

## Full Length Research Paper

# Biological parameters and parasitism capacity of *Telenomus remus* Nixon (Hymenoptera: Platygasteridae) reared on natural and factitious hosts for successive generations

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Factitious hosts are largely used in parasitoid production. However, changes in parasitism capacity may happen when hosts are switched. Therefore, the ability of a parasitoid species to be reared on factitious host and still keep high level of parasitism on the natural target pest after successive rearing can determine parasitoid quality and must be investigated. Thus, we evaluated *Telenomus remus* parasitism on *Corcyra cephalonica* eggs compared with its natural host, *Spodoptera frugiperda* eggs, for different generations. After being reared on *C. cephalonica*, *T. remus* parasitism on *S. frugiperda* was evaluated to measure different *T. remus* biological parameters and parasitism capacity (parasitoid quality). Gradual increase in *C. cephalonica* eggs parasitized was observed over the generations, stabilizing on generation F<sub>7</sub>. The number of parasitized *C. cephalonica* eggs was similar among generations (from generation F<sub>7</sub> to F<sub>19</sub>). Taking the lifetime parasitism into consideration, parasitism capacity is similar from *T. remus* reared on *S. frugiperda* eggs from those reared on *C. cephalonica* eggs (generation F<sub>19</sub>). When laboratory-produced *T. remus* on *C. cephalonica* eggs was exposed to the natural host, parasitism was higher on F<sub>5</sub> generation and stable from generations F<sub>5</sub> to F<sub>19</sub>. Therefore, parasitoids did not lose their ability to parasitize eggs of natural host assuring good quality of the laboratory-produced parasitoid using *C. cephalonica* eggs as factitious host.

**Key words:** *Spodoptera frugiperda*, *Corcyra cephalonica*, pre-imaginal condition, egg parasitoid, biological control.

## INTRODUCTION

The egg parasitoid *Telenomus remus* Nixon (Hymenoptera: Platygasteridae) is an effective biological

control agent for various pest species of the genus *Spodoptera* Guenée (Lepidoptera: Noctuidae) (Pomari et

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al., 2012), mainly due to its high reproductive capacity (Cave 2000; Bueno et al., 2008). Despite all of its favorable features for biological control, currently this parasitoid is only reared on a small scale due to both difficulties and costs inherent in rearing it on its natural host, *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) (Pomari-Fernandes et al., 2014). Among the difficulties, *S. frugiperda* cannibalism stands out (Chapman et al., 2000). It makes necessary to rear the caterpillar in individual vials as a way to decrease the pre-imaginal mortality (Chapman et al., 2000) consuming a great deal of time and resources (Perkins, 1979) what consequently raises the costs of the parasitoid rearing.

Therefore, this parasitoid has been only used for experimental purposes against *S. frugiperda* and other lepidopterans or released in small acreages as for example in Venezuela (Ferrer, 2001). In order to increase this parasitoid use in agriculture worldwide, an effective and inexpensive mass-rearing method for *T. remus* in bioindustries is crucial what could be done using a factitious host. Parasitoids can even be reared in non-preferred hosts since they are adequate to promote acceptable development of insects (Parra, 1997). In this context, *Corcyra cephalonica* (Stainton, 1865) (Lepidoptera: Pyralidae) was chosen as a factitious host candidate for *T. remus* since eggs of this moth has been extensively used in China for the production of other eggs parasitoids such as *Trichogramma* spp. (Bernardi et al., 2000) and it had previously been pointed out as a possible factitious host for *T. remus* (Kumar et al., 1986). Moreover, *C. cephalonica* is a stored-product moth, which can easily be reared in laboratory on a large scale (Bernardi et al., 2000), possibly at lower costs compared to *S. frugiperda* for which its rearing is considered to be not practical and very time and resource consuming (Perkins, 1979). These are essential requirements to enable efficient massive production of natural enemies in the laboratory.

Nevertheless, it is important to emphasize that in order to establish and keep a parasitoid rearing on factitious host assuring high quality of produced parasitoid, parasitism capacity and development of the parasitoid on the selected factitious host must be taken into consideration and further analyzed. Moreover, release success of this parasitoid, produced on the factitious host, depends on the knowledge of the bioecological characteristics of the produced parasitoid and on its interaction with the targeted host in the field (Bourchier and Smith, 1996) which can be a different species from the host used in the laboratory rearing. In such example, changes in the foraging behavior and parasitism may happen when hosts are switched.

Parasitism behavior changes happens because it can be innate, as a result of the standards set in the genotype of the species (pre-imaginal conditioning) or present patterns that can be learned as a result of experience gained during foraging and parasitism. Many parasitoids

are able to increase their search capacity due to experiences in a particular environmental situation or host species. Associating the signs learned during parasitism or during development, parasitoid female can readily locate and parasitize its host with greater efficiency and speed (Corbet, 1985, Nurindah et al., 1999), a phenomenon known as  $\alpha$  - conditioning or associative learning (Vinson, 1998; Nurindah et al., 1999).

