



## Is a diet of *Planococcus citri* nymphs and adults suitable for *Chrysoperla externa* for use in biological control?

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### ABSTRACT

Several studies have shown that the survival and reproduction of lacewings varies depending on the type of prey they consumed, especially during the first instar. In this study, we evaluated the effect of a diet consisting of *Ephesia kuehniella* (EK) and/or *Planococcus-citri* (PC) eggs offered to first instar *Chrysoperla externa* on subsequent development and survival. Larvae supplied only with *P. citri* (PC) nymphs had extended immature development, low survival, adults with lower weight and longevity, low fecundity and egg viability, and lower net reproduction rate ( $R_0$ ). Larvae submitted to the treatment EK+PC responded positively to the diet, showing results similar to those obtained in the control-EK treatment. Therefore, this mealybug supports suitable development and reproduction of *C. externa* when it is provided to second instar larvae after they fed on *E. kuehniella* eggs as first instars. As far as we know, this is the first report that demonstrates the effect of natural prey and alternatives on different larval stages of lacewings. This information is valuable in developing protocols for use of *C. externa* as a pest control agent.

### Introduction

The mealybug *Planococcus citri* (Risso, 1813) (Hemiptera: Pseudococcidae) is a common pest affecting several crops. This insect has a wide geographic distribution; it is registered in 161 countries, and is highly polyphagous, being able to feed on about 242 plant species, including roses (García Morales et al., 2016; Pillai, 2016; Polat et al., 2008; Suh, 2020). It is a sap-sucking insect (Daane et al., 2012; Mani and Shivaraju, 2016) that causes discoloration, loss of vigor and leaf fall (Santa-Cecília et al., 2020; Marília M. P. Carvalho, personal communication). Their honeydew serves as a substrate for the development of *Capnodium* fungi, known as sooty mold, which covers the plant preventing photosynthesis and reducing the commercial value of the flowers (Copland et al., 1985).

As a result, it is necessary for farmers to keep mealybug populations below the levels of aesthetic damage (Brígida Souza, personal communication). The use of biological agents and the application of phytosanitary products are among the main methods sought by producers. Moreover, there are no registered insecticides for the control

of *P. citri* in roses (Agrofit, 2021), which further increases the need to adopt biological control.

The predator *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae) plays an important role in the regulation of arthropods in a variety of crops (Rodrigues-Silva et al., 2017; Souza et al., 2019). It has essential characteristics for a natural enemy, such as high mobility, voracity, as well as high survival and reproduction rates (Carvalho and Souza, 2009). Several studies show the predatory capacity of *C. externa* associated with insects, such as thrips (Espino et al., 2017; Luna-Espino et al., 2020), bed bugs, lepidopteran eggs (Pacheco-Rueda et al., 2015; Pitwak et al., 2016; Battel et al., 2017; Cuello et al., 2019), aphids (Garzón et al., 2015; Gamboa et al., 2016), whiteflies (Castro et al., 2016), and other arthropods.

Research on the biology of control agents is an essential step for the success of an augmentative biological control program (Parra et al., 2015). In this context, some studies aiming to control *P. citri*, showed that the release of *C. externa* eggs close to hatching resulted in high larval mortality, which made it impossible to continue the studies in later stages (Bonani et al., 2009; Pedro Neto et al., 2008). However,

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there are reports on the use of this lacewing for the control of *P. citri* on roses (Brígida Souza, personal communication), which indicates that both the type of prey may affect the development and reproduction of predatory insects (Principi and Canard, 1984; Dhandapani et al., 2016) and/or that the host plant is important in this trophic relationship (Price et al., 1980; Silva et al., 2004; Adriano et al., 2010).

In general, for grower adoption and successful outcomes of biological control using predators, depends on the positive results of studies that can support the success of the releases. In the case of lacewings, there is scientific evidence that first instar larvae are very demanding in terms of type of consumed prey (Bezerra et al., 2017). Thus, in addition to the greater predatory capacity of more developed instars, the affinity with the target prey is ought to be considered. According to Nordlund et al. (2001), releases of eggs or second instar larvae are recommended.

In large-scale rearing developed in laboratories around the world, where lacewings have been used as control agents for a longer time, the larvae commonly receive eggs of *Ephesia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) (Colares et al., 2015; Nunes et al., 2017; Oliveira et al., 2016). Furthermore, it is also suitable for *C. externa* (Carvalho and Souza, 2009; Morando et al., 2014; Garzón et al., 2015; Dias et al., 2018) and used to mass-rear the species for commercial purposes in Brazil (Brígida Souza, personal communication; JB Biotecnologia Agentes Biológicos, 2021).

