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Functional Response of *Chrysoperla carnea* (Neuroptera: Chrysopidae) Larvae on *Saissetia oleae* (Olivier) (Hemiptera: Coccidae): Implications for Biological Control

Abdelkader Meni Mahzoum, María Villa, Jacinto Benhadi-Marín and José Alberto Pereira *

Centro de Investigação de Montanha (CIMO), ESA, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; abdelkadermeni@gmail.com (A.M.M.); mariavillaserrano@gmail.com (M.V.); jbenma@hotmail.com (J.B.-M.)

* Correspondence: jpereira@ipb.pt; Tel.: +351-27-330-3277

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Abstract: *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) is a voracious predator of soft-bodied insects such as juveniles of scale insects and the black scale *Saissetia oleae* (Olivier) (Hemiptera: Coccidae) is an important pest of several crops, such as the olive tree. However, the predatory efficiency of *C. carnea* on *S. oleae* has been unstudied yet. The present work aimed to study the functional response of larvae of *C. carnea* fed on *S. oleae* nymphs. In a controlled laboratory environment, increasing densities of *S. oleae* second and third nymph stages were offered to newly emerged specimens of the three larvae instars of *C. carnea*. After 24 h, the number of killed *S. oleae* was recorded and the functional response of *C. carnea* was assessed. The three larval stages of *C. carnea* displayed a type-II functional response, i.e., killed prey increased with higher *S. oleae* densities up to a maximum limited by the handling time. The attack rate did not significantly differ among the three instars while the maximum attack rate was significantly higher for the third instar. The handling time of the first larval instar of *C. carnea* was higher than that of the third instar. Our results demonstrated that *S. oleae* could act as a food resource for all larval stages of *C. carnea*. Furthermore, the third larval stage of the predator was the most efficient in reducing *S. oleae* densities. These results suggest that *C. carnea* larvae could contribute to *S. oleae* control in sustainable agriculture.

Keywords: conservation biological control; predators; attack rate; black scale; lacewings

1. Introduction

The olive tree (*Olea europaea* L.) possesses relevant socio-economic importance. Its fruits are harvested for olive oil extraction and table olive production. The olive crop is spread over an area of 10 million ha [1]. It represented a fruit production of over 15 million t in 2014 and 1499.3 kg/ha yield and the estimation of olive oil production worldwide for the 2016/2017 season was about 2 million 500 thousand tons [2].

This crop is attacked by several pests such as the black scale *Saissetia oleae* (Olivier) (Hemiptera: Coccidae). *Saissetia oleae* is a polyphagous species that feed on around 150 species of trees, shrubs, and herbaceous plants of various families [3]. In the case of the olive tree, the black scale is an important pest causing economically important damages worldwide [4,5]. This pest harms the olive tree directly by consuming the sap. Furthermore, *S. oleae* produces honeydew which damages the olive tree indirectly. This substance not only enhances the development of harmful fungi but also causes defoliation by covering the leaves' surface reducing the photosynthesis and respiration processes [6].

Moreover, the black scale honeydew may constitute a food resource for adults of other olive pests such as the olive moth *Prays oleae* (Bern) [7]. Because of recent developments in olive pest control methods, generally, *S. oleae* in the Mediterranean area became a secondary pest, causing damages occasionally [4]. However, the black scale control depends mainly on broad-spectrum chemical pesticides. Such a control method enhances insecticide residues in the olive products [8] as well as disrupts non-target organisms and natural enemies [9]. Moreover, the overuse of chemical treatment against the black scale could lead to outbreaks of this pest [8]. Alternatively, biological control uses different organisms (pathogens, parasitoids, or predators) to control damaging organisms. In the framework of Integrated Pest Management (IPM), biological control decreases the impact of chemical pesticides on the environment.

Chrysoperla carnea (Stephens) (Neuroptera: Chrysopidae) larvae are important predators in the olive agroecosystem. They feed on soft body insects such as the pests *Prays oleae* (Bernard) [10] or the young stages of *Euphyllura olivina* (Costa) [11,12] and *S. oleae* [10,13]. On the other hand, *C. carnea* adults feed on nectar, pollen, and honeydew (palino-glycophagous) [14,15]. Given that *S. oleae* excrete honeydew, its presence may benefit adults of *C. carnea*. For example, females of this species laid eggs in zones with high populations of *S. oleae* [16] and *S. oleae* honeydew enhanced *C. carnea* adult survival in laboratory conditions [15].

