Development and Reproduction of Olla v-nigrum (Coleoptera: Coccinellidae) Fed Anagasta kuehniella (Lepidoptera: Pyralidae) Eggs Supplemented with an Artificial Diet

Authors: Silva, Rafael Braga Da, Cruz, Ivan, Figueiredo, Maria De Lourdes Corrêa, Tavares, Wagner De Souza, Serrão, José Eduardo, et al.

Source: Florida Entomologist, 96(3) : 850-858

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.096.0319

DEVELOPMENT AND REPRODUCTION OF *OLLA V-NIGRUM* (COLEOPTERA: COCCINELLIDAE) FED *ANAGASTA KUEHNIELLA* (LEPIDOPTERA: PYRALIDAE) EGGS SUPPLEMENTED WITH AN ARTIFICIAL DIET

RAFAEL BRAGA DA SILVA[1,*], IVAN CRUZ[1], MARIA DE LOURDES CORRÊA FIGUEIREDO[1], WAGNER DE SOUZA TAVARES[2], JOSÉ EDUARDO SERRÃO[3] AND JOSÉ COLA ZANUNCIO[4,*]

[1]Embrapa Milho e Sorgo, Rodovia MG 424, Km 65, Caixa Postal 151, 35701-970, Sete Lagoas, Minas Gerais State, Brazil

[2]Departamento de Fitotecnia, Universidade Federal de Viçosa, 36570-000, Viçosa, Minas Gerais State, Brazil

[3]Departamento de Biologia Geral, Universidade Federal de Viçosa, 36570-000, Viçosa, Minas Gerais State, Brazil

[4]Departamento de Biologia Animal, Universidade Federal de Viçosa, 36570-000, Viçosa, Minas Gerais State, Brazil

*Corresponding authors: Rafael Braga da Silva; E-mail: rafaelentomologia@yahoo.com.br; José Cola Zanuncio; E-mail: zanuncio@ufv.br

ABSTRACT

Olla v-nigrum Mulsant (Coleoptera: Coccinellidae) preys on eggs and larvae of Coleoptera and Lepidoptera and on aphids in arboreal plants. Alternative prey supplemented with artificial diets may be used to mass rearing Coccinellidae predators for biological control programs. This study assessed the development and reproduction of *O. v-nigrum* fed *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae) eggs supplemented with an artificial diet. Adults of *O. v-nigrum* were collected from *Caesalpinia peltophoroides* Benth. (Fabales: Fabaceae) and taken to the laboratory. Three male + female couples were fed on *A. kuehniella* eggs and an artificial diet (100 g of yeast, 40 g of honey, 0.5 g of ascorbic acid and 60 mL of water). Eight male + female couples obtained from this initial generation were subjected to the same procedure in order to assess the effects on subsequent generations. The mean number of eggs per egg mass was 11.7 ± 0.7 with a viability of 54.8 ± 2.9% and an incubation period of 3.1 ± 0.02 days. The durations of instars I, II, III and IV and the durations of the larval, pre-pupal, pupa and larva to adult stages were 2.4 ± 0.3, 1.8 ± 0.2, 2.0 ± 0.3, 5.8 ± 0.1, 12.1 ± 0.3, 1.0 ± 0.01, 4.0 ± 0.2 and 17.1 ± 0.5 days, respectively. The viabilities of the larval, pre-pupal and pupal stages of this predator were 46.3 ± 4.5%, 90.0 ± 5.0% and 100%, respectively. The diet and other methods employed in this study were adequate for laboratory rearing of *O. v-nigrum* but, because of the low viabilities of the eggs and immature stages observed with these methods, further improvements will be necessary for successful mass rearing of this valuable predator.

Key Words: *Anagasta kuehniella*, biological control, *Caesalpinia peltophoroides*, ladybugs, mass rearing, predator, prey, reproduction

RESUMO

Olla v-nigrum Mulsant (Coleoptera: Coccinellidae) é um predador de ovos e larvas de Coleoptera e Lepidoptera e de pulgões em plantas arbóreas. Presa alternativa suplementada com dietas artificiais pode ser utilizada na criação massal de predadores Coccinellidae predadores para programas de controle biológico. Este estudo analisou o desenvolvimento e a reprodução de *O. v-nigrum* alimentada com ovos de *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae), suplementado com uma dieta artificial. Adultos de *O. v-nigrum* foram coletados em *Caesalpinia peltophoroides* Benth. (Fabaceae) e levados para o laboratório. Três casais foram alimentados com ovos de *A. kuehniella* e uma dieta artificial (100 g de levedura, 40 g de mel, 0,5 g de ácido ascórbico e 60 mL de água). Oito casais obtidos a partir desta primeira geração foram submetidos ao mesmo procedimento, para estudar os efeitos sobre as gerações subseqüentes. O número de ovos por postura foi de 11,7 ± 0,7 com viabilidade de 54,8 ± 2,9% e período de incubação de 3,1 ± 0,02 dias. A duração dos I, II, III e IV estádios e os estágios de larva, pré-pupa e de larva a adulto foi de 2,4 ± 0,3; 1,8 ± 0,2; 2,0 ± 0,3; 5,8 ± 0,1; 12,1 ± 0,3; 1,0 ± 0,01; 4,0 ± 0,2 e 17,1 ± 0,5 dias, respectivamente. A viabilidade dos estágios de larva, pré-pupa e pupa desse predador foi de 46,3 ± 4,5%; 90,0 ± 5,0% e 100%, respectivamente. Os métodos utilizados foram adequados para a criação em laboratório de *O. v-nigrum*, mas devido à baixa viabilidade dos ovos e dos estádios imaturos outras melhorias serão necessárias para o sucesso da criação massal desse predador.

Palavras chave: *Anagasta kuehniella*, *Caesalpinia peltophoroides*, controle biológico, criação massal, joaninhas, predador, presa, reprodução

The mass rearing and release of predators into agricultural and forest areas has been an increasing biological control strategy worldwide (Pervez & Omkar 2004; Mohaghegh & Amir-Maafi 2007; Kutuk et al. 2008). However, the provision of adequate space and labor to effectively rear Coccinellidae, especially aphidophagous species, presents difficulties (Kalaskar & Evan 2001; Phoofolo et al. 2009; Silva et al. 2013), which could be alleviated by using alternative food sources (Dong et al. 2001; Silva et al. 2009; Berkvens et al. 2010).