Then, considering possible changes in the parasitoid biology that can endanger its field efficacy after being rearing on factitious hosts for many generations, it is important to validate the use of this laboratory-produced *T. remus*. Therefore, laboratory studies investigating comparative parasitoid biology and its parasitism are needed (Hassan, 1997; Scholler and Hassan, 2001). Thus, the objective of this research was to evaluate whether it is possible to use the species *C. cephalonica* as alternate host for mass rearing of *T. remus*. Specifically, *T. remus* biology and parasitism capacity on the factitious host *C. cephalonica* was compared with that on its natural host, *S. frugiperda* for different generations. Moreover, after being reared on *C. cephalonica* eggs, *T. remus* parasitism on target pest, *S. frugiperda*, was evaluated in order to guarantee quality control of the laboratory-produced parasitoid. This research generated information which would allow future mass production of the parasitoid in the laboratory as well as the use of this egg parasitoid in extensive biocontrol programs.

## MATERIALS AND METHODS

Biological characteristics of *T. remus* reared on eggs of *C. cephalonica* eggs for different generations. The experiment was conducted in a completely randomized design with 12 treatments (*T. remus* reared on eggs of *C. cephalonica* for different generations – F<sub>0</sub>, F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>, F<sub>4</sub>, F<sub>5</sub>, F<sub>6</sub>, F<sub>7</sub>, F<sub>8</sub>, F<sub>9</sub>, F<sub>13</sub>, and F<sub>19</sub> generations) and 6 replicates (each replicate consisting of five individualized females) in a climatic chamber, adjusted at temperature of 25±2°C, relative humidity of 80±10% and photoperiod of 14/10 h (L/D). *T. remus* reared on *S. frugiperda* eggs and exposed to parasitism on *C. cephalonica* eggs made F<sub>0</sub> generation, then F<sub>1</sub> generation was the first generation of parasitoid reared on *C. cephalonica* eggs and successively afterwards.

*T. remus* females (up to 24 h old) from *C. cephalonica* eggs of different generations were placed in individual vials, each containing a honey droplet (around 100 microliters of honey). Approximately 100 eggs of unviable *C. cephalonica* (up to 24 h) were glued onto a white Bristol board paper (2.5 cm x 5 cm), previously labeled with the respective treatments. These cards were placed in individual vials, each containing a single *T. remus* female, and sealed with Polyvinyl Chloride (PVC) film. *T. remus* was allowed to parasitize for 24 h. After 24 h, the cards were removed from the tubes and transferred into new glass tubes until the emergence of adults. For each parasitoid generation (treatment), the following biological parameters were observed: number of parasitized eggs, parental female longevity (days), duration of egg-adult period (days), emergence percentage (viability) and sex ratio. To determine the duration of the egg-adult period, daily observations from emergence to the adult stage of *T. remus* were made.

Parasitism capacity of *T. remus* on *C. cephalonica* eggs compared to *S. frugiperda* eggs. The experiment was conducted in a completely randomized design with five best treatments from the previous experiment (*T. remus* reared on eggs of *C. cephalonica* for different generations – F<sub>0</sub>, F<sub>8</sub>, F<sub>13</sub>, and F<sub>19</sub> parasitizing *C. cephalonica* eggs and *T. remus* reared on eggs of *S. frugiperda* parasitizing *S. frugiperda* eggs) and 6 replicates (each replicate consisting of five individualized females) in a climatic chamber, set at a temperature of 25±2°C, relative humidity of 80±10% and photoperiod 14/10 h (L/D).

Individual mated *T. remus* (newly emerged: ≤ 24 h old) were placed into separate glass tubes (12 mm Ø x 75 mm tall) covered with PVC film. Droplets (around 100 microliters each) of pure honey on the walls of the glass tubes were offered to feed the females. Thirty glass tubes (6 replications of 5 females each) were prepared for each treatment. Around 100 eggs of *C. cephalonica* (≤ 24 h old), from the host colony, were glued onto a white Bristol board paper (2.5 cm x 5 cm). Similarly, 100 eggs of *S. frugiperda* (≤ 24 h old) were glued onto different cards also made of white Bristol board paper (2.5 cm x 5 cm). Each paper was previously labeled with the respective treatments. Then, these cards, having the eggs, were offered for parasitism for 24 h. *C. cephalonica* eggs were submitted to a process of making them unviable by exposure to ultraviolet radiation for 30 min (Stein and Parra, 1987) previously to parasitism. The cards (with the eggs) were replaced daily until the death of the female parasitoid. The eggs daily removed from the glasses were maintained inside the same controlled environmental chamber under the controlled condition until the emergence of the parasitoids. The evaluated parameters were the number of parasitized eggs per day (daily parasitism), lifetime parasitism, and parental female longevity.

Biological characteristics of *T. remus* reared on *C. cephalonica* eggs for different generations and exposed to parasitism for 24 h on *S. frugiperda* eggs. The experiment was conducted in a completely randomized design with five treatments (*T. remus* reared on eggs of *C. cephalonica* for different generations – F<sub>0</sub>, F<sub>8</sub>, F<sub>13</sub>, and F<sub>19</sub> and *T. remus* reared on eggs of *S. frugiperda* as control) and four replicates (each replicate consisting of five individualized females) in a climatic chamber, set at temperature of 25±2°C, relative humidity of 80±10% and photoperiod of 14/10 h (L/D), according to the methodology described by Pomari et al. (2012) similarly to the experiment previously described. Treatments were females of *T. remus* reared on eggs of *C. cephalonica* for five different generations (F<sub>5</sub>, F<sub>6</sub>, F<sub>8</sub>, F<sub>13</sub>, and F<sub>19</sub>) exposed to parasitism for 24 h on *S. frugiperda* eggs.

