Biological aspects of *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae) fed on different insect pests of maize (*Zea mays* L.) and sorghum [*Sorghum bicolor* L. (Moench.)]

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Abstract

Eriopis connexa (Germar) (Coleoptera: Coccinellidae) occurs in several countries of South America and its mass rearing is important for biological control programmes. This work evaluated biological aspects of *E. connexa* larva fed on eggs of *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) and *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) frozen for one day, fresh eggs of *Diatraea saccharalis* (Fabricius) (Lepidoptera: Pyralidae), *S. frugiperda* newly-hatched caterpillars, nymphs of *Rhopalosiphum maidis* (Fitch) and *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae). Duration of larva, pupa and larva to adult stages differed among prey offered, whereas the prepupa stage was similar. Larva, pupa, prepupa and larva to adult viabilities were equal or major of 87.5% in all prey, except for larva fed on newly-hatched larvae of *S. frugiperda*. *Eriopis connexa* has good adaptation to different prey corroborating its polyphagous feeding habit, which evidences the potential of this natural enemy for controlling corn and sorghum pests.

Keywords: Anagasta kuehniella, *Diatraea saccharalis*, *Rhopalosiphum maidis*, *Schizaphis graminum*, *Spodoptera frugiperda*.

Aspectos biológicos de *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae) alimentada com diferentes insetos-praga de milho (*Zea mays* L.) e sorgo [*Sorghum bicolor* L. (Moench.)]

Resumo

Eriopis connexa (Germar) (Coleoptera: Coccinellidae) ocorre em vários países da América do Sul e sua criação massal é importante para programas de controle biológico. Este trabalho avaliou os aspectos biológicos de larvas de *E. connexa* alimentadas com ovos de *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) e de *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) congelados por um dia, ovos frescos de *Diatraea saccharalis* (Fabricius) (Lepidoptera: Pyralidae), lagartas de *S. frugiperda* recém-eclodidas, ninfas de *Rhopalosiphum maidis* (Fitch) e *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae). A duração da fase larval, pupal e de larva a adulto diferiu entre as presas oferecidas; entretanto, a fase de pré-pupa foi semelhante. A viabilidade larval, pré-pupal, pupal e de adultos foi maior ou igual a 87,5% em todas as presas, com exceção para larvas alimentadas com lagartas recém-eclodidas de *S. frugiperda*. *Eriopis connexa* tem boa adaptação a diferentes presas, corroborando seu hábito alimentar polífago, o que evidencia o potencial desse inimigo natural para o controle de pragas de milho e sorgo.

Palavras-chave: Anagasta kuehniella, *Diatraea saccharalis*, *Rhopalosiphum maidis*, *Schizaphis graminum*, *Spodoptera frugiperda*.

1. Introduction

Demand for maize, *Zea mays* L. and sorghum, *Sorghum bicolor* (L.) Moench (Poales: Poaceae) has increased their cultivated area and, consequently, problems with insect pests in these crops (Sertkaya et al., 2004; Figueiredo et al., 2006a, b; Cruz et al., 2011).

Aphidophagous Coccinellidae has better development when fed on aphids than with other prey, however colonies of these prey represent ephemeral resources, which can be numerous, but for short periods (Michaud and Jyoti, 2007). Larvae of these predators may complete their development with prey that are not aphids but this is poorly studied, although eggs of Coleoptera and Lepidoptera have been used as alternative prey for them (Silva et al., 2009, 2010, 2012).

Polyphagous feeding habits of *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae) have been reported (Miller and Paustian, 1992; Miller, 1995; Sarmento et al., 2004, 2007; Silva et al., 2009), suggesting that this predator can feed on a wide number of insect pests of corn and sorghum, but food availability and sustainability are fundamental for establishing a species in a new area (Eubanks and Denno, 2000; Roger et al., 2000; Soares et al., 2004; 2005; Berkvens et al., 2008).

The aim of this study was to evaluate biological aspects of *E. connexa* fed on different insect pests of maize and sorghum and to contribute to using this predator in biological control programmes.

2. Material and Methods

The experiment was conducted in the Laboratory of Insect Breeding (LACRI) of the "Empresa Brasileira de Pesquisa Agropecuária (Embrapa Milho e Sorgo)" in Sete Lagoas, Minas Gerais State, Brazil at 25 ± 1 °C, 12 h photophase and 70 ± 10% relative humidity.

Experimental design was in completely randomised blocks with six treatments (Table 1), established from prey type (Table 2) and with four replications, each one with 10 *E. connexa* larvae, except for the treatment with nymphs of *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae), which had five *E. connexa* larvae per replication.

