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Compatibility between the predators *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae) and *Chrysoperla externa* (Neuroptera: Chrysopidae) in the control of *Planococcus citri* (Hemiptera: Pseudococcidae) associated with rose crop

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ABSTRACT

Rose crops are attacked by many pests, including mealybugs. Although *Planoccocus citri* is not registered as a main pest of roses in Brazil, it is an increasing problem on roses inside greenhouses. *Chrysoperla externa* and *Cryptolaemus montrouzieri* are options against *P. citri* and other pests on roses, however using two predators in biological control programs may face problems like intraguild predation. This work aimed to assess the consumption of 1st instar nymphs and adult females of *P. citri* by adults of *C. montrouzieri* and 3rd instar larvae of *C. externa*, as well as the interaction between these predators when confined together. The following treatments were performed with ten replications in a completely randomized design: 1 – *C. externa* + 200 nymphs of *P. citri*; 2 – *C. externa* + 10 adults of *P. citri*; 3 – *C. montrouzieri* + 500 nymphs of *P. citri*; 4 – *C. montrouzieri* + 15 adults of *P. citri*. Each replication was set on rose leaflets inside a Petri dish (9cm Ø). Intraguild interaction was assessed by releasing both predators inside dishes containing 700 nymphs of *P. citri*. Before the releases, predators stayed 24 hours without food. We evaluated the prey consumption and intraguild predation for three hours. *C. externa* consumed significantly less (85,4±2,99) nymphs than did *C. montrouzieri* (387,0±3,02). There was no difference in adult mealybugs consumed, with an average of 1,85±0,19. No intraguild predation was observed, and an increase of 11,8% in consumption was observed when predators were released together compared to the scenario of no competition.

Introduction

Rose bushes are susceptible to the attack of several pests, among which the most common are mites, thrips, whiteflies, aphids, defoliating beetles, caterpillars and, more recently, mealybugs (Carvalho et al., 2012). Although *Planoccocus citri* (Risso, 1813) (Hemiptera: Pseudococcidae) is not yet registered as a primary pest of the crop, this species has become a constant problem in the cultivation of roses in a protected environment (Messelink, 2014; Anouk et al., 2019; Mishra et al., 2021). In Brazil, large populations of *P. citri* have occurred in rose crops in the states of Minas Gerais and Ceará, requiring control measures to be taken (UFLA, personal communication).

Mealybugs live in colonies, and the nymphs and adult females suck the sap, delaying the development of the plants and causing the yellowing and fall of the leaves, with the consequent reduction of the production. *P. citri* is also associated with the injection of toxins and the occurrence of the sooty mold, *Capnodium* sp., which develops in the presence of honeydew released by the mealybug (Copland et al., 1985; Kerns et al., 2004).

In the occurrence of *P. citri* as a key pest, together with the simultaneous occurrence of other pests, the combined use of more than one species of natural enemy can be an effective way of controlling these pests. In rose crops in Ceará, some growers have empirically used lacewings (Neuroptera: Chrysopidae) to control *P. citri* in rose bushes (UFLA, personal communication). However, despite the reduction in the population density of the pest, there is a lack of information to achieve its effective control in crops. The generalist species *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae) naturally occurs in several crops of economic interest and stands out for its high reproductive potential and, mainly, for the voracity of its larvae (Freitas, 2002; Souza and Carvalho, 2002). In addition, it is relatively easy to be reared in laboratory, and presents alternative feeding habits as survival strategy (Albuquerque et al., 1994), being qualified as a biological pest control agent.

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Cryptolaemus montrouzieri Mulsant, 1853 (Coleoptera: Coccinellidae) is also a polyphagous predator, able to develop and reproduce, feeding on about 50 species from six families of Hemiptera (Kairo et al., 2013). These include several species of mealybugs, whiteflies, aphids and, to a lesser extent, they also feed on Lepidoptera eggs. However, due to its food preference, *C. montrouzieri* is popularly known as the destroyer of mealybugs and has been successfully used in biological control programs for these hemipterans in several countries (Kairo et al., 2013).

The use of more than one species of natural enemy can promote greater effectiveness in reducing pest populations (Sorribas and Garcia-Marí, 2010) and, in the case of generalist predators, this strategy can bring greater benefits as they feed on different prey species. On the other hand, the simultaneous release of two predators in a given crop can lead to changes in its biological and behavioral characteristics in a way that makes the result of its use to control target pests unpredictable (Cakmak et al., 2009; Souza et al., 2019). According to Polis et al. (2000) and Souza et al. (2008), for example, negative intraguild interactions can reduce the efficiency of natural enemies and be the cause of failures in biological control programs.

