

INFLUÊNCIA DA IDADE DE OVOS DE *TRICHOPLUSIA NI* (LEPIDOPTERA: NOCTUIDAE) EM FÊMEAS DE *TRICHOGRAMMA PRETIOSUM* (HYMENOPTERA: TRICHOGRAMMATIDAE) EM DIFERENTES TEMPERATURAS

INFLUENCE OF THE AGE OF *TRICHOPLUSIA NI* (LEPIDOPTERA: NOCTUIDAE) EGGS IN *TRICHOGRAMMA PRETIOSUM* FEMALES (HYMENOPTERA: TRICHOGRAMMATIDAE) AT DIFFERENT TEMPERATURES

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Resumo

Objetivamos avaliar a influência da idade dos ovos de *Trichoplusia ni* (Lepidoptera: Noctuidae) em fêmeas de *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae), em diferentes temperaturas. Assim, ovos de *T. ni* com idade ≤ 12 , ≤ 24 , ≤ 36 , ≤ 48 , ≤ 60 e ≤ 72 horas de desenvolvimento embrionário, separados a temperaturas de 20, 25 e 30 °C, foram oferecidos para fêmeas de *T. pretiosum* com até 24 horas de idade. O parasitismo foi inversamente proporcional ao desenvolvimento embrionário do ovo, com maiores taxas de parasitismo observadas para ovos com até 24 horas de desenvolvimento embrionário nas três temperaturas. A viabilidade do parasitismo foi influenciada pela idade dos ovos. Os ovos, com até 36 horas de idade, apresentaram viabilidade superior a 85% nas três temperaturas. A proporção sexual a 25°C apresentou a melhor taxa dentro da faixa de desenvolvimento embrionário. O número de descendentes do parasitoide por ovo foi influenciado pela temperatura e pela idade dos ovos, sendo a combinação ovos com 60-72 horas à temperatura de 30°C, a que apresentou o maior quantitativo de descendentes parasitoides por ovo. Esses resultados indicaram que a idade do hospedeiro e a temperatura ambiente podem alterar as características biológicas dos parasitoides.

Palavras-chave: Controle biológico. Brassicas. Parasitoide de ovos. Desenvolvimento embrionário.

Abstract

The aim to evaluate the influence of *Trichoplusia ni* (Lepidoptera: Noctuidae) eggs age on females of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae), at different temperatures. Thus, *T. ni* eggs

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aged ≤ 12 , ≤ 24 , ≤ 36 , ≤ 48 , ≤ 60 and ≤ 72 hours of embryonic development, separated at temperatures of 20, 25 and 30 °C, were offered to *T. pretiosum* females up to 24 hours old. The parasitism was inversely proportional to the embryonic development of the egg, with higher rates of parasitism observed for eggs with up to 24 hours of embryonic development at all three temperatures. The viability of parasitism was influenced by the eggs age. The eggs, with up to 36 hours old, presented viability over 85% in all three temperatures. The sex ratio at 25 °C showed the best rate within the range of embryonic development. The number of descendants of the parasitoid per egg was influenced by the temperature and age of the eggs, with the combination of eggs aged 60-72 hours at a temperature of 30°C, which presented the highest number of descendants of parasitoid per egg. These results indicated that the age of the host and the ambient temperature can change the biological characteristics of the parasitoids.

Keywords: Biological control. Brassicas. Eggs parasitoid. Embryonic development.

1. INTRODUCTION

The *Trichoplusia ni* (Lepidoptera: Noctuidae) is a generalist pest that mainly attacks the brassicas. Brassicas are vegetables of great consumption and economic importance in the Brazilian market, being consumed mainly in natura or minimally processed. However, the incidence of pests in crops can drastically reduce production (CARDOSO et al., 2018). The caterpillars in the final instance can consume three times its weight in a day in vegetal material, causing drastic defoliation (GALLO et al., 2002). The ability that the *T. ni* has to use a variety of hosts simultaneously and in succession is fundamental for its presence in the field. The selection of pesticide resistant populations makes this pest a great problem in the brassica producing regions (CASTELLS, 2008).

Trichogramma is a parasitoid wasp used in biological pest control and has been studied and used in biological control programs due to its effectiveness in parasitizing eggs of different pests, wide geographic distribution, ease of mass development of eggs from alternative hosts, lower cost of production, allowing for its use in inundation liberations (PARRA; ZUCCHI, 2004). In Brazil, studies with *Trichogramma* have been conducted for the control of many Lepidoptera species, as *Plutella xylostella* (Plutellidae) in Brassicaceae; *Spodoptera frugiperda* (Noctuidae) in corn; *Diaphania hyalinata* (Pyralidae) in cucurbits; *Ecdytolopha aurantiana* (Tortricidae) in *Citrus*; and *Tuta absoluta* (Gelechiidae) in tomato (MELO et al., 2007; PRATISSOLI et al., 2008; PRATISSOLI, 2015).

