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Effect of Single- And Mixed-Resource Diets of Mealybug Stages on Developmental and Reproductive Performances of the Green Lacewing, *Chrysoperla carnea*

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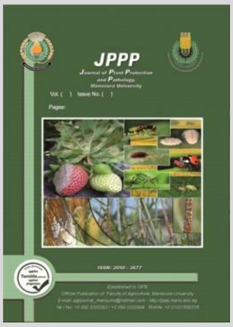
Awadalla, S. S.; M. H. Bayoumy[*] and Asma M. El-Basuony

Economic Entomology Department, Faculty of Agriculture, Mansoura University, Mansoura 35516, Egypt



ABSTRACT

The application of *C. carnea* has accounted for one third of all successful biocontrol programs worldwide. For the successful application of pest control programs that use *C. carnea* as a biocontrol agent, it is necessary to recognize high quality food/prey. Larvae of *C. carnea* were provided with either eggs of one of *Planococcus citri, Icerya aegyptiaca, I. seychellarum* and their mixing or second-instars of one of those mealybug species and one more species, *Planococcus solenopsis.* These feeding trials were conducted at 25.0±1.0 °C and 65±5% R.H. Eggs of *I. aegyptiaca* accelerated development of *C. carnea*. Eggs of mealybug species significantly affected oviposition period, female and male longevities, and fecundity. The longest female and male longevities were obtained when predator's larvae fed on mixing mealybug eggs. The highest female fecundity was when predator's larvae fed with *P. citri* eggs. Female fertility ranged between 98 and 99% in relation to mealybug eggs consumed. In case of using second-instar mealybugs as a food, the shortest developmental time and longest female longevity was when predator's larvae fed on second-instars of *P. citri*. second-instar mealybugs significantly affected female fecundity with the highest fecundity was for those their larvae fed on second-instars *P. citri* and *P. solenopsis*. Female fertility ranged between 97 and 98% in relation to type of second-instar mealybugs that provided for predator's larvae. The success of any predator majorly depends on the nutritional quality of its prey. These information will be useful for the formulation of Integrated Pest Management (IPM) programs.

Keywords: Lacewings, mealybugs, longevity, fecundity, fertility

INTRODUCTION

Mealybugs (Hemiptera: Pseudococcidae: Monophlebidae) are small sized, soft-bodied, sap-sucking insects that cause serious damage to different field crops, vegetables and fruits (Arif *et al*., 2009; Nagrare *et al*., 2009). The cotton mealybug, *Phenacoccus solenopsis* Tinsley (Pseudococcidae), the Egyptian mealybug, *Icerya aegyptiaca* Douglas (Monophlebidae), and the citrus mealybug, *Planococcus citri* (Risso) (Pseudococcidae) are highly polyphagous pests of 154, 123, 65 plant species that belonging to more than 52, 49, and 36 plant families, respectively (Arif *et al*., 2009; Abbas *et al*., 2010). The Seychelles scale, *Icerya seychellarum* (Westwood) (Monophlebidae) is another polyphagous species, with ScaleNet listing 126 genera from 57 families (García Morales *et al*., 2016). Generally, mealybugs are considered as a most desired topic for researchers because of their large populations and high stability over time. High reproductive ability of mealybugs results in occurrence of eggs and nymphs at any time of the year (Oliveira *et al*., 2014).

Lacewings (Neuroptera: Chrysopidae) are widely identified as main predators of soft-bodied insects, such as mealybugs, aphids, and psyllids (Tauber *et al*., 2000; Senior and McEwen, 2001). Among predatory species that used in biocontrol programs, lacewings gave excellent example of successful mass reared insects as a way for further inundative releases against pest species in open and closed (e.g. greenhouses) planting systems (Principi and Canard, 1984; Tauber *et al*., 2000; Senior and McEwen, 2001) systems. The green lacewing, *Chrysoperla carnea* (Stephens) is one of the most chrysopid species that highly studied because of its wide habitats, high geographical distribution, high relative occurrence frequency, high searching efficiency and easy handle for its production under controlled conditions (Tauber *et al*., 2000; Liu and Chen, 2001).

