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Functional response of *Chrysoperla carnea* (Neuroptera: Chrysopidae) to *Saissetia oleae* (Olivier) (Hemiptera: Coccidae)

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Abstract: Lacewings are common biocontrol agents against a wide range of agricultural pests. These predators are highly voracious against soft-bodied preys such as juveniles of scale insects. In this context, the present work aims to study the use of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) as biological control agent against *Saissetia oleae* (Olivier) (Hemiptera: Coccidae). For that, we studied the functional response of the larval instars of *C. carnea* to nymphs of *S. oleae*. Increasing densities (3, 5, 10, 15, 25 and 40) of *S. oleae* nymphs were offered to the three larval stages of *C. carnea* in a controlled laboratory environment. After 24 hours, the number of *S. oleae* consumed by *C. carnea* larva was recorded and the functional response calculated. The three larval stages of *C. carnea* displayed a type II functional response behavior. Prey consumption by *C. carnea* larvae increased with high *S. oleae* densities. Moreover, younger larvae showed a lower predation rate in comparison with the older ones. The search rate and handling time of the third instar larvae were lower than those of the younger larval instars, and the simulated max. attack rate of the third instar larvae was higher than those of the first and second instar larvae. Our results suggest that all larval stages of *C. carnea* could be important in *S. oleae* biological control in olive orchards, although the third larval stage of the predator was more efficient in reducing *S. oleae* densities than first and second *C. carnea* larval instars.

Key words: Olive orchard, Conservation Biological Control, predators, black scale, lacewings

Introduction

The olive tree (*Olea europea* L.) is an important crop worldwide. This crop is attacked by several pests such as the black scale, *Saissetia oleae* (Olivier) (Hemiptera: Coccidae). Many authors consider *S. oleae* among the most important pests of olive trees. It causes high economically important damages in olive groves worldwide (Ben-Dov and Hodgson, 1997; Raina, 2003; Haniotakis, 2005; Preedy and Watson, 2010). Due to recent developments in olive pest control methods, *S. oleae* became a secondary pest causing damages occasionally (Haniotakis, 2005). However, the black scale control depends mainly on broad-spectrum chemical pesticides. Such control method enhances insecticide residues in olive products

(Delrio, 1992) as well as disrupts non-target organisms and natural enemies (Bartlett, 1963; Bellows Jr. and Morse, 1988; Rimoldi et al., 2008). Moreover, the overuse of chemical treatment against the black scale leads to outbreaks of this pest (Delrio, 1992).

Chrysoperla carnea (Stephens) (Neuroptera: Chrysopidae) larvae are voracious predators of a variety of crop pests (Gautam and Tesfaye, 2002). Also, they are permanent predators of olive grove pests (Szentkirályi, 2001) and, among them, *S. oleae* immature stages (Arambourg, 1984). The ability of *C. carnea* to prey on *S. oleae* has already been studied (Beingolea, 1955; Argyriou and Katsoyannos, 1976; Bartlett, 1978). However, to our knowledge, there is no study that addresses the functional response of *C. carnea* on *S. oleae*. In this context, in the present study the objective was to evaluate the functional response of the three larval stages of *C. carnea* on different densities of *S. oleae*.

Material and methods

Insects

S. oleae juveniles were collected from olive orchards in the north-eastern region of Portugal and 2 and 3 nymph stages were used in further experiments. *C. carnea* eggs were purchased from Nutesca S. L. (Baeza, Spain). In the laboratory they were maintained isolated (to avoid cannibalism) in Petri dishes (5.5 cm diameter x 1.8 cm height) in a climatic chamber (24 ± 2 °C and 16:8 h L:D) until hatch. Then, larvae were fed *ad libitum* with *Ephestia kuehniella* Zeller eggs, purchased from Koppert Biological System (Berkel en Rodenrijs, The Netherlands).

