Biology of *Trichogramma marandobai* and *T. manicobai* (Hymenoptera: Trichogrammatidae) in eggs of *Erinnyis ello* (Lepidoptera: Sphingidae)

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**ABSTRACT**

Parasitoids of the genus *Trichogramma* are promising for the biological control of insect pests in several crops, including cassava, which is severely attacked by *Erinnyis ello* L., 1758 (Lepidoptera: Sphingidae). Evaluating the biological aspects of these parasitoids to understand their dynamics is an important step towards the implementation of this control strategy in the field. Thus, our objective was to evaluate the biology of *Trichogramma manicobai* Brun, Moraes & Soares, 1984, and *T. marandobai* Brun, Moraes & Soares, 1986 in *E. ello* eggs. The parasitoids were obtained by collecting *E. ello* eggs from a commercial production of cassava, and the host's eggs were obtained from laboratory and greenhouse rearings. The average duration of a generation (*T*), net reproduction rate (*R₀*), intrinsic rate of increase (*r₀*), and the finite rate of increase (*λ*) were estimated, and from these, the fertility life table was calculated. The results indicated that *T. marandobai* has both higher net reproduction rate and a higher intrinsic rate of increase as well as requires less time to double its population than *T. manicobai*. Thus, *T. marandobai* has potential for natural and conservative biological control of *E. ello*. In addition, its potential in applied biological control should be evaluated through studies on the viability of its mass rearing in alternative hosts and its dispersion behavior in the field.

**Introduction**

Cassava, *Manihot esculenta* Cranz, 1766 (Malpighiales: Euphorbiaceae), is one of the main crops used in human and animal food worldwide (Schons et al., 2009). Several pest insects attack this crop, reducing its production. *Erinnyis ello* L., 1758 (Lepidoptera: Sphingidae) is considered the main defoliator of cassava crops in the Neotropical region (Bellotti et al., 1999). In southern Brazil, the insect appears in the middle of October with the beginning of the rainy period and high temperatures (Gomes and Leal, 2003; Maia and Bahia, 2010), and the infestation often persists until April, when temperatures begin to decrease and the senescence of the leaves begins (EMBRAPA, 2005; Alves, 2006).

Infestation begins either with adults emerging from pupae present in the field or from populations migrating from other regions, a characteristic common to this species (Bellotti et al., 2012a). The oviposition occurs on the cassava leaf and the caterpillars appear after three to four days, with the duration of the larval phase between 12 to 15 days and life cycle from 27 to 44 days (Schmitt, 2002). The *E. ello* moth can achieve two to three generations in the western region of Paraná when the cassava crop has leaves, varying with the migratory rate of the insect, temperature, humidity, and agricultural year (Bellotti et al., 1999, Carvalho and Nakano, 1988, Farias, 2003). Successive infestations can occur in only one crop, which reduced production during the agricultural years 2014/2015 and 2015/2016 in the state of Paraná (IEA, 2016).

The *E. ello* caterpillar has a high capacity for leaf consumption, being able to feed on 1,100 cm² during the larval stage and cause up to 100% defoliation in the period of greatest accumulation of photoassimilates (Bellotti and Arias, 1988). They can also reduce root production from 26 to 45% during only one attack, varying with the phenological stage of the crop and the infestation of the pest (Bellotti et al., 1999). Generally, the second generation of the pest exhibits greater potential to injury the crop.

Fortunately, this insect can be controlled by more than 40 biological control agents (Bellotti et al., 1992; Aguiar et al., 2010; Querino and Zucchi, 2019), including entomophage and entomopathogen agents.
The main parasitoid species of *E. ello* eggs that occur naturally in Brazil are *Trichogramma pretiosum* Riley, 1879, *Trichogramma atovovirilia* Oatman & Platner, 1983, *Trichogramma manicobai* Brun, Moraes & Soares, 1984, and *Trichogramma marandobai* Brun, Moraes & Soares, 1986 (Vieira et al., 2014; Querino and Zucchini, 2019). However, no *Trichogramma* species have been released in commercial cassava crops.