The generalist predator, *C. carnea*, has been widely used and recognized as a potential predator against several serious pests including mealybugs in many agricultural systems and can easily be commercially reared to be apply against several insect pests in the field (Venkatesan *et al*., 2000; Messelink *et al*., 2012). This was because its high searching behavior, polyphagous habits, ubiquitous nature, adaptability in the field, integration with microbial agents and selected pesticides, and usability to mass production (Sengonca *et al*., 1995; Daane *et al*., 1996; Singh and Manoj, 2000; Venkatesan *et al*., 2000; Zaki and Gesraha, 2001; Duelli, 2001). The larvae of *C. carnea* are efficient stage of the predator with high voraciousness and high searching ability (Bond, 1980; Ballal *et al*., 1999). In contrast to the larval stage, the adults are free living and they only feed honey, pollen and water (Principi and Canard 1984; Ulhaq *et al*., 2006; Sarwar and Salman, 2016). The application of *C. carnea* has represented 33% of all successful biocontrol programs worldwide (Williamson and Smith, 1994; O'Neil *et al*., 1998). In Pakistan, *C. carnea* has

[*] **Corresponding author.**
E-mail address: mhmohamed@mans.edu.eg
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been released against the cotton mealybug and achieved high efficiency against this pest (Sattar *et al*., 2007). Thus, among the effective biocontrol agents, *C. carnea* is receiving a more attention than any other biocontrol agent because of its voraciousness (Sattar *et al*., 2011).

Research on biology of natural enemies is a fundamental request for the success of an augmentative biocontrol program (Parra *et al*., 2015). There is a report on the use of *Chrysoperla externa* (Hagen) for controlling *P. citri* on roses (see Carvalho *et al*., 2023), which shows that both prey types may influence growth and reproduction of predators (Principi and Canard, 1984; Dhandapani *et al*., 2016) and/or that the host plant can play a significant role in this tritrophic interaction (Price *et al*., 1980; Adriano *et al*., 2010). Indeed, several studies have demonstrated that in the availability of alternative food, a decline in rates of predation on the target pest has reported (Eubanks and Denno, 2000; Koss and Snyder, 2005; Symondson *et al*., 2006). Accordingly, both the nutritional quality and the abundance of the alternative food will affect the magnitude of control of the target pest (Eubanks and Denno, 2000; Venzon *et al*., 2002). However, biological control of pests with the generalist predators may adversely affect by the existence of multiple prey. Predators may move to more preferred alternative prey or to more abundant prey rather than feeding on the target prey (Murdoch, 1969), and availability of other food items may lead to predator satiation (Abrams and Matsuda, 1996), which may result in keep pests far from attack. In addition, predators may show lower performance on single-resource diet than on mixed diets (Evans *et al*., 1999; Oelbermann and Scheu, 2002; Toft, 2005; Marques *et al*., 2015). Thus, mixed diets may multiply populations of the predator and eventually led to effective pest control (Messelink *et al*., 2008), as well as may prolong the residence time of predators in prey colonies even if prey with suboptimal nutritional quality (Jensen *et al*., 2012; Mayntz *et al*., 2005).

Thus, for the sustainable development of biocontrol programs that use *C. carnea*, it is necessary to recognize the high quality food/prey. The first report shows the influence of eggs of the natural (*P. citri*) and alternative (*Ephestia kuehniella*) prey on different larval instars of *C. externa* has been announced by Carvalho *et al*. (2023), however few studies have been examined the effect of various prey species on growth, reproduction, and longevity of *C. carnea*, despite its importance as an efficient biocontrol agent. In addition, the nutritional value of prey for this predator is also not covered enough. Further, the relation between adult diet and egg production in *C. carnea* has been examined intensively (Hagen *et al.*, 1970; Adane and Gautam, 2002). However, less is known about the relationship between larval diet and adult's reproduction.

