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Full Length Research Paper

Impact of Bollgard® genetically modified cotton on the biodiversity of arthropods under practical field conditions in Brazil

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Using cotton cultivars that express a gene of the *Bacillus thuringiensis* (Bt) bacterium producing a protein (Cry1Ac) with an insecticide effect on the Lepidoptera pests has made it possible to reduce the number of insecticide applications during the crop cycle. Thus, the objective was to determine, in the field during the 2006/2007 harvest in Dourados/MS, Brazil, the impact of the transgenic cultivar (NuOpal®) by comparison with the isogenic, non-transgenic cultivar (DeltaOpal®) on target pests, non-target pests and natural enemies using two sampling methods (beatsheet and whole plant observation) under conventional growing conditions, with both varieties cultivated in a system incorporating the application of insecticides for non-target pests that reached the recommended threshold level for integrated pest management. It was verified that the average number of target pest specimens for both sampling methods was significantly lower in Bt-cotton than in non-Bt-cotton. However, the average number of non-target pest specimens and natural enemies presented no significant differences between the cultivars for both sampling methods assessed. The diversity of non-target pests characterized by the Shannon-Wiener index presented a significant difference between Bt-cotton and non-Bt-cotton for the whole plant sampling method, whereas for naturally occurring enemies, no difference was revealed using this sampling method.

Key words: Bt-cotton, non-target effect, diversity index, side-effect, risk assessment, cotton production, Brazil.

INTRODUCTION

Genetically modified (GM) varieties of cotton expressing the *Bacillus thuringiensis* (Bt) Cry1Ac protein (NuOpal® and DP90B) were introduced commercially in Brazil during the 2006/2007 crop season. Knowledge of the non-target species (herbivores and natural enemies) that occur in the Bt-cotton in different field conditions is still incipient in Latin America in spite of the economic importance to knowledge of the biological diversity and maintenance of biological control with the introduction of GM crops. Another important aspect in the knowledge of arthropod biodiversity is the promotion and the presser-vation of natural enemies, contributing to the integration of pest management systems with a strong biological

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control component and assessment of risk of Bt-cotton to non-target arthropods collaborating with sustainable production and preserving the environment (Romeis et al., 2006; 2008; Lövei et al., 2009).

These GM varieties resistant to pests were initially grown in countries such as the United States, Argentina, Australia, China, Mexico, South Africa and India, allowing fewer insecticide applications, reduction in the costs production, reduction of the risks in the human health (Houssain et al., 2004) and promoting the interaction between chemical control and Bt-cotton in integrated pest management (IPM) programs (Shelton et al., 2002; Fitt, 2008; Naranjo, 2009). In China, producers who adopted Bt-cotton technology reduced the application of insecticides against *Helicoverpa armigera* (Hübner, 1808) by between 60 and 80%, resulting in an increase of 24% in the population of natural enemies (Huang et al., 2002). In India, there was an increase of 29% in fiber production and a saving of 60% in insecticide applications (Barwale et al., 2004), while in Mexico, the drop in the use of pesticides was 80% (Sanvido et al., 2006). These findings suggest that there also factors, like insecticide quality, insecticide resistance and the correct choice of products and timing of sprays, that other than insecticide quanlity influenced damage control in conventional cotton and thus the yield effects of Bt technology (Quaim et al., 2006).

However, the reduction in application of insecticides against the target pests of Cry1Ac toxin promote the resurgence of secondary pests, which will be controlled with other control tactic like an insecticide. Thus, there are few published studies conducted about the impact of the Bt-cotton varieties on arthropods with adoption of chemical control, especially with respect to diversity values and indexes found. In North of China, Li et al. (2002), compared Bt-cotton (SGK321 and GK12) with non-Bt (Shyiuan 321 and Shimian 3) under non-insecticide and sprayed conditions, sampling the diversity of arthropod species (pests, natural enemies and neutral arthropods) and using the method of Shannon-Wiener's index to analyze the diversity. These authors discovered that the diversity of arthropod communities in Bt-cotton was similar to non-Bt without spraying; however, Shannon-Wiener's index for total arthropod and the neutral guild in Bt-cotton fields were significantly higher than non-Bt in sprayed plots.

Increased diversity of arthropod communities and pest sub-communities was observed in China using the same index (Men et al., 2003) in Bt and non-Bt cotton under different conditions: with and without insecticide treatments. In the insecticide treatment the diversity of communities and sub-communities of arthropods in both Bt and non-Bt cotton increased and in non-Bt cotton the natural enemies abundance decreased. However, Bt-cotton without insecticide did not significantly affect the abundance of this group.

