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Source: Florida Entomologist, 100(3) : 653-656

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.100.0324

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Biology of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) fed transgenic maize pollen

Maria F. De Sousa[1,], Marcos Gino Fernandes[2], and Thiago Alexandre Mota[2]*

Abstract

Trichogramma pretiosum Riley (Hymenoptera: Trichogrammatidae) is an important natural egg parasitoids of pest insects in the agricultural environment, being used as a form of biological control, and it may be affected by the *Bacillus thuringiensis* (Bt) toxin present in transgenic plants widely used in Brazil. The aim of this study was to evaluate the effect of Bt maize pollen fed to adults of the parasitoid *T. pretiosum* Riley (Hymenoptera: Trichogrammatidae) reared in the eggs of the alternate host *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Three treatments (diets) were compared: pollen from Bt maize, pollen from non-Bt maize, and 10% honey solution (control). Each treatment consisted of 50 *T. pretiosum* females that were freshly emerged and mated (between 24 and 36 h old). Biological characteristics indicative of the efficiency of *T. pretiosum* as a biological control agent were evaluated for 4 generations. The results suggest that the consumption of pollen did not affect the evaluated biological characteristics, such as percentage of parasitism, sex ratio, and number of individuals reared per host egg, in any of 4 generations. Thus, we showed that Bt maize is compatible with the use of *T. pretiosum* for biological control.

Key Words: integrated pest management; insect–plant interaction; egg parasitoid; Cry1Ab

Resumo

Trichogramma pretiosum Riley (Hymenoptera: Trichogrammatidae) são parasitóides de ovos naturais de insetos no ambiente agrícola e podem ser afetados pela toxina *Bacillus thuringiensis* (Bt) presente em plantas transgênicas amplamente utilizadas no Brasil. O objetivo deste estudo foi avaliar o efeito do pólen de milho Bt alimentando adultos do parasitóide *T. pretiosum* Riley (Hymenoptera: Trichogrammatidae) criados nos ovos do hospedeiro alternativo *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Três tratamentos (dietas) foram comparados: pólen de milho Bt, pólen de milho não Bt e solução de mel a 10% (controle). Cada tratamento consistiu em 50 fêmeas de *T. pretiosum* que foram recentemente emergidas e acasaladas (entre 24 e 36 h de idade). As características biológicas indicativas da eficiência de *T. pretiosum* como agente de controle biológico foram avaliadas por 4 gerações. Os resultados sugerem que o consumo de pólen não afetou as características biológicas avaliadas, como porcentagem de parasitismo, razão sexual e número de indivíduos criados por ovo do hospedeiro, em qualquer uma das 4 gerações. Assim, provamos que o milho Bt é compatível com o uso de *T. pretiosum* para o controle biológico.

Palavras Chave: manejo integrado de pragas; interação inseto–planta; parasitoide de ovos; Cry1Ab

Maize (*Zea mays* L.; Poaceae) is one of the most important crops in the world, in both social and economic contexts. Brazil is the world's 3rd largest producer of maize, with a cultivated area of nearly 16 million hectares, resulting in more than 87 million tons produced in 2016 (Conab 2017). Maize production can be compromised by several factors, such as climate fluctuations, plant diseases, and destruction by pests that can cause production losses and have a great economic impact. Maize is vulnerable to a wide range of pests, especially to larvae of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), an insect that attacks species from several plant families and is considered the main pest of maize in Brazil (Lima et al. 2012).

Among the natural enemies of *S. frugiperda*, egg parasitoids, including *Trichogramma* species (Hymenoptera: Trichogrammatidae). One of the advantages of using *Trichogramma* species is their ease of rearing under laboratory conditions, permitting production and commercialization when grown in suitable hosts (Wang et al. 2013).

Trichogramma pretiosum Riley (Hymenoptera: Trichogrammatidae) is an important egg parasitoid. Parasitoids of eggs are particularly valuable because they prevent their hosts, especially those from the order Lepidoptera, from reaching the larval stage, which is typically the stage causing maize losses (Olson & Andow 2006; Gardner et al. 2011), often eliminating the need for other control measures. The use of such biocontrol constitutes one of the tactics of integrated pest management to control the pest population, and the combination of biological control with the use of insect-resistant genetically modified plants has become a widely used approach in integrated pest management.