Larvae of some chrysopid species were observed to eat all developmental stages of *P. citri* (Bezerra *et al*., 2006). Although, the earlier reports demonstrated effective management of mealybugs with chrysopid larvae (Doutt and Hagen, 1949; Goolsby *et al*., 2000), these mealybugs may not be the most preferred and suitable prey for chrysopid larvae. Therefore, this work designed to examine the suitability of different prey stages in stages of eggs or nymphs, as a food, for accelerating development, maximizing reproduction, and prolonging longevity of *C. carnea* under controlled conditions. Such information would be useful for maximizing the mass production of the predator. The findings may also useful in determining the suitable time for releasing the predator when the prey in sufficient density and in the desired stage.

MATERIALS AND METHODS

Insect colony

The culture of *C. carnea* started by larvae, pupae, and adults that were collected from various host plants located inside and outside the campus (ficus, guava, and vegetable plants). These host plants were mainly infested with mealybugs. Leaves of these plants which infested with these insect species were transferred to laboratory for investigation. Adults were aspirated in early morning (7 Am) from those plants. Larvae and pupae were isolated in 5.5 cm Petri dishes. A filter paper was placed in the bottom of each dish to provide the walking surface for each larva. The predator's larvae were provided daily with sufficient numbers of frozen moth eggs of *Sitotroga cerealella* until pupation. The emerged adults were maintained in plastic jars (28 × 28 × 15 cm). The neck of each jar was closed with black muslin cloths using a rubber band. Adults in the jars were provided with an artificial diet composited of 1:1:1 of honey, yeast and water that was offered twice a day in form of droplets on weighted paper pieces. Adults collected from the field were added to the culture to have genetic diversity. Eggs laid on the black mesh cloths were harvested daily, by cutting their holders with scissors, collected in Petri-dishes and kept in incubator as described. Following hatching, the neonates were isolated in Petri-dishes and supplied with *Sitotroga* eggs that offered *ad libitum*, to prevent cannibalism, and water provided on a small sponge, both changed every two days, until pupation. At time of emergence, adults were introduced in the same sized jars and processed as described above. Larvae used in the trials were produced using the previous described method and were two generations old since they collected from the field.

Effect of prey stages of various mealybug species on development and reproduction of the green lacewing, *Chrysoperla carnea*

Egg stage

Following the previous technique, the *C. carnea* larvae were fed with one of three mealybug preys, *Planococcus citri, Icerya aegyptiaca,* and *Icyrya seychellarum.* The neonate larvae of *C. carnea* were supplied with eggs of each mealybug species. Every larva was fed daily with mealybug eggs in 5.5 cm Petri-dish. Thirty larvae were used for each type of eggs. Dishes were kept in an incubator set to 25.0±1.0 °C and 65±5% R.H until development completed. The larval and pupal periods, preoviposition, oviposition, postoviposition, male and female longevities, total female fecundity and fertility were estimated for each larval individual in each feeding group.

Nymphal stage

Following the previous technique, the larvae of *C. carnea* were fed with one of four mealybug preys, *Planococcus citri, Icerya aegyptiaca, Icyrya seychellarum* and *Phenococcus solenopsis.* The neonates of *C. carnea* were supplied with the second-instars of each mealybug species. Every larva was provided daily with 20 fresh nymphs in 5.5 cm Petri-dish. Thirty larvae were used in each group of feeding. Dishes were kept in incubator set to 25.0±1.0 °C and 65±5% R.H until complete its development. The larval and pupal periods, preoviposition, oviposition, postoviposition, male and female longevities, total female fecundity and fertility were estimated for each larval individual in each feeding group.

Statistical approach

Data of development, longevity, and fecundity were tested using one-way ANOVA. In case of significant, means were isolated using Fisher LSD test (α = 0.05).

RESULTS AND DISCUSSION

Results

Effect of various mealybug eggs on development and reproduction of the green lacewing *C. carnea*

Development

The data presented in Table (1) show the developmental periods of *C. carnea* stages when fed on eggs of different mealybug species (*I. aegyptiaca, I. seychellarum, P. citri* and mixed mealybug eggs). No significant effect of egg type of mealybug species on the incubation and larval periods of *C. carnea*. In contrast, it had significant effect on the pupal stage and total life cycle. Eggs of *I. aegyptiaca* accelerated pupation of *C. carnea* and gave the fastest development of the predator (Table 1).

