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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* eggs) 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.

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

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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 ($25 \pm 2\,^{\circ}C$ temperature, $70 \pm 10\%$ 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/Jaboticabal 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 kuehniella* (Lepidoptera: Pyralidae) (Parra, 1997).

Telenomus remus was originally collected in Ecuador and reared at the parasitoid rearing facilities of ESALQ/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 (F_1, F_8, F_{13}, and F_{19}) 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 F_0 generation. The F_1 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 $70 \pm 10\%$ humidity, temperature of $25 \pm 2\,^{\circ}C$, and 12/12 h photoperiod (L/D). Experimental design was completely randomized with 5 treatments (*T. remus* from *C. cephalonica* eggs of the F_1, F_8, F_{13}, and F_{19} 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* (F_1, F_8, F_{13}, and F_{19}) 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 ESALQ-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 stereoscope 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 $\sqrt{X + 0.5}$. 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