The nutritional and ecological needs of entomophagous insects have been studied in attempts to increase their effectiveness against pests (Thompson 1999; Eubanks & Denno 2000; Kagata & Katayama 2006). Studies on the development, reproduction and behavior of predators, and mass rearing techniques can improve their potential as biological control agents, but the provision of adequate natural diets presents a major problem to rearing Coccinellidae (Specty et al. 2003; De Clercq et al. 2005).

Diets based on pig (*Sus* sp. L.; Artiodactyla: Suidae) liver with amino acid solutions have been developed for predatory insects (Arijs & De Clercq 2004; Freitas et al. 2006), with *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae) being the first one reared *in vitro* to produce fertile descendants (Kariluoto 1980; Kariluoto et al. 1976). This predator achieved a larval stage viability of 86% when fed a diet based on raw pig liver supplemented with vitamins on which the adults fed voraciously, maintaining the colony for months without live prey (Attallah & Newson 1966). Diets based on fresh pig liver were subsequently used to rear *Adalia bipunctata* L., *Coccinella septempunctata* L., *Coccinella transversoguttata richardsoni* Brown, *Hippodamia tredecimpunctata tibialis* Say, and *Propylea quatuordecimpunctata* L. (Coleoptera: Coccinellidae) (Sighinolfi et al. 2008; Bonte et al. 2010). Predatory Coccinellidae have also been reared on semi-defined, meat free diets, but usually complemented with prey species and other insects (Matsuka et al. 1982; Ware et al. 2008; Silva et al. 2009). The eggs of the Mediterranean flour moth, *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae) can be produced easily and at low cost, making this prey propitious for rearing Coccinellidae (Specty et al. 2003; De Clercq et al. 2005; Silva et al. 2009). *Anagasta kuehniella* can also be used as an alternative host for parasitoids (Oliveira et al. 2000; Eliopoulos & Stathas 2008; Tavares et al. 2009), enabling the mass rearing of a variety of natural pest enemies.

Coccinellid adults may survive on artificial diets with carbohydrate solution, but females will usually not lay eggs (Hagen 1962; Lundgren 2009), showing that this type of diet can affect insect fertility and fecundity. The establishment of effective diets is a pre-requisite for progress in coccinellid rearing (Evans et al. 1999; Cabral et al. 2006; Michaud & Jyoti 2007). *Olla v-nigrum* Mulsant (Coleoptera: Coccinellidae) is frequently found on the Brazilian forest plant *Caesalpinia peltophoroides* Benth. (Fabaceae) and other legumes feeding on *Psylla* sp. (Hemiptera: Psyllidae) (Stuart et al. 2002; Michaud 2004; Mizell 2007). This predator occurs in stable populations alongside *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), indicating that it might be a possible biological control agent of this pest (Michaud 2001). *Olla v-nigrum* is attracted to, and feeds on *Toxoptera citricida* Kirkaldy and *Aphis spiraecola* Patch (Hemiptera: Aphididae), but does not complete its larva development when feeding on these prey (Michaud & Browning 1999). While females of this predator can produce fertile eggs when feeding on *T. citricida*, the mass rearing of this prey is difficult, suggesting that an alternative, more easily bred diet is necessary to improve the number of these predators that can be reared (Michaud 2000; Michaud & Grant 2004).

The objective of this work was to study the development, reproduction, behavior and characteristics of *O. v-nigrum* reared on a diet of previously frozen *A. kuehniella* eggs supplemented with an artificial diet to devise a viable laboratory rearing method to generate stock for using this natural enemy in integrated pest management (IPM) programs.

Material and Methods

The experiment was performed in an incubator at 25 ± 1 °C, relative humidity of 70 ± 10% RH and a 14:10 h L:D, in the Laboratory of Insect Rearing (LACRI) of the Brazilian Agricultural Research Corporation (EMBRAPA) in Sete Lagoas, Minas Gerais State, Brazil. This temperature was chosen because it is suitable for rearing Coccinellidae in laboratory. Moreover, it is near the average temperature in the field in Sete Lagoas (Silva et al. 2009).

Olla v-nigrum adults were collected in a *C. peltophoroides* field plantation in Sete Lagoas and placed in glass containers (12 cm diam × 18 cm high) sealed with PVC film, perforated to allow the passage of air. A 50 mL plastic cup, sealed with a perforated acrylic seal, containing a cotton ball drenched in water; 10 g of artificial diet (100 g of yeast, 40 g of honey, 0.5 g of ascorbic acid and 60 mL of water), and a 10 × 10 cm carton containing *A. kuehniella* eggs (stored as frozen for 6 months) was placed in the bottom of each container. This diet was chosen because it had the basic ingredients for rearing of Coccinellidae in the laboratory (Silva et al. 2009). The eggs of *A. kuehniella* came from LACRI breeding stock and were fixed in the cartons using a 20: 80 liquid glue: water mixture.

The sexes of *O. v-nigrum* adults were determined after copulation in the container, because this predator does not show any reliable sexual dimorphism. Three male + female couples were formed from the 10 adults collected and of the 4 remaining those identified as females were kept in the laboratory as replacements in case any female from the formed couples died.

The *O. v-nigrum* couples selected after copulation were placed in 50 mL plastic cups, and sealed with perforated transparent acrylic lids. A total of 0.5 g of artificial diet and a 3 cm^2 carton containing *A. kuehniella* eggs were placed inside the plastic cups.

Olla v-nigrum eggs were removed daily from the rearing containers to prevent cannibalism, which commonly occurs when eggs and adult Coccinellidae are kept together (Martini et al. 2009; Sato et al. 2009; Ware et al. 2009). The eggs were transferred with a brush to a moist chamber consisting of a Petri dish with paper filter and a cotton ball moistened with distilled water, and covered with PVC film. A 3-cm^2 piece of carton with *A. kuehniella* eggs was added as food for the recently hatched *O. v-nigrum* larvae.

One day after hatching, *O. v-nigrum* larvae were individually isolated each in a 50 mL plastic cup with a transparent acrylic lid. Separating the larvae on the first day of life reduces cannibalism in predatory Coccinellidae (Michaud 2000, 2005; Pervez et al. 2006). The *O. v-nigrum* larvae were fed only *A. kuehniella* eggs (previously frozen for 6 months) offered in 3 cm^2 cartons placed inside the plastic cups, until the emergence of the adults.

