

Influence of Bt Maize on Diversity and Composition of Non-target Arthropod Species

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Abstract

Non-target arthropods may be affected by toxins derived from *Bacillus thuringiensis* (Bt) expressed in transgenic maize. The objective of this study was to evaluate the possible impacts of Bt maize on the diversity and the composition of non-target arthropod species by analyzing one field cultivated with conventional maize (no expressing transgenic protein) and three fields cultivated with transgenic maize (expressing Bt proteins). In each field was sampled 50 entirely plants for the number of arthropod specimens and registred the degree of injury caused by the chewing insects. A total of 2.525 specimens of arthropods, comprising 29 species from 25 families, were recorded on 3.000 sampled plants. The most diverse family belonged to the order Hemiptera. Based on Shannon and Simpson indexes, the Bt-transgenic cultivar EXP3320YG had lower level of non-target arthropod diversity than other cultivars. From this study, it is clear that the diversity of non-target arthropods on maize crop is negatively affected by Cry1Ab protein, while the Cry1A105+Cry2Ab2+Cry1F proteins, and Cry1A105+Cry2Ab2+Cry3Bb1 proteins do not have any effect on arthropod species diversity and composition.

Keywords: biosafety, biodiversity, diversity index, GM maize

1. Introduction

The global area of transgenic crops reached 189.8 million hectares in 2017, which makes it one of the fastest adopted technologies in recent times (ISAAA, 2017). The main transgenic crops worldwide are soybean (94.1 million hectares), followed by maize (59.7 million hectares), cotton (24.1 million hectares), and canola (10.2 million hectares) (ISAAA, 2017). In Brazil, 50.2 million hectares are planted with transgenic cultivars of soybean, maize, and cotton (ISAAA, 2017). The cultivation of transgenic plants has some advantages, among which we can mention: increase of crop yield, less intensive use of labor and reduction of ecological damages (Huang et al., 2005; Naranjo, 2009; Wang et al., 2010).

Maize, with an area of about 16 million hectares and a production of over 82 million tons, is one of the most important crops in Brazilian economy (CONAB, 2018). The large-scale implementation of transgenic cultivars represents a technological innovation, and it is therefore essential to continuously evaluate their efficiency and possible effects on the environment (Bauer-Panskus & Then, 2014).

Non-target arthropods in maize ecosystems have important ecological functions such as regulation of arthropod and pest populations, decomposition and recycling of organic matter, and pollination or they may act as pests (Comas et al., 2014).

Most transgenic maize crops produce one or more Cry proteins of *Bacillus thuringiensis* (Bt) (Guo et al., 2016). Bt proteins in maize are expressed throughout the plant development (Groot & Dicke, 2002); therefore, target and non-target arthropods are exposed to these proteins directly by feeding on plant tissues (e.g., leaves, pollen or nectar) (Lovei et al., 2009; Naranjo, 2009), or indirectly through the consumption of prey or hosts that consumed plant tissues (Craig et al., 2008).

Several studies worldwide have been carried out with maize, soybean or cotton plants expressing the Bt toxins to demonstrate the possible effects of these crops on non-target species, including herbivore, predator, pollinator, and parasitoid arthropods. Most of these studies shows no effects of Bt toxins on herbivores (Habustova et al., 2014; Szenasi et al., 2014), predators (De la Poza et al., 2005; Liu et al., 2012; Guo et al., 2016; Van den Berg et al., 2017), parasitoids (Bortolotto et al., 2014; Silva et al., 2014; De Sousa et al., 2017; Tian et al., 2018), and

pollinators (Yi et al., 2018). However, other studies demonstrate some effects of the Bt crops on arthropods (Prutz & Dettner, 2004; Liu et al., 2005a, 2005b; Vojtech et al., 2005; Ramirez-Romero et al., 2007; Sanders et al., 2007; Cunha et al., 2012; Hansen et al., 2012; Pessoa et al., 2016).

