# Trophic relationship between *Chrysoperla externa* (Neuroptera: Chrysopidae) and *Planococcus citri* (Hemiptera: Pseudococcidae) associated with rose bushes

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## ARTICLE INFO

## ABSTRACT

This study aimed to determine the consumption of *Planococcus citri* (Risso, 1813) (Hemiptera: Pseudococcidae) nymphs and the predator:prey ratio required for high predatory efficacy and survival of *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae) larvae under different densities of prey, reared in rose bushes. Consumption by first-, second-, and third-instar larvae of *C. externa* was assessed. The predatory efficiency and survival of the predator were calculated for larvae in the second instar under densities of 20, 40, 80, 120, and 160 nymphs of *P. citri* and predator:prey ratios of 1:5, 1:10, and 1:20. The assessments were performed after 24 and 48 hours of exposure between species. There was increasing consumption throughout the larval development of *C. externa*. The predator:prey ratios 1:5 and 1:10 were the most efficient for *P. citri* population reduction, but the 1:5 ratio led to lower survival of larvae than the 1:10 and 1:20 ratios. There was a positive correlation between *C. externa* larval density and cannibalism ($\rho$ = 0.45) and a negative correlation between predator density and *P. citri* population density ($\rho$ = -0.81). Third-instar larvae consume more *P. citri* nymphs than second-instar larvae. Second-instar larvae of this lacewing released at a 1:20 ratio caused total mortality of mealybug nymphs within 48 hours, regardless the density of *P. citri*, with high larvae survival of *C. externa*.

## Introduction

Rose cultivation is attacked by several phytophagous species that can cause damage and compromise commercialization. Among the pests commonly associated with rose cultivation is the mealybug *Planococcus citri* (Risso, 1813) (Hemiptera: Pseudococcidae), which can cause considerable yield losses. These insects suck the sap and develop sooty mold on their excreta (Mani and Shivaraju, 2016), which prevent photosynthesis and reduce the commercial value of the flowers.

One strategy that is growing in popularity among producers for the population reduction of floriculture pests is biological control (Carvalho et al., 2019). Among the possible bioagents for use against *P. citri* is *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae), a Neotropical species naturally found in several agroecosystems (Carvalho and Souza, 2009; Cortez-Mondaca et al., 2016; Romero et al., 2019; Lara et al., 2020). Recently, *C. externa* was registered in Brazil for commercialization and control of different pest species (Agrofit, 2022).

This predator is known for its voracity against aphids, whiteflies, thrips, psyllids, mites, and mealybugs (Pedro-Neto et al., 2008; Morando et al., 2014; Castro et al., 2016; Gamboa et al., 2016; Cuello et al., 2019; Luna-Espino et al., 2020). The larvae also feed on *P. citri* (e.g., Carvalho et al., 2008), but in some cases, the prey has negative effects on the biological parameters of the predator (Pedro Neto et al., 2008).

The predatory efficiency and survival of a species depend on several factors. For *C. externa*, among the causes of the divergences found in these studied ecological variables, the prey population density, the predator:prey proportion, and the exposure time between them stand out (Barbosa et al., 2008; Gamboa et al., 2016). Many substances sequestered from plants by phytophagous organisms are used as chemical defenses against their natural enemies and can impair their development and survival (Jessie et al., 2015). Silva et al. (2004), for example, found that consumption by *C. externa* larvae varied according to the host of the ingested prey.

Strategies aimed at maximizing the efficiency of biological control of *P. citri* should be sought. Given the scarcity of such information and

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the importance of population management of this mealybug, this study aimed to assess the consumption of *P. citri* nymphs by *C. externa* larvae and the predator:prey ratio required to obtain high predatory efficacy and survival of predator larvae under different prey densities.

## Material and methods

### Obtaining insects

The rearing of *C. externa* and *P. citri*, as well as the bioassays performed, were conducted in a climate-controlled room at 25 ± 1°C and 70 ± 10% RH with a 12-hour photoperiod, as described by Carvalho and Souza (2009) and Lepage (1942). Due to the high number of *P. citri* specimens required for the bioassays, pumpkins (*Cucurbita maxima* L.) of cultivar Kabocha were used as substrates for rearing the *P. citri*. From this rearing, the laboratory colony of adult females containing an ovisac was collected for infestation of rose bushes (*Rosa* spp. cv. Avalanche - white color) grown in a greenhouse.

