Biological Control and Crop Protection

Size and flight ability of *Telenomus remus* parasitoids reared on eggs of the factitious host *Corcyra cephalonica*

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Abstract

In two independent bioassays, size and flight ability of parasitoids reared on eggs of *Corcyra cephalonica* for 19 generations and parasitoids reared on a natural host (*Spodoptera frugiperda*) for 250 generations were compared as fast quality control procedures for insect rearing. The size of parasitoids was examined by morphometric analysis using a stereoscope. Length and width of the wings, right hind tibia, and the body of 20 individuals (males and females) were measured. In the analysis of flight ability, parasitoids were divided into three groups: individuals able to fly (“flyers”), individuals that did not fly but had no visible deformation (“walkers”), and individuals with visible deformation (“deformed”). We observed that parasitoids were larger when reared on the natural host than on the factitious host for all evaluated morphological characters. However, there was no significant difference between the treatments regarding the number of “flyers”, “walkers” or “deformed” parasitoids. This indicates that even though the rearing of *T. remus* on a factitious host affects parasitoid size, it does not necessarily affect its flight ability and therefore suggests that *C. cephalonica* is suitable as a factitious host for mass rearing of *T. remus*. Other biological parameters still need to be evaluated, such as host finding ability, parasitism capacity, and parasitoid field efficacy in order to provide a more complete picture of the effects caused by a host change. However, because fast laboratory tests are needed in rearing facilities, the one used in this study might be useful to rapidly assess parasitoid quality.

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Introduction

*Telenomus remus* (Nixon, 1937) (Hymenoptera, Platygastridae) is an egg parasitoid of various Lepidoptera species (Cave, 2000, Pomari et al., 2012), currently only reared on a small scale due to the inherent difficulties of rearing it on its natural host, *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera, Noctuidae) (Pomari-Fernandes et al., 2014). *Spodoptera frugiperda* rearing is too time and resource-consuming (Perkins, 1979), mainly because of larval cannibalism, which requires the rearing of larvae in individual vials to decrease pre-imaginal mortality (Chapman et al., 2000). A possible alternative is rearing the parasitoid on a factitious host. Natural enemy rearing on factitious hosts is a determining factor for the success of many biological control programs because it reduces production costs and increases the viability for the large-scale use of the biocontrol agent (Parra, 1997).

It is crucial that laboratory-reared insects remain capable of controlling target pests in the field similar to biological control agents found in nature (Clarke and McKenzie, 1992). This has been one of the main goals of insect-rearing facilities that were created to supply biological control programs with natural enemies of target pests. Therefore, quality control of the produced insect is a key factor for the success of most biological control programs, with the overall quality of the natural enemy defined as its ability to control target pests after its release in the field (Clarke and McKenzie, 1992). However, field evaluation can be expensive and ineffective if it is not clearly defined. Thus, fast and easy-to-perform laboratory tests are of theoretical and practical interest. They can give an indication of field performance and probably make a more labor-intensive field evaluation unnecessary. For example, in order to improve rearing of the egg parasitoid *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae), the International Organization of Biological Control (IOBC Global Working Group: Quality Control of Mass Reared Arthropods) recommends the evaluation of seven different biological variables in the laboratory (van Lenteren, 2003). However, Prezotti (2001) reported that only the
variables longevity, parasitism index, and flight activity need to be assessed to maintain the high quality standards required for mass rearing of this egg parasitoid.

Flying and walking are important characteristics for a natural enemy’s performance under field conditions since they are directly related to its foraging and dispersal capacity (Gardner and Lenteren, 1986). It is important to point out that the values of these traits may change across generations during mass rearing, and therefore should be closely monitored. Thus, to ensure the quality of laboratory-produced parasitoids, it is important to develop methods that assess the ability of parasitoids to fly and walk.

In addition, insect morphology must be considered, which may be influenced by environmental variation and host changes (Grenier et al., 2001). Parasitoid size is a morphological parameter that might be impaired by host choice. Therefore, this study aimed to evaluate the size and flight ability of T. remus reared on eggs of the factitious host Corcyra cephalonica (Stainton, 1866) (Lepidoptera: Pyralidae) compared to those reared on eggs of the natural host S. frugiperda in order to determine differences between those parasitoids. This research generated information that will help to improve the quality control of future T. remus mass production in the laboratory as well as the use of this egg parasitoid in extensive biocontrol programs.

