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Note

Selection of *Trichogramma* species as potential natural enemies for the control of *Opogona sacchari* (Bojer)

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ABSTRACT: The banana moth *Opogona sacchari* (Bojer) (Lepidoptera: Tineidae) is a polyphagous pest that can cause serious damage, especially to banana crops in southern Brazil. It attacks the fruit, lowering its quality and making bananas unsuitable for export. Current control measures are limited and the use of *Trichogramma* (Hymenoptera: Trichogrammatidae) for Applied Biological Control may be an alternative for the management of this pest. In this study, we investigated the potential parasitism effectiveness of eggs of *O. sacchari* by *T. pretiosum*, *T. atopovirilia* and *T. galloi*, three species of parasitoids commonly used in Applied Biological Control programs in Brazil. Eggs of *O. sacchari* were parasitized by all three *Trichogramma* species, and *T. atopovirilia* and *T. galloi* were the most aggressive, showing greater potential for control of this pest in the banana culture.

Keywords: IPM, banana moth, biological control

Introduction

The banana moth *Opogona sacchari* (Bojer) (Lepidoptera: Tineidae) is a major pest in banana plants in different regions of Brazil. It attacks all parts of the plant except for the roots and leaves (Davis and Peña, 1990). The larva penetrates the fruit and forms galleries, reducing its commercial value (Jang et al., 2010). *O. sacchari* is polyphagous and has been reported to attack coffee, pineapple, papaya, bamboo, corn, sugarcane, stored tubers, and ornamental plants (Peña et al., 1990). The presence of this insect has hindered export of bananas, since it is an A1 or A2 quarantine pest in other countries (Cheraghian, 2013; Eppo, 2015; Senasa, 2016; USDA, 2016), including Argentina (Senasa, 2016), the major importer of Brazilian bananas (FAO, 2014).

To the best of our knowledge, biological control methods have not been used to manage this pest, although the use of microorganisms and parasitoids seem promising. Egg parasitoids of the genus *Trichogramma* have been intensively studied and used in applied biological control programs (Gomes and Parra, 2008; Parra, 2014) because of their efficiency, wide geographic distribution, easiness of laboratory rearing, and large numbers of hosts, more than 200 hosts belonging to 70 families and more than eight insect orders (Hassan, 1993). Lack of information on biological control agents for *O. sacchari* and demand for control methods that can reduce the use of agrochemicals highlight the importance of research on natural enemies that are able to control this pest. In this study, we investigated the effectiveness and parasitism potential of three species of *Trichogramma*, *T. pretiosum* Riley, *T. atopovirilia* Oatman & Platner, and *T. galloi* Zucchi, on eggs of *O. sacchari*, and evaluated the possibility of using them in the biological control of this pest.

Materials and Methods

Specimens of *O. sacchari* were collected from banana plantations in Santa Catarina (Ilhota, Luis Alves, Schröeder, Corupá, Massaranduba, and Jaraguá do Sul municipalities) and reared under laboratory conditions, using the diet developed by Greene et al. (1976), with modifications. Eggs obtained from this laboratory colony were used in the bioassays. Adults of *T. galloi*, *T. pretiosum*, and *T. atopovirilia* were obtained from the laboratory stock. The adults were reared on eggs of the factitious host *Anagasta kuehniella* (Zeller).

Females of each species of *Trichogramma* to be tested (N = 25), which were newly emerged and had already copulated, were placed in individual glass vials (Ø 1 × *h* 7 cm). One honey droplet was placed in the walls of the vials as a food source for adult females. The vials were sealed with PVC film and kept in climate-controlled chambers at 25 ± 1 °C, 75 ± 10 % relative humidity (RH), and a photophase of 14 h. Daily, about 40 eggs of *O. sacchari* were attached to a cardboard (Ø 0.5 × *h* 4 cm) and offered to the females of *Trichogramma*, isolated in the vial. This procedure was repeated daily until the females died to assess longevity.

The egg masses, less than 24 h old, were collected daily from the vials containing the females and placed in a new vial (Ø 1 × *h* 7 cm). These "new" vials with the supposed parasitized egg masses were kept at 25 ± 1 °C, 75 ± 10 % RH, and a photophase of 14 h until emergence was complete. Afterward, the dark eggs were counted to assess the total parasitism, as well as the eggs with an exit hole to assess the emergence rate. The sex ratio was determined using the proportion of males to females emerged through the equation:

$$\text{sex ratio} = \frac{\text{n. of females}}{\text{n. of females} + \text{n. of males}}$$

and males and females were distinguished by their antennae (Pinto, 1999). Parasitism viability (emergence rate) of the three *Trichogramma* species was evaluated using the proportion of the number of eggs with an exit hole for the number of darkened eggs. Longevity of females was evaluated through the daily observation of mortality. The number of insects that emerged was also counted.

For each *Trichogramma* species, 25 females (repetitions) were evaluated, and a completely randomized design was used. The Quasi-Poisson generalized linear models (Demétrio et al., 2014) were fitted to the data for parasitism rate, number of insects emerged, and number of insects per egg. A quasi-binomial generalized linear model was fitted to the data for viability and sex ratio. When the deviance analysis indicated significant treatment effects ($p < 0.05$), multiple comparisons were performed using the 95 % confidence intervals for the linear predictors. The goodness-of-fit for all models was assessed using half-normal plots with a simulated envelope (Demétrio et al., 2014). Kaplan-Meier estimates for the adult lifespan were obtained for each treatment, and the pairwise tests were performed using the log-rank tests ($p = 0.05$) (Matthews and Farewell, 2007). All analyses were performed using the statistical software "R" version 3.2.2.

