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Biology and fertility life table of *Eriopis connexa, Harmonia axyridis* and *Olla v-nigrum* (Coleoptera: Coccinellidae)

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Abstract

The coccinellids *Eriopis connexa* (Germar), *Harmonia axyridis* (Pallas) and *Olla v-nigrum* (Mulsant) are important natural biological control agents. The purpose of this paper was to study the biology and create a fertility life table of these three coccinellid species. For the biology study, 50 insects/species were used and kept in groups of 10 in glass vials (2300cm^3). For the three species studied, the viability of the total cycle varied from 45 to 50%. *O. v-nigrum* was the species which presented the longest oviposition period. However, *H. axiridis* demonstrated the best reproductive performance and ability of population growth in each generation. In conclusion, the use of commercially obtained pollen and *A. kuenhiella* eggs enables the development of coccinellids *E. connexa*, *H. axyridis* and *O. v-nigrum* under laboratory conditions, since the insects completed their biological cycle and originated adults with good reproductive performance.

Keywords: biology, coccinellids, biological control.

Biologia e tabela de vida de fertilidade de *Eriopis connexa, Harmonia axyridis* e *Olla v-nigrum* (Coleoptera: Coccinellidae)

Resumo

Os coccinelídeos *Eriopis connexa* (Germar), *Harmonia axyridis* (Pallas) e *Olla v-nigrum* (Mulsant) são importantes agentes de controle biológico natural. O objetivo deste trabalho foi estudar a biologia e a tabela de vida de fertilidade dessas três espécies de coccinelídeos. Para a biologia foram utilizados 50 insetos/espécie, mantidos em grupo de 10 em recipientes de vidro de (2300cm^3). Para as três espécies estudadas, a viabilidade do ciclo total variou de 45 a 50%. *O. v-nigrum* foi a espécie que apresentou maior período de oviposição. No entanto, *H. axiridis* foi àquela que demonstrou maior performance reprodutiva e capacidade de aumento populacional a cada geração. Em conclusão, a utilização de pólen comercial e ovos de *A. kuenhiella* possibilita o desenvolvimento dos coccinelídeos *E. connexa*, *H. axyridis* e *O. v-nigrum* em condições de laboratório, pois, os insetos completaram o ciclo biológico e originaram adultos com boa performance reprodutiva.

Palavras-chave: biologia, coccinelídeos, controle biológico.

1. Introduction

The Coccinellidae is represented by over 6000 species distributed among 360 different genera. They are predators in both their adult and larval phases, presenting an intense search for food and predatory capacity (Vandenberg, 2002; Olkowski et al., 1990; Oliveira et al., 2004). They are considered important agents in biological control helping to regulate the population of pest insects in many economically important crops (Obrycki and Kring, 1998).

Being predators, coccinellids normally have their nutritional needs met by their prey, especially aphids. However, these are opportunistic and/or generalist predators adopting different strategies to obtain their prey, feeding on lepidopteran larvae and other small arthropods. The choosing of other prey occurs when the aphid population is scarce, forcing the predators to search for an alternative food source to guarantee their survival (Sarmento et al., 2007; Evans, 2009).

Being biological control agents with a great capacity for predation, the conservation of coccinellids in crops is extremely important to Integrated Pest Management (IPM). One of the difficulties of using coccinellids for biological control is in related to the maintenance of laboratory populations for their mass rearing. For example, Kalushkov (1998), when evaluating ten species of aphids to discover essential preys for the diet of *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae), concluded that not all prey species offered as food were adequate for predator growth.

The knowledge about the bioecology of predator that aims at supplying the biological control programs is essential for optimize the mass production of these agents and thus provide insects for releases scheduled in an IPM program such as environmental conditions and diet offered, can influence in the metabolism of coccinellid to provide nutritional support and influence in different vital functions of predator.

Considering this, Rana et al. (2002) when studying the biological aspects of the predator *A. bipunctata* raised with two aphids species, *Acyrtosiphum pisum* (Harris) and *Aphis fabae* Scop.(Aphididae), observed that in temperate regions, rearings are subject to regional climatic changes, which drastically limits rearings due to reduced prey reproduction in the winter.

