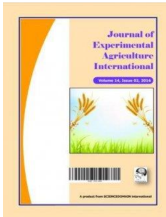

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Functional Response of Two Egg Parasitoids of *Trichogramma* (Hymenoptera: Trichogrammatidae) Genre on *Duponchelia fovealis* Zeller (Lepidoptera: Crambidae) Eggs

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Authors' contributions

This work was carried out in collaboration with all authors. Authors JPPP and DP designed the study, wrote the protocol and first draft of the manuscript. Author VLSL managed the literature searches and did a critical review of the manuscript. Author JRC managed the analyzes of the study. All authors read and approved the final manuscript

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ABSTRACT

Aims: To evaluate the potential of the parasitoids of the genus *Trichogramma* on European pepper moth, a functional response bioassay was performed with two *Trichogramma* lineages and different egg densities of the host.
Study Design: Adopting a completely randomized experimental design, 10 replications were done for each species.
Place and Duration of Study: The bioassay was conducted at the Entomology Laboratory of the

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Universidade Federal do Espírito Santo (CCAE-UFES) in Alegre, ES, Brazil, in the year 2017.
Methodology: The functional response bioassay was done for the *T. pretiosum* and *T. galloi* for the different *D. fovealis* egg densities (5, 10, 15, 20, 25 and 30 eggs). After the host eggs were glued to a paperboard, it was inserted into a flat bottom glass tube. Next, a parasitoid female was introduced into each tube. The tubes which were sealed with PVC plastic film were then placed in air-conditioned chambers keeping adjusted at 25°C temp., RH at 70 ± 10 % and a photoperiod of 14 h. Then 24 h later, the parasitoid was removed from the tube and the paperboard with the parasitized eggs was left in the tube until the offspring emerged.
Results: For both the *Trichogramma* species, the functional response was confirmed to be type II, with the Rogers model being the most fitting. An interaction between the factors of parasitoid species and host density ($F_{5,102}$ = 2.756, P = 0.022) was observed. The search efficiency for *T. pretiosum* and *T. galloi* were determined as 0.1799 ± 0.025 and 0.0976 ± 0.011 h^{-1}, while the handling time was assessed as 0.9852 ± 0.077 and 0.5751 ± 0.101 h, respectively.
Conclusion: The estimated values for the parameters search efficiency and handling time revealed that *T. pretiosum* and *T. galloi* are potential candidates for the biological control of *D. fovealis*.

Keywords: Behavioral response; biological control; egg parasitoid; European pepper moth; Lepidoptera; strawberry.

1. INTRODUCTION

The European pepper moth, *Duponchelia fovealis* Zeller (Lepidoptera: Crambidae) has been identified as a greenhouse pest in parts of Europe, Africa, the Middle East and North America [1–4]. Since 2010, *D. fovealis* has been reported as a pest in Brazil and is presently found distributed across the states of Paraná, Espírito Santo and Minas Gerais, where it has mainly caused damage in the strawberry crop [5–7]. Besides the wide spatial distribution, fast expansion in the various strawberry-producing regions and the number of hosts available, the European pepper moth quickly adapts to the environmental conditions, and can survive in a range of 18 to 30°C temperature [8]. Considering these facts and the potential damages that the European pepper moth can cause, it becomes crucial to encourage further studies that will warrant management methods for this pest, of which biological control must be underscored [7, 9, 10].

Among the most effective biotic agents are the parasitoids belonging to genus *Trichogramma* (Hymenoptera: Trichogrammatidae), as they are widespread across the world. They parasitize the eggs of more than 200 insect species and can be effortlessly reared on alternative hosts [11–13]. These parasitoids are popularly utilized as they are efficient, easy to produce in massive quantities and entail low production costs [13, 14].

The success of parasitoids in biological control programs is directly related to the knowledge of their biological characteristics, interaction with the host and influence of abiotic factors such as climatic conditions [15,16]. An important aspect to evaluate the efficiency of a natural enemy is the functional response, which is based on the relationship between host density and parasitoid search efficiency [17]. Functional response is therefore vital to the success of the biological control programs, as it is the means of evaluating the number of natural enemies that need to be released depending on the pest density. Therefore, studies that establish the requisite proportion of the *Trichogramma* females to *D. fovealis* eggs are one of the chief means of understanding the parasitoid-host interaction and the population dynamics. This study aimed at determining the type of functional response, evaluate the different models and estimate the parameters of functional response for the parasitoids *T. pretiosum* and *T. galloi* foraging on the *D. fovealis* eggs.