Descendants from this first generation were paired. However, each individual of these pairs originated from parents different from those of other individuals. This eliminated sibling pairings and inbreeding, which has been a problem in coccinellid rearing (Morjan et al. 1999; Nakayama et al. 2010). This allowed the formation of eight couples, with adults of yellow-straw color, black color and black female + yellow-straw male pairs and black male + yellow-straw female pairs (Table 1). The parameters evaluated in this first generation were: total eggs per female and per egg mass; incubation period; egg viability; duration of first, second, third and fourth instars; duration and viability of the larva stage, pre-pupa stage, pupa stage and the larva to adult period and the sex ratio.

The experiment had an entirely randomized design. Data set were subjected to ANOVA followed by the test of Tukey ($P < 0.05$) through the computer program SAS (SAS Institute 1997) (Supplier: Federal University of Viçosa).

TABLE 1. COLOR (YELLOW-STRAW OR BLACK) OF THE FIRST GENERATION OF MALE + FEMALE COUPLES OF *OLLA V-NIGRUM* AT 25 ± 1 °C, 70 ± 10% RH AND 14:10 H L:D.

Couple No.	Color of the male	Color of the female
1	black	black
2	black	black
3	black	yellow-straw
4	black	yellow-straw
5	yellow-straw	yellow-straw
6	yellow-straw	yellow-straw
7	yellow-straw	black
8	yellow-straw	black

RESULTS

Olla v-nigrum has 4 instars. The biological parameters of the progeny from the 8 couples are summarized in Table 2, and those of the progeny of each couple are summarized in Table 3. The total egg masses numbers laid per female were 10.1 ± 2.0 with 11.7 ± 0.7 eggs each. Eggs were laid in a single layer, occasionally dispersed and were elliptical in shape and light yellow in color, until near hatching, when they turned grayish. The smallest egg mass contained one egg and the largest, 33 eggs (Table 2).

The embryonic period of *O. v-nigrum* was 3.1 ± 0.02 days and its egg viability was 54.8 ± 2.9% (Table 2).

Larvae of *O. v-nigrum* are campodeiform with an elongated body, distinct abdominal segmentation and well developed legs. Soon after hatching, they remained immobile and aggregated around the eggs, feeding on the infertile ones and the remainder of the chorion. As they approached

TABLE 2. BIOLOGICAL PARAMETERS (MEAN ± STANDARD DEVIATION) AND RANGE OF *OLLA V-NIGRUM* MALE + FEMALE COUPLES FED EGGS OF *ANAGASTA KUEHNIELLA* (FROZEN FOR 6 MONTHS) SUPPLEMENTED WITH AN ARTIFICIAL DIET AT 25 ± 1 °C, 70 ± 10% RH AND 14:10 H L:D.

Parameter assessed	Mean ± SD	Range
Total eggs per egg mass	11.7 ± 0.7	1 - 33
Incubation period (days)	3.1 ± 0.02	2 - 4
Egg viability (%)	54.8 ± 2.9	10.0 - 100.0
First instar (days)	2.4 ± 0.3	2.0 - 3.0
Second instar (days)	1.9 ± 0.2	1.0 - 2.0
Third instar (days)	2.0 ± 0.3	2.0 - 3.0
Fourth instar (days)	5.8 ± 0.1	4.0 - 6.0
Larval stage (days)	12.1 ± 0.3	9.0 - 14.0
Larval viability (%)	46.3 ± 4.5	20.0 - 80.0
Pre-pupal stage (days)	1.0 ± 0.01	1.0 - 2.0
Pre-pupal viability (%)	90.0 ± 2.0	80.0 - 100.0
Pupal stage (days)	4.0 ± 0.2	3.0 - 5.0
Pupal viability (%)	100.0 ± 0.0	—
Larval/adult stage (days)	17.1 ± 0.5	17.0 - 20.0
Larval/adult viability (%)	25.0 ± 5.0	20.0 - 80.0
Sex ratio (%)	0.61 ± 0.01	0.40 - 0.65

instar change they stopped feeding, and fixed themselves by the last abdominal segment onto the surface of the acrylic lids on the sides of the 50 mL plastic cups, to await ecdysis.

The durations of the first, second, third and fourth instar were 2.4 ± 0.3, 1.9 ± 0.2, 2.0 ± 0.3 and 5.8 ± 0.1 days, respectively. The duration of the pre-pupal stage was 1.0 ± 0.01 day, the pupal stage was 4.0 ± 0.2 days, and the period from egg to adult was 20.2 ± 0.8 days (Table 2).

The viability of the larva, pre-pupa and pupa stage was 46.3 ± 4.5, 90.0 ± 5.0 and 100.0%, respectively. The embryonic period was 3.1 ± 0.02 days and the viability of the eggs was 54.8 ± 2.9% (Table 2).

The sex ratio of the hatched larvae was 0.61 ± 0.01 (Table 2). Of the emerged adults, 77% had black coloration (55% being females and 45% males) and 23% were yellow-straw (80% being females and 20% males). Black colored *O. v-nigrum* adults were obtained from all couples formed and in higher numbers than those of yellow-straw color (Table 3).

Total eggs per egg mass was higher for couples with a black male and a yellow straw female (Table 3).

The first egg mass was smaller and a tendency was found towards an increasing number of eggs in those egg masses laid later (Fig. 1A). Egg viability varied considerably, but it was higher in the last egg masses than in the first ones (Fig. 1B).

Discussion

The number of instars of *O. v-nigrum* was similar to that of *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae) feeding on *Aphis fabae* Scopoli, *Myzus persicae* Sulzer (Hemiptera: Aphididae), and *Aleyrodes proletella* L. (Hemiptera: Aleyrodidae) (Cabral et al. 2006), and, also of *Scymnus* (*Neopullus*) *sinuanodulus* Yu & Yao (Coleoptera: Coccinellidae) on leaves of *Tsuga canadensis* L. (Pinaceae) infested with *Adelges tsugae* Annand (Hemiptera: Adelgidae) (Lu et al. 2002). These comparisons show that the number of instars is not dependent on the diet. This suggests that our artificial diet plus *A. kuehniella* eggs were adequate for the development of *O. v-nigrum* instars. It is essential to hold the *O. v-nigrum* larvae singly in order to avoid cannibalism by larvae and adults, which is common in many Coccinellidae species (Michaud 2000, 2005; Pervez et al. 2006). This measure can increase the total number of insects mass reared for IPM programs.