The ability of a predator to prey embodies a primary characteristic to acknowledge when implementing biological control strategies. Effectiveness of biocontrol relies on the predator feeding rate [17] and the number of prey attacked by predators frequently depends on the prey density [18,19]. The change in the number of attacked prey derived from variations in prey densities is called functional response [19]. The ability of *C. carnea* to prey on *S. oleae* has already been studied [20]. However, to our knowledge, previous studies have not yet addressed the functional response of *C. carnea* on *S. oleae*. This constitutes a critical aspect to regulate the prey density [21] and is vital to understand the dynamics of the prey–predator system [22] as an essential characteristic of the predator competence to prey [23].

The functional response of an organism represents the intake rate as a function of food density and its study plays a significant role in the selection of natural enemies to control insect pests in agroecosystems [24]. The parameters of the functional response (e.g., attack rate and handling time) can be used to determine the ability of a potential natural enemy to limit an insect pest to a low ecologically relevant threshold [25]. There are three main types of functional response: type-I represents a linear relationship between prey density and the maximum number of killed prey; type-II is defined by the number of killed prey decreasing asymptotically with prey density, and type-III is defined by a sigmoid relationship where the amount of the killed prey is positively density-dependent [18]. Many type-II and type-III predators have shown successful results as biological control agents [26].

In the present study, we evaluated the functional response of the three larval stages of *C. carnea* fed on different densities of *S. oleae* nymphs in the laboratory to assess the potential of *C. carnea* as a biocontrol agent.

2. Materials and Methods

2.1. Insects Origin and Rearing

Infested olive leaves with *S. oleae* juveniles were collected by hand from olive orchards around the campus of the Polytechnic Institute of Bragança in north-eastern Portugal (41°47′50.6″ N; 6°46′02.4″ W). Then, they were kept in a cool box, and in the laboratory second and third nymph stages were selected for further experiments.

Chrysoperla carnea eggs were purchased from Nutesca S.L. (Baeza, Spain). Isolated eggs-to prevent cannibalism-were maintained in Petri dishes (5.5 cm diameter × 1.8 cm height) in a climatic room (24 ± 2 °C, 65 ± 5% RH, 16:8 h L:D photoperiod) until hatching. Newly emerged larvae were fed ad libitum with *Ephestia kuehniella* Zeller eggs purchased from Koppert Biological System (Berkel en Rodenrijs, The Netherlands).

2.2. Experimental Design

Each arena consisted of a glass Petri dish (5.5 cm diameter, 1.8 cm height) containing one newly emerged larva of *C. carnea* and a cotton swab moistened with tap water. Arenas were prepared for each larval stage: L1, L2, and L3 respectively. Subsequently, arenas were supplied with 3, 5, 10, 15, 25, or 40 specimens of *S. oleae* (i.e., one prey density per arena), with 20 to 25 repetitions per larval stage and prey density. Individuals of *S. oleae* were placed into the arenas together with the leaves where they were collected. The number of *S. oleae* individuals killed by *C. carnea* within each arena was recorded after 24 h. The experiment was conducted in a climatic room (24 ± 2 °C, 65 ± 5% RH, and 16:8 h L:D photoperiod).

2.3. Data Analysis

The number of prey killed during the experiment was initially fitted to the type-I functional response model:

$$\mathrm{Ne} = \mathrm{a} \times \mathrm{N} \times \mathrm{T} \tag{1}$$

In which Ne = the number of prey killed, N = the initial prey density, a = the attack rate (searching rate of a predator), and T = the time of the experiment (24 h).

