Biological aspects of *Harmonia axyridis* in comparison with *Cycloneda sanguinea* and *Hippodamia convergens*

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Abstract – The objective of this work was to evaluate the development, survival, reproductive capacity, and longevity of the Asian ladybug *Harmonia axyridis* in comparison with *Cycloneda sanguinea* and *Hippodamia convergens*. Coccinellid larvae and adults were fed daily with *Schizaphis graminum*. Ten couples of each species were isolated for evaluation of the adult phase. The duration of the larval stage of *H. axyridis* is the longest (10.2 days) and its adults are the heaviest (29.7 mg) compared with *C. sanguinea* and *H. convergens*. The three species showed similar percentages of survival during the developmental stages. An average of 82% of *C. sanguinea*, *H. axyridis*, and *H. convergens* larvae reached adulthood, which indicates that temperature (25°C) and the offered prey are favorable to coccinellid development. *Harmonia axyridis* produces a higher total number of eggs per female (1,029.2) than the other evaluated species. However, *H. axyridis*, which lives for an average of 147.2 days, does not show a significantly greater longevity than *C. sanguinea* (87.2 days) and *H. convergens* (134.3 days).

Index terms: biological control, coccinellid, development, longevity, ladybug, predator.

Aspectos biológicos de *Harmonia axyridis* em comparação a *Cycloneda sanguinea* e *Hippodamia convergens*

Resumo – O objetivo deste trabalho foi avaliar o desenvolvimento, a sobrevivência, a capacidade reprodutiva e a longevidade da joaninha-asiática *Harmonia axyridis* em comparação a *Cycloneda sanguinea* e *Hippodamia convergens*. Larvas e adultos dos coccinelídeos foram alimentados diariamente com *Schizaphis graminum*. Isolaram-se dez casais de cada espécie para as avaliações na fase adulta. A duração da fase larval de *H. axyridis* é maior (10,2 dias) e os adultos são mais pesados (29,7 mg) em comparação a *C. sanguinea* e *H. convergens*. As três espécies apresentaram percentagem de sobrevivência similar durante os estádios de desenvolvimento. Em média, 82% das larvas de *C. sanguinea*, *H. axyridis* e *H. convergens* atingiram a fase adulta, o que indica que a temperatura (25°C) e a presa oferecida foram favoráveis ao desenvolvimento dos coccinelídeos. *Harmonia axyridis* produz maior número total de ovos por fêmea (1.029,2) do que as demais espécies avaliadas. No entanto, *H. axyridis*, que vive, em média, 147,2 dias, não apresenta longevidade significativamente maior que *C. sanguinea* (87,2 dias) e *H. convergens* (134,3 dias).

Termos para indexação: controle biológico, coccinelídeo, desenvolvimento, longevidade, joaninha, predador.

Introduction

The Asian ladybug *Harmonia axyridis* (Pallas) is a notable polyphagous and effective predator (Almeida & Silva, 2002). However, the species is characterized as having a negative impact on the populations of other coccinellids, by displacing them through interspecific competition (Koch, 2003).

In Brazil, Milléo et al. (2008) observed that the population of *H. axyridis* increased, whereas those of other coccinellid species reduced in orchards with different types of fruit trees. In a secondary forest area with a predominance of ferns, *Pinus* sp. and *Baccharis* spp., Martins et al. (2009) reported low abundance of coccinellids, mainly *Cycloneda sanguinea* (Linnaeus) and *Hippodamia convergens* (Guérin-Meneville) after the introduction of *H. axyridis*.

Berthiaume et al. (2007) studied the biology of *H. axyridis* in a coniferous forest in Canada, comparing

it with that of the native species *Anatis mali* (Say). The authors found higher seasonal synchrony between the life cycles of *A. mali* and the aphid *Mindarus abietinus* (Koch) than between *H. axyridis* and *M. abietinus*. In spring, adults of *A. mali* colonized plants about two weeks earlier than exotic species, enabling their larvae to be present during the population peaks of aphid pests. Therefore, *A. mali* is better adapted than *H. axyridis* to the control *M. abietinus*.

