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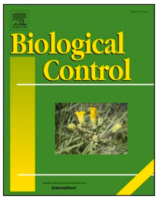

A new biocontrol agent against old enemies: The potential of *Trichogramma foersteri* for the control of *Spodoptera frugiperda* and *Spodoptera eridania*

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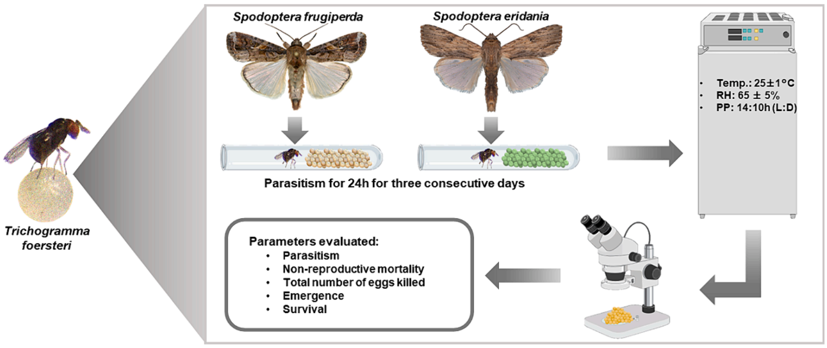
HIGHLIGHTS

- *Trichogramma foersteri* is a newly discovered species of egg parasitoid.
- *Trichogramma foersteri* showed high rates of parasitism on *Spodoptera frugiperda* and *S. eridania* eggs.
- Non-reproductive factors played an important role in egg mortality.
- For 3 days, *Trichogramma foersteri* controlled 334.2 and 174.0 *S. frugiperda* eggs laid in one and two layers, respectively, and 445.4 *S. eridania* eggs.
- *Trichogramma foersteri* showed potential as a biocontrol agent against *S. frugiperda* and *S. eridania.*

GRAPHICAL ABSTRACT



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ABSTRACT

Species of the genus *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) are widely used for pest control, highlighting the importance of exploring new species for the advancement of biological control programs. The study aimed to evaluate the efficacy of *Trichogramma foersteri* Takahashi, a recently discovered species, in controlling *Spodoptera frugiperda* (JE Smith) and *S. eridania* (Stoll), two significant pests of numerous cash crops. Over three days, the research examined parasitism, non-reproductive mortality, and emergence rates for both pest species. *Trichogramma foersteri* demonstrated effective parasitism, reducing 144.1 ± 6.0 eggs (one layer) and 55.4 ± 4.0 eggs (two layers) of *S. frugiperda*, as well as controlling 150.7 ± 5.6 eggs of *S. eridania*. Non-reproductive effects contributed significantly to mortality, causing the demise of 192.6 ± 4.7 and 118.6 ± 3.8 eggs in one and two layers of *S. frugiperda*, and 294.7 ± 7.0 eggs in *S. eridania*. Within three days, *T. foersteri* controlled approximately 334.2 ± 7.6 and 174.0 ± 4.0 eggs of *S. frugiperda* in one and two layers, respectively, and 445.4 ± 7.2 eggs of *S. eridania*. The highest emergence of *T. foersteri* occurred on the first day of evaluation, with 80.4 % for *S. frugiperda* and 23.0 % for *S. eridania*. These findings underscore the potential of *T. foersteri* as an effective control agent for both pests and provide valuable insights for the development of sustainable pest control strategies.

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1. Introduction

Biological control has been implemented globally for over a century, providing sustainable management of numerous agricultural pests (van Lenteren et al., 2018). Among the more than 230 species documented worldwide, egg parasitoids of the genus *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) are extensively used against agricultural pests in both annual and perennial crops (Querino and Zucchi, 2019; Mills, 2010; Parra et al., 2015; Oliveira et al., 2020; Zang et al., 2021). Despite the widespread use of *Trichogramma*, the prospection of new species with the potential to effectively suppress insect pests remains crucial for the advancement of biological control programs (Zhang et al., 2013; Takahashi et al., 2021). Recently, field surveys carried out on soybean crops in southern Brazil revealed a new species of *Trichogramma*, (*Trichogramma foersteri* Takahashi) parasitizing eggs of the velvet bean caterpillar, *Anticarsia gemmatalis* Hübner (Lepidoptera: Erebidae) (Takahashi et al., 2021). Screening tests showed that *T. foersteri* not only had a higher parasitism rate on eggs of *A. gemmatalis* compared to previous studies, but also effectively parasitized ten other lepidopteran pests, including *Spodoptera* species. Apart from its high fecundity, *T. foersteri* causes significant non-reproductive mortality, being capable of inviabilizing more than 200 eggs of *Anticarsia gemmatalis* during the female lifespan (Takahashi et al., 2021).