Then, we tested the functional response type using the *frair_test* function from the "frair" package [27] in R [28]. Evidence for a functional response type-II is given by a significantly negative first-order term. A type-III functional response is indicated by a significantly positive first-order term followed by a significantly negative second-order term [29]. We then used Rogers' random predator equation to fit the data with the *frair_fit* function from the same package since we did not replace killed prey during the assay [30]. The Rogers' random predator equation fits a type-II functional response as:

$$\mathrm{Ne} = \mathrm{N} \times (1 - \exp \times (\mathrm{a} \times (\mathrm{Ne} \times \mathrm{h} - \mathrm{T}))) \tag{2}$$

where Ne is the number of killed prey, N is the initial prey density, a is the attack rate, h is the handling time and T is the exposure time (24 h). To solve this equation, we used Lambert's transcendental equation [31].

The confidence limits (95%) were determined to consider the differences between the attack rate (a) and the handling time (h) between the three developmental stages using the *frair_boot* function from the same package. We calculated the maximum attack rate (T/h) and its 95% confidence limits using the function *Max_attackRates* from the simaR package [32].

3. Results

All *C. carnea* larvae used during the laboratory assays successfully preyed on immature stages of *S. oleae* under the established experimental conditions (Figure 1). The third stage was the most efficient (i.e., killing a higher number of nymphs). The first, second, and third larval instars of *C. carnea* killed respectively up to 10, 13, and 16 individuals of *S. oleae* per day.

The mean number of prey consumed increased with increasing prey density (Table 1). The highest mean number of prey consumed was 10 nymphs of *S. oleae* at a prey density of 40 individuals whereas the lowest was 1.9 at a prey density of three individuals. The three larval instars of *C. carnea* displayed a type-II functional response (Figure 2). This means that with the increase of the prey density also the time spent in handling prey increases. Therefore, the number of *S. oleae* killed is limited by the available time for handling the prey rather than by the prey availability.

The three larval instars of *C. carnea* had similar attack rates on *S. oleae* (Figure 3A) but the third larval instar had significantly lower handling time than the first instar (Figure 3B). Finally, the maximum attack rate followed an increasing pattern from the first to the third larval instar (Figure 3C). The first instar would kill a maximum of about five, the second about six and the third about 12 in 24 h.