Authorization of the NuOpal® (Bollgard®) genetically modified cotton variety for the 2006/2007 harvest in Brazil was aimed at facilitating field assessment of the impact of this technology on the biodiversity of target pests, non-target pests and their natural enemies by comparison with the isogenic, non-transgenic cultivar (DeltaOpal®) in a production system with application of insecticides for non-target pests that reached the recommended threshold level for IPM. The objective of this study is to verify in the field conditions; if arthropod abundance is affected by Bt-cotton when other non-target pests in the system not suppressed by Cry1Ac toxin require additional insecticide treatment helping IPM programs with adoption of Bt-cotton in Brazil.

MATERIALS AND METHODS

Study location

The work was carried out at the Federal University of Grande Dourados (UFGD), in the Agricultural Science Faculty (FCA), in the municipality of Dourados, state of Mato Grosso do Sul, Brazil, during the period from November 2006 to April 2007. The university is located at latitude 22°14' South, longitude 54°44' West and altitude 1,482.94 ft. The experimental area was localized near areas cultivated with soybean, maize and cotton. The climate in the region, according to the Köppen international classification, is Mesothermic Humid (Cwa). The amount of precipitation observed in the experiment area over the experimental period was higher in the ten first days of December and February months, with 210.3 and 136.3 mm, respectively. An irrigation system was installed in order to facilitate crop development during the period in which the experiment was conducted. The soil in the area is classified as very clayey-textured Typic Hapludox (65.3% clay, 17.4% silt, and 17.3% sand).

Description of sampling area

A total area 70 x 70 m (0.49 ha) was marked out and divided into subareas. Each subarea was divided into ten strips to each treatment (ten strips to NuOpal® and ten strips to DeltaOpal), each strip consisting of eight rows (7.2 x 35 m), spaced at 0.9 m, representing ten repetitions for each treatment (Bt and non-Bt cotton), in accordance with the methods proposed by Hilbeck et al. (2006) and Perrett and Higgins (2006). The useable sampling area of each strip consisted of six rows in which the two central rows were sampled, a row at each end of each strip forming a buffer for the sampling unit.

The experimental arrangement used was the strip experiment, consisting of two treatments: NuOpal® and DeltaOpal® varieties. For the purposes of data analysis, the sampling unit for whole plant sampling consisted of ten plants for each treatment, formed by the sum of individuals for the ten plants sampled separately in each of the ten strips. Whole plant sampling accounted for 430 repetitions throughout the period for which the experiment was conducted, totaling 43 samples. For the beatsheet method, the sampling unit consisted of the sum of the individuals sampled on two plants for each treatment, with 270 repetitions carried out as from the plant flowering stage (F1), totaling 27 samples.

To minimize the effects of the products applied and favor the presence of phytophagous insects (stink bugs) migrating from soybean to the cotton plants at the end of the oleaginous cycle, a buffer area of 10 m between the two cotton cultivars was allocated to growing a variety of soya, CD 219RR, which did not receive any phytosanitary treatment throughout the experimental period.

The soya seeds were pretreated with micronutrients, Co and Mo, 100g.60 kg^{-1} of seeds, and peat-based inoculant (*Bradhyrizobium*

Table 1. Application of insecticides in relation to threshold levels reached, carried out on the NuOpal® (Bt) and DeltaOpal® (Non-Bt) cultivars during the 2006/2007 harvest. Dourados, MS, Brazil.

Product (active ingredient)	Pest	Threshold level (cat/pl(*) or % damage)	Quantity (Kg or l ha^{-1})	Application date (2007)	
				NuOpal®	DeltaOpal®
Provado® (imidacloprid)	*Aphis gossypii*	40%	0.4 l	05-Jan	05-Jan
Cartap® (cartap)	*Alabama argillacea*	2 cat/pl	0.5 Kg	-	05-Jan
Abacmectin®(abamectin)	*Tetranychus urticae*	10%	0.5 l	02-Mar	02-Mar
Abacmectin®(abamectin)	*T. urticae*	10%	0.5 l	09-Mar	09-Mar
Provado® (imidacloprid)	*A. gossypii*	40%	0.4 l	19-Mar	19-Mar
Abacmectin®(abamectin)	*T. urticae*	10%	0.5 l	04-Apr	04-Apr
Abacmectin®(abamectin)	*T. urticae*	10%	0.5 l	11-Apr	11-Apr

*Caterpillars per plant.

japonicum), 100g.60kg^{-1}. 400Kg.ha^{-1} of fertilizer formula 08-20-20+Zn were applied to the seed row. Sowing was carried out on 03rd November, 2006, with emergence on 10th November, 2006.

Preparing the sampling area, sowing the cultivars and crop treatments

The experimental area was prepared so as to make the physical, chemical and biological conditions of the soil suitable for growing cotton, with the basic fertilizer and mulch applied in accordance with the soil analysis and the regional cultivation recommendations (Embrapa, 2001). The soil was prepared by plowing and harrowing, and the seeds of varieties cotton (NuOpal® and DeltaOpal) sown after desiccation of the crop area planted with soya variety RR (CD 219) using Reglone® (diquat) (3 l/ha). The crop treatments applied to both experimental areas Bt and non-Bt-cotton were N-P-K (8-20-20) (400 Kg), Fusilade® (fluazifope-P-butílico) (0.8 l), Envoke® (trifloxissulfurom-sodic) (0.005 Kg), Staple (piritiobaque-sodic) (0.15 l) and ammonium sulfate (0.15 Kg), which were made in late November, through December 2006 and beginning of January 2007.