In this study, we tested the hypothesis that a diet consisting of *E. kuehniella* eggs provided to the first instar of *C. externa* followed by a diet of *P. citri* nymphs and adults for the second and third instars promotes good development and reproduction of the predator. Thus, we aimed to determine the effect of a diet consisting of *P. citri* and/or *E. kuehniella* eggs applied to first instar larvae of *C. externa* on biological aspects of predator immature and adults.

## Material and methods

### Experiment location

The experiment was conducted in the Laboratory of Biological Control with Entomophages (LCBE), Department of Entomology (DEN), School of Agricultural Sciences of Lavras (ESAL), located at the Federal University of Lavras (UFLA), in the state of Minas Gerais. Insect rearing and bioassays were carried out at  $25 \pm 1^\circ\text{C}$ ,  $70 \pm 10\%$  RH and 12-hour photophase.

### Rose cultivation

Roses belonging to the “híbridas de chá” group (*Rosa* spp. cv Avalanche, white color) were purchased from a producer (Flora Minas, Itapeva, state of Minas Gerais) and cultivated in pots (10L) under greenhouse conditions. The substrate consisted of soil and cattle manure (1:1), in which mineral and organic fertilizers as well as irrigation were applied.

### Rearing and maintenance of insects

A laboratory colony of *P. citri* was grown on ‘Cabotchá’ pumpkins (*Cucurbita maxima* L.), which is a suitable host for rearing this insect in laboratory conditions (Lepage, 1942). Subsequently, adult females with ovisacs were transferred to roses for reproduction and use in the experiments. Sterilized *E. kuehniella* eggs were purchased from the company PROMIP – Integrated Pest Management®.

A laboratory colony of *C. externa* was maintained according to the methodology described by Carvalho and Souza (2009). The larvae were fed on eggs of the alternative prey *E. kuehniella*; adults were supplied

with water and a diet consisting of brewer’s yeast and honey. For the experiments, *C. externa* larvae from eggs up to 24 hours old were obtained from the second generation of insects submitted to the treatments.

### Experimental design

The treatments were as follows:

T1: first, second and third instars fed on *E. kuehniella* eggs (EK-control);

T2: first instars fed on *E. kuehniella* eggs and second and third instar larvae feed on *P. citri* nymphs and adults (EK+PC);

T3: first, second and third instars fed on mealybugs (PC), obtained from the rearing established on roses.

*C. externa* eggs were placed in individual wells of plates used in ELISA (Enzyme-linked immunosorbent assay) tests; each plate had 96 wells. After hatching, the larvae were transferred to plastic containers (5 cm in diameter x 4.5 cm in height) with a lid containing a 2 x 2 cm opening covered with *voile* fabric in order to allow gas exchange. These containers had the bottom covered with a filter paper disk (4.5 cm Ø), which served as shelter. Nymphs and adults of the mealybug *P. citri* and *E. kuehniella* eggs were added daily in a number greater than the consumption capacity of the predator larvae, which was determined in a preliminary test. Uneaten mealybugs and eggs were discarded and replaced and the remains of preyed specimens were discarded. The larvae remained in these containers until they reached the adult stage.

After emergence, adults were identified by sex and individually weighed on an analytical scale (0.001g) (Marte AY220, Marte Balanças e Equipamentos de Precisão Ltda). Male-female pairs were then formed and each pair was held in an individual cylindrical PVC cage (10 cm x 10 cm), internally lined with white bond paper that served as a substrate for oviposition. Water was provided in cotton supported on a plastic lid and the same diet used in maintenance was offered to the adults in the bioassay. This diet was provided on Parafilm® strips attached to the cage wall where the insects had continuous access. The upper end of the cages was closed with PVC plastic film and the base was supported on a plastic tray lined with paper towel.

In a preliminary test, a greater number of females than males emerged and, therefore, a parallel rearing was carried out, in which larvae were subjected to the same treatments and experimental conditions. This procedure aimed only at obtaining “extra” males for the formation of pairs in a sufficient number to conduct the tests with the adult phase.

To evaluate development and survival, we inspected the lacewings of all treatments daily until the end of their life for the preimaginal period, we recorded: the duration (days) and survival (%) of the embryonic stage each instar, the prepupal stage, the pupal stage, and the complete preimaginal period. For the adult phase, the following parameters were evaluated: adult weight (mg) upon emergence, sex ratio obtained by the formula ( $SR = \text{No. females} / \text{No. males} + \text{No. females}$ ) (Silveira Neto, 1976), the pre-oviposition, oviposition and post-oviposition periods (days), daily and total egg production (number of eggs), egg viability (%), longevity (days) of adults (regardless of sex), longevity and survival of females and males (days). To assess egg viability, ten eggs from each replication were collected daily from each of the treatments and held individually on ELISA plates, which were then closed with a transparent PVC film. The percentage of viable eggs was calculated based on the number of hatched larvae.

