

## Research Article

Predation behavior of *Ceraeochrysa cincta* (Schneider, 1851) (Neuroptera: Chrysopidae) on eggs parasitized by *Trichogramma galloi* Zucchi, 1988 (Hymenoptera: Trichogrammatidae)Isabela C. de O. Pimenta<sup>1✉</sup>, Carlos C. de O. Guarnieri<sup>1</sup>, Thiago N. de Barros<sup>2</sup>, Dagmara G. Ramalho<sup>1</sup>, Sergio A. De Bortoli<sup>1</sup><sup>1</sup>Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal, SP, Brazil. <sup>2</sup>Universidade Federal de Lavras, Lavras, MG, Brazil.✉Corresponding author: [isabela.pimenta@unesp.br](mailto:isabela.pimenta@unesp.br)Edited by: Elio Cesar Guzzo<sup>1</sup>

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**Abstract.** Biological control consists of regulating pest species populations through the release of natural enemies, which are often produced in the laboratory and can be carried out by more than one species of biocontrol agent. In the case of the association of two or more of these agents, studies are necessary to understand the interaction between them; as a result, additive and noncompetitive control actions can be applied in biological control. The objective of this study was to evaluate the predation behavior of *Ceraeochrysa cincta* (Schneider, 1851) (Neuroptera: Chrysopidae) on eggs of its alternative prey, *Corcyra cephalonica* (Stainton, 1865) (Lepidoptera: Pyralidae), parasitized by *Trichogramma galloi* Zucchi, 1988 (Hymenoptera: Trichogrammatidae). Preference tests (free- and no-choice) were carried out, where it was observed that the first- and third-instar larvae of the predator preferred to feed on unparasitized eggs, whereas second-instar larvae preferred parasitized eggs. The results suggest that the variation in predatory behavior of *C. cincta* among instars may affect its compatibility with *T. galloi*, representing a relevant factor in the development of integrated biological control strategies.

**Keywords:** biological control, predatory activity, parasitism, competition.

## Introduction

Biological control aims to control pests, arthropods, pathogens, and weeds using natural enemies (Stenberg et al. 2021). In integrated pest management, decisions are guided primarily by systematic monitoring. Control strategies include chemical methods and the use of genetically resistant plants, while biological control can be effectively implemented both in extensive crops, such as sugarcane, and in smaller-scale or organic plantations (Fontes et al. 2020).

In biological control, the combined use of two or more natural enemies is a common strategy to enhance pest suppression. For example, the entomopathogenic bacterium *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) has shown no adverse effects on *Trichogramma pratissolii* Querino & Zucchi, 2003 and *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae), supporting their compatibility for simultaneous use (Polanczyk et al. 2006; Sujii et al. 2020).

Associations between predators and parasitoids are also frequently observed in natural and agricultural systems, as these groups often coexist and act together in pest suppression. In surveys conducted in bean crops, several parasitoid species, such as *Aphidius* spp. (Braconidae), *Chrysocharis* sp. (Eulophidae), *Trissolcus* spp. (Platygastridae), *Telenomus* spp. (Platygastridae), and *Trichogramma* spp. (Trichogrammatidae), were recorded alongside predators such as *Solenopsis invicta* Buren, 1972 (Hymenoptera: Formicidae), *Orius* sp. (Hemiptera: Anthocoridae), *Crematogaster* sp. (Hymenoptera: Formicidae), *Anthicus* spp. (Coleoptera: Anthicidae), *Franklinothrips* sp. (Thysanoptera: Thripidae), and *Nabis* sp. (Hemiptera: Nabidae), all sharing the same environment (Fernandes et al. 2010). However, such associations may lead to competition for hosts or prey, potentially reducing the efficiency of biological control (Jahnke et al. 2016).

When natural enemies share ecological niches, resources, or

host/prey species, they may interact through intraguild predation or competition, where one species negatively affects the performance of another. These interactions can be neutral, beneficial, or harmful, depending on the ecological context. Therefore, selecting compatible species combinations is essential to avoid antagonistic interactions and to promote synergistic effects, which enhance the success of integrated biological control programs (Holt & Polis 1997; Rosenheim 2005; Rosenheim & Harmon 2006; Gardiner & Landis 2007).