The seeds of the NuOpal® (Bt) and DeltaOpal® (non-Bt) cultivars used in the experiment were supplied by MDM–Sementes de Algodão©, and pre-treated with the following fungicides: Euparen® (tolifluanid) (200 g/100 kg seeds), Monceren® (pencycuron) (200 g/100 Kg seeds) and Baytan® (triadimenol) (40 ml/20 kg seeds), aimed at controlling diseases that cause damping-off. The cultivar seeds were sown mechanically on 27th November, 2006 with a density of 13 seeds per meter and a row spacing of 0.90 m. Emergence occurred on 4th December, 2006.

Weeds were controlled by manual weeding and chemical control throughout the development cycle in the two crop areas. Special care was taken to control leaf-cutting ants of the genera *Atta* and *Acromyrmex*, during the development of the crop, and Blitz® (fipronil) bait was applied 15 and 30 days after emergence (DAE) in areas adjacent to the experiment in strips 9 and 10 of the NuOpal® cultivar.

Defining the experimental area and control decision level sampling

Insecticide applications are detailed in Table 1 and were carried out when pest infestations reached the threshold level proposed for the species present (Degrande, 2004), using a tractor with system set to constant working pressure, pesticide volume of 100 l.ha^{-1} and hollow cone nozzle. In the case of *Anthonomus grandis* (Boheman, 1843), since the pressure of attack was high, 22 applications of insecticide were necessary as from 37 DAE (11th January, 2007) until the end of the crop cycle (19th April, 2007). For controlling this insect, the following products were applied, with respective doses and application dates: Thiodan® 350CE (endosulfan) (2 l/ha) on 11, 16, 21, 26 and 31st January, 2007; on 05, 07, 12 and 17th February, 2007; Folisuper® 600CE (parathion) (1 l/ha) on 21st February, 2007, 19 and 23rd March, 2007, 02, 04 and 19th April, 2007; Bulldock® 125SC (beta-cyfluthrin) (0,1 l/ha) on 26/02/2007 and Karate® 50CE (lambda-cyhalothrin) (0,12 l/ha) on 02, 06, 09, 13 and 16th March, 2007 and on 11th April, 2007.

Sampling methods

In each plot, we observed the plants in the two central rows. Sampling was carried out every three or four days, depending on environmental rainfall conditions, starting at the VE (emergence) stage of the crop and over the period from 6th December, 2006 to 27th April 2007, assessing the presence of target and non-target pest species and natural enemies using two methods: beatsheet and whole plant observation.

The sampling process beatsheet was based on the recommendations of Degrande et al. (2003). This method involves using the inter-row space in the crop between the two central rows of each strip at two random points, giving a total of 20 points per treatment. The sheet was white to increase insect visibility, and of the same width as the crop spacing (0.90 m wide x 1 m in length). It was laid out to cover the entire area between the two rows of cotton plants sampled. In beatsheet method, the sheet was placed between two rows of plants and opened up over the soil. Next, the two rows were shaken vigorously so that both immature and adult insects fell onto the sheet and could be seen, counted and identified in terms of family and/or species while still in the field. In this method, the count for the number of *P. gossypiella* caterpillars was based on the damaged reproductive structures falling onto the beatsheet and subsequently opened up.

The whole plant assessment method was used to assess ten plants separately for each plot, counting and identifying the family and/or species of insects while still in the field, and where necessary, collecting those insects not identified in the field and placing them in bottles with 70% alcohol for later identification in the laboratory.

Fauna and statistical analysis

Fauna analysis was based on calculating indices for frequency, constancy, abundance and dominance (Silveira Neto et al., 1976),

taking into account the number of small caterpillars (smaller than1.0 cm), large caterpillars (larger than 1.0 cm), larvae, nymphs and adults. Absolute frequency was defined as the total number of specimens observed with different sampling methods.

Constancy was defined as the percentage of samples in which a given species was present (Uramoto et al., 2005). Once we had obtained the percentage constancy throughout the phenological stages of the plant, the species were classified into the following categories: Constant (w), present in more than 50% of weekly observations; accessory (y) present in 25 to 50% of observations; and accidental (z), present in fewer than 25% of observations. Abundance is the number of individuals of a given species per unit area or volume, and can vary in time and space (Southwood, 1995). To estimate abundance, we used the limits established by a confidence interval (CI) at 5 and 1% probability and the following classes were determined: Rare (r), number of species/individuals outside the lower limit of CI at 1% probability; dispersed (d), number of individuals within the lower limits of confidence intervals at 1 and 5% probability; common (c), number of individuals within the confidence interval at 5%; abundant (a), number of individuals within the upper limits of confidence intervals at 5 and 1% probability, and very abundant (va), number of individuals greater than the upper limit of CI at 1% probability.

