Biological Control and Crop Protection

Influence of host preference, mating, and release density on the parasitism of *Telenomus remus* (Nixon) (Hymenoptera, Platygastridae)

Ana Paula de Queiroz a, Adeney de Freitas Bueno b,*, Aline Pomari-Fernandes c, Orcial Ceolin Bortolotto d, Adriana Yatiemi Mikami d, Lopes Olive e

a Instituto Agronômico do Paraná, Londrina, PR, Brazil
b Empresa Brasileira de Pesquisa Agropecuária, Londrina, PR, Brazil
c Universidade Federal do Paraná, Curitiba, PR, Brazil
d Universidade Federal de Rondônia, Porto Velho, RO, Brazil
e Universidade Federal de Rondônia, Porto Velho, RO, Brazil

**A R T I C L E   I N F O**

Article history:
Received 6 September 2016
Accepted 16 December 2016
Available online 29 December 2016
Associate editor: Daniel Sosa Gómez

Keywords:
Arrhenotokous parthenogenesis
Optimal number
Phenological stages
Preimaginal conditioning

**A B S T R A C T**

We evaluated the influence of host preference, mating, and release density on *Telenomus remus* (Nixon, 1937) (Hymenoptera: Platygastridae) parasitizing eggs of *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae). First, we tested host preference of *T. remus* (free choice test) offered a choice between eggs of *Corcyra cephalonica* (Stainton, 1865) (Lepidoptera: Pyralidae) and *S. frugiperda*. Parasitism capacity and host preference (*S. frugiperda*) of *T. remus* reared on either of the two hosts did not differ. Secondly, we evaluated the influence of mating behavior of *T. remus* females on its parasitism. Only the offspring sex ratio differed between treatments, indicating that the species reproduces by parthenogenesis of the arrhenotoky type. Finally, we evaluated the influence of release density on *T. remus* parasitism. This was tested by releasing different numbers of the parasitoid per *S. frugiperda* egg using *T. remus* reared for different numbers of generations on *C. cephalonica* eggs. The regression analysis between percentage of parasitism and density of released *T. remus* females showed a quadratic effect for all tested parasitoid generations (F15, F40, and F65) with maximum parasitism from 65.07% to 71.69%. Our results allow the conclusion that (a) *T. remus* prefers *S. frugiperda* eggs, regardless of the host on which this parasitoid was reared, showing no preimaginal conditioning; (b) Mating does not affect the number of eggs parasitized by *T. remus* or the development of its offspring; and (c) The optimal *T. remus* release density when reared on *C. cephalonica* is between 0.133 and 0.150 females/S. frugiperda.

© 2016 Sociedade Brasileira de Entomologia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license [http://creativecommons.org/licenses/by-nc-nd/4.0/].

**Introduction**

*Telenomus remus* parasitizes eggs of various species of the order Lepidoptera, many of which are global crop pests (Cave, 2000). Despite possessing traits favorable for their use as biological control, this parasitoid is currently only reared on a small scale due to the difficulties of rearing it on its natural host *Spodoptera frugiperda* (Pomari-Fernandes et al., 2015). Alternatively, *T. remus* can be reared on a factitious host that may not be the parasitoid’s preference but is still adequate for its successful development (Parra, 1997). In this context, *Corcyra cephalonica*, which can be reared in the laboratory more easily and at a lower cost than *S. frugiperda* (Kumar et al., 1986), has been suggested as a possible factitious host of *T. remus* (Kumar et al., 1986; Pomari et al., 2012). However, continuous rearing of a parasitoid on a factitious host may affect its parasitism or host preference, and may directly influence its efficiency against the target pest. This is probably due to preimaginal conditioning occurring during larval development (Cobert, 1985), a biological process which needs further study for *T. remus* reared on *C. cephalonica* eggs.

