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Research Article

Can *Trichogramma atopovirilia* Oatman & Platner replaces *Trichogramma galloi* Zucchi for *Diatraea saccharalis* (Fabricius) control?

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ABSTRACT: Studies on aggressiveness of parasitoids, as assessed by their parasitism against pests, used in biological-control programs are highly important to select the most suitable species and/or strain to control insect pests. The present study investigated whether the egg parasitoid *Trichogramma galloi* Zucchi, an efficient control agent for sugarcane borer *Diatraea saccharalis* (Fabricius) in Brazil, could be replaced by *Trichogramma atopovirilia* Oatman & Platner, a parasitoid easier to mass-produce, since it has been found parasitizing *D. saccharalis* eggs in the warmest region of Brazil and Argentina. Three strains of the genus *Trichogramma* were compared: *T. atopovirilia* (ATP strain) reared on a factitious host *Anagasta kuehniella* (Zeller); *T. atopovirilia* isoline ATP-I, reared on *D. saccharalis* eggs for six generations; and *T. galloi*, reared on *A. kuehniella* eggs. We measured parasitism of each strain for 72 h and for the entire life span, parasitism rate per cluster of *D. saccharalis* eggs, number of parasitoids emerged (parasitism viability), and parasitoid life span. The results confirmed that *T. galloi* is the best species for *D. saccharalis* control, showing higher control potential, since parasitism and emergence rate were higher for this species. Although *T. atopovirilia* ATP-I performed reliably in all parameters, *T. galloi* exceeded and was the most indicated for mass-rearing in control programs for sugarcane borer.

Keywords: biological control, egg parasitoid, strain selection, sugarcane borer

Introduction

The egg parasitoid *Trichogramma galloi* Zucchi has been increasingly used in biological control (BC) programs for *Diatraea saccharalis* (Fabricius) in Brazil. From 2010 to 2018, the area treated increased 400 % reaching two million ha (Parra, 2010; Parra and Coelho Jr., 2019). *Trichogramma galloi* is advantageous in controlling *D. saccharalis* because it parasitizes the pest in the egg stage, preventing further damages to the crop. Moreover, *T. galloi* has high specificity in attacking only *D. saccharalis* eggs (Parra and Zucchi, 2004). For these reasons, interest in releasing *T. galloi* in crops has increased considerably in recent years.

Although *T. galloi* efficiently controls the sugarcane borer in different conditions, rearing this parasitoid in bio-factories is labor-intensive due to the use of factitious hosts. Adapting a population to a factitious host could be a bottleneck for commercial production once *T. galloi* accepts eggs from *A. kuehniella* with at least one week in cold storage (Spínola-Filho et al., 2014); furthermore, it has a parasitism ratio (parasitoid: egg), about 1:4 to 1:6 (Parra et al., 2004). On the other hand, the parasitoid *Trichogramma atopovirilia* Oatman & Platner is a more robust species, easy to rear in factitious hosts, it accepts eggs with up to 30 days of storage, and it parasitizes a higher proportion of eggs (1:15) (Coelho Jr., unpublished data).

Trichogramma atopovirilia has been reported parasitizing eggs of *D. saccharalis* in rice fields in the states of Tocantins and Piauí, northern and northeastern Brazil (Silva, 2018, unpublished data), and in the Tucumán province in Argentina (Isas et al., 2016). According to Browning and Melton (1987), parasitism of *T. atopovirilia* in *D. saccharalis* eggs is low compared to *T. fuentesi* Torre. However, each species of *Trichogramma* Westwood varies widely between strains. Several authors have reported large differences between *Trichogramma* strains in biological parameters, especially fertility (Sorati et al., 1996; Thomson and Hoffmann, 2002; Coelho Jr. et al., 2016). According to Parra et al. (2015), a BC program that does not take intraspecific characteristics of *Trichogramma* into account is likely to fail.