Material and methods

Parasitoid and host colonies

Corcyra cephalonica eggs, S. frugiperda eggs and T. remus females used in the experiments came from insect colonies kept at Embrapa Soybean, Londrina, State of Paraná, Brazil. Spodoptera frugiperda was originally collected from maize plants in Rio Verde, State of Goiás, and has been kept in the laboratory for approximately five years. This species is reared under laboratory-controlled environmental conditions (25 ± 2 °C temperature, 70 ± 10% relative humidity, and 14/10 h photoperiod [L/D]) and fed on the artificial diet described by Greene et al. (1976) and Parra (2001). Corcyra cephalonica was supplied by UNESP/Ibatobical and has been kept in the laboratory for approximately three years. Corcyra cephalonica is reared on its natural diet, using a methodology adapted from Zeller (1879) for rearing Anagasta kuehieli (Lepidoptera: Pyralidae) (Parra, 1997).

Telenomus remus was originally collected in Ecuador and reared at the parasitoid rearing facilities of ESAQL/USP (Luiz de Queiroz College of Agriculture/University of São Paulo), from where some specimens were transferred to Embrapa Soybean seven years (around 250 generations) ago. At Embrapa Soybean laboratory, T. remus was reared on both S. frugiperda egg masses and on unviable C. cephalonica (up to 24 h) eggs in order to provide two distinct colonies (reared on different hosts). In each colony, host eggs were glued onto white Bristol board (2.5 cm × 5 cm) and placed with eggs previously parasitized by T. remus. Small drops of honey were added to the inside of these tubes to feed the adults as soon as they emerged. The tubes were then closed, and the eggs were allowed to be parasitized for 24 h. The adults that emerged from these eggs were used for trials or colony maintenance.

Morphological characters of Telenomus remus

The experiment was carried out in a 5 × 2 factorial randomized block design (5 parasitoids × 2 parasitoid genders – female or male) with 10 replicates consisting of one adult that was measured individually. Therefore, 10 male and 10 female parasitoids reared on C. cephalonica eggs from four different generations (F1, F8, F13, and F19) were analyzed and compared with parasitoids reared on S. frugiperda. Telenomus remus reared on S. frugiperda eggs and exposed to parasitism on C. cephalonica eggs formed the F0 generation. The F1 generation was the first generation of parasitoids reared on C. cephalonica eggs, and successively afterwards.

For each replicate (adult insect), morphometric evaluations of length and width of the right anterior wing, length of the right hind tibia, and body length (head to the tip of the abdomen) were performed. To measure morphological characters, each specimen was photographed in a stereoscopic microscope (Leica Application Suite, Version 1.6.0). Images were used for morphometric analysis with the software Image J (Version 1.47).

Flight ability of Telenomus remus

The trial was carried out in controlled environmental conditions inside a Biochemical Oxygen Demand (BOD) climate chamber (ELETROLab®, model EL 212, São Paulo, SP, Brazil) set at 70 ± 10% humidity, temperature of 25 ± 2 °C, and 12/12 h photoperiod (L/D). Experimental design was completely randomized with 5 treatments (T. remus from C. cephalonica eggs of the F1, F8, F13, and F19 generations and T. remus from eggs of the natural host, S. frugiperda) and 10 replicates. Each replicate contained 100–150 pupae of T. remus reared on C. cephalonica (F1, F8, F13, and F19) or S. frugiperda eggs. Around the time of emergence, those T. remus pupae were put on a plastic plate of 2.5 cm diameter and 1 cm height, which was placed on the bottom of each replicate. This protocol was originally proposed by Dutton and Bigler (1995) and adapted in ESAQL-USP (Prezotti et al., 2002), as briefly described in the following.

Replicates consisted of a cage made of a PVC cylinder (18 cm high and 11 cm in diameter). The interior of the cage was painted with black ink on a white acrylic latex layer to facilitate attachment. The bottom of the cage was sealed with flexible black plastic (larger than the tube diameter) fitted tightly by a Styrofoam disk approximately one centimeter thick and of the same diameter as the tube. After fitting, the protruding portion of the plastic was fixed to the tube by elastic bands, creating a perfect seal and preventing the escape of parasitoids. Then, entomological adhesive (composed of polybutene and synthetic silica) was spread over the walls of the cage (3.5 cm from the bottom), to serve as a trap for “walkers” (parasitoids that were unable to fly but could walk and had no visible deformation). A transparent Petri dish sprayed with entomological adhesive was placed on top of the cylinder to serve as a trap for flying parasitoids.

The position and the number of parasitoids in the adhesive ring (“walkers”), in the Petri dish (“flyers”), and “deformed” were recorded and used to calculate their percentages of the total number of emerged adults. The parasitoids considered “non-flyers” were observed under a microscope to determine the percentage of individuals with wing deformities (“deformed”) (Prezotti et al., 2002).