Knowledge of the biology and ecology of insects and their natural enemies is a prerequisite for the success of biological control in Integrated Pest Management (IPM) (Cave, 2000). Adult mating can impact parasitism, and should be taken into account when testing the use of an egg parasitoid in massive field releases (Prattisoli et al., 2009). Males of *T. remus* have one larval instar less than females (Cave, 2000), and therefore emerge earlier than females from the same host egg mass. The newly emerged males guard egg masses to ensure their mating with females as soon as they emerge (Cave, 2000). Because parasitism capacity may differ between mated and unmated *T. remus* females, the influence of mating at the time of emergence should be assessed prior to adopting the recently

* Corresponding author.

E-mail: adeney.bueno@embrapa.br (A.F. Bueno).
developed technology of aerial release of individual pupae close to emergence.

Additionally, superparasitism can decrease the number of parasitized eggs, and may occur when an excessive number of parasitoids per host egg is released (Cave, 2000). Research is needed to test the influence of release density on parasitism with the long-term goal of determining the optimal number of parasitoids to be released into the field (Sá and Parra, 1993). To this end, this study evaluates the influence of host preference, mating, and release density on *T. remus* parasitism on eggs of *S. frugiperda*. The results yield crucial information for the success of rearing *T. remus* and its release in the field.

### Material and methods

The studies (bioassay 1 and 2) were carried out under controlled laboratory conditions (25 °C ± 2 °C, relative humidity 80% ± 10%, photoperiod 14/10 h [light/dark]) and in a greenhouse (bioassay 3) at Embrapa Soybean, Londrina, State of Paraná, Brazil. This work involved three independent bioassays. In the first bioassay we assessed host preference of the parasitoid *T. remus* offered a choice between eggs of *C. cephalonica* and *S. frugiperda*. In the second bioassay we evaluated the influence of mating on *T. remus* parasitism of *S. frugiperda* eggs. In the third bioassay we determined the optimal number of parasitoids to be released for the successful control of *S. frugiperda* in maize. All hosts and parasitoids used in the experiments were obtained from the rearing laboratory at Embrapa Soybean.

**Bioassay 1: host preference of Telenomus remus**

All *T. remus* colonies evaluated in the host preference test originated from insects reared on *C. cephalonica* eggs at the F40 generation and *S. frugiperda* eggs at the F35 generation (Parra, 1997) in order to compare the two insect populations. The experiment was carried out in a completely randomized 2 × 2 factorial design (2 parasitoid colonies by 2 host eggs) and 15 replicates. Each replicate consisted of an arena according to Thuler et al. (2007): a polyethylene bottle (4 and 2 cm in diameter) for parasitoid release was placed in the middle of the arena around which four tubes (volume 1.5 mL) containing the host specimens were arranged at equal distances.

Approximately 150 eggs of a single host (*C. cephalonica* or *S. frugiperda*) were glued to labeled, white cardboard cards (2.5 × 5 cm) with white glue (Tenax®). Two cards per host were individually introduced into the tubes located on opposite sides of the arena. Next, four newly emerged (24 h) *T. remus* females fed on honey were released into the arena from the central bottle. After 24 h, the cards were removed and placed individually in 1.5 mL Duran tubes until the emergence of adults. At this step, the number of parasitized eggs was recorded.

The results (Table 1) were analyzed for normality (Shapiro and Wilk, 1965) and homogeneity of variance of treatments (Burr and Foster, 1972) and, whenever necessary, transformed to perform ANOVA. The number of parasitized eggs was transformed by log (x + 1). The treatment means were compared using Tukey’s test at a probability level of 5% (SAS Institute, 2009).

**Bioassay 2: influence of parasitoid mating on its parasitism**

Mated and unmated *T. remus* females reared on *C. cephalonica* eggs (F40 generation) were offered eggs of *S. frugiperda* for a 24 h period. Each female was placed in a Duran type tube (1.5 mL) containing a droplet of honey as food, and offered approximately 100 *S. frugiperda* eggs (up to 24 h old) glued to white cardboard cards (2.5 × 5 cm) (six replicates per treatment). Afterwards, cards were removed and placed individually in Duran-type tubes until the emergence of adults. The number of parasitized eggs, parasitoid emergence (%), sex ratio, and longevity of parental females was recorded. The results (Table 2) were analyzed for normality (Shapiro and Wilk, 1965) and homogeneity of variance of treatments (Burr and Foster, 1972) and, whenever necessary, transformed to perform ANOVA. The number of parasitized eggs was transformed by log (x + 1) and parasitoid emergence (%) transformed by arcsin √x/100. Then, treatment means were compared by Tukey’s test at a probability level of 5% (SAS Institute, 2009).