Research into new biocontrol agents is particularly important when dealing with species of the genus *Spodoptera* Guenée, as most studies report low parasitization rates of *Trichogramma* spp. on eggs of these species. This can be attributed to the behavior of certain female *Spodoptera* species, which usually lay their eggs in multiple layers and subsequently cover them with protective scales during oviposition (Beserra et al., 2005; Li et al., 2023), creating a barrier that hampers parasitism by *Trichogramma* (Greenberg et al., 1998). Female of *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) deposit egg masses with multiple layers covered by scales. Along with the southern armyworm, *Spodoptera eridania* (Stoll) (Lepidoptera: Noctuidae), both species are economically important pests that attack various crops. In the Neotropical region, *S. eridania* has gained increased attention due to the damage it causes to important and widely grown crops such as soybeans and cotton (Montezano et al., 2014), while *S. frugiperda* has long been recognized as the most prominent corn pest (Parra et al., 2022). Furthermore, both species were recently detected for the first time in several countries in Western and Central Africa (Goergen et al., 2016; Goergen, 2018). In 2019, *S. frugiperda* was identified in China, where it is considered a highly destructive invasive pest (Wu et al., 2019). Recently, *S. eridania* has been classified as an A1 quarantine pest in Europe due to a significant risk of spreading to other regions of the continent (EPPO, 2020). The highly polyphagous behavior of *S. eridania* and *S. frugiperda* facilitates their establishment and spread in the invaded regions. The fall armyworm is known to feed on more than 350 plant species (Kenis et al., 2023), while the southern armyworm has been observed feeding on more than 200 plant species belonging to 58 different families (Montezano et al., 2014).

In addition to their polyphagous behavior, the lack of effective control techniques against *S. eridania* and *S. frugiperda*, their wide temperature tolerance range, and the presence of resistant populations to various insecticides are the primary factors contributing to their widespread distribution and control failures (Sosa-Gómez and Omoto, 2012; Durigan et al., 2017; Yang et al., 2020; Wan et al., 2021). Furthermore, the *Spodoptera* species complex naturally exhibits tolerance to genetically modified plants expressing toxic proteins from *Bacillus thuringiensis* Berliner (Eubacteriales: Bacillaceae) (Storer et al., 2010; Yang et al., 2020; Nascimento et al., 2022). These conditions highlight the importance of employing different control practices to manage these pests (Wan et al., 2021; Colmenarez et al., 2022).

Screening tests performed in the laboratory have shown that *T. foresteri* can successfully parasitize the eggs of *S. eridania* and *S. frugiperda*. However, further studies are needed to fully understand the potential of this species in controlling these pests. Like other *Trichogramma* species, *T. foersteri* can negatively impact hosts without successful offspring development or direct feeding - a characteristic referred to as mortality due to non-reproductive effects (Cebolla et al., 2017; Abram et al., 2019. These effects do not offer clear benefits to parasitoids in terms of current or future reproduction but entail negative consequences for hosts, such as mortality (Abram et al., 2019). Despite that, host mortality by non-reproductive effects continues to be a factor often overlooked in many studies (Marchioro et al., 2015; Cebolla et al., 2017; Abram et al., 2019). Ideally, all factors contributing to host mortality should be considered in biological control programs (Cebolla et al., 2017). With this in mind and given the (1) recognized role of *Trichogramma* parasitoids in maintaining pest populations below the economic injury level, (2) the increasing significance of *Spodoptera* species as pests in various cultivated plants worldwide, and (3) the successful parasitism of *Spodoptera* eggs observed in screening tests, the objective of this study was to assess the potential of *T. foersteri* to control *S. frugiperda* and *S. eridania*. Our hypothesis is that *T. foersteri* can effectively kill eggs of *S. frugiperda* and *S. eridania*, indicating its potential to be used in biological control programs against these pests.

2. Material and methods

2.1. Laboratory rearing of the parasitoid and its hosts

Colonies of *T. foersteri*, its host (*A. gemmatalis*), as well as the target pests (*S. frugiperda* and *S. eridania*), were maintained in the Laboratory of Integrated Insect Control at the Federal University of Paraná under controlled temperature, humidity, and photophase conditions (25 ± 1 °C, 70 ± 10 % RH, and a photophase of 14:10 h). Larvae of *A. gemmatalis* and *S. eridania* were collected from commercial soybean areas between the crop seasons 2017/18 and 2019/20 in São José dos Pinhais (25°36′49.0″S, 49°08′01″W), Paraná, Brazil, and *S. frugiperda* was collected during samplings conducted in the 2019 maize season at the Experimental Farm of Canguiri in Pinhais city (25°24′01″S, 49°07′01″W), Paraná, Brazil.

Anticarsia gemmatalis was kept in the laboratory following the methodology described by Hoffman-Campo et al. (1985), and *S. frugiperda* and *S. eridania* were reared using the methods outlined by Parra (2001) and fed an artificial diet proposed by Marchioro and Foerster (2012). The parasitoids used in the experiments were obtained from a population originally collected from *A. gemmatalis* eggs sampled on soybean during the 2017/18 season in São José dos Pinhais (Takahashi et al., 2021). The rearing of *T. foersteri* followed the methodology described by Parra (2010) with adaptations and was conducted using eggs of its natural host, *A. gemmatalis*.