Results and Discussion

All three *Trichogramma* species parasitized the eggs of *O. sacchari*; however, the parasitoids differed in the number of eggs parasitized (F = 3.71, df = 2, P = 0.03), viability (F = 7.4307, df = 2, P = 0.0012), number of emerged parasitoids (F = 6.62, df = 2, P = 0.002), and sex ratio (F = 3.1903, df = 2, P = 0.025) (Table 1). In contrast, the longevity data (χ^2 = 2.1 df = 2, P = 0.345) and number of insects per egg (F = 2.3047, df = 2, P = 0.1081) did not differ between *T. galloi*, *T. pretiosum*, and *T. atopovirilia*.

The total parasitism observed was 36 to 57 eggs, on average; *T. atopovirilia* and *T. galloi* were the most aggressive species. These species parasitized about 20 % more eggs than *T. pretiosum* (Table 1) did. More individuals of *T. atopovirilia* emerged than *T. pretiosum*, while *T. galloi* was statistically similar to both. The parasitism viability (emergence percentage) ranged from 50 % for *T. pretiosum* to 60 % for *T. atopovirilia* and *T. galloi*. The sex ratio differed between the species. *T. pretiosum* had a higher proportion of males (F = 3.1903, df = 2, P = 0.025). According Russel and Stouthamer (2010), it is highly desirable that a biocontrol agent has a higher proportion of females. Low proportion of females in a population of parasitoids can occur due to variation in ecological variables such as host size or nutritional facts (Luck et al., 2001).

To the best of our knowledge, this is the first report on parasitism of species of *Trichogramma* on eggs of *O. sacchari*. The eggs were parasitized by all three *Trichogramma* species. *T. atopovirilia* and *T. galloi* showed the highest parasitism capacity as well as larger number of parasitoids emerged.

The parasitism capacity of *T. atopovirilia*, that is, the total number of eggs parasitized (eggs that became dark) by one of *Trichogramma* species, was higher than that of *T. pretiosum* for eggs of *Spodoptera frugiperda* (JE Smith) (Beserra et al., 2005) and of *Stenoma catenifer* Walsingham (Nava et al., 2007). In general, *T. galloi* has a higher parasitism capacity than that of *T. pretiosum* for *Diatraea saccharalis* (Fabricius) eggs, as these eggs are rarely parasitized by *T. pretiosum* (Monje, 1995).

Zucchi et al. (1991) found that *T. galloi* was the most abundant species on *D. saccharalis* eggs, which, similarly to *O. sacchari* eggs, are arranged in clusters and are flattened. This shape could explain why *T. galloi* parasitizes more intensively eggs of *O. sacchari*.

The parasitism capacity and viability of parasitoids is related to the size and nutritional quality of the host eggs (Bai et al., 1992; Cônsoli and Vinson, 2012), host age (Vinson, 2010; Zhang et al., 2014; Song et al., 2015) and physical barriers to oviposition (Beserra et al., 2005). These factors directly affect population maintenance of natural enemies, a key factor for natural or classical biological control (Flint and Driestadt, 1998). However, the

Table 1 – Number of parasitized eggs, number of emerged parasitoids, parasitism viability parasitoid sex ratio, parasitoids emerged per egg, and longevity of *Trichogramma galloi*, *T. pretiosum* and *T. atopovirilia* on eggs of *Opogona sacchari* reared at 25 ± 1°, 75 ± 10 % RH and a photophase of 14 h.

Species	Number of parasitized eggs[a] (± SE)[c]	Number of emerged parasitoids[a] (± SE)	Viability[b] (± SE)	Sex ratio[a] (± SE)	Parasitoids emerged per egg[b] (± SE)	Longevity[b] (± SE)
T. galloi	51.43 ± 5.17 ab	19.6 ± 2.0 ab	58.0 ± 5.6 a	0.73 ± 0.019 a	1.1 ± 0.04	8.0 ± 0.9
T. pretiosum	36.45 ± 5.17 b	14.1 ± 2.3 b	50.9 ± 6.8 b	0.50 ± 0.06 b	1.1 ± 0.03	9.0 ± 1.0
T. atopovirilia	57.74 ± 4.57 a	26.8 ± 2.3 a	58.6 ± 5.9 a	0.66 ± 0.04 a	1.03 ± 0.01	8.0 ± 0.6

[a] Means within a column followed by the same letter are not significantly different according to the Tukey HSD test ($p < 0.05$); [b] non-significant difference; [c] Standard error.

most important factor in selecting a parasitoid species for applied biological control programs (programs where a large number of natural enemies were released and control level is reached faster) would be the mortality rate generated by the parasitoid after release, even if it does not become established in the area (Flint and Driestadt, 1998).

In this study, *T. atopovirilia* and *T. galloi* caused high mortality of eggs of *O. sacchari*. Overall, the data confirmed that *T. atopovirilia* and *T. galloi* were promising biocontrol agents for *O. sacchari*, better than *T. pretiosum*, having potential to be used in applied biological control programs targeted at this insect pest species.

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