Efficiency of Three Egg Parasitoid Species on Fall Armyworm (Lepidoptera: Noctuidae) in Laboratory and Field Cages[1]

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Abstract Egg parasitoids are the most used natural enemies in biological control of *Spodoptera frugiperda* (J.E. Smith), a pest of gramineous plants native to the Americas that recently invaded Africa and some countries of Asia. Although *Trichogramma pretiosum* (Riley) is one of the main species used against this pest, there are other species with equal or greater parasitism potential. The objective of this work was to compare, in laboratory and in field cages, the parasitism of *Telenomus remus* Nixon, *Trichogramma atopovirilia* (Oatman and Platner), and *T. pretiosum* on *S. frugiperda* eggs. *Telenomus remus* and *T. atopovirilia* reached the highest percentages of parasitism in laboratory (>70%) which did not differ statistically from each other. *Trichogramma pretiosum* had the lowest percentage of parasitism (29%) of the three. In the field cage assays, *Te. remus* parasitized 30% of the *S. frugiperda* egg masses, and *T. pretiosum* parasitized about 7.5% of the egg masses. These results underscore the importance of adequate selection of egg parasitoids for their use in biological control programs by augmentation against *S. frugiperda*.

Key Words *Telenomus remus, Trichogramma atopovirilia, Trichogramma pretiosum,* augmentative biological control

Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae) is a polyphagous pest that feeds on gramineous plants such as maize (*Zea mays* L.), sorghum (*Sorghum bicolor* [L.] Moench), rice (*Oryza sativa* L.) but can attack other important crops such as soybean (*Glycine max* [L.] Merrill), cotton (*Gossypium hirsutum* L.), and tomatoes (*Solanum lycopersicum* L.) (Montezano et al. 2018). It is native to the Americas (Ashley et al. 1989), but in 2016 it invaded Africa (Goergen et al. 2016) and was detected in 17 Asian countries including India (Chormule et al. 2019), Malaysia (Early et al. 2018), and China (Wu et al. 2019). It also was recently reported in Australia (IPPC 2020). Blanco et al. (2014) estimated that in underdeveloped locales, two to three applications of insecticides are used to combat this pest during a growing season. The estimated cost of making two applications of insecticide (approximate cost of US$30), without including costs of actual application, is approximately US$225 million on 7.5 million ha of maize in

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Mexico alone (FIRA 2020). Due to the importance of *S. frugiperda* as a pest and its ability to develop resistance to insecticides (Boaventura et al. 2020, Gutiérrez-Moreno et al. 2018), the use of natural enemies should be included in integrated management programs for this pest.

There are more than 350 species of natural enemies of *S. frugiperda* including parasitoids, predators, fungi, viruses, bacteria, and nematodes (Bahena and Cortez 2015, Molina-Ochoa et al. 2003). Despite this diversity, wasps of the genus *Trichogramma* and the entomopathogen *Bacillus thuringiensis* Berliner are the most used in biological control of this pest, mainly through augmentative methods (Malo and Hore 2020). Some authors question the use of certain species of *Trichogramma* due to their inability to parasitize egg masses with overlapping layers, especially if they contain scales, as occurs in *S. frugiperda* (Beserra and Parra 2003). Other authors consider that *Telenomus remus* Nixon (Hymenoptera: Platygastridae) has greater potential to successfully parasitize *S. frugiperda* eggs due to its size and biology (Bueno et al. 2010, Cave 2000).

Telenomus remus is an egg parasitoid of several Lepidoptera species, but it specifically attacks those in the genus *Spodoptera* (Wojcik et al. 1976). In the Americas, it was first imported to Barbados for the control of *Spodoptera* spp., and it was released later in Venezuela, Honduras, and the US state of Florida (Cave 2000). It is reared in seven countries of Latin America for use in controlling *Spodoptera* species (Cave 2000). In Mexico, *Te. remus* was introduced to corn–bean–squash polycultures in La Frailesca, Chiapas, resulting in parasitism >90% of *S. frugiperda* (Gutierrez-Martínez et al. 2012). However, the establishment and spread of this species was not subsequently monitored.