Experimental design

New emerged *C. carnea* larvae (Larva 1 – L1; Larva 2 – L2; Larva 3 – L3) were released individually into each Petri dish (5.5 cm in diameter x 1.8 cm height) together with different densities of *S. oleae* (3, 5, 10, 15, 25 and 40) placed along with olive leaves in the Petri dishes. They were provided with cotton swabs moistened with tap water. The number of replicates per density was 20 to 25. The number of *S. oleae* consumed by *C. carnea* after 24 h was recorded. The experiments were conducted in a climatic temperature-controlled chamber 25 ± 1 °C, 65 ± 5% RH and 16L:8D photoperiod.

Data analysis

Firstly, we tested the functional response type of our data using the *frair_test* function from the "frair" package (Pritchard et al., 2017) in R (R Core Team, 2017). The evidence for a type-II functional response is a significant negative result of the first order term. A type III functional response is indicated by a first order term with a significant positive result as well as a second order term with a significant negative result (Juliano, 2001). We then used Rogers' random predator equation to describe Type II functional response using the *frair_fit* function from the same package. We used Rogers' random equation as we did not replace the prey consumed by the predator (McCoy et al., 2012; Barrios-O'Neill et al., 2014). The confidence limits (95%) was determined by the *frair_boot* function to consider the differences between searching rate (a), as well as handling time (h) for all three developmental stages. We calculated the estimated maximum feeding rate with the function *Max_attackRates* from the "simaR" package (Benhadi-Marín, 2017) in R.

Results

The functional responses indicated that *C. carnea* prey consumption rate increased with the *S. oleae* densities increase. L1 consumed up to 10 *S. oleae* individuals per day, L2 larvae consumed up to 13 *S. oleae* individuals per day and L3 consumed up to 16 *S. oleae* individuals per day. Then, consumption leveled off (Figure 1).

Search rate and handling time of the third instar larvae were lower than those of the first and second instar larvae. Additionally, simulated max attack rate of the third instar larvae was higher than that of the younger instar larvae (a = 0.087, Th = 2.104, Simulated max attack rate = 12.202, Table 1).

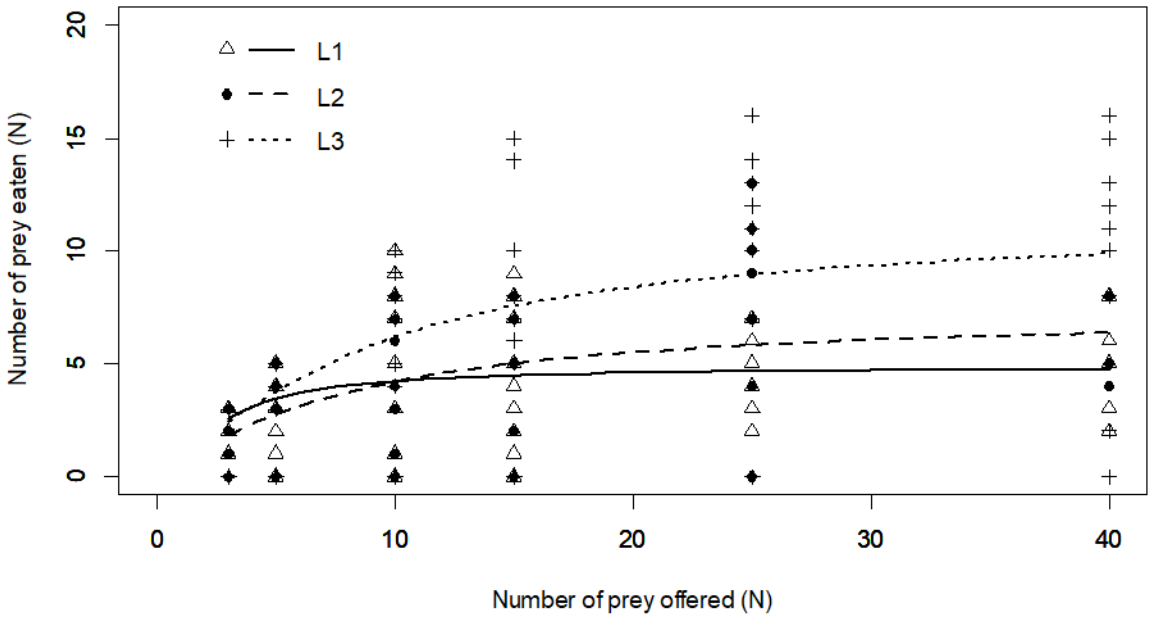

Figure 1. Functional responses of first (L1), second (L2) and third (L3) *C. carnea* larval stages feeding on *S. oleae*.