**Bioassay 3: influence of *T. remus* release density on its parasitism**

The influence of the release density of *T. remus* on its parasitism was determined by releasing different numbers of *T. remus* in relation to a given number of eggs of *S. frugiperda*, using *T. remus* reared for a different number of generations (F35, F40, and F45) on *C. cephalonica* eggs. We chose *T. remus* reared on *C. cephalonica* eggs because this parasitoid is planned to be released in the field on a large scale for biological control programs. An independent trial was carried out for each parasitoid generation under greenhouse conditions, using a fully randomized experimental design with ten treatments (0, 0.017, 0.033, 0.050, 0.067, 0.083, 0.100, 0.117, 0.133, and 0.150 *T. remus* females per *S. frugiperda* egg) and five replicates. Eggs of the pest species were obtained from laboratory rearing and exposed to treatments inside iron-framed cages (40 cm × 40 cm × 120 cm) covered with voile fabric in each replicate. A pot of 40-cm diameter with two hybrid IPR 114 maize plants was placed inside each of these cages, attaching a white cardboard card with 150 eggs to the whorl of each plant. Different numbers (5, 10, 15, 20, 25, 30, 35, 40, and 45) of previously mated females (Pomari et al., 2013) were released, representing the proportions of 0.017, 0.033, 0.050, 0.067, 0.083, 0.100, 0.117, 0.133, and 0.150 *T. remus* females per *S. frugiperda* egg. No parasitoids were released in the control treatment. Parasitism was allowed for 24 h, and temperature and humidity were recorded using a digital data logger (Instrutherm HT-500) (Table 3). Next, eggs were collected and maintained in Petri dishes at 25 °C until they darkened and parasitoids emerged for subsequent evaluation. We recorded total percent of parasitism per replicate. This procedure was repeated

### Table 1

<table>
<thead>
<tr>
<th>Host</th>
<th>Parasitoid colony</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. cephalonica</em></td>
<td><em>T. remus</em> reared on <em>C. cephalonica</em></td>
<td>2.29 ± 1.03</td>
</tr>
<tr>
<td><em>S. frugiperda</em></td>
<td><em>T. remus</em> reared on <em>S. frugiperda</em></td>
<td>94.36 ± 12.71</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>48.32 ± 10.85 A</td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td>39.15</td>
</tr>
<tr>
<td><em>Fparasitoid</em></td>
<td></td>
<td>1.57</td>
</tr>
<tr>
<td><em>Fhost</em></td>
<td></td>
<td>259.46</td>
</tr>
<tr>
<td><em>Fparasitoid</em></td>
<td></td>
<td>1.73</td>
</tr>
<tr>
<td><em>Prasitoid</em></td>
<td></td>
<td>0.2161</td>
</tr>
<tr>
<td><em>Phost</em></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><em>Fparasitoid</em></td>
<td></td>
<td>0.1950</td>
</tr>
<tr>
<td><em>DFparasitoid</em></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em>DFhost</em></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em>DFparasitoid</em></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em>DFtotal</em></td>
<td></td>
<td>53</td>
</tr>
</tbody>
</table>

Means (±SE) followed by identical upper-case letters within a row (different parasitoid colonies), and lower-case letters within a column (different egg hosts) did not statistically differ according to Tukey’s Studentized range test at 5% probability. Original data followed by statistics performed on data transformed in log (x + 1).