Trichogramma atopovirilia is easier to rear and is reported to occur naturally in *D. saccharalis* eggs, besides the genus *Trichogramma* shows intraspecific variability, especially in fertility. Therefore, this study compared the efficiency of parasitism in *D. saccharalis* eggs of three strains of *Trichogramma*: *T. atopovirilia* reared on a factitious host *A. kuehniella*; *T. atopovirilia* isoline ATP-I, reared on *D. saccharalis*; and *T. galloi*, reared on *A. kuehniella*.

Materials and Methods

Rearing *Diatraea saccharalis*

Eggs of *Diatraea saccharalis* were obtained from a laboratory rearing. Four larvae from these eggs were inoculated into individual flat-bottom glass tubes (25 mm Ø × 85 mm *h*), containing approximately 20 mL of the artificial diet proposed by Hensley and Hammond (1968). The tubes were kept in a climate-controlled room

at 25 ± 2 °C, 60 ± 10 % RH, and 14-h photophase, until the pupa stage. Approximately 25 days after inoculation, pupae of both sexes were transferred to emergence cages of a PVC tube (10 cm Ø × 20 cm *h*) with a Petri dish (15 cm Ø) lined with moistened filter paper on the bottom and a second Petri dish of the same size to cover the cage top. The PVC tube was lined inside with white paper, changed, and moistened daily, where *D. saccharalis* eggs were laid.

The eggs collected were stored in Petri dishes (15 cm Ø) lined with moistened filter paper to keep humidity. Some egg-clusters were used in the experiments (80 %) and others were used to continue the rearing, kept in the laboratory at 25 ± 2 °C, 60 ± 10 % RH, and 14-h photophase.

Rearing *Trichogramma* strains

The *Trichogramma* strains used in the study were kept in a rearing colony in the laboratory. The strains were reared on eggs of *Anagasta kuehniella*, the most suitable factitious host (Parra et al., 2015). These eggs were also obtained from the rearing colony kept at laboratory, which uses a diet based on 97 % whole-wheat flour and 3 % brewer's yeast, according to the method described by Parra (1997).

The eggs of *A. kuehniella* from the rearing colony were placed on a sheet of white cardboard (17 mm *w* × 77 mm *h*), affixed with double-sided tape, and rendered unviable by exposure to germicidal light for 50 min (Stein and Parra, 1987). At the egg-card top, the strain code to which the eggs would be offered and the date of parasitism exposure were noted.

Unviable eggs were offered for parasitism to adults of the different *Trichogramma* strains, in flat-bottom glass tubes (25 mm Ø × 85 mm *h*) for 48 h. For food, the adults were provided a droplet of pure honey, deposited at the egg-card top with the No. 00 entomological pin. The tubes were kept in a controlled room at 25 ± 2 °C, 60 ± 10 % RH, and 14-h photophase while the parasitoids developed. After 48 h, the parasitized egg-cards were transferred to new tubes without *Trichogramma* and approximately 10 days after the eggs were offered for parasitism, parasitoids emerged.

New egg-cards with killed eggs were offered to the adults to continue the rearing.

Trichogramma species and strain selection

Three strains were selected for the experiment. *Trichogramma atopovirilia* (ATP strain) and *T. galloi* reared in eggs of the factitious host *A. kuehniella* were used. The third strain was an isoline termed ATP-I, selected from *T. atopovirilia* (ATP) developed in eggs of *D. saccharalis* for six generations. Taxonomy of the species was confirmed by Dr. Jaci Mendes Vieira, a specialist in *Trichogramma*.

The *T. galloi* strain used was originally collected from *D. saccharalis* eggs in Santa Vitória, Minas Gerais, Brazil (18°50′20″ S, 50°07′15″ W, altitude 498 m) (tropical climate with dry winters, Köppen *Aw*) (Kottek et al., 2006). The ATP strain, *T. atopovirilia*, was collected in the municipality of São José dos Pinhais, Paraná, Brazil (25°32′06″ S, 49°12′21″ W, altitude 906 m) (temperate climate with mild summers, Köppen *Cfb*) in eggs of corn earworm *Helicoverpa zea* (Boddie).

