DEVELOPMENT OF *Coleomegilla maculata* DE GEER (COLEOPTERA: COCCINELLIDAE) WITH PREY AND ARTIFICIAL DIET

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ABSTRACT - *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae) is an important predator and has the potential to be used as a biological control agent of insect pests of maize (*Zea mays* L.) and sorghum [*Sorghum bicolor* (L.) Moench]. This study aimed to evaluate the development of *C. maculata* larvae fed eggs *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) alone or associated with artificial diet, eggs of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) and nymphs of *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae). The experiment was conducted in a complete randomized design with four replications, each with 10 larvae of *C. maculata* in an acclimatized room (25 ± 1 ºC, 70 ± 10% RH and 12 hours photophase). The duration of the stages of *C. maculata* was similar in all treatments, except for one that used nymphs of *S. graminum*, resulting in a shorter period for the larval stage. The viability of larval, prepupal and pupal stages and larval to adult *C. maculata* was high and similar in all treatments. No effect of diets on the weight of *C. maculata* adults occurred, except for the heavier insects fed on eggs of *A. kuehniella*, whose males and females weighed 9.8 ± 0.3 mg and 13.3 ± 0.5 mg, respectively.

Therefore, based on laboratory results, the predator can be evaluated in the field in pest management program against *S. frugiperda* and *S. graminum*. Advance in the methodology of rearing the predator in the laboratory can be obtained through the use of artificial diet (honey, yeast, ascorbic acid, propionic acid, nipagin and water) associated with eggs of *A. kuehniella*.
Key words: biological control, fall armyworm, aphids, lady beetles, rearing methodology.

DESENVOLVIMENTO DE *Coleomegilla maculata* DE GEER (COLEOPTERA: COCCINELLIDAE) COM PRESAS E DIETA ARTIFICIAL

RESUMO - *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae) é um predador importante e com potencial para o controle biológico de insetos-praga de milho (*Zea mays* L.) e de sorgo [*Sorghum bicolor* (L.) Moench]. Este trabalho teve como objetivo avaliar o desenvolvimento de larvas de *C. maculata* alimentadas com ovos de *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae), isoladamente ou associados à dieta artificial, ovos de *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) e ninfas de *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae). O delineamento estatístico foi inteiramente casualisado, com seis tratamentos e quatro repetições, sendo cada uma composta por 10 larvas de *C. maculata,* em sala climatizada (25 ± 1 ºC, 70 ± 10% UR e fotofase de 12 horas). A duração dos estádios de *C. maculata* foi semelhante em todos os tratamentos, à exceção daquele em que foram utilizadas ninfas de *S. graminum*, resultando em menor período para o estádio larval. A viabilidade do estádio larval, pré-pupal, pupal e de larva a adulto de *C. maculata* foi alta e semelhante em todos os tratamentos. Não houve efeito das dietas sobre o peso de insetos, exceto para aqueles que se alimentaram com ovos de *A. kuehniella*, cujos machos e fêmeas pesaram 9,8 ± 0,3 mg e 13,3 ± 0,5 mg, respectivamente. Portanto, pelos resultados de laboratório, o predador pode ser avaliado no campo, em programas de manejo, visando tanto *S. frugiperda,* quanto *S. graminum*. Avanços na metodologia de criação deste inseto pode ser obtido através do uso de dieta artificial (mel, levedo de cerveja, ácido ascórbico, ácido propiônico, nipagin e água) associada a ovos de *A. kuehniella*.
Palavras-chave: controle biológico, lagarta-do-cartucho, pulgões, joaninhas, metodologia de criação.

Many studies have been conducted to establish nutritional needs, environmental condition and potentiality as biocontrol agents of entomophagous insects (Thompson, 1999).

The knowledge of the biology, behavior and breeding techniques can improve the potential of predatory insects, but food suitability in laboratory rearing still represents major limitation toward the use of Coccinellidae aphidophagous as biological control agent (Kato et al., 1999ab; Silva et al., 2009).