Among the predatory insects, *Ceraeochrysa cincta* (Schneider, 1851) (Neuroptera: Chrysopidae), stands out for its ecological role in various agroecosystems. Its larvae, commonly known as "trash bugs" due to the debris they carry on their backs, are voracious predators of several agricultural pests, including aphids, mites, lepidopterans, leafhoppers, thrips, and whiteflies (Oliveira et al. 2016a; 2016b; Nunes et al. 2017; Pimenta et al. 2020). Among parasitoids, *Trichogramma galloi* Zucchi, 1988 (Hymenoptera: Trichogrammatidae) is widely used in sugarcane pest management in Brazil due to its ability to parasitize eggs of the sugarcane borer *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae). This parasitoid is mass-reared and released over extensive crop areas (Oliveira et al. 2014).

The combined use of *C. cincta* and *T. galloi* may contribute to more effective pest management strategies (Varshney & Ballal 2018). However, it is essential to understand the interaction between these agents to ensure their compatibility and avoid interspecific competition that could reduce their efficacy. Therefore, the objective of this study was to evaluate the interaction between *C. cincta* and *T. galloi* through preference tests, to verify the occurrence or absence of competitive interference between them.

## Material and Methods

**Bioassay site.** The bioassays were carried out at the Insect Biology and Breeding Laboratory (LBCL) of the Phytosanitary Department of



FCAV-Unesp, Jaboticabal, SP, Brazil, under controlled conditions of temperature ( $25 \pm 2^\circ\text{C}$ ), relative humidity ( $70 \pm 10\%$ ) and photoperiod (12 h light/12 h dark).

**Obtaining parasitized eggs.** *Corcyra cephalonica* (Stainton, 1865) (Lepidoptera: Pyralidae) eggs parasitized by *T. galloi* used in the experiments were obtained from Biofábrica da Usina São Martinho, Pradópolis, SP and kept under refrigeration ( $15^\circ\text{C}$ ) in the LBCI until the start of the bioassays.

***Corcyra cephalonica* rearing.** *Corcyra cephalonica* eggs were obtained from a rearing conducted at LBCI, where a diet containing 1.25 g of wheat germ and 81.52 g of brewer's yeast is used to feed the caterpillars, with the wheat germ undergoing a sterilization process at  $150^\circ\text{C}$  for 1 h and 30 min. After cooling to room temperature, the germ was placed in transparent plastic boxes measuring 47.0 cm long, 29.5 cm wide, and 10.5 cm high, and brewer's yeast was added. Subsequently, the diet was homogenized and evenly distributed throughout the box (Parra et al. 2021). Three shallow grooves were made in the diet along the length of each box to facilitate the uniform distribution of 0.27 g of *C. cephalonica* eggs. After distributing the eggs, the container is closed with a lid that has a rectangular opening measuring 24.0 cm  $\times$  14.0 cm, covered by a "voile" fabric.

After emergence, adults were collected daily using a vacuum cleaner adapted with a suction device consisting of a capture chamber made from plastic bottles and polyvinyl chloride (PVC) tubes. The collected adults were placed in cylindrical glass cages measuring 15.0 cm in diameter  $\times$  25.0 cm in height, with 60 females and 40 males. Pieces of Sombrite® type mesh were placed inside the cages, serving as a substrate for oviposition. The plastic lid of the "cage" had a circular hole (10.0 cm in diameter), covered with mesh, to improve the internal aeration of the container. The eggs were collected daily, with the cage inverted on a white plastic tray and lightly tapped into the bottom with the palm of the hand; as a result, the eggs that adhered to the laying substrate detached and fell into the tray. The eggs were then sieved to eliminate impurities and kept under refrigeration ( $10^\circ\text{C}$ ) for 15 d, until they were used, with the surplus returned to maintenance farming.