Although the literature discusses many natural enemies of *E. ello*, considering the planting system in a large area, pest dynamics have required increased applications of chemical products, mainly due to the increase in population of other pests (Bellotti et al., 2012a; Bellotti et al., 2012b). To reduce the negative impacts of these applications, efforts have been made to implement an Integrated Pest Management (IPM) program, in which biological control is the main strategy. A successfully applied biological control program requires basic knowledge, such as taxonomy, biology, parasitism rate, intra- and interspecific interaction, economic viability, and rearing and multiplication techniques (Parra et al., 2015).

Initially, these studies are carried out in the laboratory to determine which agents exhibit potential. Then semi-field and field experiments can be conducted to verify whether the data obtained in the laboratory are consistent with the parasitism efficiency and capacity obtained in the field and/or greenhouse.

Although the species of *Trichogramma* that parasitize *E. ello* eggs are known in Brazil (Vieira et al., 2014; Querino and Zucchini, 2019), researchers have still not studied the biology of these parasitoids in this host. One of the main obstacles to obtaining this information is the difficulty of mass rearing *E. ello*. No adequate artificial diet is available to rear this lepidopteran, and the use of its natural food would require too many cassava plants. Thus, the required space, manpower, and expense make natural rearing unfeasible. In addition, knowledge about alternative rearing hosts is limited for *T. marandobai* and *T. manicobai* (Milanez et al., 2009). The few field surveys conducted in Brazil (Oliveira et al., 2010; Souza et al., 2016; Noronha et al., 2020) indicate that *T. marandobai* is the predominant species and has a high capacity for parasitism.

No basic comparative studies have been conducted on the fertility life table that would indicate which species of *Trichogramma* might be the most efficient for the applied biological control of *E. ello* in Brazil (Brun et al., 1986; Botelho, 1997; Oliveira et al., 2010). Several *Trichogramma* species are widely used in mass releases in several commercial crops throughout the world (Wajnberg and Hassan, 1994; Pratissoli et al., 2003). An example of successful biological control in Brazil is the use of *T. pretiosum* and *T. galloi* to control the sugarcane borer, *Diatrea saccharalis* (Parra et al., 2010).

For these reasons, determination of life table of each parasitoid species is necessary to select the most appropriate species for biological control of the *E. ello* caterpillar. In this work, we obtained the fertility life table of *T. marandobai* and *T. manicobai*, as egg parasitoids of *E. ello*, under a controlled environment, for the first time in Brazil. This research will contribute to the implementation of both augmentative and conservative biological control within the Brazilian cassava IPM program.

**Material and methods**

*Erinyis ello* rearing

Depending on the life stage, the rearing was maintained in the laboratory or greenhouse. Adults were maintained in 6 × 4 × 2 m screened cages in a greenhouse. Each cage contained 4-L pots with cassava plants for oviposition. When the presence of eggs was verified, the plants were removed from the cage and kept on benches in the greenhouse until the caterpillars hatched. Then they were transferred to the laboratory.

In the laboratory, 3 to 4 caterpillars were placed on cassava plants in 1-L plastic pots and maintained in a semi-climatic room with a 12-h photophase and a temperature of 25 ± 2 °C. The caterpillars were fed daily with cassava leaves, previously disinfected by washing in 3% hypochlorite solution and then rinsed in distilled water. The caterpillars were kept in the pots until they pupated. Then they were transferred to plastic trays with moistened vermiculite and later placed in a climate-controlled chamber (B.O.D.) with 70 ± 10% RH and 14-h photophase. The temperature was adjusted to between 19 and 28 °C to accelerate or decelerate, respectively, the emergence of adults, according to the requirements for the tests. Four generations were obtained in the laboratory before installation of the test.

*Trichogramma marandobai* and *T. manicobai*

*Erinyis ello* eggs were collected from commercial cassava crops in the municipality of Marechal Cândido Rondon, PR (24°69′ 20.9″ S e 54°13′ 99.3″ W), taken to the laboratory and kept under controlled conditions. After emergence, the parasitoids were reared and maintained in the laboratory, in test tubes (13 × 100 mm), which contained a drizzle of pure honey to feed the parasitoids. Some of the first specimens were mounted (Hoyer’s mounting medium with Canada balsam) for microscope identification according to the methodologies proposed by Querino and Zucchini (2011). After identification, specimens were isolated and grouped by species, to initiate laboratory rearings.

