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Protocol & Techniques

Impact of a Bt-based bioinsecticide on the predatory capacity of *Ceraeochrysa cincta* (Schneider, 1851) (Neuroptera: Chrysopidae) on *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae)

Sarah C. da Silva, Danrley da R. Pacheco, Isabela C. de O. Pimenta, Dagmara G. Ramalho, Sergio A. De Bortoli

Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal, SP, Brazil.
Corresponding author: isalpj@hotmail.com

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Abstract. Green lacewings (Neuroptera: Chrysopidae) are predatory insects during their larval stages, feeding on a variety of insect pests including psyllids, miner bugs, whiteflies, mealybugs, and the eggs and larvae of lepidopterans. These predators are integral to various agricultural systems. *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae), commonly known as the fall armyworm, is a significant corn pest in Brazil and damages other crops like soybeans, cotton, and tomatoes. To control this pest, strategies such as *Bacillus thuringiensis* Berliner, 1915 (Bt)-based bioinsecticides and Bt transgenic plants are used. However, the rapid development of resistance hinders control, making the integration of different management tactics essential. This study examines the impact of the Bt-based bioinsecticide on the predatory behavior of the first, second, and third larval instars of *Ceraeochrysa cincta* (Schneider, 1851) (Neuroptera: Chrysopidae) through functional response analyses, using neonate *S. frugiperda* larvae as prey. Distinct functional responses were observed among larval instars. First-instar larvae exhibited a Type III response in both control and Bt-based bioinsecticide (Crystal®) treatments. In contrast, second- and third-instar larvae showed a Type I response in the control and a Type III response under bioinsecticide exposure. The highest attack rates occurred in first- and third-instar larvae in the control treatment, while handling time did not differ among instars or treatments. In conclusion, although the Bt-based bioinsecticide altered the functional response pattern of second- and third-instar larvae, it did not compromise the overall predatory capacity of *C. cincta*, supporting its compatibility with integrated pest management programs against *S. frugiperda*.

Keywords: green lacewing, biological control, fall armyworm, functional response.

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae), is a major pest of corn in Brazil and damages crops such as soybean, tomato, and cotton by feeding on vegetative and reproductive organs. Originally distributed throughout the Americas, it has become a significant global threat over the last decade (Kenis et al. 2022). In Brazil, annual losses to corn production are estimated at approximately US$ 400 million (Figueiredo et al. 2005), a problem aggravated by the rapid evolution of resistance to chemical insecticides and *Bacillus thuringiensis* (Bt)-based bioinsecticides (Diez-Rodriguez & Omoto 2001; Carvalho et al. 2013; Okuma et al. 2018; Boaventura et al. 2020).

Biological control relies on both microorganisms (fungi, bacteria, and viruses) and macroorganisms (predators and parasitoids). Microorganisms act as entomopathogenic agents capable of infecting and killing insects, whereas macroorganisms feed on or develop within their hosts, often causing mortality or debilitation (Alves et al. 2011). Among predators, *Ceraeochrysa cincta* (Schneider, 1851) (Neuroptera: Chrysopidae) is widely distributed in agricultural systems and exhibits high voracity and a broad prey range, including aphids, mites, lepidopterans, mealybugs, thrips, and whiteflies (Nunes et al. 2017; Pimenta et al. 2020; Koutsoula et al. 2023). Interactions between predators and entomopathogenic agents, such as Bt, may modify predatory performance and potentially enhance biological control outcomes (Pimenta et al. 2020).

Bacillus thuringiensis is a gram-positive bacterium that produces crystal (Cry) proteins toxic mainly to Lepidoptera, Coleoptera, and Diptera. After ingestion, these toxins are activated in the insect midgut, causing pore formation in the gut epithelium, paralysis, and septicemia (Vachon et al. 2012; Domínguez-Arrizabalaga et al. 2020).

The combined use of biological control agents can result in synergistic or antagonistic effects, depending on factors such as species, dose, application method and timing, pest developmental stage, and environmental conditions (Sedaratian-Jahromi 2021; Niño & Cave 2023). Therefore, this study aimed to evaluate whether Bt-based bioinsecticide affects the predatory behavior of *C. cincta* through functional response analysis when feeding on neonate *S. frugiperda* larvae, and to assess its potential contribution to fall armyworm management.

Bioassay site. The study was conducted at the Laboratory of Insect Biology and Rearing (LBCI), Department of Fitossanidade, FCAV/Unesp, Jaboticabal, São Paulo, Brazil. Insects were reared and bioassays performed under controlled conditions (25 ± 2 °C, 70 ± 10% RH, and 12: 12 h L:D photoperiod).