We evaluated the following population parameters: intrinsic growth rate ( $r_m = \sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1$ ), finite growth rate ( $\lambda = e^r$ ), net reproduction rate ( $Ro = \sum_{n=0}^{\infty} l_n m_n$ ) and mean generation time

$(T = \frac{\ln(Ro)}{r})$  (Birch, 1948; Goodman, 1982; Chi and Liu, 1985; Chi, 1988). Means and standard errors of population parameters were estimated using the Bootstrap method, with 100,000 resamplings, and the existing differences were analyzed by the paired Bootstrap test using the statistical software TWOSXMSChart (Efron and Tibshirani, 1993; Huang et al., 2018; Chi, 2020).

A completely randomized design with three treatments with 80 replications each was used. All parameters were subjected to analysis of variance (ANOVA) assumptions, such as the normality of the residuals and homoscedasticity of the variance, using the Anderson-Darling and Bartlett tests, respectively, and the means were analyzed by the Tukey test. Data that did not meet the ANOVA assumptions were analyzed using the generalized linear model (GLM) or the nonparametric Kruskal-Wallis test and, when significant, the means were analyzed using the Tukey and Dunn tests, respectively. A significance level of  $p < 0.05$  was adopted to detect differences between treatments. For the weight and longevity of adults, the factorial scheme (3 types of diet x 2 genders) was used. Data obtained for longevity were transformed into square roots.

Duration data were analyzed by the Kruskal-Wallis test and survival data for instars, immature stages and sex ratio were analyzed by (GLM) with binomial model, logit link and F test. Egg viability and adult weight were also analyzed by GLM, but the Gamma and inverse link model were used. The Kaplan-Meier survival curves referring to the immature phases as well as those referring to the survival of *C. externa* males and females were compared using the log-rang test. All analyses were performed using the statistical software R 3.3.1 (R Core Team, 2019). The statistical packages used were "nortest" (Gross and Ligges, 2015), "rstatix" (Kassambara, 2021), "hnp" (Moral et al., 2018) and "emmeans" (Lenth et al., 2021).

## Results

### Development and preimaginal survival

The consumption of *E. kuehniella* eggs and/or *P. citri* nymphs and adults did not affect the duration of the embryonic stage of *C. externa* ( $p = 0.369$ ), which was, on average, five days. When larvae were fed exclusively on mealybugs in all instars (T3-PC), there was an extension in the duration of the first ( $K = 151.95$ ;  $df = 2$ ;  $p < 0.001$ ), second ( $K = 74.862$ ;  $df = 2$ ;  $p < 0.001$ ) and third instars ( $K = 142.12$ ;  $df = 2$ ;  $p < 0.001$ ), as well as in the prepupal ( $K = 28.992$ ;  $df = 2$ ;  $p < 0.001$ ) and pupal ( $K = 6.2691$ ;  $df = 2$ ;  $p < 0.05$ ) stages, compared with those that were supplied with

mealybugs only from the second instar (T2-EK+PC) and those that fed only on *E. kuehniella* eggs (T1-EK) (Figure 1). Consequently, there was a longer duration of the egg-pupa period ( $31.34 \pm 0.53$  days) when the larvae were fed only on *P. citri* when compared with those who consumed only *E. kuehniella* eggs ( $26.17 \pm 0.21$  days) or the combination of eggs and mealybugs ( $24.05 \pm 0.06$  days) ( $K = 127.8$ ;  $df = 2$ ;  $p < 0.001$ ). The survival of larvae from the first ( $F = 14.94$ ;  $df = 2$ ;  $p < 0.001$ ) and third instars ( $F = 5.91$ ;  $df = 2$ ;  $p < 0.01$ ) as well as the prepupal stage ( $F = 4.46$ ;  $df = 2$ ;  $p < 0.05$ ) and the egg-pupa period ( $F = 24.61$ ;  $df = 2$ ;  $p < 0.001$ ) varied according to the supply of *E. kuehniella* eggs and/or *P. citri* nymphs (Table 1). The survival of these instars and developmental stage were lower when the larvae consumed exclusively *P. citri* (T3-PC). There was a mortality rate close to 24% of first instar larvae fed on mealybug. On the other hand, there was no significant effect of the diet on the survival of the embryonic stage ( $F = 1.00$ ;  $df = 2$ ;  $p = 0.369$ ) of the second instar ( $F = 1.63$ ;  $df = 2$ ;  $p = 0.194$ ) or the pupal stage ( $F = 0.222$ ;  $df = 2$ ;  $p = 0.800$ ) (Table 1).