***Ceraeochrysa cincta* rearing.** The rearing of *C. cincta* was carried out according to Freitas (2001); the adults were kept in cylindrical PVC tubes (20.0 cm in diameter and 20.0 cm in length), internally covered with white bond paper (21.0 cm  $\times$  29.7 cm), which served as the substrate for oviposition. The upper ends of the tubes were sealed with a "voile" fabric to allow better internal aeration; the fabric was fixed with elastic, while the lower parts were placed on plates, normally used as supports for plant pots (number 4). An opening approximately 2 cm in diameter was made in the fabric to supply food to adults and packaged in centrifuge (Falcon) tubes with a round bottom (capacity of 30.0 mL), to which a disposable plastic cup lid (10.0 mL) was attached, to keep the bottle with the food suspended inside the cage. This bottle was filled with water, and a piece of foam was inserted where the diet was placed.

Every 48 hours, the adults were removed from the cages and transferred to flat-bottom test tubes (2.5 cm in diameter  $\times$  8.5 cm in height) to facilitate egg collection from the oviposition substrate. After egg collection, the adults were returned to the oviposition cages. The eggs were removed using a knife blade attached to a razor and placed in Petri dishes (14.0 cm in diameter and 3.0 cm in height), with approximately 30 eggs transferred per dish, where they remained until the larvae hatched. The larvae were transferred to plates similar to those described above, and folded strips of paper towel were inserted to serve as refuges and minimize cannibalism among them. The plates were sealed with PVC plastic film, and the larvae were fed three times a week *ad libitum*, with *C. cephalonica* eggs. The pupae obtained were transferred to flat-bottom test tubes (2.5 cm in diameter and 8.5 cm in height), where they remained until adults emerged.

**Experimental design.** To evaluate the possible competition between *C. cincta* and *T. galloi*, preference tests were carried out using Petri dishes measuring 15.0 cm in diameter and 3.0 cm as arenas. Two types of bioassays were performed: (1) free-choice and (2) no-choice, with 15 repetitions for bioassay 1 and 30 repetitions for bioassay 2. The eggs were adhered to cardboard paper (3.0 cm  $\times$  1.5 cm) using

a slightly moistened brush, placing 130 eggs per card, which were then used in the tests. In bioassay 1, two cards (parasitized eggs vs. unparasitized eggs) were placed per arena to allow the predator to choose. In bioassay 2, cards containing parasitized or unparasitized eggs were placed, depriving the predator of choice. Larvae of *C. cincta* from all larval instars were used, with one larva being individually released into the center of each test arena. Before performing the bioassays, the insects were left without food for 12 h. Egg consumption was recorded after 24 h, with those that withered and/or damaged considered to be predated. Unparasitized eggs were rendered unviable by exposure to ultraviolet radiation, while parasitized eggs were used only after parasitism was confirmed — indicated by a dark coloration that appeared approximately 3–4 days after parasitism.

**Statistical analysis.** Statistical analysis was carried out using SAS (SAS Institute 2023), with frequency analysis results being carried out using Proc FREQ and interpreted using the  $\chi^2$  test, where a ratio of 1:1 was assumed when *C. cincta* showed no difference in predation preference.

## Results and Discussion

The results indicate that *C. cincta* larvae exhibit instar-dependent feeding preferences (Tab. 1). First-instar larvae consistently preferred unparasitized eggs of *T. galloi*, consuming approximately 70% in the free-choice tests and 68% in the no-choice tests. A similar pattern was observed for third-instar larvae, with consumption rates of 74% and 53% for unparasitized eggs in the free- and no-choice tests, respectively. In contrast, second-instar larvae showed a clear preference for parasitized eggs, consuming 76% in the free-choice and 64% in the no-choice tests (Fig. 1).

These findings partially align with those of Varshney & Ballal (2018), who reported that *Geocoris ochropterus* (Fieber, 1844) (Hemiptera: Geocoridae) preferred unparasitized eggs of *Tuta Absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) in both free- and no-choice tests. Similarly, first- and third-instar larvae of *C. cincta* in the present study preferred unparasitized eggs of *T. galloi*. However, second-instar larvae deviated from this pattern by favoring parasitized eggs in both test conditions. This shift suggests that, under certain circumstances, the combined use of *C. cincta* and *T. galloi* could lead to intraguild predation, potentially reducing the effectiveness of the parasitoid.