To standardize the developmental stage of the mealybug, first-instar nymphs were transferred to Petri dishes (15 cm) containing rose leaflets arranged on a 1% agar-water layer, according to the method adopted by Garzón et al. (2015). The bioassays were conducted in plastic containers (5 cm in diameter and 4.5 cm in height), with a bottom lined with filter paper to serve as a substrate for mouth parts cleaning and as a place of refuge for the chrysopid larvae. The plastic containers were sealed with a hollow plastic lid, and the opening was covered with *voile* fabric.

### Consumption of Planococcus citri *nymphs by* Chrysoperla externa *larvae*

The consumption by larvae of first-, second-, and third-instar *C. externa* was assessed by making second-instar nymphs of *P. citri* available in quantities greater than the predatory capacity according to preliminary tests. To assess consumption in the second and third instars, the larvae were previously fed (*ad libitum*) eggs of the flour moth *Ephestia kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae) until reaching the instar to be studied. This prey was used because it is a food option commonly used in the breeding of lacewing (Carvalho and Souza, 2009).

To standardize the prey age, 20 adult females were added to leaflets of rose bushes supported on a layer of agar:water (1%). After two days, the females were removed, leaving only the eggs. After approximately four days of the embryonic period and seven days of the first instar, the nymphs of *P. citri* met in the second instar (De Paula, 2017). The instar change was verified by the presence of the exuvia.

The insects were confined in plastic containers. Daily, all mealybugs that were not consumed were replaced by others in the second instar, which ensured the consumption of prey of the same instar. Throughout the larval stage of *C. externa*, the dead mealybugs that had a twisted or dehydrated appearance, characteristic of predatory action, were counted daily, yielding the total number of nymphs consumed per instar.

### Predatory efficiency and survival of Chrysoperla externa *larvae as a function of the predator:prey ratio and density of* Planococcus citri *nymphs*

Twenty, 40, 80, 120, or 160 second-instar nymphs of *P. citri* were added to the plastic containers, and the larvae of *C. externa* in the second instar were released in predator:prey ratios of 1:5, 1:10, and 1:20, according to the initial densities of mealybugs. Thus, for the respective proportions, 4, 8, 16, 24, and 32; and 2, 4, 8, 12, and 16; and 1, 2,

4, 6, and 8 and 8 *C. externa* larvae were released, respectively. At 24 and 48 hours after larval release, we counted dead mealybugs with signs of predation (twisted and dehydrated aspects) and the surviving *C. externa* larvae. The standardization nymphs age was similar to the previous test. Larvae killed by cannibalism, characterized by injuries (signs of predation) on the predator's body, were also counted. No initial nymphs were replaced, but the survivors were replaced by others at the same developmental stage to enable further assessments with nymphs of the same instar. The proportion of mealybug nymphs consumed (PMNC) was determined by the following formula, adapted from Abbott (1925): PMNC = PM/DMIP, where PM = predated mealybugs and DMIP = density of mealybugs initially provided.

### Data analysis

The consumption data were analyzed by the nonparametric Kruskal–Wallis test, and the means were compared by Dunn's multicomparison test ($p < 0.05$), with 20 replicates per treatment. To assess the effect of the density of *P. citri* nymphs and the proportion of *C. externa* larvae on predatory efficiency and survival, the generalized linear model with the quasibinomial distribution, the *clog log* link, and the F test, with 15 replicates per treatment, were used. In the cases of significance ($p < 0.05$), the means were compared using the Tukey test. All analyses were performed using R 3.6 software (R Core Team, 2019) using the packages lme4 (Bates et al., 2015), emmeans (Lenth et al., 2021), and hnp (Moral et al., 2017). Spearman's correlation coefficient ($\rho$) was calculated to find any relationship between larval density and cannibalism and between the densities of *C. externa* larvae and *P. citri* nymphs.
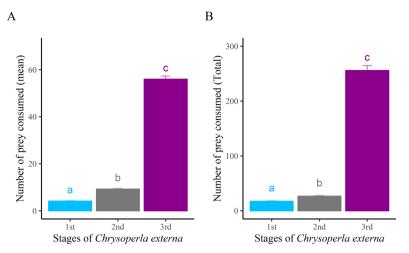
## Results and discussion