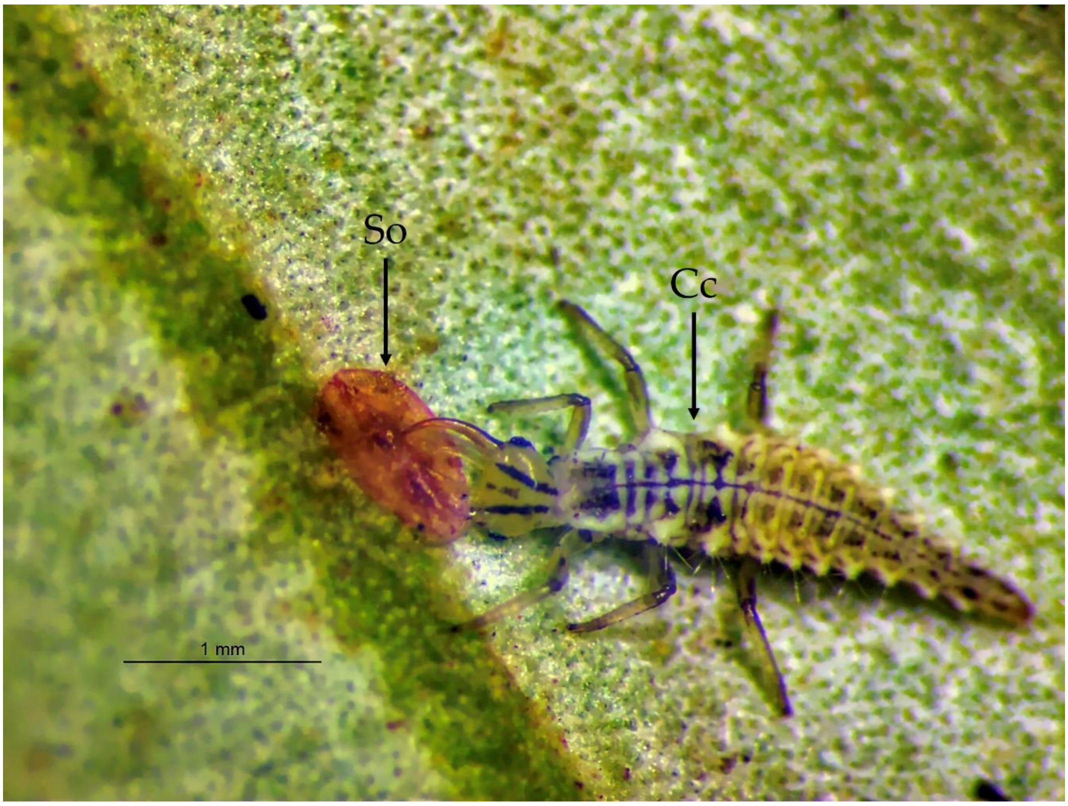

Figure 1. Larvae of *Chrysoperla carnea* (Cc) preying on *Saissetia oleae* (So) during the experiment.

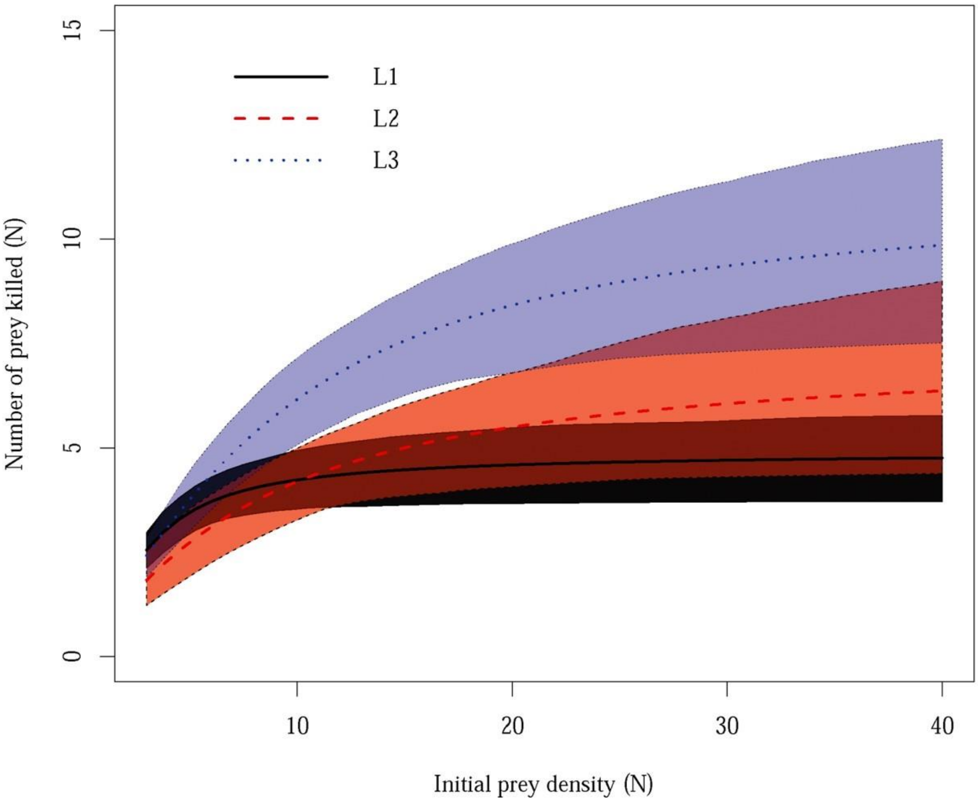

Figure 2. Functional response displayed by the first (L1), the second (L2), and the third (L3) larval instar of *Chrysoperla carnea* fed on different densities (3, 5, 10, 15, 25 and 40 individuals) of *Saissetia oleae*. Thick lines represent the fitted values of empirical data and the shaded areas that surround them represent the limits of the confidence interval based on 999 bootstrap replicates.

