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Inter - and intraspecific relationships between *Macrosiphum rosae* (Hemiptera: Aphididae) and *Chrysoperla externa* (Neuroptera: Chrysopidae)

Marília Mickaele Pinheiro Carvalho[1*], Daniel Amorim Vieira[2], Marvin Pec[3], Brígida Souza[1]

[1] *Universidade Federal de Lavras, Departamento de Entomologia, Laboratório de Controle Biológico com Entomófagos, Lavras, MG, Brasil.*
[2] *Universidade Federal de Lavras, Departamento de Biologia, Laboratório de Ecofisiologia Vegetal, Lavras, MG, Brasil.*
[3] *Escola Superior de Agricultura Luiz de Queiroz (ESALQ), Departamento de Entomologia e Acarologia, Piracicaba, SP, Brasil.*

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ABSTRACT

The predator *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae) is a polyphagous species that has, among its wide range of acceptable prey, the aphid *Macrosiphum rosae* (Linnaeus, 1758) (Hemiptera: Aphididae), an important pest of rose plants. We assessed the consumption of nymphs and adults of *M. rosae* by *C. externa* larvae, the predatory efficiency, and larval survival under different predator:prey ratios, densities of aphids, and ages of aphids. The bioassays were performed at 25 ± 1°C and 70 ± 10% RH under a 12-hour photoperiod, with aphids taken from roses of the cultivar Avalanche. Consumption was assessed by making young aphids (2nd and 3rd instars) and more developed aphids (4th instar and adult) available throughout the larval stage of the predator. The predatory efficiency and survival were studied for second-instar larvae offered *M. rosae* nymphs at densities of 20, 40, 80, 120, and 160 using predator:prey ratios of 1:5, 1:10, and 1:20. The assessments were performed 24, 48, and 72 hours after putting the species together. All of the *C. externa* instars consumed younger than older aphids. Larvae in the third instar consumed 80% of the young aphids and 77% of the older aphids. The higher the predator:prey ratio and larval density, the higher the predatory efficiency and the lower the larval survival. The ratios 1:5 and 1:10 led to the elimination of younger and older aphid populations 72 hours into the interaction between species.

Introduction

The aphid *Macrosiphum rosae* (Linnaeus, 1758) (Hemiptera: Aphididae) is dispersed on all continents except Antarctica and mainly attacks rose bushes (Cabi, 2021). These insects cause wilting, leaf fall, anomalies of flower buds, and stunting of plants. Dirt adheres to its excreta and promotes the development of black fungi (sooty mold) that cover parts of the plant, preventing photosynthesis, in addition to reducing the commercial value of the marketed product (Singh and Singh, 2016). In addition, aphids are usually vectors of viruses to their hosts, which can lead to the death of the plant (Kirk et al., 1991; Chau and Heinz, 2004; Jevremović et al., 2016).

In recent years, rose producers have adopted more sustainable control measures for the management of pest arthropods, including the use of entomophagous and entomopathogenic agents (Souza and Marucci, 2021). The predator *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae) occurs naturally in cultivated areas, which makes its application as a control agent viable (Rodrigues-Silva et al., 2017; Martins et al., 2019; Lara et al., 2020). The larvae of this lacewing have a high capacity for population reduction of several pests, including caterpillars, whiteflies, thrips, psyllids, eggs, and small caterpillars of moths (Fonseca et al., 2015; Castro et al., 2016; Battel et al., 2017; Cuello et al., 2019; Luna-Espino et al., 2020). The aphid *M. rosae* is one of the biological targets included in the recent record for the commercialization of *C. externa* in Brazil (Agrofit, 2022).

Although studies have proven the ability of *C. externa* larvae to control *M. rosae* populations, several gaps of knowledge still need to be filled. These include information on intrinsic and extrinsic factors to the species that can affect its efficiency as a control agent. In general, the amount of prey ingested by predators can be measured by the intake capacity and predatory efficiency, which depend on several criteria, such as prey density and suitability, predator search and handling capacity, architecture and morphology of plant structures, and food preference (Souza et al., 2019).

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[*] Corresponding author.
E-mail: marilia.mickaele@gmail.com (M.M.P. Carvalho).