An organism is considered dominant when it is subjected to an environmental impact and adapts to it (Silveira Neto et al., 1976). For this study, a species was considered dominant when it presented a relative frequency higher than 1/S, where S is the total number of species found during the sampling period. To compare differences in the averages for groups of target pests, non-target pests and natural enemies, we took into account the species' development stages for Bt and non-Bt treatments. Since the normality assumptions (Kolmogorov-Smirnov Z test) and homogeneity of variances (Levene test) were not satisfied for both the original values and the values transformed by the formula $\sqrt{X+0,5}$, we used the non-parametric statistics Mann-Whitney U test as an alternative to the Student t-test. All results were analyzed based on a significant level of α = 5%.

The diversity of target pests, non-target pests and natural enemies in "Bt-cotton" and "non-Bt cotton" environments was studied using the Shannon-Wiener index as a correction factor and natural logarithm (Poole, 1974), based on specimen frequency. This index measures the degree of uncertainty in predicting which species an individual belongs to when chosen at random from a sample of S species and N individuals (Neto et al., 1976).

The lower the Shannon index value, the lower the degree of uncertainty and, therefore, the lower the diversity of the sample. Diversity tends to be higher as the index value increases (Uramoto et al., 2005). The Student t- test was used to test the difference in species diversity between cultivars based on a significant level of α = 5%.

RESULTS

Biodiversity and fauna indices

During the sampling period, we observed a total of 39 species distributed over 10 orders and 25 families which were split into three groups: Target pests, non-target pests and natural enemies (Table 2). For the whole plant assessment method, of the 1958 specimens sampled on NuOpal®, 17 (0.86%) belonged to target pest species. There were 1270 individuals belonging to non-target pest species, representing 64.8% of the sample, and 671 natural enemy individuals, representing 34.2%. On Delta Opal®, of the 2502 specimens sampled, 674 (26.9%) belonged to target pest species, 1328 (53.0%) to non-target pest species and 500 (19.9%) to natural enemy species.

For the beatsheet method on Bt-cotton, of the 1288 specimens sampled, 16 (1.24%) belonged to the target pest group, 601 (46.6%) were non-target pests and 671 (52.0%) natural enemy individuals. In contrast, for non-Bt cotton, of the 2144 specimens sampled, 982 (45.4%) belonged to target pest species, 614 (28.6%) to non-target pest species and 548 (25.5%) to natural enemy species.

Whole plant and beatsheet sampling

Considering the numbers of insecticide application to control of non-target species like *A. grandis*, the target pest species for Bollgard® technology *Alabama argillacea,* showed itself to be dominant on both NuOpal® and DeltaOpal®, and in both whole plant and beatsheet samples (Table 2).

The non-target herbivorous species classified as very abundant, dominant and constant on both NuOpal® and DeltaOpal® for both sampling methods was *A. grandis* (Table 3). Non-target sucking species *Bemisia tabaci* (Gennadius, 1889) was classified as very abundant, dominant and constant on both Bt-cotton and on non-Bt cotton in the whole plant method. However, using the same sampling method, a species of genus *Frankliniella* was dominant only on Bt-cotton. While, non-target herbivores *Euschistus heros* (Fabr, 1798), *Dysdercus* sp. and *Spodoptera frugiperda* (Smith, 1797) were dominant on both NuOpal® and DeltaOpal® for beatsheet sampling. However, using this sampling method, *Lagria villosa* (Fabr, 1783) was dominant only on non-Bt cotton (Table 2).

In the natural enemies group, Araneae and *Doru luteipes* (Scudder, 1876) were found to be abundant and dominant predators on both Bt-cotton and non-Bt-cotton for both sampling methods. For whole plant sampling, the predator *Scymnus* sp. was abundant only on non-Bt-cotton and dominant on both cultivars. However, the predator hymenopteran *Solenopsis invicta* (Buren) was very abundant only on Bt-cotton using this sampling method, whereas using the beatsheet method, *Scymnus* sp. was dominant only on non-Bt cotton and *S. invicta* was very abundant and dominant on both cultivars (Table 2).

In regard to constancy parameter of diversity observed throughout the sampling period, Araneae was constant on both Bt-cotton and on non-Bt cotton for both sampling methods. While *Scymnus* sp. was constant on both NuOpal® and DeltaOpal® only for whole plant sampling, and predator species *D. luteipes* was constant only on NuOpal® for this sampling method; for the beatsheet method, *D. luteipes* was constant on both types of cotton (Table 2).