Obtaining *S. frugiperda*. Egg masses and artificial diet of *S. frugiperda* were obtained from Corteva Agriscience (Mogi Mirim, SP, Brazil). Upon arrival, egg masses were placed in glass containers with artificial diet and maintained under controlled conditions until hatching, which occurred within 24 h. For the control treatment, neonates were transferred directly to untreated diet. For the Bt treatment, neonates were fed for 24 h on an artificial diet treated with a Bt-based bioinsecticide (Crystal®, *B. thuringiensis* subsp. *tolworthi*) at the manufacturer's recommended field rate (1000 mL ha^{-1}). Considering a spray volume of 200 L ha^{-1}, this corresponded to 5 mL L^{-1}, obtained by diluting 2.5 mL of product in 500 mL of deionized water. Diet cubes were dipped in the solution for 10 s, air-dried at room temperature, and offered to larvae. After feeding on the treated diet for 24 h to ensure ingestion, larvae were used in the experiments. This procedure ensured synchronized neonates and minimized developmental variability.

Rearing *C. cincta*. The rearing of *C. cincta* followed the protocol described by Freitas (2001). Adults were maintained in PVC cages with paper substrates for oviposition and fed a honey and brewer's yeast diet (1:1). Eggs were collected every 48 h and transferred to Petri dishes until hatching. Larvae were reared individually, provided with paper shelters to reduce cannibalism, and fed *Corcyra cephalonica* (Stainton, 1866) (Lepidoptera: Pyralidae) eggs ad libitum. Pupae were isolated in test tubes until adult emergence.

Experimental design. The functional response of first-, second-, and third-instar *C. cincta* larvae was evaluated using neonate S. frugiperda as prey under two treatments: control (untreated prey) and Bt-treated prey (Crystal®, *B. thuringiensis* subsp. *tolworthi* isolate 344). Prey densities were 2, 4, 8, 16, 32, and 64 larvae for first instars, and 2, 4, 8, 16, 32, 64, and 128 larvae for second and third instars, with ten replicates per density. One predator larva was used per replicate. Prior to bioassays, predators were starved for 24 h. Prey consumption was assessed after 24 h by counting partially or fully consumed larvae.

Estimation of functional response parameters. Attack rate (a) and handling time (Th) were estimated using Rogers' random predator equation, appropriate for experiments without prey replacement (Rogers 1972):

$$Na = N0\,\{1 - \exp\,[\mathrm{a}\,(\mathrm{Th}\ \mathrm{x}\ Na - \mathrm{T})]\},$$

where Na is the number of preys consumed, N0 the initial prey density, and T the exposure time (24 h). Parameters were estimated by non-linear regression; no direct behavioral measurements were performed.

Statistical analysis. The functional response of predators was analyzed using logistic polynomial regression implemented with the PROC NLIN procedure in SAS® (SAS Institute 2023). The relationship between the number of preys consumed (N_a) and the number of preys offered (N_0) was described by the following model:

$$\frac{Na}{N0} = \frac{\exp\,(P_0 + P_1N_0 + P_2{N_0}^2 + P_3{N_0}^3)}{1 + \exp\,(P_0 + P_1N_0 + P_2{N_0}^2 + P_3{N_0}^3)}$$

where N_a represents the number of prey larvae consumed, N_0 is the initial prey density, and P_0, P_1, P_2, and P_3 are the intercept, linear, quadratic, and cubic coefficients, respectively. Parameters were estimated using the maximum likelihood method. Functional responses were classified according to Juliano (2001). A Type I response is characterized by a constant proportion of prey consumed as prey density increases. A Type II response shows a decreasing proportion of prey consumed at higher prey densities, typically due to handling time limitations. A Type III response exhibits a sigmoidal pattern, with low consumption at low prey densities followed by a rapid increase. Statistically, a Type II response was indicated by a significantly negative linear coefficient ($P_1 < 0$), whereas a Type III response was identified by a significantly positive linear coefficient ($P_1 > 0$) combined with a negative quadratic coefficient ($P_2 < 0$). Attack rate (a) and handling time (Th) were estimated using non-linear regression of Rogers' random predator equation (Rogers 1972), also implemented in PROC NLIN. Parameter estimates and their 95% confidence intervals were obtained for each treatment and larval instar, and differences between treatments were considered significant when confidence intervals did not overlap ($P < 0.05$) (Di Stefano 2005).

Analysis of the data revealed distinct functional responses among the larval instars of *C. cincta* (Tab. 1; Fig. 1). First-instar larvae exhibited a Type III functional response under both control and *Bacillus thuringiensis* Berliner, 1915 (Bt)-based bioinsecticide treatments, characterized by a sigmoidal increase in prey consumption with increasing prey density, as indicated by significant linear and quadratic terms in the logistic regression.