The *Trichogramma* was found in many other pests and agroecosystems, highlighting its potential to be used commercially, which has been explored in other countries (PARRA; ZUCCHI, 2004, COSTA et al., 2011). When well implemented, the biological control with the use of parasitoid of the *Trichogramma* genus can be an alternative to obtain more satisfactory and long-term results in face of the chemical control recommendations. The high number of studies, that cite the natural enemies complex in the different brassica production regions, show the importance of these insects for maintenance of low *T. ni* populations to avoid economic damage (PARRA; ZUCCHI, 2004).

Some factors can be responsible for the success or failure in the use of *Trichogramma* in the control of pests, such as knowledge of biological parameters when associated to certain target hosts and the embryonic phase of its eggs (PRATISSOLI et al., 2007; PASTORI et al., 2010; POLTRONIERI et al., 2014). The biological aspects of *Trichogramma pretiosum* have been researched in different hosts and at different temperatures (PRATISSOLI et al., 2007; ZAGO et al., 2007; POLTRONIERI et al., 2014). However, studies about this parasitoid's biological aspects in *T. ni* eggs are scarce.



The study of biological characteristics of *T. pretiosum*, in function of the age of eggs of the host and the temperature, may provide relevant information of this parasitoid for implementing of phytosanitary programs for *T. ni*. Therefore, in this study, aimed to obtain basic information about biological aspects of *T. pretiosum* raised in *T. ni* eggs of different ages and at three temperatures.

2. MATERIAL AND METHODS

The experiment was conducted in the *Núcleo de Desenvolvimento Científico e Tecnológico em Manejo Fitossanitário* (NUDEMAFI), *Centro de Ciências Agrárias e Engenharias* (CCAEE), *Universidade Federal do Espírito Santo* (UFES) in climatized chambers.

2.1 Maintenance of *Trichoplusia ni*

The adults of *T. ni* were kept in a cage (60 x 50 x 50 cm) and a 10% solution of honey was offered every 48 hours in 20 mL containers with a cotton ball in contact with the solution. The collard (*Brassica* sp.) green leaves petioles were submerged in water, in 300 mL containers, for oviposition of females. Every morning, the collard greens were substituted for new ones and those with eggs were kept in plastic containers. The newly hatched caterpillars were transferred from the leaves to a modified artificial diet proposed by Greene et al. (1976) with a bean, beer yeast, and wheat germ base, kept in glass tubes (8.0 x 2.5cm) with up to three caterpillars until pupae phase.

2.2 Maintenance of alternative host *Anagasta kuehniella* (Lepidoptera: Pyralidae)

The maintenance of *Anagasta kuehniella* consisted in the use of plastic boxes (30 x 25 x 10 cm), where a diet based on whole wheat flour (60%), corn meal (37%) and beer yeast (3%) were given after being homogenized. About 0.3 grams of eggs were distributed in the diet which was used as substrate for the larvae. After the emergence of adults, a daily process was started for collection using an adapted vacuum. The moths collected were transferred into plastic containers with nylon screens, folded in zig-zag, where oviposition occurred. The upper extremity of the cage was closed with phylum cloth.

The eggs were collected daily for a period of 5 days. They were kept and conserved in refrigerators at a temperature of 4 ± 1 °C for a maximum period of 20 days.

2.3 Maintenance of *Trichogramma pretiosum*

The alternative host eggs were collated in rectangular sky-blue cardboard (8.0 x 2.0 cm) with arabic gum diluted at 30% and posteriorly unviable by exposure to germicidal light for a period of 45 minutes. After unviability, the eggs were offered to females of *T. pretiosum* in glass tubes and kept in climatized chambers with temperatures regulates at 25 ± 1 °C, $70 \pm 10\%$ relative humidity (RH) and photophase of 14 hours (L:D).