Despite the benefits, there are possible risks associated with genetically modified plants used in integrated pest management programs. Maize pollen, for example, is an important nutritional resource for some biocontrol agents, including *Trichogramma* species.

Several studies have been carried out with non-target species including predators, pollinators, and parasitoids to demonstrate the possible effects of the *Bacillus thuringiensis* (Bt) toxin expressed in genetically modified maize on these organisms (Silva et al. 2014; Tian et al. 2014). To complement these studies, our objective was to evaluate the effect of Bt maize pollen on selected biological characteristics of *T.*

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pretiosum, which feeds as an adult on maize pollen. We evaluated the percentage of parasitism, number of individuals reared per host egg, parasitoid viability, sex ratio, and longevity of female offspring because these characteristics are indicative of the efficiency of *T. pretiosum* as a biological control agent.

Materials and Methods

EXPERIMENT LOCATION

The present study was conducted at the Laboratory of Insect Rearing of the Faculty of Biological and Environmental Sciences of the Federal University of Grande Dourados, State of Mato Grosso do Sul, Brazil.

INSECTS ORIGIN AND REARING

The *T. pretiosum* adult parasitoids used in this study originated from cultures kept for several generations (more than 10). They were acclimatized in biochemical oxygen demand chambers (model EL 222, Electrolab, Mumbai, India) at a constant temperature of 25 ± 1 °C, 70% relative humidity measured in a digital thermohigrometer model 7666 (Incoterm, China), and a photoperiod of 14:10 h L:D in the laboratory. The rearing process of *T. pretiosum* parasitoids was based on the method of Parra (1997). All the adult females used in the bioassays were fed a 10% honey solution.

For these experiments, a haplodiploid strain of *T. pretiosum* was used. The parasitoids were grown in the eggs of the alternative host *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). The eggs used in the experiments were harvested on the day of experiments.

POLLEN SOURCE

Maize pollen was directly harvested from Bt transgenic maize (MON 89034), which expresses the Cry1A105 and Cry2Ab2 proteins, and conventional non-Bt maize (DKB177) at the Experimental Farm of the Federal University of Grande Dourados, Brazil. The crop area comprised 0.5 ha and was cultivated in a conventionally managed cultivation system, without the addition of insecticides during the pollen harvesting period.

Pollen processing, aiming at preparing the diets, was conducted as done in Wang et al. (2007). Briefly, pollen was harvested by carefully placing paper bags over the tassel of plants during anthesis, stapling the bags closed, and removing the bags after 48 h. After collection, the pollen was sieved at the Laboratory of Insect Cultivation, transferred to plastic containers, and stored in a refrigerator at 4 °C.

BIOASSAYS

Three treatments (diets) were applied as was done in Wang et al. (2007): pollen from Bt maize (20 mg pollen suspended in a 10% honey solution), pollen from non-Bt maize (20 mg pollen suspended in a 10% honey solution), and a 10% honey solution (control). For each treatment, 50 newly emerged and mated female *T. pretiosum* wasps (24 to 36 h old) were transferred to individual microcentrifuge tubes (1.5 mL, 4.5 cm; Eppendorf, Hamburg, Germany) that contained a piece of cardboard (4.5 × 0.9 cm) with 30 *E. kuehniella* eggs (Pereira et al. 2004; Zart et al. 2012) and 1 drop of the respective diet solution. This procedure was repeated during 4 parasitoid generations. Parasitism was allowed for 24 h, after which the females were sacrificed and the cardboard pieces that contained parasitized eggs were transferred to closed plastic bags (23 × 4 cm) and kept in a climatized chamber at 25 ± 1 °C, 70% relative humidity, and a 14:10 h L:D photoperiod, until offspring emergence. This experiment was repeated 4 times.