Eriopis connexa larvae were obtained from the laboratory colony where its adults are fed on *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs frozen for one week plus artificial diet (Table 2) (Silva et al., 2009).

Anagasta kuehniella, *Diatraea saccharalis* (Fabricius) (Lepidoptera: Pyralidae) and *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) eggs were supplied *ad libitum* in 50 mL plastic vials covered with transparent lids, according to the treatment (Table 1). Newly-hatched larvae of *S. frugiperda* were similarly supplied to this predator, but with 20, 30, 50 and 70 larvae for the first, second, third or fourth instars of *E. connexa*, respectively. Non-consumed prey were disposed and the consumption rate calculated.

Rearing cages for *E. connexa* larvae in the treatment with nymphs of *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae) and *R. maidis* were 50 ml plastic cups with a paper filter moistened in 2 mL of water, 2 cm^2 sections leaves of *S. bicolor* BRS 310 and nymphs until the third instar of these aphids *ad libitum*. These cages were sealed with transparent covers where *E. connexa* larvae remained until the adult emergence of the predator.

Table 1. Food supplied to *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae) larvae at 25 ± 1 °C, 70 ± 10% relative humidity and 12-hour photophase in Sete Lagoas, Minas Gerais State, Brazil.

Treatments	Diets
T1	Eggs of *Anagasta kuehniella* (frozen for one day)
T2	Eggs of *Spodoptera frugiperda* (frozen for one day)
T3	Eggs fresh of *Diatraea saccharalis*
T4	*Spodoptera frugiperda* newly-hatched larvae
T5	Nymphs of *Schizaphis graminum*
T6	Nymphs of *Rhopalosiphum maidis*

Table 2. Artificial diet for feeding of *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae) at 25 ± 1 °C, 70 ± 10% relative humidity and 12 hour-photophase in Sete Lagoas, Minas Gerais State, Brazil.

Compound	Amount (g)
Honey	100
Brewer's yeast	90
FeSO4	1.5
Ascorbic acid	1.5
Propionic acid	0.50
Sorbic acid	0.25
Nypagin	0.25
Water	60

Eriopis connexa adults were sexed, body weight obtained just after emergence and transferred to cages (glass recipient 12 cm diameter and 18 cm height) closed with plastic wrap (PVC) and fed according to treatment.

The period of larva hatching to adult emergence of *E. connexa* was daily analysed to determine the number (n = 20), duration (n = 20) and survival (n = 40) of instars and of the prepupa, pupa and larva to adult stages and adult weight and sex ratio (n = 40) of this predator. All larvae of the treatment with *R. maidis* were used to obtain the parameters described, due to the low number of replications in this treatment (n = 20).

Data were submitted to variance analysis (ANOVA) and compared using the Tukey test at 5% probability (Russel, 1989).

3. Results

Eriopis connexa had four instars, in all treatments. Duration instars and the larva stage of *E. connexa* differed

Table 3. Duration (days) of each instar and larval stage (mean ± standard error) of *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae) fed on different prey at 25 ± 1 °C, 70 ± 10% relative humidity and 12 hour-photophase in Sete Lagoas, Minas Gerais State, Brazil.

Treatments	First	Second	Third	Fourth	Larval phase
T1	3.3 ± 0.09^{a}	2.9 ± 0.25^{a}	2.7 ± 0.17^{ab}	3.6 ± 0.12^{ab}	12.5 ± 0.4^{a}
T2	3.4 ± 0.12^{a}	2.2 ± 0.00^{bc}	2.4 ± 0.08^{ab}	3.2 ± 0.17^{bc}	11.2 ± 0.2^{b}
T3	2.5 ± 0.05^{b}	2.5 ± 0.05^{ab}	2.8 ± 0.05^{ab}	2.8 ± 0.05^{a}	10.6 ± 0.1^{bc}
T4	3.2 ± 0.08^{a}	2.8 ± 0.19^{a}	2.9 ± 0.27^{a}	3.9 ± 0.18^{a}	12.8 ± 0.4^{a}
T5	2.4 ± 0.08^{a}	2.4 ± 0.05^{a}	2.2 ± 0.09^{ab}	2.8 ± 0.09^{a}	9.8 ± 0.2^{c}
T6	2.1 ± 0.06^{b}	1.9 ± 0.05^{c}	2.0 ± 0.00^{a}	2.5 ± 0.17^{d}	8.5 ± 0.2^{d}

Means followed by the same letter, per column, do not differ by Tukey test ($p < 0.05$).

between treatments (Table 3) but with similar values for this predator fed on eggs of *S. frugiperda* (T2) or *D. saccharalis* (T3) as well as with *A. kuehniella* and *S. frugiperda* newly-hatched larvae (T4) (Table 3).