Data analyses

Prior to ANOVA, experimental results were subjected to exploratory analyses to assess the assumptions of normality of residuals (Shapiro and Wilk, 1965) and homogeneity of variance of the treatments (Burr and Foster, 1972) and, if necessary, transformed for ANOVA. For “Deformed” parasitoids, data was transformed by √(X + 0.5). The treatment means were then compared by the Tukey test at the 5% probability level (SAS Institute, 2001).

Results

Morphological characters of Telenomus remus

There was no interaction between parasitoids and gender in the factorial analyses (Table 1), and therefore both factors were
analyzed independently. In the parasitoid analysis, differences were observed in all evaluated morphological characters: wing length, wing width, body length, and right hind tibia length (Table 1). Overall, morphological measurements (mm) of T. remus had higher values when the parasitoid was reared on the natural host, S. frugiperda. Wing length and width values were significantly higher of T. remus reared on S. frugiperda than of parasitoids reared on C. cephalonica eggs (F1, F8, F13, and F19 generations). Body length did not differ between parasitoids reared on eggs of S. frugiperda or C. cephalonica F8, F13, and F19. Also, the right hind tibia length was similar between parasitoids from S. frugiperda and C. cephalonica F8 (Table 1). In the analysis of the factor gender, males had longer wings and tibiae than females but shorter body length. In contrast, wing width did not differ between the genders (Table 1).

### Flight ability of Telenomus remus

The total number of parasitoids emerging from the 100 to 150 T. remus pupae was similar for the two hosts, varying from 114.8 (C. cephalonica F1) to 127.6 (S. frugiperda) (Table 2). The number of T. remus "flyers" reared on S. frugiperda eggs (102.8) did not differ from that of parasitoids reared on C. cephalonica eggs in the F1 (96.8), F8 (96.4), F13 (101.9), and F19 (106.3) generations (between 80.33% and 84.43% "flyers", Table 2). Likewise, the number of parasitoids without any visible deformation that did not fly ("walkers") were similar between treatments. In addition, "deformed" parasitoids were less than 1% in each treatment, and did not differ between treatments (Table 2).

### Discussion

The observed reduction in morphological characters of T. remus when switching from egg of a natural to those of a factitious host might be associated with the different forms and sizes of both host eggs. Moreover, different host eggs might have different surfaces, chorion structures, and other egg properties during embryonic development (Cónsoli et al., 1998). Those host features were previously pointed out as important factors for T. remus parasitism and development by Bueno et al. (2014). There is an important difference in shape between eggs of C. cephalonica, which are ellipsoidal (mean length 573.5 mm and mean width 346.1 mm) and eggs of S. frugiperda, which are almost spherical (mean length 454.9 mm

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Morphological characters (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wing length</td>
</tr>
<tr>
<td>Parasitoid</td>
<td></td>
</tr>
<tr>
<td>S. frugiperda</td>
<td>0.546 ± 0.008 a</td>
</tr>
<tr>
<td>C. cephalonica F1</td>
<td>0.485 ± 0.007 b</td>
</tr>
<tr>
<td>C. cephalonica F8</td>
<td>0.490 ± 0.006 b</td>
</tr>
<tr>
<td>C. cephalonica F13</td>
<td>0.487 ± 0.007 b</td>
</tr>
<tr>
<td>C. cephalonica F19</td>
<td>0.476 ± 0.007 b</td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>0.483 ± 0.005 B</td>
</tr>
<tr>
<td></td>
<td>0.155 ± 0.002</td>
</tr>
<tr>
<td>Statistics</td>
<td>CV (%)</td>
</tr>
<tr>
<td></td>
<td>Ftotal/generation</td>
</tr>
<tr>
<td></td>
<td>Fgender</td>
</tr>
<tr>
<td></td>
<td>Ftotal/gender*gender</td>
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<tr>
<td></td>
<td>Fgender</td>
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<tr>
<td></td>
<td>Ftotal/gender*gender</td>
</tr>
</tbody>
</table>

* Means ±SE followed by the same lower case letter in host/generation and capital letters in gender parameters did not statistically differ (Tukey test, p > 0.05) for each morphological character.