2.2. Parasitism capacity of *Trichogramma foersteri*

The experiment was conducted using a completely randomized design. Females *S. eridania* typically lay their eggs in a single layer (Sampaio et al., 2023); on the other hand, egg masses of *S. frugiperda* typically contain one to four layers in the field (Beserra et al., 2005; Li et al., 2023), and in this case, both one-layer and two-layer egg masses were offered for parasitism. Before the experiments, the parasitoids were reared for two consecutive generations in *S. frugiperda* and *S. eridania* eggs. Approximately 100 eggs of each species, less than 24 h old, were provided for parasitism by *T. foersteri* females from the laboratory colony. After 24 h, the females were removed and the eggs were kept in a climate-controlled chamber (25 ± 1 °C, 70 ± 10 % RH, and a photophase of 14:10 h) until the parasitoids emerged. Following the emergence of the first generation, this procedure was replicated to obtain a second generation of parasitoids, and the adults emerged from each host species were used in the experiments. Parasitoids reared in *S. frugiperda* eggs for two generations were employed in experiments with *S. frugiperda*, and the same protocol was followed for experiments with

S. eridania.

Parasitism was assessed over three consecutive days for both species, with 20 replicates for each treatment. For each host species, 20 copulated females of *T. foersteri*, less than 24 h old, were individually placed in glass tubes measuring 7.5 cm height and 1.0 cm diameter. The tubes were closed with a cotton ball and placed in chambers at 25 ± 1 °C, 70 ± 10 % RH, and a photoperiod of 14:10 h. Droplets of pure honey were provided as food inside the tubes. Approximately 250 eggs, less than 24 h old, were offered for parasitism for 24 h within each glass tube. After this period, the egg masses were transferred to new glass tubes. New egg masses were offered for parasitism for two additional consecutive days. In the experiments, the egg masses were handled with minimal manipulation to ensure the preservation of the existing scale, which varied in thickness from 0 to 71.6 μm, based on the classification introduced by Hou et al. (2022).

The number of eggs parasitized per female was recorded for each species by counting the darkened host eggs. During preliminary laboratory tests, it was observed that parasitism drastically decreases starting from the third day. Based on this information, we opted to evaluate parasitism during the first three days of the female's life. Larvae of *S. frugiperda* and *S. eridania* that hatched from non-parasitized eggs were counted and removed from the egg masses twice a day to avoid egg cannibalism.

2.3. Non-reproductive mortality caused by *Trichogramma foersteri*

Because previous studies indicated that *T. foersteri* can cause host mortality through non-reproductive means (Takahashi et al., 2021), the methodology employed by Vásquez et al. (1997) and Marchioro et al. (2015), based on the procedure outlined by Abbott (1925), was used to assess the total mortality caused by the parasitoid, as follows:

$$TM = \left(\frac{PVE - PHL}{PVE}\right)*100$$

where, TM = total mortality, PVE = percentage of viable eggs, and PHL = percentage of hatched larvae in each treatment. To determine egg viability, 10 cards containing 50 eggs of each species were placed individually in glass tubes and maintained under the same controlled experimental conditions. These eggs had the same origin as those used in the experiments. Mortality caused by parasitism was directly assessed by the visual inspection of the host eggs offered to parasitism. Non-reproductive mortality was determined by subtracting the total number of eggs controlled by the parasitoid (TM) from the mortality caused by parasitism.

Five days after parasitism, photographs of the egg masses were taken using cameras connected to a stereoscopic microscope. This allowed the identification and counting of the number of eggs killed by parasitism (dark eggs) and by non-reproductive causes (greenish eggs). The counting of the egg masses was performed using the ImageJ software (Schneider et al., 2012). To ensure the accuracy of the results obtained from the photographs, each parasitized egg was individually evaluated under a stereoscope microscope after the emergence of the parasitoids. This additional evaluation served as confirmation and validation of the findings derived from the photographic analysis.

2.4. Emergence, longevity, and survival of *Trichogramma foersteri*

Following the emergence of the parasitoids, the parasitized egg masses were examined to determine the emergence rate. This was calculated by dividing the number of eggs with an exit hole by the total number of darkened eggs. To assess the longevity of *T. foersteri*, 20 newly emerged couples were kept in 7.5 x 1.0 cm glass tubes. Droplets of pure honey were provided as food source, while no eggs were offered in this setup. Daily observations were conducted to record the date of death for both females and male, enabling the calculation of the survival rate.

2.5. Statistical analysis

The parameters evaluated in the study were analyzed using generalized linear models (GLM) in the R computing environment (R Core Team, 2022). The ability of *T. foersteri* to control *S. eridania* and *S. frugiperda* was analyzed separately, as our objective was not to compare parasitism between different hosts. For *S. frugiperda*, factors such as the day of parasitism and the number of egg layers were considered when analyzing parasitism rate, non-reproductive mortality, and the total number of eggs killed by the parasitoid. For *S. eridania*, since egg masses typically consist of only one layer, only the day of parasitism was considered as a factor. The number of eggs controlled by parasitism and by non-reproductive causes, as well as the total number of eggs killed by the parasitoid, were evaluated with a quasi-poisson distribution with a logarithmic link function. The emergence rate was evaluated using logistic regression with a quasi-binomial distribution and logit link function. Longevity was assessed using a Gaussian distribution with an identity link function. When significant differences were observed among treatments ($P < 0.05$), Tukey's test was employed to compare the means at a significance level of 5 %. Additionally, survival curves were constructed for female and male of *T. foersteri* individuals developing on both hosts. These curves were compared using the non-parametric Kaplan-Meier method (Kaplan and Meier, 1958) implemented in the survival R package. All mentioned averages in the text are accompanied by their respective standard errors.