Telenomus remus is able to parasitize overlapping and scaled egg masses (Gerling and Schwartz 1974), which contributed to 80 or 100% parasitism in *Spodoptera* species (Wojcik et al. 1976). On the other hand, *Trichogramma pretiosum* (Riley), the most released parasitoid against lepidopteran pests in Mexico (Bahena and Cortez 2015, Jaraleño-Teniente et al. 2020), does not exhibit these high levels of parasitism and has provided variable results (Murillo-Cruz 1977, Peralta 1980). Due to the biological traits of *Te. remus,* such as size, host specificity, and ability to parasitize egg masses covered by scales, the objective of the work was to compare the parasitism rates of *Te. remus, T. pretiosum*, and *Trichogramma atopovirilia* (Oatman and Platner) on *S. frugiperda* eggs in laboratory and field cages.

Materials and Methods

Insect rearing. *Telenomus remus* and *T. atopovirilia* were reared on *S. frugiperda* eggs (≤24 h old) at the Biological Control Laboratory of the Colegio de Postgraduados in Texcoco, Estado de Mexico. The host *S. frugiperda* was reared on a meridic diet according to Jaraleño-Teniente et al. (2020). Naturally occurring *Te. remus* were collected initially with sentinel eggs (mass exposure of *S. frugiperda* eggs in the field) from maize production fields in Tapachula, Chiapas, during the fall of 2018. *Trichogramma atopovirilia* was collected from maize and sorghum fields in the fall of 2017 and 2018 in Guanajuato (Jaraleño-Teniente et al. 2020), and *T. pretiosum* was obtained from a commercial rearing facility (Organismos Benéficos para la Agricultura S.A., Autlán, Jalisco, México) where it has been reared for more than 30 generations on *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) eggs. The parasitoid adults were fed with a solution of water and honey (10:1). Parasitoid rearing, *S. frugiperda* rearing, and all experimental assays were conducted in a rearing chamber at 25 ± 2°C, 75 ± 5% relative humidity (RH), and on a 12:12-h light/dark photo regime.

Laboratory experiment. For each of the three species of parasitoids, females aged between 24 and 36 h and with no oviposition experience were used. The *S. frugiperda* eggs used were ≤24 h old. To evaluate the percentage of parasitism of each species, a mass of single-layer, scaleless eggs (with 45 to 68 eggs) was placed in a 3-cm-diameter petri dish. Inside each petri dish, a female and a male parasitoid were released. The egg masses were exposed for 24 h to the parasitoids. After this period, the eggs were held in a bioclimatic chamber. Six days later, the percentage of parasitism was evaluated. Forty such arenas were performed for each species. To evaluate parasitism, pictures were taken with a digital camera (Nikon Body D5600®) of each egg mass. Later, each image was analyzed with the Java-based ImageJ program (Softonic International S.A., Barcelona, Spain) (Schneider et al. 2012) to count the total number of eggs in the mass and those that had signs of parasitoid emergence.

Field cage experiment. After analyzing the results of the laboratory assays, two species (*Te. remus* and *T. pretiosum*) were selected for the field cages assay with three release densities (i.e., one, two, or three parasitoid adults per one host egg mass). The experimental design was factorial (3 × 2) with a control of no parasitoid release. Each experimental unit was of a cage (3 × 3 × 1.8 m) made of organza fabric that covered three rows (12 plants per row) of H20 hybrid maize (30 d old) in which the egg masses (sentinels) were placed. The treatments were randomly assigned to the cages, and treatments were replicated four times with five sampling units (sentinels).

In the field, cages were first established to isolate plants. Then, using a motorized vacuum cleaner, the unwanted organisms (phytophagous and entomophagous) were eliminated inside the cages. Subsequently, within each cage, five single-layer, scaleless *S. frugiperda* egg masses were randomly placed. The masses were attached to the underside of the leaves with a stapler, one mass on each plant. After placement of the egg masses, the parasitoids were released. Sentinels were exposed for 48 h. At the end of this period, the masses were removed and held in a bioclimatic chamber at the environmental conditions already described. Subsequently, the number of parasitized eggs was evaluated as previously described.