*T. remus* females (up to 24 h old) from *C. cephalonica* eggs of different generations (F<sub>5</sub>, F<sub>6</sub>, F<sub>8</sub>, F<sub>13</sub>, and F<sub>19</sub>) were placed in individual vials, each containing a honey droplet. Approximately 100 eggs of *S. frugiperda* (up to 24 h) were glued onto a white Bristol board paper (2.5 cm x 5 cm), previously labeled with the respective treatments. These cards were placed in individual vials, each containing a single *T. remus* female, and sealed with PVC film. *T. remus* was allowed to parasitize for 24 h. After 24 h, the cards were removed from the tubes and transferred to new glass tubes until the emergence of adults. For each parasitoid generation (treatment), the following biological parameters were observed: number of parasitized eggs, parental female longevity (days), duration of egg-adult period (days), emergence percentage (viability) and sex ratio. To determine the duration of the egg-adult period, daily observations from emergence to the adult stage of *T. remus* were made.

### Statistical analysis

Prior to ANOVA, the experimental results were submitted to exploratory analysis to test the normality of the residuals (Shapiro and Wilk, 1965), the homogeneity of variance of the treatments

(Burr and Foster, 1972), and the additivity of the model. Means were compared using the Tukey test (5% error probability) through the SAS statistical analysis program (SAS Institute, 2001).

## RESULTS

Biological characteristics of *T. remus* reared on eggs of *C. cephalonica* eggs for different generations. The number of *C. cephalonica* parasitized eggs differed over the parasitoid generations ( $F_{11, 36} = 2.79$ ,  $P < 0.0001$ ) (Table 1). There was a gradual increase in parasitism (%) from generation F<sub>0</sub> up to F<sub>7</sub> ranging from 2.98 parasitized eggs to 62.88 parasitized eggs in 24 h of parasitism, respectively (Table 1). There was similar parasitism (%) from generation F<sub>7</sub> (62.88%) to F<sub>19</sub> (61.5%) (Table 1).

The average length of the egg-adult period (days) differed between generations ( $F_{11, 36} = 36.96$ ,  $P < 0.0001$ ), being initially lower (F<sub>0</sub> to F<sub>2</sub>) with an increase of 1 or 2 days in the other evaluated generations (Table 1). Egg-adult period (days) was from 12 to 13.2 days in the first 3 generations (F<sub>0</sub> to F<sub>2</sub>) and from 14 to 15 days from F<sub>3</sub> to F<sub>19</sub> (Table 1). Parasitism viability (%) was greater than 78% in all evaluated generations (Table 1). It was similar and around 80% from F<sub>0</sub> to F<sub>4</sub> and increased to around 90% from F<sub>5</sub> to F<sub>19</sub> ( $F_{11, 36} = 13.94$ ,  $P < 0.0001$ ). Sex ratio did not differ among the evaluated generation ( $F_{11, 36} = 1.33$ ,  $P = 0.2510$ ). Even though parental female longevity changed in the different generations ( $F_{11, 36} = 9.16$ ,  $P < 0.0001$ ), the observed variation seemed randomly and no pattern could be observed (Table 1).

Parasitism capacity of *T. remus* on *C. cephalonica* eggs compared to *S. frugiperda* eggs. The lifetime number of parasitized *C. cephalonica* eggs by *T. remus* reared on *C. cephalonica* eggs increased over the generations ( $F_{4, 20} = 38.30$ ,  $P < 0.0001$ ). The higher amount of parasitized *C. cephalonica* eggs was found at generation F<sub>19</sub>. These values were similar to the control treatment, which are parasitoid from natural host (*S. frugiperda* eggs) parasitizing the same natural host (*S. frugiperda* eggs) (Table 2). More than 80% of this lifetime parasitism of *T. remus* on eggs of *C. cephalonica* from generations F<sub>13</sub> (Figure 1D) and F<sub>19</sub> (Figure 1E) was reached, respectively, at 4<sup>th</sup> and 6<sup>th</sup> days of parasitism. This is similar to *T. remus* from natural host (*S. frugiperda* eggs) parasitizing the same natural host (*S. frugiperda* eggs) (Figure 1A) in which 80% of lifetime parasitism was reached at the 4<sup>th</sup> day. *T. remus* from generations F<sub>0</sub> and F<sub>8</sub> on *C. cephalonica* eggs took longer to reach 80% of lifetime parasitism, 10 and 9 days, respectively (Figure 1B and C). The number of parasitized eggs per day varied with parasitoid generations and hosts, but it was higher on the first 24 h on both studied variables (host and generation). In these results it is important to emphasize for all treatments parasitoid decreased the number of eggs daily parasitized on the studied host as a function of the time of parasitism (Figure 1A and E).

**Table 1.** Biological parameters of *T. remus* reared on *C. cephalonica* eggs for different generations and exposed to parasitism for 24 h on *C. cephalonica* eggs under controlled conditions [temperature of 25±2°C, relative humidity of 80±10% and photoperiod of 14/10 h (L/D)].