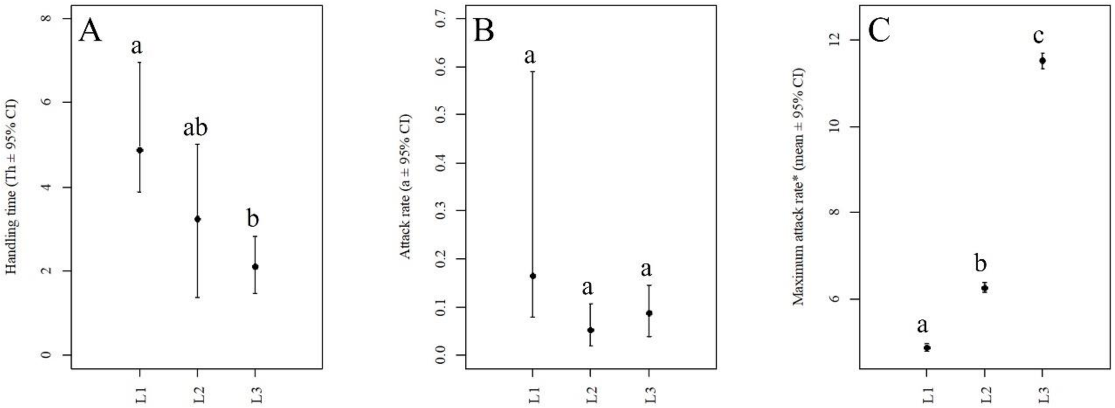

Figure 3. Parameters of the functional response of *Chrysoperla carnea* larvae fed for 24 h on different densities of *Saissetia oleae*. **A**: Handling time (h); **B**: attack rate (a), and **C**: simulated maximum attack rate (T/h). Dots characterize the original data. Bars represent the 95% confidence intervals given by 999 bootstrap replicates. Different letters over bars indicate significant differences based on the overlap of confidence intervals.

Table 1. Mean number of consumed prey (*S. oleae*) at different prey densities during immature stages of *C. carnea*. n: number of replicates.

Prey Density	*n*	Mean Number of Consumed Prey (Mean ± SE)		
		1st instar	2nd instar	3rd instar
3	25	2.7 ± 0.20	1.9 ± 0.39	2.4 ± 0.38
5	25	2.8 ± 0.51	3.2 ± 0.54	3.6 ± 0.60
10	24	5.6 ± 1.12	3.9 ± 0.90	6.4 ± 0.93
15	22	4.2 ± 0.90	2.9 ± 1.03	8.0 ± 1.67
25	23	4.3 ± 0.51	8.2± 1.12	8.3 ± 1.86
40	20	4.5 ± 0.59	5.25 ± 0.82	10.0 ± 1.59

4. Discussion

The number of *S. oleae* killed achieves the plateau of the functional response when all the available time is spent in handling prey [33]. Similarly, *C. carnea* larvae have been generally found to kill most of the presented prey (e.g., aphids, moths, and whiteflies) at low prey densities whereas reduction of killed individuals occurred at higher densities. Furthermore, the third instar was found to be the most efficient against spider mites, Lepidoptera, and aphids, which agrees with our results for *S. oleae* [18,34–42]. Moreover, the attack rate was similar for the three larval instars and the handling time of the third instar was the lowest when *C. carnea* was fed on *Hyalopterus pruni* (Geoffer) nymphs [34].

Since almost all the scales were killed at the lowest densities, our results support that *C. carnea* could be a successful natural enemy of *S. oleae* during the early stages of infestation in olive orchards. Previous studies showed the importance of *C. carnea* as a predator of olive pests, mainly *S. oleae*, *E. olivina*, and larva and eggs of *P. oleae* [43–46]. Several field-collected coccinellid species *Chilocorus bipustulatus* L., *Scymnus* (*Mimopullus*) *mediterraneus* Iablokoff-Khnzorian, *Scymnus* (*Pullus*) *subvillosus* (Goeze) and *Scymnus* (*Scymnus*) *interruptus* (Goeze) are predators of *S. oleae* [47]. Moreover, the Percentage of *S. oleae* killed by *Scutellista cyanea* Motsch. and *Metaphycus lounsburyi* (How.) combined is higher compared to when they occurred alone [48]. *S. oleae* prey density had a significant effect on attack rate. The attack rate of 3rd instar of *C. carnea* larvae was higher than its other instars. Similarly, 3rd instar is more effective than the younger instars of *C. carnea* larvae to attack other prey species such as *Hyalopterus pruni* (Geoffer) [34], *Myzus persicae* (Sulz.) [49], *Aphis gossypii* Glov. [50] and *Lipaphis erysimi* (Kaltenbach) [51].