Coleomegilla maculata De Geer (Coleoptera: Coccinellidae) is a polyphagous cosmopolitan species (Munyaeza & Obrycki, 1998) with a great potential to be used in integrated pest management programs due to some outstanding qualities such as highly biotic potential and predation habit in both larval and adult stage.

Diets based on pig liver have been developed for predators. *Coleomegilla maculata* was one of the first species to produce fertile offspring under *in vitro* conditions. Viability around 86 percent was obtained from insect larvae reared on raw pork liver based artificial diet and vitamin supplement (Attallah & Newson, 1966). Fresh pig liver has been also suitable for rearing other Coccinellidae species, including *Adalia bipunctata* (L.), *Coccinella septempunctata* (L.), *Coccinella transversoguttata ricardsoni* (Brown), *Hippodamia tredecimpunctata tibialis* (Say) and *Propylea quatuordecimpunctata* (L.) (Kariluoto et al., 1976; Kariluoto, 1980). Semi defined diets, without meat, but supplemented with insect preys have been evaluated to rear species of Coccinellidae (Attallah & Newson, 1966; Kariluoto, 1980; Matsuka et al., 1982, Silva et al., 2009). Alternative food such as eggs of *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) has been considered as a replacement of natural prey for Coccinellidae due to low production cost and suitability in rearing procedures (Kato et al., 1999ab; Silva et al., 2009).

Larval or adult food source can influence the biological aspects of insects, such as fecundity and fertility. For example, artificial diets based on carbohydrate solution although enabled the maintenance of adult Coccinellidae, drastically reduce the female fecundity (Hagen, 1962). Easy disposability and nutritional suitability for larvae and adult development are essential to progressing in biological control program using Coccinellidae species, considering the wide range of consumed preys (Hodek, 1973).

Quantity and quality of food can influence the physiological status of females of the genus *Hippodamia* (Coleoptera: Coccinellidae) (Hodek, 1967). For example, *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae) requires the amino acid cystine to normal pupation and metamorphosis (Racioppi et al., 1981). The lack of such compound could explain female infecundity when feed only on eggs of *A. kuehniella* (Kato et al., 1999b).

Coleomegilla maculata has its development influenced by food source (Hodek, 1973). Faster development and higher survival rates were obtained when the insect received as food source a mix of corn leaf aphid, *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae) and maize (*Zea mays* L.) pollen than with only one of these sources (Cottrell & Yeargan, 1998; Lundgren & Wiedenmann, 2004).

Production system can affect the efficiency of predator. Predation rate of *C. maculata* on eggs of *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) was higher in a monoculture corn than in maize/beans/squash mixed production system (Andow & Risch, 1985). This was explained by the fact that predators spend more time foraging on plants without prey (beans and squash), which decreases their efficiency (Risch et al., 1983). *Coleomegilla* species were observed feeding on young larvae of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) in maize (Hoballah et al., 2004). Eggs of *S. frugiperda* also can be a food source for *C. maculata* on maize plants (Pereira, 1997). *Spodoptera frugiperda* is a key pest of maize in America and can limit the yield of maize plants.

In general, aphids are recognized as a preferential prey for Coccinelidae. Two important aphid species in South America agribusiness are associated to maize and sorghum, *Sorghum bicolor* (L.) Moench plants: the corn leaf aphid, *R. maidis* and the greenbug, *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae).

Rhopalosiphum maidis occurs in economically important crops such as barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), rye (*Secale cereale* L.), sugar cane (*Saccharum officinarum* L.), wheat (*Triticum vulgare* Vill.), maize and sorghum, and can survive in wild Poaceae. Intense attacks of this insect can cause direct damage to host plant by sucking sap, affecting the quality of seeds (Maia et al., 2005; Fonseca et al., 2006). This insect is a vector of several diseases, caused mainly by viruses (Stoetzel & Miller, 2001), such as dwarf mosaic in sugar cane, maize and sorghum and the common mosaic, considered among the most important disease in maize in Brazil (Fonseca et al., 2006).