Thus, this work aimed to evaluate the predatory capacity of *C. montrouzieri* and *C. externa* on *P. citri*, to investigate the occurrence of intraguild interactions and the behavior of these predators alone and when combined.

Materials and methods

Rose plants

Rose plants (*Rosa* sp. cv. Avalanche) were obtained from the company Flora Minas, located in Itapeva, MG, Brazil, and planted in 10L pots containing a substrate composed of soil and tanned bovine manure (1:1) plus fertilization with NPK 8 28 16. The roses were cultivated in a greenhouse at the Department of Entomology at the Federal University of Lavras (DEN/UFLA). The leaflets used in the bioassays were removed from the middle third of the plant, taken to the laboratory and cleaned in a solution of water and sodium hypochlorite.

Insects

Planoccocus citri

The rearing of *P. citri* was set in an acclimatized room at 25±1ºC, relative humidity of 70±10% and photophase of 12 hours, at DEN/UFLA, using insects from the Laboratory of Biological Control of Pests, from the Company of Agricultural Research of Minas Gerais, EPAMIG Sul, Lavras, MG. The mealybugs were multiplied on kabocha squashes (*Cucurbita maxima* L.), a host typically used for laboratory rearing (Lepage, 1942), following the methodology described by Sanches and Carvalho (2010), with some modifications.

Chrysoperla externa

The individuals of *C. externa* were obtained from the existing rearing at DEN/UFLA, which is conducted according to the methodology described by Carvalho and Souza (2009) and kept at 25±1ºC, relative humidity of 70±10% and photophase of 12 hours.

Cryptolaemus montrouzieri

Around 25 couples were donated by the Brazilian Agricultural Research Corporation, Embrapa Semiárido Unit, Petrolina, PE, in order to implement the rearing at DEN/UFLA. The adults were reared in PVC cages (10 cm high X 10 cm in diameter) sealed with laminated PVC film and kept in an acclimatized room at 25±1°C, RH of 70±10%, photophase of 12 hours. Every two days, the adults were offered a waxy mass of *P. citri* containing eggs, nymphs, and adults as food source. For water supply, a moistened cotton was placed inside the cage.

As the eggs were usually found in the cotton provided to the adults, they were collected every two days when the food was changed. Eggs were transferred to 15 cm diameter Petri dishes, which were sealed with laminated PVC film. Insects remained on these plates during the egg, larva, and pupal stages. The larvae were fed with mealybugs every two days and, after emergence, the adults were transferred to a new cage.

Predatory capacity of *Cryptolaemus montrouzieri* and *Chrysoperla externa* against *Planococcus citri*

The number of mealybugs, in their three instars and adult stage, predated by adults of *C. montrouzieri* and third instar larvae of *C. externa* during 3 consecutive hours was evaluated. The assessment of consumption during 3 hours was due to the high predatory capacity of adults of *C. montrouzieri* when supplied with first instar nymphs, which consumed more than 800 nymphs per adult in 24 hours. This preliminary evaluation showed the impracticality of counting enough nymphs to feed the predator in this period of time, as well as evaluating the number of prey consumed. For standardization purposes, the same evaluation time was used for *C. externa* larvae.

The consumption by adults of *C. montrouzieri* and by third instar larvae of *C. externa* was chosen because these are the stages of greatest voracity for both predators (Murata et al., 2006; Rosas-García et al., 2009). The number of mealybug nymphs and adults used in each treatment was established in preliminary trials. Newly emerged adults of *C. montrouzieri* were randomly taken from the rearing, without sex separation. Both coccinellid adults and lacewing larvae were kept for 24 hours without food before being released in the test. The experiment was carried out in an acclimatized room (25±1ºC, 70±10% RH and 12 hours photophase).

Predators were released in Petri dishes with a diameter of 5 cm, containing rose leaflets supported on a 5 mm layer of agar water solution (1%), with the abaxial surface facing upwards, and infested with *P. citri* adults and nymphs in each instar. The tests were carried out in a 4x2 factorial system, as detailed below: 1 *C. montrouzieri* adult + 500 1st instar nymphs; 1 adult of *C. montrouzieri* + 200 2nd instar nymphs; 1 *C. montrouzieri* adult + 100 3rd instar nymphs; 1 adult of *C. montrouzieri* + 15 adult females of *P. citri*; 1 3rd instar larva of *C. externa* + 200 1st instar nymphs; 1 3rd instar larva of *C. externa* + 100 2nd instar nymphs; 1 3rd instar larva of *C. externa* + 30 3rd instar nymphs; 1 3rd instar larva of *C. externa* + 10 adult females of *P. citri*.