Reproduction

Eggs of different mealybug species significantly affected certain reproductive outputs. No significant effect of mealybug eggs, used as a food during the larval stage, was reported on preoviposition and postoviposition periods, but there were significant effects on oviposition period, adult longevities, and fecundity. The longest female and male longevities were obtained when predator's larvae provided with mixture of mealybug eggs. Whereas the highest female fecundity was obtained for females that their larvae fed eggs of *P. citri*. Fertility percentages ranged between 98 and 99% in relation to mealybug eggs (Table 2).

Data obtained are difficult to explain in the light of the available research that dealt with effect of mealybug egg types on developmental and reproductive performances. Further, the data obtained in this study did not provide sufficient information to determine if *C. carnea* larvae prefer a mixed prey items over a single-resource diets, or vice versa. Eggs of *I. aegyptiaca* yielded the fastest development and eggs of *P. citri* maximized the reproductive outputs of female predator. Mixing mealybug eggs was not suitable for accelerating development, but prolonged adult longevity of the predator. Therefore, it is generally clear that most of mealybug prey are not suitable food for lacewings, at least when larvae of *C. carnea* are subjected to a diet encompassing this prey. Messelink *et al*. (2016) found that mixing mealybugs with supplemental food led to increase survival and developmental rate of larvae. This results are inconsistent with the current study. Adding eggs of high quality prey, *E. kuehniella* to mealybug diet of lacewing may be the reason for such difference between this study and ours. Several predators exhibited higher development, survival, or egg production rates on mixed diets than on single-resource item (Evans *et al*., 1999; Oelbermann and Scheu, 2002; Toft, 2005; Marques *et al*., 2015). In contrast, the development of *C. carnea* larvae on a diet of mixed mealybug eggs was longer than on a single-mealybug eggs. This is more likely because the predator is truly generalist predator and switch between various prey to gain a single advantage from each prey species.

Table 1. Influence of various mealybug eggs and their mixing eggs on development of the green lacewing *Chrysoperla carnea*

Stage (days)		*Icerya aegyptiaca*	*Icerya Seychellarum*	*Planococcus citri*	Mixed eggs
Egg		3.1 ± 0.06 a	3.1 ± 0.06 a	3.1 ± .09 a	3.2 ± 0.10 a
Larval instar	First	3.2 ± 0.10 a	3.1 ± 0.09 a	3.1 ± 0.06 a	3.1 ± 0.09 a
	Second	3.3 ± 0.11 a	3.2 ± 0.10 a	3.2 ± 0.17 a	3.3 ± 0.11 a
	Third	3.4 ± 0.13 a	3.5 ± 0.13 a	3.1 ± 0.09 a	3.3 ± 0.16 a
Larval		9.7 ± 0.19 a	9.8 ± 0.22 a	9.4 ± 0.23 a	9.5± 0.24 a
Pupal		9.5 ±0.29 b	10.7 ± 0.30 a	9.5 ± 0.32 b	11.5 ± 0.33 a
Total development	(egg-adult)	22.5 ± 0.38 bc	23.5 ± 0.43 ab	22 ± 0.37 c	24.5 ± 0.35 a

Means bearing by the same letters in a row are not significantly different at 0.05 probability level.

Table 2. Influence of various mealybug eggs and their mixing eggs on adult longevity and female reproduction of the green lacewing *Chrysoperla carnea.*

Reproduction			*Icerya aegyptiaca*	*Icerya Seychellarum*	*Planococcus citri*	*Mixed eggs*
Adult longevity (days)	Female Longevity	Pre-oviposition period	3.4 ± 0.15 a	3.5 ± 0.15 a	3.4 ± 0.15 a	3.5 ± 0.15 a
		Oviposition period	31.0 ± 0.76 ab	29.4 ± 0.9 b	30.6 ± 0.60 b	33.6 ± 0.60 a
		Post-oviposition period	5.6 ± 0.41 a	5.3 ± 0.41 a	6.9 ± 0.73 a	5.6 ± 0.41 a
		Total	40.0 ± 0.84 ab	38.2 ± 1.22b	40.9 ± 1.05 ab	42.7 ± 0.90 a
	Male longevity		30.1 ± 0.75 a	21 ± 1.15 b	31.3 ± 0.25 a	32.0 ± 1.7 a
Fecundity			243.6 ± 7.98 b	199.3 ± 8.57 c	300.7 ± 8.29 a	265.0 ± 11.16 b
Fertility (%)			98	98	99	98