### Table 2

<table>
<thead>
<tr>
<th>Host</th>
<th>Total parasitoids*</th>
<th>Flyers*</th>
<th>Walkers*</th>
<th>Deformed†</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. frugiperda</td>
<td>127.6 ± 3.9**</td>
<td>102.8 ± 2.7 (80.83%)</td>
<td>23.9 ± 2.4 (18.49%)</td>
<td>0.9 ± 0.2 (0.68%)</td>
</tr>
<tr>
<td>C. cephalonica</td>
<td>114.8 ± 4.2</td>
<td>96.8 ± 3.4 (84.43%)</td>
<td>17.3 ± 1.8 (14.92%)</td>
<td>0.7 ± 0.2 (0.64%)</td>
</tr>
<tr>
<td>C. cephalonica</td>
<td>116.4 ± 2.0</td>
<td>96.4 ± 1.9 (92.89%)</td>
<td>19.7 ± 1.7 (16.86%)</td>
<td>0.3 ± 0.2 (0.25%)</td>
</tr>
<tr>
<td>C. cephalonica</td>
<td>122.7 ± 4.2</td>
<td>101.9 ± 3.0 (83.33%)</td>
<td>20.2 ± 2.7 (16.17%)</td>
<td>0.6 ± 0.3 (0.50%)</td>
</tr>
<tr>
<td>C. cephalonica</td>
<td>106.3 ± 2.4</td>
<td>106.3 ± 2.3 (83.84%)</td>
<td>20.0 ± 1.9 (15.70%)</td>
<td>0.6 ± 0.2 (0.47%)</td>
</tr>
<tr>
<td>CV (%)</td>
<td>9.03</td>
<td>8.39</td>
<td>17.68</td>
<td>21.38</td>
</tr>
<tr>
<td>p</td>
<td>2.87</td>
<td>2.47</td>
<td>1.09</td>
<td>0.98</td>
</tr>
</tbody>
</table>

* Generation of parasitoids used in the treatment (T. remus was reared on eggs of S. frugiperda for approximately 250 generations and on eggs of C. cephalonica for 1 (F1), 8 (F8), 13 (F13), and 19 (F19) generations).

† Total number of parasitoids emerged in the treatment (Flyers + Walkers + Deformed).

‡ Number of parasitoids able to fly, and its proportion (%) of the total number of parasitoids emerged.

§ Number of parasitoids that did not fly but had no visible deformation, and its proportion (%) of the total number of parasitoids emerged.

¶ Number of parasitoids with visible deformation, and its proportion (%) of the total number of parasitoids emerged.

†† Statistics performed on transformed data by √X + 0.5.

** Non-significant.
and mean width 390.2 mm) (Cósoli et al., 1999). Host size is reported to be directly related to parasitoid dimensions (Gautam, 1986). For example, adults of *T. remus*, reared on Agrotis spinerfa (Hubner, 1808) (Lepidoptera: Noctuidae) eggs were larger than parasitoids from Spodoptera littura (Fabricius, 1775) (Lepidoptera: Noctuidae) eggs, which are smaller. However, both *C. cephalonica* and *S. frugiperda* eggs have a similar volume of around 0.036 mm³ (Cósoli et al., 1999). This indicates that the host influence on the morphology of the produced parasitoids is not due to egg size alone.

For example, different time spans of co-evolution between *T. remus* and its various hosts may have resulted in different degrees of adaptation to each specific host egg (Bueno et al., 2009). The studied *T. remus* colony has been reared on *S. frugiperda* eggs under laboratory conditions for about 250 generations, possibly leading to better adaptation of the parasitoid strain to this host compared with *C. cephalonica*, which was reared for only 19 generations. As a consequence, memory of the original host may be involved in host-locating ability and host acceptance and therefore may have influenced the results reported here. However, it is important to point out that parasitoids display interspecific variation in learning rate and memory dynamics that reflect variation in the foraging task and largely determines their fitness (Hoedjes et al., 2011). Host preference innate to the species (pre-imaginal conditioning) might be altered by learning rate and memory dynamics as a result of experience gained during foraging and parasitism (Pomari-Fernandes et al., 2015). Although learning is usually considered as more important for generalists, it is also a crucial factor for specialist parasitoid wasps (Hoedjes et al., 2011) such as *T. remus*.

Parasitoids can change their innate preferences for odor cues that guide them to patches of hosts (Hoedjes et al., 2011). Associating the signs learned during parasitism or during development enables parasitoid females to locate and parasitize its host with greater efficiency and speed (Cober, 1985; Nurindah et al., 1999; Hoedjes et al., 2011), a phenomenon known as α-conditioning or associative learning (Vinson, 1998; Nurindah et al., 1999). Therefore, although female parasitoids have an innate preference for certain odors, foraging efficiency of most species is optimized by associative learning (Hoedjes et al., 2011). Parasitoids which had experienced the presence of a certain host species, thereafter narrow their (olfactory) ‘search image’ by learning, as a form of temporal specialization (Hoedjes et al., 2011).