2. MATERIALS AND METHODS

The *D. fovealis, T. pretiosum* and *T. galloi* insects employed in the bioassays were drawn from the inventories of the Laboratory of Entomology (NUDEMAFI) of the Universidade Federal do Espírito Santo (UFES-CCAE), in Alegre, ES, Brazil.

Based on the methodology proposed by Paes et al. [8] the *D. fovealis* was reared; the parasitoids, on the other hand, were reared on the eggs of the alternative host *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae), according to the method of Carvalho et al. [18]. Both insects were reared in the laboratory at 25 ± 1°C, 70 ± 10% RH and a photoperiod of 14 h.

The functional response bioassay was done for the *T. pretiosum* and *T. galloi* for the different *D. fovealis* egg densities (5, 10, 15, 20, 25 and 30 eggs). Egg densities were defined in preliminary tests that resulted in a potential for maximum parasitism around 20 parasitized eggs. After the host eggs (up to 24 hours old) were glued to a paperboard (2.5 x 0.5 cm) using gum arabic (20 %), it was inserted into a flat bottom glass tube (8.5 x 2.5 cm). Next, a parasitoid female less than 6 h old was introduced into each tube; honey droplets were then deposited on the inner wall of the tube, as food. The tubes which were sealed with PVC plastic film were then placed in air-conditioned chambers keeping the temperature adjusted at 25°C, RH at 70 ± 10% and a photoperiod of 14 h. Then 24 h later, the parasitoid was removed from the tube and the paperboard with the parasitized eggs was left in the tube until the offspring emerged. The freshly hatching caterpillars of the non-parasitized eggs were removed every day and the number of parasitized eggs was carefully noted.

Adopting a completely randomized experimental design, 10 replications were done for each species. The data were subjected to the analysis of variance to determine the effects of the species, host densities and the interaction among these factors on the number of parasitized *D. fovealis* eggs. The means were compared using Tukey test ($P < .05$).

The functional response was analyzed using the number of parasitized eggs. The functional response was verified by submitting the data to the *friar test*() function of the *frair* package [19] using the R software version 3.4.0 [20]. The data were then verified to reveal type II functional response (results not displayed). The type II functional response models proposed by Holling et al. (1959) (Equation 1) and Rogers (1972) (Equation 2) were thus tested [21,22].

$$N_a = \frac{a \cdot N_0 \cdot P \cdot T_t}{1 + a \cdot T_h \cdot N_0} \quad \text{(Eq. 1)}$$

$$N_a = N_0 \left[1 - \exp\left(- \frac{a \cdot P \cdot T_t}{1 + a \cdot T_h \cdot N_0} \right) \right] \quad \text{(Eq. 2)}$$

Where:

N_a: number of parasitized eggs; N_0: host egg density; a: search efficiency; P: number of parasitoids (P = 1); T_t: experiment duration (T_t = 24 h); and T_h: handling time.

The models were compared using the Logarithm of maximum likelihood (LogLik), Akaike information criterion (AIC) and Chi-square test.

The coefficients of search efficiency and handling time were determined using the method of maximum likelihood with the functions *emdbook* [23] and *bbmle* [24] packages using the R software version 3.4.0 [20].

3. RESULTS

The interaction between the parasitoid species and host density factors ($F_{5, 102}$ = 2.756; P = .022) showed significance for the analysis of variance (Table 1). For all the *D. fovealis* egg densities no significant difference was observed among the *Trichogramma* species for the number of parasitized eggs, barring for the density of 10 host eggs (Table 1). The number of parasitized eggs increased, according to the rise in host egg density for both the *Trichogramma* species (Table 1).