Sorghum is the preferred host of *S. graminum*, but this aphid can attack various other grasses, with the damage characterized by large amount of sap extracted which limits the supply of water and nutrients. In the process of feeding, *S. graminum* injects toxin, which causes enzymatic destruction of the cell wall of leaves, causing chlorosis and finally necrosis. Besides the direct damage, the insect can transmit viruses or predispose the plant to stem rot and the depreciation of the grains (Cruz & Vendramim, 1989).

The objective of this study was to identify a suitable diet for rearing *C. maculata* in the laboratory, using eggs of *A. kuehniella* or *S. frugiperda* and nymphs of *S. graminum* as food source, and association with artificial diet.

Material and Methods

The experiment was conducted in acclimatized room (25 ± 1 ºC, 70 ± 10% RH and photophase of 12 hours) in the Insect Rearing Lab (LACRI) of the Brazilian Agricultural Research Corporation (EMBRAPA Maize and Sorghum) in Sete Lagoas, Minas Gerais State, Brazil.

The statistical model was a randomized complete design with six treatments and four replications, each one composed by 10 larvae of *C. maculata*. All larvae were obtained from adults maintained in routine rearing lab procedure fed on one week frozen eggs of *A. kuehniella* associated with artificial diet (Table 1) (Silva et al., 2009). One day after hatching, the larvae of *C. maculata* were isolated in 50 mL plastic cups, sealed with transparent acrylic lids and kept fixed in polystyrene support. The diets were offered *ad libitum* to the larvae of *C. maculata* according to treatment (Table 1).

After emergence, adults were sexed, weighed on an electronic scale (resolution of 0.1 mg) and transferred to breeding cages (glass container with 12 cm diameter and 18 cm tall) capped with plastic wrap and fed according to treatment.

Insects were observed daily to assess the stadium number (n = 20), development period of larval, prepupal, pupal and larval-adult stages (n = 20), larval, prepupa, pupa and larva to adult viabilities (n = 40) in addition to adult weight and sex ratio (n = 40).

TABLE 1. Food offered to the larvae of *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae) at 25 ± 1 ºC, 70 ± 10% RH and 12 hours photophase.

Treatments	Food source
T1	Eggs of *A. kuehniella* (frozen for one day)
T2	Eggs of *A. kuehniella* (frozen for one day) + artificial diet[1]
T3	Eggs of *S. frugiperda* (fresh)
T4	Eggs of *S. frugiperda* (fresh, without scales)
T5	Eggs of *S. frugiperda* (fresh, with scales)
T6	Nymphs of *S. graminum*

[1]Honey (100g), Beer yeast (100g), Ascorbic acid (1.5g), Propionic acid (0.5g), Sorbic acid (0.25g), Nipagim (0.25g) and water (60g)

The data were submitted to analysis of variance (ANOVA) and means compared by the Scott-Knott at 5% probability by the program SISVAR (Ferreira, 2000).

Results

Coleomegilla maculata presented four larval stages, and each stage was not affected by the food source (Table 2). First to fourth stages takes 2.9, 2.4, 2.5 and 3.3 days to complete, respectively. However, total life stage was shorter (10.2 days) when the larvae were fed on nymphs of *S. graminum* (T6). There was no significant difference in the mean larval period (11.2 days) among the other treatments.

Prepupa, pupa and larva to adult periods and respective viability were not affected by food source (Tables 3 and 4).

Sex ratio (0.51) of *C. maculata* was not affected by food source. However, food source did affect the insect weight (Table 5). Heavier adult females were obtained when larvae fed on fresh fall armyworm eggs (T3, T4 and T5).

TABLE 2. Development time (days) of each stage and the larval phase (mean ± standard error) of *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae) with different diets at 25 ± 1 ºC, 70 ± 10% RH and 12 hours photophase.