Generation	Number of <i>C. cephalonica</i> parasitized eggs <sup>1</sup>	Parental female longevity (days) <sup>1</sup>	Progeny		
			Egg-adult period (days) <sup>1</sup>	Parasitism viability (%)	Sex ratio
F <sub>0</sub>	2.98 ± 0.22 <sup>g</sup>	10.5 ± 1.3 <sup>c</sup>	12.1 ± 0.4 <sup>e</sup>	82.58 ± 1.26 <sup>bcd</sup>	0.56 ± 0.03 <sup>ns</sup>
F <sub>1</sub>	5.63 ± 0.17 <sup>fg</sup>	13.3 ± 1.2 <sup>bc</sup>	13.2 ± 0.1 <sup>d</sup>	81.87 ± 1.23 <sup>cd</sup>	0.67 ± 0.04
F <sub>2</sub>	8.55 ± 0.11 <sup>efg</sup>	10.4 ± 1.7 <sup>c</sup>	12.0 ± 0.0 <sup>e</sup>	80.17 ± 1.29 <sup>cd</sup>	0.65 ± 0.02
F <sub>3</sub>	12.76 ± 0.17 <sup>ef</sup>	20.7 ± 1.5 <sup>a</sup>	14.0 ± 0.0 <sup>c</sup>	78.87 ± 1.94 <sup>d</sup>	0.65 ± 0.02
F <sub>4</sub>	16.00 ± 0.24 <sup>de</sup>	19.0 ± 2.6 <sup>ab</sup>	15.0 ± 0.0 <sup>a</sup>	81.04 ± 2.39 <sup>cd</sup>	0.64 ± 0.04
F <sub>5</sub>	22.19 ± 0.25 <sup>d</sup>	8.6 ± 0.7 <sup>c</sup>	14.0 ± 0.0 <sup>c</sup>	86.24 ± 0.75 <sup>abc</sup>	0.62 ± 0.02
F <sub>6</sub>	38.72 ± 0.39 <sup>c</sup>	11.0 ± 0.2 <sup>c</sup>	14.6 ± 0.3 <sup>abc</sup>	90.26 ± 1.05 <sup>a</sup>	0.60 ± 0.04
F <sub>7</sub>	62.88 ± 0.88 <sup>ab</sup>	9.0 ± 0.9 <sup>c</sup>	14.9 ± 0.1 <sup>ab</sup>	91.78 ± 1.59 <sup>a</sup>	0.66 ± 0.02
F <sub>8</sub>	66.15 ± 0.95 <sup>a</sup>	10.0 ± 0.7 <sup>c</sup>	14.6 ± 0.1 <sup>abc</sup>	89.63 ± 0.32 <sup>a</sup>	0.68 ± 0.04
F <sub>9</sub>	63.15 ± 0.82 <sup>ab</sup>	10.5 ± 0.2 <sup>c</sup>	14.1 ± 0.1 <sup>bc</sup>	88.46 ± 0.69 <sup>ab</sup>	0.62 ± 0.01
F <sub>13</sub>	55.50 ± 0.97 <sup>b</sup>	11.6 ± 0.3 <sup>c</sup>	14.0 ± 0.0 <sup>c</sup>	89.64 ± 0.29 <sup>a</sup>	0.64 ± 0.01
F <sub>19</sub>	61.50 ± 1.17 <sup>ab</sup>	10.8 ± 0.3 <sup>c</sup>	14.1 ± 0.1 <sup>bc</sup>	90.65 ± 0.14 <sup>a</sup>	0.65 ± 0.03
<i>p</i>	<0.0001	<0.0001	<0.0001	<0.0001	0.2510
F	2.79	9.16	36.96	13.94	1.33
DF <sub>error</sub>	36	36	36	36	36
CV(%)	10.26	9.72	2.32	2.94	8.88

Means (Mean ± Standard Error) follow by the same small letters in the columns are not statistically different (Tukey test, P≤0,05). <sup>1</sup>Original data followed by analysis performed on data transformed into  $\sqrt{X}$ . <sup>ns</sup>ANOVA non-significant.