Values bearing by the same letters in a row are not significantly different at 0.05 probability level

Effect of second-instar mealybug species on development and reproduction of the green lacewing *C. carnea.*

Development

The results presented in Table (3) show the developmental time of *C. carnea* when fed with the second-instars of different mealybug species (*P. citri, P. solnpsis, I. aegyptiaca*, and *I. seychellarum*). Mealybug species had no significant effect on the incubation period of eggs. Whereas, it had significant effects on periods of the larval and pupal stages and total life cycle (egg-adult). The shortest development of larval stage, pupal stage, and total life cycle were when predator's larvae fed on second-instar *P. citri*.

Table 3. Effect of second-instar mealybug species on development of the green lacewing *Chrysoperla carnea*

Mealybug species / Stage (days)		Planococcus citri	Icerya aegyptiaca	Icerya Seychellarum	Planococcus solnpsis
Egg		3.1 ± 0.06 a	3.1 ± 0.06 a	3.1 ± 0.05 a	3.1 ± 0.04 a
Larval instars	First	3.3 ± 0.12 c	4.6 ± 0.12 a	4.6 ± 0.16 a	3.6 ± 0.08 b
	Second	3.8 ± 0.14 b	3.6 ± 0.13 b	5.04 ± 0.15 a	3.8 ± 0.10 b
	Third	4.8 ± 0.29 b	8.7 ± 0.28 a	7.8 ± 0.26 a	5.7 ± 0.22 b
Larval		11.9 ± 0.25 c	16.9 ± 0.26 a	17.4 ± 0.25 a	13.2 ± 0.25 b
Pupal		9.4 ± 033 c	14.7 ± 0.41 a	12.8 ± 0.47 b	10.6 ± 0.36 c
Total development (Eggs-Adult)		24.4 ± 0.45 c	34.7 ± 0.46 a	33.4 ± 0.36 a	26.9 ± 0.44 b

Means bearing by the same letters in a row are not significantly different at 0.05 probability level.

Reproduction

second-instars of different mealybug species significantly affected different reproductive outputs. The shortest preoviposition, oviposition, and postoviposition periods were when larvae of the predator fed with second-instars of *P. solnpsis*, *I. seychellarum*, and *P. citri* nymphs, respectively. The shortest female longevity was for females reared during their larval stage on *I. seychellarum* nymphs. As well, mealybug species had significant effect on male longevity with the shortest longevity was for males fed during their larval stage on *I. seychellarum* nymphs. Also, female fecundity significantly affected by type of mealybug. The highest female fecundity was obtained for females that their larvae fed with second instars of *P. citri* and *P. solnpsis* without significant differences between both mealybug species. Fertility percentages ranged between 97 and 98% in relation to type of mealybug (Table 4).

Table 4. Effect of second-instar mealybug species on adult longevity, and female reproduction of the green lacewing *Chrysoperla carnea*

Reproduction			*Planococcus citri*	*Icerya aegyptiaca*	*Icyrya Seychellarum*	*planococcus solnpsis*
Adult longevity (days)	Female longevity	Pre-oviposition period	3.5 ± 0.22 bc	4.2 ± 0.24 b	5.4 ± 0.31 a	3.3 ± 0.12 c
		Oviposition period	28.9 ± 0.99 a	24.4 ± 0.75 b	22.3 ± 0.58 b	27.9 ± 0.50 a
		Post-oviposition period	6.6 ± 0.60 a	7 ± 0.60 a	7.2 ± 0.49 a	7.6 ± 0.44 a
		Total	39.0 ± 1.01 a	35.6 ± 0.63 b	34.9 ± 0.75 b	38.6 ± 0.65 a
	Male longevity		23.5 ± 1.13 b	23.7 ± 0.80 ab	19.2 ± 1.53 c	27.3 ± 0.71 a
Fecundity (no. of eggs/ female)			316.3 ± 7.47 a	164.4 ±4.82 b	168.9 ±9.79 b	302.7±10.77 a
Fertility (%)			98	98	97	98

Means bearing by the same letters in a row are not significantly different at 0.05 probability level.