For the selection of ATP-I strain, an egg-card containing several egg-clusters of *D. saccharalis* was exposed to parasitism by female of *T. atopovirilia* (ATP) in a 600-mL glass tube. Regarding feed for adults, a droplet of pure honey was deposited on the tube wall with an No. 00 entomological pin. Parasitism was allowed until the insects died. After nine days, egg-clusters containing darkened eggs, indicating parasitism by *Trichogramma*, were cut from the cardboard and transferred to a new glass tube for emergence and a new set of egg-clusters was exposed to parasitism. This process was repeated for six generations thus genetic variability (nuclear background) for the strain was expected to be low (Li, 1955).

Fifteen selected females were placed individually in flat-bottom glass tubes (25 mm Ø × 85 mm *h*) to create an isoline (isofemale line). Each of the 15 females was offered a *D. saccharalis* egg-cluster and a droplet of pure honey on the tube wall for food. Parasitism was allowed until they died. The most-parasitized egg-cluster was chosen to breed the isoline and an *A. kuehniella* egg-card was offered for the emerged adults to parasitize.

The most parasitized *D. saccharalis* egg-cluster was chosen to rear the isoline and adults emerged from the egg-card were then reared on *A. kuehniella* egg-cards for two generations in order to prevent pre-imaginal conditioning. Adults of the selected ATP-I strain emerged from the egg-card containing *A. kuehniella* eggs were used as one of the treatments for the experiment, along with *T. atopovirilia* (ATP strain) and *T. galloi*, setting up the three experimental strains.

Trichogramma parasitism efficiency on *Diatraea saccharalis* eggs

To conduct the experiment, 30 newly emerged and mated females from each strain were placed in individual flat-bottom glass tubes (25 mm Ø × 85 mm *h*). Again, a droplet of pure honey was deposited on the tube wall as food for the adults. The glass tubes were closed with plastic film and kept in a climate-controlled chamber at 25 ± 1 °C, 75 ± 10 % RH, and 14-h photophase. Egg-clusters with about 40 *D. saccharalis* eggs were offered daily to *Trichogramma* females in the tubes. This process was repeated until all females died in order to assess their life span.

The egg-clusters offered for parasitism were less than 24 h old. The parasitized egg-clusters were collected daily from the tubes containing the females and placed in a new tube of the same dimensions. The new tubes, containing the presumably parasitized egg-

clusters, were kept in the same controlled chamber until the end of parasitoid development.

Total parasitism (fertility) was evaluated based on the number of parasitized (darkened) eggs. Parasitism by the 3rd day (72 h), considered the action time of *Trichogramma* in the field (Kazmer and Luck, 1995), was also assessed. Parasitism viability (emergence) was obtained based on the proportion between dark eggs and the number of eggs with an exit hole. Non-parasitized eggs were also counted until the 3rd day to obtain the parasitism rate per egg-cluster. Life span was assessed by daily observation of female mortality.

Statistical analysis

For each *Trichogramma* strain, 30 females were evaluated using a completely randomized design. The quasi-Poisson generalized linear model (GLM) (Demétrio et al., 2014) was applied to the data for total parasitism and parasitism until the 3rd day. For the analysis of parasitism rate and viability (emergence), the quasi-binomial generalized linear model was used. Quality of the model adjustments was verified by fitting half-normal envelope curves (Moral et al., 2017). When differences were found, the Tukey test was used to compare the means with a 95 % reliability index, designed for the GLM analysis using the GLHT package (Hothorn et al., 2008). A survival curve was used to assess life span. All analyses used the statistical software "R", version 3.2.2.