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The size of both prey and predator and the time of exposure between species and predator:prey ratios are criteria that can also influence the amount of prey ingested (Barbosa et al., 2008; Pacheco-Rueda et al., 2015; Gamboa et al., 2016; Elango and Sridharan, 2017; Moreira et al., 2019).

We hypothesized that the densities of the prey *M. rosae* and predator *C. externa* as well as the stage of development (size) of the prey, influence the intake capacity, predatory activity, and lacewing survival in the interaction between these two insects. Thus, this study aimed to determine the consumption of nymphs and adults of *M. rosae* by larvae of this lacewing, the predatory efficiency, and the larval survival under different predator:prey ratios and densities and ages of aphids.

Materials and methods

Obtaining insects

The specimens of *C. externa* (Generation F3) were obtained from the rearing in the Laboratory of Biological Control with Entomophages, Department of Entomology of the Lavras School of Agricultural Sciences / Federal University of Lavras - ESAL/UFLA, as described by Carvalho and Souza (2009). The aphids were multiplied in rose bushes, *Rosa* spp. cv Avalanche, white, grown in a greenhouse at DEN/ESAL/UFLA. The bioassays were conducted in a climate-controlled room at 25 ± 1°C and 70 ± 10% RH with a 12-hour photoperiod. Aiming to standardize the stage of aphid development, adults were transferred from rose bushes to Petri dishes (15 cm) containing leaflets arranged on an agar-water layer (1%). Agar was used to maintaining leaf turgor, according to the method of Garzón et al. (2015). Experiments were started with the nymphs produced in these containers.

Eggs of sterilized *Ephestia kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae), acquired from the Integrated Pest Management company PROMIP - Manejo Integrado de Pragas® were used to feed the larvae of *C. externa* before performing the bioassays. Eggs of this pyralid have been used as alternative prey for lacewing larvae in general, either for research purposes or for large-scale rearing, because they meet the nutritional requirements of these predators (Carvalho and Souza, 2009). The experimental units consisted of plastic containers (5 cm in diameter and 4.5 cm in height), with a bottom lined with filter paper to serve as a place of refuge for the larvae and as a substrate for cleaning the oral parts.

Consumption of Macrosiphum rosae nymphs and adults by Chrysoperla externa larvae

The consumption of two groups of aphids of different ages by *C. externa* larvae of the first, second, and third instars was assessed. One group included young aphids (second and third instars), and the other consisted of older aphids (fourth instar and adults). To assess the consumption by the second and third instars of the predator, the larvae were fed *E. kuehniella* eggs made available *ad libitum* until they reached the instar to be studied. The number of aphids offered to the larvae was higher than their intake capacity, as determined in preliminary tests. The insects were confined to plastic containers. Daily, all uneaten aphids were replaced by others of the same age to ensure that the study included individuals of the same age. The dead aphids were counted daily per instar of *C. externa*, characterized by the twisted and dehydrated morphology resulting from the predatory action.

Effect of the relationship between Chrysoperla externa and Macrosiphum rosae on the predation and survival of lacewings

This bioassay was performed with second-instar larvae of *C. externa* fed young (second- and third-instar) and older (fourth-instar and adult) aphids. Twenty, 40, 80, 120, and 160 young and older specimens were added separately to the plastic containers to characterize the two groups composed of different ages. The second-instar larvae of *C. externa* were released in these containers at predator:prey ratios of 1:5, 1:10, and 1:20, according to the initial density of aphids. Thus, for the 1:5 ratio, 4, 8, 16, 24, and 32 larvae were added; for the 1:10 ratio, 2, 4, 8, 12, and 16 were added; and for the 1:20 ratio, 1, 2, 4, 6, and 8 larvae were added, similar to the method adopted by Gamboa et al. (2016) and Carvalho et al. (2022). In the period before release, the larvae were fed (*ad libitum*) *E. kuehniella* eggs.

At 24, 48, and 72 hours after the release of aphids and lacewing larvae, dead specimens, victims of predation, which were characterized by their twisted and dehydrated appearance, were counted. Larval survival was also assessed because cannibalism is an important mortality factor, especially at higher densities. The larvae killed by conspecifics were not replaced. The surviving aphids were replaced by others of the same age to ensure the stage of development chosen for study; however, the initial number of prey provided was not replaced. The predatory efficiency (PE) (%) of *C. externa* toward *M. rosae* was determined by the following formula, adapted from Abbott (1925): PE = PA/IDA, where PA = predated aphids and IDA = initial density of aphids.