In addition, morphological characters are listed in the literature as good indicators of the fitness and fertility of adult parasitoids from different hosts and rearing facilities. Changing host species can clearly impact parasitoid morphology, as reported for different species of the genus Trichogramma (Kazmer and Luck, 1991; Grenier et al., 2001). Differences among hosts can be due to various reasons. For example, *S. frugiperda* lays its eggs in superposed masses while *C. cephalonica* lays its eggs individually. This can affect not only parasitism but also the host’s suitability for parasitoid development (Cósoli et al., 1999), directly affecting the quality of the produced parasitoids. Therefore, to study these differences in order to determine an appropriate host for parasitoid mass rearing that maximizes production, reduces costs, and does not change the morphological characters of natural enemies might be important for the success of a biological control program (Vaz et al., 2004).

Even though individuals of *T. remus* reared on *C. cephalonica* were smaller (mainly in their wing dimensions) than individuals reared on *S. frugiperda* eggs, no impairment in flying capacity was observed in the laboratory test (short distance). The similarity in the percentages of flying individuals from *C. cephalonica* (generations F1, F2, F3, and F10) and individuals from the natural host *S. frugiperda* indicates that while the reduction in wing size was significant, it was not sufficient to compromise the flight ability of the insect. The average percentage (≈83%) of parasitoids captured on the cover (‘flyers’) was similar to that found by other authors using the same protocol for other parasitoid species. Rodrigues et al. (2009) found averages from 85.9 to 97.7% of flying individuals and Prezotti et al. (2002) reported mean percentages between 74.7 and 90.6% for *T. pretiosum*. The similarity of results suggests that the protocol tested can also be used to study the flight activity of *T. remus* for quality control in mass rearing facilities.

The average percentage of ‘walkers’ caught in the glue ring (≈16%) was higher than that obtained in other studies using *T. pretiosum* (Prezotti et al., 2002; Rodrigues et al., 2009), which is probably related to behavioral differences between species. The percentage of these individuals with deformities such as stunted or folded wings was less than 1%. This confirms that the visual observation of the percentage of individuals with deformed wings alone is insufficient to characterize the quality of the parasitoid, since almost all of the ‘walkers’ apparently had normal wings (Prezotti et al., 2002). These non-flying individuals should be better evaluated before determining whether they could have a negative effect on biological control programs. Gardner and Lenteren (1986) considered that both flying and walking are important features for the performance of natural enemies in field conditions, as they relate to foraging and dispersal.

Considering both the morphometry and the flight test, our results suggest that the host-specific size differences of *T. remus* especially regarding wing length and width, did not necessarily affect the flight activity of the parasitoid since the percentage of “flyers” did not significantly differ between hosts. In the future, the most appropriate number of insects to be released should be studied, based on the parasitism capacity of *T. remus*. In order to test the hypothesis that releasing a greater number of *T. remus* reared on *C. cephalonica* will be as effective as releasing a smaller number of the same parasitoid reared on *S. frugiperda*.

Other biological characteristics may also be affected due to changes in parasitoid size. For example, in nature it is common to find increasing fertility with increasing adult size. Chau and Mackauer (2001) reported that the parasitoid Monoctonus paulensis (Ashmead) (Hymenoptera: Braconidae) preferred larger aphids, which was understood as a process of selection to maximize their fertility (number, size, and quality of eggs). In addition, the quality of the host is not simply related to size, reflecting the biomass available to be consumed by the parasitoid, but also to the development period. To maximize its size in low-quality hosts, the parasitoid can reduce its growth rate and increase the development period (Sequeira and Mackauer, 1992). Adult parasitoids can also increase their lifespan during the absence of suitable hosts (Carneiro et al., 2009) or even develop mechanisms of oocyte absorption to physiologically synchronize egg formation with host availability (Chabi-Olaye et al., 2001). Therefore, other biological parameters still need to be evaluated such as parasitoid finding ability, parasitism capacity, and parasitoid field efficacy in order to have a more complete picture of host change effects on parasitoid biology. However, fast laboratory tests are needed in rearing facilities and the one used in this study might be useful to rapidly indicate parasitoid quality. In addition, taking into consideration the results discussed here, there is a clear indication that *C. cephalonica* can be used as a factitious host for mass rearing of *T. remus*.

Conflicts of interest

The authors declare no conflicts of interest.

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