The consumption of *P. citri* nymphs by *C. externa* larvae increased throughout the development of the predator (first to the third instar). Significant differences were found in the mean (K = 47.75; df = 2; $p < 0.001$) and a total number of mealybugs predated (K = 44.84; df = 2; $p < 0.001$) as a function of the lacewing instars (Fig. 1).

The larvae of the first, second, and third instars of *C. externa* consumed an average of 4.1, 9.2, and 55.9 nymphs per day and 17.4, 26.6, and 255.8 specimens of the pseudococcid along each instar, respectively. The consumption of *P. citri* nymphs by third-instar larvae represented 81% of the total observed for the entire larval phase of the predator. These results reiterate the greater voracity of this instar about the others, as usually reported in the scientific literature for larvae of this chrysopid (Fonseca et al., 2015; Cuello et al., 2019) or other species of Chrysopidae (Elango and Sridharan, 2017; Rahman et al., 2017; Farhan et al., 2019; Moreira et al., 2019).

Similar findings were obtained by Bezerra (2014) on the consumption of larvae of *C. externa* fed with second-instar nymphs of *P. citri* reared in potato (*Solanum tuberosum* L.) cultivar Monalisa sprouts. However, the author observed a total consumption low of mealybugs by first and second instar larvae (7.9 and 15.2 nymphs, respectively), then we found in our study. As the host plant of the phytophagous insect usually affects the third trophic level in various ways (Price, 1980; Sujii et al., 2020), the difference in the number of nymphs consumed may be related to the species used in the rearing of the mealybug, which in our study were rose bushes of cultivar Avalanche.

One of the greatest difficulties encountered by lacewing larvae in the predation of pseudococcids is the sticky exudates released by exocrine glands in the body of these insects. These secretions adhere to the mouthparts of predator larvae at the time of prey capture and, when contacting air, solidify, making it difficult to capture other prey.

A

B



**Figure 1** Mean (A) and total (B) number of second-instar nymphs of *Planococcus citri* consumed by first-, second-, and third-instar larvae of *Chrysoperla externa* (mean ± SE). Different letters in a column indicate significant differences by Dunn's multicomparison test ($p < 0.05$).
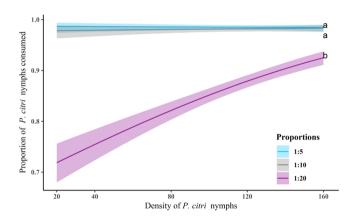
This is a defense strategy presented by pseudococcids that leads, in most cases, to the death of the predator (Santa-Cecília et al., 2020).

The *C. externa* first-instar larvae were the most vulnerable to the impregnation of the mouth parts by these exudates. Our observations showed that the smaller size and greater fragility of the mouth parts apparatus were the characteristics responsible for the 25% mortality rate of first-instar predators. On the other hand, the larvae of the second and third instars, due to their larger size and more resistant oral parts, are more likely to survive. In this respect, in natural environments, the larvae find various types of substrates (parts of the host plant, for example) that can be used to help clean the oral apparatus. In these situations, these exudates most likely do not represent such an important mortality factor for larvae that feed on mealybug colonies of the family Pseudococcidae.

Although the third-instar larvae required a greater number of mealybugs because they are bigger (Fig. 1), they spend a relatively short time in contact with prey as they soon pupate. Second-instar larvae also had high predatory efficiency on second-instar nymphs of the mealybug, so we considered the possibility of using larvae of this instar as the agents of *P. citri* population reduction. This efficiency depended on the density of prey offered initially (F = 39.100; df = 4; $p < 0.001$), the ratio between the number of larvae and the number of nymphs (F = 344.793; df = 2; $p < 0.001$), and the interaction between these factors (F = 13.014; df = 8; $p < 0.001$) (Fig. 2).