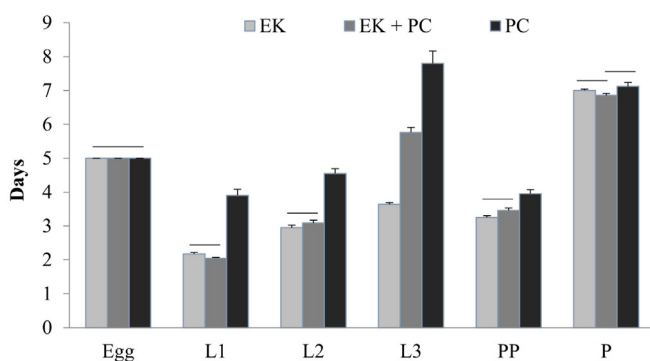
The duration ( $K = 127.8$ ;  $df = 2$ ;  $p < 0.001$ ) and survival ( $\chi^2 = 46.40$ ;  $df = 2$ ;  $p < 0.001$ ) of the preimaginal period for larvae subjected to different feeding regimes varied significantly (Figure 2).

The shortest preimaginal period (24.05 days) was obtained for larvae fed on *E. kuehniella* eggs (EK), and the longest (31.34 days) for those fed on the mealybug *P. citri* (PC) (Figure 2). This extended development period is consistent with the reduction observed for the survival of immature stages (50%) when larvae were supplied only with mealybug, unlike the results observed when they received *E. kuehniella* eggs at least in the first instar (EK or EK+PC), which resulted in a survival of up to 94%.

### Adult development

The larval diet significantly affected the body weight of subsequent females and males. There were significant differences between diets ( $F = 80.61$ ;  $df = 2$ ;  $p < 0$ ), genders ( $F = 88.43$ ;  $df = 1$ ;  $p < 0$ ) and the interactions between them ( $F = 3.38$ ;  $df = 2$ ;  $p = 0.036$ ) (Figure 3).

Females and males showed lower body weight when larvae were fed only on mealybug (PC) (female = 5.41 mg and male = 4.75 mg). The weight was intermediate when they consumed *E. kuehniella* eggs only in the first instar (EK+PC) (female = 6.81 mg and male = 5.33 mg) and higher when they were supplied with *E. kuehniella* eggs throughout the entire larval period (EK) (female = 8.20 mg and male = 6.59 mg) (Figure 3). Females obtained greater weight than males for all treatments. After weighing, 40, 41 and 22 pairs were formed from larvae that received EK, EK+PC and PC, respectively. Diet did not significantly interfere with sex ratio ( $F = 0.585$ ;  $df = 2$ ;  $p = 0.558$ ), which ranged from 0.53 to 0.62. The sex ratio was close to 1:1 (Table 2).



**Figure 1.** Duration of instars and preimaginal phases (days) of *Chrysoperla externa* as a function of feeding on *Ephestia kuehniella* eggs and/or *Planococcus citri* nymphs and adults. Means  $\pm$  SE corresponding to the columns paired under the horizontal bar do not differ by the Dunn test (Kruskal-Wallis,  $p < 0.05$ ). L1= 1st instar larvae; L2= 2nd instar larvae; L3= 3rd instar larvae; PP= Prepupae; P= Pupae. EK= *E. kuehniella* eggs; EK + PC= *E. kuehniella* eggs in the first instar and *P. citri* in subsequent instars; PC= *P. citri* in all instars.

**Table 1**

Survival (%) of *Chrysoperla externa* instars and preimaginal stages in response to feeding on *Ephestia kuehniella* eggs and/or *Planococcus citri* nymphs and adults.