So, to complement these studies, we carried out this research with the objective of evaluating the possible effects of Bt maize on insect diversity and composition of species, analyzing one field cultivated with conventional maize (no expressing transgenic protein) and three fields cultivated with transgenic maize (expressing Bt proteins).

2. Materials and Method

2.1 Experimental Areas

The present study was carried out in the 2017 crop season in four fields used for commercial maize production located in the region of Dourados, state of Mato Grosso do Sul (22.19 84466° N, 51.8901629° W). They were evaluated three commercial transgenic maize cultivars: EXP3320YG (Monsanto, EUA) expressing Cry1Ab that confers resistance to Lepidoptera, MG600PW (Monsanto, EUA e Dow Agrosociences, EUA) expressing Cry1A.105+Cry2Ab2+Cry1F that confer resistance to Lepidoptera and AG9030VTpro3 (Monsanto, EUA) expressing Cry1A.105+Cry2Ab2+Cry3Bb1 that confer resistance to Lepidoptera and Coleoptera; it was also evaluated a fourth field with the conventional cultivar SW5560 (no expressing transgenic protein). All fields were cultivated in a distance of 500m between them, and cultural practices in the sample fields followed the good agricultural practice recommended for the region.

2.2 Sampling Methods

In each of the 4 fields were sampled 50 plants (sample units) randomly selected plants for the careful visual counting of the insects present (this way avoiding the flight of the insects) and the degree of injury caused by the chewing insects in each plant. The sampling period was from the April 4, 2017 until July 11, 2017 (comprising 15 weeks). In this time period, that comprised the time of occurrence of all key pests from the maize crops in this region, were taken samplings of the pest, predator, parasitoid and flower-visiting insects. In the four fields, the weekly evaluations were carried out on the same day.

The arthropod samples were collected for identification to the lowest possible taxonomic level with the aid of identification keys and upon consultation with taxonomists who were specialists in the taxonomic groups found. Some collected individuals could not be identified to the species level, reaching the gender or family level. But all of them were classified at least to the morphospecies level.

2.3 Data Analysis

Species richness and uniformity for each plot was determined using the Shannon diversity index, that was calculated by $\sum pi \cdot \ln pi$, where, pi is the probability of occurrence of the individual i , and \ln is logarithm neperian (Shannon & Weaver, 1949), and species dominance was assessed with the Simpson's index (Pinto-Coelho, 2002). Also was made graphic analysis about the number of individuals from each population of insect pest and natural enemies in each sampling date.

3. Results

3.1 Species Richness and Abundance

A total of 2.525 individuals of arthropods, comprising 29 species (Table 1), were recorded in 3.000 plants during the entire sample period. The highest number of individuals was found on EXP3320YG (736), but the lowest on MG600PW. The latter also had the lowest diversity of species (16 species), while the greatest diversity was observed in the conventional cultivar (21 species) (Table 1).

The cultivar EXP3320YG harbored a large number of arthropods, with the following species being more abundant as compared with the other evaluated cultivars: *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae), *Diabrotica speciosa* (Germar) (Coleoptera: Chrysomelidae), *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), *Dalbulus maidis* (DeLong & Wolcott) (Hemiptera: Cicadellidae), and Acrididae sp. (Orthoptera). On the cultivar MG600PW, nymphs of Pentatomidae, *Doru luteipes* (Scudder) (Dermaptera: Forficulidae), and Araneae were present in greater number when compared to the other cultivars. *Helicoverpa zea* (Boddie) (Lepidoptera, Noctuidae), *Leptoglossus zonatus* (Dallas) (Hemiptera: Coreidae), Aphididae (Hemiptera), *Solenopsis* sp. (Hymenoptera), and Syrphidae sp. (Diptera) were more abundant on AG9030 VTpro3. Compared to other cultivars, SW5560, harbored more *Euschistus heros* (Fabricius) (Heteroptera: Pentatomidae), Chrysopidae sp. (Neuroptera), and *Musca domestica* (L.) (Diptera: Muscidae) (Table 1).

