller ) at 25 and 30 °C were only 62.6 and 63.9%, respectively. It is noteworthy that the meager survival rates of the different *C. externa* instars and the total larvae stage, as well as the 100% pupal mortality at 12 and 16 °C shows that these temperatures are not suitable for the development of the immature stages of *C. externa* when fed on *R. padi*. Similarly, Oliveira et al. (2010) found that *C. externa* larvae did not complete the third instar at 12 °C

when fed on *S. flava*. Fonseca et al. (2001) using *S. graminum* as prey and Figueira et al. (2000) using *A. argillacea* eggs as prey registered *C. externa* pupal survival rates of 100% and 61% at 15 °C, respectively. The above-mentioned results indicate that besides temperature, the prey offered can affect the survival of *C. externa*; and this confirms the observations of Kubo (1993) who reported that for *C. externa* and other species of Chrysopidae the type of prey influences the duration and survival of larval stage. Since lacewings are important predators and prefer several aphid species (Gravena & Cunha 1991), research related to the interaction of various predators with *R. padi* is justified. By means of the aforementioned comparisons regarding the biological parameters of *C. externa* fed on various prey species, we suggest that *R. padi* is satisfactory as its prey for use in conservation biological control systems in forage crops.

Several species of lacewings and coccinellids have common prey, such as aphids and eggs of Lepidoptera, and these results in the co-occurrence of these diverse predators in the same macro and micro habitats (Hagen 1986; Coderre et al. 1987; Majerus, 1994). However, temperature levels change the response of these biocontrol agents, thereby causing one species to be superior to other depending on the temperature regimen. However, studies comparing the response of a native predator (*C. externa)* with an exotic one (*H. axyridis*) are nonexistent in Brazil.

*Rhopalosiphum padi* is an excellent food for the development of *C. externa* and *H. axyridis*. However, the durations of development of *H. aryxidis* in its total larval stage at 24 and 28 °C and its pupal stage at 20-32 °C were less than those of *C. externa*. Likewise the survival rate of the *H. aryxidis* larval stage at 28° C was greater than that of *C. externa*. The longest duration and greatest survival rate of the pre-imaginal phase

(Lanzoni et al. 2004), and the longest larval duration (Michaud 2000) were recorded for *H. axyridis* compared with other coccinellids maintained at 25 °C.

It is noteworthy that the range 12 to 28 °C is favorable for the development of *R. padi* (Auad et al. 2009), and temperatures included in this range are also ideal for the development of the immature stages of *H. axyridis* and *C. externa* when fed on *R. padi*. Thus, it can be stated that of these 2 predator species and this prey species proceed through their respective the life cycles at corresponding rates in the above temperature range. In addition, the LTT and the thermal constant are important for predicting the number of annual generations of the insect in a given location depending on weather conditions. Values of this parameter close to those found in this study were recorded for *H. axyridis* by Stathas et al. (2001) and for *C. externa* by Albuquerque et al. (1994) and Maia et al. (2000).

Despite the similar LTTs for both species of predators, the development period from larvae to adult of *C. externa* was 1.7-fold greater than that of *H. axyridis* (GD 227.27). By adopting 9.93 and 8.46 as lower thermal limits, the numbers of degree-days (DD) to reach adulthood are 227.3 and 384.6 for *H. axyridis* and *C. externa*, respectively. Thus, as the region of the Juiz de Fora, Brazil has a mean monthly temperature of 17 °C in dry season (Apr to Sep), one can estimate the mean number of generations/month of 2.2 for *H. axyridis* and 1.3 for *C. externa*. Whereas, in the rainy season, when the mean monthly temperature is 24 °C (Oct to Mar) one can estimate the mean number of generations/month of 3.2 for *H. axyridis* and 1.9 for *C. externa*. These results are important for planning of rearing in the laboratory, as well as predicting the number of annual generations of predators that may occur in the field according to climatic conditions. Importantly, in addition to the greater number of generations obtained by *H. axyridis*, it is predatory in both the larval and adult stages causing potentially greater pest suppression, compared to lacewings that are predatory only as larvae.

By comparing basic biological parameters, it is suggested that *H. axyridis* has intrinsic advantages over *C. externa*. This fact associated with the characteristics of *H. axyridis*, such as high fecundity, aggressive behavior, increased body size (Michaud 2002), high mobility (With et al. 2002), high resistance to insecticides (Michaud 2001), indicates that this predator may be the best choice for augmentative release against *R. padi* in pastures. However, other aspects, such as cost - benefit ratio, ease of mass production, market supply, and technology transfer to the users, must be considered when choosing the predator.

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