

## ***Telenomus remus* (Hymenoptera: Platygasteridae) parasitism on *Spodoptera frugiperda* (Lepidoptera: Noctuidae) eggs: different parasitoid and host egg ages**

### **Parasitismo de *Telenomus remus* (Hymenoptera: Platygasteridae) em ovos de *Spodoptera frugiperda* (Lepidoptera: Noctuidae): diferentes idades do parasitoide e do ovo hospedeiro**

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#### **Abstract**

Release of egg parasitoids for biological control of pests is a promising technique in integrated pest management (IPM). However, there is a lack of information on the performance of parasitoid females of different ages, and specifically on the behavior of the parasitoid *Telenomus remus* towards pest eggs at different stages of embryonic development. Thus, the relationships between host age, parasitoid age, and parasitism by *T. remus* on *Spodoptera frugiperda* eggs were evaluated. Three separate bioassays were performed, each in a completely randomized design. In the first bioassay, *T. remus* females grouped by age in days (ranging from 1 to 10 days old) were offered 100 ± 20 eggs of *S. frugiperda* for 24 hours. In the second bioassay, 100 ± 20 eggs of *S. frugiperda* (24, 48 or 72 hours old) were offered to females of *T. remus* for 24 hours. In the third bioassay, 24, 48- and 72-hour-old host eggs of *S. frugiperda* were offered to *T. remus* females in a choice test. The variables evaluated were: number of parasitized eggs, parasitoid emergence (%), and sex ratio of progeny in bioassays 1 and 2, and the number of eggs parasitized in bioassay 3. The age of *T. remus* females did not affect the number of *S. frugiperda* eggs parasitized or emergence of the progeny. However, the sex ratio was more male-biased in the progeny of 1- and 2-day-old females compared to older wasps. In bioassay 2, the highest parasitism was observed in 24- and 48-hour-old eggs. Percentage emergence and sex ratios were not influenced by the ages of the eggs tested. *Telenomus remus* preferred to parasitize 24-hour-old eggs in bioassays 3. Overall, the age of *T. remus* females tested did not affect the parasitism of *S. frugiperda* eggs, but the number of eggs parasitized decreased with increasing host age.

**Key words:** Fall armyworm. Biological control. Egg parasitoid. Embryonic development.

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## Resumo

A liberação de parasitoides de ovos para o controle biológico de pragas é uma técnica promissora no manejo integrado de pragas (IPM). Entretanto, há uma carência de informações sobre o desempenho de fêmeas do parasitoide em suas diferentes idades assim como do comportamento e parasitismo de *Telenomus remus* em ovos da praga em diferentes estádios de desenvolvimento embrionário. Avaliou-se a relação entre idade do hospedeiro, idade do parasitoide e parasitismo de *T. remus* em ovos de *Spodoptera frugiperda*. Três bioensaios separados foram realizados, cada um em um delineamento inteiramente casualizado. No primeiro bioensaio, as fêmeas de *T. remus* agrupadas por idade em dias (variando de 1 a 10 dias de idade) foram oferecidas 100 ± 20 ovos de *S. frugiperda* por 24 horas. No segundo bioensaio, 100 ± 20 ovos de *S. frugiperda* (24, 48 ou 72 horas de idade) foram oferecidos às fêmeas de *T. remus* por 24 horas. No terceiro bioensaio, ovos hospedeiros de 24, 48 e 72 horas de idade de *S. frugiperda* foram oferecidos para fêmeas de *T. remus* em teste de escolha. As variáveis avaliadas foram: número de ovos parasitados, emergência do parasitoide (%) e razão sexual da progênie nos bioensaios 1 e 2 e o número de ovos parasitados no bioensaio 3. A idade das fêmeas de *T. remus* não afetou o número de ovos de *S. frugiperda* parasitados ou a emergência da progênie. No entanto, a razão sexual foi mais tendenciosa no sexo masculino na progênie de fêmeas de 1 e 2 dias de idade em comparação com as vespas mais velhas. In bioassay 2, the highest parasitism was observed in 24- and 48-hour-old eggs. A porcentagem de emergência e a razão sexual não foram influenciadas pela idade dos ovos testados. *Telenomus remus* preferiu parasitar ovos de 24 horas no bioensaios 3. No geral, a idade das fêmeas de *T. remus* testadas não afetou o parasitismo dos ovos de *S. frugiperda*, mas o número de ovos parasitados diminuiu com o aumento da idade do hospedeiro.