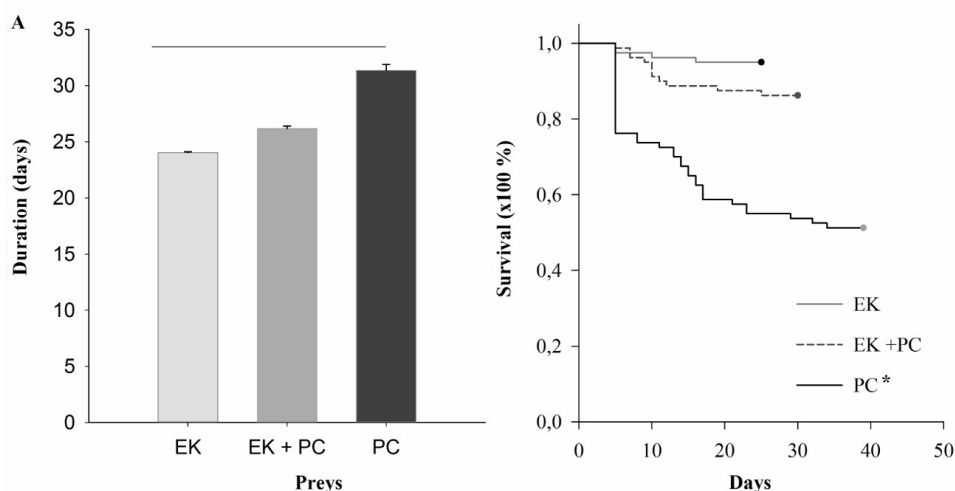
Phases / Instars	T1-EK	T2-EK+PC	T3-PC
	Mean $\pm$ SE*	Mean $\pm$ SE	Mean $\pm$ SE
Egg	100 $\pm$ 0.00 a	100 $\pm$ 0.00 a	100 $\pm$ 0.00 a
1 <sup>st</sup> instar	97.50 $\pm$ 1.75 a	98.75 $\pm$ 1.25 a	76.25 $\pm$ 4.78 b
2 <sup>nd</sup> instar	100.00 $\pm$ 0.00 a	97.5 $\pm$ 1.75 a	97.5 $\pm$ 1.75 a
3 <sup>rd</sup> instar	98.75 $\pm$ 1.25 a	92.50 $\pm$ 2.96 ab	85.00 $\pm$ 4.01 b
Pre pupa	100.00 $\pm$ 0.00 a	100.00 $\pm$ 0.00 a	95.00 $\pm$ 2.45 b
Pupa	98.75 $\pm$ 1.25 a	97.50 $\pm$ 1.75 a	97.50 $\pm$ 1.75 a
Egg-Pupa	95.00 $\pm$ 2.45 a	86.25 $\pm$ 3.87 a	51.25 $\pm$ 5.62 b

\*Means  $\pm$  SE followed by different letters on the same line differ from each other by the Tukey test ( $p < 0.05$ ), through the GLM-binomial analysis. EK= *E. kuehniella* eggs; EK + PC= *E. kuehniella* eggs in the first instar and *P. citri* in the subsequent instars; PC= *P. citri* in all instars.

**Table 2**  
Sex ratio, reproductive parameters and egg viability of *Chrysoperla externa* adults from larvae fed on *Ephesthia kuehniella* eggs and/or *Planococcus citri* nymphs and adults.

Parameters	T1-EK		T2-EK+PC		T3-PC	
	N	*Mean ± SE	N	Mean ± SE	N	Mean ± SE
RZ <sup>1</sup>	75	0.53 ± 0.06 a	66	0.62 ± 0.06 a	40	0.55 ± 0.08 a
PPREO <sup>2</sup> (days)	40	4.02 ± 0.08 b	41	4.34 ± 0.09 ab	22	4.77 ± 0.15 a
PO <sup>3</sup> (days)	40	62.37 ± 2.61 a	41	56.46 ± 3.167 a	22	32.68 ± 3.07 b
OD <sup>3</sup> (n° of eggs)	40	17.47 ± 0.61 a	41	17.02 ± 0.59 a	22	15.35 ± 0.90 a
OT <sup>3</sup> (n° of eggs)	40	1076.90 ± 48.92 a	41	958.15 ± 58.19 a	22	513.73 ± 53.03 b
PPOSO <sup>2</sup> (days)	40	1.52 ± 0.36 a	41	2.49 ± 0.56 a	22	4.73 ± 0.96 a
OV <sup>4</sup> (%)	40	98.33 ± 0.22 a	41	97.93 ± 0.24 a	22	96.30 ± 0.45 b

\*Means ± SE followed by different letters on the same line differ from each other by the Tukey test ( $p < 0.05$ ) (GLM binomial<sup>1</sup>, ANOVA<sup>2</sup> and GLM Gamma<sup>3</sup>) and the Dunn test (Kruskal–Wallis<sup>2</sup>) ( $p < 0.05$ ). N= Number of individuals. RZ= Sex ratio; PPREO and PPOSO= Pre and post-oviposition period; PO= Oviposition period; OD and OT= Daily and total oviposition; OV= Viable eggs. EK= *E. kuehniella* eggs; EK + PC= *E. kuehniella* eggs in the first instar and *P. citri* in subsequent instars; PC= *P. citri* in all instars.