---

**References**


at various developmental stages of the plants (V2/V3, V4/V5, and V8/V9 according to Magalhães and Durães, 2006) because different leaf surfaces might provide different sizes of area for a parasitoid to find eggs and therefore may impact its parasitism. The average parasitism observed for each parasitoid density during different plant stages was used in the analyses (Fig. 1). The number of T. remus females per egg of S. frugiperda and percentage of parasitism were used in a regression analysis (SAS Institute, 2009). Parasitism data was analyzed for normality (Shapiro and Wilk, 1965) and homogeneity of variance of treatments (Burr and Foster, 1972), and whenever necessary transformed to perform ANOVA. Parasitism values of T. remus generations F40 and F45 were transformed by (x+1)^(1/2). The treatment means were then compared by Tukey’s test at a probability level of 5% (SAS Institute, 2009).

Results

Bioassay 1: host preference of Telenomus remus

There was no interaction between T. remus colonies (T. remus reared on C. cephalonica or S. frugiperda eggs) and parasitized hosts (C. cephalonica or S. frugiperda) (Pparasitoid*host = 0.1950, Fparasitoid*host = 1.73) (Table 1). Telenomus remus reared on C. cephalonica eggs and S. frugiperda eggs exhibited similar parasitism capacity regardless of the host species they were reared on. The number of parasitized eggs was similar for both parasitoid colonies (48.32 and 40.53 eggs for parasitoids reared on C. cephalonica and S. frugiperda, respectively, Pparasitoid = 0.2161, Fparasitoid = 1.57) (Table 1). In contrast, higher numbers of S. frugiperda eggs (87.96) were parasitized compared with C. cephalonica eggs (1.19) (Phost = 0.0001, Fhost = 259.46) (Table 1), indicating a clear preference for S. frugiperda eggs regardless of the host the parasitoid was reared on.

Bioassay 2: influence of parasitoid mating on its parasitism

No differences were found between mated and unmated females with respect to the number of parasitized eggs, parasitoid emergence (%) and longevity of parental females (days) (Table 2). In contrast, offspring sex ratio differed between treatments (0.61 for mated females and 0.00 for unmated females, Table 2), indicating that T. remus reproduces parthenogenetically with characteristics of the arrhenotokous type.

Bioassay 3: influence of the release density of T. remus on its parasitism

For S. frugiperda eggs attached to maize leaves, we found a quadratic effect for the regression analysis between the percentage of parasitism and density of released T. remus females (number of parasitoid per pest egg) in all parasitoid generations tested (Fig. 1). Maximum parasitism was 71.69%, 71.69%, and 65.07% for F35, F40, and F45, respectively. The maximum parasitism rate was reached at densities between 0.133 and 0.150 of T. remus females per S. frugiperda egg (Fig. 1). Throughout the study, climatic data were recorded with a data logger at greenhouse conditions. Differences in temperature and relative humidity between all evaluated growth stages are shown in Table 3.

Discussion

Parasitoid rearing on factitious hosts is essential for the success of its mass release in augmentative biological control programs (Bueno et al., 2008) and requires the establishment of procedures to monitor the quality of the produced insect. According to Cobert (1985), continuous rearing of parasitoids on factitious hosts can cause the loss of their ability to recognize and choose a host and therefore reduce their efficiency against the targeted pest species. In our study, this negative effect was not observed for T. remus reared on C. cephalonica eggs. Parasitism of eggs of the target field pest (S. frugiperda) was similar in parasitoid females reared on both C. cephalonica and S. frugiperda. However, the influence of the factitious host on parasitoid quality might also depend on the number of generations the parasitoid is reared on the same host. Pratissoli et al. (2004) reported that parasitism capacity of species reared on factitious hosts was inversely proportional to the number

Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of parasitized eggs</th>
<th>Parasitoid emergence (%)</th>
<th>Sex ratio</th>
<th>Longevity of parental females (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mated females</td>
<td>53.33 ± 5.68^a</td>
<td>99.13 ± 0.26^a</td>
<td>0.61 ± 0.04 a</td>
<td>5.63 ± 0.34^a</td>
</tr>
<tr>
<td>Unmated females</td>
<td>46.00 ± 5.27</td>
<td>98.78 ± 0.81</td>
<td>0.00 ± 0.00 b</td>
<td>6.08 ± 0.18</td>
</tr>
<tr>
<td>CV (%)</td>
<td>11.84</td>
<td>4.46</td>
<td>19.88</td>
<td>10.52</td>
</tr>
<tr>
<td>p</td>
<td>0.3506</td>
<td>0.9264</td>
<td>&lt;0.0001</td>
<td>0.2835</td>
</tr>
<tr>
<td>F</td>
<td>0.98</td>
<td>0.01</td>
<td>253.13</td>
<td>1.32</td>
</tr>
<tr>
<td>DF residual</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

^a Means (±SE) followed by identical upper-case letters within a row (different parasitoid colonies), and lower-case letters within a column (different egg hosts) did not statistically differ according to Tukey’s Studentized range test at 5% probability Original data followed by statistics performed on data transformed in log(x+1).

^a ANOVA not significant.

Table 3

<table>
<thead>
<tr>
<th>Generation – stadium</th>
<th>Phenological</th>
<th>Temperature</th>
<th>Relative humidity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
<td>Average</td>
</tr>
<tr>
<td>F35 – V2/V3</td>
<td>19.8</td>
<td>27.1</td>
<td>23.45</td>
</tr>
<tr>
<td>F35 – V4/V5</td>
<td>23.2</td>
<td>36.8</td>
<td>30</td>
</tr>
<tr>
<td>F35 – V8/V9</td>
<td>25.6</td>
<td>38.4</td>
<td>32</td>
</tr>
<tr>
<td>F40 – V2/V3</td>
<td>23.3</td>
<td>29.3</td>
<td>26.3</td>
</tr>
<tr>
<td>F40 – V4/V5</td>
<td>21.4</td>
<td>36.4</td>
<td>28.9</td>
</tr>
<tr>
<td>F40 – V8/V9</td>
<td>19.3</td>
<td>26.7</td>
<td>23</td>
</tr>
<tr>
<td>F45 – V2/V3</td>
<td>21.8</td>
<td>38.8</td>
<td>30.3</td>
</tr>
<tr>
<td>F45 – V4/V5</td>
<td>24.9</td>
<td>37.3</td>
<td>31.1</td>
</tr>
<tr>
<td>F45 – V8/V9</td>
<td>21.8</td>
<td>38.8</td>
<td>30.3</td>
</tr>
</tbody>
</table>
individuals (Kaiser et al., 1989; Nurindah et al., 1999). The absence of preimaginal conditioning or learning was previously reported for *T. remus* reared on *S. frugiperda* eggs and, when tested for different hosts (*S. frugiperda* versus *S. cosmiodes*, *S. frugiperda* vs. *S. albula*, and *S. cosmiodes* vs. *S. albula*) this species preferred *S. cosmiodes* as a host (Goulart et al., 2011). This supports the use of *T. remus* in biological control because of the possibility of rearing it on a single host for several generations without reducing its effectiveness against different target pests in the field.

This host preference of *T. remus* is probably related to the nutritional quality of the host. Adding more complexity to this phenomenon, Molina et al. (2005) claim that not only the nutritional quality of the future host but also of the previous host on which the parasitoid was reared might influence host preference. Egg surface, color and other traits of the host can also influence host preference in a more complex decision-making process (Cônsoli et al., 1999). However, the preference of *T. remus* for *S. frugiperda* eggs when reared on *C. cephalonica* may be more closely related to the superior nutritional value of *S. frugiperda* compared to *C. cephalonica*.

The importance of adult mating must also be considered when rearing *T. remus*, because it can compromise the maintenance of the parasitoid in the field (Pratissoli et al., 2009; Farrokhi et al., 2010). A recent strategy to release egg parasitoids in the field has been to use isolated pupae that are sprayed on plants, for example of *Trichogramma* spp. in Brazil. However, males of *T. remus* which emerge earlier than females due to their shorter lifecycle await females to emerge from the same parasitized egg mass (Cave, 2000), allowing for high mating rates directly after emergence. Therefore, the spraying of *T. remus* individual pupae in the field could negatively impact the reproductive behavior of the parasitoid, highlighting the importance of evaluating mating deprivation.