**Palavras-chave:** Lagarta-do-cartucho. Controle biológico. Parasitoide de ovos. Desenvolvimento embrionário.

## Introduction

*Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) can be responsible for significant yield reductions in corn, and is considered the most important corn pest in Brazil and other countries worldwide (CRUZ, 2008; MENDES et al., 2011). This lepidopteran is polyphagous, feeding on approximately 180 plant species (CASMUZ et al., 2010), notably including corn, rice, and cotton (BUSATO et al., 2004; MARTINELLI et al., 2007). This characteristic, as well as its ability to adapt to different climates, results in *S. frugiperda* having a broad geographical distribution, and it can cause problems in all Brazilian agricultural areas at different periods of the year (POGUE, 2002).

Management of this pest relies mainly on spraying chemical insecticides, which is the most common tool used by farmers. However, inappropriate use of insecticides can lead to problems such as elimination of natural enemies (SILVA et al., 2016; GRANDE et al., 2018),

selection of insecticide-resistant pest populations, environmental contamination, and increases in production costs, among others (FERNANDES et al., 2010). Therefore, the use of more sustainable pest control tactics to keep pest population below the economic damage level without the exclusive use of chemicals (VAN LENTEREN et al., 2017) is fundamental to reducing the negative effects agriculture might have on the environment.

Biological control through release of egg parasitoids is a promising tool for integrated pest management (IPM) (VAN LENTEREN et al., 2017). In this context, *Telenomus remus* (Nixon) (Hymenoptera: Platygasteridae) deserves special attention for control of pests of the genus *Spodoptera* (POMARI et al., 2013). These wasps can be used in augmentative releases with great potential for success due to their high reproductive capacity and efficiency against the target pest (CAVE, 2000). However, one of the factors that can reduce the efficiency of this parasitoid in the field is the lack

of synchronization between occurrence of the most susceptible age of the pest egg and the age at which adult parasitoids released into the environment have their highest activity of parasitism (CINGOLANI et al., 2014). There is a lack of information on the performance of parasitoid females of different ages, and on the behavior of *T. remus* towards pest eggs at different embryonic development stages. The published papers in this field (VENTURA et al., 2001; POLANCZYK et al., 2007; PIZZOL et al., 2012) are restricted to the genus *Trichogramma*, and have shown that the age of female parasitoids affects their ability to control the target pest. Understanding this information is essential to provide appropriate recommendations for use of parasitoids in augmentative biological control (ABC) programs, but is still absent in the literature for *T. remus*.

According to Zuim et al. (2017), the performance of a parasitoid in an ABC program partially depends on its host selection behavior. The advance in embryonic development can reduce egg acceptance by parasitoids, consequently reducing parasitism (LOPES; PARRA, 1991; OLIVEIRA et al., 2003; ROCHA et al., 2006; ZUIM et al., 2017). Therefore, studies evaluating responses of *T. remus* females of different ages to *S. frugiperda* eggs at different stages of embryonic development can provide essential information to optimize the use of this parasitoid in ABC programs. Thus, the objective of this study was to evaluate the influence of *T. remus* female age on parasitism of *S. frugiperda* eggs, and preferences of *T. remus* for *S. frugiperda* eggs of different ages.

## Material and Methods

### Host and parasitoid rearing

Eggs of *S. frugiperda* and adult *T. remus* females used in the experiments came from insect colonies kept at Embrapa Soja, Londrina, State of Paraná, Brazil. These species were reared under controlled

laboratory conditions following Greene et al. (1976) (for *S. frugiperda*) and Bueno et al. (2008) (for *T. remus*) in acclimatized rooms at  $25 \pm 2^\circ\text{C}$ ,  $70 \pm 10\%$  humidity, and 14/10 h (L/D) photoperiod, as briefly described in the following.

Adults of *S. frugiperda* were kept in cages (45 cm length  $\times$  33 cm width  $\times$  35 cm height) lined with white sulfite paper on their sides as a substrate for oviposition. For adult feeding, a solution containing honey (10%) and distilled water was offered. Egg masses were removed daily and were either used for experiments, or used for maintenance of the colony by keeping the *S. frugiperda* larvae on artificial diet (GREENE et al., 1976) in plastic cups (50 mL) until the pupal stage.