For the mass rearing of *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae), 17 different artificial diet combinations were tested, but the predator's biological development was adequate when the diet included eggs from *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae) (Silva et al., 2009). In another study different diets were tested, including lyophilized beef, eggs from *Ephestia kuehniella* Zeller (Lepidoptera: Phycitidae), pollen and cists from *Artemia franciscana* Kellogg (Branchiopoda: Artemiidae), with the purpose of obtaining a diet for mass rearings of *A. bipunctata*. Adults were produced with all tested diets; however, there was variability in relation to viability and duration of stages and in adult weight. The best development occurred when fed with *A. kuehniella* eggs (Bonte et al., 2010)

For other coccinellids, variations in biological parameters also was observed when the energy provided by prey is changed, as shown by Bado and Rodriguez (1997) when various species of aphid was used to feed *Olla v-nigrum* (Mulsant), or even to Santos et al. (2009) which observed reproductive performance of *Harmonia axyridis* (Pallas) when to fed with aphids and moth eggs.

In this context, knowing the biological aspects of coccinellids is primordial in optimizing laboratory rearings of these insects thus strengthening the strategies of biological control programs. The aim of this paper was to study the biology and make a fertility life table for *Eriopis connexa* (Germar), *H. axyridis* (Pallas) and *O. v-nigrum*, using commercially obtained pollen and *A. kuehniella* eggs for food.

2. Material and Methods

Adult *E. connexa*, *H. axyridis* and *O. v-nigrum* were collected in an experimental peach and strawberry orchard belonging to Embrapa Clima Temperado, Pelotas, Rio Grande do Sul (latitude 31°42'S and longitude 52°24'W). After the collection, the insects were transported to a laboratory, kept in plastic cages (25.5cm in length × 5.5cm in height × 15.5cm in width), closed at the top with voile to allow for aeration, and maintained in a climatized room at 25±1°C, relative humidity of 60±10% and a 14h photophase. Slightly moist filter paper was used to maintain humidity and toilet paper (odorless) was used as a substrate for oviposition. The cages were checked daily to collect eggs, clean and change food.

The eggs were placed in Petri dishes (9cm in diameter) containing a disc of slightly moist filter paper. Following eclosion, the larvae were transferred in groups of 10, with the help of a small brush, to new growth chambers (glass vials 2300cm^3), containing a piece of filter paper folded in "zigzag", where *A. kuehniella* eggs were placed. Eggs up to 48h old were offered to the larvae "ad libitum". In addition, pollen was offered in plastic vials (2cm in diameter × 1cm in height). The insects were kept in environmental chambers at 25±1°C, relative humidity of 60±10% and photophase of 14h. Fifty insects of each species were used; each repetition was composed of 10 insects (kept in glass vials, as previously stated). For each of the species studied, the following parameters were evaluated: duration and viability of egg, larva and pupal stages, egg to adult period, sex, duration of pre-oviposition and oviposition periods, longevity and fertility. The sexual ratio was calculated by the formula: sr = female/(male + female). The duration and viability of the egg, larva and pupa stages and egg-adult period were determined by daily observations.

The longevity, fecundity and fertility of the three coccinellid species studied were evaluated from the pairing of 20 couples of each species, which were separated in transparent PVC cages (20cm in height × 10cm in diameter), containing a disc of wet filter paper, pollen and *A. kuehniella* eggs "*ad libitum*," along with the substrate for oviposition (toilet paper). The number of eggs and adult mortality were evaluated daily. The second egg laying of each couple was used to determine the embryonic period, the eggs were placed in flat-bottomed glass tubes (8.5×2.5cm). A piece of filter paper (2×1cm) was placed inside each tube, moistened daily with distilled water, and closed off at the top with plastic film. Eggs laid were counted daily as was the number of larval eclosions.

