

## Research Paper

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# Functional response of *Chrysoperla externa* (Neuroptera: Chrysopidae) to two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae): Implications for biological control

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## Abstract

The predator *Chrysoperla externa* (Neuroptera: Chrysopidae) has great potential for its use in biological pest control programs. In order to assist future biological control programs that use Chrysopidae as a control agent, this research aims to study the behaviour of the green lacewing, *C. externa*, consuming two-spotted spider mites, *Tetranychus urticae* (Acari: Tetranychidae). In the laboratory, experiments were carried out to determine the predation behaviour of *C. externa* on different densities of adults of the two-spotted spider mite, *T. urticae* (1, 2, 4, 8, 16, 32, and 64 prey). For comparison purposes, the behaviour of *C. externa* was also studied using eggs from the alternative prey *Ephestia kuehniella* (Lepidoptera: Pyralidae). The functional response was determined by logistic regression of the number of mites consumed as a function of the initial number of prey using polynomial logistic regression. The random equation was used to describe the parameters of the functional response. The predator *C. externa* showed a type II functional response consuming both *E. kuehniella* eggs and *T. urticae* adults. The results obtained will allow to define the best strategy for the use of green lacewings in the biological control of the two-spotted spider mite, *T. urticae*.

## Introduction

Lacewings belonging to the *Chrysoperla* genus are recognised as crucial natural predators and are frequently employed in the biological management of agricultural pests (Figueiredo *et al.*, 2021; Liu and Chen *et al.*, 2024). *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae) holds significance as one of the prominent lacewing species within the genus across the Americas, spanning from the southern USA to Argentina (Albuquerque *et al.*, 2001; Toledo-Hernández *et al.*, 2024). This species possesses a remarkable adaptability to diverse climates, facilitating its widespread distribution (Silva *et al.*, 2024).

Research on the biology of *C. externa* and its utilisation as a augmentative biological control has been documented since the 1970s (Albuquerque *et al.*, 1994). Despite extensive investigations into the biology of *C. externa* over the years (Braghini *et al.*, 2024), its predatory capability against two-spotted mites remains inadequately explored. In various South American countries such as Argentina, Peru, Mexico, and Colombia, *C. externa* is reared in laboratories and subsequently released into agricultural environments (Acevedo *et al.*, 2024; Cruces *et al.*, 2024; Toledo-Hernández *et al.*, 2024). In Brazil, five companies are officially registered with the Ministry of Agriculture, Livestock, and Supply for the production of *C. externa*, primarily targeting the management of whiteflies and certain aphid species. However, approval for the control of two-spotted mites is still pending. Presently, lacewing releases in Brazil encompass an area of 750 thousand hectares annually, with expectations of additional companies receiving approval by the end of 2024. This approval process will facilitate the broader commercial availability of these predators.

Moreover, its broad spectrum of prey, encompassing delicate insects like aphids, whiteflies, thrips, moths, and mites, renders it suitable for various biological control initiatives (Braghini *et al.*, 2024; Carvalho *et al.*, 2022; Luna-Espino *et al.*, 2020; Saraiva *et al.*, 2024). The predatory effectiveness of Chrysopidae members escalates during the larval stage's molting processes (Palomares-Perez *et al.*, 2019; Santos *et al.*, 2024). Additionally, adults sustain themselves

on nectar, pollen, or insect-produced honey (Martins *et al.*, 2025). Furthermore, they are easily cultivated in laboratory settings (Braghini *et al.*, 2024).

Incorporating a natural antagonist, be it a predator or a parasitoid, into a biological control scheme necessitates a comprehensive grasp of its predation patterns. Insight into prey consumption holds pivotal significance for the effective integration of these organisms into biological control and integrated pest management strategies. Such understanding serves as a cornerstone for deploying efficient biological control measures (Bueno *et al.*, 2023).