For *T. remus* rearing, *S. frugiperda* egg masses were glued to pieces of cardboard (10 cm  $\times$  12 cm) and introduced into plastic recipients (2 L) containing newly emerged *T. remus* adults ( $\leq 24$  h since emergence). Honey droplets were placed on the inner wall of the recipients to provide food for the adults. The cardboards were kept in the recipient for 24 hours to allow parasitism. They were then collected and stored in new plastic recipient (2 L) until the emergence of adults, which were then used for experiments or for colony maintenance.

### Bioassays

Three independent experiments were conducted. In the first experiment, parasitism of *S. frugiperda* eggs by *T. remus* females of different ages was studied. In the second, the susceptibility of *S. frugiperda* eggs of different ages to parasitism by *T. remus* was evaluated. Finally, in the third, preference of *T. remus* for *S. frugiperda* eggs of different ages was studied. All tests were performed under controlled conditions within biochemical oxygen demand (BOD) climatic chambers (ELETROLab®, EL 212 model, São Paulo, SP, Brazil) at a temperature of  $25 \pm 2^\circ\text{C}$ , relative humidity of  $80 \pm 10\%$ , and photoperiod of 14/10 h (L/D).

### *Parasitism of S. frugiperda eggs by T. remus females of different ages*

The experiment was conducted in a completely randomized design with 10 treatments (adult females from 1 to 10 days old from the same laboratory generation) and 4 replicates (N = 40). Each replicate consisted of five females individualized in plastic microtubes (12 mm diameter × 75 mm height) (four replicates × five females per replicate = 20 females evaluated per treatment) following methodology proposed by Queiroz et al. (2017a). Rather than using a single female parasitoid per replicate, a group of females was used to increase the representativeness of the replicate to the species under study. Female parasitoids of insect eggs are small and fragile, and thus vulnerable to tiny injuries during experimental manipulation which could affect their behavior. Using a set of parasitoids for each replicate can partly mitigate this potential negative effect of experimental manipulation.

To obtain adult female parasitoids used in this bioassay, eggs of *S. frugiperda* parasitized by *T. remus* were stored in plastic recipients (12 cm diameter × 25 cm height) and sealed with PVC film until emergence of adult parasitoids. After emergence, droplets (approximately 100 µl each) of pure honey were placed on the walls of the tubes for adult feeding. After 24 hours, 200 females of *T. remus* were then transferred into plastic microtubes (12 mm diameter × 75 mm height) and sealed with plastic PVC film. A droplet of pure honey (approximately 100 µl) was also used for adult feeding. Then, eggs of *S. frugiperda* (100 ± 20 eggs aged ≤24 h) were glued onto pieces of white cardboards (1 cm × 6 cm), labeled with their treatment, and one card was placed in each microtube containing female parasitoids. This procedure was performed for each 20 females (4 replicates of 5 female parasitoids) during 10 days. It offered the treatments of parasitoid females ranging from one to 10 days old. The cards were exposed to parasitism for 24 hours. After that, the females were removed, leaving only the cardboard in the

microtubes, which were sealed with PVC plastic film and kept under the same conditions, until the emergence of adults (9 ± 2 days). The variables evaluated for each card were: number of parasitized eggs, parasitoid emergence (%), and sex ratio of emerged parasitoids.

### *Susceptibility of S. frugiperda eggs of different ages to T. remus parasitism*

The experiment was conducted in a completely randomized design with three treatments (*S. frugiperda* eggs at 24, 48, and 72 hours old) and 4 replicates (N = 12). Each replicate consisted of five individualized females (4 replicates × 5 females per replicate = 20 females evaluated per treatment). The eggs were kept in BOD climatic chambers under the conditions previously described for 48 or 72 hours before setting up the experiment.

Females of *T. remus* (≤24 since emergence) were transferred into microtubes (12 mm diameter × 75 mm height) containing pure honey droplets (approximately 100 µl each) for adult feeding. Afterwards, white pieces of cardboard (1 cm × 6 cm) containing *S. frugiperda* eggs (100 ± 20 eggs) of different ages (24, 48, or 72 hours) were inserted into the microtubes, which each contained one *T. remus* female, and parasitism was allowed for 24 hours. After this period, females were removed and microtubes containing the cardboards were sealed with plastic PVC film and kept under the same conditions until adult emergence (9 ± 2 days). The parameters evaluated were: number of parasitized eggs, parasitoid emergence (%), and sex ratio of emerged parasitoids.