The dishes were sealed with laminated PVC film and the evaluations were performed under a stereoscopic microscope. A completely randomized design was used, and each treatment had 10 replications.

Intraguild interaction between *Cryptolaemus montrouzieri* and *Chrysoperla externa* in the presence of *Planococcus citri*

C. montrouzieri adults and *C. externa* 3rd instar larvae were kept for 24 hours without food before being released in the test. They were released into Petri dishes with a diameter of 9 cm containing rose leaflets supported on a 5 mm layer of agar-water solution (1%), with the abaxial surface facing upwards, and infested with *P. citri* in the following combinations: 1 larva of 3rd instar of *C. externa* + 10 adult females of *P. citri*; 1 adult of *C. montrouzieri* + 15 adult females of *P. citri*; 1 3rd instar larva of *C. externa* + 1 adult of *C. montrouzieri* + 25 adult females of *P. citri*.

The plates were sealed with laminated PVC film and kept in an acclimatized room (25±1ºC, 70±10% RH and 12 hours photophase). After 24 hours, evaluations were carried out under a stereoscopic microscope, counting the surviving prey and predators. The difference between the initial number of prey available and the number of prey found alive at the time of the evaluations was considered a result of extraguild predation. A completely randomized design was used, and each treatment had 10 replications.

Behavior of the predators

We observed lower activity of *C. montrouzieri* against adult females of *P. citri*, and a parallel experiment was carried out to investigate whether prey size affects the interaction between predators. Here, first instar nymphs of coccinellid were used, proceeding the evaluations after 3 hours of exposure to the prey. The same methodology described for the previous bioassay was used, substituting the prey development stage, as detailed below: 1 3rd instar larva of *C. externa* + 200 1st instar nymphs of *P. citri*; 1 adult of *C. montrouzieri* + 500 nymphs of 1st instar of *P. citri*; 1 3rd instar larva of *C. externa* + 1 adult of *C. montrouzieri* + 700 1st instar nymphs of *P. citri*.

The behavior of predators was monitored in all replications of all bioassays and their respective treatments, using the Etholog 2.2 Software. The observation time was 30 minutes and the following categories were used: Standing (predator does not move); Seeking (search for prey); Preying (prey consumption); Cleaning (cleaning the mouthparts); Walking (predator moves randomly). The time obtained in each category was transformed into seconds, and later, into percentage.

Statistical analysis

Data were analyzed using the R statistical program (R Core Team, 2016). Data referring to predatory capacity were transformed to square root in order to meet the assumptions of normality and homogeneity of variances, and then submitted to the analysis of variance (ANOVA). Means were separated by Tukey's test. Data corresponding to intraguild interaction were submitted to the Bartlett and Shapiro Wilk test to verify homogeneity and normality, respectively. Subsequently, they were submitted to ANOVA and compared using the Tukey test. To evaluate the behavior, the data were transformed to square root in order to meet the assumptions of normality and homogeneity of variances, before being submitted to ANOVA. Means were separated by Tukey's test. The significance level for all tests was 0.05.

Results

Predatory capacity of *Cryptolaemus montrouzieri* and *Chrysoperla externa* against *Planococcus citri*

Significant differences were observed in the consumption by both predators fed on *P. citri* in the different instars (Table 1).

Cryptolaemus montrouzieri consumed a greater number of first instar nymphs of *P. citri*, and gradually reduced its consumption with the development of the mealybug. Against second instar nymphs, the coccinellid showed a 64% reduction in consumption compared to first instar nymphs. In relation to the third instar and adult females of *P. citri*, *C. montrouzieri* consumed 78% and 95% less when compared to first instar nymphs.

Although in smaller proportions when compared to *C. montrouzieri*, *C. externa* also exhibited a significant gradual reduction in the number of prey consumed as *P. citri* developed. Consumption of the second instar of the mealybug was reduced by 28% when compared to the first instar. For the third instar and adult females, the reduction was 76% and 85%, respectively. When offered adult females of *P. citri*, the performance of *C. externa* did not differ statistically from *C. montrouzieri*, however, in general, the coccinellid was more efficient in controlling the pest. Considering the total number of nymphs and adults consumed by both predators, the coccinellid consumed 54.5% more, but considering that *C. montrouzieri* is a natural predator of mealybugs, a greater predatory capacity was expected.