In contrast, second- and third-instar larvae showed different response patterns depending on treatment. Under control conditions, both instars displayed a Type I functional response, with a linear increase in prey consumption across the tested prey densities. However, when fed Bt-exposed prey, both instars shifted to a Type III functional response (Fig. 1).

Table 1. Parameters of the logistic regression of the proportion of neonate larvae of *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae) consumed by the different larval instar of *Ceraeochrysa cincta* (Schneider, 1851) (Neuroptera: Chrysopidae).

Instars	Treatments	Parameter*	Estimate*	SE*	χ^2*	P*
1st	Control	Intercept	-0.4101	0.3981	1.06	0.3029
		Linear	0.2746	0.0651	17.81	<0.0001
		Quadratic	-0.0114	0.00250	20.76	<0.0001
		Cubic	0.000112	0.000025	20.66	<0.0001
	Bt-treated	Intercept	-0.2600	0.3784	0.47	0.4921
		Linear	0.1693	0.0584	8.39	0.0038
		Quadratic	-0.00824	0.00222	13.84	0.0002
		Cubic	0.000089	0.000022	16.47	<0.0001
2nd	Control	Intercept	2.4957	0.4127	36.56	<0.0001
		Linear	-0.0216	0.0309	0.49	0.4847
		Quadratic	0.000287	0.000580	0.24	0.6208
		Cubic	-1.81E-6	2.848E-6	0.40	0.5256
	Bt-treated	Intercept	1.5137	0.3844	15.50	<0.0001
		Linear	0.1208	0.0331	13.31	0.0003
		Quadratic	-0.00299	0.000645	21.43	<0.0001
		Cubic	0.000016	3.196E-6	24.02	<0.0001
3rd	Control	Intercept	2.1716	0.4300	25.51	<0.0001
		Linear	-0.0344	0.0450	0.58	0.4453
		Quadratic	0.00273	0.00116	5.57	0.0183
		Cubic	-0.00002	6.599E-6	8.85	0.0029
	Bt-treated	Intercept	1.1358	0.3298	11.86	0.0006
		Linear	0.0966	0.0292	10.96	0.0009
		Quadratic	-0.00189	0.000577	10.72	0.0011
		Cubic	8.926E-6	2.887E-6	9.56	0.0020

* Parameter: regression coefficient type (intercept, linear, quadratic, cubic term); Estimate: parameter value estimated by the model; SE: standard error of the estimate; χ^2: Wald chi-square statistic used to test parameter significance; P: probability value associated with χ^2.

Estimates of attack rate (a) and handling time (Th), obtained using Rogers' random predator equation, are presented in Tab. 2. The highest attack rates were observed for first- and third-instar larvae in the control treatment. Exposure to Bt significantly reduced attack rates

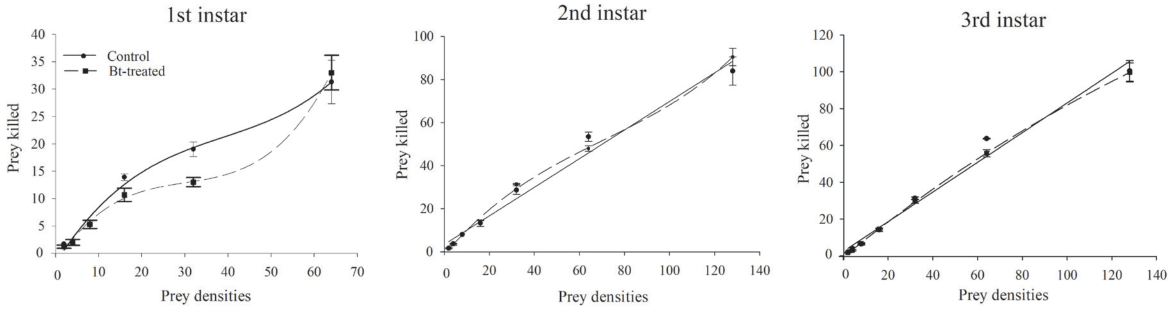

Figure 1. Functional response of larval instar of *Ceraeochrysa cincta* (Schneider, 1851) (Neuroptera: Chrysopidae) preying on neonate larvae of *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae). a) control and b) *Bacillus thuringiensis* Berliner, 1915 (Bt)-based bioinsecticide.

for first- and third-instar larvae, whereas no significant differences were detected for second-instar larvae. Handling time did not differ significantly among instars or between treatments.

Table 2. Attack rate[1] and handling time[1] values (95% CI) for the larval instar of *Ceraeochrysa cincta* (Schneider, 1851) (Neuroptera: Chrysopidae) preying on neonate larvae of *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae). Number of preys attacked estimated during the observation period (T/T_h; T=24h).

