## Scientific Notes

# Functional response of *Trichogramma pretiosum* on *Trichoplusia ni* eggs at different temperatures and egg densities

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Abstract – The objective of this work was to determine the functional response of the parasitoid *Trichogramma pretiosum* on *Trichoplusia ni* eggs at different temperatures (20, 25, and 30ºC) and egg densities (5, 10, 15, 20, 25, and 30 eggs). The logistic regression showed a type-II functional response for all temperatures. The search efficiency of *T. pretiosum* was reported as 0.049±0.0019, 0.069±0.0042 and 0.068±0.0033 per hour, and the estimated handling times were 1.82±0.0424, 1.69±0.0398, and 1.54±0.0498 hour at 20, 25 and 30ºC, respectively. Females of *Trichogramma pretiosum* show greater efficiency at 30ºC and a type-II functional response. The parasitism rate decreases, when host density increases.

Index terms: behavioral response, biological control, egg parasitoid, Noctuidae.

# Resposta funcional de *Trichogramma pretiosum* sobre ovos de *Trichoplusia ni* a diferentes temperaturas e densidades de ovos

Resumo – O objetivo deste trabalho foi determinar a resposta funcional do parasitoide *Trichogramma pretiosum* sobre ovos de *Trichoplusia ni* a diferentes temperaturas (20, 25 e 30ºC) e densidades de ovos (5, 10, 15, 20, 25 e 30 ovos). A regressão logística revelou resposta funcional tipo II para todas as temperaturas. Os valores de eficiência de busca de *Trichogramma pretiosum* foram de 0.049±0.0019, 0.069±0.0042 e 0.068±0.0033 $h^{-1}$, e os tempos de manipulação estimados foram de 1.82±0.0424, 1.69±0.0398 e 1.54±0.0498 h a 20, 25 e 30ºC, respectivamente. Fêmeas de *Trichogramma pretiosum* apresentam mais eficiência a 30ºC e resposta funcional do tipo II. Ocorre decréscimo da taxa de parasitismo, quando a densidade do hospedeiro aumenta.

Termos para indexação: resposta comportamental, controle biológico, parasitoide de ovos, Noctuidae.

The cabbage looper [*Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae)] is one of the major pests of cruciferous crops, such as cabbage, cauliflower and broccoli. It can also infest host plants as cotton, soybean, tomato, and potato, which are of economic importance (Capinera, 2014). An efficient alternative of biological control is to release *Trichogramma* parasitoids (Hymenoptera: Trichogrammatidae) against *Trichoplusia ni* (Carvalho et al., 2014). These parasitoids are one of the most important biotic agents, and are widely distributed around the world parasitizing eggs of more than 200 species of insects (Jalali, 2013).

For the successful release in the field, it is essential to know the required proportion of parasitoids for the number of host eggs present in the agroecosystem under consideration. The parasitoid efficiency can be reduced by intraspecific competition because when the parasitoid density intensifies, the probability of an individual to locate an unparasitized egg decreases; such a situation will result in superparasitism (Chen et al., 2016). Therefore, the functional response emerges as a very significant factor to assess the efficiency of a natural enemy, which depends on the balance between host density and efficiency of parasitoid search.

The functional response is significantly affected by various factors such as host, age, and exposure time of the host to the parasitoid (Nikbin et al., 2014). Temperature exerts a great influence on parasitoid development and behavior (Carvalho et al., 2014), as

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well as on the functional response of these natural enemies (Nikbin et al., 2014). Studies that had determined the required proportion of *Trichoplusia ni* eggs parasitized by *Trichogramma pretiosum* (Riley, 1879) females, at different temperatures, could offer useful data on the population dynamics, which are relevant to the enforcement of biological control programs.

Therefore, to further improve our knowledge regarding the effect of temperature on the parasitoid-host interactions, the objective of this work was to determine the type of functional response *Trichogramma pretiosum* displays to various *Trichoplusia ni* egg densities at different temperatures.

Both *Trichoplusia ni* and *Trichograma pretiosum* specimens were purchased from the Núcleo de Desenvolvimento Científico e Tecnológico em Manejo Fitossanitário de Pragas (Nudemafi), of the Centro de Ciências Agrárias e Engenharias of Universidade Federal do Espírito Santo, in the municipality of Alegre, in the state of Espírito Santo, Brazil. The host *Trichoplusia ni* was reared using the methodology of Altoé et al. (2012). To rear *Trichogramma pretiosum*, the alternative host *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) was used, according to the methodology adopted by Stein & Parra (1987).