The hypothesis of this research is that *C. externa* is a predator of the two-spotted mite *Tetranychus urticae* (Koch, 1836) (Acari: Tetranychidae) and consumes a high number of individuals at elevated prey densities. To test this hypothesis, the results were compared with the alternative prey *Ephesia kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae), commonly used in the mass breeding of natural enemies. Therefore, considering the potential of *C. externa* for biological pest control programs, this research was conducted to study the behaviour of the predator *C. externa* consuming the two-spotted mite, with the aim of producing results that can be applied in future biological control programs for this pest in strawberry cultivation.

## Materials and methods

### *Chrysoperla externa* rearing

Individuals from field collections conducted in organic coffee-growing areas in the region of Franca, São Paulo, Brazil, were transferred to the laboratory and housed in a climate-controlled room (temperature of  $25 \pm 1^\circ\text{C}$ , photoperiod of 12L:12D, and relative humidity of  $70 \pm 10\%$ ). Adults from the rearing were sent to a taxonomist, Prof. Dr. Francisco José Sosa Duque, UFRA, Capitão Poço, PA, Brazil, to confirm the species and were later deposited at the Goeldi Museum, Belém, PA, Brazil. The rearing of the predator was conducted following a methodology adapted from Freitas (2001), as described by Dami *et al.* (2023). The predator larvae were provided with *E. kuehniella* eggs as prey. The eggs were supplied by the Insect Rearing Laboratory of the Minas Gerais Association of Cotton Growers (AMIPA) in Uberlândia, MG, Brazil.

### *Tetranychus urticae* rearing

A spider mite colony was obtained from organic strawberry plantations in Claraval, MG, Brazil. The colony was maintained on strawberry plants (var. Festival) placed in transparent plastic cages (60 cm wide  $\times$  60 cm long  $\times$  30 cm high). The cages were covered with voile fabric, secured with elastic gum, allowing for air circulation. The plants were kept under laboratory conditions with two 60-watt incandescent lamps providing a photoperiod of 14 h light to 10 h dark, a temperature of  $25 \pm 1^\circ\text{C}$ , and a relative humidity of  $70 \pm 10\%$ . The plants received approximately 250 mL of water three times a week. New plants were introduced into the cages weekly.

### Experimental trial

The bioassays were conducted in a room with controlled abiotic conditions (temperature of  $25 \pm 1^\circ\text{C}$ , photoperiod of 12L:12D, and relative humidity of  $70 \pm 10\%$ ). The experimental arena consisted of an acrylic container (15 cm in diameter  $\times$  3 cm in height) closed with a plastic lid to prevent mites from escaping. In each arena, strawberry leaves were added with prey. The strawberry cultivar

(*Fragaria*  $\times$  *ananassa* Duch. 'Albion') used was a cultivar widely employed by organic strawberry producers and susceptible to the target pest.

To conduct the experiments, third-instar larvae of *C. externa*, aged 12–24 h, were utilised. These larvae were obtained from the laboratory rearing, which was maintained according to the previous description. Lacewing larvae were kept without prey for a period of 12 h before the commencement of the tests. Adults of *T. urticae* were used as prey at densities of 1, 2, 4, 8, 16, 32, and 64 individuals. For comparison purposes, eggs of *E. kuehniella*, a pest of stored products like flour, were also employed as prey. These eggs are utilised for lacewing rearing in the laboratory, and the same densities mentioned for the spider mite were used to *E. kuehniella* eggs. Each arena contained strawberry leaves with the specified mite densities. Arenas with *E. kuehniella* eggs did not contain leaves. A single third-instar lacewing larva was introduced into each arena. Mite adults obtained from rearing were used to infest strawberry leaves. Subsequently, leaves containing mite adults were used in the experiments based on the mentioned densities. The experiment was conducted using a completely randomised design.

Assessments of predation behaviour were conducted 24 h after the start of the experiment, counting the number of prey consumed per replicate at each density for the predator *C. externa*. Ten replicates were observed for each prey density (mites and eggs). Both the *T. urticae* adults and the *E. kuehniella* eggs used in the experiments were not replaced until the end of the evaluations. To verify prey survival in the absence of the predator, the same number of replicates without a predator was established for each prey density.