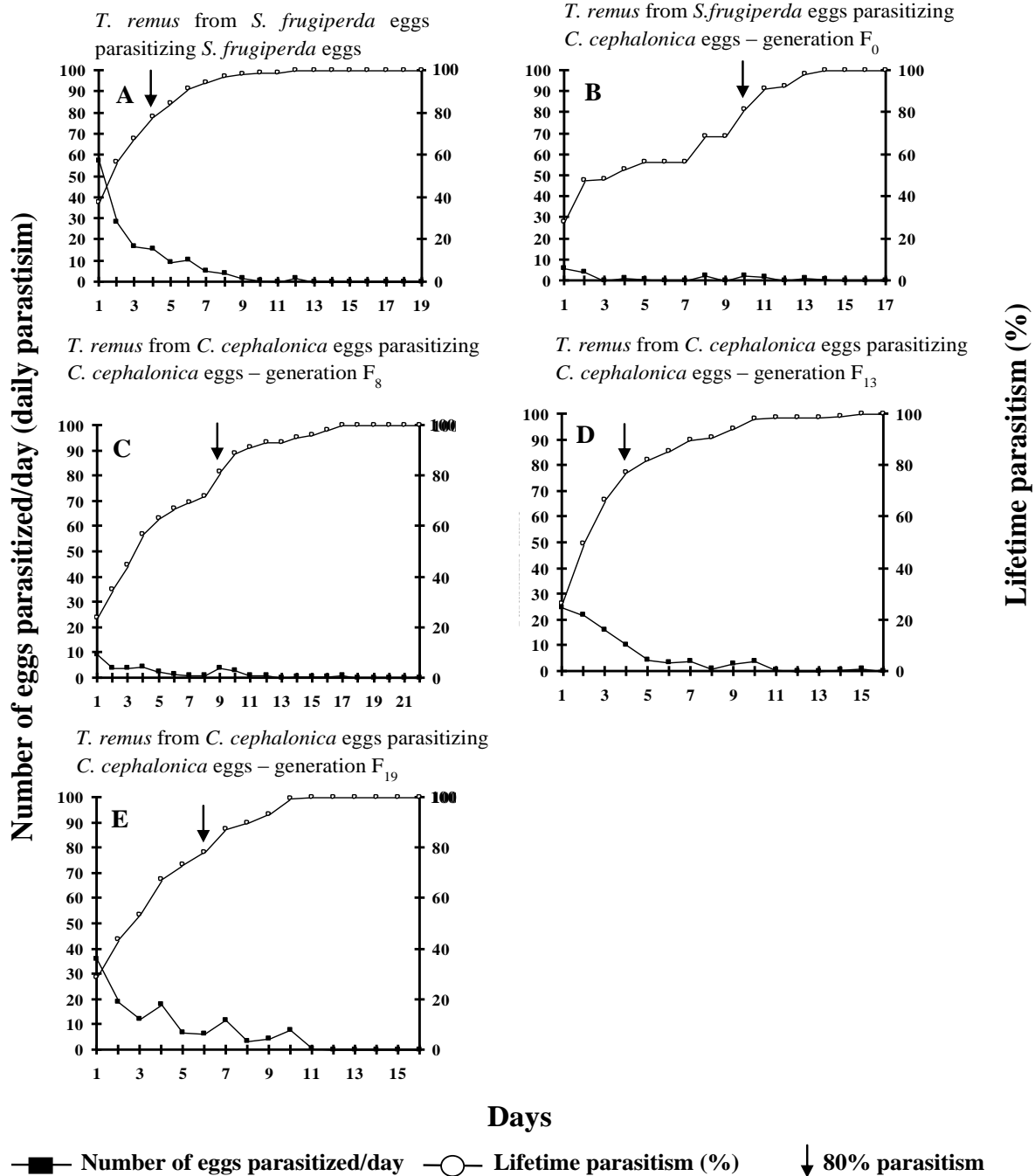
**Table 2.** Lifetime number of parasitized eggs, parasitism viability, sex ration and parental female longevity of *T. remus* reared on different hosts/generations under controlled conditions [temperature of 25±2°C, relative humidity of 80±10% and photoperiod of 14/10 h (L/D)].

Treatment	Lifetime number of parasitized eggs	Parental female longevity (days)	Progeny	
			Parasitism viability (%)	Sex ratio
1) <i>T. remus</i> from <i>S. frugiperda</i> eggs on <i>S. frugiperda</i> eggs	140.8 ± 10.9 <sup>a</sup>	8.3 ± 0.8 <sup>b</sup>	87.8 ± 1.6 <sup>a</sup>	0.67 ± 0.02 <sup>b</sup>
2) <i>T. remus</i> from <i>S. frugiperda</i> eggs on <i>C. cephalonica</i> eggs – generation F <sub>0</sub>	30.3 ± 4.2 <sup>c</sup>	13.1 ± 0.4 <sup>a</sup>	15.0 ± 1.9 <sup>c</sup>	0.69 ± 0.02 <sup>b</sup>
3) <i>T. remus</i> from <i>C. cephalonica</i> eggs on <i>C. cephalonica</i> eggs – generation F <sub>8</sub>	51.47 ± 7.71 <sup>c</sup>	15.3 ± 0.7 <sup>a</sup>	75.0 ± 3.0 <sup>b</sup>	0.75 ± 0.01 <sup>a</sup>
4) <i>T. remus</i> from <i>C. cephalonica</i> eggs on <i>C. cephalonica</i> eggs – generation F <sub>13</sub>	107.4 ± 6.5 <sup>b</sup>	13.5 ± 0.3 <sup>a</sup>	77.9 ± 0.9 <sup>b</sup>	0.71 ± 0.01 <sup>ab</sup>
5) <i>T. remus</i> from <i>C. cephalonica</i> eggs on <i>C. cephalonica</i> eggs – generation F <sub>19</sub>	122.4 ± 7.5 <sup>ab</sup>	13.5 ± 0.3 <sup>a</sup>	70.6 ± 1.2 <sup>b</sup>	0.69 ± 0.01 <sup>ab</sup>
<i>p</i>	<0.0001	<0.0001	<0.0001	0.0146
F	38.30	24.69	238.90	4.24
DF <sub>error</sub>	20	20	20	17
CV (%)	18.92	9.21	6.39	4.14

Means (Mean ± Standard Error) follow by the same small letters in the columns are not statistically different (Tukey test, P≤0,05). <sup>ns</sup>ANOVA non-significant.