After offspring emergence, we recorded the following variables: parasitoid viability (*E. kuehniella* eggs with an orifice), percentage of parasitism (proportion of parasitized eggs [blackened eggs] × 100), number of adults emerged per egg (number of emerged individuals per number of eggs with an orifice), longevity of female offspring, and sex ratio (number of females / total number of emerged individuals). The adults were sexed according to Querino & Zucchi (2011). The longevity of female wasps fed each experimental diet was determined for each generation by transferring 50 newly emerged, mated females (24 to 36 h old), to individual microcentrifuge tubes (1.5 mL, 4.5 cm; Eppendorf, Hamburg, Germany) that contained 1 drop of the respective nutritional source, where they remained until dying. Longevity was determined by recording the number of days the female wasps lived after transfer to the tubes.

STATISTICAL ANALYSES

We used an experimental design with 3 treatments (honey + Bt maize pollen, honey + conventional maize pollen, and honey) and 50 repetitions for each of 4 parasitoid generations. The treatments and the generations of the parasitoid were subjected to analysis of variance and the means were compared by the Tukey test ($P \leq 0.05$) in the Assistat program version 7.7 2016 (Silva 2016).

Results

The percentage of parasitism by *T. pretiosum* was high, with percentages ranging from 52.9 to 92.3%. The percentage of parasitism by wasps fed Bt pollen and control diets were significantly different in the 1st and 3rd generations, with lower percentage of parasitism in the control-fed wasps (Table 1). In contrast, no significant differences were observed in the 2nd and 4th generations fed Bt pollen and the control diet. There were no significant differences between the Bt pollen and non-Bt pollen treatments, except during the 4th generation that presented lower percentage of parasitism in Bt pollen-fed wasps (about 84.1% compared with about 92.3% in the non-Bt pollen and the control honey solution; Table 1).

The percentage of parasitism by both Bt pollen- and non-Bt pollen-fed *T. pretiosum* wasps were significantly lower in the 2nd generation, and the percentage of parasitism by honey-fed wasps was higher in the 4th generation (Table 2).

Parasitoid viability reached values of over 70% in all treatments, and although there was a significant difference between the Bt pollen and non-Bt pollen treatments in the 2nd generation, no significant differences were observed between the treatments in the 1st, 3rd, and 4th generations (Table 1). However, when the generations were compared within treatments, the viability of wasps fed Bt pollen in the 4th generation and the viability of non-Bt pollen-fed wasps in the 2nd and 4th generations were significantly different from the respective viability of wasps in the 1st generation (Table 2).

The sex ratio ranged from 0.82 and 0.93 and was similar between the treatments in all of the 4 generations (Tables 1 and 2).

The number of individuals emerging per egg varied between 0.95 and 1.58, with no differences between treatments in the first 3 generations; however, for the honey treatment, the number of individuals per egg in the 4th generation was significantly greater than the numbers in the other treatments (Table 1).

The longevity of female offspring was similar among the treatments of the 1st, 2nd, and 4th generations; however, in the 3rd generation,

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Table 1. Reproductive biology of *Trichogramma pretiosum* fed on transgenic Bt maize pollen, conventional maize pollen, or honey solution (control).