Treatments	Instars development time (days)[1]				Larval stage (days)
	First	Second	Third	Fourth	
T1	3.2±0.1 A	2.3±0.1 A	2.5±0.1 A	3.3±0.1 A	11.3±0.3 B
T2	3.0±0.1 A	2.6±0.1 A	2.6±0.1 A	3.1±0.1 A	11.3±0.1 B
T3	2.9±0.1 A	2.2±0.2 A	2.5±0.2 A	3.5±0.1 A	11.1±0.3 B
T4	2.9±0.1 A	2.3±0.1 A	2.6±0.1 A	3.6±0.3 A	11.4±0.2 B
T5	2.9±0.1 A	2.3±0.1 A	2.5±0.1 A	3.5±0.2 A	11.2±0.2 B
T6	2.7±0.1 A	2.3±0.1 A	2.3±0.1 A	2.9±0.1 A	10.2±0.2 A
CV (%)	7.3	9.3	7.2	10.0	4.1

[1]Means followed by the same letters in column do not differ (p <0.05) according to the Scott-Knott test.

TABLE 3. Development time (days) of pre-pupa, larva and pupa to adult (mean ± standard error) of *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae) with different diets at 25 ± 1 ºC, 70 ± 10% RH and 12 hours photophase.

Treatments	Development time (days)[1]		
	Prepupa	Pupa	Larva to adult stage
T1	1.0±0.0 A	3.9±0.1 A	16.2±0.4 A
T2	1.0±0.0 A	3.7±0.1 A	16.0±0.4 A
T3	1.0±0.0 A	3.5±0.1 A	15.6±0.2 A
T4	1.0±0.0 A	3.6±0.2 A	16.0±0.2 A
T5	1.0±0.0 A	3.7±0.1 A	15.9±0.3 A
T6	1.0±0.0 A	3.7±0.1 A	14.9±0.2 A
CV (%)	0	5.2	3.6

[1]Means followed by the same letter in column do not differ (p <0.05) according to the Scott-Knott test.

The lowest weight of female was obtained from larvae fed on one day frozen *A. kuehniella* eggs. The weight of adult males was significantly lower when larvae were fed on one day frozen *A. kuehniella* eggs compared to the other treatments. Females were significantly heavier than males independently of food source.

Discussion

Larvae of *C. maculata* are characterized by elongated body with distinct abdominal segmentation and well developed legs. Close to each stadium change the insect stops eating, stands on the surface or sides of the rearing container using the last abdominal segment and then molts.

Inadequate food can increase the number of stages (Scriber & Slansky, 1981). It seems not to be the case of food sources offered to *C. maculata* in this research. In all treatments the insect presented the same number of stages.

The same number of larval stages presented by *C. maculata* was also reported to other Coccinellidae, such as *Coccinella undecimpunctata* (L.) feeding on aphids species *Megoura persicae* (Buckton) (Hemiptera: Aphididae) and *Aphis fabae* (Scopoli) (Hemiptera: Aphididae) (Cabral et al., 2006) and *Eriopis connexa* (Germar) feeding on preys and artificial diets (Silva et al., 2009).

The period of time spent on first, second, third and fourth stages of *C. maculata* was similar among treatments. However, considering the total larval stage, shorter period of time was verified for those larvae receiving nymphs of *S. graminum* as food source. Apparently this prey could be a target in biological control program using the predator once good food quality increases the suitability of Coccinellidae larvae (Isikber & Copland, 2002).

The longest period of time spent on the fourth stage of *C. maculata*, in all treatments, may be due to the fact of larvae require more nutrients at this stage to ensure pupation and metamorphosis, as reported for other insects (Scriber & Slansky, 1981).

The viability of the larvae of *C. maculata* was similar between the different treatments, highlighting the fact that the eggs of *S. frugiperda* were evaluated with or without scales. Overlapping layers of eggs and scales also did not affect the consumption or the development rate of other natural enemies with the same prey (Armas & Ayala, 1993).