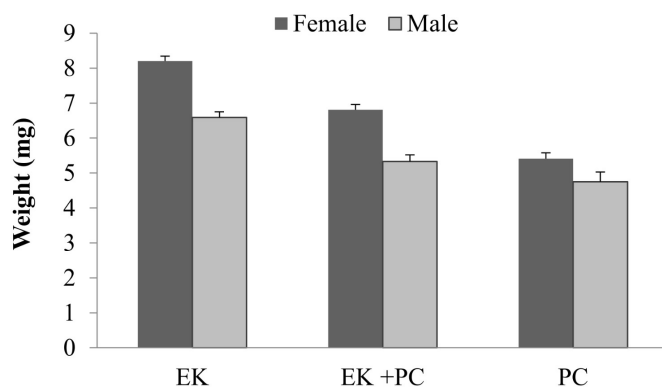


**Figure 2.** (A) Duration (days) and (B) survival (x100%) of the preimaginal period of *Chrysoperla externa* for larvae fed on *Ephesthia kuehniella* eggs and/or *Planococcus citri* nymphs and adults. Means ± SE under the horizontal bar differ from each other by the Dunn test (Kruskal–Wallis,  $p < 0.05$ ). Means containing an asterisk\* differ from each other by the log-rank test (Kaplan–Meier). EK= *E. kuehniella* eggs; EK + PC= *E. kuehniella* eggs in the first instar and *P. citri* in subsequent instars; PC= *P. citri* in all instars.

Soon after mating, the females took 3 to 6 days to start oviposition (PPREO). This variation was affected by the type of diet ingested in the larval stage ( $K = 18.10$ ;  $df = 2$ ;  $p < 0$ ). A similar response did not occur for the post-oviposition period (PPOSO), which was not significantly affected by the diet consumed in the larval stage ( $K = 2.06$ ;  $df = 2$ ;  $p = 0.355$ ). The oviposition period (PO) was significantly longer ( $F = 20.78$ ;  $df = 2$ ;  $p < 0$ ) when the larval diet was exclusively or partially *E. kuehniella* eggs (EK) or (EK+PC) (Table 2).

The type of diet available to the larvae did not statistically affect the average daily egg production of subsequent adults ( $F = 2.16$ ;  $df = 2$ ;  $p = 0.121$ ). However, the food consumed during the larval period influenced the total number of eggs produced throughout the reproductive period and egg viability (Fecundity:  $F = 22.03$ ;  $df = 2$ ;  $p < 0$ ; Egg viability:  $F = 11.84$ ;  $df = 2$ ;  $p < 0.001$ ). Fecundity was about twice as high when larvae were fed only on *E. kuehniella* eggs (EK) or when they received *E. kuehniella* eggs and mealybug (EK+PC). Regardless of diet, egg viability was greater than 95% (Table 2).

There were differences in longevity as a function of diet ( $F = 19.35$ ;  $df = 2$ ;  $p < 0.001$ ) and gender ( $F = 8.61$ ;  $df = 1$ ;  $p < 0.01$ ). However, there was no interaction between these factors ( $F = 1.04$ ;  $df = 2$ ;  $p = 0.399$ ). The greatest longevity occurred for treatments EK and EK+PC ( $71.29 \pm 1.95$  and  $65.82 \pm 2.66$  days). For PC, longevity was  $49.97 \pm 3.53$  days. With regard to gender, it was found that males lived longer ( $69.59 \pm 1.97$  days) than females ( $60.80 \pm 2.53$  days). There was an influence of the diet consumed by *C. externa* larvae on the survival of adults ( $\chi^2 = 17.58$ ;  $df = 2$ ;  $p < 0.001$ ) and on the survival of females and males ( $\chi^2 = 7.315$ ;  $df = 1$ ;  $p < 0.01$ ) (Figure 4).