To study the functional response of *Trichogramma pretiosum,* different *Trichoplusia ni* egg densities (5, 10, 15, 20, 25, and 30 eggs) were used. Host eggs up to 24 hours of age were fixed on light-blue paper (2.5x0.5 cm) with 0.5% gum arabic (m $v^{1}$), and moistened with distilled water using a brush no. 000. The carton with *Trichoplusia ni* eggs was put into a glass tube (8.5x2.5 cm). A freshly emerged *Trichogramma pretiosum* female (age < 6 hours) was introduced into each tube, and fed on the honey droplets introduced in the tube. After sealing with PVC plastic film, the tubes were placed in climatic chambers. The temperatures were adjusted to 20, 25, and 30ºC, with 70±10% relative humidity, and 14-hour photoperiod. For each temperature, 15 replicates were performed for each host density. Twenty-four hours later, the parasitoids were removed from the tube. The paperboard with the parasitized eggs was maintained undisturbed in the tube until the offspring emerged. Newly hatched caterpillars of the nonparasitized eggs were removed daily. The number of parasitized eggs was carefully noted.

The experimental design was completely randomized with 15 replicates. The data were subjected to the analysis of variance, to verify the effects of temperatures, host densities, and of the interaction between these factors on the number of eggs parasitized by *Trichogramma pretiosum*. The number of parasitized eggs was used to analyze the functional response. These data were used to verify the functional response through the function frair_test() of the frair package (Pritchard et al., 2016) via R software (R Core Team, 2009). Following this procedure, the data were verified to show the type II functional response. Thus, the type II functional response models, suggested by Holling (1959) and Rogers (1972), respectively, were tested:

$$N_a = \frac{a \times N_0 \times P \times T_t}{1 + a \times T_h \times N_0} \text{ and}$$

$$N_a = N_0\left[1 - \exp\left(-\frac{a \times P \times T_t}{1 + a \times T_h \times N_0}\right)\right],$$

in which $N_a$ is the number of parasitized eggs; $N_0$ is the host egg density; a is the search efficiency; P is the number of parasitoids (p=1); $T_t$ is the experiment duration (24 hours); and $T_h$ is the handling time.

The comparison of the models was done employing the Akaike's information criterion (AIC).

After the model was selected, a comparison between the parameters a and $T_h$ was done and, based on the methodology of Juliano (2001), it was adapted to the Holling's model:

$$N_a = \frac{\left[a + D_a \times (j)\right] \times P \times N_0 \times T_t}{1 + \left\{\left[a + D_a \times (j)\right] \times \left[T_h + D_{Th} \times (j)\right]\right\} \times N_0},$$

in which Da is the difference between the values of a the temperatures i and j; and $D_{Th}$ is the difference between the values of $T_h$ the temperatures i and j.

To determine the coefficients of the search efficiency and handling time, and their respective differences for the temperatures, a nonlinear least square regression was used, adopting the nls() function with the R software.

In the analysis for the effects of temperature and host density on *Trichogramma pretiosum* parasitism, the interaction was shown to be significant ($p<0.05$). In the testing of the functional response models, a high coefficient of determination was observed ($R^2$ >80%). Both models (Holling's and Roger's) fitted to the data, although the analysis of AIC indicated that the Holling's model was the most adequate for the three temperatures (T20˚C = 310.3 and 126.1, T25˚C = 364.7 and 181.3, and T30˚C = 431.2 and 240.0, for Roger's and Holling's model, respectively).

The temperature significantly influences the functional response of *Trichogramma pretiosum* parasitizing *Trichoplusia ni* eggs. At all temperatures assessed, the typical type II functional response curve was noted (Holling, 1959), with the characteristic monotonic decrease in the rate of parasitism, when the host density increased (Figure 1). Trichogrammatides may exhibit all three functional response types, with type II and type III occurring more frequently (Wang & Ferro, 1998; Kalyebi et al., 2005; Moezipour et al., 2008; Nikbin et al., 2014).