Several studies have demonstrated that predators tend to avoid parasitized eggs. Oliveira et al. (2004), for example, found that *Podisus maculiventris* (Say, 1832) (Hemiptera: Pentatomidae) preferred unparasitized eggs of *Ephesia huehniella* (Zeller, 1879) (Lepidoptera: Pyralidae) over those parasitized by *Trichogramma brassicae* Bezdenko, 1968 (Hymenoptera: Trichogrammatidae). Similarly, Ciociola Júnior et al. (1999) reported that *Chrysoperla externa* Hagen, 1861 (Neuroptera: Chrysopidae) showed a clear preference for unparasitized *Helicoverpa armigera* Hübner, 1805) (Lepidoptera: Noctuidae) eggs rather than those parasitized by *Trichogramma pretiosum*. In the same vein, Gupta & Ballal (2007) observed that both nymphs and adults of *Orius tantillus* (Motschulsky, 1863) (Hemiptera: Anthocoridae) significantly preferred eggs of *H. armigera* and *C. cephalonica* unparasitized by *Trichogramma chilonis* Ishii, 1941 (Hymenoptera: Trichogrammatidae), indicating potential compatibility between anthocorid predators and trichogrammatids in integrated biological control programs.

The lack of preference for parasitized eggs observed in first- and third-instar larvae of *C. cincta* may be explained by several factors: (1) physiological or chemical changes in the egg caused by parasitism; (2) deposition of urate salts, which darken and harden the chorion; and (3) alterations in egg morphology and development time (Gelman et al. 2002; Chailleux et al. 2013). In contrast, the preference for parasitized eggs exhibited by second-instar larvae may be associated with stage-specific nutritional requirements, which vary throughout the insect's development (Panizzi & Parra 2009).

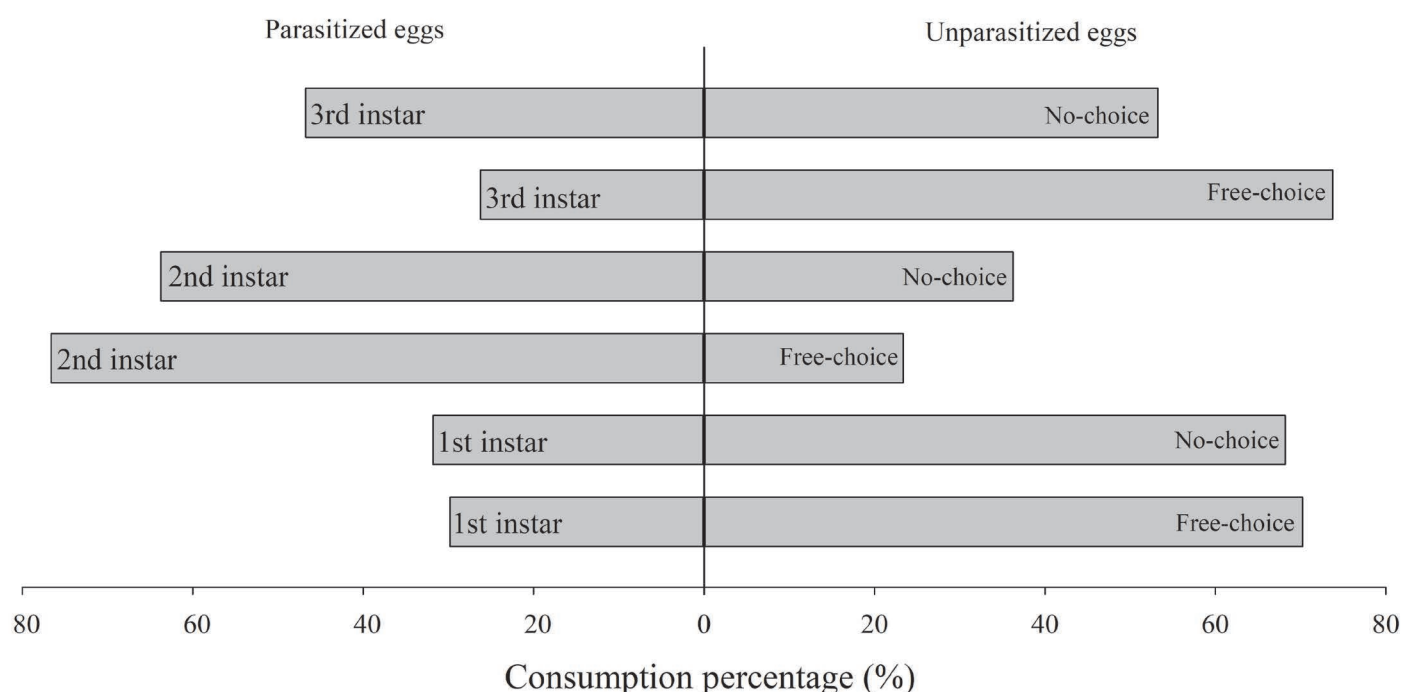
One possible explanation is that second-instar larvae experience a nutritional deficit carried over from the first instar and, therefore, require more nutrient-rich prey to support continued development. In this context, parasitized eggs may offer greater nutritional value due to