Data analysis. The total number of parasitized eggs from the laboratory assay, per species of parasitoid, was subjected to analysis of variance. Where a significant difference was found due to the effect of the treatments, a multiple comparison of means was performed by Tukey test ($\alpha = 0.05$). The parasitism data from the field trial did not meet the assumption of normality; therefore, they were analyzed by logistic regression at a level of significance of $\alpha = 0.05$. The comparison of means was conducted using contrasts. All analyses were performed with the R program, version 3.6 (R Core Team 2013).

Results and Discussion

Laboratory experiment. In general, successful parasitism was recorded on *S. frugiperda* eggs by the three species of parasitoids in the laboratory assay.

Telenomus remus parasitized the eggs at a higher rate than the two *Trichogramma* species with significant differences among treatments ($F = 42.6$; df = 2, 108; $P <$ 0.001). Parasitism by *T. atopovirilia* and *Te. remus* reached 70.1% and 77.6%, respectively, with no difference between the two. However, both percentages were higher than parasitism by *T. pretiosum* (29.2%).

Other authors have reported similar results with *Te. remus* and different species of *Spodoptera*. For example, Morales et al. (2000) reported 75% parasitism on *S. frugiperda*. Wojcik et al. (1976) tested different hosts and reached up to 100% parasitism. Furthermore, five species of *Spodoptera*, *Heliothis zea* (Boddie), and *Feltia subterranea* (F.) had the highest percentages of parasite emergence. Gómez (1988) reported 79% parasitism on *Spodoptera litura* (F.) eggs. These differences could be due, in part, to the number of exposed eggs and the reproductive capacity of the parasitoid. There is evidence that *T. pretiosum* has lower daily fecundity (23 eggs/d) compared to *T. atopovirilia* (38 eggs/d) (Beserra and Parra 2004), and this is lower than the reproductive potential of *Te. remus*, which can parasitize more than 120 eggs/d with an average of 51.5 eggs/d on *S. frugiperda* (Bueno et al. 2010, Gomez 1988).

Although in laboratory assays a favorable numerical trend was detected for *Te. remus*, it was not different from that of *T. atopovirilia*. Seemingly, the preference of the host is less apparent in *Te. remus* than in wasps of the genus *Trichogramma* (Goulart et al. 2011), but this work cannot dismiss the possibility that the rearing host had influenced the success of parasitism. *Telenomus remus* and *T. atopovirilia* were reared on *S. frugiperda*, and *T. pretiosum* was reared on eggs of *Sitotroga cerealella*, a species that is used as a factitious host in most commercial facilities. This could explain, at least hypothetically, why *T. atopovirilia* performed better than *T. pretiosum*.

Field cage experiment. *Telenomus remus* reached a higher percentage of parasitism than *T. pretiosum* regardless of number of parasitoids released in the cages. Treatments of one and two parasitoids of *Te. remus* per egg of *S. frugiperda* resulted in greater parasitism, significantly different from the other treatments ($P \leq$ 0.001), but they did not differ from each other. As the sex ratio of the parasitoids in rearing conditions was 1:1, the release level can be expressed as 0.5 females of *Te. remus* per egg of *S. frugiperda*, and 30% parasitism was reached (Fig. 1).

The percentage of parasitism in this work (30%) seems slightly low when compared to that of Pomari et al. (2013). They reported that 0.165 females of *Te. remus* per egg of *S. frugiperda* provided 70% parasitism. Queiroz et al. (2017) recorded 71% parasitism using 0.13 to 0.15 female parasitoid per egg of the pest. If surface area is considered, the best result (Fig. 1) would be equivalent to 14 females/m^2 and 30% parasitism. Figueiredo et al. (2002), using 12 females/m^2, reached 88.3% parasitism.

From 80 egg masses exposed in this work, *Te. remus* parasitized 42.5% while *T. pretiosum* did so in 10%. To standardize the size of the mass, the upper layers of eggs were removed as well as the scales left by the female of *S. frugiperda*. This probably affected the amount of stimulus that was emitted from *S. frugiperda* scales. Several species of egg parasitoids use the kairomones emitted by *S. frugiperda* to locate their host (Fatouros et al. 2008). Furthermore, it has been shown that the compounds (Z)-9-tetradecene-1-ol acetate and (Z)-9-dodecene-1-ol acetate, components of the sex pheromone of *S. frugiperda*, increase parasitism in