Parasitoid adults were provided with *E. ello* eggs from the laboratory rearing. Parasitized *E. ello* eggs were maintained in a climate-controlled chamber (B.O.D.) at a constant temperature of 25 ± 1 °C and photophase of 14 h, until the third generation, when a sufficient number of insects were obtained to conduct the test.

Biological parameters and fertility life table determination of *T. manicobai* and *T. marandobai*

The biological study was conducted using a completely randomized design with two species of parasitoids – *T. manicobai* and *T. marandobai* – and 25 replicates per species, maintained under controlled conditions (B.O.D.: 25 ± 1 °C, 70 ± 10% RH, and 14 h photophase). Each replicate contained a 24-h-old mated female, individualized in a transparent glass tube (2.5 × 8.5) containing a drop of pure honey as a food source and closed with a cotton ball. Every day, five *E. ello* eggs, up to 24-h old, were offered to each female until her death. Parasitism was allowed for 24 h. After that, the eggs from each replicate were transferred to gelatin capsules to avoid dehydration, where they remained until the emergence of adults.

The variables analyzed were: Percentage of parasitism (obtained through the equation: P (%) = (number of eggs parasitized) / (total number of eggs exposed to parasitism) × 100); total number of parasitized eggs (average number of eggs parasitized per female); parasitism viability (obtained by the ratio of the number of eggs with an emergence hole to the number of parasitized eggs); individuals per egg (count of the emerged adults per egg); number of offspring (count of the emerged adults); number per egg female and male (count of female and male adults per egg); sex ratio (obtained by the equation: [♀/(♂+ ♀)]; period egg-adult, in days (conducted through daily observations, always at the same time, in a 24-h interval); female longevity (period, in days, between emergence and death).

From the longevity, survival, and oviposition data of each female, the fertility life table was constructed according to the methodology cited...
by Silveira Neto et al. (1976). The net reproduction rate ($R_0$), intrinsic rate of increase ($r_m$), population time (Dt), average generation time (T), and finite rate of increase ($\lambda$) were calculated, using the formulas:

\[ R_0 = \sum l_x m_x \]
\[ T = \sum (l_x m_x x) \]
\[ r_m = \text{log } R_0 / T.0.4343 \]
\[ Dt = \ln (2) / r_m \]
\[ \lambda = e^{rm} \]

where, $x$ is the age of individuals in days, $l_x$ is the age-specific survival, and $m_x$ is the age-specific number of female offspring. The maximum rate of population growth is when the lines of specific fertility ($m_x$) and survival rate ($l_x$) intersect in a graph.

### Statistical Analyses

The parameters of the fertility life table were estimated using the "Jackknife" technique (Meyer et al., 1986) and the means compared by the unilateral t-test (P<0.05), using the software “Lifetable.sas” (Maia et al., 2000) in the “SAS System” environment (SAS Institute, 2009). The other parameters were tested for normality by the Shapiro-Wilk test and for homoscedasticity by the Bartlett test. Was used test U Mann-Whitney, nonparametric test was performed at 5% probability using the program Statistica 7 (StatSoft Inc., 2004).

### Results

The species of *E. ello* egg parasitoid identified and successfully reared in the laboratory were *T. marandobai* and *T. manicobai*. The biological parameters of both *Trichogramma* species when using *E. ello* as host are summarized in Table 1.

The percentage of *T. marandobai* and *T. manicobai* parasitism did not differ statistically (Table 1). However, the number of parasitized eggs was statistically different, and *T. marandobai* parasitized about 1.5 times more eggs than *T. manicobai*. This led to 34.35% more descendants of *T. marandobai* than *T. manicobai*. However, neither the percentage of parasitism, which was greater than 80%, nor the number of offspring per egg, which was approximately 12 individuals, differed between the two species (Table 1). *Trichogramma marandobai* and *T. manicobai* exhibited a high capacity to produce offspring, despite the small number of parasitized eggs.