Data analysis

The data on consumption were transformed by a logarithmic function before being subjected to ANOVA in a bifactorial scheme consisting of three *C. externa* instars and two groups of aphids (younger and older), with 20 replicates/treatment. The means were compared by Tukey's test ($p < 0.05$).

The predatory efficiency included five aphid densities, three predator:prey ratios, and two populations composed of different ages, assessed every 24 hours for 3 days. The generalized linear model (GLM) was used in a bifactorial scheme, quasibinomial distribution, cloglog link, and F test, with 15 replicates per treatment. The same tests were used to analyze the survival of the larvae, but the binomial distribution was used. The means were compared by Tukey's test. All bioassays were analyzed using R 3.6 software (R Core Team, 2019) with the statistical packages emmeans (Lenth et al., 2018) and hnp (Moral et al., 2017). Spearman's correlation coefficient (ρ) was calculated to quantify the relationship between larval density, aphid density, and cannibalism, as well as the influence of the exposure time between predator and prey on predatory efficiency and larvae survival.

Results

Consumption of Macrosiphum rosae nymphs and adults by Chrysoperla externa larvae

All larvae of *C. externa* consumed *M. rosae* of different stages (Fig. 1). The number of predated young aphids in each lacewing instar was significantly higher than that observed for older aphids (F = 1246.999; df = 1; $p < 0.001$). Consumption increased significantly with the development of lacewing larvae (from the first to the third instar) (F = 2479.545; df = 2; $p < 0.001$). There was an interaction between the consumption of aphids at the different stages and the developmental stages of *C. externa* (F = 5.927; df = 2; $p < 0.01$) (Fig. 1).

C. externa first-, second-, and third-instar larvae had an average daily intake of 5.38, 10.87, and 66.95 second- and third-instar nymphs and 2.19, 4.18, and 21.63 fourth-instar/adult *M. rosae*, respectively (Fig. 1). The daily intake of young nymphs by first- and second-instar lacewing larvae was double that of fourth-instar nymphs and adults.

Third-instar larvae consumed young specimens at three times the rate of older specimens (Fig. 1). Third-instar larvae were responsible for 80% of the average daily intake of young aphids and 77% of the consumption of older aphids.

Effect of the ratio between Chrysoperla externa and Macrosiphum rosae specimens on the predation and survival of lacewings

The predatory efficiency and larval survival of *C. externa* varied statistically as a function of the initial density of aphids offered, predator:prey ratio, age of aphids, and the interaction between the three factors 24, 48, and 72 hours after the beginning of the test (Table 1).

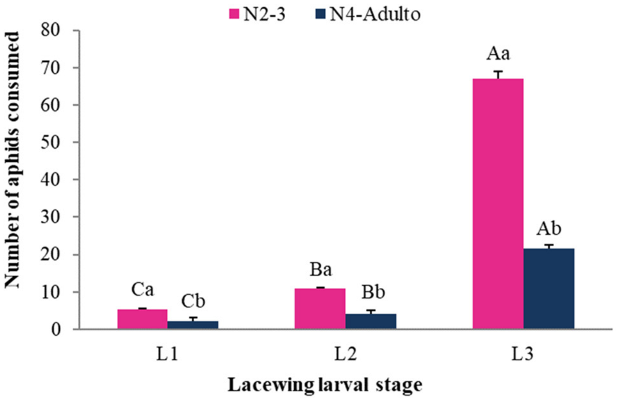

Figure 1 Mean daily number (± SE) of younger (second- and third-instar) and older (fourth-instar and adult) *Macrosiphum rosea* specimens consumed by first-, second-, and third-instar larvae of *Chrysoperla externa.* Different letters above the columns, uppercase for the larval stages of the chrysopid and lowercase for the aphid ages, indicate significant differences between the treatments according to Tukey's multicomparison test ($p < 0.05$). L1, L2, and L3 = larvae of the first, second, and third instars of the chrysopid; N2-3 = second- and third-instar nymphs of the aphid; N4-Adult = fourth-instar and adult aphids.