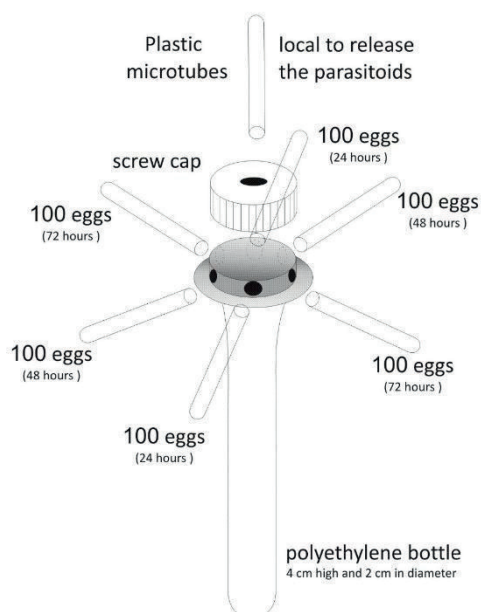
### *Preference of T. remus for S. frugiperda eggs of different ages*

The experiment was conducted in a completely randomized design with 3 treatments (*S. frugiperda* eggs at 24, 48, and 72 hours old) and 15 replicates (one arena per replicate). This host preference

test was conducted using arenas adapted from Thuller et al. (2007) as modified by Queiroz et al. (2017b). Arenas consisted of polyethylene bottles (2 cm diameter x 4 cm height) containing six plastic

microtubes (12 mm diameter × 75 mm height) arranged equidistantly apart in the lower part of the bottle, and one microtube (12 mm diameter × 75 mm height) placed at the top of the arena (Figure 1).

**Figure 1.** Arenas adapted from Thuller et al. (2007) as modified by Queiroz et al. (2017b) used in the host preference test for the parasitoid *Telenomus remus*.



Eggs for each treatment ( $100 \pm 20$  eggs of *S. frugiperda* 24, 48 or 72 hours old) were glued on white cardboards (1 cm × 6 cm). Then, two cardboards of each treatment were introduced into the microtubes, with cards of the same treatment placed on opposite sides (Figure 1). Six newly emerged *T. remus* females ( $\leq 24$  since emergence, with no previous parasitism experience) were released into the top each arena (hence, a rate of one female per  $100 \pm 20$  eggs) and parasitism was allowed for 24 hours. After the 24-hour period, cardboards were removed and kept in BOD climatic chambers until emergence of adults. The number of parasitized eggs in each treatment was evaluated, which allowed for calculation of the percentage parasitized in each treatment in relation to total parasitism observed in the arena (parasitism distribution %).

#### Statistical analysis

Response variables from all three bioassays were evaluated for normality of residuals (SHAPIRO; WILK, 1965) and homogeneity of variance between treatments for ANOVA (BURR; FOSTER, 1972). For analysis of *S. frugiperda* egg parasitism by *T. remus* females of different ages, and for susceptibility of *S. frugiperda* eggs of different ages, percentage emergence was subjected to arcsine transformation before ANOVA. For analysis of *T. remus* preference for *S. frugiperda* eggs of different ages, the number of parasitized eggs was transformed by  $\log(X + 1)$  before ANOVA. Then, the means were compared by Tukey test ( $\alpha = 0.05$ ) using SAS statistical software (SAS INSTITUTE, 2009).

## Results

### *Parasitism of S. frugiperda eggs by T. remus females of different ages*

The age of female *T. remus* wasps did not affect the number of parasitized *S. frugiperda* eggs, or the percentage emergence of adult parasitoids. Parasitoid sex ratios were affected by the age of

wasps, as parasitism by 1- and 2-day-old *T. remus* females generated a lower proportion of females in the second generation (0.61 and 0.62, respectively) when compared to progeny from 9-day-old females (0.88) (Table 1). However, the other treatments did not differ among themselves and all had a sex ratio higher than 0.60 (Table 1).

**Table 1.** The number of parasitized eggs, parasitoid emergence, and sex ratios of wasps emerging from *Spodoptera frugiperda* eggs parasitized by *Telenomus remus* females of different ages. The bioassay was performed under controlled conditions with a temperature of  $25 \pm 2^\circ \text{C}$ , relative humidity of  $80 \pm 10\%$ , and a photoperiod of 14/10 h (L/D).