Generation	Treatment	Parasitism (%)	Viability of parasitoids	Sex ratio	No. of individuals per egg	Female longevity (d)
1	Honey + Bt maize pollen	75.45 ± 3.34 a	0.71 ±0.02 a	0.93 ± 0.02 a	1.04 ± 0.03 a	10.42 ± 0.32 a
	Honey + conventional maize pollen	72.66 ± 4.19 ab	0.78 ± 0.03 a	0.87 ± 0.03 a	0.96 ± 0.05 a	9.90 ± 0.39 a
	Honey	61.59 ± 4.15 b	0.78 ± 0.04 a	0.86 ± 0.03 a	1.02 ± 0.04 a	10.66 ± 0.35 a
2	Honey + Bt maize pollen	56.06 ± 3.71 a	0.75 ± 0.03 b	0.89 ± 0.02 a	1.07 ± 0.05 a	10.52 ± 0.20 a
	Honey + conventional maize pollen	57.52 ± 2.78 a	0.88 ± 0.01 a	0.83 ± 0.02 a	0.95 ± 0.02 a	10.44 ± 0.27 a
	Honey	52.86 ± 2.64 a	0.83 ± 0.03 ab	0.82 ± 0.03 a	0.96 ± 0.03 a	10.60 ± 0.26 a
3	Honey + Bt maize pollen	76.99 ± 3.10 a	0.78 ± 0.02 a	0.91 ± 0.02 a	1.06 ± 0.04 a	11.76 ± 0.35 a
	Honey + conventional maize pollen	75.46 ± 3.53 ab	0.85 ± 0.02 a	0.86 ± 0.03 a	1.01± 0.03 a	10.16 ± 0.28 b
	Honey	64.59 ± 3.66 b	0.82 ± 0.02 a	0.88 ± 0.02 a	1.59 ± 0.58 a	9.22 ± 0.32 b
4	Honey + Bt maize pollen	84.06 ± 3.06 b	0.91 ± 0.01 a	0.90 ± 0.01 a	0.98 ± 0.01 b	10.68 ± 0.32 a
	Honey + conventional maize pollen	92.26 ± 1.99 a	0.93 ± 0.01 a	0.91 ± 0.01 a	1.00 ± 0.01 b	10.04 ± 0.41 a
	Honey	91.99 ± 2.11 ab	0.89 ± 0.02 a	0.89 ± 0.01 a	1.58 ± 0.57 a	10.24 ± 0.37 a

Means followed by the same letter in a column did not differ by the Tukey test at 5% probability.

longevity was longest in Bt pollen-fed offspring (Table 1). We found that the longevity of females of both Bt pollen- and honey-fed offspring in the 3rd generation was significantly different from the longevity of wasps in the 1st and 2nd generations, with the 3rd generation females living longer in the Bt pollen treatment and shorter in the honey (control) treatment (Table 2).

Discussion

The efficiency of parasitism can be affected by various factors, including the species of parasitoid host, number of generation of the parasitoid maintained in the laboratory, and alternative hosts used for rearing (Pratissoli & Oliveira 1999), as well as biological characteristics of the host itself (Pratissoli et al. 2004a). However, the present study has demonstrated that the consumption of pollen from Bt maize had little effect on the biological characteristics of the *T. pretiosum* parasitoid, over 4 generations.

The high viability of the parasitoids in the experiments conducted here indicates that the different diets tested and the use of an alternative host had little effect on the capacity of parasitism of the parasitoid. The results observed here are similar to the high levels of parasitism observed by Nicoli et al. (2004) in their study of the viability of *Trichogramma atopovirilia* Oatman & Platner (Hymenoptera: Trichogrammatidae) in the eggs of *E. kuehniella*.

In the present study, the proportion of females (i.e., sex ratio) was also high, which is highly desired in biological control programs, because it indicates a higher potential to effectively control pests (Pratissoli et al. 2004b; Wakeil et al. 2008). Longevity of females is important for biological control programs, because parasitoids that live longer can be more effective natural enemies and remain in culture for longer periods than short-lived individuals (Sorensen et al. 2012).

Several studies have been carried out with parasitoids in many crops of economic interest, such as soybean and corn, to identify possible effects of the Bt toxin on these organisms, as they are of extreme agricultural importance because they control insects considered pests. For example, a study with toxin Cry1F in corn, the parasitoid *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae), and the host *S. frugiperda* demonstrated that Cry1F maize does not affect the development, parasitism, survival, sex ratio, longevity, and fecundity of *C. marginiventris* females when they parasitize the host

Table 2. Reproductive biology of *Trichogramma pretiosum* fed on transgenic Bt maize pollen, conventional maize pollen, or honey solution (control), considering the comparison of 4 generations of the parasitoid maintained in laboratory.