The highest predatory efficiency was obtained at a 1:5 ratio for larvae fed young aphids 24 hours after putting the species together, regardless of the initial prey density (Fig. 2a and Table S1). In contrast, at 1:10 and 1:20, the mean consumption was lower and increased with the number of aphids offered (20 to 160), regardless of whether they were younger or older. There was greater predation of young aphids than older aphids under all studied ratios (Fig. 2a).

The 1:5 ratio resulted in a 99% reduction in the number of young aphids, and this number varied between 74.7 and 99% for older aphids (Fig. 2a). After 48 hours, the consumption of all available aphids, regardless of density (Tables S1 and S2), was achieved with ratios of 1:5 and 1:10 with young aphids and with the ratio of 1:5 with older aphids, which were statistically equal to each other (Fig. 2c). After 72 hours, there was a total predation of the young aphids available at the 1:20 ratio, which was equal to all the *M. rosae* ratios and densities tested (Fig. 2e and Table S1). Statistically similar results were obtained for fourth-instar nymphs and adults of *M. rosae* at ratios of 1:5 and 1:10 (Fig. 2e and Table S2). The 1:20 ratio was the only one that allowed the survival of older aphids, as it required an additional 24 hours for all prey to be consumed. There was a negative correlation between larval density and the number of surviving aphids (ρ = -0.51; p = 0.001) and a positive correlation between exposure time and predatory efficiency (ρ = 0.55; p = 0.001).

The survival of larvae at the 1:10 and 1:20 ratios, when the predator was fed younger or older aphids, was higher than 95% after 24 hours of exposure, regardless of *M. rosae* density. Under the 1:5 ratio, survival was significantly lower (Fig. 2b and Tables S3 and S4). After 48 hours, larval survival was even lower under the 1:5 ratio when the predator was supplied with young aphids, differing statistically from the survival under the other ratios tested (1:10 and 1:20) (Fig. 2d and Table S3). The survival of larvae fed young aphids at a 1:10 ratio and those fed older aphids at a 1:5 ratio was statistically equal (Fig. 2d). Larvae fed older aphids at 1:10 and 1:20 ratios and lower aphid densities (20, 40, and 80) had higher survival (Fig. 2d and Table S4).

After 72 hours of interaction between prey and predator, larval survival dropped under all ratios studied as the density of aphids offered increased (Fig. 2f and Tables S3 and S4). The highest mortality

Table 1
Three-way analysis of variance (ANOVA) of the accumulated predatory efficiency (%) and survival (%) of *Chrysoperla externa* larvae according to the effect of density (D), predator:prey ratio (Pr), and age (A) of *Macrosiphum rosae*, and the interaction between the factors 24, 48, and 72 hours after the release of the species. Values from the anova table: *Df*= degree of freedom; *F*= F test value; *p*=p-value.

Effect	Accumulated predatory efficiency (%)								
	24 hours			48 hours			72 hours		
	Df	*F*	*p*	*Df*	*F*	*p*	*Df*	*F*	*p*
D	1	289.68	<0.001	1	117.63	<0.001	1	47.92	<0.001
Pr	2	1727.96	<0.001	2	961.91	<0.001	2	240.26	<0.001
A	1	1780.15	<0.001	1	1151.75	<0.001	1	209.07	<0.001
D*Pr	2	38.15	<0.001	2	17.76	<0.001	2	8.00	<0.001
D*A	1	4.43	<0.03	1	1.65	0.199	1	17.94	<0.001
Pr*A	2	12.51	<0.001	2	45.44	<0.001	2	75.29	<0.001
D*Pr*A	2	19.87	<0.001	2	21.90	<0.001	2	2.55	0.078
Effect	Larval survival (%)								
	24 hours			48 hours			72 hours		
	Df	*F*	*p*	*Df*	*F*	*p*	*Df*	*F*	*p*
D	1	12.61	<0.001	1	61.96	<0.001	1	88.95	<0.001
Pr	2	29.88	<0.01	2	196.88	<0.001	2	235.06	<0.001
A	1	9.64	<0.001	1	382.27	<0.001	1	291.30	<0.001
D*Pr	2	5.73	<0.01	2	3.54	<0.05	2	3.05	<0.048
D*A	1	0.50	0.475	1	1.00	0.323	1	0.58	<0.444
Pr*A	2	3.31	<0.05	2	6.76	<0.01	2	15.79	<0.001
D*Pr*A	2	0.03	0.970	2	0.69	0.498	2	0.29	0.744