The experimental design was completely randomized. The data from each biological parameter was tested in relation to homogeneity of variance of error, and with relation to this test, no data transformations were performed. As a method to discern the different treatments, the averages of each biological parameter and each species were compared using the Tukey test (P<0.05) in SAS® (PROC: ANOVA) (SAS Institute, 2000). The possible deviation in sexual proportions (1 male for 1 female) was compared using the Chi-squared test (χ^2) (P<0.05) carried out in the SAS® (PROC FREQ) (SAS Institute, 2000). Additionally, a life and fertility table was made estimating average time between generations (T), the net reproduction rate (R_o), the intrinsic growth rate (r_m) and the finite growth rate (λ). The parameters from the life and fertility table and their respective standard errors were estimated via computer program "Lifetable.sas" (Maia et al., 2000) and compared by the bilateral t test (P<0.05) with SAS® (SAS Institute, 2000).

3. Results and Discussion

The duration of the egg phase was significantly higher for *H. axyridis* than for *E. connexa* and *O. v-nigrum* (F=604.99, df=2,10, P<0.0001). *Olla v-nigrum* was the species with the shortest larval duration (≈7 days), significantly less than *H. axyridis* and *E. connexa* (≈10 days) (F=9.35, df=2,10, P=0.0005). On the other hand, the pupal phase duration of the three coccinellid species did not differ significantly from each other (F=4.25, df=2,10, P=0.0562). The duration of the egg-adult period was longer for *H. axyridis* (≈18 days), differing significantly from that of *O. v-nigrum* (≈16 days) (F=6.45, df=2,10, P=0.0159) (Figure 1A), while *E. connexa* and *O. v-nigrum* had similar durations for the period. The duration of the cycle (larva to adult) of *E.connexa* when fed *Acyrtosiphum pisum* (Harris) (Aphididae) was 14 days at 27°C, and about 13.4 days while the prey was *Schizaphis graminum* (Rondani) (Aphididae) (Gyenge et al., 1998), if added to incubation eggs period (2.5 days), the total cycle observed is similar to our results. In contrast, the cycle period (larva to adult) of *O. v-nigrum* was 25 days when the prey was *S. graminum* (Bado and Rodríguez, 1997) in 22±4°C temperature.

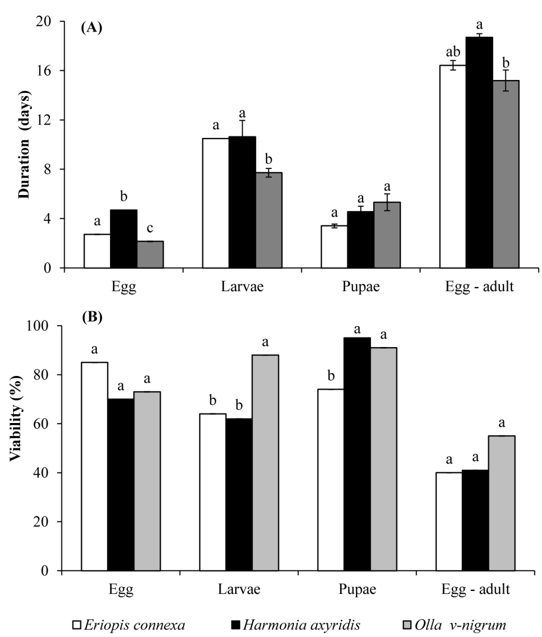

Figure 1. Duration (A) and viability (B) of egg, larval and pupal stages and egg-adult period of *E. connexa*, *H. axyridis* and *O. v-nigrum* fed with pollen and *A. kuehniella* eggs (25°C±1; RH 60±10%; 12h photophase). Means followed by same letter within the stages of development are not significantly different, as assessed by Tukey's test (P<0.05).