Instars	Treatments	a (h^{-1})	T_h (h)
1st	Control	0.00481 (0.00212–0.00751) a*	0.7286 (0.6148 – 0.8424) a*
	Bt-treated	0.00154 (0.000834 – 0.3766) b	0.5239 (0.3766 – 0.6712) a
2nd	Control	0.00415 (0.00225 – 0.00605) a	0.2645 (0.2398 – 0.2891) a
	Bt-treated	0.00286 (0.00201 – 0.00371) a	0.2331 (0.2135 – 0.2526) a
3rd	Control	0.00900 (-0.00021 – 0.0182) a	0.2255 (0.2081 – 0.2430) a
	Bt-treated	0.00386 (0.00242 – 0.00530) b	0.2115 (0.1938 – 0.2293) a

*Different letters within the same instar indicate statistical differences between treatments ($p < 0.05$), based on 95% confidence interval non-overlap. [1]Attack rate = a (h^{-1}); handling time = T_h (h).

Prey consumption increased with prey density for all larval instars in both treatments. In general, first-instar larvae showed lower consumption under Bt exposure compared with the control, whereas second- and third-instar larvae exhibited similar consumption levels in both treatments. When expressed as percentage of prey consumed, reductions under Bt exposure were evident mainly for first-instar larvae, while older instars maintained comparable consumption rates across treatments, particularly at medium and high prey densities.

Overall, prey exposure to the Bt-based bioinsecticide primarily affected early instars of *C. cincta*, reducing attack rates and prey consumption, whereas later instars maintained high predatory performance regardless of treatment.

Exposure of *S. frugiperda* neonates to the Bt-based bioinsecticide altered the functional response of *C. cincta* without reducing predation levels, particularly in second- and third-instar larvae. Although the mechanisms underlying this response remain uncertain, Bt-induced changes in prey quality, mobility, or chemical cues may have altered predator–prey interactions. Similar effects have been reported for arthropod natural enemies feeding on Bt-exposed prey (Desneux et al. 2007; Silva et al. 2019). Previous studies also demonstrated that Bt-based products can modify the functional response of *C. cincta* larvae (Pimenta et al. 2020), reinforcing the stage-specific nature of predator–prey interactions under microbial insecticide exposure.

First-instar larvae exhibited a sigmoidal predation curve consistent with a Type III functional response under both treatments. This pattern is often associated with limited prey detection or handling efficiency at low prey densities (Juliano 2001; Ail-Catzim et al. 2019). Although learning or prey switching was not directly assessed, early instars may face constraints related to prey defenses or limited foraging capacity (Zanuncio et al. 2008). The absence of a clear saturation plateau, however, restricts a definitive classification of this response (Jeschke et al. 2002).

In contrast, second- and third-instar larvae in the control treatment showed linear increases in prey consumption, consistent with a Type I functional response. This pattern likely reflects the greater mobility, voracity, and energetic demands of advanced instars (Griswold & Lounibos 2006; Aljetlawi et al. 2004). Although Type I responses are uncommon in invertebrate predators and generally require minimal handling time (Holling 1959), the lack of density-dependent variation in handling time partially supports this interpretation.

Under Bt treatment, second- and third-instar larvae shifted toward a Type III functional response. This shift may result from Bt-mediated alterations in prey behavior or chemical signaling, which can influence predator efficiency (Lawo & Romeis 2008; Silva et al. 2019). However, because attack rate and handling time estimates did not show consistent treatment-related trends, the mechanisms driving this shift remain unresolved and warrant further behavioral investigation (Rogers 1972).

Although predation curves did not reach clear saturation at high prey densities, this limitation does not invalidate the treatment- and stage-specific differences observed. Instead, it suggests constraints on prey handling or consumption, particularly in early instars. Future studies incorporating broader prey density ranges and direct behavioral observations may help clarify these limits (Symondson et al. 2002).

Overall, our results demonstrate that *B. thuringiensis* subsp. *tolworthi* can stage-specifically alter the pattern of predation and early-instar predation intensity by *C. cincta*. These findings highlight the importance of considering prey condition and predator developmental stage when integrating microbial insecticides and natural enemies in IPM programs, particularly in systems affected by widespread insecticide resistance in *S. frugiperda* populations (Diez-Rodríguez & Omoto 2001; Omoto et al. 2016; Stenberg et al. 2021).

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Authors' Contributions

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

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

Ethical Approval

This article does not contain studies involving human participants or vertebrate animals. The research was conducted exclusively with invertebrate insects, which do not require institutional ethical approval under current Brazilian legislation and university guidelines.

Data Availability

The data supporting the findings of this study are included in the article. Raw data are available from the corresponding author upon request.

Generative AI Statement

The authors declare that generative artificial intelligence (AI) tools, including ChatGPT, were used only to assist with language revision and text editing. No AI tools were used to generate scientific content, analyze data, or draw conclusions. The authors take full responsibility for the accuracy and integrity of the manuscript.

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