Interestingly, the functional response type of the parasitoid can vary according to density, lineage, or age of the parasitoid, and the host, as well as with environmental changes, most often because of the influence of temperature (Wang & Ferro, 1998; Moezipour et al., 2008; Nikbin et al., 2014). In the present study, *Trichogramma pretiosum* displayed the type II functional response at all tested temperatures. Similarly, the response types (all type I) of six trichogrammatide species on *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) was not influenced by temperature (Kalyebi et al., 2005). However, no behavioral pattern appears to emerge for the *Trichogramma* species exposed to different temperatures. *Trichogramma ostriniae* (Pang & Chen) exhibited functional response type II at the lower temperatures (17 and 20°C), and the type III, at higher temperatures (27°C) (Wang & Ferro, 1998). However, *Trichogramma brassicae* (Bezdenko) expressed type II functional response at 25°C, and type III, at 20 and 30°C temperatures (Moezipour et al., 2008). These facts encouraged the authors to suggest that temperature affects the parasitoid foraging behavior, influencing the learning process in distinguishing between parasitized and nonparasitized eggs.

The parameters of search efficiency (a) and manipulation time ($T_h$) were both significant ($p<0.001$), implying that the relationship between the search efficiency and the initial number of hosts was gradually rising at different temperatures (Table 1). The maximum search efficiency (0.069±0.0042 $h^{-1}$) was recorded at 25°C, followed by the temperatures of 30ºC (0.068±0.0033 $h^{-1}$) and 20°C (0.049±0.0019 $h^{-1}$) (Table 1 A). However, the search efficiency showed no significant difference between 25 and 30°C (p=0.904) (Table 1 B). Handling time was seen to drop as the temperature rose, ranging from 1.82±0.0424 h at

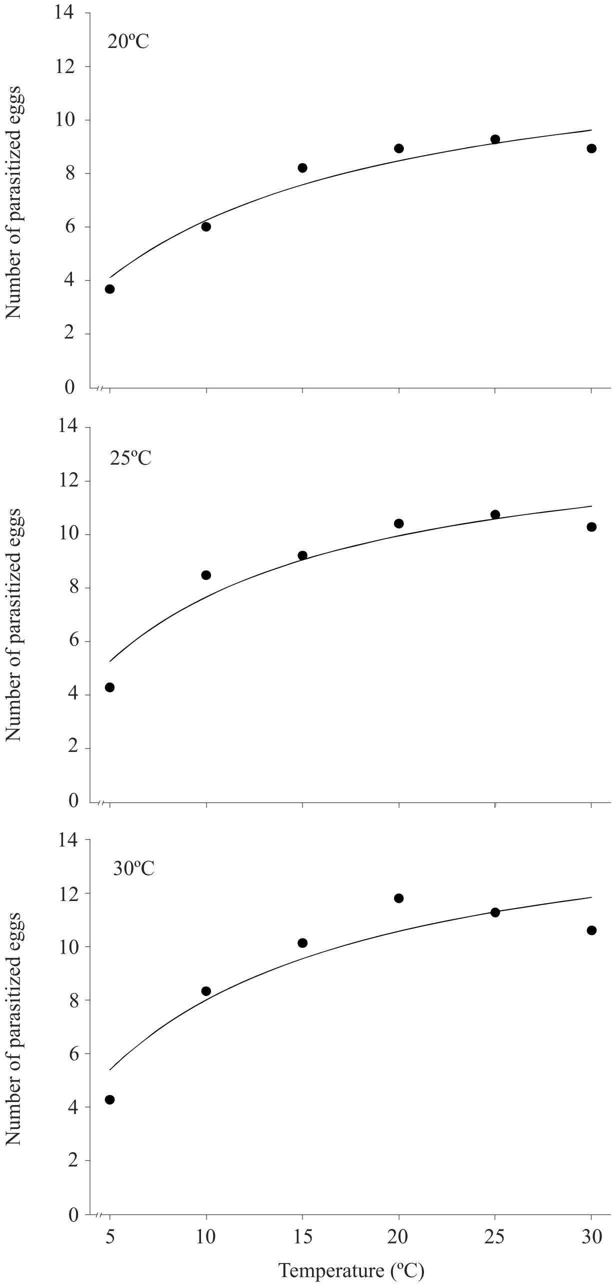

**Figure 1.** Type II functional response of *Trichogramma pretiosum* to *Trichoplusia ni* egg density, at three temperatures.