The oviposition behavior and egg color of *O. v-nigrum* were similar to those of *Hippodamia variegata* Goeze (Coleoptera: Coccinellidae) reared on *Dysaphis crataegi* Kaltenbach (Hemiptera: Aphididae) (Kontodimas & Stathas 2005) and *Rhyzobius lophanthae* Blaisdell (Coleoptera: Coccinellidae) fed on *Aspidiotus nerii* Bouché (Hemiptera: Diaspididae) (Stathas 2000), showing that these are characteristics of the family Coccinellidae.

The low viability of the *O. v-nigrum* eggs and larvae from Sete Lagoas, Minas Gerais State, Brazil may be explained by inbreeding, because eggs from healthy and heterogeneous couples may have higher viability and more eggs per egg mass (Morjan et al. 1999; Nakayama et al. 2010). Indeed a total of 40 eggs per egg mass with 94.5% viability was reported for healthy *O. v-nigrum* females from Polk County, Florida (Michaud & Grant 2004).

The behavior and characteristics of *O. v-nigrum* larvae were similar to those of *C. maculata*, *Cycloneda munda* Say, *Hippodamia convergens* Guérin-Méneville, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) (Cottrell 2007) and *S. sinuanodulus* fed on *A. tsugae* (Lu et al. 2002), showing that the characteristics documented are those common to coccinellid predators.

The durations of the instars and of larval stage of *O. v-nigrum* were comparable to other Coccinellidae, but these parameters may vary with the prey provided. The duration of larval development of *C. septempunctata* was shorter when fed *Lipaphis erysimi* Kaltenbach (Hemiptera: Aphididae) than when fed 5 other species of aphids, which was attributed to *L. erysimi* having a higher level of protein (Omkar & Srivastava 2003). The longer duration of *O. v-nigrum* larva stage indicates the need to accumulate nutrients, perhaps because of increased metabolism in the following stage (Scriber & Slansky Jr. 1981; Thompson 1999). Food quality may affect the development and survival of coccinellid larvae (Kalushkov & Hodek 2001, 2004; Isikber & Copland 2002). However, differences in the morphology, behavior and chemical composition of prey can affect the development of these predators, as seen in *Propylea dissecta* Mulsant (Coleoptera: Coccinellidae) and *C. septempunctata* fed on different aphid species (Omkar & Srivastava 2003; Omkar & Mishra 2005).

The durations of the pre-pupal, pupal and egg to adult stages of *O. v-nigrum* were considered satisfactory compared to other coccinellids. The pre-pupal stage started when the larvae completed their development and stopped feeding and fixed themselves by the last abdominal segment to the surface of the acrylic lids or on the sides of the 50 mL plastic cups. In these positions they remained immobile, but when taunted they reacted with abrupt movements. The pre-pupa stage of *O. v-nigrum* presents a different pattern than that of *S. sinuanodulus*, whose pre-pupal stage starts when the larvae stop feeding, release a great quantity of liquid from the anal region and become immobile for 1 to 2 days (Lu et al. 2002). This behavior was displayed by some *O. v-nigrum*

TABLE 3. BIOLOGICAL PARAMETERS (MEAN ± STANDARD DEVIATION) OF *OLLA V-NIGRUM* MALE + FEMALE COUPLES FED *ANAGASTA KUEHNIELLA* EGGS (FROZEN FOR 6 MONTHS) SUPPLEMENTED WITH AN ARTIFICIAL DIET AT 25 ± 1 °C, 70 ± 10% RH AND 14:10 H L:D.

Parameter assessed	Couple No.								CV (%)
	1	2	3	4	5	6	7	8	
Total eggs per egg mass	12.8 ± 2.9 a	8.5 ± 2.2 b	12.2 ± 1.7 a	13.3 ± 1.4 a	10.6 ± 2.7 ab	7.5 ± 1.7 c	12.1 ± 1.4 a	6.0 ± 2.6 c	26.1
Incubation period (days)	3.2 ± 0.0 a	3.0 ± 0.0 a	3.0 ± 0.0 a	3.0 ± 0.0 a	3.0 ± 0.0 a	3.0 ± 0.0 a	3.0 ± 0.04 a	3.0 ± 0.0 a	3.3
Egg viability (%)	65.3 ± 5.9 a	50.0 ± 11.0 b	51.0 ± 6.6 b	46.6 ± 8.4 b	50.9 ± 15.0 b	61.5 ± 14.0 a	52.5 ± 5.4 b	57.7 ± 15.9 ab	11.7
First instar (days)	2.4 ± 0.6 ab	2.0 ± 0.3 b	2.8 ± 0.2 a	2.5 ± 0.5 a	2.1 ± 0.1 b	2.6 ± 0.3 a	2.4 ± 0.3 ab	2.4 ± 0.3 ab	10.6
Second instar (days)	1.9 ± 0.1 a	1.9 ± 0.1 a	1.8 ± 0.2 a	1.9 ± 0.1 a	2.0 ± 0.0 a	1.8 ± 0.2 a	1.9 ± 0.1 a	2.0 ± 0.0 a	3.9
Third instar (days)	2.0 ± 0.0 a	2.0 ± 0.1 a	2.0 ± 0.6 a	2.0 ± 1.0 a	2.0 ± 0.4 a	2.0 ± 0.0 a	2.0 ± 0.1 a	2.0 ± 0.2 a	0.0
Fourth instar (days)	5.8 ± 0.2 a	4.9 ± 0.8 b	5.9 ± 0.1 a	5.9 ± 0.1 a	6.0 ± 0.0 a	6.0 ± 0.0 a	5.9 ± 0.1 a	6.0 ± 0.0 a	6.3
Larval stage (days)	12.1 ± 0.2 a	10.8 ± 0.3 b	12.5 ± 0.3 a	12.3 ± 0.4 a	12.1 ± 0.1 a	12.4 ± 0.1 a	12.2 ± 0.1 a	12.4 ± 0.1 a	4.5
Larval viability (%)	40.5 ± 6.0 c	48.3 ± 1.5 b	67.8 ± 2.9 a	30.9 ± 9.1 c	39.5 ± 4.5 c	27.0 ± 7.5 c	76.4 ± 3.0 a	40.0 ± 1.5 c	37.4
Pre-pupal stage (days)	1.0 ± 0.0 a	1.2 ± 0.0 a	1.0 ± 0.0 a	1.0 ± 0.0 a	1.0 ± 0.0 a	1.0 ± 0.0 a	1.0 ± 0.0 a	1.0 ± 0.0 a	6.8
Pre-pupal viability (%)	100.0 ± 0.0 a	90.0 ± 5.0 b	80.0 ± 5.0 c	80.0 ± 2.0 c	100.0 ± 0.0 a	90.0 ± 2.0 b	90.0 ± 2.0 b	90.0 ± 1.0 b	8.3
Pupal stage (days)	3.6 ± 0.2 b	4.2 ± 0.2 a	4.8 ± 0.1 a	3.0 ± 0.1 b	4.5 ± 0.3 a	4.0 ± 0.7 a	4.9 ± 0.1 a	3.0 ± 0.2 b	18.6
Pupal viability (%)	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	0.0
Larval/adult stage (days)	16.7 ± 0.1 b	16.2 ± 0.1 b	18.3 ± 0.2 a	16.3 ± 0.2 b	17.6 ± 0.1 a	17.4 ± 0.4 a	18.1 ± 0.1 a	16.4 ± 0.1 b	4.8
Larval/adult viability (%)	30.0 ± 5.0 a	20.0 ± 6.0 b	30.0 ± 7.0 a	35.0 ± 10.0 a	20.0 ± 5.0 b	20.0 ± 4.0 b	25.0 ± 1.0 ab	30.0 ± 2.0 a	22.1
Sex ratio[1]	0.60 ± 0.0 b	0.54 ± 0.0 b	0.52 ± 0.0 c	0.70 ± 0.0 a	0.60 ± 0.0 b	0.62 ± 0.0 a	0.60 ± 0.0 b	0.70 ± 0.0 a	10.6