In relation to the presence of natural enemies sampled in the areas, MG600PW harbored a large number of *Cycloneda sanguinea* (L.) (Coleoptera: Coccinellidae) and *D. luteipes*; followed by AG9030VTpro3, which hosted a large number of *C. sanguinea* and Chrysopidae sp., and EXP3320YG that accommodated a large number of *C. sanguinea* and *D. luteipes* (Table 1).

Regarding the flower-visiting insects, the species *D. speciosa* and Syrphidae sp. were the most abundant among the species known as potential pollinators (Coleoptera, Diptera, Hymenoptera, and Lepidoptera). *D. speciosa* was most abundant on EXP3320YG, while its lowest incidence was registered for MG600PW; the smallest number of Syrphidae sp. individuals was found on EXP3320YG, and the highest number of those individuals was observed on AG9030VTpro3 (Table 1).

The data on population dynamics of the main pest and predator species show that the number of individuals was highly different among the four cultivars. In general, the pests showed higher population on EXP3320YG cultivar and smaller population on MG600PW cultivar (Table 1). The number of *S. frugiperda* caterpillars was higher in the EXP3320YG cultivar during the whole occurrence period, reaching a peak of 80 individuals in the seventh evaluation. The total number of *D. speciosa* adults was high in the cultivar EXP3320YG, showing a peak of 69 individuals per 50 plants in the sixth evaluation and 48 individuals in the tenth evaluation. Cultivar AG9030VTpro3 also presented a high number of individuals with a maximum of 21 individuals in the tenth evaluation. For the MG600PW cultivar, the number of this pest was not high during the whole period of its occurrence (Figure 1A).

The population of *C. sanguinea* and *D. luteipes* was high in the cultivar MG600PW, both reaching high number of individuals in this cultivar. In cultivar AG9030VTpro3 only *C. sanguinea* reached high population in the final period of the occurrence (after tenth sampling) of this predator (Figure 1B).

Table 1. Species of pest, predator, parasitoid and flower-visiting insects on three resistant insect transgenic maize cultivars and one conventional maize cultivar. Diversity index Shannon-Wiener (H), Simpson (D) and number of individuals. Dourados, MS, 2017