The sex ratio did differ statistically (Table 1), for the two species studied, in which the number of females per egg was 3.6 and 7.8 times greater than that of males for *T. marandobai* and *T. manicobai*, respectively. No statistical difference was observed the number of females per egg between the two species, however there was a statistical difference for the number of males (Table 1). The egg-adult period also similar for both species.

Charting the production of offspring throughout the female's life elucidated that the highest daily fertility rates occurred on the first day of life, for both parasitoids species (Fig. 1). *T. manicobai* had the highest fertility rate with 20.60 individuals per female (Fig. 1b), whereas for *T. marandobai*, this value was 17.7 (Fig. 1a). However, the oviposition period for *T. manicobai* was shorter at seven days, while *T. marandobai* oviposited until the tenth day.

The values obtained for the fertility life table, except for the average interval between generations (T), differed statistically between the species studied, and was more favorable for *T. marandobai* (Table 2).

### Table 1

**Biological parameters of *Trichogramma* species (Hymenoptera: Trichogrammatidae) in eggs of *Erinyx ello* (Lepidoptera: Sphingidae).**

<table>
<thead>
<tr>
<th>Species</th>
<th>Parasitism (%)</th>
<th>Total parasitized eggs*</th>
<th>Viability (%)</th>
<th>Individuals per egg*</th>
<th>Number of offspring*</th>
<th>Number per egg*</th>
<th>Sex ratio</th>
<th>Period egg-adult (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. marandobai</em></td>
<td>17.5 (18.3)</td>
<td>7.0 (7.7) a</td>
<td>85.0 (84.7)</td>
<td>11.5 (11.9)</td>
<td>73.0 (74.3) a</td>
<td>9.8 (9.17) a</td>
<td>1.8 (2.5) a</td>
<td>0.8 (0.8) b</td>
</tr>
<tr>
<td><em>T. manicobai</em></td>
<td>14.3 (15.4)</td>
<td>5.0 (5.0) b</td>
<td>100.0 (87.9)</td>
<td>12.0 (12.2)</td>
<td>49.0 (48.8) b</td>
<td>11.2 (10.9) b</td>
<td>1.0 (1.4) b</td>
<td>0.9 (0.9) a</td>
</tr>
<tr>
<td>U</td>
<td>184.0</td>
<td>130.0</td>
<td>207.0</td>
<td>227.5</td>
<td>122.5</td>
<td>200.5</td>
<td>154.0</td>
<td>140.5</td>
</tr>
<tr>
<td>Z</td>
<td>1.35</td>
<td>2.63</td>
<td>0.84</td>
<td>0.32</td>
<td>2.79</td>
<td>0.96</td>
<td>2.06</td>
<td>2.37</td>
</tr>
<tr>
<td>P-value</td>
<td>0.176</td>
<td>0.008</td>
<td>0.400</td>
<td>0.0742</td>
<td>0.005</td>
<td>0.335</td>
<td>0.039</td>
<td>0.017</td>
</tr>
</tbody>
</table>

* Medians followed by the same letter in the column do not differ by Mann-Whitney U test (p<0.05). Values in parentheses represent Means. ** Not significant.

### Table 2

**Mean generation time (T), doubling time (Dt), net reproduction rate ($R_0$), intrinsic rate of increase ($r_m$), and finite rate of increase ($\lambda$) of two species of *Trichogramma* in *Erinyx ello* eggs.**

<table>
<thead>
<tr>
<th>Species</th>
<th>T (days)</th>
<th>Dt (days)</th>
<th>$R_0$ (♀/♀)</th>
<th>$r_m$ (♀/♀/day)</th>
<th>$\lambda$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. marandobai</em></td>
<td>9.49 (9.14 - 9.83)</td>
<td>1.68 (1.60 - 1.76) b</td>
<td>49.29 (40.93 - 57.65) a</td>
<td>0.41 (0.39 - 0.42) a</td>
<td>1.50 (1.48 - 1.53) a</td>
</tr>
<tr>
<td><em>T. manicobai</em></td>
<td>9.63 (9.36 - 9.89)</td>
<td>1.84 (1.73 - 1.95) a</td>
<td>36.87 (28.93 - 43.91) b</td>
<td>0.37 (0.35 - 0.39) b</td>
<td>1.45 (1.42 - 1.48) b</td>
</tr>
</tbody>
</table>