[1]Number of females/number of females + males. Averages followed by the same small case letter in each row, do not differ by Tukey's test ($P < 0.05$). CV = Coefficient of variation.

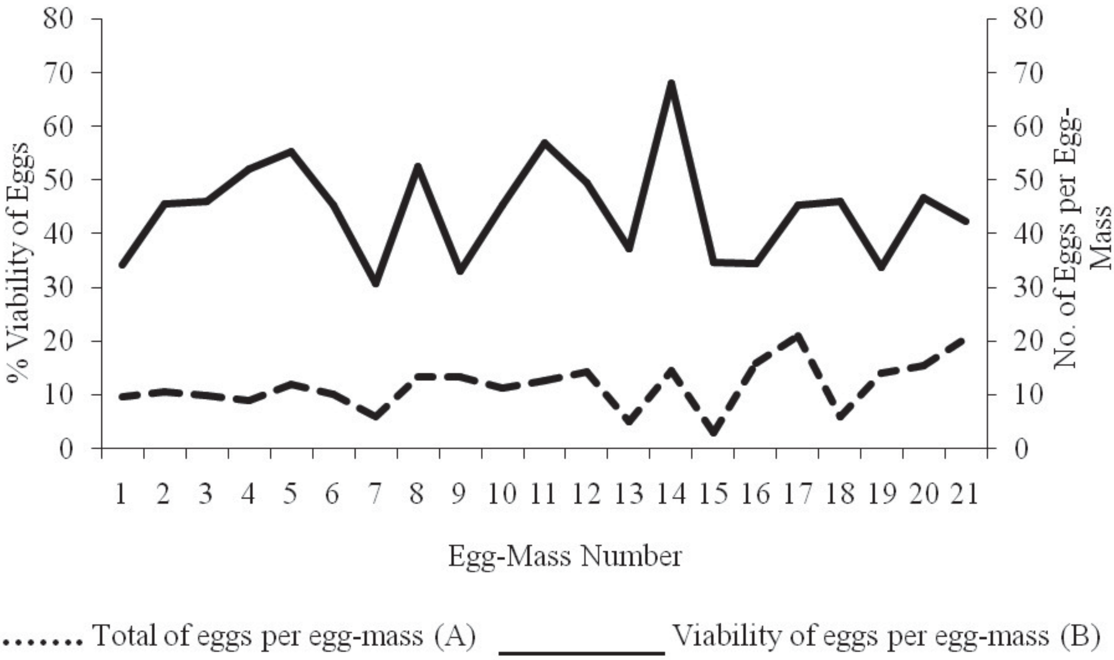

Fig. 1. Production and viability of eggs in successive egg-masses laid by of *Olla v-nigrum* fed on eggs of *Anagasta kuehniella* (frozen for 6 mo) supplemented with an artificial diet at 25 ± 1 °C, 70 ± 10% RH and 14:10 h L:D. Line A (dashed line) shows the total eggs/egg-mass. Line B (solid line) shows percent viability of eggs per egg-mass.

larvae, but *S. sinuanodulus* larvae may display yet another different behavior (Lu et al. 2002) in that after the immobile period, they crawl and only after this period are the pupae formed. However, a high percentage of *S. sinuanodulus* pupae showing this behavior died soon after (Lu & Montgomery 2001; Lu et al. 2002). The crawling behavior of *S. sinuanodulus* larvae is associated with a dispersal mechanism to seek places to feed and to hide for pupation (Lu et al. 2002).

The greater viability of the larval, pre-pupal and pupal stages of *O. v-nigrum* that fed on an artificial diet and *A. kuehniella* eggs than the viability of those that fed on recently-hatched *S. frugiperda* armyworm larvae may be due to nutritional factors, because the growth rates, larval survival and reproduction of Coccinellidae are associated with prey quality. The best larval performance of Coccinellidae is achieved with prey having a high level of protein and that are eaten in large quantities (Omkar & Srivastava 2003; Zhang et al. 2007). The viabilities of pre-pupal and pupal stages of *O. v-nigrum* were not negativel affected by the artificial diet and *A. kuehniella* eggs, in contrast to the reduced viability of eggs, and larvae. This suggests that additional nutrients are needed in this artificial diet, such as essential amino acids and dietary minerals, so as to reflect the outcome of the generalist feeding behavior of Coccinellidae in the wild, where a variety of prey and pollen grains are eaten (Isikber & Copland 2002; Ragkou et al. 2004; Berkvens et al. 2008). Coccinellid larvae rarely complete the first instar with inadequate and/or of poor quality food, such as when lacking a specific nutrient (Michaud 2005). This was observed in *Coleomegilla maculata fuscilabris* Mulsant (Coleoptera: Coccinellidae) whose development was not completed when fed only on *T. citricida*, but it survived when this prey was supplemented with a suitable artificial diet fed to the first instar (Michaud 2000). *Anagasta kuehniella* eggs, previously frozen for 6 months, may have lost some of their nutritional value thus compromising the development of *O. v-nigrum*. However, this food has great advantages in insect rearing programs due to its ease of storage and lower associated costs compared to alternative fresh prey (Adams 2000; Mohaghegh & Amir-Maafi 2007).