### Data analysis

The natural mortality of the prey without the presence of a predator was less than 5% across all experiments. Therefore, we ran our analyses without correcting for natural mortality. For each prey density combination, we calculated the number of prey attacked ( $N_a$ ) as a function of the initial number of prey available ( $N_0$ ).  $N_a$  and  $N_0$  were then used to determine the functional response of the predator *C. externa* for each prey species, which was estimated to the description by Dami *et al.* (2023). The type of functional response was then determined by the significance and slope of the linear term (Juliano, 2001). The parameters of the attack rate ( $a'$ , expressed per hour ( $\text{h}^{-1}$ )) and handling time ( $T_h$ , expressed in h (h)) of the functional response were estimated as described by Juliano *et al.* (1993).

## Results

Logistic regression models using the predation data were estimated to determine the type of functional response of the predator *C. externa* to both prey species (*T. urticae* and *E. kuehniella*). For the prey *T. urticae* adult, the linear parameter presented a value of  $-0.5125 \pm 0.1011$ , and  $P < 0.0001$  (Table 1). Since the linear parameter was negative and significant, this indicates that the predator *C. externa*, when consuming mites, exhibited a type II functional response. For *E. kuehniella* eggs as prey, the linear parameter had a value of  $-0.1550 \pm 0.0357$ , and  $P < 0.0001$  (Table 1). Thus, the type of functional response of *C. externa* preying on *E. kuehniella* eggs was also determined to be type II (Table 2).

The average number of prey attacked was proportional to the density provided to the predator *C. externa*. As the number of *T. urticae* adults and *E. kuehniella* eggs offered increased, the number of prey attacked also increased (Fig. 1). However, the proportion of

**Table 1.** Estimated mean of the logistic regression parameters of *Tetranychus urticae* adults and *Ephestia kuehniella* eggs predated by third-instar *Chrysoperla externa* larva with prey densities between 1 and 64 individuals

Treatments	Parameters	Values $\pm$ SE	$\chi^2$ (df = 1)	P	Functional response type
<i>T. urticae</i>	Intercept	4.9488 $\pm$ 0.7932	38.93	<0.0001	II
	Linear	-0.5125 $\pm$ 0.1011	25.71	<0.0001	
	Quadratic	0.0186 $\pm$ 0.00352	27.78	<0.0001	
	Cubic	-0.00018 $\pm$ 0.000034	28.42	<0.0001	
<i>E. kuehniella</i>	Intercept	4.1262 $\pm$ 0.5398	58.43	<0.0001	II
	Linear	-0.1550 $\pm$ 0.0357	18.88	<0.0001	
	Quadratic	0.00286 $\pm$ 0.000635	17.86	<0.0001	
	Cubic	-0.00001 $\pm$ 0.000003	17.10	<0.0001	

**Table 2.** Mean values (95% confidence limits (CL)) of attack rate ( $a'$ , expressed in  $h^{-1}$ ), handling time ( $t_h$ , expressed in h) and estimated number of prey attacked during the observation period (24 h/ $t_h$ ) of third-instar *Chrysoperla externa* larva when consuming *Tetranychus urticae* adults or *Ephestia kuehniella* eggs

Treatments	$a'$	$T_h$	24 h/ $T_h$
<i>T. urticae</i>	0.00466 (0.00275–0.00656)	0.3725 (0.3115–0.4336)	64.43 (55.35–77.05)
<i>E. kuehniella</i>	0.00293 <sup>ns</sup> (0.00153–0.00433)	0.1737* (0.1433–0.2040)	138.17* (117.64–167.48)

<sup>ns</sup>indicates that there is no significant difference between treatments based on overlapping 95% confidence intervals.

\*Indicates significant difference between treatments based on non-overlapping 95% confidence intervals.

the average number of prey attacked was inversely related to the initial density of prey offered, tending to decrease as the prey density increased (Fig. 1).

