

# Parasitism of *Trichogramma Pretiosum* in Eggs of *Corcyra Cephalonica* on the Age of the Parasitoid and the Density of the Host

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## Research Article

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# Abstract

Several biological control programs use *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae). The parasitism capacity and the functional response of *T. pretiosum* parasitizing eggs of *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) were studied. To determine the parasitism capacity, females of the parasitoid were individualized, being offered to each female eggs from the host. Daily observations determined the number of parasitized eggs per female, accumulated percentage of parasitism, female longevity and percentage of parasitoid emergence. To establish the functional response, *T. pretiosum* females were individualized and offered *C. cephalonica* eggs. Number of parasitized eggs and percentage of adults' emergence were evaluated, being calculated handling time (Th) and attack rate (a). On the first day of the parasitoid's life there was parasitism in more than 80% of the host eggs, similar to the percentage of adult emergence throughout the life cycle, and the daily percentage of parasitism. The percentages of eggs of *C. cephalonica* parasitized were statistically different, with the quadratic model better describing the parasitoid-host relationship, with a positive value of 0.00594, since with the increase in the availability of eggs of *C. cephalonica*, parasitism was increased. The attack rate was approximately 0.00158, while the handling time of the host eggs was 0.6884 h.

## Introduction

In the current global agricultural scenario, several management tactics are employed, either alone or in association, with the objective of combating pest insects capable of producing economic damages in different crops. Among the control tactics, the biological one has the ability to significantly reduce the impact of chemicals on the environment (Parra et al. 2015), and the use of parasitoids as control agents can be highly effective, particularly due to their high host specificity (Nasrin et al. 2016).

The genus *Trichogramma* spp., among the parasitoids, is one of the most studied due to its wide geographical distribution and ease of rearing in the laboratory (Nasrin et al. 2016). More than 210 species of *Trichogramma* occur in the world, of which 28 are cited in South America, with Brazil having the largest number of records, with 28 species (Pinto 2006). The females of these insects parasitize especially pest lepidopteran eggs, preventing their hosts from passing to larval stage (Haji et al. 1995).

*Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae), a species of Mexican and American origin, is the most distributed worldwide, with the occurrence of its parasitism in 18 hosts of approximately 13 crops of economic importance (Zucchi and Monteiro 1997). It is an idiobionte species, since it parasitizes eggs of different insect orders, including Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Neuroptera and Thysanoptera, preventing their hosts from reaching the larval stage (Monnerat et al. 2007; Carvalho et al. 2014; Amaro et al. 2015).

For the massal rearing of *Trichogramma* spp. alternative or replacement hosts have been used, that is, hosts that are not parasitized in natural conditions, the main objective being to reduce costs and increase

efficiency, but with the guarantee of adequate development (Parra 1997), as has happened with the lepidopterans *Anagasta kuehniella* (Zeller) (Pyralidae), *Sitotroga cerealella* Oliver (Gelechiidae) and *Corcyra cephalonica* (Stainton) (Pyralidae) (Zagatti et al. 1987; Chandrawanshi et al. 2018).

Various characteristics of host eggs, such as size, chorion thickness, nutritional value and age can affect the development and quality of the parasitoids produced, directly influencing the percentage of parasitism, the sex ratio and the number of individuals generated per egg host, relations that may vary according to the species (Hoffmann et al. 2001; Roriz et al. 2006; Rukmowati-Brotodjojo and Walter 2006).

*Corcyra cephalonica* has been shown to be easy to rear in the laboratory, providing a large quantity of eggs, which favors the numerical success for the multiplication of parasitoids in massal laboratory rearing, with low production cost, in whose diet only two ingredients are used, wheat germ and yeast (De Bortoli et al. 2012).

For the success of a biofactory in the massal rearing of insects aiming at the applied biological control, it is essential to understand the parasitoid x host relationship, particularly regarding its biological, ecological, behavioral aspects and, especially, the qualitative nutritional aspects (Pastori et al. 2010). Some authors such as Bernardi, Haddad and Parra (2000) and Chaudhuri and Senapati (2017) have already demonstrated that *C. cephalonica* is a potential host for the rearing of *T. pretiosum*.