Eriopis connexa consumed 28.0 ± 5.5, 55,8 ± 5,2, 125.7 ± 9.9 and 275.9 ± 11.2 newly-hatched larvae of *S. frugiperda* during the first, second, third and fourth instars, respectively, with total consumption of 485.4 ± 14.8 larvae of this prey, during the larva stage of this predator.

Duration of *E. connexa* prepupa stage was similar among treatments (Table 4). Survival of this stage was also similar between treatments, except for the lower survival with *S. frugiperda* newly-hatched larvae (Table 5).

Prey type affected the duration of *E. connexa* pupa stage, with variation from 3.1 to 3.7 days (Table 4), whereas pupa survival of *E. connexa* was similar between treatments (Table 5).

Duration of larva stage was shorter with nymphs of *R. maidis* (T6); 8.5 days (Table 3). The duration from larva to adult of *E. connexa* ranged from 12.6 to 17.2 days, differing among treatments (Table 4). The percentage of adult emergence of this predator was high in all treatments (>90%) except with *S. frugiperda* newly-hatched larvae (Table 5).

The sex ratio of *E. connexa* was similar between treatments, whereas the weight of females of this predator was higher than males (Table 6).

Table 4. Duration (days) of prepupa, pupa and larva for the adult period (mean ± standard error) for *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae) fed on different prey at 25 ± 1 °C, 70 ± 10% relative humidity and 12 hour-photophase in Sete Lagoas, Minas Gerais State, Brazil.

Treatments	Prepupa	Pupa	Larva-adult
T1	1.0 ± 0.00^{a}	3.7 ± 0.01^{a}	17.2 ± 0.44^{ab}
T2	1.1 ± 0.10^{a}	3.7 ± 0.18^{a}	16.0 ± 0.11^{bc}
T3	1.1 ± 0.05^{a}	3.4 ± 0.08^{ab}	15.1 ± 0.09^{c}
T4	1.0 ± 0.00^{a}	3.6 ± 0.06^{a}	17.4 ± 0.30^{a}
T5	1.0 ± 0.00^{a}	3.6 ± 0.06^{a}	14.4 ± 0.26^{c}
T6	1.0 ± 0.00^{a}	3.1 ± 0.06^{b}	12.6 ± 0.23^{e}

Means followed by the same letter, per column, do not differ by Tukey test ($p < 0.05$).

4. Discussion

The number of *E. connexa* instars was the same as that of this predator fed on *Coccinella undecimpunctata* (L.) (Coleoptera: Coccinellidae), *Megoura persicae* (Buckton) and *Aphis fabae* (Scopoli) (Hemiptera: Aphididae) (Cabral et al., 2006); *Diuraphis noxia* (Mordvilko) and *R. maidis*, *D. noxia*, *Acyrnothosiphon pisum* (Harris) and *Myzus persicae* (Sulzer), *Cinara atlantica* (Wilson) (Hemiptera: Aphididae) (Miller and Paustian, 1992; Miller, 1995; Oliveira et al., 2004) and with *Scymnus* (*Neopullus*) *sinuanodulus* Yu and Yao (Coleoptera: Coccinellidae) (Lu et al., 2002). Generalist predators obtain better nutritional balance by including different sources to complement their diet (Roger et al., 2000; Snyder and Clevenger, 2004). Inadequate diets, generally, increase the number of instars (Scriber and Slansky, 1981; Thompson, 1999). Those supplied to *E. connexa* were of good quality, since this predator had the same number of instars with all of them and the prey type is important for the maintenance of its number of predatory insects (Nava and Parra, 2005; Phoofolo et al., 2007, 2009; Silva et al., 2009).

The larvae period of *E. connexa* fed on *S. graminum* (T5) had a similar duration to those obtained with *D. saccharalis*. This predator fed on *R. maidis* nymphs (T6) had a shorter larva stage than with other diets. The longer duration of first and fourth instars of *E. connexa* suggest the need for nutrient storage of this predator in these instars (Scriber and Slansky, 1981; Thompson, 1999). The longer duration of the fourth instar was also found for *Pseudodorus clavatus* (Fabricius) (Diptera: Syrphidae) fed on *S. graminum*, which is interesting since there is higher predation rate in this instar (Auad, 2003).