Table 2. Fauna analysis of target pest, non-target pest and natural enemy groups per order, family and species, sampling method and type of cotton. Dourados/MS, 2007.

Group	Order/Family	Species	Stage*	Sampling method			
				Whole plant		Beatsheet	
				NuOpal®	DeltaOpal®	NuOpal®	DeltaOpal®
				F C (A) D**	F C (A) D**	F C (A) D**	F C (A) D**
Target pests	Lepidoptera/ Noctuidae	*Alabama argillacea*	LP+LG	11z(c)s	613w(c)s	11z(c)s	967w(c)s
	Lepidoptera/ Noctuidae	*Heliothis virescens*	SC+LG	6z(c)n	14z(c)n	5z(c)n	9z(c)n
	Lepidoptera/ Gelechiidae	*Pectinophora gossypiella*	CAT	0	47z(c)n	0	6z(c)n
Total				17	674	16	982
Non-target pests	Coleoptera/ Chrysomelidae	*Cerotoma arcuata*	Ad	0	0	2z(d)n	0
	Coleoptera/ Chrysomelidae	*Diabrotica speciosa*	Ad	3 z(c)n	7 z(c) n	15y(c)n	26y(c)n
	Coleoptera/ Chrysomelidae	*Jansonius boggianii subaeneus*	Ad	1 z(c)n	1 z (c) n	2 z(d)n	5z(d)n
	Coleoptera/ Curculionidae	*Aracanthus* sp.	Ad	0	0	16 y(c)n	8 z(c)n
	Coleoptera/ Curculionidae	*Anthonomus grandis*	L+Ad	390 w(ma)s	330 w(ma)s	201 w(ma)s	210 w(ma)s
	Coleoptera/ Lagriidae	*Lagria villosa*	Ad	7 z(c)n	7 z (c) n	28 z(c)n	38 y(c)s
	Coleoptera/ Melyridae	*Astylus variegatus*	Ad	14 z(c)n	10 z(c)n	16 z(c)n	5 y(d)n
	Hemiptera/ Aleyroidade	*Bemisia tabaci*	Ad	583 w(ma)s	761 w(ma)s	0	0
	Hemiptera/ Cicadellidae	*Agallia albidula*	Ad	43 y(c)n	44 z (c) n	4 z(d)n	7 z(c)n
	Hemiptera/ Coreidae	*Hypselonotus* sp.	Ad	0	1 z(c)n	0	1z(d)n
	Hemiptera/ Coreidae	*Leptoglossus zonatus*	Ad	1z(c)n	2z(c)n	10z(c)n	6z(c)n
Non-target pests	Hemiptera/Miridae	*Horciasoides nobilellus*	Ad	4 z(c)n	11 z (c) n	9z(c)n	6 z (c) n
	Hemiptera/ Pentatomidae	*Edessa meditabunda*	N+Ad	1 z(c)n	0	2 z(d)n	2 z(d)n
	Hemiptera/ Pentatomidae	*Euschistus heros*	N+Ad	35 y(c)n	35 z (c) n	153 w(ma)s	149 w(ma)s
	Hemiptera/ Pentatomidae	*Nezara viridula*	N+Ad	0	0	1 z(d)n	1 z(d)n
	Hemiptera/ Pyrrochoridae	*Dysdercus* sp.	N+Ad	8 z(c)n	10 z (c) n	39 y(c)s	43 y(c)s
	Lepidoptera/ Noctuidae	*Spodoptera eridania*	LP+LG	11 z(c)n	4 y (c) n	2 z(d)n	10 y(c)n
	Lepidoptera/ Noctuidae	*Spodoptera frugiperda*	LP+LG	71 y(c)n	28w(c) n	82w(ma)s	65w(a)s
	Lepidoptera/ Noctuidae	*Pseudoplusia includens*	LP+LG	12 z(c)n	9 y(c)n	19 y(c)n	31 z(c)n
	Orthoptera/ Tettigonidae	Tettigonidae sp.1	Ad	1 z(c)n	0	0	1 z(d)n
	Thysanoptera/ Thripidae	*Frankliniella* sp.	Ad	85y(c)s	68 z(c)n	0	0
Total				1270	1328	601	614

Table 2. Contd.