Behavior of *Cryptolaemus montrouzieri* in the presence of *Planococcus citri*

The behavior of *C. montrouzieri* was affected by the developmental stage of *P. citri* (Figure 1). The coccinellid showed almost no walking behavior when in the presence of first instar mealybug nymphs, with only 0.13% of the time spent in walking, without searching for prey. The walking time was similar compared to the other instars and adults of the prey. Search activity differed significantly across all developmental and adult stages of *P. citri* and was higher when the predator fed on the first instar of the pest.

In general, the immobility time of *C. montrouzieri* was inversely proportional to the search time, while the time spent in cleaning the mouthparts was similar to the time spent in the predation activity, except

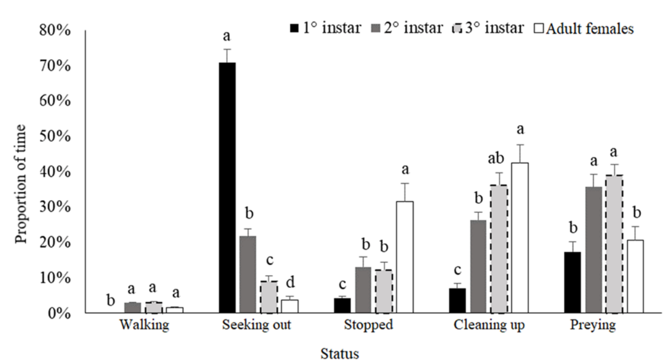

Figure 1 Behavior of *Cryptolaemus montrouzieri* against nymphs and adult females of *Planococcus citri*. Time averages (%) followed by the same letter do not differ by Tukey's test, P < 0.05.

Table 1
Predatory capacity (mean ± standard error) of adults of *Cryptolaemus montrouzieri* and 3rd instar larvae of *Chrysoperla externa* against different instars of *Planococcus citri*, during 3 hours of exposure to prey.

Predator	Nº of predated mealybugs			
	Planococcus citri instar			Adult females
	1st	2nd	3rd	
C. montrouzieri	387.0 ± 3.02 aA	137.2 ± 2.64 bA	30.4 ± 1.23 cA	1.5 ± 0.17 dA
C. externa	85.4 ± 2.99 aB	61.5 ± 1.59 bB	14.8 ± 0.85 cB	2.2 ± 0.20 dA

Means followed by the same letter, lowercase in the line and uppercase in the column, do not differ from each other by the Tukey's test, P < 0.05.

on adult females. We observed that *C. montrouzieri* showed less interest in preying on mealybug adults, differently from what was verified for the nymphal stage, when the predator started consumption as soon as it was released. In addition, when it started feeding on adults, did not consume them completely and sometimes stopped and resumed after a variable time.

Behavior of *Chrysoperla externa* in the presence of *Planococcus citri*

Similar to *C. montrouzieri*, there were no significant differences in the walking time of *C. externa* (Figure 2). The search time was shorter than that of the coccinellid in all *P. citri* development stages; on the other hand, the predation time was higher than that verified for *C. montrouzieri*, regardless of the stage of development of the mealybug. The longer time spent by *C. externa* larvae during predation was due to the lower effective search activity, in addition to the lacewing taking longer to consume the prey. The average time spent in the consumption of the prey by this predator was 38.2 seconds, 1.3 minutes and 6.3 minutes, on first, second and third instar nymphs of *P. citri*, respectively. In relation to adult females, when a greater difference in predation time was observed between *C. externa* and *C. montrouzieri*, the lacewing exhibited a different behavior to that of the coccinellid. When capturing the female, *C. externa* consumed it in its entirety, which resulted in an average duration of 24.5 minutes, a relatively long time considering the evaluation period of 30 minutes.

The time spent by *C. externa* in cleaning the mouthparts did not differ significantly when feeding on the three instars of *P. citri*. There was no cleaning of the mouthparts by larvae preying on adult females during the 30 minutes of evaluation, due to the constant feeding of the lacewing on the prey.

Since the time spent searching for prey is directly related to the time of immobility of the predator, *C. externa* showed more time without performing any action when it presented less search activity, which occurred when facing adult females of *P. citri*. As described for *C. montrouzieri*, there was less search activity when predating adults of the mealybug, however, *C. externa* remained immobile around 10% less than *C. montrouzieri*. This result, together with the shorter time cleaning the mouthparts, could have favored the performance of *C. externa* against females of *P. citri*, however, the final consumption of the lacewing did not differ significantly from that obtained for the coccinellid, showing that regardless of behavior, the presence of adult females of *P. citri* similarly affects consumption by predators.