2.4 Conduction of the experiment

Eggs of *T. ni* were taken from breeding in laboratory with ages ≤ 12 , ≤ 24 , ≤ 36 , ≤ 48 , ≤ 60 and ≤ 72 hours, being collated in rectangular sky-blue cardboard (2.5 x 0.5 cm) with help from a humid brush and isolated in glass tubes (8.5 x 2.5 cm), and separated in temperatures of 20, 25 and 30 °C. Twenty eggs of *T. ni* were used for each combination of age and temperature, respectively,



being considered as a repetition of the combination of treatments (egg age x temperature). A newly emerged female of *T. pretiosum* was added to each tube for the formation of treatments. As food supply, the females received a drop of honey deposited in the internal wall of the tube. After 24 hours of parasitism, the parasitoids were removed. The cards containing parasitized eggs were kept in glass tubes of 8.5 x 2.5 cm sealed in PVC plastic in the conditions of temperature of the parasitism, humidity, and photophase as previously mentioned. The newly hatched unparasitized caterpillars were removed in order to avoid affecting the development of parasitized eggs.

After decease of parasitoids, the number of eggs, eggs with orifice, number of males and females were evaluated. The number of parasitized eggs and viability were expressed in percentages. The total number of parasitoids was divided by the number of eggs with orifice to determine number of parasitoids per egg. The sexual rate was determined by the number of females to the total number of individuals in the population.

The experiment was conducted in a factorial 6 x 3 (six levels of host age and three levels of temperature) with 15 repetitions in a totally randomized design. Each repetition was represented by a card containing 20 eggs of *T. ni*. The data were analyzed statistically at a 5% error, and significant values were used in regression analysis which adopted a response surface method from linear and quadratic models with two independent variables, adjusted to the data based on the coefficient of determination (R^2) and significance of regression through the *F* test (up to 5% probability), using SIGMAPLOT 2001 software.

3. RESULTS AND DISCUSSION

The percentage of parasitism, viability, sexual rate, and number of individuals of *T. pretiosum* per *T. ni* egg were influenced by the combinations in age and temperature, as can be observed in Figures 1, 2, 3, and 4, where the regression equations were established, as well as the respective response surfaces.

3.1 Parasitism

In the linear response surface, the percentage of parasitism at the three temperatures decreased according to the increase of host age (Fig. 1). The *T. pretiosum* obtained the best responses for parasitism when exposed to eggs with embryonic development of up to 24 hours, with observed rates of 57% [n = 270 (5,400 eggs)] at the three temperatures. In eggs with development above 48 hours, it was found a sharp decrease in parasitism and those with 72 hours of age presented averages lower than 20% at the three temperatures.



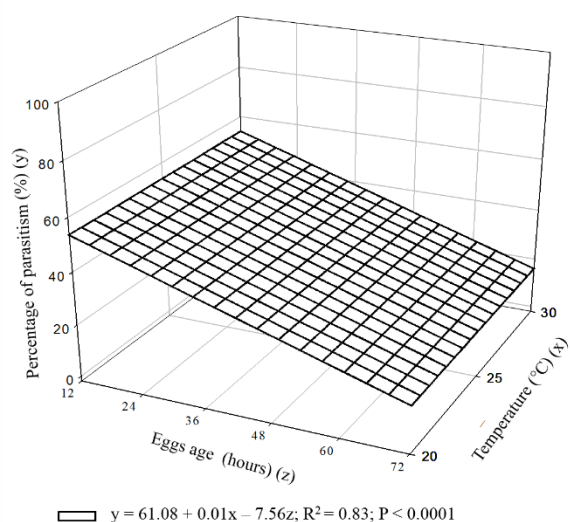


Figure 1. *Trichogramma pretiosum*'s percentage of parasitism in function of *Trichoplusia ni* eggs age and temperature at 70±10% relative humidity and photophase of 14 hours (L:D).

Trougakos and Margaritis (2002) and Boivin (2010) highlight that depending on the host, embryonic development causes physical and chemical changes over time that can influence host acceptance by the parasitoid *T. pretiosum* and, consequently, cause a reduction in the rate of parasitism in eggs with advanced development age, as observed in the present study. The temperature is another factor that may influence the performance of the parasitoid indirectly, once low temperatures such as 20°C induce to slower development of the egg and, therefore, the acceptance by the parasitoid in advanced ages is higher at higher temperatures such as 25°C a 27°C (BOIVIN, 2010; VENKATESAN; JALALI, 2013). The acceptance at this condition would be due to faster embryonic development and would alter physical states such as hardening of corion, and chemical such as change in nutritional value of the egg. The host factor was reported as a major inference on the development of parasitoid (BOIVIN, 2010; VENKATESAN; JALALI, 2013).