Table 1. Searching rate (a), handling time (Th) and simulated max attack rate (S), as well as 95% confidence intervals in the Holling disc equation for *C. carnea* larvae feeding on *S. oleae*.

Larval stage	a (nymph/h)	95% CL		Th (h/nymph)	95% CL		S	95% CL	
		Lower	Upper		Lower	Upper		Lower	Upper
1st	0.164	0.08	0.589	4.882	3.884	6.952	5.263	5.153	5.374
2nd	0.052	0.019	0.108	3.250	1.381	5.014	6.681	6.503	6.864
3rd	0.087	0.04	0.146	2.104	1.46	2.812	12.487	12.202	12.770

Discussion

Our results follow well to those defined by Holling (1959). *C. carnea* larvae exhibited type II functional response after preying on *S. oleae* being that *S. oleae* density influenced *C. carnea* consumption rate. It confirms previous results of *C. carnea* larvae displaying functional responses type II after feeding on different preys such as aphids, moths and whiteflies (Stark and Whitford, 1987; Atlihan et al., 2004; Montoya-Alvarez et al., 2010; Hassanpour et al., 2011; Sultan and Farhanullah Khan, 2014; Rios-Velasco et al., 2017). Our findings also indicated that search rate and handling time of the *C. carnea* third instar larvae were lower than those of the first and second instar larvae. Similarly, Atlihan et al. (2004) found that the *C. carnea* third instar larvae handling time was lower than those of the younger instars after feeding on *H. pruni* nymphs while the searching rates (a) were equal for the three instar larvae. Moreover, *C. carnea* expressed a lower handling time (h) and a higher attack rate in comparison with the predator *C. nipponensis* (Montoya-Alvarez et al., 2010).

C. carnea prey consumption increased with the increasing number of offered preys. The number of consumed preys by the first instar of *C. carnea* larvae (10 prey individuals per day) was lower than that the consumed by the second (13 prey individuals per day) and the third instar (16 prey individuals per day). Therefore, all larval stages should be considered in order to estimate the general predation ability of *C. carnea*. These results are in line with those of Hassanpour *et al.* (2009) which suggest that the number of spider mite preys consumed by *C. carnea* third instar larvae was higher than that consumed by first and second larvae instars. Similarly, the L3 of *C. carnea* showed more consumption rate on Lepidoptera preys (Klingen et al., 1996; Huang and Enkegaard, 2010; Batool et al., 2014), and consumed a higher number of Aphids (Atlihan et al., 2004) than the younger larval instars. Furthermore, *S. oleae* honeydew have shown very good results as alternative food for adults of *C. carnea* and for other biological control agents including *Elasmus flabellatus*, *Episyrphus balteatus* and even three parasitoid species of *S. oleae*: *Metaphycus lounsburyi*, *Coccophagus semicircularis* (Förster) and *Coccophagus lycimnia* (Pinheiro et al., 2015; Marrao, 2017; Villa et al., 2017). These results indicate that even when *S. oleae* occurs in numbers below its damage threshold could act as alternative prey for natural control agents.

The present study has improved our understanding of the importance of *C. carnea* in *S. oleae* biological control. Therefore, the use of chemical pesticides in olive orchards should likely be limited to enhance the augmentation of *C. carnea* in IPM control programs of the black olive scale. Nevertheless, future studies under field conditions are required in order to validate the efects of *C. carnea* on *S. oleae* populations.

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