Intraguild interaction between *Cryptolaemus montrouzieri* and *Chrysoperla externa* against *Planococcus citri* and behavioral aspects of predators

No predation was observed between the predators and no mortality was observed in the treatments. Relative to the effect of intraguild interaction on the consumption of adult females of *P. citri* by *C. montrouzieri* and *C. externa*, the combined predatory action did not differ significantly on the sum of individual consumption of each one (Table 2).

However, when analyzing the action of combined predators against first instar nymphs, a synergistic effect on pest consumption was observed (Table 3), indicating that the presence of a competitor can benefit the population reduction of this mealybug.

Regarding behavior, in both analyzes of predators acting in combination, the category "Intraguild" was excluded, since no intraguild predation was observed. Analyzing the individual behavior of each predator among the categories studied, we found that the predation time exhibited by *C. externa* against *P. citri* adults was significantly longer than that recorded for the other categories. For *C. montrouzieri*, cleaning the mouthparts was the activity that required the most time (Figure 3).

Although the averages obtained for the consumption of *C. montrouzieri* and *C. externa* against adults of *P. citri* are not statistically different (when taken together) (Table 2), the records for the category "predating" show greater activity for *C. externa* in relation to *C. montrouzieri*. The same time was recorded for walking and searching behaviors for both predators in the presence of *P. citri* adults, however, the coccinellid presented longer immobility and cleaning time, contributing to reduce the predation time, which was superior for *C. externa*.

The behavior of *C. externa* and *C. montrouzieri* against the first instar of *P. citri* (Figure 4), although in different proportions, was similar to that observed when adults of the pest were offered. The time that both walked did not differ for first instar nymphs and adult females and, also, *C. externa* spent less time in a state of immobility and cleaning the mouthparts and more time feeding on prey, compared to the two stages of development of *P. citri* studied.

When nymphs were supplied, *C. montrouzieri* showed a longer search time than *C. externa*. We also observed that the predation time demanded when the predators are combined is shorter when they feed on nymphs.

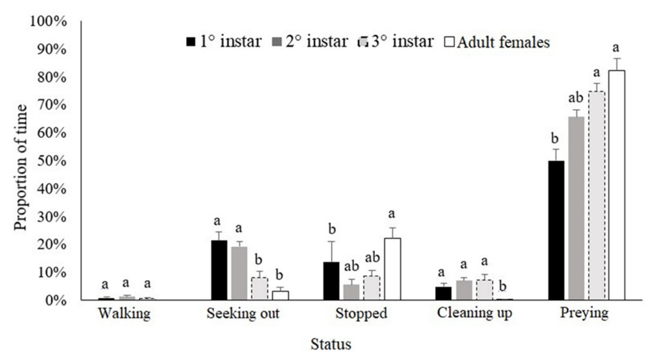

Figure 2 Behavior of Chrysoperla externa *against nymphs and adult females of* Planococcus citri. Time averages (%) followed by the same letter do not differ by Tukey's test, P < 0.05.

Table 2
Consumption of adult females of *Planococcus citri* (mean ± standard error) by *Chrysoperla externa* and *Cryptolaemus montrouzieri* acting alone and in combination.

Predators	Number of adult females predated
C. montrouzieri	4.7 ± 0.45b
C. externa	5.5 ± 0.45b
C. montrouzieri + *C. externa* (Sum of individual consumptions)	10.2 ± 0.79a
C. montrouzieri + *C. externa* (Combined consumption by the predators)	9.4 ± 0.37a

Means followed by the same letter do not differ from each other by Tukey's Test, P < 0.05.

Table 3
Consumption of first instar nymphs of *Planococcus citri* (mean ± standard error) by *Chrysoperla externa* and *Cryptolaemus montrouzieri* acting alone and in combination.

Predators	Number of nymphs predated
C. montrouzieri	387.0 ± 3.03c
C. externa	117.5 ± 4.88d
C. montrouzieri + *C. externa* (Sum of individual consumptions)	504.5 ± 5.30b
C. montrouzieri + *C. externa* (Combined consumption by the predators)	563.9 ± 3.22a

Means followed by the same letter do not differ from each other by Tukey's Test, P < 0.05.

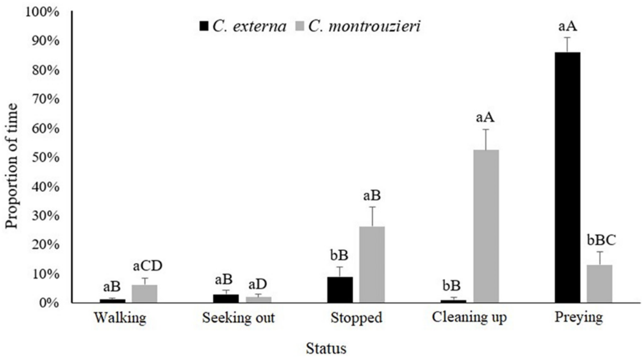

Figure 3 Behavior of Chrysoperla externa *and* Cryptolaemus montrouzieri *acting in combination, against adult females of* Planococcus citri. *Time averages (%) followed by the same letters do not differ by Tukey's Test, $P < 0.05$. Lowercase letters compare predators within each category; uppercase letters compare each predator individually across categories.