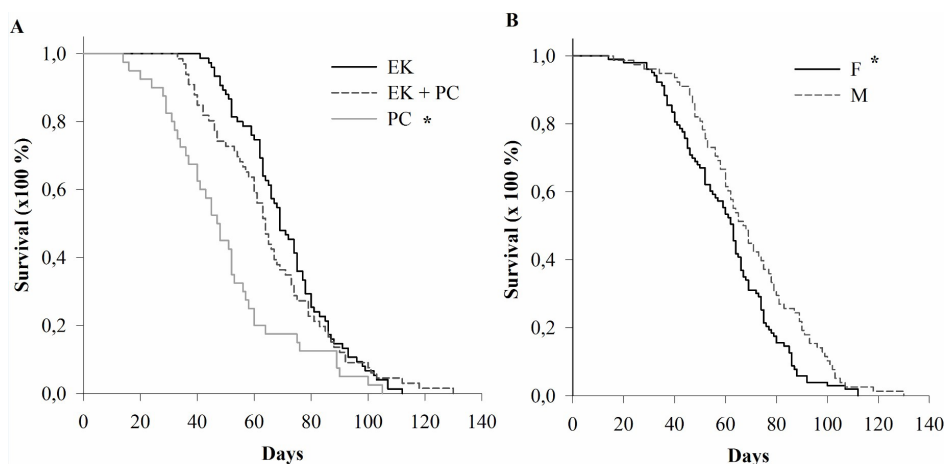


**Figure 3.** Weight (mg) of *Chrysoperla externa* males and females from larvae fed on *Ephesthia kuehniella* eggs and/or *Planococcus citri* nymphs and adults. Means ± SE followed by different letters, uppercase for prey types and lowercase for females and males, differ from each other by the Tukey test ( $p < 0.05$ ). EK= *E. kuehniella* eggs; EK + PC= *E. kuehniella* eggs in the first instar and *P. citri* in subsequent instars; PC= *P. citri* in all instars.

#### Population parameters

There was no effect of the diet experienced in the larval stage on the intrinsic growth rate ( $r_m$ ), finite growth rate ( $\lambda$ ) or mean generation time ( $T$ ) ( $p < 0.05$ ) (Table 3). However, the diet to which the larvae were submitted significantly reflected the net reproduction rate ( $R_0$ ) ( $p < 0.05$ ) (Table 3), which was lower when only mealybugs were provided to the





**Figure 4.** Survival (x100%) of *Chrysoperla externa* adults from larvae fed on (A) *Ephestia kuehniella* eggs and/or *Planococcus citri* nymphs and adults, as a function of gender (B), female and male. Curves with an asterisk (\*) differ by the log-rank test (Kaplan-Meier,  $p < 0.05$ ). EK= *E. kuehniella* eggs; EK + PC= *E. kuehniella* eggs in the first instar and *P. citri* in subsequent instars; PC= *P. citri* in all instars. F= females; M= males.

three larval stages of the predator. When larvae received *E. kuehniella* eggs in the first instar and mealybugs in the second, the results were similar to those obtained with the supply of only *E. kuehniella* eggs.

## Discussion

Our research demonstrated that the use of *E. kuehniella* eggs for rearing first instar larvae and *P. citri* mealybugs for the other instars changed the life history of *C. externa*. It ensured the preimaginal and adult development of the lacewing, with results similar to those obtained for larvae fed only on *E. kuehniella* eggs. *C. externa* specimens were able to develop, survive and reproduce after the larvae were fed on *E. kuehniella* eggs and/or *P. citri* nymphs and adults. However, the mealybug provided throughout the larval period of *C. externa* was not entirely suitable for the immature and adult development of the predator. These results confirm those obtained by Pedro Neto et al. (2008) and Bonani et al. (2009).

The durations of the first and second instars as well as that of the prepupal and pupal stages were similar to the results obtained by Bonani et al. (2009). Nevertheless, the duration of the third instar found in our research (about 8 days) was longer than that verified by the previous authors (5 days). The survival obtained for first instars (24%) resulting from a diet consisting only of mealybugs was similar to that found by Pedro Neto et al. (2008). The highest mortality of larvae fed only on *P. citri* was caused by the waxy and sticky secretion produced and released by mealybugs during predation. In contact with air, this secretion quickly solidifies in the larval mouthparts, making it difficult for them to feed or even causing their death by starvation, since they are prevented from feeding, as also reported by Gillani and Copland (1999). It was observed that the larvae had difficulties in continuing their predatory activity and some of them ended up dying attached to the rearing container. It was also found that first instar larvae are more sensitive to this wax impregnation in the mouthparts when compared with those from later instars. In addition to the predatory act itself, this secretion interferes with the ability of searching and parasitism by natural enemies (Bugila et al., 2014).

The reduction in survival throughout the preimaginal period (51%) verified for *C. externa* larvae fed only on *P. citri* nymphs and adults was also observed by Tapajós et al. (2016), after this lacewing larvae consumed *Pseudococcus jackbeardsleyi* Gimpel & Miller, 1996 (Hemiptera: Pseudococcidae) nymphs. *P. citri* nymphs from roses were poorly suited

**Table 3**

Population parameters of *Chrysoperla externa* from larvae fed on *Ephestia kuehniella* eggs and/or *Planococcus citri* nymphs and adults.