Female age (days)	Parasitized eggs (n)	Emergence (%) <sup>1</sup>	Sex ratio <sup>2</sup>
1	64.23 $\pm$ 5.01 <sup>ns</sup>	98.25 $\pm$ 0.69 <sup>ns</sup>	0.61 $\pm$ 0.06 b
2	63.35 $\pm$ 6.73	97.68 $\pm$ 0.49	0.62 $\pm$ 0.06 b
3	52.50 $\pm$ 6.38	96.07 $\pm$ 1.18	0.77 $\pm$ 0.03 ab
4	70.85 $\pm$ 5.29	97.33 $\pm$ 0.69	0.80 $\pm$ 0.05 ab
5	67.58 $\pm$ 3.12	98.22 $\pm$ 0.68	0.83 $\pm$ 0.04 ab
6	79.96 $\pm$ 6.36	99.45 $\pm$ 0.21	0.81 $\pm$ 0.04 ab
7	75.90 $\pm$ 4.40	97.91 $\pm$ 0.60	0.70 $\pm$ 0.03 ab
8	60.43 $\pm$ 8.18	96.50 $\pm$ 2.30	0.76 $\pm$ 0.08 ab
9	64.81 $\pm$ 5.81	97.91 $\pm$ 0.72	0.88 $\pm$ 0.01 a
10	56.05 $\pm$ 9.48	96.66 $\pm$ 0.90	0.75 $\pm$ 0.07 ab
CV (%)	19.27	3.9	13.16
F	1.78	1.09	3.17
P	0.1132	0.4003	0.0083
df <sub>total</sub>	39	38	39

Means  $\pm$  standard error followed by the same letter in the column do not differ according to Tukey test ( $\alpha = 0.05$ ) following ANOVA. <sup>1</sup>Data were transformed by arcsine for analysis by ANOVA; untransformed means are presented. <sup>2</sup>Sex ratio = number of females/(number of males + females). <sup>ns</sup>Anova not significant.

### *Susceptibility of S. frugiperda eggs of different ages to T. remus parasitism*

Eggs of different ages differed in their susceptibility to parasitism by *S. frugiperda*. Eggs 24- and 48-hour-old were equally parasitized, but both were more parasitized than 72-hour-old eggs (Table 2). On the other hand, *S. frugiperda* egg age did not affect percentage *T. remus* emergence or its sex ratio (Table 2).

### *Preference of T. remus for S. frugiperda eggs of different ages*

*Spodoptera frugiperda* egg age influenced parasitism distribution (%) of *T. remus* (host preference). Significantly more 24-hour-old eggs were parasitized (mean = 116.81 eggs) than 48-hour-old eggs (18.18), which in turn was higher than the number of 72-hour-old parasitized eggs (only 0.36) (Table 2).

**Table 2.** The number of parasitized eggs, percentage emergence, and sex ratios of wasps emerging from *Spodoptera frugiperda* eggs of different parasitized by *Telenomus remus* in choice tests, and parasitism preference for different ages of eggs. The bioassay was performed under controlled conditions with a temperature of  $25 \pm 2^\circ \text{C}$ , relative humidity of  $80 \pm 10\%$ , and a photoperiod of 14/10 h (L/D).

Egg age (h)	Biological characteristics			Parasitism preference
	Parasitized eggs (n)	Emergence (%) <sup>1</sup>	Sex ratio	Number of parasitized eggs <sup>2</sup> (parasitism distribution %)
24	57.95 ± 2.83 a	98.48 ± 0.79 <sup>ns</sup>	0.69 ± 0.06 <sup>ns</sup>	116.81 ± 12.70 a (89.95%)
48	56.83 ± 12.64 a	99.66 ± 0.34	0.74 ± 0.09	18.18 ± 9.28 b (9.81%)
72	16.83 ± 10.86 b	95.66 ± 4.34	0.83 ± 0.14	0.36 ± 0.28 c (0.24%)
CV (%)	35.04	5.9	23.47	48.87
F	6.89	0.55	0.54	49.93
P	0.0222	0.5968	0.6008	<0.0001
df <sub>total</sub>	9	10	10	32

Means ± standard error followed by the same letter in the column do not differ according to Tukey test ( $\alpha = 0.05$ ). <sup>1</sup>Data were transformed by arcsine for analysis by ANOVA; untransformed means are presented. <sup>2</sup>Data were transformed by log (X+1) for analysis by ANOVA; untransformed means are presented. <sup>ns</sup>Anova not significant.