Order and Family	EXP3320 YG			MG600PW			AG9030 VTpro3			Conventional SW5560		
	Individuals number	pi·lnpi	pi ²	Individuals number	pi·lnpi	pi ²	Individuals number	pi·lnpi	pi ²	Individuals number	pi·lnpi	pi ²
Lepidoptera												
<i>Spodoptera frugiperda</i>	321	0.190219	-0.36191	68	0.024103	-0.28919	133	0.032566	-0.30899	170	0.076659	-0.35556
<i>Helicoverpa zea</i>	4	2.95E-05	-0.02834	0	0	0	78	0.011201	-0.23769	4	4.24E-05	-0.03279
Sphingidae	0	0	0	0	0	0	1	1.84E-06	-0.00896	0	0	0
Coleoptera												
<i>Diabrotica speciosa</i>	197	0.071644	-0.35279	17	0.001506	-0.1261	68	0.008513	-0.21988	126	0.042112	-0.325
<i>Cycloneda sanguinea</i>	29	0.001553	-0.12742	67	0.023399	-0.2872	65	0.007778	-0.21416	41	0.004459	-0.18072
<i>Lagria villosa</i>	6	6.65E-05	-0.03921	0	0	0	2	7.36E-06	-0.01604	6	9.55E-05	-0.04523
Hemiptera												
<i>Euschistus heros</i>	0	0	0	0	0	0	4	2.95E-05	-0.02831	7	0.00013	-0.05101
<i>Dichelops</i> sp.	1	1.85E-06	-0.00897	0	0	0	0	0	0	0	0	0
<i>Leptoglossus zonatus</i>	4	2.95E-05	-0.02834	2	2.09E-05	-0.02461	5	4.60E-05	-0.03387	3	2.39E-05	-0.026
Reduviidae	0	0	0	0	0	0	0	0	0	1	2.65E-06	-0.01046
Ninfas Pentatomidae	10	0.000185	-0.05841	16	0.001334	-0.1209	11	0.000223	-0.06276	10	0.000265	-0.06706
Aphididae	84	0.013026	-0.24771	95	0.047043	-0.33149	144	0.038176	-0.31902	73	0.014135	-0.25319
<i>Bemisia tabaci</i>	10	0.000185	-0.05841	0	0	0	2	7.36E-06	-0.01604	3	2.39E-05	-0.026
<i>Dalbulus maidis</i>	7	9.05E-05	-0.04428	4	8.34E-05	-0.04289	2	7.36E-06	-0.01604	3	2.39E-05	-0.026
<i>Notozulia entreriana</i>	1	1.85E-06	-0.00897	0	0	0	0	0	0	0	0	0
Hymenoptera												
<i>Solenopsis</i> sp.	1	1.85E-06	-0.00897	7	0.000255	-0.06611	9	0.000149	-0.0538	0	0	0
<i>Ata</i> sp.	0	0	0	0	0	0	1	1.84E-06	-0.00896	0	0	0
<i>Apis mellifera</i>	0	0	0	1	5.21E-06	-0.01389	0	0	0	0	0	0
Vespidae	0	0	0	0	0	0	0	0	0	1	2.65E-06	-0.01046
Neuroptera												
Chrysopidae sp.	2	7.38E-06	-0.01605	13	0.000881	-0.10439	16	0.000471	-0.08315	17	0.000767	-0.09931
Dermaptera												
<i>Doru luteipes</i>	20	0.000738	-0.09798	80	0.03336	-0.31054	15	0.000414	-0.07926	19	0.000958	-0.10755
Forficulidae sp.	0	0	0	1	5.21E-06	-0.01389	0	0	0	0	0	0
Araneae												
Araneae	0	0	0	3	4.69E-05	-0.03413	1	1.84E-06	-0.00896	2	1.06E-05	-0.01865
Orthoptera												
Acrididae sp.	4	2.95E-05	-0.02834	2	2.09E-05	-0.02461	0	0	0	1	2.65E-06	-0.01046
Gryllidae sp.	0	0	0	0	0	0	0	0	0	1	2.65E-06	-0.01046
Diptera												
<i>Musca domestica</i>	4	2.95E-05	-0.02834	2	2.09E-05	-0.02461	0	0	0	5	6.63E-05	-0.03917
Syrphidae sp.	30	0.001661	-0.13044	60	0.018765	-0.27231	180	0.05965	-0.34428	120	0.038197	-0.31906
Tachinidae sp.	0	0	0	0	0	0	0	0	0	1	2.65E-06	-0.01046
Odonata												
Libellulidae sp.	1	1.85E-06	-0.00897	0	0	0	0	0	0	0	0	0
Total individuals	736			438			737			614		
Total species	19			16			18			21		
Shannon-Wiener	1.692801			2.086845			2.060156			2.024573		
Simpson	0.721			0.849148			0.840755			0.822019		