Natural enemies	Araneae	Araneae	Ad	215 w(ma)s	210 w(ma)s	194 w(ma)s	132 w(ma)s
	Coleoptera/ Carabidae	*Callida* sp.	Ad	1 z(d) n	3 z (c) n	13 y (d) n	16 y (c) n
	Coleoptera/ Coccinellidae	*Cycloneda sanguinea*	L+Ad	20 y (c) n	20 y (c) n	16 y (d) n	10 z (c) n
	Coleoptera/ Coccinellidae	*Eriopsis connexa*	L+Ad	4 z(d)n	6 z(c)n	1 z(c)n	1 z(d)n
	Coleoptera/ Coccinellidae	*Hippodamia convergens*	L+Ad	0	0	2 z(d)n	1 z(d)n
	Coleoptera/ Coccinellidae	*Scymnus* sp.	L+Ad	90 w(c)s	114 w(ma)s	59 y(d)n	73 y(c)s
	Dermaptera/ Forficulidae	*Doru luteipes*	Ad	111 w(a)s	76 y(a)s	130 w(a)s	125 w(ma)s
	Diptera/Syrphidae	*Toxomerus* sp.	L+Ad	13 z(c)n	4 z(c)n	0	2 z(d)n
	Hemiptera/ Anthocoridae	*Orius* sp.	Ad	6 z(d)n	10 z(c)n	4 z(d)n	1 z(d)n
	Hemiptera/ Lyageidae	*Geocoris* sp.	Ad	6 z(d)n	2 z(c)n	0	5 z(c)n
	Hemiptera/ Pentatomidae	*Podisus* sp.	N+Ad	0	2 z(c)n	0	0
	Hemiptera/ Reduviidae	*Zelus longipes*	Ad	12 z(c)n	2 z(c)n	0	1 z(d)n
	Hymenoptera/ Formicidae	*Solenopsis invicta*	Ad	192 y(ma)s	44 z(c)s	248 y(ma)s	179 y(ma)s
	Neuroptera/ Chrysopidae	*Chrysoperla* sp.	L	1 z(d)n	7 z(c)n	3 z(d)n	2 z(d)n
	Neuroptera/ Hemerobiidae	*Nusulala* sp.	L	0	0	1 z(c)n	0
Total				671	500	671	548
Overall Total				1958	2502	1288	2144

* SC = Small caterpillar; LG = large caterpillar; CAT = caterpillar; L = larvae; N = nymph; Ad = adult; **(F) total number observed under different sampling conditions; (C) constancy: (w) constant (y) accessory (z) accidental; (A) abundance: (ma) very abundant (a) abundant (c) common (d) dispersed (r) rare; (D) dominance: (s) dominant (n) non-dominant.

Table 3. Shannon-Wiener diversity index (variance) and number of non-target pest and natural enemy species present in Bt-cotton and non-Bt cotton environments.

Whole plant	**Bt-cotton* (n = 430)**	**Non-Bt cotton* (n = 430)**	**t-student**	**p**
Non-target pests	1.52(0.001)(17)a	1.34(0.001)(16)b	3.718	0.000
Natural enemies	1.67(0.001)(12)a	1.64(0.002)(13)a	0.418	0.675
Beatsheet	**Bt-cotton* (n = 270)**	**Non-Bt cotton* (n = 270)**		
Non-target pests	1.94(0.001)(17)a	1.97(0.001)(18)a	-0.515	0.606
Natural enemies	1.51(0.001)(11)a	1.60(0.001)(13)b	-2.092	0.037

*The same letters on same row represent non-significant values at 5%, assuming the same variances for the Levene test; Dourados/MS, 2007.

Diversity index (Shannon-Wiener)

In regard to the diversity of non-target pests, using the whole plant method, the Shannon-Wiener index for Bt-cotton was 1.52, as opposed to 1.34 for non-Bt cotton, showing a statistically significant difference (t = 3.718, df = 429, P = 0.000). For the beatsheet method, the diversity index was 1.94 for Bt-cotton and 1.97 for non-Bt cotton, and hence did not show any significant difference (t = 0.515, df = 269, P = 0.606) (Table 3).

Table 4. Average number of specimens (standard deviation) of arthropods present in the sample for each sampling method and type of cotton,

Whole plant	Bt-cotton* (n = 430)	Non-Bt cotton* (n = 430)	Mann-Whitney U [ns]	p
Target pests	0.04(0.21)a	1.57(3.80)b	-12.821	0.000
Non-target pests	2.95(4.02)a	3.08(5.67)a	-0.985	0.325
Natural enemies	1.56(3.28)a	1.16(1.73)a	-0.569	0.570
Beatsheet	**Bt-cotton* (n = 270)**	**Non-Bt cotton* (n = 270)**		
Target pests	0.06(0.31)a	3.64(8.28)b	-13.012	0.000
Non-target pests	2.22(2.61)a	2.27(2.28)a	-1.368	0.171
Natural enemies	2.48(3.98)a	2.02(3.12)a	-0.144	0.886

[ns]The averages of two-by-two treatments do not differ significantly; p > 0.05; *the same letters on the table row represent non-significant values at 5%, assuming the same variances for the Levene test; Dourados/MS, 2006/2007.

Table 5. Total number and percentage of eggs with and without parasites for target pests on NuOpal® and DeltaOpal® cultivars for the whole plant sampling method. Dourados/MS, 2007.