The consumption increase from newly-hatched larvae to last instars of *E. connexa* corroborates data for *Propylea dissecta* (Mulsant) (Coleoptera: Coccinellidae) showing that neonate larvae of Coccinellidae have lower foraging ability (Hemptinne et al., 1992), consumption rate (Ponsonby and Copland, 2000) and voracity (Pervez and Omkar, 2004), perhaps due to the small size and mobility of initial instars of the predator.

The higher consumption of *S. frugiperda* larvae by fourth instar larvae of *E. connexa* may indicate the necessity of nutrients for pupation as showed for *Rhyzobius lophanthae*

Table 5. Survival (%) of larva, prepupa, pupa and larva to adult periods (mean ± standard error) for *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae) fed on different prey at 25 ± 1 °C, 70 ± 10% relative humidity and 12 hour-photophase in Sete Lagoas, Minas Gerais State, Brazil.

Treatments	Larva	Prepupa	Pupa	Larva-adult
T1	92.5 ± 4.8[a]	100.0 ± 0.0[a]	100.0 ± 0.0[a]	92.5 ± 4.8[a]
T2	95.0 ± 4.8[a]	97.5 ± 2.5[a]	95.0 ± 2.9[a]	92.5 ± 4.8[a]
T3	100.0 ± 0.0[a]	90.0 ± 4.1[a]	100.0 ± 0.0[a]	90.0 ± 4.1[a]
T4	57.5 ± 8.5[b]	76.0 ± 5.8[b]	87.5 ± 7.9[a]	37.5 ± 7.5[b]
T5	100.0 ± 0.0[a]	97.5 ± 2.5[a]	100.0 ± 0.0[a]	97.5 ± 2.5[a]
T6	100.0 ± 0.0[a]	100.0 ± 0.0[a]	95.0 ± 0.0[a]	95.0 ± 2.9[a]

Means followed by the same letter, per column, do not differ by Tukey test ($p < 0.05$).

Table 6. Adult sex rate (%) and weight (mg) (mean ± standard error) of *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae) from larvae fed on different prey at 25 ± 1 °C, 70 ± 10% relative humidity and 12 hour-photophase in Sete Lagoas, Minas Gerais State, Brazil.

Treatments	Sex ratio (%)	Weight (mg)	
		Females	Males
T1	0.48 ± 0.05[a]	11.0 ± 0.56[b]	7.0 ± 0.26[c]
T2	0.48 ± 0.04[a]	10.9 ± 0.20[b]	7.7 ± 0.17[bc]
T3	0.53 ± 0.04[a]	12.4 ± 0.08[a]	7.9 ± 0.07[a]
T4	0.62 ± 0.13[a]	8.6 ± 0.12[c]	5.1 ± 0.20[d]
T5	0.61 ± 0.05[a]	13.3 ± 0.04[a]	8.9 ± 0.07[a]
T6	0.52 ± 0.05[a]	13.2 ± 0.11[a]	8.9 ± 0.06[a]

Means followed by the same letter, per column, do not differ by Tukey test ($p < 0.05$).

(Blaisdell) (Coleoptera: Coccinellidae) fed on *Parlatoria pergandii* Comstock (Hemiptera: Diaspididae) (Stathas, 2000) and growth and development (Sharma et al., 1997). The food consumption increasing in the fourth instar Coccinellidae also shows food suitability, because the size of larvae of the last instar determines that of the adult (Phoofolo et al., 2007, 2009; Honek et al., 2008).

Duration of *E. connexa* larva stage with eggs of *A. kuehniella*, *D. saccharalis* or eggs or newly-hatched larvae of *S. frugiperda* was similar to that reported for *E. connexa* with *C. atlantica* (Oliveira et al., 2004), whereas those obtained with nymphs of *S. graminum* and *R. maidis* had similar values to those with *D. noxia* and *R. padi* (Miller and Paustian, 1992) and with *D. noxia*, *A. pisum* and *M. persicae* (Miller, 1995). Survival of *E. connexa* larva stage was similar between treatments, as reported for this predator with *D. noxia* and *R. padi* (Miller and Paustian, 1992) and *M. persicae* and *D. noxia* (Miller, 1995), respectively, except for the lower survival in the treatment with *S. frugiperda* newly-hatched larvae.