The authors Pastori et al. (2010) evaluated the behavior of parasitism of *T. pretiosum* in *Bonagota salubricola* (Lepidoptera: Tortricidae) eggs and verified that the occurrence of interaction between the factors age of parasitoid and age of host eggs, where the parasitoid showed preference for eggs in different embryonic and development stages. The 24-48 hours were the ones with the highest rates of parasitism.

The non-acceptance of the parasitoid to a particular host is sometimes not exclusive to a single species, or strain, as in the case of *T. pretiosum* and *T. exiguum* in eggs of *Mocis latipes* (Lepidoptera: Noctuidae), in which both species presented reduced rates of parasitism (STINGUEL et al., 2013; ZUIM et al., 2013) and Milanez et al. (2009) for several *Trichogramma* species and strains on *T. ni*. According to age of the host egg, change in its physical and chemical characteristics is the factor that has been more influential in the development of the parasitoid, as it was demonstrated in this study. The authors Vargas et al. (2017) observed similar results. However, Polanczyk et al. (2007) found that *T. exiguum* presented a better rate of parasitism when eggs of crucifer moths had 72 hours in embryonic development.

3.2 Viability

In the quadratic responses, the viability at the three temperatures decreased according to the increase of host age (Fig. 2). The *T. pretiosum* showed better averages in viability when exposed to eggs with embryonic development of up to 36 hours, obtaining a percentage of emergence of descendants over 86.96% [n = 270 (3,780 parasitized eggs)]. The lower viability rates were found in eggs with over 48 h in embryonic development, with viability between 70.87% (25 °C) and 65.88% (20 °C), respectively.

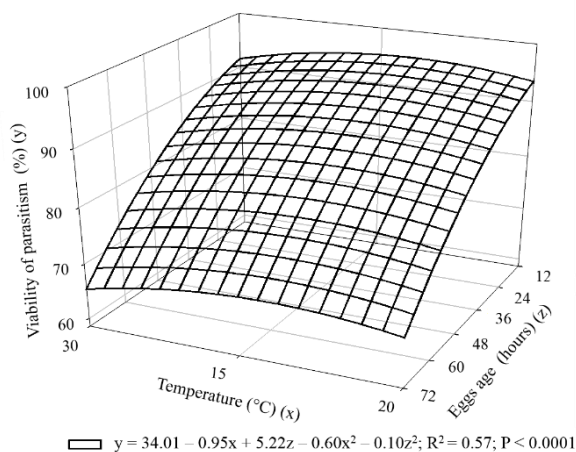


Figure 2. *Trichogramma pretiosum*'s viability of parasitism in function of *Trichoplusia ni* eggs age and temperature at 70±10% relative humidity and photophase of 14 hours (L:D).

The results showed that, with embryonic development, there are modification in the nutritional content of eggs, which negatively influences the viability of parasitism in older eggs, as highlighted by Trougakos and Margaritis (2002) and Boivin (2010). Other researchers such as Navarro and Marcano (1999) and Pratisoli et al. (2007) obtained the same results when working with *Helicoverpa zea* and *P. xylostella*. However, it is not consistent, because alterations in physical-chemical characteristics that occur during the embryonic development of the egg, for each host, may or not interfere in the viability of parasitoids in the *Trichogramma* genus. This fact can be diagnosed in studies by Zuim et al. (2013) and Stinguel et al. (2013) when working with *M. latipes* as a host, in which the egg age did not influence on viability of parasitoids *T. exiguum* and *T. pretiosum*.

3.3 Sexual ratio

In the linear response surface, the sexual ratio at the three temperatures decreased according to increased host age (Fig. 3). The eggs with younger age at 20°C temperature had the highest average, close to 0.8 [n = 270 (2,676 viable parasitized eggs)]. The lower values for sexual ratio were found in more advanced phases of embryonic development (60-72 hours) with an average of 0.6 for temperatures at 25 and 30°C.

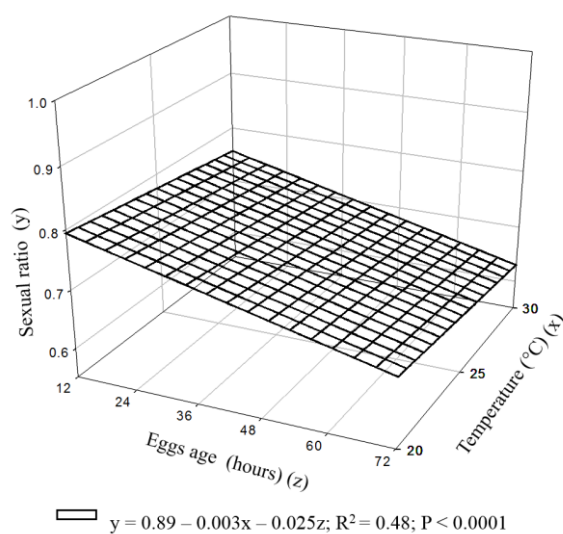


Figure 3. *Trichogramma pretiosum*'s sexual ratio in function of *Trichoplusia ni* eggs age and temperature at 70±10% relative humidity and photophase of 14 hours (L:D).