Prepupa period of *C. maculata* showed no difference between treatments and had values similar to those observed for *H. convergens* fed with eggs of *A. kuehniella* (Kato et al., 1999b) and for *Cycloneda sanguinea* L. (Coleoptera: Coccinellidae) with *M. persicae*, *Megoura viciae* (Buckton) (Hemiptera: Aphididae), *A. fabae* or *Aphis gossypii* (Glover) (Hemiptera: Aphididae) (Isikber & Copland, 2002)

The initial phase of prepupa of *C. maculata* was set when the larvae stopped feeding and set by the last abdominal segment in various parts of the rearing container. When touched, pre-pupa moved sharply. Similar

TABLE 4. Viability (%) of the larval, prepupal, pupal and larvae to adult (mean ± standard error) of *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae) with different diets at 25 ± 1 ºC, 70 ± 10% RH and 12 hours photophase.

Treatments	Viability (%)[1]			
	Larval	Prepupal	Pupal	Larva to adult stage
T1	100.0±0.0 A	90.0±5.8 A	93.7±6.2 A	85.0±9.6 A
T2	100.0±0.0 A	97.5±2.5 A	100.0±0.0 A	97.5±2.5 A
T3	95.0±2.9 A	100.0±0.0 A	97.4±2.6 A	91.9±2.7 A
T4	92.5±4.8 A	100.0±0.0 A	100.0±0.0 A	92.5±4.8 A
T5	95.0±8.5 A	100.0±0.0 A	100.0±0.0 A	95.0±2.9 A
T6	97.5±2.5 A	94.9±2.4 A	97.3±2.4 A	94.2±3.3 A
CV (%)	5.7	5.2	6.0	10.7

[1]Means followed by the same letter in column do not differ (p <0.05) according to the Scott-Knott test.

TABLE 5. Sexual ratio (%) and adult weight (mg) (mean ± standard error) of *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae) originated from larvae with different diets at 25 ± 1 ºC, 70 ± 10% RH and 12 hours photophase.

Treatments	Sexual ratio (%)[1]	Weight (mg)[1]		
		Females	Males	Mean
T1	0.45±0.03 A	13.3±0.5 A	9.8±0.3 A	11.6
T2	0.48±0.04 A	15.5±0.5 B	12.0±0.3 B	14.9
T3	0.54±0.07 A	17.8±0.2 C	12.4±0.5 B	15.1
T4	0.55±0.06 A	17.6±0.1 C	12.4±0.1 B	14.9
T5	0.48±0.04 A	17.3±0.3 C	12.3±0.3 B	14.8
T6	0.54±0.04 A	15.2±0.2 B	11.9±0.1 B	13.6
Mean	0.51±0.02	16.4±0.3 a	11.9±0.2 b	
CV (%)	18.7	4.3	4.6	

[1]Means followed by the same letters in column do not differ (p <0.05) according to the Scott-Knott test.

behavior was also observed in *Olla v-nigrum* (Mulsant) (Coleoptera: Coccinellidae) (Kato et al., 1999a).

Prepupa stage of *C. maculata* showed different pattern from that reported for *S. sinvanodulus* because the prepupa of this species was seen when the larvae stopped feeding and released the anal region liquids in large numbers and became still for one to two days (Lu et al., 2002), a fact also observed for *C. maculata*. However, some larvae of *S. sinvanodulus* after the period of immobility, began to crawl, forming a pupa after this period of creep, and a high percentage of those that died exhibited this behavior (Lu et al., 2002). This behavior of *S. sinvanodulus* was associated with a mechanism of the larvae of this predator to seek suitable sites for feeding or protection for pupation (Lu et al., 2002).

Coleomegilla maculata is a holometabolous insect, whose pupa is classified as drawn up by not showing appendages applied on the body, but free and visible. The pupa of this species had light coloring and darkening slowly acquiring spots of the sub family Coccinellinae (Hodek, 1973). The larval and pupal viability were similar between treatments, showing that the foods offered were sufficient to meet their nutritional needs.