The similar duration of the prepupa stage between treatments shows that prey were adequate for *E. connexa*, because inadequate resources for growth and development can compromise insect development (Scriber and Slansky, 1981; Thompson, 1999; Michaud and Jyoti, 2007). Duration of prepupa was similar to that of *C. sanguinea* with *M. persicae, Megoura viciae* (Buckton), *Aphis gossypii* (Glover) and *Aphis fabae* (Scopoli) (Hemiptera: Aphididae) (Isikber and Copland, 2002). Prepupa behaviour of *E. connexa* differed from of *S. sinvanodulus*, because it released fluids from the anal region in great quantity when its larvae stopped feeding and became static for one to two days (Lu et al., 2002). This was also observed for *E. connexa*, but is not recorded for *S. sinvanodulus* larvae (Lu et al., 2002), which, after the period of immobility, crawled and formed the pupa afterwards. However, a high percentage of *S. sinvanodulus* pupa with this behaviour died (Lu and Montgomery, 2001; Lu et al., 2002). The crawling of *S. sinvanodulus* larvae was associated to a mechanism for dispersion and seeking adequate sites for feeding and pupation (Lu et al., 2002).

The duration of pupa stages with *D. saccharalis* eggs (T3) was similar to those of other treatments, whereas no differences were found with nymphs of *R. maidis* (T6) or *D. saccharalis* eggs, being of 3.1 and 3.4 days, respectively. This was similar to that of this predator with *D. noxia* and *R. padi* (Miller and Paustian, 1992). The shorter duration of the pupa stage with fresh eggs of *D. saccharalis* (T3) and nymphs of *R. maidis* (T6) is important, because the pupa is almost static and longer duration of this stage can increase of predation and parasitism. On the other hand, increasing duration of this stage in the other treatments can be beneficial for pupa storage in mass rearing procedures, to wait for more appropriate periods for their release for pest control (Auad, 2003). Viability of *E. connexa* pupa was similar to that of this predator with *C. atlantica* (Oliveira et al., 2004), *D. noxia* and *R. padi* (Miller and Paustian, 1992; Miller, 1995). High pupa survival of *E. connexa*, in all treatments, suggests that prey tested supplied the metabolic needs of this predator in the previous stages. This is important because prey type affects fertility and other biological features of predators (Ferkovich et al., 2007).

The shorter duration from larva to adult of *E. connexa* with *R. maidis* suggests better nutritional quality for this aphid, since inadequate food can extend the life cycle of insects (Scriber and Slansky, 1981; Thompson, 1999). The development period of Coccinellidae can be higher with poor nutritional quality diets or with unsatisfactory quantity (Michaud and Jyoti, 2007). In addition, the lack of some nutrients such as aminoacids may increase the duration of the immature stage of insects (Hacker and Bertness, 1996; Bottrell et al., 1998). Our findings suggest that aphidophagous Coccinellidae develop more rapidly when fed on aphids than with other prey, as also reported for *C. maculata* (Michaud and Jyoti, 2007).

The similar sex ratio of *E. connexa* fed on different prey agrees with the finding for this predator with *M. persicae*, *D. noxia* and *A. pisum* (Miller 1995) or *C. atlantica* (Oliveira et al., 2004), for *Diomus austrinus* (Gordon) (Coleoptera: Coccinellidae) with *Phenacocus madeirensis* (Green) and *Planococus citri* (Risso) (Hemiptera: Pseudococcidae) (Chong et al., 2005), and for *Stethorus punctillum* (Weise) (Coleoptera: Coccinellidae) (Heimpel and Lundgren, 2000). The negative rate between duration of larva stage and adult weight of Coccinellidae is known (Michaud and Jyoti, 2007) as for *E. connexa*, with shorter development period and higher weight with *R. maidis* and *S. graminum*. *Eriopis connexa* females were heavier with nymph*s* of *S. graminum* and *R. maidis* (T5 and T6), which is desirable for mass rearing, because heavier females can produce more descendents (Zanuncio et al., 2002, Omkar and Srivastava, 2003).

The weight of *E. connexa* adults can be used as sex dimorphism, because females were always heavier than males, as reported for *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) and *H. convergens* with *Myzus persicae nicotianae* (Sulzer) (Hemiptera: Aphididae) (Katsarou et al., 2005).

High survival of immature stages and heavier weight of *E. connexa*, in all treatments, except with *S. frugiperda* newly-hatched larvae, corroborates the polyphagous feeding habit of this predator. This shows its feeding plasticity and ability to adapt to different diets, which is interesting for the management of corn and sorghum pests and evidences its potential for biological control. Our results were relevant, but the potential of *E. connexa* in biological control programmes needs to be better studied in the field. This predator is not common in corn and sorghum crops, and that is why further studies are necessary to assess its adaptation and role in these crops. This can contribute to correct releasing and improving efficiency, and to reducing problems, such as intraguild predation and competition that can compromise native natural enemies of insect pests in these crops.

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