The sexual ratio were not directly associated with egg age, but to strain and/or species of *Trichogramma* and physical-chemical characteristics of the host. This correlation can be found in studies by Stinguel et al. (2013) between 0.82 and 1, Zuim et al. (2013) between 0.57 and 0.91, where sexual ratio in *M. latipes* eggs presented a ratio varying from 0.82 to 1 when the parasitoid was *T. pretiosum*, and 0.57 and 0.91 for *T. exiguum*. However, Vargas et al. (2017) used *T. pretiosum* in *S. frugiperda* host and the rates varied from 0.46 to 0.50.

The sexual ratio presented in the study are superior to 0.5. Therefore, it was considered satisfactory for pest control, since rates equal to 1 would mean absence of males in the population, which is not desirable, since reproduction would not be viable in this population. Thus, the presence of males in a population is desired so there is sexed reproduction, genetic viability and improved conditions for the maintenance of the population in the field.

3.4 Individual per Egg

The number of individuals per egg had an interaction between the age of the eggs and the three temperatures, similarly to the increasing age of the host, the number of individuals per egg increased in a quadratic response surface (Fig. 4). The parasitized eggs by *T. pretiosum* with 12 hours had no statistical differences at the tested temperatures, with approximately 1.7 individuals per egg [n = 270 (2,676 viable parasitized eggs)]. However, in the embryonic development phases of 48-60 and 60-72 hours at 30 °C, the numbers of individuals per egg (2.20 and 2.40, respectively) were statistically higher than at other temperatures (20 and 25 °C).

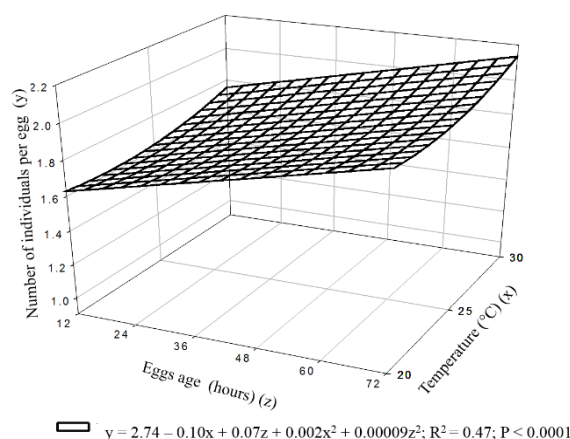


Figure 4. *Trichogramma pretiosum*'s number of individuals per egg in function of *Trichoplusia ni* egg age and temperature at 70±10% relative humidity and photophase of 14 hours (L:D).

The *T. pretiosum* exposed to eggs with over 48 hours of embryonic development tends to allocate a higher number of descendants in the same host, which can lead to super parasitism and loss in quality of descendants, evidenced by the lower viability rates in this embryonic development stage, but that warrants the survival of the species under adverse conditions. The *T. ni* eggs have enough capacity for the development superior to 1 individual per egg, since no deformed descendants were found, even in upper emergence eggs with over 2 individuals per egg. A similar result was found by Oliveira et al. (2003), where average emergence of 3.61 and 2.93 individuals of *T. maxacalii* in *Oxydia vesulia* (Cramer) (Lepidoptera: Geometridae) eggs were observed at 1 and 2 days old, respectively. Pratisoli (1999) studied *T. pretiosum* in *H. zea* eggs at 1, 2, 3, and 4 days old, in which was observed higher number of individuals in eggs at 1 and 2 development days with a value of 1.19 and 1.28, respectively.

The embryonic development of *T. ni* eggs interfered in the biological characteristics of *T. pretiosum*, with temperature as a highly influential factor in this relation. The greater parasitism, viability, and sexual ratio of *T. pretiosum* were for *T. ni* eggs with up to 24 hours of embryonic development. Therefore, in case of using the *T. pretiosum* egg parasitoid in *T. ni* integrated management programs, it is necessary to release the parasitoid as soon as the presence of pest eggs is detected in the field, seeking to maximize the efficiency of the agent biological.

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