Soon after the emergence, the adults of *C. maculata* remained motionless, with color changing gradually from pale, then pink hue, darkening to become red, with black spots.

The sex ratio of *C. maculata* was similar to other Coccinellidae, as *H. convergens*, *Cryptolaemus montrouzieri* (Mulsant), *Delphastus pusillus* (LeConte), *Harmonia axyridis* (Pallas), *Lindorus lophanthae* (Blais) and *S. punctillum* (Heimpel & Lundgren, 2000).

Females had higher weight than males, which seems to be a pattern of Coccinellidae (Kato et al., 1999a; Silva et al., 2009). The greater weight of females of *C. maculata* in all treatments suggests that these may be distinguished from male conspecifics by their weight, as reported for *H. convergens* and *Coccinella septempunctata* (L.) (Coleoptera: Coccinellidae) on *Myzus persicae nicotianae* (Sulzer) (Hemiptera: Aphididae) (Katsarou et al., 2005).

Female fecundity is determined by the insect oogenesis, a physiological process regulated by the availability of nutrients in the body of the insect (Wheeler, 1996). Any factor that affects the incorporation of nutrients can affect oogenesis and hence the rate of laying insects. Generally, larger females are more fecund than smaller ones, as reported for *C. sanguinea*, which is heavier and fruitful with *Toxoptera citricida* (Kirkaldy) (Hemiptera: Aphididae) than with *Aphis spiraecola* (Patch) (Hemiptera: Aphididae) (Michaud, 2000).

The greater weight of adult *C. maculata*, which fed on eggs of *A. kuehniella* (frozen for a day) alone or associated with artificial diet, and eggs of *S. frugiperda*, shows that these foods offer the best way for the development of predator than nymphs of *S. graminum*. This is an important factor because the weight is indicative of the nutrients and energy stored, which may influence copulation, flight dispersal and fecundity (Omkar et al., 2006). In fact, Coccinellidae predators need food nutritional supplementation, such as pollen and nectar, which sustain metabolism and reproductive development of certain species (Hagen, 1962; Hodek, 1967, 1973). Specifically, *C. maculata* showed faster development and higher survival rates when fed on a mixture of *R. maidis* and corn pollen than when fed on only one of these sources (Cottrell & Yeargan, 1998; Lundgren & Wiedenmann, 2004).

Michaud & Jyoti (2008) reported the consequences of changing the diet of larvae and adult *C. maculata*. Females fed on eggs of *A. kuehniella* that had their diet replaced by nymphs of *S. graminum* had increased its fecundity compared to females which continued eating only eggs from *A. kuehniella*. Also, the predator can survive either on egg or young larvae of *S. frugiperda* (Pereira, 1997; Hoballah et al., 2004).

Eggs of *A. kuehniella* (one day of freezing) or *S. frugiperda* and nymphs of *S. graminum* were appropriate for different developmental stages of *C. maculata*, which facilitates rearing in the laboratory. Although stored frozen eggs can be used as a food source for rearing insect predators with initial lower production cost than with fresh eggs, one should consider the balance between nutritional quality of the eggs of *A. kuehniella* and storage period of time (Mohaghegh & Amir-Maafi, 2007).

Coleomegilla maculata has great potential use for biological control of *S. frugiperda* and *S. graminum*, due to its large feeding plasticity and ability to adapt to different foods, which is interesting for the management of these pests in maize and sorghum. However, to be used in field, laboratory massal rearing will be needed. One of the difficulties in rearing Coccinellidae predator in the laboratory is to obtain their natural diet throughout the year, particularly natural hosts such as aphids, whose populations in the field usually have a nature ephemeral and unpredictable in time and space. However the use of *A. kuehniella* eggs as an alternative food source has proven favorable in the replacement of several natural preys of Coccinellidae. This insect can be easily and cheaply reared in the lab.

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