Lanzoni et al. (2004) compared the biological aspects of *H. axyridis* with those of the native coccinellids *H. variegata* (Goeze) and *Adalia bipunctata* (Linnaeus) and observed differences in the durations of the egg, larva, and pupa stages between these predators. *Harmonia axyridis* showed a longer larval period (10.4 days) than the other species. According to the authors, the biological traits of *H. axyridis* do not seem to contribute to its success as an invasive species.

These studies are essential in determining the population growth potential of the species because they consider the competitive capacity of its natural enemies (Lanzoni et al., 2004). However, no comparative biological studies among *H. axyridis*, *H. convergens*, and *C. sanguinea*, which are established species in Brazilian agroecosystems, have been conducted to date.

Schizaphis graminum (Rondani) is recognized as an important pest of cereal crops (Nuessly & Nagata, 2005; Loeck et al., 2006) as it transmits viruses that cause plant death (Al-Mousawi et al., 1983; Nuessly & Nagata, 2005). In Brazil, *S. graminum* causes damage to wheat (*Triticum aestivum* L.) and sorghum (*Sorghum bicolor* L.) crops (Rubin-de-Celis et al., 1997). Therefore, this hemipteran species is a relevant target for studies aimed at biological control using coccinellids.

Because the records on the Asian ladybug in Brazil are relatively recent, information about its behavior, potential for predation, biology, food preference, and competition with other coccinellid species is limited.

The objective of this work was to evaluate the development, survival, reproductive capacity, and longevity of the Asian ladybug *H. axyridis* in comparison with *C. sanguinea* and *H. convergens*.

Materials and Methods

The study was carried out from March 2009 to September 2011 at the Laboratory of Insect Ecology of Departamento de Fitossanidade, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista (FCAV/Unesp), Jaboticabal, state of São Paulo, Brazil (21°15'22"S, 48°18'58"W).

Larvae of each predator species were obtained by rearing coccinellids from the Laboratory of Insect Ecology according to Santos et al. (2009). The rearing of *S. graminum* began by using aphids collected from leaves of sorghum plants (*S. bicolor*) grown at the experimental area of FCAV/Unesp. The aphids were kept on 45 cm sections of sorghum stems of the BRS 310 cultivar – susceptible to attack by this hemipteran –, packed in glass containers with 300 mL of water, and sealed with PVC film. Water and stems were replaced weekly.

Newly hatched coccinellid larvae were individualized and fed daily, ad libitum, *S. graminum* nymphs and adults, and the treatments were represented by the ladybug species. Each experimental block consisted of a Petri dish of 9.0 cm in diameter, containing the predator species and prey. The insects were maintained in a climatic chamber at a constant temperature of 25±1.0°C, under a 12 hour photoperiod and 70±10% relative humidity. Thirty replicates per treatment were performed, and observations were made daily. The duration and viability of the instars were determined during the immature phase, including the pre-pupal phase in the fourth instar, the pupal phase, and the egg-adult period. The fresh mass of the coccinellids was obtained by weighing 15 larvae of each species, 24 hours after each instar change, 1 day after the emergence of adults.

For evaluations of the adult stage, individuals originated from the corresponding experiment with the immature stages of coccinellids were used. Ten couples of each species were isolated, each representing one replicate. These couples were kept in transparent 350 mL plastic cups and fed *S. graminum* daily. Observations were conducted daily, and the periods of pre-oviposition and oviposition, daily and total oviposition capacity, and longevity were determined. To determine the incubation period and egg viability of the species of coccinellids, laid eggs were separated daily for a period of seven days for each couple. The number of eggs in each laying was counted, and the number of eggs hatched and the time to hatching (in days) were assessed.