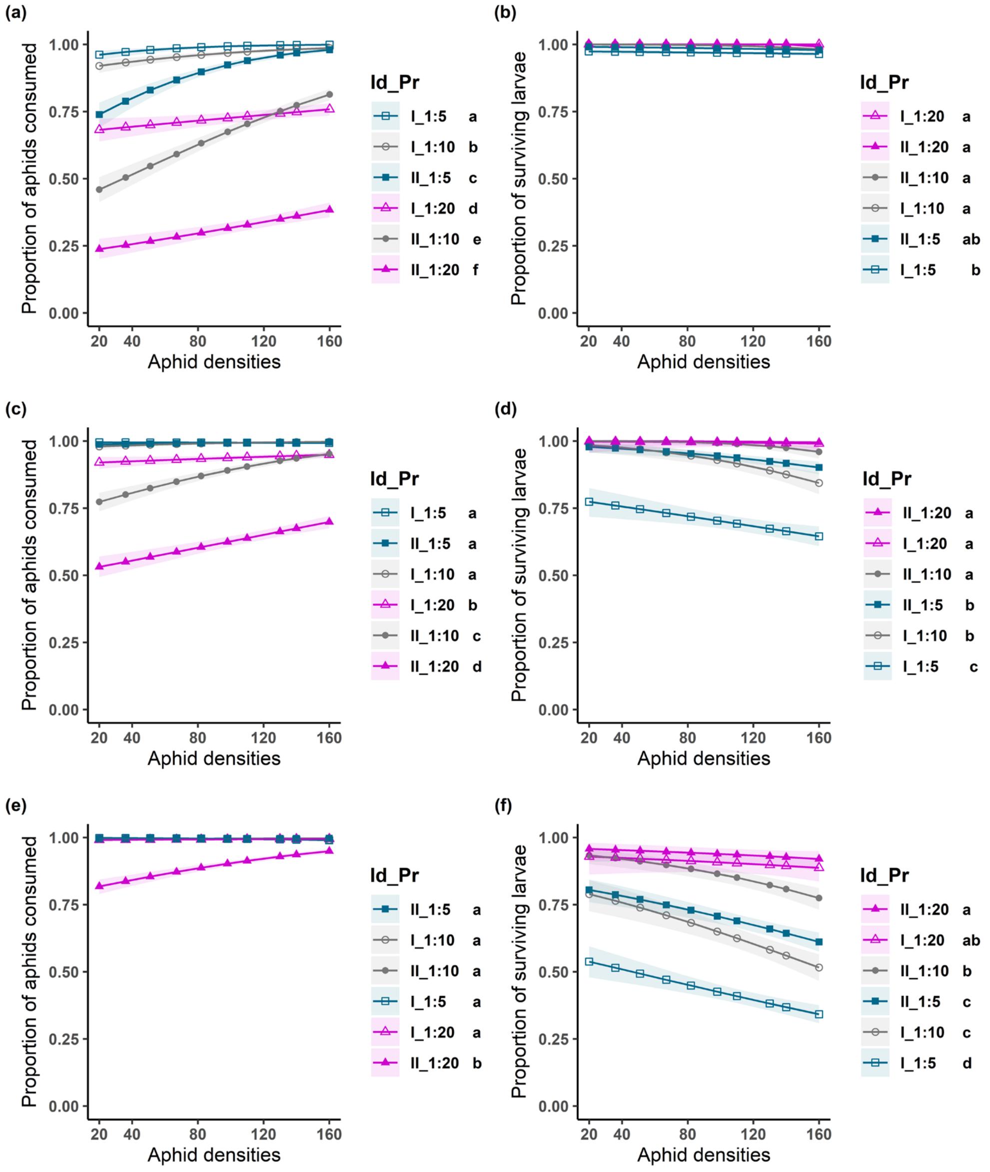

Figure 2 Accumulated predatory efficiency and survival of *Chrysoperla externa* larvae fed aphids at different ages (A) (I - 2nd and 3rd instars; II - 4th instar and adults) of *Macrosiphum rosae*, according to the predator:prey ratio (Ra) and aphid density 24 (a, b), 48 (c, d), and 72 hours (e, f) after the release of the species. Different letters indicate significant differences between treatments according to Tukey's multicomparison test (*p* < 0.05) (GLM, quasibinomial).