The attack rate of *C. externa* preying on *T. urticae* adults and *E. kuehniella* eggs over a 24-h period was not influenced by the type of prey (Table 2). The attack rate for *T. urticae* adults was 0.00466 (0.00275–0.00656)  $h^{-1}$ , and for *E. kuehniella* eggs, it was 0.00293 (0.00153–0.00433)  $h^{-1}$ . The handling time was influenced by the types of prey tested (Table 2). The handling time for *T. urticae* adults was 0.3725 (0.3115–0.4336) h, and for *E. kuehniella* eggs, it was 0.1737 (0.1433–0.2040) h for pupae. Consequently, a higher number of eggs were consumed within 24 h (138.17).

## Discussion

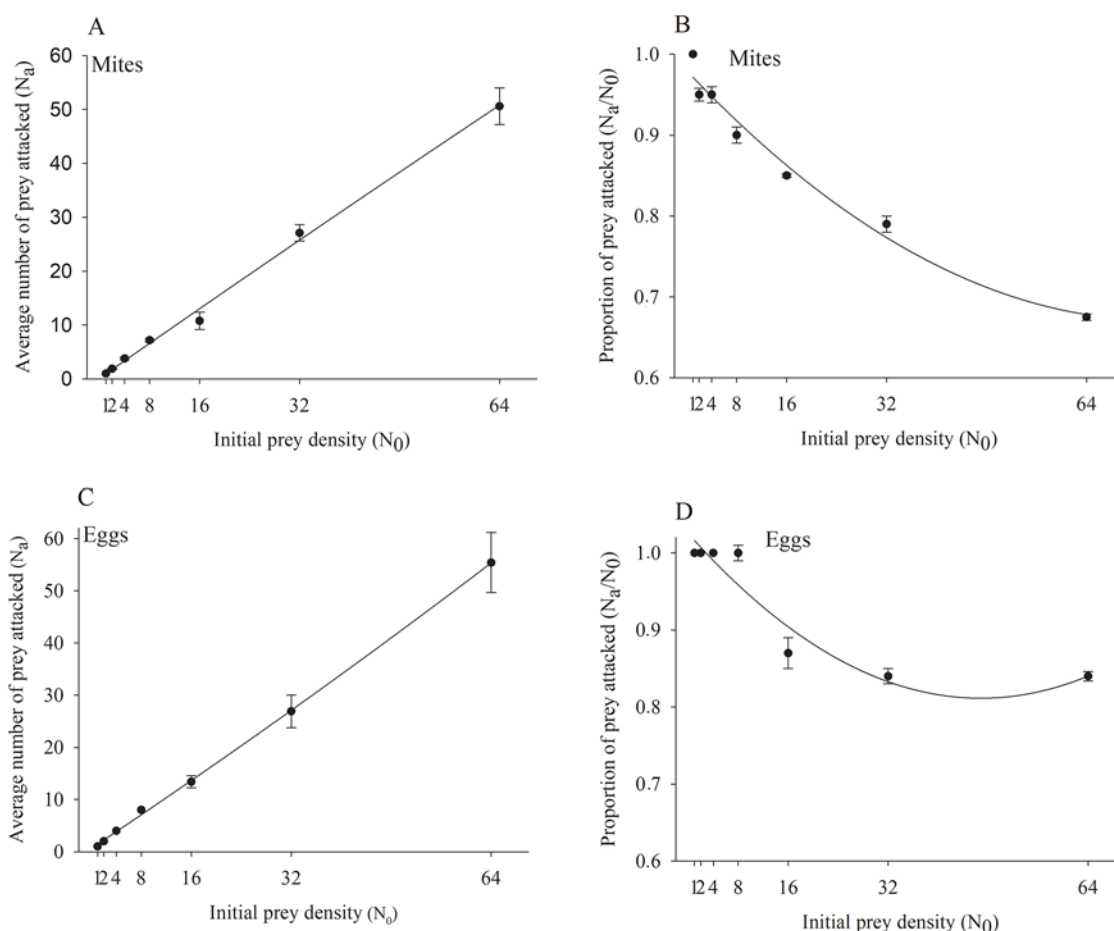
This study presents the initial evidence supporting the capability of the lacewing species *C. externa* to readily consume adult two-spotted spider mites under laboratory conditions. Concerning the feeding behaviour of *C. externa* on both *T. urticae* adults and *E. kuehniella* eggs, it was observed that the functional response followed a type II pattern. This means that as the prey population increases, consumption tends to stabilise at higher densities over a 24-h period for both types of prey. At lower prey densities, fewer prey were subdued, and consumption increased with higher prey densities but did not reach a stable level. Thus, future investigations should focus on higher prey densities. This pattern is attributed to the voracious appetite of the predator *C. externa* (Dami *et al.*, 2023) and the small size of the mites, necessitating substantial daily consumption to satisfy the predator. Additionally, the abundance or scarcity of prey influences the behaviour of predatory arthropods