However, studies on the parasitism capacity for the genus *Trichogramma* are necessary, in order to measure the rate of predation or parasitism as a function of host density, contributing to the understanding of the process of predation or parasitism, allowing to infer comparisons and estimates that indicate the potential of success of the studied agent, both in massal rearing, as in applied biological control (Hassell 1978). In this sense, the present study evaluated the effect of the parasitoid's age on its reproductive capacity, the availability of host eggs on the potential of parasitism and the viability of *T. pretiosum*, an important contribution to the use of the alternative host *C. cephalonica* in biofabrics of massal rearing of the parasitoid, as well as, in the decision making of its release in the field to control pest insects.

## Results

### Parasitism capacity

The percentages of *C. cephalonica* eggs parasitized by *T. pretiosum* females on the different days of their longevity (1 to 15 days) showed significant differences, with emphasis on the first day of life with more than 80% of the parasitized eggs. Between the 2nd and the 5th and on the 10th, 11th, 12th and 13th day of life of the females the percentages of parasitized eggs ranged from 33.3 to 53.2%, while in the others they were always below 30% (Table 1).

Table 1  
 Percentage of *Corcyra cephalonica* eggs parasitized by *Trichogramma pretiosum* during 15 days of female longevity.

Female age	Means ± Standard error
01 day	85.4 ± 4.77a <sup>1</sup>
02 days	53.2 ± 4.44b
03 days	39.4 ± 4.93bcd
04 days	44.0 ± 3.40bc
05 days	33.4 ± 3.33bcd
06 days	27.1 ± 2.28cd
07 days	23.1 ± 2.42cd
08 days	24.3 ± 2.45cd
09 days	27.2 ± 2.42cd
10 days	38.4 ± 6.86bcd
11 days	37.8 ± 8.82bcd
12 days	33.3 ± 8.56bcd
13 days	36.2 ± 8.28bcd
14 days	16.6 ± 3.87d
15 days	29.3 ± 6.40cd
<sup>1</sup> Means ± standard error followed by the same letter in the column do not differ by Student Newman Kells (0.05%), (F = 1.43; df = 14; p < 0.001).	

The percentages of emergence of adults of *T. pretiosum* from the eggs of *C. cephalonica* parasitized by females at different ages were similar in practically the entire period of life (ranging from 95.8 to 69.1%), differing only in the 5th and 7th day of female longevity (64.0 and 53.6%, respectively) (Table 2).

Table 2

Percentage of emergence of *Trichogramma pretiosum* from eggs of *Corcyra cephalonica* parasitized during 15 days of female longevity.

Female age	Means $\pm$ Standard error
01 day	95.8 $\pm$ 3.32a <sup>1</sup>
02 days	87.8 $\pm$ 5.48ab
03 days	79.5 $\pm$ 8.13ab
04 days	94.3 $\pm$ 1.44 <sup>a</sup>
05 days	64.0 $\pm$ 6.19bc
06 days	89.1 $\pm$ 5.69ab
07 days	53.6 $\pm$ 7.41c
08 days	85.8 $\pm$ 5.78ab
09 days	97.7 $\pm$ 1.72 <sup>a</sup>
10 days	97.3 $\pm$ 1.45 <sup>a</sup>
11 days	83.0 $\pm$ 4.96ab
12 days	92.0 $\pm$ 5.81 <sup>a</sup>
13 days	89.5 $\pm$ 2.80ab
14 days	76.1 $\pm$ 9.73ab
15 days	69.1 $\pm$ 11.10abc
<sup>1</sup> Means $\pm$ standard error followed by the same letter in the column do not differ by Student Newman Kells (0.05%), (F = 2.78; df = 14; $p < 0.001$ ).	

The daily percentage of parasitism practically uniform during the 15 days of longevity of the females, with emphasis on the first two days, with values close to 50% (Fig. 1).

#### Functional response

Among the models used to determine the functional response, the quadratic described the best arrangement, with a positive value of 0.00594 (Table 3). With the increase in the availability (density) of *C. cephalonica* eggs, there was also an increase in parasitism by *T. pretiosum* (Fig. 2). The attack rate of the parasitoid was approximately 0.00158 eggs h<sup>-1</sup>, while the handling time was 0.6884 h (Table 4).