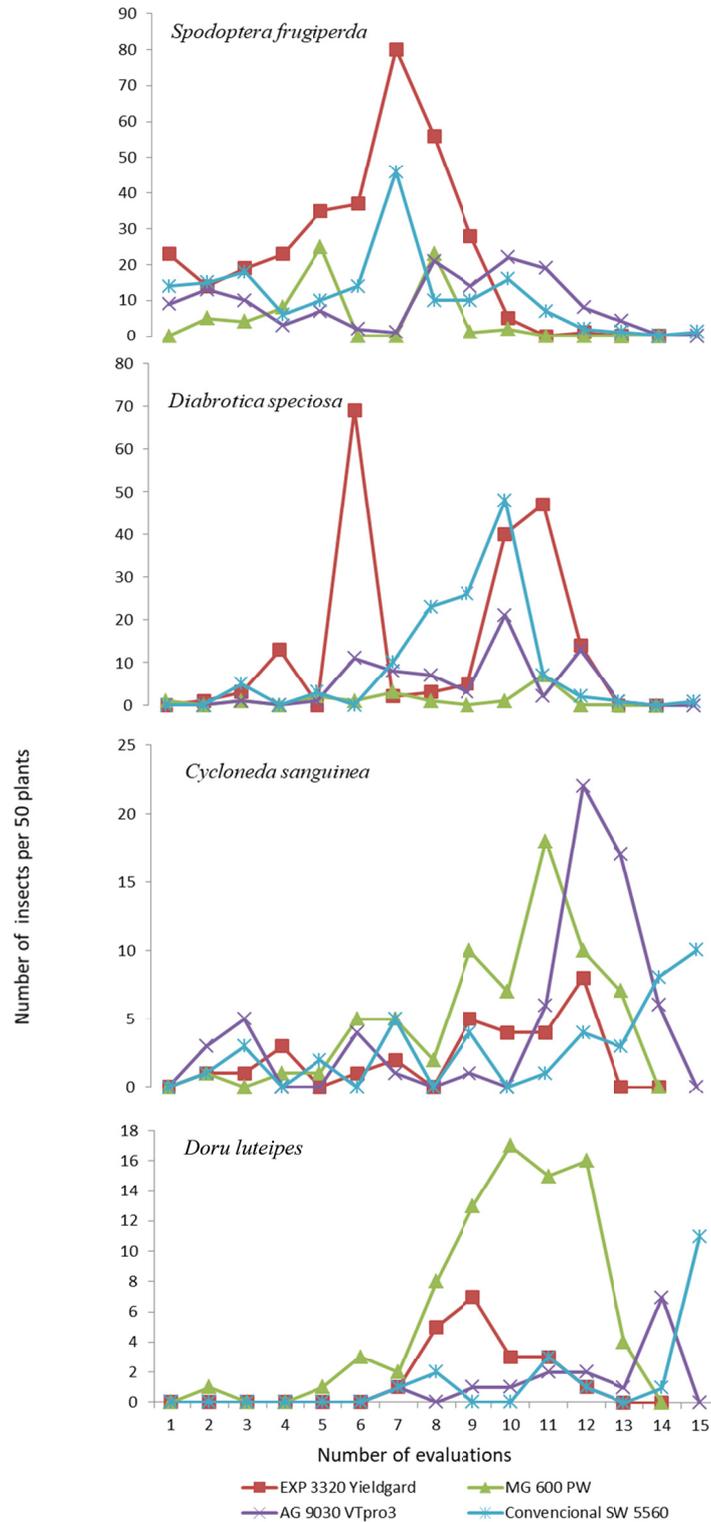


Figure 1. Fluctuation of pests (A) and natural enemies (B) in the four evaluated areas

3.2 Species Diversity

The four cultivars studied hosted a total of 20 families of herbivorous insects (Noctuidae, Sphingidae, Crysomelidae, Lagriidae, Pentatomidae, Coreidae, Reduviidae, Aphididae, Aleyrodidae, Cicadellidae, Cercopidae, Formicidae, Apidae, Vespidae, Araneae, Acrididae, Gryllidae, Muscidae, Syrphidae, Libellulidae) and 5 families of natural enemies (Coccinellidae, Ichneumonidae, Crysopidae, Forficulidae, Tachinidae),

belonging to 10 orders. Hemiptera (Pentatomidae, Coreidae, Reduviidae, Aphididae, Aleyrodidae, Cicadellidae, Cercopidae) and Hymenoptera (Formicidae, Apidae, Ichneumonidae, Vespidae) were the orders with the highest total number of sampled families.

The lowest values of the Shannon and Simpson indexes were calculated for EXP3320YG, even though both indexes showed little difference in relation to the other cultivars. The diversity of non-target arthropods on maize crop was negatively affected by EXP3320YG, while the MG600PW and AG9030VTpro3 did not have any effect on non-target arthropods diversity when compared with the conventional maize.