The obtained results for using second-instar nymphs of different mealybug species in feeding trials of *C. carnea* larvae are more clear than using eggs of mealybug species for explanation. second-instars of *P. citri* yielded the fastest development, the highest female fecundity, and the longest female longevity. This means that second-instars of *P. citri* seems to be more suitable prey for development and reproduction of *C. carnea*. The growth rates of the predator stages may be increased by feeding on high quality prey (Torres *et al*., 2004, Barbosa *et al*., 2014). This finding agrees with our results for *C. carnea* fed second-instar *P. citri*.

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تأثير البيئة الغذائية وحيدة المصدر والبيئة الغذائية الخليطة لأطوار البق الدقيقي علي الأداء النموي والإنتاجي لمفترس أسد المن الأخضر

سمير صالح عوض الله، محمد حسن بيومي، أسماء محمد البسيوني

قسم الحشرات الاقتصادية- كلية الزراعة- جامعة المنصورة- المنصورة ٣٥٥١٦- مصر

الملخص

تطبيقات مفترس أسد المن الاخضر مثلت حوالي ثلث برامج المكافحة البيولوجية الناجحة علي مستوي العالم. التطبيق الناجح لبرامج مكافحة البيولوجية للافة بأستخدام مفترس اسد المن الاخضر كعامل مكافحة حيوية يتطلب تحديد جودة الفريسة الأعلي. تم تغذية يرقات المفترس أما علي بيض بق الموالح الدقيقي او البق الدقيقي المصري او بق السيشلارم او خليط من من بيضهم، او علي حوريات العمر الثاني لواحد من هذه الانواع الثلاث من البق الدقيقي وبق القطن الدقيقي. أجريت التجارب علي حرارة ٢٥±١م° ورطوبة نسبية ٦٥±٥ %. التغذية علي بيض البق الدقيقي المصري سرعت من نمو المفترس وأثرت بصورة معنوية علي فترة وضع البيض وعمر الحشرة الكاملة وخصوبة إناث المفترس. تغذية يرقات المفترس علي خليط من بيض الثلاث أنواع من البق الدقيقي اطال فترة عمر انثي وذكر المفترس. في حين سجلت الإناث أعلي خصوبة في حالة تغذية يرقاتها علي بيض بق الموالح الدقيقي. تراوحت نسبة الفقس في بيض المفترس من ٩٨-٩٩% تبعاً لنوع بيض البق الدقيقي المستخدم في التغذية. في حالة أستخدام حوريات العمر الثاني من أنواع البق الدقيقي كغذاء وجد أن أقصر فترة للنمو وأطول فترة لعمر إناث المفترس سجلت في حالة تغذية يرقاتها علي حوريات بق الموالح الدقيقي. اثرت حوريات العمر الثاني للبق الدقيقي بصورة معنوية علي الخصوبة الكلية لاناث المفترس مع أعلي خصوبة كانت لإناث المفترس التي تغذت يرقاتها علي حوريات بق الموالح الدقيقي وبق القطن الدقيقي. تراوحت نسبة فقس البيض بين ٩٧-٩٩% تبعاً لنوع حوريات العمر الثاني المستخدمة في التغذية. يعتمد نجاح اي مفترس بصورة رئيسية علي جودة الفريسة الغذائية. هذه المعلومات سوف تكون مفيدة في إعداد برامج الإدارة المتكاملة للافة.

الكلمات الدالة: اسد المن- البق الدقيقي الابيض- عمر الحشرة الكاملة- الخصوبة الكلية- الخصوبة الفعلية