Table 3  
Parameters estimated by logistic regression between the proportion of *Corcyra cephalonica* eggs parasitized by *Trichogramma pretiosum*.

Parameters	Values $\pm$ SE*	DF**	$\chi^2$	P
Intercept	-0.3239 $\pm$ 0.2459	1	1.73	0.1878
Linear	-0.1106 $\pm$ 0.0388	1	8.12	0.0044
Quadratic	0.00594 $\pm$ 0.00149	1	15.84	< 0.0001
Cubic	-0.00007 $\pm$ 0.000015	1	19.76	< 0.0001
*Standard error				
**Degree of freedom				

Table 4  
Attack rate and handling time of *Corcyra cephalonica* eggs by *Trichogramma pretiosum*, in different densities and during 24 h of exposure.

Attack rate (a')	Handling time (Th)	a'/Th
0.00158 $\pm$ 0.000310	0.6884 $\pm$ 0.0670	34.86
(0.000966–0.8210) <sup>1</sup>	(0.5588–0.8210)	(42.9–29.2)
<sup>1</sup> Means $\pm$ 95% confidence interval.		

## Discussion

The females of *T. pretiosum* responded to the availability of host eggs during their longevity, maintaining an average daily parasitism with the exception of the first 24 h which was higher, and without significant losses to the total fecundity of the females. That way, females of *T. pretiosum* were able to perform postures from the first day to the last day of life (15th day), differently from what was observed in the works of Pastori et al. (2007), dos Santos (2007) and Carvalho et al. (2014) for *T. pretiosum* and other species of the genus, where daily parasitism was higher in the first 24 h, decreasing after this period. According to Queiroz et al. (2020), for the parasitoid with one to five days of longevity, the efficiency of parasitism remained the same in eggs of *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Noctuidae). Another point to be highlighted is the temperature at which the bioassay was carried out, 25  $\pm$  2°C, which may have disadvantaged the percentage of parasitism. Kumari et al. (2020) reported that the percentage of parasitism of *Trichogramma chilonis* Ishii in *C. cephalonica*, at this same temperature, was 58.67%, while Oliveira et al. (2017) reported that at 28 °C, 100% of the parasitized eggs of *Neoleucinodes elegantalis* (Gueneé) (Lepidoptera: Crambidae) resulted in the emergence of adults from the parasitoid.

Parveen and Sultan (2012) also found the maximum parasitism achieved (84.3%) in eggs of *C. cephalonica* at a temperature of 28 °C. In addition, the species of lineage of the parasitoid and host used can influence the parasitism capacity (Hassan and Guo 1991; Hansen and Jensen 2002; Pratisoli et al. 2004).

With 14 days old females there was instability in the value of the percentage of parasitism. Laurentis et al. (2019) describe those irregularities in parasitism rates are common, as these insects, when reared in the laboratory, tend to modify this behavior and develop adaptations throughout the entire life cycle, affecting the efficiency of parasitism. The accumulated percentage of parasitism steadily increased until the end of the assessment, resulting in an increase in that rate until the end of the experiment (Subandi et al. 2017). However, it is important to consider that *T. pretiosum* has its population reduced throughout its longevity.

As for the percentage of *T. pretiosum* emergence, only with females of five, seven and 15 days of longevity was not found an ideal emergency for *Trichogramma*, which is above 85%, according to Navarro (1998). Viability is directly related to host characteristics, such as egg quantity and nutritional quality of them. Thereby, it was found that *C. cephalonica* eggs are suitable for the development of *T. pretiosum*. In this sense, Pratisoli and Oliveira (1999) and Gonçalves et al. (2003), evaluating the viability of *T. pretiosum* in eggs of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) and *S. cerealella*, obtained values above 91% and 89%, respectively, corroborating with the percentages found in this work. According to the International Organization for Biological Control (IOBC), for parasitoids of the genus *Trichogramma* the average indexes recommended as a quality standard are: parasitism  $\geq 25$  eggs/female in 48 hours; emergence  $\geq 80\%$ ; sex ratio  $\geq 0.5$  and longevity  $\geq 7$  days (Van Lenteren 1994). In this work, the first day of parasitism with 48 hours was considered, since in the first 24 hours the parasitoids were mated, this suggesting that *T. pretiosum* in the host *C. cephalonica* fits the IOBC quality indexes, even though the sex ratio was not observed.