4. Discussion

The Shannon and Simpson diversity indexes were useful as indicators of the distribution of natural enemy communities (Guo et al., 2014) and non-target herbivores by Bt technologies. The values of both diversity indexes were similar between Bt (MG600PW, AG9030VTpro3) and the non-Bt cultivar (conventional SW5560), but they were the lowest in EXP3320YG (Bt cultivar). These similar indexes (MG600PW, AG9030VTpro3 and SW5560) indicate that the two groups (Bt and non-Bt) of cultivars shared similar species richness and abundance patterns. On the other hand, we hypothesized that lower species richness found in the transgenic cultivar EXP3320YG was caused by the evolution of the resistance of the target pests to this technology (Omoto et al., 2016).

The high density of the pests *S. frugiperda* and *D. speciosa* found on the cultivar EXP3320YG (Cry1Ab) throughout the sample period suggested that these pests have probably acquired resistance to the Cry1Ab toxin. According to McGaughey and Whalon (1992), the continuous expression of cry genes in transgenic plants exerts a strong selection pressure on target pests, leading to the emergence of their resistance. In fact, several studies indicate that resistance of *S. frugiperda* to the Cry1Ab protein expressed in maize crop was broken (Omoto et al., 2016; Horikoshi et al., 2016; Sousa et al., 2016). The evolution of resistance in populations of target insects may reduce the economic and environmental benefits of Bt-based transgenic crops (Farias et al., 2014).

Other studies also reported the non-effect of Bt toxins on a large number of non-target insects. For example, Guo et al. (2014) evaluated the effects of maize expressing the Cry1Ac toxin (not present in Brazilian cultivars) on non-target arthropod community, and found no evidence that Bt maize negatively affected the communities of non-target herbivores, predators, and parasitoids. Similarly, Truter et al. (2014), Svobodová et al. (2015) and Resende et al. (2016) found no significant differences between Bt and non-Bt maize fields expressing different Bt proteins on the diversity and abundance of non-target arthropods.

The results of the present study are similar to those found in other field studies that assess the abundance and diversity of arthropods in Bt and non-Bt maize fields, demonstrating no significant effects of Bt on the population of sampled arthropods. However, there are several other studies that showed some effects of the Bt toxins on arthropod communities (Prutz & Dettner, 2004; Liu et al., 2005a, 2005b, Vojtech et al., 2005; Ramirez-Romero et al., 2007; Sanders et al., 2007; Cunha et al., 2012; Hansen et al., 2012; Pessoa et al., 2016), probably because field conditions are complex and several factors (e.g. climate, temperature, different types of prey and hosts) cannot be controlled. Moreover, preference for a particular prey or host, and competition between enemies are factors that may influence the final results (Guo et al., 2016).

In the present study, the composition and diversity of beneficial arthropod species were affected by the EXP3320YG cultivar. The lowest number of Chrysopidae sp., Araneae, and Syrphidae sp., for instance, were found in this cultivar; on the other hand, the number of natural enemies found on others transgenic cultivars (MG600PW and AG9030VTpro3) and on conventional cultivar was very similar.

The most abundant species of flower-visiting insects were *Lagria villosa* (Fabricius) (Coleoptera: Lagriidae) and Syrphidae sp. The abundance of *L. villosa* did not differ between the cultivars, while the abundance of Syrphidae sp. differed between the cultivars, with the smallest number of individuals found on EXP3320YG and the highest on AG9030VTpro3.

The abundance of beneficial species on EXP3320YG was lower than that of other transgenic cultivars, probably due to the large number of *S. frugiperda* caterpillars that occurred in this cultivar. Considering that, among the three transgenic cultivars, EXP3320YG was the one with the highest number of caterpillars and the highest damage levels during all evaluations, a possible reduction in the cultivar's resistance for Lepidoptera is indicated.

We conclude from this study that the abundance and diversity of non-target arthropods on maize crops is negatively affected by cultivar expressing Cry1Ab protein, while the Cry1A105+Cry2Ab2+Cry1F, and Cry1A105+Cr2Ab2+Cry3Bb1 proteins do not have any effects on arthropods diversity when compared with the

non-Bt maize cultivar. It is also possible to affirm that the cultivar expressing Cry1Ab is much more attacked by pests than the cultivars expressing other Bt proteins.

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