The absence of mating directly influenced offspring sex ratio, leading to the production of males only. However, other biological parameters were not affected. The observed reproduction mode of *T. remus* via parthenogenesis of the arhenotoky type (i.e. fertilized females give rise to diploid females, whereas unfertilized females give rise to haploid males), has been described as the most common reproductive type for insects of the order Hymenoptera (Pratissoli et al., 2014).

In contrast to sex ratio, the numbers of parasitized eggs per mated and unmated female were similar, as well as parasitoid emergence (%) and longevity of parental females. It should be noted that in our study the longevity of the parental females was similar to that observed for *T. remus* of generation F15 reared on *C. cephalonica* eggs as reported by Pomari-Fernandes et al. (2015). These results differ from Pratissoli et al. (2014) who observed an influence of mating on female longevity of the egg parasitoid *Trichogramma pretiosum* (Riley, 1879) (Hymenoptera: Trichogrammatidae). In addition, Southamer (1993) reports that field releases of parasitoids (genus *Trichogramma*) reproducing by parthenogenesis of the thelytoky type were more efficient compared with parasitoids with those reproducing by parthenogenesis of the arhenotoky type, illustrating the importance of studying the reproductive mode of each parasitoid species.

Our results suggest that although parasitism of *T. remus*, and therefore its control efficiency, was not affected by mating, parasitoid permanence in the field may be impacted when unmated females are released. Some studies reported the failure to establish *T. remus* in the field after its release (Van Waddill and Whitcomb, 1982; Figueiredo et al., 1999). Since additional release might be always necessary when pest population increase, the releasing of mated females might not be important considering that the parasitism of the available eggs in the field would be the same (Carneiro et al., 2009).

Additionally, the success of *T. remus* as biological control depends on the appropriate parasitoid density per *S. frugiperda*.
egg which can be evaluated by releasing different numbers of parasitoids in relation to a given number of pest eggs. We studied parasitoid release in maize, using different densities of T. remus females of the F1, F20, and F45 generation. Parasitism of females of all tested generations was positively related to the density of females per S. frugiperda egg, reaching maximum parasitism between 0.133 and 0.150 T. remus/S. frugiperda egg (40–45 T. remus females per 300 eggs of S. frugiperda). In a similar study, the optimal density was almost 50% less than in our study (25 T. remus females/300 S. frugiperda eggs of T. remus reared on S. frugiperda reported by Pomari et al. 2013). Given that parasitism can strongly depend on the parasitoid species and/or strains (Sá and Parra, 1993), these discrepancies may be due to different parasitoid colonies used in both trials. The rearing of T. remus on C. cephalonica eggs for many generations seems to generate parasitoids with lower parasitism capacity compared with those reared on S. frugiperda eggs, requiring a higher density of T. remus females to effectively control the pest. Although a greater number of parasitoids per pest egg are needed when the parasitoid is reared on a factitious host, several other factors, such as the ease of rearing and low cost of the parasitoid, should be considered. Overall, our results allow the conclusion that (a) T. remus prefers to parasitize S. frugiperda to C. cephalonica eggs, despite the absence of preimaginal conditioning; (b) mating does not affect parasitism capacity and development of T. remus; (c) the optimal release density of T. remus reared on C. cephalonica is between 0.133 and 0.150 females/S. frugiperda eggs, which is higher than the optimal release density of T. remus reared on its natural host S. frugiperda. These results importantly contribute to existing knowledge on the successful rearing of and field release strategies for T. remus.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The authors would like to thank Embrapa Soja and the sponsor agencies CAPES and CNPq for their financial support and scholarships. This paper was approved for publication by the Editorial Board of Embrapa Soja.

References