3. Results

3.1. Parasitism by *Trichogramma foersteri*

Trichogramma foersteri was capable of parasitizing both host species throughout the three-day evaluation period. Parasitism of *S. frugiperda* eggs was significantly affected by the number of egg layers ($\chi^2 = 38.4$; df = 1; $p < 0.05$) and the parasitism day ($\chi^2 = 151.9$; df = 2; $p < 0.05$). However, the interaction between these two factors did not significantly influence parasitism ($\chi^2 = 5.5$; df = 2; $p = 0.06$). Parasitism levels were higher on egg masses with a single layer compared to two-layer egg masses. When considering data from all three evaluation days, *T. foersteri* parasitized an average of 144.1 ± 6.0 eggs on one-layer egg masses and 55.4 ± 4.0 eggs on egg masses with two layers (Fig. 1a). Irrespective of the number of egg layers, the parasitism was notably higher on the first day of evaluation in comparison to the second and third days (Fig. 1a).

Parasitism of *S. eridania* eggs was significantly influenced by the evaluation days ($\chi^2 = 35.7$; df = 2; $p < 0.05$). The average number of parasitized eggs was higher on the first day (89.8 ± 9.4 eggs), followed by the second (33.2 ± 7.0 eggs) and third evaluation days (27.6 ± 5.7 eggs) (Fig. 1b), totalling an average of 150.7 ± 5.6 parasitized eggs of *S. eridania* over the three evaluated days.

3.2. Non-reproductive mortality

Trichogramma foersteri showed a remarkable ability to render the eggs of the examined hosts unviable through non-reproductive causes. The incidence of this type of mortality in *S. frugiperda* eggs exhibited significant variation in relation to the day of parasitism ($\chi^2 = 7.8$; df = 2; $p < 0.05$), number of layers ($\chi^2 = 17.3$; df = 1; $p < 0.05$), and the interaction between these two factors ($\chi^2 = 23.6$; df = 2; $p < 0.05$). In one-layer egg masses, the observed number of unviable eggs by non-reproductive causes was higher on the first two days (69.9 ± 9.0 eggs on the first and 71.2 ± 6.8 eggs on the second day) compared to the third day of parasitism (48.9 ± 7.9 eggs) (Fig. 1c). A different pattern was observed when two-layer egg masses were offered to parasitism. In this case, the number of eggs killed by non-reproductive causes was higher on the second (53.4 ± 5.2 eggs) and third day (49.3 ± 6.7 eggs) in comparison to the first day (15.8 ± 4.3 eggs) (Fig. 1c). Similar to the