Olla v-nigrum is a holometabolic insect with exarate pupae with free and visible appendages. Initially the pupa has a light coloration that slowly darkens, acquiring the characteristic spots of the Coccinellinae subfamily (Hagen 1962). Soon after emergence the adult develops a lighter color and remain immobile within the exuvia until the normal color develops. Black colored adults continue to darken to a glossy black, the macula of their elytra becoming orange. Adults with the yellow-straw coloration increase the light tone of their maculas on the black-colored elytra. Common characteristics of *O. v-nigrum* adults are the gray-colored membranous wings, capitada-shaped antennae and black legs. The color pattern of *O. v-nigrum* does not differ between the sexes, but this species is classified as dichromatic, due to sex-linked frequencies of the alleles of a single gene. This was observed for *H. axyridis* in which color variations are caused by 5 alleles of a single gene (Morjan et al. 1999).

Olla v-nigrum females were on average more robust than males and this feature could be used to sex individuals, but more robust males are not uncommon. Copulations were frequent, despite the different color combinations of the eight couples that were formed from the first generation.

Cannibalism was observed in the adult stage; both males and females eat eggs, making it essential to keep individuals separated or isolated. Adults were also observed to cannibalize other adults with physical anomalies. The sex ratio of *O. v-nigrum* was similar to those reported for *H. convergens* and *C. septempunctata* fed on *Myzus persicae nicotinae* Sulzer (Hemiptera: Aphididae) (Katsarou et al. 2005).

The total percentage of *O. v.nigrum* with black or yellow-straw coloration was similar to that of *Psylla* sp. (Kato et al. 1999), but the sex ratio differed from that of these authors, who obtained 50% *O. v-nigrum* male or female with black coloring and 46.7% of males and 53.3% females of this predator with straw-yellow color.

The diet and other methods employed in this study were adequate for the laboratory rearing of *O. v-nigrum*, but because of the low viabilities of the eggs and immature stages, further improvements are necessary for successful mass rearing this valuable predator.

Acknowledgments

To Dr. J. P. Michaud (Department of Entomology, Agricultural Research Center, Kansas State University, United States) and Dr. S. N. Thompson (Department of Entomology, College of Natural and Agricultural Sciences, University of California, United States) for reviewing the manuscript. To "Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)", "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)" and "Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG)" for financial support. To the Group Solucion for translating this manuscript. To Asia Science Editing of the Republic of Ireland for reviewing and editing this manuscript.

References Cited

ADAMS, T. S. 2000. Effect of diet and mating status on ovarian development in a predaceous stink bug *Perillus bioculatus* (Hemiptera: Pentatomidae). Ann. Entomol. Soc. Am. 93(3): 529-535.

ARIJS, Y., AND DE CLERCQ, P. 2004. Liver-based artificial diets for the production of *Orius laevigatus*. BioControl 49(5): 505-516.

ATTALLAH, Y. H., AND NEWSON, L. D. 1966. Ecological and nutritional studies on *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae). I. The development of an artificial diet and laboratory rearing technique. J. Econ. Entomol. 59(5): 1173-1179.

BERKVENS, N., LANDUYT, C., DEFORCE, K., BERKVENS, D., TIRRY, L., AND DE CLERCQ, P. 2010. Alternative foods for the multicoloured Asian lady beetle *Harmonia axyridis* (Coleoptera: Coccinellidae). Eur. J. Entomol. 107(2): 189-195.

BERKVENS, N., BONTE, J., BERKVENS, D., DEFORCE, K., TIRRY, L., AND DE CLERCQ, P. 2008. Pollen as an alternative food for *Harmonia axyridis*. BioControl 53(1): 201-210.

BONTE, M., SAMIH, M. A., AND DE CLERCQ, P. 2010. Development and reproduction of *Adalia bipunctata* on factitious and artificial foods. BioControl 55(4): 485-491.

CABRAL, S., SOARES, A. O., MOURA, R., AND GARCIA, P. 2006. Suitability of *Aphis fabae*, *Myzus persicae* (Homoptera: Aphididae) and *Aleyrodes proletella* (Homoptera: Aleyrodidae) as prey for *Coccinella undecimpunctata* (Coleoptera: Coccinellidae). Biol. Control 39(3): 434-440.

COTTRELL, T. E. 2007. Predation by adult and larval lady beetles (Coleoptera: Coccinellidae) on initial contact with lady beetle eggs. Environ. Entomol. 36(2): 390-401.

DE CLERCQ, P., BONTE, M., VAN SPEYBROECK, K., BOLCKMANS, K., AND DEFORCE, K. 2005. Development and reproduction of *Adalia bipunctata* (Coleoptera: Coccinellidae) on eggs of *Ephestia kuehniella* (Lepidoptera: Phycitidae) and pollen. Pest Mgt. Sci. 61(11): 1129-1132.

DE OLIVEIRA, H. N., ZANUNCIO, J. C., PRATISSOLI, D., AND CRUZ, I. 2000. Parasitism rate and viability of *Trichogramma maxacalii* (Hym.: Trichogrammatidae) parasitoid of the *Eucalyptus* defoliator *Euselasia apison* (Lep.: Riodinidae), on eggs of *Anagasta kuehniella* (Lep.: Pyralidae). Forest Ecol. Mgt. 130(1-3): 1-6.

DONG, H., ELLINGTON, J. J., AND REMMENGA, M. D. 2001. An artificial diet for the lady beetle *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). Southwest. Entomol. 26(3): 205-213.

ELIOPOULOS, P. A., AND STATHAS, G. J. 2008. Life tables of *Habrobracon hebetor* (Hymenoptera: Braconidae) parasitizing *Anagasta kuehniella* and *Plodia interpunctella* (Lepidoptera: Pyralidae): Effect of host density. J. Econ. Entomol. 101(3): 982-988.