20°C to 1.54±0.0498 h at 30°C (Table 1 A), showing significant difference ($p<0.0001$) (Table 1 B). Although the type II functional response was observed at all tested temperatures, the findings of the present study suggest that *Trichogramma pretiosum* parasitizes *Trichoplusia ni* eggs at the optimal temperature of 30°C, depending on the briefer handling time ($T_h$) and the higher search efficiency (a) of the parasitoid, similarly to other trichogrammatides, which also shows the optimal temperature to be 30°C (Kalyebi et al., 2005; Moezipour et al., 2008). In the present study, although the *Trichogramma pretiosum* search efficiency at 25 and 30°C was statistically identical, the handling time was less at 30°C. A similar result was reported for *Trichogramma brassicae* on eggs of *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) (Moezipour et al., 2008). One of the characteristics supporting trichogrammatide selection as an agent of biological control is the handling time, or the time that a parasitoid requires to chase, subjugate, oviposit, and rediscover a host (Holling, 1959).

To determine the ability of the natural enemy to parasitize various host densities, comparative studies have been performed as to the parasitoid strains/species (Kalyebi et al., 2005), the most suitable age of attack (Nikbin et al., 2014), and the favorable environmental conditions (Kalyebi et al., 2005; Moezipour et al., 2008), in order to find a candidate, natural enemy. From among the three types of functional responses, the parasitoids showing type III response are assumed to have better opportunities for regulation of their populations (Holling, 1959). *Trichogramma pretiosum* displayed type II response, observed by the increased attack rate which gradually plateaued, a condition most frequently determined by the limitation of the parasitoid eggs. The endocrine system controls oviposition, and the refusal to oviposit can be regarded as a specific state not under the control of oogenesis, in such a manner that the stability of the parasitism is dependent on neurohormonal regulation (Reznik et al., 2003).

The results were able to establish a clear relationship between temperature and the functional response of *Trichogramma pretiosum* eggs. This parasitoid females have a greater efficiency at 30ºC, and show type II functional response. The rate of parasitism decreases, when the host density increase.

The present study can facilitate the optimization of laboratory conditions for the insect rearing, and present a starting point to enhance the control of *Trichoplusia ni* in the field. Besides the laboratory studies, a greater attention should be focused on the semi-field and field research in host cultures of *Trichoplusia ni*, in order to obtain results closer to field conditions.

**Table 1.** Parameters estimated by the Holling's model (A), and test of differences of functional response parameters (B) of *Trichogramma pretiosum* to *Trichoplusia ni* egg density at three temperatures.

| Temperature (°C) | Parameter(1) | Estimate(2) | SE | 95% CI | | t-value | p-value |
|---|---|---|---|---|---|---|---|
| | | | | Lower | Upper | | |
| | | | | Estimated parameters (A) | | | |
| 20 | a | 0.04947 | 0.0019 | 0.0457 | 0.0532 | - | - |
| | $T_h$ | 1.82112 | 0.0424 | 1.7379 | 1.9043 | - | - |
| 25 | a | 0.06928 | 0.0033 | 0.0627 | 0.0758 | - | - |
| | $T_h$ | 1.69104 | 0.0398 | 1.6129 | 1.7691 | - | - |
| 30 | a | 0.06862 | 0.0042 | 0.0603 | 0.0769 | - | - |
| | $T_h$ | 1.54093 | 0.0498 | 1.4433 | 1.6385 | - | - |
| | | | Test of differences of the functional response parameters (B) | | | | |
| 20 and 25 | $D_a$ | 0.0198 | 0.0037 | 0.0125 | 0.0271 | 5.290 | < 0.0001 |
| | $D_{Th}$ | -0.1301 | 0.0615 | -0.2507 | -0.0094 | -2.114 | 0.0359 |
| 20 and 30 | $D_a$ | 0.0191 | 0.0044 | 0.0104 | 0.0278 | 4.297 | < 0.0001 |
| | $D_{Th}$ | -0.2802 | 0.0754 | -0.4279 | -0.1325 | -3.717 | 0.0003 |
| 25 and 30 | $D_a$ | -0.0006 | 0.0055 | -0.0114 | 0.0101 | -0.120 | 0.9044 |
| | $D_{Th}$ | -0.1501 | 0.0648 | -0.2772 | -0.0230 | -2.315 | 0.0217 |

(1)Parameters: a, search efficiency ($h^{-1}$); $T_h$, handling time (h); $D_a$, indicator variable that estimates the differences between temperature in the parameter value a; $D_{Th}$, indicator variable that estimates the differences between temperature in the parameter value $T_h$. (2)Significant by the t-test, at 1% probability .

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