*Means followed by the same letter in the column do not differ between them by the Jackknife method (P>0.05). Values in parentheses represent confidence interval (CI).*
This species took less time to double its population (DT), achieved higher net reproduction rates ($R_0$), had a higher intrinsic rate of increase ($r_m$), and exhibited a higher finite rate of increase ($\lambda$), which indicates better performance by *T. marandobai* than *T. manicobai*.

*Trichogramma manicobai* required 1.09-fold more time to double its population, and its net reproduction rate ($R_0$) was 25% less than *T. marandobai*. The intrinsic rate of increase ($r_m$), which indirectly represents the daily contribution of each female in relation to the number of females in the population, of *T. marandobai* was 1.10 times greater than that of *T. manicobai* (Table 2). Such results indicate the superiority of *T. marandobai* in increasing its population, because with more females, its population will double in less time.

Among the biological parameters evaluated, *T. marandobai* achieved greater longevity and higher population increase in a shorter period than *T. manicobai*, under laboratory conditions.

**Discussion**

Considering that *E. ello* produce a relatively large egg, about 1.5 mm in diameter, which has a high level of nutrients, it provides the species *T. marandobai* and *T. manicobai* excellent capacity to produce offspring. Thus, more individuals develop per host, depending on the volume and nutrition of the egg. This reflects in lower energy expenditure in the search for a host to ensure their offspring.

The parasitism capacity of *Trichogramma* species varies depending on the host in which it multiplies. However, it has no direct reflection on the number of offspring, since the number of individuals per egg depends on the size of the host’s egg (Beserra and Parra, 2004; Meira et al., 2011).

Female parasitoids use chemical, physical, and visual clues to select hosts (Consoli and Grenier, 2010; Van Atta et al., 2015; Gardner and Hoffmann, 2020). Studies conducted on the preference of *T. pretiosum* for a particular host found that the chemical characteristics, shape, size, and age of the host may influence the acceptance (Bourchier et al., 1994; Hassan, 1994; Brotoedojo and Walter, 2006). Therefore, the parasitoid’s preference for a particular host should be tested first in the laboratory. Data from the fertility life table are essential to analyze the biological parameters and dynamics between host parasitoids, indicating whether the species exhibits good performance and could be a potential candidate for biological control. After these results, the next steps can be taken towards the implementation of a biological control program, an important control method to begin IPM.

The biological parameters obtained from the two main species of *Trichogramma* that parasitize *E. ello* – *Trichogramma marandobai* and *T. manicobai* – found that the number of descendants per egg is important. Although the number of parasitized eggs per female and the percentage of parasitism did not present an expressive quantity, the ability to generate offspring was high.

This ability to generate more descendants was demonstrated by Vianna et al. (2011), who obtained an average parasitism of 19 eggs per female of *T. pretiosum* in eggs of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae), which was more than the *Trichogramma* species in the present study. However, the number of individuals *T. manicobai* and *T. marandobai* that emerged from *E. ello* eggs was much higher than emerged from *A. gemmatalis* eggs (1.38), which resulted in a significant difference in the final number of individuals.

Evaluation of biological parameters of *T. marandobai* in an alternative host, *Chloridea vitaecsens* (Lepidoptera: Noctuidae), which is smaller than *E. ello*, found that the number of offspring and individuals per egg was low (Vieira et al., 2015), and different from the data obtained in the natural host verified in this work. Thus, these species, by parasitizing larger eggs, can reduce the energy expended searching for more of the host’s eggs and instead invest their energy to increase their reproductive potential, with larger oviposition.

The size of the host egg has been reported as one of the most important factors affecting the parasitism taxa of mass-reared *Trichogramma* species. This factor influences the size of the descendants and consequently their reproductive performance (Xu et al., 2020). From large hosts, more *Trichogramma* emerge and they develop in less time, compared to smaller hosts, due to the higher nutritional quantity of the host. These stronger and more abundant descendants have greater capacity to search and disperse; thus, larger *Trichogramma* are more fertile and produce more offspring (Hohmann et al., 1988; Bai et al., 1992).