Results and Discussion

Based on the parasitism capacity of the different species and strains over 72 h (three days), *T. galloi* was superior, parasitizing 11.68 ± 0.94 eggs ($F_{2;86}$ = 91.63; p < 0.001) (Table 1), a much higher number than that found for *T. atopovirilia* strains ATP-I and ATP. Strain ATP-I parasitized more eggs than ATP, indicating that the selection on *D. saccharalis* eggs increased efficiency; however, both strains were inferior to *T. galloi*, parasitizing only 2.63 ± 0.50 and 1.07 ± 0.25 eggs, respectively. Total parasitism per female differed significantly ($F_{3;116}$ = 55.84; p < 0.001) between the two *T. atopovirilia* strains and *T. galloi*. Again, *T. galloi* (GA) was superior to ATP-I (3.03 eggs) and ATP (2.13 eggs) and, in the latter, two strains did not differ from each other in the total number of parasitized eggs (Table 1). Regarding parasitism rate (72 h) per *D. saccharalis* egg-cluster, *T. galloi* showed higher parasitism than the other strains, with a mean of 9.24 ± 0.6 % parasitized eggs per egg-cluster, higher than percentages for ATP-I I (1.87 ± 0.3 %) and ATP (0.74 ± 0.2 %), which differed from each other ($F_{2;87}$ = 86.46, p = 0.001) (Table 1).

Life spans of the three strains showed that, differently from parasitism data, strain ATP-I showed the highest longevity, with a mean of 11.0 ± 0.55 days, although without a major difference from strain ATP (9.1 ± 0.62). Life span of *T. galloi* GA was significantly shorter (8.5 ± 0.50 days) (X^2_3 = 11.1; p = 0.01) (Table 2).

Regarding parasitism viability, the three strains and species showed no significant differences, ranging from 20.8 to 52.1 % ($F_{2;43}$ = 2.96, p = 0.06) (Table 2).

All three species and strains parasitized eggs of *D. saccharalis*, which suggests that in nature, if it does not locate its natural host, *T. atopovirilia* can use eggs of *D. saccharalis* as a host. Each parasitoid species has distinct characteristics; however, there are also intraspecific differences among strains. Several authors have described *T. pretiosum* strains by showing differences in their biological parameters, such as flight capacity, both in the laboratory and in the field (Cerutti and Bigler, 1995; Coelho Jr. et al., 2018), as well as in parasitism efficiency and dispersal in field conditions (Bourchier and Smith, 1996; Fournier and Boivin, 2000; Coelho Jr. et al., 2016).

In a study conducted in Australia, *Trichogramma brassicae* Bezdenko showed different reproductive performances in field and laboratory conditions, since strains kept in the laboratory were selected for the artificial environment, losing their potential as BC agents. This characteristic was reconstituted, obtaining isolines, and drastically reduce the genetic variability of these insects (Sorati et al., 1996). According to Coelho Jr. et al. (2016), 45 *T. pretiosum* isolines showed reproductive performances (parasitism) in the laboratory that clearly differed from one another, similar to other traits, such as spatial distribution, superparasitism, sex ratio, and development rate.

Table 1 – Number of *Diatraea saccharalis* eggs parasitized by females of *Trichogramma* spp. for 72 h, total parasitism over the life span, and parasitism rate per egg-cluster of the three *Trichogramma* strains and species (mean ± standard error). Temperature 25 ± 1 °C, RH: 75 ± 10 % and photophase: 14 h.

Strains	Parasitized eggs in 72 h*	Total parasitized eggs*	Parasitism % per egg-cluster*
ATP-I	2.63 ± 0.50 b	3.03 ± 2.89 a	1.87 ± 0.33 a
ATP	1.07 ± 0.25 a	2.13 ± 2.27 a	0.74 ± 0.17 b
GA	11.68 ± 0.94 c	13.03 ± 4.98 b	9.24 ± 0.61 c

*Means followed by same letter did not differ by the Tukey test p < 0.05; ATP-I = *T. atopovirilia* isoline, reared on *D. saccharalis* eggs for six generations; ATP = *T. atopovirilia* strain, reared on *A. kuehniella* eggs; GA = *T. galloi* strain, reared on *A. kuehniella* eggs.