**Table 1.** Predation (means  $\pm$  standard error) by *Ceraeochrysa cincta* (Schneider, 1851) larvae in *Corcyra cephalonica* (Stainton, 1865) eggs parasitized and unparasitized by *Trichogramma galloi*, in free- and no-choice tests.

Predator larvae	Eggs	Free-choice	No-choice
1st instar	Parasitized	4.67 $\pm$ 2.18 b*	6.9 $\pm$ 1.04 b
	Unparasitized	11.0 $\pm$ 2.65 a	14.9 $\pm$ 3.75 a
		$\chi^2 = 20.02$	$\chi^2 = 22.39$
2nd instar	Parasitized	48.9 $\pm$ 8.82 a	66.7 $\pm$ 9.78 a
	Unparasitized	14.9 $\pm$ 8.44 b	37.9 $\pm$ 7.47 b
		$\chi^2 = 146.10$	$\chi^2 = 60.58$
3rd instar	Parasitized	11.9 $\pm$ 4.13 b	76.0 $\pm$ 8.48 b
	Unparasitized	33.4 $\pm$ 9.83 a	86.5 $\pm$ 10.33 a
		$\chi^2 = 59.64$	$\chi^2 = 5.12$
		$P < 0.0001$	$P = 0.02$

\* Means followed by different letters in the column, within each instar, differ by Tukey's test ( $P < 0.05$ ).



**Figure 1.** Percentage of *Corcyra cephalonica* (Stainton, 1865) eggs parasitized and unparasitized by *Trichogramma galloi* Zucchi, 1988 predated by *Ceraeochrysa cincta* (Schneider, 1851) larvae, in free- and no-choice tests.

the advanced development of the parasitoid larva within, in contrast to recently laid, unparasitized eggs that contain only the host's original reserves. Previous studies have shown that generalist predators tend to avoid parasitized eggs of *Trichogramma* spp., although such preference may vary depending on the age of the parasitized egg (Pehlivan et al. 2017; Bueno et al. 2023), a factor not evaluated in the present study.

These findings have important practical implications for integrated pest management programs involving the simultaneous release of predators and parasitoids. The observed preference of second-instar larvae of *C. cincta* for parasitized eggs suggests that intraguild predation may occur, potentially compromising the efficacy of *T. galloi*. In this context, the joint release of both agents may be contraindicated when second-instar larvae predominate in the field. Conversely, the predominance of first- and third-instar larvae, which showed a preference for unparasitized eggs, would reduce the risk of intraguild interactions and may allow for the effective coexistence of both biocontrol agents. These results emphasize the importance of synchronizing the developmental stages of natural enemies when designing integrated biological control strategies.

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## Authors' Contributions

ICOP: Conceptualization, Investigation, Methodology, Formal Analysis, Data Curation, Writing - original draft, Writing - review and editing; CCOG: Investigation, Writing - original draft, Writing - review and editing; TNB: Investigation, Writing - review and editing; DGR: Conceptualization, Methodology, Formal Analysis, Validation, Writing - review and editing; SADB: Project administration, Supervision, Validation, Writing - review and editing.





## Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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