Among the tested statistical models, the quadratic one described the best arrangement. This model has also been observed in previous research with *T. pretiosum* in *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) eggs in tomato in natural field conditions (Pratisoli et al., 2005), and with *Trichogramma atopovirilia* Oatman and Platner (Hymenoptera: Trichogrammatidae) in *H. zea* eggs at 25°C (Paron et al. 1998). Cabello and Vargas (1985) also obtained the same model working with *Trichogramma cordubensis* Vargas and Cabello (Hymenoptera: Trichogrammatidae) on eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) at 20°C, inferring that the percentage of parasitized eggs stabilized with the increase of parasitoid density from ten females. This fact was explained by the numerical superiority of females able to parasitism, because the lower the density of females, the greater was the number of parasitized eggs.

The type II functional response, considered quadratic, shows that, according to the increase in the availability of *C. cephalonica* eggs, there is an increase in parasitism by *T. pretiosum*. The limit reached in this work is approximately 25 parasitized eggs, even though there are more than 60 available eggs. This

behavior is also mentioned by Price (1975) as Holling type II functional response, as parasitism increases at a decreasing rate with the increase in the number of hosts, a behavior that is commonly observed in parasitoids and insect predators (Holling 1961). Milanez et al. (2018) also recorded the same type of functional response, with the same parasitoid, in eggs of *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), concluding that the decrease in oviposition due to the limitation of parasitoid eggs is due to its endocrine oviposition control system, even if eggs are available, not due to the absence of oogenesis, but due to the regulation of neurohormones (Reznik et al. 2003).

The attack rate of *T. pretiosum* in *C. cephalonica* eggs was lower than that mentioned by Godfray (1994), with a variation between 0.002 and 0.030. h<sup>-1</sup>, generally for parasitoids. Changes in the parameters of the functional response are frequent for parasitoids and predator, according to factors such as host and environmental conditions, which particularly affect the foraging capacity and, consequently, the foraging rate (Mackauer 1983). The lower attack rate found in this work demonstrates that the parasitoid exploits a smaller area to find the host, and that although there is a reduction in the area explored, the expected average number of parasitized eggs is reached.

*Trichogramma pretiosum* showed good biological performance in eggs of *C. cephalonica*, with high values for the potential for parasitism and percentage of emergence in the first 48 hours of life.

*Trichogramma pretiosum* has reproductive capacity throughout its longevity.

High density of available host eggs induces *T. pretiosum* to parasitize a greater number of eggs (between 25 to 30 eggs).

## Methods

The bioassays, as well as the rearing of *T. pretiosum* and *C. cephalonica*, were conducted in the Laboratório de Biologia e Criação de Insetos (LBCI) of the Departamento de Ciências da Produção Agrícola (Fitossanidade), Faculdade de Ciências Agrárias e Veterinárias, UNESP, Jaboticabal, SP, in controlled conditions of temperature (25 ± 2°C), relative humidity (70 ± 10%) and daily photoperiod (12 h light and 12 h dark).

### Rearing of the host *Corcyra cephalonica*

The eggs of *C. cephalonica* were obtained from the LBCI stock rearing, and a parallel rearing was conducted to supply the eggs needed for bioassays using a diet consisting of wheat germ (94%), sterilized at 150°C for two hours, and beer yeast (6%) (Bernardi et al. 2000). The diet was arranged in 47.0 cm x 29.5 cm x 10.5 cm plastic containers, distributed in four parallel grooves and in direction of the length of the container 0.15 g of eggs, with subsequent closure with plastic lids containing central holes covered with voile-type fabric, measuring 6.0 cm x 8.0 cm. The newly emerged adults were collected daily using a vacuum cleaner adapted with a capture chamber built with a PET bottle and PVC pipe. The collected adults were transferred to cylindrical glass containers (11.0 cm in diameter x 17.0 cm in height)