EUBANKS, M. D., AND DENNO, R. F. 2000. Health food versus fast food: the effects of prey quality and mobility on prey selection by a generalist predator and indirect interactions among prey species. Ecol. Entomol. 25(2): 140-146.

EVANS, E. W., STEVENSON, A. T., AND RICHARDS, D. R. 1999. Essential versus alternative foods of insect predators: benefits of a mixed diet. Oecologia 121(1): 107-112.

FREITAS, S. P. C., EVANGELISTA JÚNIOR, W. S., ZANUNCIO, J. C., AND SERRÃO, J. E. 2006. Development, survival and reproduction of *Podisus nigrispinus* (Dallas, 1851) (Heteroptera: Pentatomidae) with salt and amino acids solutions supplementary diet. Braz. Arch. Biol. Technol. 49(3): 449-455.

HAGEN, K. S. 1962. Biology and ecology of predaceous Coccinellidae. Annu. Rev. Entomol. 7(35): 289-326.

ISIKBER, A. A., AND COPLAND, M. J. W. 2002. Effects of various aphid foods on *Cycloneda sanguinea*. Entomol. Exp. Appl. 102(1): 93-97.

KAGATA, H., AND KATAYAMA, N. 2006. Does nitrogen limitation promote intraguild predation in an aphidophagous ladybird? Entomol. Exp. Appl. 119(3): 239-246.

KALASKAR, A., AND EVAN, E. W. 2001. Larval responses of aphidophagous lady beetles (Coleoptera: Coccinellidae) to weevil larvae versus aphids as prey. Ann. Entomol. Soc. Am. 94(1): 76-81.

KALUSHKOV, P., AND HODEK, I. 2001. New essential aphid prey for *Anatis ocellata* and *Calvia quatuordecimguttata* (Coleoptera: Coccinellidae). Biocontrol Sci. Technol. 11(1): 35-39.

KALUSHKOV, P., AND HODEK, I. 2004. The effects of thirteen species of aphids on some life history parameters of the ladybird *Coccinella septempunctata*. BioControl 49(1): 21-32.

KARILUOTO, K. T. 1980. Survival and fecundity of *Adalia bipunctata* (Coleoptera: Coccinellidae) and some other predatory insect species on an artificial diet a natural prey. Ann. Entomol. Fenn. 46(4): 101-106.

KARILUOTO, K. T., JUNNIKKALA, E., AND MARKKULA, M. 1976. Attempts at rearing *Adalia bipunctata* L. (Col., Coccinellidae) on different artificial diets. Ann. Entomol. Fennici 42(2): 91-97.

KATO, C. M., AUAD, A. M., AND BUENO, V. H. P. 1999. Biological and ethological aspects of *Olla v-nigrum* (Mulsant, 1866) (Coleoptera: Coccinellidae) on *Psylla* sp. (Homoptera: Psyllidae). Cienc. Agrotec. 23(1): 19-23.

KATSAROU, I., MARGARITOPOULOS, J. T., TSITSIPIS, J. A., PERDIKIS, D. C., AND ZARPAS, K. D. 2005. Effect of temperature on development, growth and feeding of *Coccinella septempunctata* and *Hippodamia convergens* reared on the tobacco aphid, *Myzus persicae nicotianae*. BioControl 50(4): 565-588.

KONTODIMAS, D. C., AND STATHAS, G. J. 2005. Phenology, fecundity and life table parameters of the predator *Hippodamia variegata* reared on *Dysaphis crataegi*. BioControl 50(2): 223-233.

KUTUK, H., YIGIT, A., AND ALAOGLU, O. 2008. The effect of season on the levels of predation by the ladybird *Serangium parcesetosum* Sicard (Coleoptera: Coccinellidae) on the cotton whitefly *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae), a serious pest of eggplants. J. Pest Sci. 81(4): 207-212.

LU, W. H., AND MONTGOMERY, M. E. 2001. Oviposition, development, and feeding of *Scymnus* (*Neopullus*) *sinuanodulus* (Coleoptera: Coccinellidae): a predator of *Adelges tsugae* (Homoptera: Adelgidae). Ann. Entomol. Soc. Am. 94(1): 64-70.

LU, W. H., SOUPHANYA, P., AND MONTGOMERY, M. E. 2002. Descriptions of immature stages of *Scymnus* (*Neopullus*) *sinuanodulus* Yu and Yao (Coleoptera: Coccinellidae) with notes on life history. Coleopts. Bull. 56(1): 127-141.

LUNDGREN, J. G. 2009. Nutritional aspects of non-prey foods in the life histories of predaceous Coccinellidae. Biol. Control 51(2): 294-305.

MARTINI, X., HACCOU, P., OLIVIERI, I., AND HEMPTINNE, J. L. 2009. Evolution of cannibalism and female's response to oviposition-deterring pheromone in aphidophagous predators. J. Anim. Ecol. 78(5): 964-972.

MATSUKA, M., WATANABE, M., AND NIIJIMA, K. 1982. Longevity and oviposition of vedalia beetles on artificial diets. Environ. Entomol. 11(4): 816-819.

MICHAUD, J. P. 2000. Development and reproduction of ladybeetles (Coleoptera: Coccinellidae) on the citrus aphids *Aphis spiraecola* Patch and *Toxoptera citricida* (Kirkaldy) (Homoptera: Aphididae). Biol. Control 18(3): 287-297.

MICHAUD, J. P. 2001. Numerical response of *Olla v-nigrum* (Coleoptera: Coccinellidae) to infestation of Asian citrus psyllid, (Hemiptera: Aphididae) in Florida. Fla Entomol. 84(4): 608-612.

MICHAUD, J. P. 2004. Natural mortality of Asian citrus psyllid (Homoptera: Psyllidae) in central Florida. Biol. Control 29(2): 260-269.

MICHAUD, J. P. 2005. On the assessment of prey, suitability in aphidophagous Coccinellidae. Eur. J. Entomol. 102(3): 385-390.

MICHAUD, J. P., AND BROWNING, H. W. 1999. Seasonal abundance of the brown citrus aphid, *Toxoptera citricida* (Homoptera: Aphididae) and its natural enemies in Puerto Rico. Fla Entomol. 82(3): 424-447.

MICHAUD, J. P., AND GRANT, A. K. 2004. Adaptive significance of sibling egg cannibalism in coccinellidae: Comparative evidence from three species. Ann. Entomol. Soc. Am. 97(4): 710-719.