The proportion of males and females is important, because a higher number of females can increase the parasitoid population and maintain the species (Borba et al., 2006). A high quantity of females is an important characteristic, because the efficiency of parasitism is high when a higher number of females are produced. These detect and oviposit in the host, which reflects in the effectiveness of biological control (Pereira et al., 2009). The sex ratio of the parasitoid species is influenced, in addition to other factors, by the nutritional quality of the host. The female *Trichogramma* can assess the nutritional quality and quantity of its host, to determine the proportion of males and females that it will oviposit (Rodrigues, 2013). Hosts with lower nutritional levels usually lead to more males, as they require less nutrients to develop (Vinson, 1997).

The sex ratio results of both species analyzed in this study suggest, according to Navarro (1998) and Van Lenteren et al. (2003), their potential for biological control of *E. ello*. Because the sex ratio of *T. marandobai* and *T. manicobai* presented satisfactory values, which is one of the important parameters when assessing species for use in biological control programs (Pereira et al., 2019). Study with *Trichogramma marandobai* achieved values close to those obtained in an alternative host (Vieira et al., 2015). However, as the species *T. manicobai* does not have an alternative host, there are no studies in the literature about the biological parameters for this species, which makes our work pioneering.

In *Trichogramma*, the length of the period from egg to adult mainly depends on temperature but can also be influenced by the origin of the insect, the host, the culture that it was collected, and the adaptation of the species or lineage (Pratissoli et al., 2003; Poorjavad et al., 2011). The highest fertility rates in *Trichogramma* spp. were observed at the beginning of adulthood and with age (days) they decrease. Loss of female fertility is natural behavior, which is directly influenced by age (Zago et al., 2008).

The values of net reproduction rate ($R_0$) are important to determine the behavior of a parasitoid population, since lower $R_0$ values indicate population decline (Bellows Junior et al., 1992). This is an important parameter to consider for the control potential of the parasitoid.

The intrinsic growth rate is the main parameter of a $r_m$ fertility life table (Pedigo and Zeiss, 1996). The higher this value, the more successful the species will be in a given environment (Andrewartha and Birch, 1954).

*Trichogramma marandobai*, in general, presented a better performance than *T. manicobai*, with a higher number of individuals, a higher percentage of parasitism, shorter development time for the earlier life phases, and more female progeny. As a result, it achieved better rates in the fertility life table. Studies on the fluctuation of parasitoids in cassava field indicate that *T. marandobai* exhibits higher percentages than *T. manicobai*, which may be because this species presents differences in preference for host, crop, and search behavior (Schmidt and Smith, 1985; Hassan and Guo 1991; Wührer and Hassan, 1993).

This study performed the first steps by demonstrating, under laboratory conditions, that the species *T. marandobai* and *T. manicobai* have potential as biological control agents. However, selectivity tests,
flight capacity, adaptability to the laboratory environment, suitability for alternative hosts that are easy to handle, and economic viability, among other tests are essential for quality control of a biological control program (Parra, 2002).

Therefore, additional laboratory studies as well as semi-field and field tests must be conducted to verify the parasitism capacity and efficiency of each species, because the dynamics of the host parasitoid must be tested for efficient management. Then research can determine if these are really good biological control agents to be used in a program to manage *E. ello*.

In addition to the use of the parasitoid as a controlling agent, studies on how to optimize rearing in the laboratory with the development of alternative host diets are other challenges that must be studied and solved. Another challenge for future research and possible use in a management program is that these species have few alternative hosts, and the species that they parasitize are difficult or economically unfeasible to raise in the laboratory.

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**Conflicts of interest**

There was no conflict of interest regarding the preparation and submission of this manuscript.

**Author contribution statement**

LCC, JASF, PWRC and JJW performed the bioassays and participated in all data collection. LCC performed the statistical analysis. LCC, APGSW and VP conceived and designed the research. LCC, APGSW and VP interpreted data and wrote the paper. All authors read and approved the manuscript.

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