The experimental design was completely randomized. Data were subjected to analysis of variance (Anova), and the means were compared by the Tukey test, at 5% probability. Residue analysis was carried out to verify the assumptions of Anova. The SAS software, version 9.0 (SAS Institute, Cary, NC, USA), was used.

Results and Discussion

The duration of the developmental stages differed significantly among the three coccinellid species (Table 1). The period of egg incubation in *H. axyridis* and *H. convergens* was approximately 1.5 days shorter than in *C. sanguinea*. Conversely, with the exception of the second larval stage, *H. axyridis* and *H. convergens* showed a longer duration for the other phases of development than *C. sanguinea*.

The fourth-instar stage was longer in *H. axyridis* compared with the other evaluated species. According to Santa-Cecília et al. (2001), during the fourth instar, coccinellid larvae require higher amounts of nutrients for pupal development and differentiation into adults. This is also the instar in which the larvae have the greatest voracity, since, during this stage, individuals consume 60 to 80% of their total prey (Hodek & Honěk, 1996). For purposes of biological control of the aphid *S. graminum*, the extension of the fourth instar in the Asian species is advantageous because larvae at this stage may be equally or more voracious than adults and have the additional advantage of, once released, showing a reduced capacity for dispersion. Lanzoni et al. (2004) compared the development of *H. axyridis*, *H. variegata* (Goeze), and *A. bipunctata* (Coleoptera: Coccinellidae) fed with the aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) and recorded a 1 day extension in the larval phase of *H. axyridis* in comparison with the other species.

In the present study, the duration of the larval phase was similar to that found by Michaud (2000) (9.0 days) and Lanzoni et al. (2004) (10.4 days) for *H. axyridis* fed with *Toxoptera citricida* (Kirkaldy) and *M. persicae*, respectively, and maintained under the same temperature conditions, i.e., 25°C (Table 1). However, the duration of the phases was longer in *H. axyridis* (14.0 days) when the species was subjected to a temperature of 30ºC and fed with *Acyrthosiphon pisum* (Harris) (Lamana & Miller, 1998). Because temperature increases usually result in a higher development rate in insects (Speight et al., 2008), the shorter developmental time observed for *H. axyridis* suggests that the aphid *S. graminum* was a favorable food for its growth. This highlights the nutritional quality of *S. graminum* as a prey for *H. convergens* and *C. sanguinea*, which completed the developmental phases in shorter times, 17.2 and 17.5 days, respectively, than when fed with nymphs of the aphid *Cinara* spp., at 25ºC (Cardoso & Lázzar, 2003). The duration of the immature phases of *C. sanguinea* was similar to that reported by Santos et al. (2003) (8.1 days), at 25ºC, using *S. graminum* as food.

The duration of larval development is unique in each coccinellid species; however, factors such as room temperature and quality and quantity of food strongly influence this variable (Nedvěd & Honěk, 2012). The reduction in the developmental period during the immature phase may represent a strategy for the species to remain in that particular phase for a short time, particularly when these are more vulnerable to predation than others, increasing their likelihood of survival. Therefore, because adults of *C. sanguinea* emerged earlier than those of *H. axyridis* and

Table 1. Duration of immature stages (mean±standard error) of *Cycloneda sanguinea*, *Harmonia axyridis*, and *Hippodamia convergens* fed *Schizaphis graminum* at 25±1°C, under 12 hours photophase and 70±10% relative humidity[(1)].