The proportions 1:5 and 1:10 resulted in a significant reduction in the number of mealybugs (minimum 97%), regardless of the initial prey density or the number of larvae of the predator, differing statistically from the 1:20 ratio. Generally, higher predator:prey ratios, in which the difference between the number of predators and the number of prey is smaller, are more efficient in controlling the pest (Alghamdi et al., 2018; Li et al., 2020). The greater voracity of predators under higher densities is attributed to the competitive effect and the greater opportunity to encounter prey, which stimulates their food search and leads to greater efficiency of control (Cheng et al., 2012; Souza et al., 2019). These considerations explain the negative correlation between larval density and the population density of *P. citri* ($\rho = -0.81$; $p < 0.001$).

The predatory efficiency observed at the 1:20 ratio, although statistically lower, was considered high, since the values ranged between 71.8% and 95.8%, showing that the level of predation was directly proportional to the supply of mealybugs (densities of 20 to 160 nymphs). Gamboa et al. (2016) obtained a similar result using a 1:10 ratio in a study involving larvae of *C. externa* and the aphid
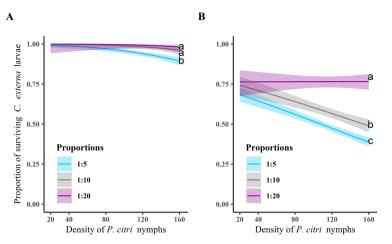


**Figure 2** Proportion of second-instar nymphs of *Planococcus citri* consumed by second-instar larvae of *Chrysoperla externa* as a function of the nymph density of the mealybug and predator:prey ratio. Different letters at the end of the curves indicate significant differences by Tukey's multicomparison test ($p < 0.05$).

*Macrosiphum euphorbiae* (Thomas, 1878) (Hemiptera: Aphididae). In any case, regardless of prey density or predator:prey ratio, after 48 hours of interaction between the insects of the two trophic levels, 100% mortality of *P. citri* nymphs was observed.

The main way lacewings find their prey is through physical contact (Canard and Duelli, 1984). According to Cheng et al. (2012), larvae of these predators can more efficiently forage prey with the gregarious distribution. Our results show that, at a 1:20 ratio, with a greater amount of prey available, *C. externa* larvae do not need to search as long, finding food more quickly. Attention should be given to the fact that, in the case of applied biological control, regardless of the type of intervention, whether through modification of the agricultural system or release of the mortality agent, the measure must still be taken at the beginning of infestation by the targeted phytophagous organism. According to Easterbrook et al. (2007), under high pest densities, control with chrysopids may fail.

When selecting the predator:prey ratio to be used in a release, the cost/benefit of the natural enemy should be considered, in addition to the control efficiency, since the release of larger numbers of larvae will result in additional costs to the producer. Knowing that ratios 1:5 and 1:10 provided similar predatory efficiencies, ratio 1:10 would require a smaller amount of chrysopids. Whether in the field or a greenhouse, predatory efficiency can vary due several of factors, such as intraguild

**A**                                          **B**



**Figure 3** Proportion of second-instar larvae of *Chrysoperla externa* survivors as a function of the density of second-instar nymphs of *Planococcus citri* supplied as prey and as a function of the predator:prey ratio after 24 (A) and 48 hours (B) of interaction between species. Different letters indicate significant differences by Tukey's multicomparison test ($p < 0.05$).

predation and cannibalism; the presence, quantity, quality, type, and age of the prey; and the effects of abiotic factors (temperature, among others) (Cheng et al., 2012; Rodrigues-Silva et al., 2017; Marcossi et al., 2020; Ye and Li, 2020; Zarei et al., 2020). Even so, it is believed that larval density is the main factor affecting the predatory efficiency of *C. externa* (Barbosa et al. 2008).

In the survival analysis of *C. externa*, the proportion of surviving larvae depended on the predator:prey ratio (F = 19.430; df = 2; $p < 0.001$) and the initial density of mealybugs offered (F = 10.739; df = 4; $p < 0.001$). There was no interaction between these factors (F = 0.169; df = 8; $p = 0.995$) 24 hours or more after larval release (Fig. 3A).