Parameters	T1-EK	T2-EK+PC	T3-PC
	*Mean ± SE	Mean ± SE	Mean ± SE
$r_m$ (days <sup>-1</sup> )	0.15 ± 0.003 a	0.14 ± 0.003 a	0.10 ± 0.005 a
$\lambda$ (days <sup>-1</sup> )	1.16 ± 0.004 a	1.15 ± 0.003 a	1.11 ± 0.005 a
$R_0$ (descendants/ individual)	537.86 ± 64.568 a	491.05 ± 61.235 a	141.27 ± 29.378 b
T (days)	42.39 ± 0.377 a	44.65 ± 0.503 a	48.60 ± 1.148 a

\*Means ± SE followed by different letters on the same line differ from each other by the Bootstrap test paired with 100,000 resamplings ( $p < 0.05$ ).  $r_m$ = intrinsic growth rate;  $\lambda$ = finite growth rate;  $R_0$ = net reproduction rate; T= average time of a generation. EK= *E. kuehniella* eggs; EK + PC= *E. kuehniella* eggs in the first instar and *P. citri* in subsequent instars; PC= *P. citri* in all instars.

for the development of *Chrysoperla lucasina* (Lacroix, 1912) (Neuroptera: Chrysopidae) (Messelink et al., 2016).

Therefore, it was observed that not all mealybug species are suitable prey for lacewings in general, at least when the larvae are subjected to a diet consisting exclusively of this prey. However, the survival percentage was higher than 92% for preimaginal stages, when considering a sex ratio of 1:1 [commonly found for *C. externa* (Bezerra et al., 2006; Trivellato et al., 2012)] and female weight relatively high (Silva et al., 2004) with a diet consisting of first instar *E. kuehniella* eggs and mealybugs in the subsequent (EK + PC).

Considering the characteristics sought in a biological control agent, the EK+PC diet yielded a short pre-oviposition period, high fecundity and egg viability, long oviposition period and adult longevity. The means obtained for these parameters were higher than those by Costa et al. (2012), when *C. externa* larvae fed on *Neotoxoptera formosana* (Takahashi, 1921) (Hemiptera: Aphididae). The post-oviposition period was similar to that found by Pitwak et al. (2016) for larvae fed on *E. kuehniella* eggs and other prey involved in their study.

The mixed diet tested in our study yielded *C. externa* population parameters, with  $R_0$  and T values higher than those obtained by Palomares-Pérez et al. (2020), although  $\lambda$  and  $r_m$  were lower. These differences may be related to the prey itself, the experimental methodology adopted in each of the studies, as well as the different host plants of the prey, which can influence predator performance (Price et al., 1980; Sujii et al., 2020).

The predator biological response is one of the measures of the adequacy of the trophic interaction among plant-herbivore-natural enemy, and should be considered when recommending the predator

for population reduction of the target arthropod. Thus, our results reiterate the importance of offering *E. kuehniella* eggs to first instar *C. externa* larvae, already emphasized by Bezerra et al. (2017), who studied the effects of an artificial diet on the larval and reproductive development of *C. externa*. The nutritional quality of *E. kuehniella* eggs was also considered responsible for the reduction in development time, high survival and egg production by *C. externa* (Carvalho and Souza, 2009; Morando et al., 2014; Garzón et al., 2015). It is believed that, the release of larvae can be more efficient than that of eggs, due to the immediate ability to seek and consume prey. The eggs can be preyed upon by ants because they are still and without defense (Hayashi and Nomura, 2014).

Therefore, it is recommended that larvae be fed on *E. kuehniella* eggs and released in the second stage of development, not only because of the lower voracity of first instar larvae, but also the greater sensitivity of this instar to the sticky honeydew secreted by *P. citri*. This research provides basic information for decision making regarding the most suitable stage of *C. externa* for the control of *P. citri* in roses and it may also contribute to the development of subsequent research with the aim of enabling the use of *C. externa* in population reduction of *P. citri* and other crop pests.

## Conclusion

The mealybug *P. citri* reared from 'Avalanche' roses is a suitable prey for the development and survival of immature and *C. externa* adults only if first instar larvae are fed on *E. kuehniella* eggs.

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## Conflicts of interest

The authors declare no conflicts of interest.

## Author contribution statement

All authors contributed materially to this scientific research. MMPC and BS planned the work and wrote the manuscript. MMPC, LACR, MLCP, MMM and DAV performed the experiments. MMPC conducted data analyses. MMPC, BS and DAV Revised and edited the manuscript. All authors accepted the final version of this work.

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