Parasitism viability was higher for the progeny of parasitoid from *S. frugiperda* eggs parasitizing *S. frugiperda* eggs (control treatment) compared to the other

treatments (*T. remus* reared on eggs of *C. cephalonica* for different generations – F<sub>0</sub>, F<sub>8</sub>, F<sub>13</sub>, and F<sub>19</sub>) which did not differ among themselves ( $F_{4, 20} = 238.90$ ,



**Figure 1.** Number of eggs parasitized per day and lifetime parasitism (%) of *S. frugiperda* and *C. cephalonica* eggs by *T. remus* reared on different hosts/generations under controlled conditions [temperature of  $25\pm 2^\circ\text{C}$ , relative humidity of  $80\pm 10\%$  and photoperiod of 14/10 h (L/D)].

$P < 0.0001$ ). *T. remus* from *S. frugiperda* eggs parasitizing *S. frugiperda* eggs presented 87.8% of parasitism viability while these values varied from 70.6 to 77.9% for *T. remus* reared on *C. cephalonica* eggs and parasitizing *C. cephalonica* eggs (Table 2). The sex ratio of progeny differed among treatments ( $F_{4, 17} = 4.24$ ,  $P = 0.0146$ ). However, all of them were higher than 0.6 (Table 2),

values greater than 67% of females, while female longevity ( $F_{4, 20} = 24.69$ ,  $P < 0.0001$ ) was higher with eggs from *C. cephalonica*, regardless of generation (Table 2).

Biological characteristics of *T. remus* reared on *C. cephalonica* eggs for different generations and exposed to parasitism for 24 h on *S. frugiperda* eggs. The number of *S. frugiperda* parasitized eggs were similar among  $F_6$ ,

**Table 3.** Biological parameters of *T. remus* reared on *C. cephalonica* eggs for different generations and exposed to parasitism for 24 h on *S. frugiperda* eggs under controlled conditions [temperature of  $25\pm 2^\circ\text{C}$ , relative humidity of  $80\pm 10\%$  and photoperiod of 14/10 h (L/D)].

Generation	Number of <i>S. frugiperda</i> parasitized eggs <sup>1</sup>	Parental female longevity (days)	Progeny		
			Egg-adult period (days) <sup>1</sup>	Parasitism viability (%) <sup>1</sup>	Sex ratio <sup>1</sup>
F <sub>5</sub>	170.10 ± 9.85 <sup>a</sup>	15.75 ± 0.90 <sup>a</sup>	10.00 ± 0.00 <sup>c</sup>	88.44 ± 5.32 <sup>ab</sup>	0.40 ± 0.04 <sup>b</sup>
F <sub>6</sub>	65.18 ± 10.08 <sup>b</sup>	13.50 ± 0.98 <sup>a</sup>	11.59 ± 0.14 <sup>a</sup>	73.16 ± 5.91 <sup>b</sup>	0.63 ± 0.03 <sup>a</sup>
F <sub>8</sub>	81.65 ± 7.00 <sup>b</sup>	7.15 ± 0.41 <sup>b</sup>	11.25 ± 0.14 <sup>ab</sup>	91.14 ± 2.66 <sup>a</sup>	0.66 ± 0.01 <sup>a</sup>
F <sub>13</sub>	49.45 ± 6.30 <sup>b</sup>	7.85 ± 0.79 <sup>b</sup>	11.00 ± 0.00 <sup>b</sup>	96.92 ± 2.22 <sup>a</sup>	0.75 ± 0.03 <sup>a</sup>
F <sub>19</sub>	51.60 ± 6.89 <sup>b</sup>	6.60 ± 0.55 <sup>b</sup>	11.10 ± 0.00 <sup>b</sup>	97.40 ± 0.72 <sup>a</sup>	0.72 ± 0.03 <sup>a</sup>
<i>p</i>	<0.0001	<0.0001	<0.0001	0.0033	<0.0001
F	37.40	30.25	51.07	6.40	17.54
DF <sub>error</sub>	15	15	15	15	15
CV(%)	19.58	14.90	1.51	8.71	10.34

Means (Mean ± Standard Error) follow by the same small letters in the columns are not statistically different (Tukey test,  $P\leq 0.05$ ). <sup>ns</sup>ANOVA non-significant.

F<sub>8</sub>, F<sub>13</sub>, and F<sub>19</sub> generations (Table 3). Differences were reported for egg-adult period ( $F_{4, 15} = 51.07$ ,  $P < 0.0001$ ) among the tested generations. For example, F<sub>6</sub> and F<sub>8</sub> generations took little longer (less than 2 days) to complete immature development compared to F<sub>5</sub> generation, which had the faster development (10.0 days) among the tested generations (Table 3). Parasitism viability (%), which is the percentage of parasitized eggs that presented parasitoid emergence, differed among parasitoid generations ( $F_{4, 15} = 6.40$ ,  $P = 0.0033$ ), but was only smaller than 80% for parasitoid generation F<sub>6</sub>. Generations F<sub>8</sub>, F<sub>13</sub>, and F<sub>19</sub> presented values above 90% (Table 3).