Eggs without parasites	NuOpal®	DeltaOpal®
Heliothis virescens	3183 (99.9%)	3202 (99.1%)
Alabama argillacea	4873 (72.9%)	6222 (77.2%)
Eggs with parasites		
Heliothis virescens	2 (0.06%)	28 (0.86%)
Alabama argillacea	1805 (27%)	1834 (22.7%)

The average number of individuals in the non-target pest and natural enemy (mainly predators) groups did not show any significant differences between NuOpal® and DeltaOpal® for either of the sampling methods used (Non-target: whole plant – U = 0.985, df = 429, P = 0.325 / beatsheet – U = 1.368, df = 269, P = 0.171; natural enemy: whole plant – U = 0.569, df = 429, P = 0.570 / beatsheet – U = 0.144, df = 269, P = 0.886) (Table 4).

In regard to the diversity of natural enemies (mainly predators), for the whole plant method, the Shannon-Wiener index for Bt-cotton was 1.67 and 1.64 for non-Bt cotton, showing no significant difference (t = 0.418, df = 429, P = 0.675). For the beatsheet method, the index was 1.51 for Bt-cotton and 1.60 for non-Bt cotton, showing a marginally significant difference (Table 3) (t = 2.092, df = 269, P = 0.037). This result shows that the diversity of predators was slightly higher on non-Bt cotton than on Bt-cotton using the beatsheet method.

Average number of target pest, non-target pest and natural enemy individuals

NuOpal® cotton presented a significantly lower number of *A. argillacea*, *H. virescens* and *P. gossypiella* target pest specimens than observed on DeltaOpal® cotton for both sampling methods (Whole plant – U = 12.821, df = 429, P = 0.000 / Beatsheet – U = 13.012, df = 269, P = 0.000) (Table 4). Mainly in relation to species *A. argillacea* and *H. virescens,* this observation could be due to the total number of eggs, both with and without parasites, sampled on DeltaOpal® (Table 5).

DISCUSSION

As expected, both the “whole plant” and “beatsheet” sampling methods showed that the target Lepidoptera were controlled by the Cry1Ac toxin inserted into NuOpal®. This result shows it was possible to detect the presence of neonate caterpillars of the target species *A. argillacea* on the NuOpal® cultivar, with no detection of large caterpillars or pupae. The control of target species, like *H. virescens* was also observed by Head et al. (2005) in USA and the fact that *A. argillacea* proved to be dominant on both NuOpal® and DeltaOpal® for both sampling methods could be attributed to the control by Cry1Ac toxin demonstrating the entomocide efficacy of the NuOpal® on this insect.

Fauna analysis of non-target species sampled in the NuOpal® and DeltaOpal® environments showed that the abundance, dominance and constancy of these species could be attributed to factors such as (a) The possible absence of insecticide activity on these insects of the Cry1Ac toxin present in the transgenic cultivar, as was confirmed in this study for *A. grandis*, or (b) the reduction of food competition between non-target herbivores and the target insects controlled by the Bollgard® technology, suggesting that drops in the populations of these Lepidoptera could also affect the dynamics of predators, as in the case of *Nabis* sp. (specie sampled in this study), since this was also observed by Daly and Buntin (2005) and Whitehouse et al. (2005) on maize and Bt-cotton, respectively. Another fact in the case of *A. grandis* is the stems from migration of its population from experimental areas with non-Bt cotton stubble adjacent to the experiment, causing this population to attack both types of cotton.

The results showed the importance of choosing the sampling method for monitoring each genus and/or species of insect on the transgenic cultivar (Wade et al.,

2006), which could help in verifying cases of possible development of resistance to the Cry1Ac toxin and elevation of the non-target species to categories like principal pests of cotton. In this study, this was observed with sample of *B. tabaci* (whitefly), which was facilitated in the whole plant method. This is an insect of low mobility, with the result also being observed by Sisterson et al. (2004). This result would orient future research around seeking an adequate sampling method for monitoring insects on transgenic cultivars.

Another fact around the choosing of the sample method, is that this choice was related to the behaviour of insect, mainly mobility, and the application of chemical control (mainly pyrethroids) on non-target pests of Bt (Men et al., 2003; Naranjo, 2009), such as *A. grandis*, could also have affected these fauna parameters, where the survival of insect pests combined with the action of the insecticides applied could have negatively affected populations of their natural enemies (Hagerty et al., 2000), reducing their populations and promoting greater uniformity in the abundance of the populations of surviving individuals. Another aspect of the application of chemical control was that the introduction of Bt cotton did not help meet the expected reduction in insecticide use in Brazil. This fact was mentioned by Hofs et al. (2005) in a study conducted in South Africa.

The observation that non-target Lepidoptera species *S. frugiperda* was very abundant on NuOpal® cotton by comparison with DeltaOpal® in beatsheet sampling was not verified by Head et al. (2005), in which caterpillars of this Lepidoptera occurred sporadically. This result could be attributable to the feeding and movement behavior of this pest, which was observed feeding in the bolls of cotton which dropped in the beatsheet method, protecting this pest from the action of insecticides applied to the control in both Bt and non-Bt cotton.