Regarding the viability of the different developmental stages of the coccinellids studied, all three species had similar egg viability, superior to 70% (F=1.35, df=2,10, P=0.3015) (Figure 1B). In contrast, there were significant differences on the viability of the larval stage (F=8.29, df=2,10, P=0.0075), *O. v-nigrum* had the highest viability (greater than 80%), while *E. connexa* and *H. axyridis* had similar larval viabilities (≈65%). The survival of *H. axyridis* (larval period) was greater than 70% when fed on eggs and *S. graminum* + *A. kuehniella* eggs (Santos et al., 2009). Studying different diets and time to freeze eggs of *A. kuehniella* offered as food for to *E. connexa*, Silva et al. (2009) observed that larval viability of predators was above 75% when food was diet + *A. kuehniella* eggs frozen for 24 hours. For the pupal stage, *H. axyridis* and *O. v-nigrum* presented high pupal viability (>80%), whereas *E. connexa* viability (≈70%) was significantly lower (F=2.65, df=2, 10, P=0.1194). In spite of the differences which occurred in larval and pupal viabilities among the species evaluated, the viability of the total cycle (egg-adult) did not differ significantly for all three species, varying from 45 to 55% (F=2.46, df=2,10, P=0.1348). According Silva et al. (2009), the viability of *E. connexa* ranges from 25 to 100% depending on the offered diet (artificial diet + frozen eggs *A. kuehniella*). This may be explained by the fact that working with larvae grouped together in a chamber, in an attempt to come close to the predator's habitat, leads to a competition for space or food. The exploration of the patch by the predator, as well as the levels of resources available, causes a control of the population growth due to the availability of resources and space. This was demonstrated by Grez et al. (2012) while analyzing intraguild competition between *Hippodamia variegata* (Goeze) (Coccinellidae) and *E. connexa*, where *E. connexa* turned out to be more susceptible to the changes in environment and prey size.

The longevity of *O. v-nigrum* males and females was significantly higher than that of *E. connexa* males and females (Figure 2). On the other hand, *H. axyridis* had longevity similar to that of the other two species studied. *O. v-nigrum* adults raised on *Psylla cubana Crawford* (Hemiptera: Psyllidae) presented approximately 60 days of longevity (Chazeau et al., 1991) which reveals the increase in longevity for this species when fed with *A. kuehniella* eggs. No significant differences among the species studied were seen regarding the pre-oviposition period (F=0.26, df=2,33, P=0.7708). However, *O. v-nigrum* females presented a significantly longer oviposition period than *E. connexa* and *H. axyridis* (F=20.59, df=2,33, P<0.0001). The longest oviposition period was observed for *O. v-nigrum* with about 110 days. Similarly to the pre-oviposition period, the post-oviposition periods of all three species were alike (F=0.98, df=2,33, P=0.3856) (Figure 2).

Regarding reproductive performance, *H. axyridis* had the greatest daily and total fecundity compared to *E. connexa* and *O. v-nigrum* (F=3.94, df=2,32, P=0.0296) (Table 1). The daily fecundity of *H. axyridis* was 23 eggs while for *E. connexa* and *O. v-nigrum* it was about 11 and 14 eggs day^{-1}, respectively. The sex ration was the same for all species (≈0.5).

The greater daily oviposition of *H. axyridis* had a direct effect on the total fecundity of this species, which demonstrated the capacity of laying over 1500 eggs in a life cycle, significantly higher than the oviposition capacity of *E. connexa* and *O. v-nigrum* (F=6.18, df=2,38, P=0.0047). The variation observed on fecundity and oviposition duration may be directly related to the nutritional input offered by their diet. This has been demonstrated by Santos et al. (2009), observing a change in duration of egg, larva and pupa stages and survival of *H. axyridis* when raised on

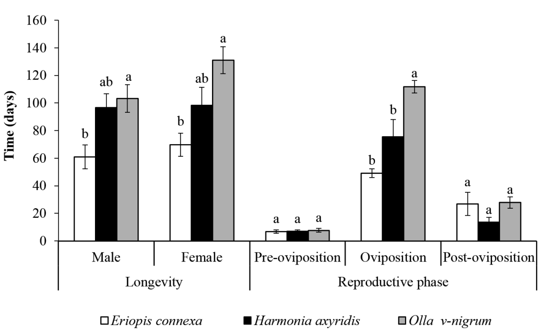

Figure 2. Longevity of males and females and duration of pré-oviposition, oviposition and post-oviposition phases (days) of *E. connexa*, *H. axyridis* and *O. v-nigrum* fed with pollen and *A. kuehniella* eggs (25°C±1; RH 60±10%; 12h photophase). Means followed by same letter within the stages of development are not significantly different, as assessed by Tukey's test (P<0.05).