was observed under the 1:5 ratio and, mainly, the densities of 120 and 160 young aphids. The highest survival observed for larvae fed aphid nymphs and adults was observed at the 1:20 ratio. There was a negative correlation between aphid exposure time and predator larval survival (ρ = -0.66; *p* = 0.001), and there was a positive correlation between larval density and cannibalism (ρ = 0.59; *p* = 0.001).

Discussion

All of the *C. externa* instars consumed more young aphids (second and third instars) than older aphids (fourth instar and adult), the third larval stage being the most voracious, in line with earlier results on this predator (Fonseca et al., 2015; Cuello et al., 2019). The lower consumption of fourth-instar/adult *M. rosae* is related to the greater body volume of the prey at this developmental stage, which needs to be eaten in smaller numbers to satiate the predator. Working with these same species of predator and prey, Tamashiro (2016) found similar results, observing lower consumption of third-instar nymphs of *M. rosae* than first-instar nymphs by second-instar larvae of *C. externa*. Adults of *M. rosae* can measure up to 4 mm in length (Alford, 2012), almost twice the size of adults of other aphids (Hemiptera: Aphididae), such as *Myzus persicae* (Sulzer, 1776) (1.9 to 2.3 mm) and *Cinara atlantica* (Wilson, 1919) (1.7 to 2.6 mm) (Penteado et al., 2016; Tiwari and Singh, 2018). Therefore, the higher consumption of specimens of these aphids by *C. externa* larvae (Cardoso and Lazzari, 2003; Barbosa et al., 2006) may be related to the difference in size intrinsic to the species.

In addition to the size, the lower consumption of fourth-instar nymphs and adults of *M. rosae* observed in this study may have been due to the behavior of these aphids of performing sudden movements with their legs (similar to kicks), which were observed to hinder capture and feeding by lacewing larvae. This *M. rosae* defense strategy was responsible for causing mortality among first-instar larvae (20%), either by starvation or injury. On the other hand, the survival of all first-instar larvae supplied with young aphids was recorded. According to Canard and Duelli (1984), smaller specimens of a prey species may be easier to capture by lacewings than those of larger size, which generally have a greater ability to escape from their natural enemies. Defensive responses such as kicks, agile movements, dropping from the plant, and secreting substances from the cornicle (Fan et al., 2017; Francesena et al., 2020; Gish, 2021), among others, are skills that can ensure the survival of the prey and that depend on the developmental stages of the organisms involved (Dixon, 1958).

Regarding the predator:prey ratios and density of tested aphids, our results showed that all combinations promoted the total mortality of younger and older aphids within 96 hours; however, with the 1:5 ratio, control was achieved faster. Similar results were obtained by Mathew et al. (1999), who considered this ratio ideal for providing greater predatory efficiency to the second-instar larvae of *Chrysoperla carnea* (Stephens, 1836) (Neuroptera: Chrysopidae) against the aphid *Pentalonia nigronervosa* (Coquerel, 1859) (Hemiptera: Aphididae), although it was the ratio that caused the highest larval mortality. We also found that the predatory efficiency of second-instar *C. externa* associated with *M. rosae*, whether they were younger or older specimens, was directly proportional to the density of predators, but on the other hand, higher densities increased larval mortality. These results indicate a negative relationship between the predator:prey ratio and the larval survival variable but a positive relationship between this ratio and predatory efficiency. In general, high predator:prey ratios are more efficient at pest control (Zaki et al., 1999; Figueira and Lara, 2004; Opit et al., 2004; Rahman et al., 2011; Saleh et al., 2017) due to the higher probability of encounters between species, as well as the competitive effect among the conspecifics for the available food (Souza et al., 2019).