containing a piece of sombrite type-screen folded in “Z” (substrate for oviposition), the container being closed with the same type of screen. The eggs were collected daily by inverting the adults’ container on a white plastic tray, tapping the bottom of the container with the palm of the hand, so that the eggs adhered to the laying substrate would detach and fall into the tray. The eggs were sieved to remove impurities, part of which was used to maintain the rearing itself and another part to carry out the bioassays. The eggs produced and momentarily not used were stored in a refrigerator (temperature close to 10 °C) (Jacob and Cox 1977).

#### Rearing of the parasitoid *Trichogramma pretiosum*

The parasitoids used in the bioassays came from the LBCI stock rearing, and their maintenance was carried out according to the methodology described by Parra (1997). Before making the cards with *C. cephalonica* eggs, they underwent a non-viability process with exposure to the germicidal lamp for 45 minutes. Then, the unviable eggs adhered to cards of sky-blue paperboard (3.5 cm x 1.5 cm), with fixation on double-sided adhesive tape (2.5 cm x 1.2 cm), were exposed to parasitism, with the cards positioned inside flat-bottom test tubes (2.0 cm in diameter x 8.0 cm in high), containing adults of *T. pretiosum* newly emerged. On the surface of the inner side of the tube, a droplet of honey was added to feed the parasitoids. The card was replaced every 24 hours, for five days, and those with parasitized eggs were transferred to another similar tube, with the tubes always being sealed with PVC film, with the emergence of adult insects in these tubes.

#### Assessment of parasitism capacity

Females of *T. pretiosum* were individualized in 30 Duran glass tubes (3.0 cm in length x 0.90 mm in diameter) and sealed with PVC film. For each female, 30 eggs of *C. cephalonica* were offered, aged between 0 and 24 h, adhered to cards of sky-blue paperboard. The cards were replaced every 24 hours for the entire longevity of the females, being identified and maintained in the same conditions. Daily it was determined: the number of parasitized eggs, accumulated percentage of parasitism, total number of parasitized eggs per female, female longevity and percentage of emergence of adults. The design used was completely randomized design (CRD), and the data analysis was conducted with the SAS® Software. For the analysis, the data were submitted to Bartlett’s tests to verify homoscedasticity (PROC GLM), and Cramer von Mises test for normality (PROC UNIVARIATE). As the data did not present such requirements, the transformation at  $x + 0.5$  was used for the Analysis of Variance (PROC ANOVA). The means, when significant, were compared using the Student Newman Kells test ( $p > 0.05$ ).

#### Functional response of *Trichogramma pretiosum* with *Corcyra cephalonica* eggs

Females of *T. pretiosum* were individualized in 140 flat-bottom test tubes (2.0 cm in diameter x 8.0 cm in high) and sealed with PVC film. For each female, eggs were offered between 0 and 24 h of laying and in densities of 1, 2, 4, 8, 16, 32 and 64, adhered to cards of sky-blue paperboard. The cards were removed after 24 h of exposure to parasitism, with 20 repetitions per egg density. The assessments were carried out after 10 days, determining the number parasitized eggs and the percentage of emergence of adults.

Subsequently, the random equation of functional response proposed by Rogers (1972) was used, calculating the handling time ( $T_h$ ) and attack rate ( $a$ ), with the construction of the functional response equation. The parameters were determined by the graphical linearization of the values of the number of parasitized eggs (x-axis) and the natural logarithm ( $\ln$ ) of the proportion of remaining eggs (y-axis). Using the slope of the straight line divided by 24 h, the attack rate ( $a$ ) (eggs/h) was obtained in hours. Dividing this value by the slope of the line gave the handling time ( $T_h$ ), also measured in hours. The estimated parameters of attack rate ( $a$ ) and handling time ( $T_h$ ) were compared between densities using the confidence interval (CI) at 95% probability, differing the means in which the CI did not overlap.