Generation	Parasitism (%)	Parasitoid viability	Sex ratio	No. of individuals per egg	Female longevity (d)
Honey + Bt maize pollen					
1	75.45 ± 3.34 a	0.71 ± 0.02 b	0.93 ± 0.02 a	1.04 ± 0.03 a	10.42 ± 0.32 b
2	56.06 ± 3.71 b	0.75 ± 0.03 b	0.89 ± 0.02 a	1.07 ± 0.05 a	10.52 ± 0.20 b
3	76.99 ± 3.10 a	0.78 ± 0.02 b	0.91 ± 0.02 a	1.06 ± 0.04 a	11.76 ± 0.35 a
4	84.06 ± 3.06 a	0.91 ± 0.01 a	0.90 ± 0.01 a	0.98 ± 0.01 a	10.68 ± 0.32 ab
Honey + conventional maize pollen					
1	72.66 ± 4.19 b	0.78 ± 0.03 b	0.87 ± 0.03 a	0.96 ± 0.05 a	9.90 ± 0.39 a
2	57.52 ± 2.78 c	0.88 ± 0.01 a	0.83 ± 0.02 a	0.95 ± 0.02 a	10.44 ± 0.27 a
3	75.46 ± 3.53 b	0.85 ± 0.02 ab	0.86 ± 0.03 a	1.01 ± 0.03 a	10.16 ± 0.28 a
4	92.26 ± 1.99 a	0.93 ± 0.01 a	0.91 ± 0.01 a	1.00 ± 0.01 a	10.04 ± 0.41 a
Honey					
1	61.59 ± 4.15 b	0.78 ± 0.04 a	0.86 ± 0.04 a	1.02 ± 0.04 a	10.66 ± 0.35 a
2	52.86 ± 2.64 b	0.83 ± 0.03 a	0.82 ± 0.03 a	0.96 ± 0.03 a	10.60 ± 0.26 a
3	64.59 ± 3.66 b	0.82 ± 0.02 a	0.88 ± 0.02 a	1.59 ± 0.58 a	9.22 ± 0.32 b
4	91.99 ± 2.11 a	0.89 ± 0.02 a	0.89 ± 0.01 a	1.58 ± 0.57 a	10.24 ± 0.37 ab

Means followed by the same letter in a column did not differ by the Tukey test at 5% probability.

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S. frugiperda fed with Bt corn containing the Cry1F toxin (Tian et al. 2014). Soybean expressing the Cry1Ac protein does not affect the development time or the survival of the nymphal stages of *Euschistus heros* (F.) (Hemiptera: Pentatomidae), or weight, pronotum size, sex ratio, and fecundity of adults, and egg viability. *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae), an *E. heros* egg parasitoid, did not show any differences in ability to parasitize hosts, egg-to-adult period, sex ratio, and longevity when reared on *E. heros* fed Bt soybean as compared with *T. podisi* reared on *E. heros* fed non-Bt soybean (Silva et al. 2014).

Bt crops may have direct and indirect effects on non-target organisms (Lovei et al. 2009; Naranjo, 2009). Direct effects may occur by ingestion of plant tissues by non-target organisms, whereas indirect effects may occur through multi-trophic interactions (various interrelationships that occur between organisms that are present in the environment) (Craig et al. 2008). However, similar to our findings, Pratissoli et al. (2006) reported that providing Bt suspensions as a food source to adult *Trichogramma pratissoli* Querino & Zucchi (Hymenoptera: Trichogrammatidae) had no effect on parasitism, although an indirect effect on offspring emergence was observed with some treatments of different isolates of Bt.

Wang et al. (2007) also did not observe any difference in offspring longevity, number of parasitized eggs, viability, or sex ratio of *Trichogramma ostriniae* Pang & Chen (Hymenoptera: Trichogrammatidae) adult females fed with pollen from transgenic and non-transgenic maize. Santos et al. (2011) observed that biological parameters of *T. pretiosum*, such as parasitism capacity, parasitoid viability, and sex ratio, were not affected by treatment with Bt toxin. The findings of the present study are in agreement with previous studies and demonstrate that the use of Bt maize is compatible with the use of *T. pretiosum* for biological control.

Acknowledgments

We thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for granting a Master scholarship to the first author.

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