Regarding to sex ratio ( $F_{4, 15} = 17.54$ ,  $P < 0.0001$ ) differences were only noted from generation F<sub>5</sub> (0.40) to F<sub>6</sub> (0.63). Generations F<sub>6</sub>, F<sub>8</sub>, F<sub>13</sub>, and F<sub>19</sub> did not differ among themselves and all presented sex ratio higher than 0.5 (Table 3). Parental female longevity also differed among parasitoid generations ( $F_{4, 15} = 30.25$ ,  $P < 0.0001$ ). Longevity was higher at F<sub>5</sub> and F<sub>6</sub> generations presenting 15.75 and 13.5 days, respectively. The values decreased for F<sub>8</sub>, F<sub>13</sub>, and F<sub>19</sub> generations in which parental female longevity was 7.15, 7.85, and 6.60 days, respectively (Table 3).

## DISCUSSION

The observed increase in *T. remus* parasitism over the generations reared on *C. cephalonica* eggs might suggest parasitism behavior changes as a result of selection of *T. remus* genotypes which are able to parasitize the factitious host. This learning process occurs in different parasitoid species at the time of emergence and may influence future host preference (Corbet, 1985; Hérard et al., 1988; Vet and Groenewold,

1990) which would mean the existence of pre-imaginal conditioning. Different parasitoid species are able to increase their parasitism in non-preferable hosts due to past experiences (Corbet, 1985; Nurindah et al., 1999). Associating the signs learned during parasitism, *T. remus* reared on *C. cephalonica* eggs over different generations could more readily locate and parasitize this factitious host with greater efficiency and speed compared to *T. remus* reared on *S. frugiperda* eggs, a phenomenon known as  $\alpha$  - conditioning or associative learning (Vinson, 1998; Nurindah et al., 1999). Regardless of the form of learning exerted by the parasitoid through the generations, Kumar et al. (1986) reported that *T. remus* development in eggs of *C. cephalonica* was low at first generations increasing up to 100% parasitism on the seventh generation, which is similar to our results ratifying the existence of a adapting process in this parasitoid species over successive generations in order to develop on the factitious host egg.

These conclusions differ from Goulart et al. (2011) who had previously reported non-preference of *T. remus* for *S. frugiperda* eggs after the parasitoid be reared on those eggs for several generations. Accordingly to these authors it would demonstrate that the host acceptance or preference behavior of the *T. remus* females could not be attributed to the pre-imaginal conditioning described by Cobert (1985) and Kaiser et al. (1989). However, it is important to point out that Goulart et al. (2011) compared *T. remus* preference among *Spodoptera* spp. eggs. Eggs from moths of the same genus might not exhibit enough differences for *T. remus* be able to differentiate among them. Peculiarities of each parasitoid host and their relative differences on the thickness of the chorion of the egg, especially the exochorion that is the most protein-rich layer, can affect not only the handling time and exploitation by egg parasitoids, but also the host

suitability (Pak et al., 1990). Eggs differences between *S. frugiperda* and *C. cephalonica* are certainly higher than when compared eggs inside *Spodoptera* genus. It could help to explain these differences observed when both findings are compared.

Our results, on the other hand, suggest that some generations are required for the parasitoid to adapt to *C. cephalonica* eggs. After this adaptation process, this factitious host could be used in *T. remus* massive rearing. However, it is important to confirm if the *T. remus* adapted to parasitize and develop on *C. cephalonica* eggs do not lose its capacity of parasitizing target field pests such as *S. frugiperda* eggs. Therefore, the number of *S. frugiperda* eggs parasitized by *T. remus* massive reared on *C. cephalonica* eggs for different generations is an important evaluation of the quality of this laboratory-produced parasitoid intended for field releases.

The number of parasitized *S. frugiperda* eggs in 24 h and the lifetime parasitism indicates a parasitism increase (recovery) after the decrease observed in the first 24 h. It is also important to observe that this noted lifetime parasitism is also similar those found when the parasitoid was reared on eggs of *S. frugiperda* reported in previous work (Pomari et al., 2012). Therefore, even though it might be necessary to release a higher number of *T. remus* when the parasitoid is from factitious host, this laboratory-produced parasitoid, reared on *C. cephalonica* eggs, did not lose its capacity to control *S. frugiperda* eggs. It is true even when the eggs are laid on superposed layers. Similar findings were reported by Kumar et al. (1986) who found that *T. remus* reared by 75 generations on *C. cephalonica* eggs was always able to parasitize the natural host *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) over all tested generations.

Parental female longevity is also an important biological feature related to parasitoid success in the field. Longer longevity is desirable and could mean a higher parasitism or at least a longer time in which the crop is protected against the target pest. It is correlated with the energy expenditure required for parasitism (Almeida, 2004) which can differ depending upon the host used. Differences in this parameter, when *T. remus* was exposed to the different hosts and generations, may indicate a higher or lower energy expense for the parasitism. This can explain the usually observed smaller lifetime directly related to higher parasitism rates. Moreover, this lower energy expenditure can be coupled with the fact *C. cephalonica* eggs be offered isolate in single layer, which may have led to lower metabolic rate, consequently increasing female longevity compared to *S. frugiperda* eggs that are laid on superposed masses.