Among the studies that have assessed the predatory efficiency and larval survival of *C. externa* associated with pest arthropods (Figueira and Lara, 2004; Barbosa et al., 2008; Gamboa et al., 2016, for example), we did not find any that addressed the effect of the age of the prey. We found high predatory efficiency of second-instar larvae of *C. externa* toward *M. rosae*, regardless of aphid age. The predator:prey ratio of 1:5, within 48 hours after exposure between species, was the most efficient but resulted in lower larval survival. The 1:10 ratio promoted moderate predatory efficiency and larval survival, with total elimination of aphids within 72 hours. With the 1:20 ratio, the lowest predatory efficiency was obtained, but the highest larval survival was obtained, with total predation of aphids in up to 96 hours. The highest number of surviving larvae found at the 1:20 ratio was due to the greater availability of prey to the predator, reiterating the findings of Canard and Duelli (1984) that cannibalism among satiated larvae is sporadic.

We found that cannibalism was the only cause of mortality of second-instar larvae of *C. externa*. Larval survival decreased throughout the assessment period, especially at the higher predator–prey ratios, due to the greater number of larvae about the number of available prey, which intensified cannibalism. It should be noted, however, that confined larvae are more likely to be found, since there is no possibility of escape. In rose cultivars, as well as in other crops, either in the field or in a protected environment, *C. externa* larvae usually find other types of prey that are associated with the crop (Carvalho et al., 2012; Gamboa et al., 2016). Chen et al. (2014) found that even in protected cultivation, where there is a greater likelihood of dispersal, the density of *Mallada basalis* (Walker, 1853) (Neuroptera: Chrysopidae) larvae gradually decreased over time due to cannibalism and intraguild predation, and the predation of lacewing eggs and larvae by ants was observed. Along these lines, several studies have concluded that the predatory activity of lacewings is also affected by the occurrence of other natural enemies (Adly, 2016; Hagler et al., 2020), the type of culture (Alghamdi et al., 2018), weather conditions such as temperature (Fonseca et al., 2015) and species of aphids (Rana et al., 2017; Batista et al., 2022). Prey quality has a great influence on predator performance, aphids, for example, have a higher content of carbohydrates than of proteins and lipids, a condition opposite to that found in eggs of E. kuehniella (Specty et al., 2003), standard food for rearing *C. externa* (Carvalho and Souza, 2009). So, depending on the nutritional quality of the aphid, it may only partially meet the nutritional needs of the lacewing.

Based on these considerations, the results of this study show that a predator:prey ratio of 1:10 is the most appropriate for the control of *M. rosae* because it ensures the consumption of all specimens of younger and older aphids and provides a balance between predatory efficiency and the survival of *C. externa* larvae. They also reveal the inverse relationship between intake capacity and prey size, since younger aphids than larger aphids were eaten.

Conclusion

All larval instars of *C. externa* consumed more young aphids than the older ones, and the third instar larvae consumed more aphids than the other instars. Predator:prey relationship of 1:5 and 1:10 promoted total control of nymphs and adults of *M. rosae* after 72 hours of interaction between species, with low and moderate larval survival, respectively.

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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 and DAV performed the experiments. MMPC and MP 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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Supplementary Material

The following online material is available for this article:

Table S1 - Accumulated predatory efficiency (%) of Chrysoperla externa larvae fed second- and third-instar nymphs of Macrosiphum rosae according to the predator:prey (P:P) ratio and aphid density after 24. 48. and 72 hours of interaction between species.

Table S2 - Accumulated predatory efficiency (%) of Chrysoperla externa larvae fed fourth-instar nymphs and adult Macrosiphum rosea according to the predator:prey (P:P) ratio and aphid density after 24. 48. and 72 hours of interaction between species.

Table S3 - Accumulated larval survival (%) of Chrysoperla externa fed second- and third-instar Macrosiphum rosae nymphs according to the predator:prey (P:P) ratio and initial aphid density after 24, 48, and 72 hours of interaction between species.

Tabela S4 - Accumulated larval survival (%) of Chrysoperla externa fed fourth instar nymphs and adults of Macrosiphum rosae according to the predator:prey (P:P) ratio and initial aphid density after 24, 48, and 72 hours of interaction between species.