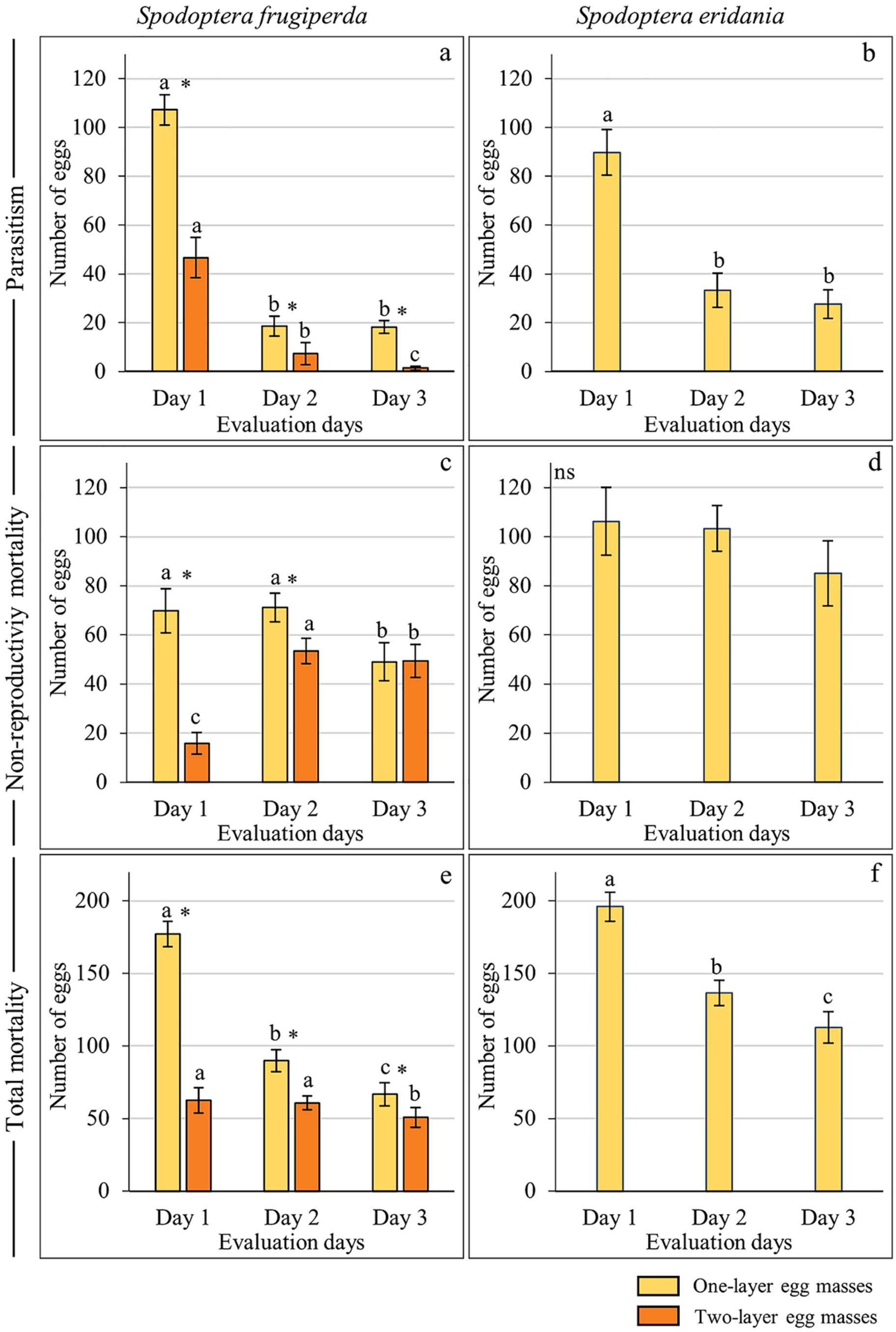

Fig. 1. Parasitism, non-reproductive mortality and total number of eggs killed (parasitism + non-reproductive mortality) by *Trichogramma foersteri* in eggs of *Spodoptera frugiperda* (a, c, and e) and *S. eridania* (b, d, and f) over a period of three consecutive days. Lowercase letters represent the differences between days of parasitism, while asterisks indicate differences between *S. frugiperda* egg masses with one and two egg layers.

observed parasitism trend, the non-reproductive mortality was higher in one-layer compared to two-layer egg masses. In total, *T. foersteri* rendered an average of 190.1 ± 4.7 eggs unviable in one-layer egg masses of *S. frugiperda* over the three-day evaluation period, and 118.6 ± 3.8 eggs in egg masses with two layers. On the second and third days, non-reproductive mortality was higher than parasitism when *T. foersteri* was exposed to one- and two-layer egg masses (Fig. 2).

Conversely, the day of parasitism did not have a significant influence on the mortality through non-reproductive causes in eggs of *S. eridania* ($\chi^2 = 1.7$; df = 2; p = 0.4, Fig. 1d). The number of eggs rendered unviable due to non-reproductive causes tended to be higher on all evaluation days, with average values of 106.2 ± 13.7, 103.3 ± 9.3, and 85.1 ± 13.2 eggs on the first, second, and third days, respectively. For *S. eridania*, the total number of eggs rendered unviable due to non-reproductive causes during the three-day evaluation was 294.7 ± 7.0. Non-reproductive mortality accounted for most of the observed mortality for *S. eridania* on all evaluated days (Fig. 2).

3.3. *Total number of eggs killed by Trichogramma foersteri*

The total number of *S. frugiperda* eggs controlled by *T. foersteri*, which includes eggs killed by parasitism and non-reproductive causes, was significantly influenced by the evaluation day ($\chi^2 = 60.8$; df = 2; P < 0.05), number of egg layers ($\chi^2 = 68.0$; df = 1; $p < 0.05$), and the

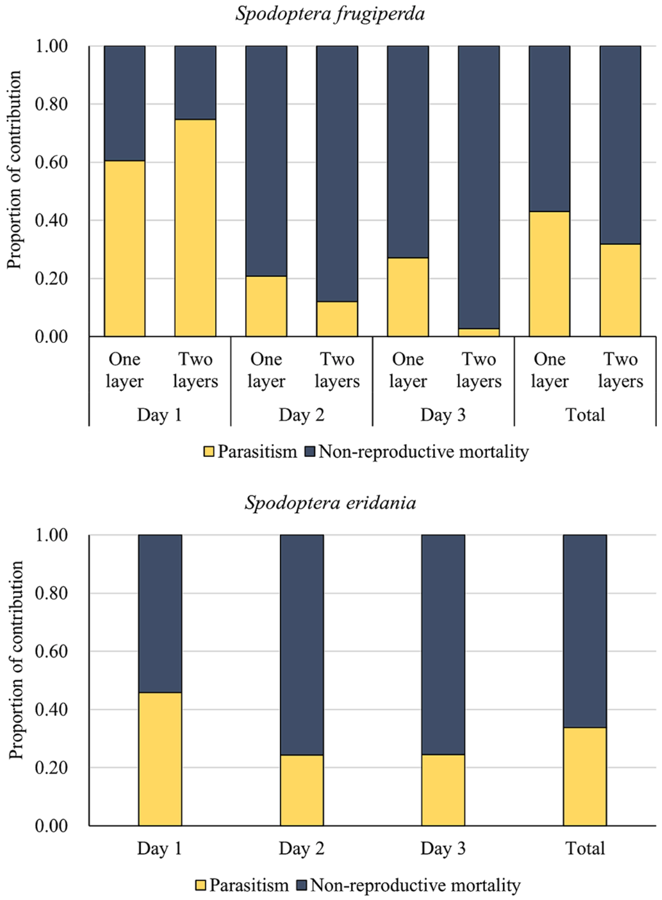

Fig. 2. Decomposition of the total mortality caused by *Trichogramma foersteri* on eggs of *Spodoptera frugiperda* and *S. eridania*.

interaction between these two factors ($\chi^2 = 18.4$; gl = 2; $p < 0.05$). One-layer egg masses had a higher number of eggs controlled by *T. foersteri* compared to two-layer egg masses. In total, the parasitoid controlled an average of 334.2 ± 7.6 eggs of *S. frugiperda* in one-layer egg masses and 174.0 ± 4.0 eggs in two-layers egg masses. The total number of killed eggs decreased with the day of parasitism, starting from 177.0 ± 8.6 eggs on the first day to 66.7 ± 7.8 on the third day in one-layer egg masses (Fig. 1e). Conversely, there were no significant differences in the total number of eggs controlled by *T. foersteri* in two-layers egg masses between the first and second days of parasitism. However, on the third day, a significant decrease was observed, with only 50.7 ± 6.8 eggs being controlled (Fig. 1e).