(Udayakumar *et al.*, 2024). The predator's attack, successful prey ingestion, and subsequent conversion are crucial processes indicating the predator's biological performance and its potential to regulate pest populations (Pocius and Kersch-Becker, 2024; Wang *et al.*, 2024).

In this initial experiment involving the predator species *C. externa*, observations were made regarding its ability to prey on *T. urticae*, revealing that the predators could consume mite adults even in the presence of a web (as noted during laboratory assessments). Regardless of the density of the prey population under investigation, *C. externa* exhibited substantial consumption of *T. urticae* within a 24-h period (averaging 64.43 prey). Predators actively foraged until they located their prey on the leaf, even when confronted with webs, a characteristic defence mechanism of this mite species. Previous findings suggested that while lacewings could potentially consume mites, the presence of webs might pose a challenge to consumption (Mena *et al.*, 2020), and it was previously believed that adult *T. urticae* might not be suitable prey for lacewings (Pappas *et al.*, 2007).

The feeding behaviour of *C. externa* showed no significant difference between *T. urticae* and *E. kuehniella*, indicating that even when reared on *E. kuehniella* eggs, the predator readily preys on mites when released into the field. *C. externa* demonstrated the ability to consume over 50 prey within a 24-h period. There are limited published studies on the biology and predatory habits of *Chrysoperla* species feeding on *T. urticae*, with only one study focusing on *C. externa*. Morando *et al.* (2014) investigated the biology of *C. externa* feeding on two-spotted spider mites, highlighting its potential for controlling this pest species. Hassanpour *et al.* (2009) reported the functional response of a closely related species, *Chrysoperla carnea* (Stephens, 1836), against *T. urticae*. They observed an estimated consumption of up to 187.5 mites, with a type II functional response for the first and second instars and a type III response for the third instar of the predator.

Experiments investigating functional responses in laboratory settings with elevated prey densities have been subject to criticism due to concerns that predation rates derived from such studies may not accurately represent natural field conditions (Choo *et al.*, 2021). Wiedenmann and O'Neil (1991) explored the theory that, in controlled laboratory environments, the attack rate is mainly constrained by consumption behaviour (such as handling time), whereas in natural settings, the attack rate is more influenced by search behaviour. However, various factors including host plants, climate, competition from other predators or parasitoids, the presence of alternative prey, among others, can impact the efficiency



**Figure 1.** Functional response of third-instar *Chrysoperla externa* larva (A and C) and the proportion of prey attacked (B and D) towards *Tetranychus urticae* adults and *Ephestia kuehniella* eggs. Mean ( $\pm$ SE) number ( $n_a$ ) of mites and eggs consumed in relation to initial prey density ( $n_a/n_0$ ) by third instar of predator after 24 h of exposure.

of predators (Cicero *et al.*, 2024; Rostami *et al.*, 2024; Su *et al.*, 2024). Thus, experiments conducted under laboratory conditions offer insights into potential predator predation, contributing to the understanding of fundamental mechanisms underlying predator–prey interactions in field settings.

## Conclusion

The predator *C. externa* exhibits a type II functional response when consuming *T. urticae* adults. This underscores the potential of this natural enemy as an effective biological control agent for the two-spotted spider mite, a key pest in strawberry plants.

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