The survival of larvae of *C. externa* was high under all predator:prey ratios and all densities of mealybugs. Twenty-four hours after the beginning of the interaction between the species, the survival of the predator gradually fell in the 1:5 ratio group, with significant mortality at the density of 160 *P. citri* nymphs (Fig. 3A). This density provided survival of 89% of the larvae, whereas, at the 1:10 and 1:20 ratios, the survival was 96.2% and 100%, respectively, which did not differ statistically from each other.

Larval mortality was due solely to the cannibalistic behavior of the species, and survival was negatively proportional to the density of predators. There was a positive correlation between larval density and cannibalism after 24 ($\rho = 0.475$; $p < 0.05$) and 48 hours ($\rho = 0.66$; $p < 0.01$) of interaction between species. In contrast to our results, Barbosa et al. (2008) found that predator:prey ratio (1:5, 1:10, and 1:20) did not affect the rate of cannibalism of larvae of *C. externa* fed *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) in sweet pepper plants. The confinement of larvae by itself generates a higher probability of encounters between them compared to situations where larvae have opportunities to move and/or shelter in niches, as in an open environment (Duelli, 1981). As discussed above, higher densities provide a higher probability of encounters between them and, likewise, lower food availability, which translates into greater competition and increased cannibalism (Bayoumy and Michaud, 2015; Lima, 2016; Chathuranga et al., 2020).

Forty-eight hours after the release of *C. externa* larvae along with *P. citri* nymphs, there were significant differences in the percentage of surviving larvae as a function of *P. citri* density (F = 34.219; df = 4; $p < 0.001$), predator:prey ratio (F = 97.325; df = 2; $p < 0.001$), and the interaction between these factors (F = 7.288; df = 8; $p < 0.001$) (Fig. 3B). Larval survival under the proportions of 1:5 and 1:10 varied from 38.83% to 70.83% and from 50.00% to 80.00%, respectively, according to the density of *P. citri*. At these proportions, the survival of the mealybug nymphs was also very low.

The scarcity of prey was a determinant for the intensification of cannibalism, characteristic of chrysopid larvae, and was the main thing responsible for the reduction in predator survival. This behavior is important when food resources become scarce in the agricultural environment since these resources are responsible for keeping predators in the area (El-Wakeil and Gaafar, 2020). Without enough food, many of them abandon the cultivated area or end up dying (Chen et al., 2014). In this situation, food supplementation can be used to maintain or attract chrysopids to cropping systems and increase their survival and reproduction (Messelink et al., 2016; Resende et al., 2017).

Unlike the other proportions, the 1:20 ratio provided enough prey in the interval between 24 and 48 hours to ensure the survival of *C. externa* larvae (between 65.55% and 100%) and prevent mortality by cannibalism. The release rate of 1:20 was the most adequate for the control of *P. citri* nymphs because it was able to reduce 100% of the mealybugs in 48 hours after the release of the larvae and the high survival of *C. externa*. However, the amount of *C. externa* larvae to be released is based on the level of infestation of the pest, thus in high infestation, it is recommended to use smaller proportions (Agrofit, 2022). Few studies have aimed to understand the interaction of this predator with the *P. citri* mealybug to determine the consumption capacity, predatory efficiency, and survival of larvae under different prey and predator densities. Such information would be valuable for the establishment of a biological control program with *C. externa* as the control agent. The tolerable densities of *P. citri* and its level of control in rose bushes have not yet been studied, which hinders the implementation of this method with this predator. The present study shows the potential of *C. externa* for the control of *P. citri* populations. The integration of these results with future results will assist in the effective use of this chrysopid in the integrated pest management of rose bushes.

## Conclusions

Third-instar larvae of *C. externa* consume more second-instar nymphs of *P. citri* than in other stages. Second-instar larvae of this lacewing released at a 1:20 ratio caused total mortality of mealybug nymphs within 48 hours, regardless the density of *P. citri*, with high larvae survival of *C. externa*.

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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, MMM, DAV, LACR and MEFS performed the experiments. MMPC and MMPH 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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