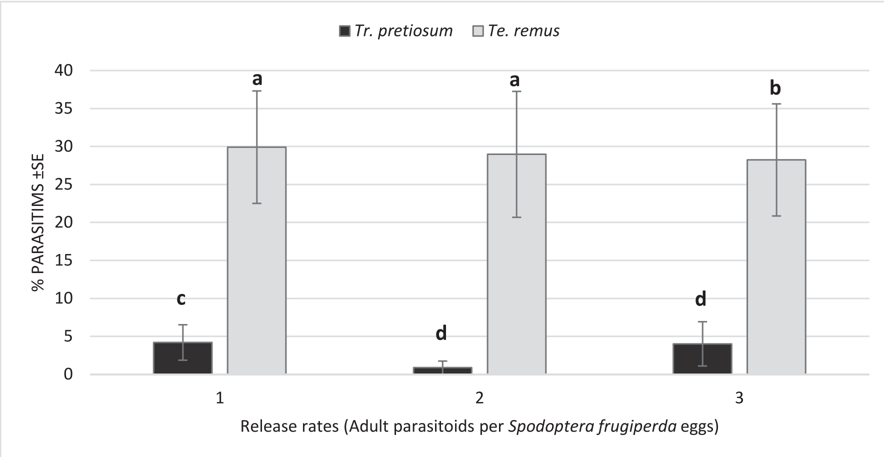

Fig. 1. Parasitism of *Trichogramma pretiosum* and *Telenomus remus* on fall armyworm eggs in field cages ($P \leq 0.001$). Release rates: one, two, and three adults of the parasitoid per *S. frugiperda* egg. Means with the same letter are not significantly different from each other ($P < 0.05$).

Te. remus (Nordlund et al. 1983). It is likely that the low level of parasitism in field cages in this study was linked to the handling of egg masses.

Summary. *Telenomus remus* is a parasitoid native to Malaysia. Its hosts include noctuids that are important pests such as *Agrotis ipsilon* (Hufnagel), *Anticarsia gemmatalis* (Hübner), *Mythimna unipuncta* (Haworth), and *S. frugiperda* (Cave 2000). On the American continents, its use has been limited to countries in the Neotropical zone (Cave 2000). For example, in Venezuela, regular releases of *Te. remus* have been used against *S. frugiperda* since 1979, and up to 90% parasitism has been reported in some regions of the country. This reduced the use of pesticides by up to 80% in some locations (Ferrer 2001). Its presence had been reported on lepidopteran eggs in countries of Asia from which it originates, and recently it was reported on *S. frugiperda* in African countries, where this pest has become an important pest for small farmers (Elibariki et al. 2020, Kenis et al. 2019).

In Mexico, the use of *Te. remus* has only been documented in La Frailesca, Chiapas, in polycultures of corn–beans–squash. The results showed preference towards a maize genotype, and parasitism reached up to 99% (Gutierrez-Martínez et al. 2012). Another study analyzed the cost of producing *Te. remus* on two different hosts. The cost of producing it on *S. frugiperda* eggs was US$0.40/thousand. Producing it on *Corcyra cephalonica* (Stainton), a factitious host, was estimated at half the price, at US$0.20/thousand (Vieira et al. 2017). In contrast, the production of *T. pretiosum* is US$0.052/thousand. We must consider that, though its production cost is low, further evaluations have yet to confirm whether it is effective against *S. frugiperda*.

The parasitism of *T. atopovirilia* and *Te. remus* in different assays, their biological traits, and the limited formal evaluations in North America reinforces the need for additional studies under field conditions. We must consider that the biological traits and the parasitism of the native parasitoid, *T. atopovirilia*, as well as the already established mass rearing methodology for its reproduction, are tools for evaluating and determining what parasitism it can offer in the field through different levels of release or systems of liberation and distribution. *Telenomus remus* provides effective activity on *S. frugiperda* populations in some Neotropical areas of the Americas, its biological traits make this parasitoid a favorable biological control agent for *S. frugiperda*. Before ensuring that it is the ideal candidate for the pest, however, it should be evaluated further. One should be aware that North America has, due to the division and transition zone between the Neotropical and Nearctic zones, incredible diversity of agro-ecological zones, and it is necessary to know in which ones this natural enemy could perform best.

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