Significant differences between evaluation days were also recorded for *S. eridania* ($\chi^2 = 33.8$; df = 2; $p < 0.05$). The highest number of eggs killed by *T. foersteri* in this host occurred on the first day (196.0 ± 10.0 eggs), while the lowest number was recorded on the third day (112.7 ± 10.9 eggs) (Fig. 1f). For *S. eridania*, a total of 445.4 ± 7.2 eggs were killed by *T. foersteri* during the three evaluation days.

3.4. Emergence and survival of Trichogramma foersteri

The emergence rate was significantly influenced by the host species ($\chi^2 = 51.7$; df = 1; $p < 0.05$) and evaluation days ($\chi^2 = 115.0$; df = 2; $p < 0.05$). However, there was no significant interaction between these two factors ($\chi^2 = 0.17$; df = 2; p = 0.9). A higher emergence rate of 80.5 % was observed on the first evaluation day when *T. foersteri* developed on *S. frugiperda* eggs. There was a sharp decline in parasitism in the second and third evaluation days, with only a few parasitoids emerging from the parasitized eggs. The recorded emergence rates for these days were 26.0 % and 2.8 %, respectively. Lower emergence rates were observed for parasitoids developed on eggs of *S. eridania*. In this host, an emergence rate of 23.0 % was observed on the first day of parasitism, and only 2.2 % of the parasitoids emerged on the second day. No emergence of parasitoids was observed from egg masses offered for parasitism on the third day.

Parasitoid longevity from egg masses of *S. frugiperda* was influenced by the sex of parasitoids ($\chi^2 = 50.9$; df = 1; $p < 0.05$). The average longevity of female *S. frugiperda* (8.3 days ± 0.1) was higher than males (5.7 days ± 0.3). A similar pattern was observed for *S. eridania* ($\chi^2 = 10.1$; df = 1; $p < 0.05$), with females (7.6 ± 0.4) living longer than males (5.5 days ± 0.4). These findings were further supported by the survival analysis (Fig. 3), which demonstrated significant differences in survival between female and male parasitoids for both *S. frugiperda* ($\chi^2 = 41$; df = 1; $p < 0.05$) and *S. eridania* ($\chi^2 = 8.9$; df = 1; $p < 0.05$).

4. Discussion

In this study, the parasitism of a newly discovered species of *Trichogramma* was evaluated on eggs of *S. frugiperda* and *S. eridania*, two relevant agricultural pests of growing importance. This is the first investigation examining the efficacy of *T. foersteri* in controlling these two *Spodoptera* species. The results of our study revealed that *T. foersteri* successfully parasitizes eggs of both *S. frugiperda* and *S. eridania* and shows a substantial capacity to induce host mortality through non-reproductive means. These findings provide valuable and fundamental insights, suggesting that *T. foersteri* has the potential to be incorporated into integrated pest management programs for the effective control of *S. frugiperda* and *S. eridania*.

Most of the parasitism occurred on the first day of evaluation; however, subsequent days exhibited effective control through parasitism. The number *S. frugiperda* and *S. eridania* eggs parasitized by *T. foersteri* within the first 24 h is remarkably high compared to other *Trichogramma* species evaluated at the same temperature and photoperiod conditions as in our study. For instance, Yang et al. (2022) reported that *Trichogramma chilonis* Ishii, *T. dendrolimi* Matsumura, and *T. pretiosum* Riley parasitized approximately 16, 30, and 26 eggs of *S. frugiperda*, respectively, within the same time frame. In another study, Bueno et al. (2010) found that *T. pretiosum* parasitized only 6.5 eggs of *S. frugiperda* within the same period, while Tian et al. (2020) reported that *T. dendrolimi* and *T. bilingensis* He and Pang parasitized 20 and 9.6 eggs of *S. frugiperda*, respectively. Similar disparities in parasitism between *T. foersteri* and other *Trichogramma* species were also observed on eggs of *S. eridania*. Carvalho et al. (2012) reported that *T. pretiosum* parasitized 9.9 eggs of *S. eridania* within 24 h, *T. pratissolii* Querino and Zucchi parasitized 9.2, *T. galloi* Zucchi parasitized 7.3 eggs, *T. exiguum* Pinto and Platner parasitized 5.0 eggs, and *T. atopovirilia* Oatman and Platner parasitized only 3.8 eggs. When compared to other *Trichogramma* species, these findings clearly demonstrate the high parasitization capacity of *T. foersteri* on eggs of both *S. frugiperda* and *S. eridania*.