Species	Egg	1st instar	2nd instar	3rd instar	4th instar	Larval period	Pupae	Larva-adult
	(day)							
C. sanguinea	3.4±0.13a	1.9±0.22a	1.1±0.31a	1.2±0.22a	3.0±0.30a	7.3±0.66a	3.3±0.19a	10.6±0.79a
H. axyridis	2.0±0.09b	2.8±0.06b	1.8±0.09b	1.8±0.09b	3.8±0.10c	10.2±0.11c	4.2±0.06b	14.5±0.10b
H. convergens	2.0±0.00b	2.9±0.11b	1.3±0.09a	1.7±0.08b	3.4±0.11b	9.4±0.10b	3.9±0.09c	13.3±0.09c
F	209.4*	22.2*	18.5*	18.7*	12.9*	152.4*	24.3*	238.4*
CV (%)	11.6	24.3	29.0	26.2	17.1	7.2	12.9	5.2

[(1)]Means followed by equal letters, in the columns, do not differ by the Tukey test, at 5% probability.

H. convergens, this species should be favored when competing with the other two species for prey. It must be emphasized that this biological characteristic of *C. sanguinea* increases the number of generations per year and favors adaptation to Brazilian agroecosystems, since it is considered one of the major aphid predatory insects in cotton, sugarcane, citrus, mango, soybean, and sorghum crops (Veloso et al., 1995). However, after the introduction of *H. axyridis*, established species, such as *C. sanguinea* and *H. convergens*, have been potentially susceptible to intraguild predation due to overlapping habitats, which are similar to those of exotic species (Martins et al., 2009). As reported by Santos-Cividanes et al. (2012), *H. axyridis* is the predominant competitor of *C. sanguinea* and *H. convergens*, acting as an intraguild predator, even in the presence of other preferred prey such as aphids.

No significant differences in coccinellid survival were observed during the development of the different phases (Table 2). The first instar of *H. axyridis* showed the highest susceptibility and lowest percentage of survival. Santos et al. (2009) also observed a similar trend, reporting a 73.3% survival during this instar when the species was fed with *S. graminum*. However, this biological characteristic of *H. axyridis* is compensated for by the higher average number of eggs produced per female when compared with the other species (Table 3). *Cycloneda sanguinea* and *H. convergens* showed the highest fragility during the second instar, as revealed by the lowest rate of survival for this phase. This reduction in survival is probably related to the fact that the larval stage is of short duration in these two species (approximately 1 day), leaving no time for the larvae to store nutrients that are essential for energy expenditure during ecdysis. Rapid development, therefore, becomes a burden, resulting in a reduction in survival. Both species showed significantly shorter durations in this phase in comparison with that observed for *H. axyridis* (Table 1). Approximately 74.3% of *C. sanguinea*, 82.9% of *H. axyridis*, and 88.6% of *H. convergens* larvae reached adulthood, indicating that the temperature and the prey provided were favorable for their development.

The survival rate was similar to those reported by Santa-Cecília et al. (2001) for the immature phases of *C. sanguinea* fed with *S. graminum* at 25ºC: 100, 85, 100, 94, 80, and 100% for the first, second, third, fourth instars, and the larval and pupal periods, respectively. However, these were lower than those found by Santos et al. (2003) in *C. sanguinea* fed with *S. graminum* at 25ºC: 100% viability in all development phases. The variation in the survival rate observed in previous studies, which were conducted using the same temperature and prey, is probably due to manipulation

Table 2. Survival of immature stages (mean±standard error) of *Cycloneda sanguinea*, *Harmonia axyridis*, and *Hippodamia convergens* fed *Schizaphis graminum* at 25±1°C, under 12 hour photophase and 70±10% relative humidity[(1)].

Species	Egg	1st instar	2nd instar	3rd instar	4th instar	Larval period	Pupae	Larva-adult
	(%)							
C. sanguinea	88.0±2.00a	97.1±2.86a	88.6±8.57a	97.1±2.86a	93.6±4.18a	77.1±2.53a	97.1±2.86a	74.3±8.08a
H. axyridis	82.0±4.00a	94.3±3.69a	97.1±2.86a	100.0±0.00a	100.0±0.00a	88.6±4.04a	93.6±4.18a	82.9±5.22a
H. convergens	89.0±1.00a	97.1±2.86a	94.3±3.69a	96.4±3.57a	100.0±0.00a	91.4±5.95a	97.1±2.86a	88.6±5.95a
F	2.13*	0.3*	0.6*	1.0*	2.4*	1.6*	0.4*	0.8*
CV (%)	4.4	12.5	20.2	7.7	10.8	20.4	13.7	22.3

[(1)]Means followed by equal letters, in the columns, do not differ by the Tukey test, at 5% probability.