MICHAUD, J. P., AND JYOTI, J. L. 2007. Dietary complementation across life stages in the polyphagous lady beetle *Coleomegilla maculata*. Entomol. Exp. Appl. 126(1): 40-45.

MIZELL, R. F. 2007. Impact of *Harmonia axyridis* (Coleoptera: Coccinellidae) on native arthropod predators in pecan and crape myrtle. Florida Entomol. 90(3): 524-536.

MOHAGHEGH, J., AND AMIR-MAAFI, M. 2007. Reproduction of the predatory stinkbug *Andrallus spinidens* (F.) (Heteroptera: Pentatomidae) on live and frozen prey. Appl. Entomol. Zool. 42(1): 15-20.

MORJAN, W. E., OBRYCKI, J. J., AND KRAFSUR, E. S. 1999. Inbreeding effects on *Propylea quatuordecimpunctata* (Coleoptera: Coccinellidae). Ann. Entomol. Soc. Am. 92(2): 260-268.

NAKAYAMA, S., SEKO, T., TAKATSUKI, J. I., MIURA, K., AND MIYATAKE, T. 2010. Walking activity of flightless *Harmonia axyridis* (Coleoptera: Coccinellidae) as a biological control agent. J. Econ. Entomol. 103(5): 1564-1568.

OMKAR, AND MISHRA, G. 2005. Preference-performance of a generalist predatory ladybird: a laboratory study. Biol. Control 34(2): 187-195.

OMKAR., AND SRIVASTRA, S. 2003. Influence of six aphid prey species on development and reproduction of ladybird beetle, *Coccinella septempunctata*. BioControl 48(4): 379-393.

PERVEZ, A., AND OMKAR. 2004. Prey-dependent life attributes of an aphidophagous ladybird beetle, *Propylea dissecta* (Coleoptera: Coccinellidae). Biocontrol Sci. Technol. 14(4): 385-396.

PERVEZ, A., GUPTA, A. K., AND OMKAR. 2006. Larval cannibalism in aphidophagous ladybirds: Influencing factors, benefits and costs. Biol. Control 38(3): 307-313.

PHOOFOLO, M. W., ELLIOTT, N. C., AND GILES, K. L. 2009. Analysis of growth and development in the final instar of three species of predatory Coccinellidae under varying prey availability. Entomol. Exp. Appl. 131(3): 264-277.

RAGKOU, V. S., ATHANASSIOU, C. G., KAVALLIERATUS, N. G., AND TOMANOVIC, Z. 2004. Daily consumption and predation rate of different *Stethorus punctillum* instars feeding on *Tetranychus urticae*. Phytoparasitica 32(2): 154-159.

SAS, INSTITUTE SAS. 1997. User's guide: statistics. SAS Institute Cary, NC, USA.

SATO, S., YASUDA, H., EVANS, E. W., AND DIXON, A. F. G. 2009. Vulnerability of larvae of two species of aphidophagous ladybirds, *Adalia bipunctata* Linnaeus and *Harmonia axyridis* Pallas, to cannibalism and intraguild predation. Entomol. Sci. 12(2): 111-115.

SCRIBER, J. M., AND SLANSKY JR., F. 1981. The nutritional ecology of immature insects. Annu. Rev. Entomol. 26(1): 183-211.

SIGHINOLFI, L., FEBVAY, G., DINDO, M. L., REY, M., PAGEAUX, J. F., BARONIO, P., AND GRENIER, S. 2008. Biological and biochemical characteristics for quality control of *Harmonia axyridis* (Pallas) (Coleoptera, Coccinellidae) reared on a liver-based diet. Arch. Insect Biochem. 68(1): 26-39.

SILVA, R. B., ZANUNCIO, J. C., SERRÃO, J. E., LIMA, E. R., FIGUEIREDO, M. L. C., AND CRUZ, I. 2009. Suitability of different artificial diets for development and survival of stages of predaceous ladybird beetle *Eriopis connexa* (Coleoptera: Coccinellidae). Phytoparasitica 37(2): 115-123.

SILVA, R. B., CRUZ, I., ZANUNCIO, J. C., FIGUEIREDO, M. D. C., ZANUNCIO, T. V., AND SERRÃO, J. E. 2013. *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) eggs as alternative food for rearing of lady beetles *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae). Biol. Control 64(2): 101-105.

SPECTY, O., FEBVAY, G., GRENIER, S., DELOBEL, B., PIOTTE, C., PAGEAUX, J. F., FERRAN, A., AND GUILLAUD, J. 2003. Nutritional plasticity of the predatory ladybeetle *Harmonia axyridis* (Coleoptera: Coccinellidae) comparison between natural and substitution prey. Arch. Insect Biochem. 52(2): 81-91.

STATHAS, G. J. 2000. The effect of temperature on the development of the predator *Rhyzobius lophanthae* and its phenology in Greece. BioControl 45(4): 439-451.

STUART, R. J., MICHAUD, J. P., OLSEN, L., AND MCCOY, C. W. 2002. Lady beetles as potential predators of the root weevil *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in Florida citrus. Fla Entomol. 85(3): 409-416.

TAVARES, W. D., CRUZ, I., PETACCI, F., DE ASSIS, S. L., FREITAS, S. D., ZANUNCIO, J. C., AND SERRÃO, J. E. 2009. Potential use of Asteraceae extracts to control *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and selectivity to their parasitoids *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) and *Telenomus remus* (Hymenoptera: Scelionidae). Ind. Crop Prod. 30(3): 384-388.

THOMPSON, S. N. 1999. Nutrition and culture of entomophagous insects. Annu. Rev. Entomol. 44(1): 561-592.

WARE, R., YGUEL, B., AND MAJERUS, M. 2009. Effects of competition, cannibalism and intra-guild predation on larval development of the European coccinellid *Adalia bipunctata* and the invasive species *Harmonia axyridis*. Ecol. Entomol. 34(1): 12-19.

WARE, R. L., YGUEL, B., AND MAJERUS, M. E. N. 2008. Effects of larval diet on female reproductive output of the European coccinellid *Adalia bipunctata* and the invasive species *Harmonia axyridis* (Coleoptera: Coccinellidae). Eur. J. Entomol. 105(3): 437-443.

ZHANG, S. Z., ZHANG, F., AND HUA, B. Z. 2007. Suitability of various prey types for the development of *Propylea japonica* (Coleoptera: Coccinellidae). Eur. J. Entomol. 104(1): 149-152.