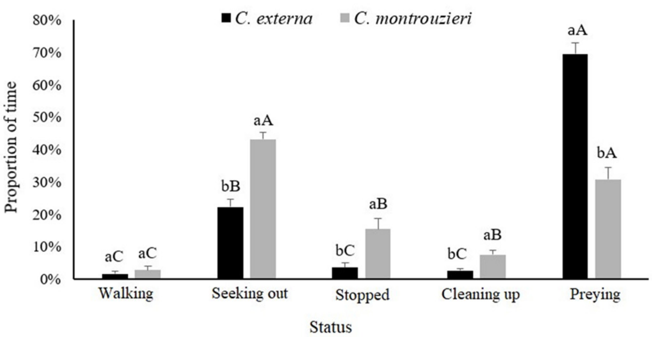

Figure 4 Behavior *of* Chrysoperla externa *and* Cryptolaemus montrouzieri *acting in combination, against first instar nymphs of* Planococcus citri. *Time averages (%) followed by the same letters do not differ by Tukey's Test, $P < 0.05$. Lowercase letters compare predators within each category; uppercase letters compare each predator individually across categories.

When used against *P. citri* adults, we observed that both predators spent less time still when combined, with a reduction of 5.3% and 4.9% in immobility time, respectively. The time spent cleaning the mouthparts by *C. montrouzieri* in the presence of *C. externa* had an increase around 10% of the time and, consequently, the predation time was reduced by 7.7%, against an increase of 3.5%. 4.0% in *C. externa* predation time. These results suggest that competition may have stimulated consumption by *C. externa*. Another evidence that shows that *C. externa* is more competitive refers to the 19.5% increase in its predation time, as well as the 18.7% reduction in immobility time, when the predators were combined against the nymphs of first instar of *P. citri*. However, *C. montrouzieri* also increased predation time by 13.4%, although it reduced prey search time by 27.7%.

The results of the present work are not sufficient to state that there is no intraguild predation between *C. montrouzieri* and *C. externa* in the control of *P. citri*. Since both have potential as intraguild predators, further studies should be carried out considering the larval stage of *C. montrouzieri*, as well as all stages of development of *P. citri*, since opposite interactions of predators were observed when against first instar nymphs and adults of the mealybug.

Discussion

The reduction in the predatory capacity of *C. montrouzieri* on the advanced stages of development of the citrus mealybug can be explained by the morphological characteristics of *P. citri*. In the first instar, in addition to the small size of the prey (up to 6 times smaller than an adult female), the nymphs do not have wax on the body, facilitating their ingestion. As the nymphs develop, there is an increase in the waxiness that covers the body, which makes it difficult for the predator to feed, and with the increase in waxy filaments, the palatability of the mealybug is reduced (Kerns et al., 2004; Santa-Cecília et al., 2007). In addition, greater prey body surface implies greater predator satiety. Higher consumption of first instar nymphs by *C. montrouzieri* was also observed by Kaur and Virk (2011), on *P. solenopsis*. Attia et al. (2011) reported that this coccinellid feeds more on nymphs than on adults of *P. citri*, with a daily consumption of about 180 nymphs, without informing which instar, against the consumption of 17 adults.

In a study of the predation potential of *C. montrouzieri* against different instars of *P. citri* reared on *Cucurbita pepo* L. squash, Rosas-García et al. (2009) reported the consumption of 1055 first instar nymphs, 443.3 second instar nymphs, 28.3 third instar nymphs and 12.7 adult females per a single predator adult, for 24 hours. If we compare the average number of mealybugs of each instar preyed per hour, the consumption obtained in the present work is much higher in relation to the results of the aforementioned authors. In addition, according to Rosas García et al. (2009), the number of third instar nymphs preyed on by *C. montrouzieri* in 24 hours is lower than that obtained in the present work, in 3 hours of evaluation (Table 1). However, it should be considered that after 24 hours without food, the predator is more voracious in the first hours in contact with prey. This period of abstinence was not pointed out by the authors and may not have been used by them. According to Pereira et al. (2008), the predation rate of a predator depends on the success of the attacks, the time of exposure of the prey and the time of manipulation.