Table 3. Fecundity and longevity (mean±standard error) of *Cycloneda sanguinea*, *Harmonia axyridis*, and *Hippodamia convergens* fed *Schizaphis graminum* at 25±1°C, under 12 hour photophase and 70±10% relative humidity[(1)].

Species	Pre-oviposition	Oviposition	Post-oviposition	Total number	Daily number	Adult female longevity (day)	Adult male longevity (day)
	(egg per female)						
C. sanguinea	3.8±0.37a	70.5±9.80b	2.4±0.68b	1,021.0±76.49b	15.3±1.87a	87.2±13.72a	133.0±13.62a
H. axyridis	3.2±0.20a	102.4±14.43ab	3.2±0.66b	1,029.2±228.26a	8.8±1.38b	147.2±20.79a	130.0±15.83a
H. convergens	1.6±0.24b	116.2±10.61a	15.4±2.50a	484.5±35.82b	4.7±0.53b	134.3±17.18a	111.3±14.03a
F	16.2**	3.9*	22.2*	4.93*	20.4**	3.2*	0.6*
CV (%)	22.1	29.9	49.3	40.7	30.3	34.8	28.5

[(1)]Means followed by equal letters, in the columns, do not differ by the Tukey test, at 5% probability.

techniques used in the laboratory. Coccinellid larvae present a soft integument and may suffer irreversible damage when manipulated with a brush or transferred to a new container. Larval handling must, therefore, be carried out only when extremely necessary, particularly during the initial instars. In the present study, the survival of *H. axyridis* was superior to that reported by Lanzoni et al. (2004) (49.9%), who fed the species with *M. persicae* at 25ºC. However, this biological parameter was similar to that observed by Santos et al. (2009) (86.7%), who fed the insects with *S. graminum* at 27ºC. The instar survival of *H. convergens* was lower than that found by Cardoso & Lázzar (2003), which was of 100% survival at the fourth instar when fed with nymphs of *Cinara* spp. aphids at 25ºC.

During the second ($F=0.92$; $p<0.05$; CV=25.4%) and third instars ($F=2.32$; $p<0.05$; CV=19.0%), *C. sanguinea*, *H. axyridis*, and *H. convergens* larvae showed similar average weights (Figure 1). However, significant differences were observed in the weight of fourth-instar larvae ($F=5.64$; $p<0.01$; CV=18.6%) and during the adult phase ($F=32.4$; $p<0.01$; CV=15.5%). Adults of *H. axyridis* were heavier than those of the other two species, weighing approximately 6.2 and 11.6 mg more than the adults of *H. convergens* and *C. sanguinea*, respectively. According to Michaud (2000), *H. axyridis* larvae and adults were larger than those of *C. sanguinea*. The larger size of *H. axyridis* in relation to the other coccinellid species is a key feature that favors the Asian ladybug in displacing native coccinellid species (Pell et al., 2008).

Reproductive parameters varied among the evaluated species (Table 3). *Hippodamia convergens* showed a shorter pre-oviposition period and a longer post-oviposition period than *C. sanguinea* and *H. axyridis*. The oviposition period in *H. convergens* was on average 116.2 days, which was longer than that observed for *C. sanguinea*, but similar to that of *H. axyridis*.