Certain species of *Spodoptera*, such as *S. frugiperda*, often lay egg masses with multiple layers. This characteristic is recognized as an important factor that limits parasitism by *Trichogramma* species (Beserra et al., 2005). Typically, female *S. frugiperda* lay egg masses with one to four layers, with the majority of egg masses containing two layers (Hou et al., 2022). However, many studies did not provide information about the number of layers in the egg masses used for parasitism tests. Moreover, some studies performed parasitism tests using single-layer egg masses without scales, claiming comparability with other host species (e.g. Jin et al., 2021). In this study, we examined the parasitism of *T. foersteri* on *S. frugiperda* egg masses containing one and two layers, without the removal of scales. Surprisingly, *T. foersteri* exhibited a high level of parasitism even in two-layer egg masses.

Although the parasitism in two-layer egg masses of *S. frugiperda* was lower compared to one-layer egg masses, it was relatively higher than the ones reported for other *Trichogramma* species (e.g. Bueno et al., 2010; Tian et al., 2020; Jin et al., 2021; Yang et al., 2022). For instance,

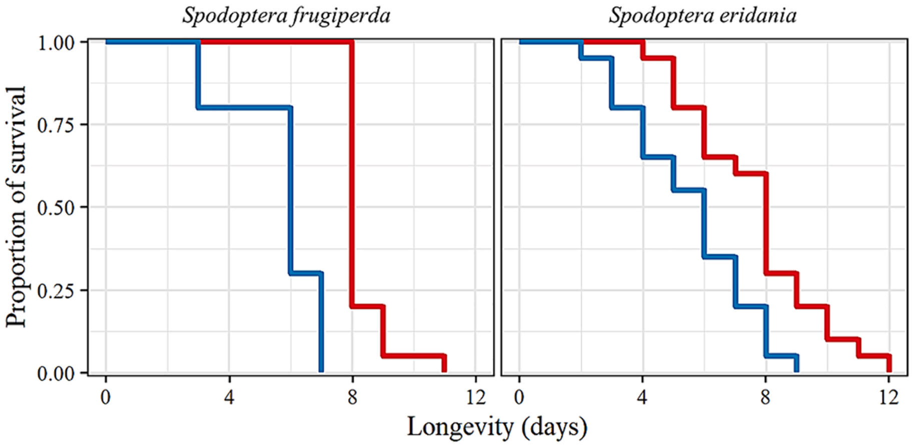

Fig. 3. Survival curves constructed according to the Kaplan-Meier method for adults of *Trichogramma foersteri* reared on eggs of *Spodoptera frugiperda* and *S. eridania*. The blue and red lines indicate the survival curves of males and females, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Jin et al. (2021) evaluated the parasitism of seven *Trichogramma* species in one-layer egg masses of *S. frugiperda* with the scales artificially removed. Even under those conditions, the parasitism by the seven species was significantly lower than the observed for *T. foersteri* in this study. When exposed to one- and two-layer egg masses, the parasitism by *T. foersteri* was 11 to 27, and 4.5 to 12 times higher, respectively, than the parasitism reported by Jin et al. (2021). As a recently discovered species, *T. foersteri* requires further studies to fully understand its bioecology. The observed high reproductive capacity of *T. foersteri* may be attributed to its larger size compared to other species commonly found in Brazil. Laboratory observations confirmed that *T. foersteri* is larger in size than both *T. atopovirilia* and *T. pretiosum*, which exhibited lower parasitism rates on *S. frugiperda* eggs. Previous studies have demonstrated a positive correlation between parasitoid size and fecundity (Boivin, 2010), as well as the existence of physiological and behavioral advantages over their smaller counterparts (Godfray, 1994). Furthermore, our results also indicate that *T. foersteri* females tend to rapidly lay most of their eggs upon contact with a potential host. Therefore, investigating *T. foersteri* parasitism throughout the female lifespan may shed light on whether the observed differences are associated to this behavior or to other factors, such as parasitoid size.

Interestingly, the visual observations of the egg masses after 24 h of parasitism indicated that *T. foersteri* primarily parasitized the outer eggs while failing to reach the inner eggs of the clutch. While this finding might suggest that the number of egg layers, rather than the presence of scales, played a more significant role in limiting the parasitism by *T. foersteri*, further investigation considering that previous studies have reported that the thickness of scales on egg masses had a significant impact on the parasitism success of different *Trichogramma* species (Dong et al., 2021; Hou et al., 2022). Therefore, it is crucial to delve deeper into this matter to gain a comprehensive understanding of the parasitism behavior of *T. foersteri* when parasitizing egg masses with multiple layers.

While it is widely recognized that parasitoids of the genus *Trichogramma* primarily induce host mortality through parasitism (Abram et al., 2019), it has been documented that these parasitoids can also cause host death through non-reproductive means (Asgari and Rivers, 2011; Marchioro et al., 2015; Abram et al., 2016; Cebolla et al., 2017; Kaser et al., 2018). Throughout the three-day evaluation period, *T. foersteri* consistently induced mortality in eggs of both *S. frugiperda* and *S. eridania* through non-reproductive factors. Notably, the mortality caused by non-reproductive factors surpassed that caused by parasitism for both host species. This outcome aligns with previous findings that demonstrate the potential for non-reproductive mortality to exceed reproductive mortality in certain instances (Kaser et al., 2018). Throughout the three days of parasitism examined in this study, *T. foersteri* played a significant role in the high number of eggs affected by non-reproductive mortality in both single and double-layer egg masses of *S. frugiperda*. Notably, the impact of this effect on the total mortality of *S. eridania* was even more pronounced, reaching almost 300 eggs. Parasitoids can induce non-reproductive mortality through various mechanisms, including (1) mechanical damage to the host egg, (2) death of the embryo during egg perforation, (3) larval feeding before parasitoids' eventual death, (4) release of chemical compounds or teratocytes without subsequent parasitoid development, or (5) adult feeding (Asgari and Rivers, 2011; Abram et al., 2013; Strand, 2014; Huang et al., 2017; Abram et al., 2019). This diverse range of mechanisms leading to host mortality through non-reproductive means helps to explain the efficacy of biological control programs, even when low rates of parasitism are observed (Kaser et al., 2018). It remains to be determined the cause of the high non-reproductive mortality levels of *T. foersteri* on eggs of both hosts.