The higher consumption of first instar nymphs by *C. externa* coincides with the results of Rashid et al. (2012) and Hameed et al. (2013) who reported better performance of *Chrysoperla carnea* (Stephens, 1836) (Neuroptera: Chrysopidae) against the first developmental stage of *Phenacoccus solenopsis* Tinsley, 1898 (Hemiptera: Pseudococcidae). The morphological characteristics of *P. citri* certainly influence consumption by the predator. The larvae of *C. externa* have a mandibular sucking mouthpart, however, when introducing it in the prey, the wax covering the body of *P. citri* adheres to its mouthparts, making it difficult to feed. Awadallah et al. (1975) observed that *C. carnea* larvae had its mouthparts partially agglutinated in the wax secretion of *Icerya purchasi* Maskell, 1879 (Hemiptera: Monophlebidae) nymphs and adults. Furthermore, we observed that, in certain situations, the adult female of *P. citri* secreted a gelled substance through its lateral ostioles. This substance, which was attributed by Williams (1978) to the release of alarm pheromones, may have increased the difficulty of *C. externa* in feeding on adult *P. citri* females. Another factor related to the successive reduction in consumption as a function of the development of *P. citri* nymphs, as reported for *C. montrouzieri*, is the prey's body size. According to Canard (2001), prey body size has a direct influence on the predation rate of Chrysopidae.

Khan et al. (2012) found greater voracity of *C. montrouzieri* compared to *C. carnea* against all stages of *P. solenopsis*. *C. montrouzieri* was considered the best biological control agent for the pink hibiscus mealybug, *M. hirsutus* (Kairo et al., 2000).

Although *C. montrouzieri* and *C. externa* are reported as intraguild predators in coccydophagous guilds (Dinesh and Venkatesha, 2014; Cardoso, 2015), no intraguild predation was observed. This result may be due to the use of predators at different stages of development (adult of *C. montrouzieri* and larvae of *C. externa*), which reduces the chances of intraguild predation (Polis et al., 1989). However, Golsteyn et al. (2021) found that *C. carnea* larval stages were prone to attack by *C. montrouzieri* adults under laboratory conditions. These authors indicate frequent events of asymmetric intraguild predation, in favor of *C. carnea* in most cases, between the different stages of the predators.

Against adult females, the combined predatory action of predators did not stand out in relation to the sum of the individual consumption of each one, which can be supported by the analysis of the behavior results, where the predators, in front of the adults of *P. citri*, are less active. On the other hand, when predating first instar mealybug nymphs, the combined action of predators promoted a synergistic effect. As far as we are aware, the contrast between the interactions between predators in face of different stages of the prey is unprecedented in the scientific literature and suggests that extraguild prey size can interfere in the interaction between two predators. Additive interaction between predators was observed by Chang (1996), where *Chrysoperla plorabunda* (Fitch, 1855) (Neuroptera: Chrysopidae) and *Coccinella septempunctata* Linnaeus, 1758 (Coleoptera: Coccinellidae) maintained the populations of *Aphis fabae* Scopoli, 1763 (Hemiptera: Aphididae) at low densities, without any evidence of negative interaction.

Regarding the behavior, the search activity was higher when the first instar of *P. citri* was offered to *C. montrouzieri*, which seems contradictory since the highest consumption of the predator was observed at this stage of development (Table 1), however, due to their small size, the first instar mealybug nymphs are quickly consumed. It was found that the coccinellid takes, on average, 1.5 seconds to consume a nymph at this stage and move to another. Furthermore, the nymphs tend to cluster close to the midrib of the rose leaflet, thus, when moving, *C. montrouzieri* consumed its prey concomitantly, making it difficult to record its action in Etholog.

The progressive reduction of *C. montrouzieri* foraging activity was directly affected by the age of the prey. Against larger nymphs, the predator, in addition to consuming less prey, takes longer to predate, requiring less time in the search for the next victim. According to Van Driesche et al. (2007), the search activity of a predator can be influenced by chemical signals released by the prey, chemical and physical properties of the host plant, by the sex of the predator, by the type and spatial distribution of the prey, as well as by the presence of alternative prey, and habitat complexity. As the adults of *C. montrouzieri* locate their prey from chemical and visual stimuli (Heidari and Copland, 1992), when in the presence of adult mealybugs, which are less preferred (Attia et al., 2011), the coccinellid has lower search activity.