The highest fertility rates were observed in *C. sanguinea* and *H. axyridis*, which produced on average a total of 1,025.0 eggs, twice the value for *H. convergens* females (Table 3). The number of eggs produced daily by *C. sanguinea* was three-fold higher than that observed for *H. convergens* and two-fold higher than that for *H. axyridis*. This characteristic allows *C. sanguinea* to produce a larger number of descendants within a shorter period than the other two studied species, resulting in a rapid population growth that may favor competition with *H. axyridis* and *H. convergens* for prey. However, *H. axyridis* females showed a distinct strategy by producing the highest total number of eggs compared with the other species. The higher oviposition capacity combined with egg viability, which is similar to that of the other evaluated coccinellids, comparatively favors an increase in Asian ladybug populations at each generation. This strategy has been shown to be efficient since, according to Martins et al. (2009), *H. axyridis* was dominant in the coccinellid community, representing 90% of the species sampled in Curitiba, state of Paraná, Brazil. Conversely, the most abundant species in 1999 and 2000, namely *C. sanguinea* and *H. convergens*, were affected by the introduction of exotic species, which represented less than 3% of the coccinellid community in 2006 and 2007. Mignault et al. (2006) reported that *H. axyridis* produced three to five times more eggs than the coccinellids *Propylaea quatuordecimpunctata* L. and *Coleomegilla maculata* (De Geer) when fed with the soybean aphid *Aphis glycines* Matsumura, at 24ºC. According to Nedvěd & Honěk (2012), the number of eggs produced by Coccinellidae is characteristic of each species. This number increases with body size; however, it can also be influenced by

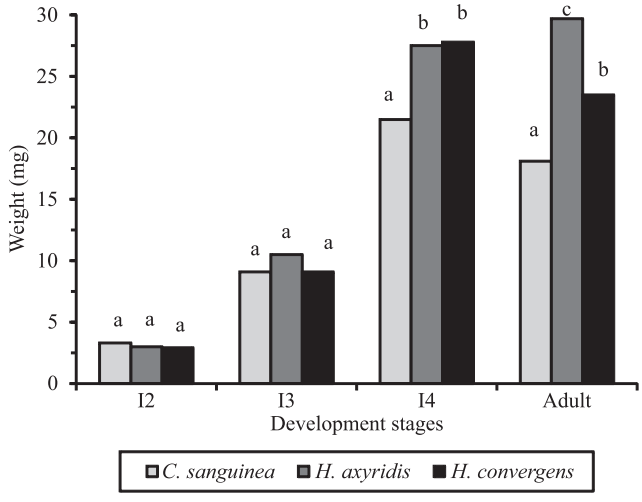

Figure 1. Average larvae weight of *Cycloneda sanguinea*, *Harmonia axyridis*, and *Hippodamia convergens* at the second (I2), third (I3), and fourth (I4) instars and adult stage fed *Schizaphis graminum* at 25°C, under 12 hour photophase and 70±10% relative humidity.

environmental temperature, as well as by the quality and quantity of food consumed during the preimaginal development and reproductive periods.

The longevity of females and males was similar (Table 3). Therefore, under the same conditions of humidity, temperature, and food, the Asian ladybug did not show significantly greater longevity than *C. sanguinea* and *H. convergens*. This result corroborates those reported by Lanzoni et al. (2004), who observed similar longevity among *H. axyridis* (27.5 days), *H. variegata* (36.9 days), and *A. bipunctata* (30.7 days) fed with the aphid *M. persicae* at 25ºC.

Conclusions

1. The aphid *Schizaphis graminum* and a temperature of 25°C favor the development of *Harmonia axyridis*, *Cycloneda sanguinea*, and *Hippodamia convergens*.

2. The longer duration of larval phase and the greater adult weight observed in *H. axyridis* confer advantages to this species in relation to *C. sanguinea* and *H. convergens*.

3. *Harmonia axyridis* produces a higher total number of eggs per female than the other evaluated species.

4. *Harmonia axyridis* does not show a significantly greater longevity than *C. sanguinea* and *H. convergens* under the same conditions of humidity, temperature, and food.

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