## Discussion

Factors that potentially affect efficiency of egg parasitoids in ABC have been well documented. Among these factors, the most well-studied include temperature (OZDER; KARA, 2010; PIZZOL et al., 2010) and the host species used for rearing the parasitoids (OZDER; KARA, 2010). However, there are few studies to date on the potential effects of host egg age (PAK et al., 1986; MOREAU et al., 2009; MORENO et al., 2009) and the age of the parasitoids (GARCIA et al., 2001; PIZZOL et al., 2012) on parasitism. In addition, most of these studies focused on trichogrammatids and none of them studied *T. remus*. Thus, as far as we know, this study is the first to report relationships between parasitoid and host ages affecting parasitism by *T. remus* on *S. frugiperda* eggs. The understanding of these effects could contribute significantly to improvements in rearing and use of this parasitoid successfully for ABC programs. Timing the release parasitoids in the field to match the period when their hosts are more susceptible to parasitism is one of the main challenges for success in ABC (VAN LENTEREN et al., 2017). Therefore, studies that evaluate different biological aspects, including host

dynamics, are important to determine the parasitism capacity and potential for success of the biological control agent under study (OLIVEIRA et al., 2016). Thus, knowledge of the biological aspects presented in this article, such as parasitism by females of different ages, and susceptibility of eggs at different stages of embryonic development to parasitism, is essential to evaluate the potential of *T. remus* in the field. In the field, various conditions such as host deprivation, eggs of different ages, and others could influence parasitism. In addition, storage tactics and parasitoid utilization in the laboratory can be improved with the knowledge obtained in this work, aiming for better functioning of laboratory production to meet input needs in the field.

Considering the relationship between the age of the parasitoid and level of parasitism by *T. remus*, there is great potential for this parasitoid to contribute to ABC strategies compared to trichogrammatids. Parasitism by *T. remus* was similar for females between 1 to 10 days old, whereas for *Trichogramma* spp., parasitism is generally concentrated in the first days of life (BUENO et al., 2012). This is usually because most species of the genus *Trichogramma* have the ability to store a complement of mature

eggs in their ovaries or oviducts, and complete oogenesis before or shortly after the emergence of adults (*i.e.*, they are pro-ovigenic parasitoids) (MILLS; KUHLMANN, 2000). Thus, adults emerge ready to oviposit. Under conditions of host deprivation, *Trichogramma* spp. females can eventually reabsorb their oocytes, reducing their parasitism and period of fertility (HOUGARDY et al., 2005). In contrast, some other species of egg parasitoids, such *T. remus* as observed in the present study, are able to retain eggs in the absence of hosts and thus maintain their rates of parasitism even after several days of host deprivation. This improves their chances to successfully parasitize hosts in the field, especially in areas of constant fluctuation population size of the target pest (CHABI-OLAYE et al., 2001; CARNEIRO et al., 2009). The ability of females to adjust egg production according to host availability can provide a reproductive advantage (FLEURY; BOULETREAU, 1993; HOUGARDY et al., 2005). For example, the ability to parasitize even after ten days of host deprivation is clearly an advantage for the use of *T. remus* in ABC programs. In field conditions, it is possible for females to not find eggs immediately, resulting in a need to forage for longer (BIEVER, 1972). Thus, considering our results, which showed that during the first ten days of parasitoid adult life there was no decrease in reproductive potential, *T. remus* will probably be capable of foraging longer in the search of hosts compared with trichogrammatids.

Greater flexibility in parasitism by *T. remus*, which even in the absence of hosts for up to 10 days did not have reduced ability to parasitize, is not only an advantage for ABC in the field, but also for mass rearing in the laboratory. To meet needs for insect rearing or for ABC in the field, our results suggest that *T. remus* adults can be stored at 25°C for later use without any impairment of their subsequent parasitism performance.