When supplied with second and third instar nymphs of *P. citri*, *C. montrouzieri* has a longer predation time due to the greater number of prey consumed. In these stages, nymphs have a smaller body surface and a less dense waxy body covering than adults. Consequently, less wax is ingested and less need to clean the mouthparts. Due to the dense waxiness that coats the body of adult females, together with the release of secretions that adhere to the predator's mouthparts, *C. montrouzieri* adults spent most of their time cleaning themselves when fed by mealybug adults. In a study on the relationship between species and prey size on the functional response of *Nephus includens* (Kirsch, 1870) (Coleoptera: Coccinellidae), Milonas et al. (2011) found a higher rate of attack on smaller nymphs of *P. citri* and *Planococcus ficus* (Signoret, 1875) (Hemiptera: Pseudococcidae), as well as partial consumption of adult females. According to Riechert and Maupin (1998), this behavior may be an adaptive response in order to provide the selection of parts that are more easily digested or with greater nutritional value. Complementing, Maupin and Riechert (2001) suggested that, in situations of high prey density, this behavior reflects the predator's aggressiveness.

Unlike *C. montrouzieri*, larvae of *C. externa* sucked the entire body content of adult mealybug females. Although this behavior seems advantageous, from the point of view of pest control, it does not offer any advantage, considering that when consuming only a portion, the prey will no longer survive, as verified for *C. montrouzieri*. Similar to the observed for the coccinellid, there was an increase in the duration of predation by *C. externa* larvae as a function of greater prey development (Figure 2). According to Persson et al. (1998), the handling time by the predator increases with the size of the prey and, according to Sundby (1966), the time spent to consume a prey interferes with the predator's search capacity and efficiency.

The time spent cleaning the mouthparts by the larvae of *C. externa* was lower than that observed for *C. montrouzieri*, suggesting that the waxy covering of the mealybug is less uncomfortable for the lacewing, at least at the beginning of feeding. Mantoanelli and Albuquerque (2007) found that third instar larvae of *Leucochrysa* (*Leucochrysa*) *varia* (Schneider, 1851) (Neuroptera: Chrysopidae) require less time cleaning mouthparts and more time feeding on *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) eggs, in comparison to the mobility, immobility and camouflage categories, during 45 minutes of laboratory observation.

The performance of *C. externa* and *C. montrouzieri* in combination, against adult females of *P. citri*, evidenced greater predatory activity for *C. externa*. The coccinellid is less active and does not completely consume the adult prey after predation has started, sometimes moving away, and resuming consumption after a variable time. This behavior played a significant role in reducing the predation time recorded during the evaluation. In addition, *C. montrouzieri* spent more time cleaning the mouthparts, which corroborates the lower consumption of adults verified when predators were combined (Table 2), given that more time cleaning the mouthparts implies a lower contribution to the total consumption of *P. citri*.

When first instar nymphs were available, *C. montrouzieri* presented a longer search time due to its faster and more efficient predation, unlike *C. externa*, which presented a longer prey handling time and, consequently, a shorter search time.

Conclusions

Adults of *C. montrouzieri* and third instar larvae of *C. externa* showed decreasing predatory capacity as *P. citri* developed. The first stages of *P. citri* are more vulnerable to both predators, therefore, it is recommended that the releases for pest control be carried out at the beginning of its development, otherwise the predators would not be so efficient to control large populations of the pest. Although *C. montrouzieri* has performed better than *C. externa* on the young stages of *P. citri*, this lacewing occurs naturally in several agroecosystems and feeds on a wide variety of agricultural pests and may have a complementary action to the coccinellid in controlling *P. citri*. The coccinellid is able to consume a greater number of nymphs, however, on adult females, there is no difference between predators.

Cryptolaemus montrouzieri adults and *C. externa* larvae do not show significant intraguild interaction when confined with *P. citri* adult females. However, against first instar nymphs, predators interact positively, resulting in synergistic consumption. The behavior of predators is influenced by the stage of development of the mealybug and the presence of a competitor.

From the point of view of augmentative biological control, the combined use of adults of *C. montrouzieri* and third instar larvae of *C. externa* is viable and can satisfactorily reduce *P. citri* populations in rose crops. However, from the perspective of predator establishment, it is necessary to evaluate the occurrence of intraguild predation between the larval instars of *C. montrouzieri* and *C. externa*.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

FFP contributes to the definition, installation and conduction of experiments and data collection; tabulation, statistical analysis of data and creation of tables and figures; writing of the text and standardization of norms according to the journal. CESB contributes to the definition of experiments, guidance in conducting, statistical analysis of data, revising the text and addition significant parts. BS contributes to the definition of experiments, guidance in conducting, revising the text and addition significant parts.

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