Table 2 – Adult life span (in days) and parasitism viability (%) (mean ± standard error) on *Diatraea saccharalis* eggs of the three *Trichogramma* strains and species. Temperature 25 ± 1° C, RH: 75 ± 10 %, and photophase: 14 h.

Strains	Life span (days)*	Viability*
ATP-I	11.0 ± 0.55 a	28 ± 16.5 a
ATP	9.1 ± 0.62 ab	20.8 ± 11 a
GA	8.5 ± 0.50 b	52.1 ± 6.8 a

*Means followed by same letter did not differ by the Tukey test, p < 0.05; ATP-I = *T. atopovirilia* isoline, reared on *D. saccharalis* eggs for six generations; ATP = *T. atopovirilia* strain, reared on *A. kuehniella* eggs; GA = *T. galloi* strain, reared on *A. kuehniella eggs*.

The unique characteristics of different *Trichogramma* strains are evident, considering that the same species may have different strains with very particular and divergent inherent characteristics. This suggests that another strain could perhaps accomplish a higher parasitism rate of *D. saccharalis* eggs by *T. atopovirilia*, more adapted to the target host (*D. saccharalis*). In the present study, after the first three days of parasitism, differences were apparent between the isolines selected in *D. saccharalis* eggs (ATP-I) and the non-selected strain. This finding suggested that the results could be different if the *T. atopovirilia* strain assessed was the same collected in Brazil (states of Tocantins and Piauí) or Argentina, where it naturally parasitized *D. saccharalis* (Silva et al., 2018 unpublished data; Isas et al., 2016) rather than the strain kept at labotratory condition, which was originally collected in the state of Paraná (Brazil) parasitizing *H. zea* eggs. Alternatively, if the initial population of *T. atopovirilia* had higher genetic variability, although kept in laboratory for a few generations, the possibility of finding a more aggressive haplotype toward *D. saccharalis* is greater. Comparing different species, Fournier and Boivin (2000) found that the flight activity of a *T. pretiosum* strain from Canada was higher than a strain of *Trichogramma evanescens* Westwood introduced from Egypt, because the Egyptian strain was already adapted to the climatic conditions in Canada where the study was conducted. The specific differences that lead to biological differences, according to the authors, are due to evolutionary pressures.

The rearing of *T. atopovirilia* is easier compared to *T. galloi* for BC programs. The first one adapts better to laboratory conditions and it naturally parasitizes eggs of *D. saccharalis* in rice-fields in Brazil and sugarcane in Argentina. In addition, *T. atopovirilia* shows characteristics of accepting eggs from a factitious host with a longer storage time (Spínola-Filho et al., 2014). All these points motivated this comparative study of the two species, using different strains, which confirmed the superiority of *T. galloi* in parasitizing *D. saccharalis*.

The data from this study indicate that the Paraná strain of *T. atopovirilia* collected on *H. zea* eggs is not a good candidate to replace *T. galloi* for mass rearing of *D. saccharalis* in BC programs. New experiments should be conducted, using different strains of *T. atopovirilia*, particularly strains collected in the field parasitizing *D. saccharalis* eggs. The species of *Trichogramma* show wide intraspecific variations; therefore, strains of *T. atopovirilia* that naturally parasitize this pest should have a closer parasitoid-host relationship, due to the natural processes of strain selection.

Authors' Contributions

Conceptualization: Camarozano, C.T.; Coelho Jr., A.; Silva, R.B.Q.; Parra, J.R.P. **Data acquisition:** Camarozano, C.T.; Coelho Jr., A. **Data analysis:** Camarozano, C.T.; Coelho Jr., A. **Design of methodology:** Camarozano, C.T.; Coelho Jr., A.; Parra, J.R.P. **Writing and editing:** Camarozano, C.T.; Coelho Jr., A.; Silva, R.B.Q.; Parra, J.R.P.

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