In olive orchards, at least 32 species of Chrysopids were reported in Mediterranean olive orchards [46]. In Spanish olive groves ten species of five genera of Chrysopids were identified [52].

Moreover, the ability of different lacewings species to prey on pest can vary. For example, *C. carnea* fed on *Aphis gossypii* Glover express a lower handling time and a higher attack rate in comparison with *Chrysoperla nipponensis* (Okamoto) [39]. Therefore, further studies should analyze the predatory potentiality of different species of Chrysopids on *S. oleae* and other olive pests.

Coccids are available as prey in agroecosystems for longer periods than other prey such as aphids [53]. Thus, beyond the potential of *C. carnea* to limit the pest, *S. oleae* could represent a food resource for this important predator. Low levels of the pest in the olive grove do not represent a risk for olive production because *S. oleae* is generally a secondary pest that causes low economic damages [4]. Particularly, in the northeast of Portugal, the second and third nymphs of *S. oleae* develop from early summer to early spring [54]. During this period, damages caused by *S. oleae* are usually low on the olive tree crop. Additionally, this pest produces honeydew which could be used by other natural enemies as an alternative food. In field conditions, chrysopid adults and eggs increased with a higher infestation of *S. oleae* and honeydew production [55]. Previous studies showed that different natural enemies such as the *P. oleae* parasitoid *Elasmus flabellatus* (Fonscolombe) (Eulophidae), the generalist predator *Episyrphus balteatus* (DeGeer) (Syrphidae) and the *S. oleae* parasitoids *Metaphycus lounsburyi* (Howard) (Encyrtidae), *Coccophagus semicircularis* (Förster) and *Coccophagus lycimnia* (Walker) (Aphelinidae) fed successfully on *S. oleae* honeydew in laboratory conditions [56–58]. Therefore, *S. oleae* could provide nymphs as prey for predators and honeydew for other natural enemies.

The number of *S. oleae* nymphs killed by *C. carnea* increased with increasing prey density up to 40 prey in 24 h, at which the highest consumption was observed. The number of prey killed by *C. carnea* was the highest at 160 prey per day for *Hyalopterus pruni* Geoffer [34] and 128 for *Aphis gossypii* Glover [39]. This may be explained by different traits of the prey such as body size or palatability. Moreover, both the prey and the predator were confined in small Petri dishes and this may amplify the activity *of C. carnea* under large prey densities. Besides, the attack ability of *C. carnea* in field conditions may decrease due to an increased time taken by the predator seeking its prey [34]. The results reported in this study should hence be used carefully to make comparisons with results under field conditions.

Other outputs could result under field or more complex conditions in laboratory experiments, e.g., implementing food webs with a gradient of prey biodiversity for *C. carnea* larvae or including other guild interactions, such as other natural enemies of *S. oleae*. The relative importance of *C. carnea* as predator of *S. oleae* can change depending on a variety of factors, e.g., the prey community composition (richness and/or diversity), its mean body size [59], the trophic complexity [60], the relative preference of the predator for prey or habitat complexity [61]. For example, the predatory efficiency of a generalist predator on a target prey species can shift in the presence of alternative prey [62]. Furthermore, predator persistence is maximized when the minimum prey size in the community is intermediate, but as prey diversity increases, the minimum body size could adopt a broader range of values [59]. Moreover, at different habitat complexities, the prey availability and foraging strategies can change and affect the preference and consumption patterns of predators [61].

5. Conclusions

Summarizing, these results show the potentiality of *C. carnea* larva as a predator of young stages of *S. oleae* and inversely, the potentiality of *S. oleae* as a food resource for *C. carnea* in the laboratory and simplified conditions as well as arise new queries about this interaction in more complex systems. Therefore, future studies in the field or in more complex laboratory conditions should identify the balance between damages in the crop caused by *S. oleae* and its benefits as food resource-prey and honeydew-provider.

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