Table 1. Mean of parasitized eggs (± SE) by *Trichogramma pretiosum* and *T. galloi* on the different *Duponchelia fovealis* egg densities

Egg density	*T. pretiosum* [1]	*T. galloi* [1]
5 ^{ns}	4.80 ± 0.20 B	4.40 ± 0.16 D
10 *	9.75 ± 0.16 aB	8.00 ± 0.50 bD
15 ^{ns}	12.20 ± 1.21 AB	12.00 ± 0.82 CD
20 ^{ns}	16.20 ± 0.77 AB	16.40 ± 0.65 BC
25 ^{ns}	17.11 ± 1.45 AB	18.55 ± 0.53 AB
30 ^{ns}	19.09 ± 0.64 A	20.09 ± 0.67 A

[1] Means followed by the same letter, lowercase in the line and uppercase in the column, do not differ by Tukey test (P < .05); ^{ns} Not significant.*

The Rogers model revealed a better fit for both *Trichogramma* species, registering higher maximum likelihood log values, lower AIC values and higher weights (Table 2).

Both the *Trichogramma* species revealed functional response type II (Fig. 1 A, B). However, the functional response curves for the Rogers model highlighted the differences between the *Trichogramma* species for the parameters of search efficiency and handling time (Table 3). The *T. pretiosum* species registered a higher search efficiency (0.1799 ± 0.025 h^{-1}) than did the *T. galloi* (0.0976 ± 0.011 h^{-1}), with difference significant (Table 3). However, the handling time of the host egg by *T. pretiosum* (0.9852 ± 0.077 h) was greater than that noted for *T. galloi* (0.5751 ± 0.101 h) (Table 3).

4. DISCUSSION

In the current work, the parasitoids *T. pretiosum* and *T. galloi* revealed a typical type II functional response curve [21]. The parasitoids of the Trichogrammatidae family exhibit three types of functional response, of which types II and III are the more common [25–29]. According to Holling et al. (1959) the general expression of the functional response was classified based on the shape of the response curve below the upper limit - type I response showed a linear increase; type II response revealed a monotonic deceleration kind of increase; and type III response showed a sigmoidal increase in the attacked hosts. In this study, the type II functional response that was identified implies that the parasitoids *T. pretiosum* and *T. galloi* increased their rate of parasitism when the host density increased until the parasitoids achieved maximum reproductive capacity. This limitation in oviposition depends on the endocrine system. Therefore, the withholding of ovulation that was exhibited can be regarded as a particular state which is managed by the neurohormonal regulation of the insect and not by oogenesis [30].

The functional response of the parasitoid can vary in type, depending on the parasitoid density, lineage or age [25,29,31], host [32] and ambient conditions [26,33]. In the same way that *T. pretiosum* and *T. galloi* presented type II functional response in our study, type II responses were observed in *T. pretiosum*

Table 2. Comparison between the Roger's and Holling's models in different *Duponchelia fovealis* egg densities parasitized by *Trichogramma pretiosum* and *T. galloi*

Species	Model type II	LogLik	AIC	Weight	χ^{2} [a]	*P*
T. pretiosum	Rogers	-120.52	245.04	0.948	0.0990	.9998
	Holling II	-123.42	250.84	0.052	0.2064	.9990
T. galloi	Rogers	-108.12	220.24	0.65	0.1125	.9998
	Holling II	-108.75	221.50	0.35	0.1466	.9996