A. kuehniella eggs and the aphid *S. graminum*. The direct influence of diet on the daily fecundity of the species was also demonstrated by Rivero et al. (2005) while testing different proportions between artificial and natural food for *O. v-nigrum*. The duration and viability of the larva, pupa and larva-adult stages were different when *E. connexa* was fed with different artificial diets (Silva et al., 2009).

For the parameters on the life table, there were significant differences among the coccinellid species studied. For the mean interval between generations (T), *E. connexa* and *H. axyridis* presented a similar duration (≈40 days). In contrast, *O. v-nigrum* presented in average 12 days longer (T). For the net reproductive rate (R_o) after 39.2 days (T) about 346 females are expected to result from each *H. axyridis* female (R_o), while *E. connexa and O. v-nigrum* are capable of generating 126 and 223 females/female/generation, respectively. Besides, the intrinsic growth rate (R_m) was similar between *E. connexa* and *O. v-nigrum* and superior for *H. axyridis* (Table 2). Regarding the finite rate of increase (λ), for all species the population presents a growth rate greater than 1, the population growth may, however, be considered slow. And this slow growth rate may be due to the confinement of these predators with generalist habits. The restraining of these insects to a reduced amount of space could affect their capacity of search and dispersion directly influencing their biological parameters. Another important aspect to be considered is that coccinellids have their population growth in a density-dependent relationship where the predator's growth is intimately related to the prey's population growth – a difficult condition to be simulated in laboratory experiments. This aspect may also affect the bioecology of these insects. Another important aspect noted in this study is that *H. axyridis* demonstrated greater reproductive and population growth capacities which may explain their greater reproductive success in the environment. This species may be displacing other coccinellids as seen by the high number of specimens collected in some years of collecting and the reduced number of other coccinellids

Table 1. Daily and total fecundity of *Eriopis connexa*, *Harmonia axyridis* and *Olla v-nigrum* fed with pollen and *Anagasta. kuehniella* eggs (25°C±1; RH 60±10%; photophase 12h).

Biological parameter	*E. connexa*(1)	*H. axyridis*(1)	*O. v-nigrum*(1)
Daily fecundity	14.93±2.58b	22.92±2.67a	11.51±1.90b
Total fecundity	584.0±96.50b	1565.71±236.57a	846.89±170.23b

Means followed by the same letter in the same line do not differ according to the Tukey's test (P<0.05). (1)Values represent means ± SE.

Table 2. Fertility life table of *Eriopis connexa*, *Harmonia axyridis* and *Olla v-nigrum* fed with pollen and *Anagasta. Kuehniella* eggs (25°C±1; RH 60±10%; photophase 12h).

Biological parameter	*E. connexa*(1)	*H. axyridis*(1)	*O. v-nigrum*(1)
T (days)	38.45±1.76a(1)	39.62±0.95a	50.32±3.24b
R_o	126.15±20.48b	346.55±52.40a	223.27±45.64ab
R_m	0.126±0.006b	0.148±0.004a	0.107±0.008b
λ	1.134±0.006b	1.159±0.004a	1.13±0.009c

Means followed by the same letter in the same line are not significantly different, as assessed by the "t" bilateral test (P≤0.05). (1)Values represent means ± SE.

collected in the same areas, since *H. axyridis* presents greater inter and intraguild competition and predation making it an excellent colonizer (Milléo et al., 2008). The polyphagous and voracious *H. axyridis* is also a bigger than most native coccinellid species, granting it a certain advantage in competing for food (Koch, 2003).

Rearing *E. connexa*, *H. axyridis* and *O. v-nigrum* with commercial pollen and *A. kuenhiella* eggs was viable in the laboratory since the insects completed their biological cycle with a relatively high larval viability and originated adults with good reproductive performance. The rearing of coccinellids in laboratory conditions with natural feeding is a viable alternative.

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