Sex ratio is another important biological characteristic of parasitoids in biological control programs. Specifically, higher production of

females is desirable since they are responsible for the direct suppression of the target pest (BUENO et al., 2009). As reported by Schwartz and Gerling (1974), the proportion of males in the progeny of *T. remus* increases with female age, especially after adults are 6 days old. Such inversion in the sex ratio of parasitoid offspring as the age female increases may be due to a decrease or lack of sperm in the spermatheca, which consequently decreases the number of fertilized eggs and thus the number of females in the next generation (HOUSEWEART et al., 1983). However, in the absence of host eggs, sperm remained viable for the 10 days evaluated in our experiment, and the sex ratio always remained higher than 0.60, which is considered adequate for ABC programs (VAN LETEREN; BUENO, 2003). Notably, with regard to sex ratio, despite the statistical difference presented in the treatments with 1- and 2-day-old females compared to 9-day-old females, there was no difference between sex ratios in the progeny of wasps of other ages. Progeny of the 1- and 2-day-old females had similar sex ratios as the progeny of 3- to 8-day-old and 10-day-old females. Similarly, progeny of 9-day-old females had similar sex ratios as progeny 3- to 8- and 10-day-old females. Nevertheless, there was tendency for younger females (1 and 2 days old) in the experiment to have a lower sex ratio than older wasps, which might be explained by the type of reproduction in this insect. Reproduction of *T. remus* occurs by arrhenotokous parthenogenesis, (PRATISSOLI et al., 2014), in which unfertilized females produce haploid males, and fertilized females produce both females and males. Therefore, unfertilized females are restricted to producing male progeny (BOIVIN, 2010). The trend of greater numbers of males in the progeny of 1- and 2-day-old females may have been due to less time available for mating for these wasps, which had less contact with males because they transferred away from them after only 24 and 48 hours of age, respectively. However, it is difficult to explain why 10-day-old females did not also show an increase in proportion of males in their progeny.



Host age is a limiting factor in the performance of egg parasitoids in the field and directly affects the choice of the parasitoid species used in ABC. In the present study, *T. remus* preferred to parasitize 24- and 48-hour-old *S. frugiperda* eggs over 72-hour-old eggs. Their preference for younger eggs may be related to internal and external characteristics of the eggs. When parasitoid females approach eggs of a pest, they examine and drum on the eggs with their antennae to discriminate whether the eggs are suitable for oviposition. As embryonic development advances, there is a reduction in egg nutrient content, making them lower quality for the parasitoid. Such changes in the internal characteristics of host eggs could result in a decrease in parasitism (NAVARAJAM, 1979; SCHMIDT; SMITH, 1987; STRAND; VINSON, 1986). This may explain the negative relationship between host egg age and parasitism potential of *T. remus* females. In addition, occurrence of parasitism in older eggs forces the larva of the parasitoid to develop more rapidly, which can generate deformed individuals unable to emerge (PIZZOL et al., 2012).

Lower parasitism by *T. remus* on 72-hour-old *S. frugiperda* eggs may also be related to hardening of the corium as embryonic development occurs, limiting the penetration capacity of the parasitoid ovipositor (PAK et al., 1986). Thus, parasitoids require more time to oviposit in older eggs, resulting in fewer parasitized (FARIA et al., 2000). This behavior might have relevance in the field, where there could be an overlap of different ages of the pest eggs, hence influencing the performance of the parasitoid, the results of the present study suggest that parasitoids would prefer to oviposit in and have higher performance in younger eggs. However, despite the lower parasitism of 72-hour-old eggs, there was no influence of *S. frugiperda* egg age of on the parasitoid emergence (%) and sex ratio of *T. remus*. Similar findings were reported by Borges Filho et al. (2017), who also did not observe an influence of *Pachycoris torridus* (Scopoli) (Hemiptera: Scutelleridae) egg age on the

emergence and sex ratio of *Telenomus pachycoris* (Johnson) (Hymenoptera: Scelionidae).

To the best of our knowledge, the present study is the first report on the biological characteristics of *T. remus* and their potential importance for the successful use of this parasitoid in biological control programs. The absence of a reduction in parasitism among females from 1 to 10 days of age is a clear indication that in a host deprivation field situation, adults can maintain their parasitism and efficiency potential. This is unlike other species of egg parasitoids in which reabsorption of eggs occurs. In addition, this result suggests that it is possible to have a 10 day time delay between parasitoid adult production and use in the field without losses in biological control efficiency. This is a beneficial characteristic, as it means laboratory production capacity can be flexible with the demand for release of biological control agents in the field. We observed the influence of host egg age on the behavior of the *T. remus* females, as there was reduced acceptance of older hosts and consequently a reduction in parasitism. Such knowledge is crucial to maintaining the quality standard of *T. remus* in the laboratory, and also for optimizing field releases of parasitoids for the biological control of *S. frugiperda*.

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