The findings of our study carry significant implications, as the non-reproductive effects induced by egg parasitoids are often under-evaluated and overlooked in research assessing the impacts of *Trichogramma* species on their hosts (Abram et al., 2016; Cebolla et al., 2017; Kaser et al., 2018). Neglecting this aspect not only underestimates the true potential of biological control agents but can also influence the accuracy of insect population models in understanding the host-parasitoid dynamics (Cebolla et al., 2017). Currently, detailed information regarding the mechanisms employed by *T. foersteri* in causing this type of mortality is lacking. Conducting behavioral studies and biochemical analyses of the contents of eggs rendered unviable by non-reproductive causes could offer more definitive insight into this process.

The genus *Trichogramma* exhibits a complex relationship with respect to the nutritional quality of host eggs (Goulart et al., 2011; Zang et al., 2021), and larval survival serves as a crucial indicator when assessing the nutritional suitability of hosts. For instance, eggs of *Chilo suppressalis* Walker (Lepidoptera: Crambidae) were found to be suitable for *T. dendrolimi*, *T. japonicum* and *T. chilonis*, but not for *T. ostriniae*, which exhibited low rates of parasitism and progeny survival in this particular host (Zhang et al., 2013). Similarly, our results indicate significant variations in emergence rates depending on the host species, with *S. eridania* eggs displaying a lower emergence rate compared to *S. frugiperda*. Based on this biological parameter, one could infer that *S. eridania* eggs are less nutritious for *T. foersteri* than *S. frugiperda* eggs. This assumption is further supported by the shorter lifespan of *T. foersteri* when parasitizing *S. eridania* compared to *S. frugiperda*. One factor that might explain these findings is the potential presence of a more robust immune response of *S. eridania* against the parasitoid, as in certain situations the host's immune system can eliminate the parasitoid eggs (Abram et al., 2016). The extended lifespan of female parasitoids is a desirable trait as it promotes the search for food sources, mates, and hosts (Foerster et al., 2014; Tian et al., 2016). Females of both species exhibited long lifespans, particularly in the case of *S. frugiperda*, where all evaluated females survived for at least eight days.

Together, parasitism and non-reproductive mortality exerted control over a significant proportion of the eggs available to female *T. foersteri*. Understanding the mechanisms through which the parasitoid eliminates its host is crucial in determining the most effective strategy for biological control programs (Huang et al., 2017). For instance, during the three-day evaluation period, we observed that *T. foersteri* successfully controlled a total of 334 and 174 eggs in one- and two-layer egg masses of *S. frugiperda*, respectively, along with 445 eggs of *S. eridania*. These results indicate that *T. foersteri* is a potential candidate to be employed in inundative biological control which involves the mass production and release of parasitoids to rapidly control the pest through the action of the released parasitoids rather than their offspring (Abram et al., 2016). The non-reproductive mortality plays a pivotal role in reducing the pest population and, in certain instances, constitutes the primary cause of host mortality (Abram et al., 2019), as observed for *T. foersteri*. On the other hand, if the goal is to use the inoculative biological control, entailing the periodic release of parasitoids to restore an imbalanced system that has not been naturally maintained, preference should be given to parasitoids displaying high parasitization capacity and emergence rates.

In conclusion, this study represents the first assessment of the potential of the newly discovered egg parasitoid *T. foersteri* for controlling species of *Spodoptera*. Our laboratory experiments demonstrate that *T. foersteri* has the potential to control *S. frugiperda* and *S. eridania*, both through parasitism and non-reproductive mechanisms. In fact, a significant portion of the mortality inflicted on the hosts by *T. foersteri* was due to non-reproductive factors, highlighting the importance of considering this aspect when evaluating the impact of *Trichogramma* species on insect pests. Moreover, *T. foersteri* exhibited a notably higher capacity for parasitization compared to other *Trichogramma* species. Given the global demand for sustainable agricultural production, the prospection of new parasitoids like *T. foersteri* can serve as a vital tool in the development of Integrated Pest Management Programs. This information holds even greater significance for pests such as *S. frugiperda* and *S. eridania*, which continue to expand their distribution range and cause economic losses in various regions worldwide. While the number of eggs killed by *T. foersteri* demonstrates its potential as a biocontrol agent against these pests, further studies should be conducted to evaluate the feasibility of its mass rearing using factitious hosts, as well as its ability to parasitize hosts under field conditions.

CRediT authorship contribution statement

Fábio Sampaio: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Cesar A. Marchioro:** Conceptualization, Writing – original draft, Writing – review & editing. **Tamara A. Takahashi:** Conceptualization, Writing – review & editing. **Luís A. Foerster:** Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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