[a] *Chi-square test.*

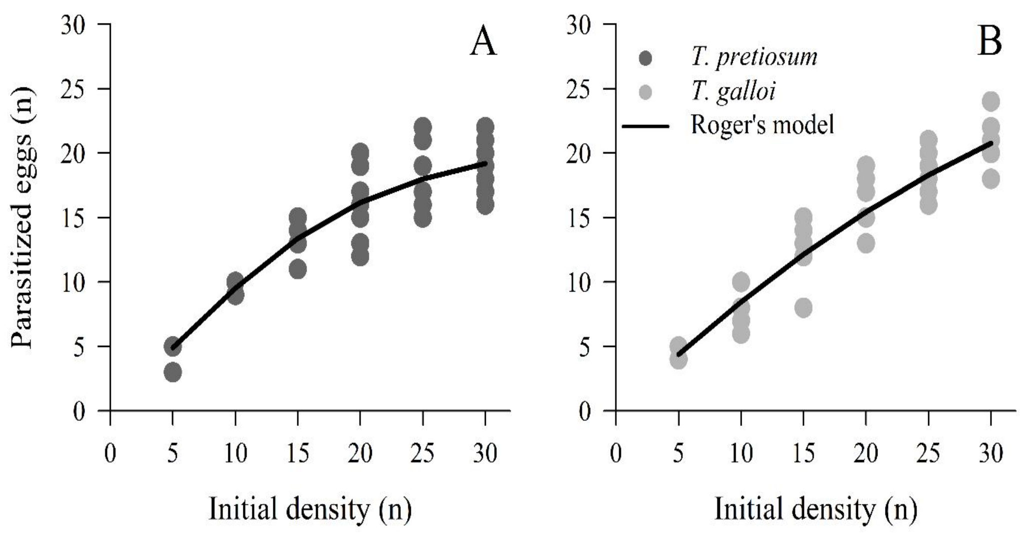

Fig. 1. Parasitism and type II functional response by the Rogers model for *Trichogramma pretiosum* (A) and *T. galloi* (B) in response to the different *Duponchelia fovealis* egg densities

Table 3. Estimated parameters using the Rogers model of the *Trichogramma pretiosum* and *T. galloi* parasitizing the different densities of the *Duponchelia fovealis* eggs

Species	Parameter [1]	Estimate [2]	SE	95 % CI	
				Lower	Upper
T. pretiosum	*a*	0.17994	0.02586	0.136299	0.239682
	T_h	0.98524	0.07739	0.824746	1.130943
T. galloi	*a*	0.09765	0.01169	0.077023	0.123218
	T_h	0.57512	0.10148	0.355747	0.759023

[1] Parameters: a = search efficiency (h^{-1}); T_h = handling time (h); [2] Significant (P < .001).

foraging eggs of *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) [25] and *T. brassicae* (Bezdenko) on the *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) eggs [32]. However, other species of the genus *Trichogramma* presented a type III functional response, such as *T. chilonis* (Ischii) foraging *Galleria mellonella* L. (Lepidoptera: Pyralidae) [31], *T. brassicae* on the *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) and *T. ostriniae* on the *Ostrinia nubilalis* (Lepidoptera: Pyralidae) eggs [26]. Therefore, the *Trichogramma* genus does not seem to exhibit any specific functional response pattern, as it is obviously affected by several factors. For example, in a study using *T. brassicae*, while type II functional response was noted at 25°C, type III was recorded at 20 and 30°C. The authors ascribe these findings to the effect of temperature on the learning process, which influences the foraging behavior of this parasitoid and its capacity to distinguish between the parasitized and non-parasitized eggs [33].

Knowledge regarding the type of functional response is crucial in selecting a natural enemy; however, there are a few other desirable attributes like short handling time, high degree of search efficiency, high intrinsic rate of population increase and tendency to aggregate in locations frequented by the target pest [34]. In this study, the parasitoids *T. pretiosum* and *T. galloi* showed an estimated handling time and search efficiency of 0.98 and 0.57 h and 0.17 and 0.09 h^{-1}, respectively. These results are potentially higher than those noted in other studies [31,32,35,36]. *Trichogramma brassicae* foraging the eggs of *H. armigera, E. kuehniella* and *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) displayed handling time and search efficiency of 1.48, 1.33, 0.96 h and 0.03, 0.12, 0.10 h^{-1}, respectively [32]. Therefore, the short handling time and the high search efficiency recorded in this study can be considered a good result in comparison to the results reported in other studies. In addition, short handling times and high search efficiency lead to optimum parasitoid foraging and population stability between parasitoids and hosts [34].

5. CONCLUSION

Functional response studies are essential to estimate the ability of the natural enemy to parasitize different host densities. In this study, we were able to estimate the type of functional response that the parasitoids *T. pretiosum* and *T. galloi* exhibited when parasitizing the *D. fovealis* eggs. Besides, assessing the search efficiency and handling time the results were considered potentially useful in identifying these parasitoids as a biological control agent. The findings given here offer a good starting point for enhancing the control of *D. fovealis* with *Trichogramma* in the field.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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