In addition, more active females can increase body temperature due to the parasitism activity. Female longevity is usually impacted by temperature being lower at warmer environment (Gerling, 1972). This relationship was previously reported by Almeida (2004), evaluating

*Trichogramma atopovirilia* (Oatman and Platner, 1983) (Hymenoptera: Trichogrammatidae) parasitism. According to them higher energy expenditure of females is due to parasitism, firstly with the introduction of the ovipositor and oviposition, followed by a review of the host by contact with the antennae (Almeida, 2004). Besides these factors, one should take into account that *T. remus* females have the maximum number of eggs in their ovaries between 2 to 3 days of age (van Welzen and Waage, 1987), and produce more than 76% of their offspring during the first 5 days of adulthood (Schwartz and Gerling, 1974). Thus, longevity of parental females, although important, is not a determining factor between the hosts, since parasitoids from both hosts lived longer than 5 days.

*T. remus* parasitism period (which is the time for how long *T. remus* females are active) might differ from female longevity. Parasitism period varies due to differences in temperature (Reznik and Vaghina, 2006), hosts (Reznik et al., 2001), or parasitoid species/strain (Pratissoli and Parra, 2000; Pizzol et al., 2010) and can influence the success of biological control programs using egg parasitoids (Smith 1996). Also, whether parasitism is more active in the first days of life or evenly distributed throughout adulthood is an important characteristic to be considered in choosing the best parasitoid release strategy (Bueno et al., 2010). Our results showed that parasitism activity of *T. remus* always peaked in the first 24 h of parasitism regardless of host species or generation, similarly to what has been reported for this species when attacking *S. frugiperda* (Hernández and Díaz, 1996; Bueno et al., 2010).

The concentration of the parasitism activity in the first days of life is not a characteristic only common to *T. remus*, but also to other egg parasitoids, which need to quickly find their hosts and assure the allocation of their progeny. Oviposition peak of different species of egg parasitoids from the genus *Trichogramma*, for example, have been already reported in the literature on the first day after adult emergence (Bai et al., 1992; Volkoff and Daumal, 1994). This is usually a consequence of most of these parasitoids to have the capacity to store a full complement of mature eggs in the ovaries or oviducts and complete oogenesis either before or shortly after adult emergence (pro-ovigenic parasitoids) (Mills and Kuhlmann, 2000) and, thus, adults emerge ready to lay eggs. In contrast, other studies indicate that some parasitoid species emerge with an egg load that accounts for only a fraction of their potential parasitism, which exceeds their capacity to store mature eggs in their ovaries or oviducts (synovigenic parasitoids) (Kuhlmann and Mills, 1999).

Parasitoid development (egg-adult period) is another biological trait important to consider when comparing host suitability. This parameter is dependent on different biotic and abiotic factors (Pomari et al., 2012). Nutritional requirements from *T. remus* immature development

determines the need for a longer or shorter period of development. Most *T. remus* egg-adult periods in this study did not differ more than 2 days apart, indicating that these biological parameter is not affected over the different *T. remus* generations on *C. cephalonica* eggs. Moreover, similar egg-adult period (days) had been reported when *T. remus* parasitized *S. frugiperda* eggs (Bueno et al., 2008; Pomari et al., 2012). This is a clear indication that *T. remus* is adapted to parasitized *C. cephalonica* eggs. Its relationship has been demonstrated for other egg parasitoids such as *Trichogramma pretiosum* (Pratissoli and Parra, 2000; Bueno et al., 2009).

It is important to emphasize intrinsic characteristics of the egg as nutritional quality may be the key factor in the acceptance of the host and development of the parasitoid (Shipp et al., 1998; Pratissoli and Parra, 2001). Therefore, *C. cephalonica* seems to have enough nutritional quality for *T. remus* development. Other host eggs and/or parasitoid species may have different results for durations of the egg-adult period (Gerling, 1972; Gerling and Schwartz, 1974; Hernández and Díaz, 1996). The sex ratio is another important biological feature in biological control programs since greater proportion of females is desirable because they are responsible for parasitism (Navarro, 1998; Bueno et al., 2008). In this context, all tested generations supported more than 50% females indicating that the use of egg parasitoid *C. cephalonica* as factitious host in massive rearing does not affect the development of *T. remus* females. Additionally, the percentages of females obtained were similar to those observed for the host establishment and others of the same genus (Pomari et al., 2012). Thus, the smaller size of the *C. cephalonica* eggs in relation to the preferred hosts did not influence this biological parameter.

In conclusion, the results obtained with *T. remus* on eggs of *C. cephalonica* were satisfactory enough to state that egg from this stored-grain moth can be successfully used as *T. remus* factitious host. It is important to mention the parasitoid reared by successive generations in eggs of this factitious host has not lost its ability to parasitize eggs of the natural host. Although there was a decrease in the number of parasitized eggs, sex ratio and parasitism viability were not affected. The reduced number of parasitized eggs is not a problem since it can be circumvented with an increase in the number of parasitoids to be released. Also, parasitoids reared on factitious host can go throughout a generation on its target species from time to time in order to keep or even increase its parasitism aggressiveness. This method was used by Gautum (1994) using egg *Agrotis spinifera* (Hubner, 1808) (Lepidoptera: Noctuidae) as a factitious host for a generation/year to increase the effectiveness of *T. remus*, since these eggs were adults reared larger, longer-lived and more fertile than those obtained from *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae)

eggs. Nevertheless, massive *T. remus* rearing on *C. cephalonica* eggs is already a reality can be successfully performed since the adaptation period of at least 13 generations is taken before starting releasing this parasitoids in the field.

### Conflict of Interest

The authors have not declared any conflict of interest.

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