The predation data were submitted to Student's test to determine the significance among treatments, using the SAS® Software, with the functional response determined by means of the logistic regression of the proportion of eggs consumed according to the original densities, using different models, using PROC CATMOD, with the random parasitism equation adjusted to the results by PROC NLIN (Juliano 2001).

## Declarations

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### AUTHOR CONTRIBUTIONS

G.G.M., S.J.J., A.A.F.L. and N.A.S. wrote the main manuscript text and prepared all figures and tables. All authors reviewed the manuscript.

### ADDITIONAL INFORMATION

The authors declare no competing interests.

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## Figures

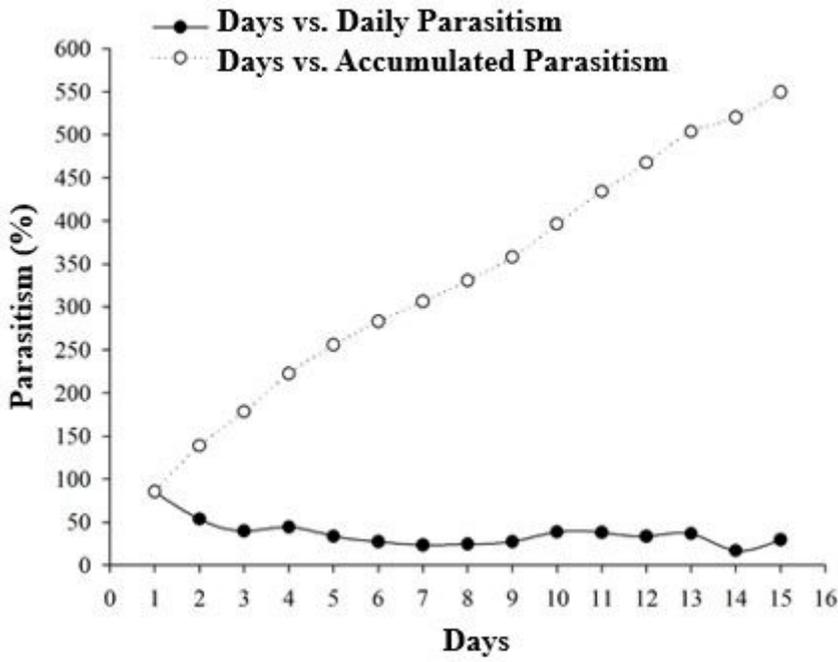


Figure 1

Average daily and accumulated number of parasitized eggs of *Corcyra cephalonica* by *Trichogramma pretiosum*.

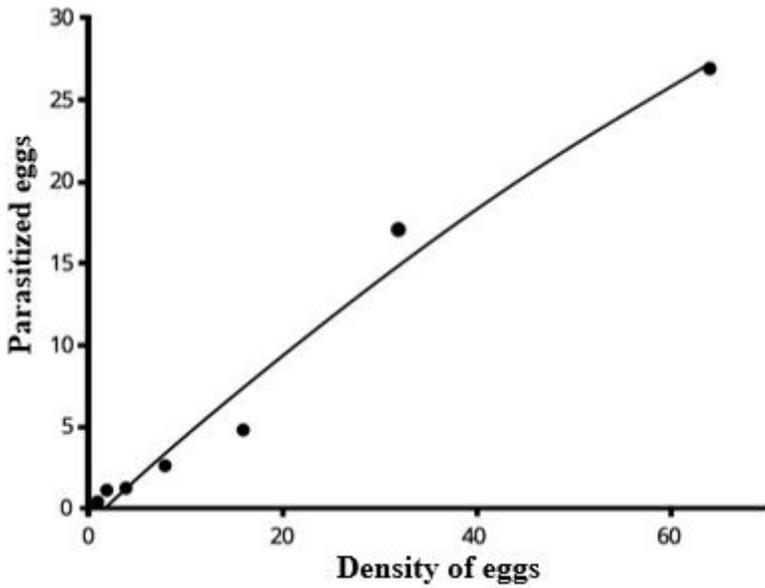


Figure 2

Average number of *Corcyra cephalonica* eggs, in different densities, parasitized by *Trichogramma pretiosum*, in 24 h of exposure.