Despite there had being no difference in the abundance of Araneae and *D. luteipes* predators between the NuOpal® and DeltaOpal® cultivars for both sampling methods, the results showed that the diversity of predators was slightly higher on non-Bt cotton than on Bt-cotton using the beatsheet method. This result could stem from the action of insecticides on these beneficial insects, as well as a reduction in the population of target caterpillars that serve as a food source for the prey of these natural enemies on Bt-cotton.

In the present study, there was a difference in the *Scymnus* sp. and *S. invicta* predators. This was observed in USA by Head et al. (2005) and in Australia by Whitehouse et al. (2007), in which the beatsheet method showed a small but consistent difference for Araneae and *S. invicta* between Bt-cotton (Vip) and non-Bt. This difference between the results could be attributable to the type of sampling used, and also probably to the different number of insecticide applications carried out on Bt-cotton and non-Bt.

The fact that the diversity of individuals belonging to the Coccinellidae family was not negatively affected by the Cry1Ac toxin in a production system involving chemical control for pests reaching the threshold level was also observed by Men et al. (2004) and Torres and Ruberson (2005). This verification could possibly be explained by the fact that ladybugs on Bt-cotton are more exposed to insecticides on non-Bt cotton. This result was also observed by Head et al. (2005). In another study conducted in South Africa, the diversity of these insects in untreated plots could not be compared with Bt and non-Bt cotton (Hofs et al., 2005), shown in small-scale farming fields where pesticides are still sprayed for controlling other pests like sucking pests. The adoption of Bt-cotton may help meet the expected reduction in insecticide use, like in this study with the applications to control *A. grandis* in Brazil.

The fact that the non-target pest diversity calculated using the Shannon-Wiener index was higher on NuOpal® for the whole plant method can be explained by the reduction in competition for food with the target pests controlled by the Bt cultivar, and this explanation is also given in studies conducted by Naranjo (2005) and Naranjo et al. (2005).

The fact that there was no significant difference in the diversity of predators using the Shannon-Wiener index between NuOpal® and DeltaOpal® for whole plant sampling could be explained by these insects having found conditions favorable to their development on both the Bt and non-Bt cultivar, as well as by the fact that Cry1Ac does not have any negative impact on this group of insects.

However, contrary result was obtained in Australia by Whitehouse et al. (2005) where the diversity of beneficial insects was observed to be lower, as in the case of Araneae on non-Bt cotton as opposed to Ingard®, both in a production system with chemical control. This result could be attributed to the fact that many non-target species were controlled by insecticides affecting the populational dynamics of these predators (Men et al., 2003; Naranjo, 2005).

In conclusion, the biodiversity study conducted with two cultivars (NuOpal® and DeltaOpal®) applying insecticides when necessary and in accordance with IPM under the conditions in Brazil made it possible to verify the efficiency with which the NuOpal® cultivar controls target lepidoptera *A. argillacea* and *P. gossypiella*, even with 22 applications of insecticide to control *A. grandis*, while *H. virescens* was very low in non-Bt as well as Bt-cotton. These applications affect the resurgence of other non-target pests and the efficiency of natural control by existing predators in the Bt-cotton and non-Bt cotton environments. Using fauna analysis by Shannon Wiener's index, the study also confirmed the absence of any negative effect of Bt-cotton on non-target pest and natural enemy (mainly predators) diversity, demonstrating which beneficial arthropod species could continually control the pest species in a production system represented by Bt-

cotton and chemical control, promoting the preservation of these natural enemies and the environment.

The main novelty in this research is the choice of the sample method used to monitor the arthropods species in the agroecosystem constituted by GM plants. This is due to the fact that depending on the insect species sampled, a specific sampling method should be used considering the bioecology and behaviour of this species, which may influence the number of individuals sampled between Bt and non-Bt cotton, leading to wrong interpretations about the real effect of the GM plant on the arthropod, and consequently on the biological control, which may be potentialized with the adoption of Bt-crops and insecticide use patterns when necessary.

The present study also points the way for new research concerning oviposition preferences of target insects on Bt-cotton and non-Bt cultivars, and also the possible phagodeterrent effects of the Cry1Ac toxin on non-target herbivores, such as in the case of *L. villosa*, as well as the need for future experiments on the effects of different transgenic cotton production systems on the population dynamics of non-target insects (mainly predators) under Brazilian cultivation conditions.

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ABBREVIATIONS

Bt, *Bacillus thuringiensis*; **GM,** genetically modified; **IPM,** integrated pest management; **DAE,** days after emergence; **w,** constant; **y,** accessory; **z,** accidental; **CI,** confidence interval; **r,** rare; **d,** dispersed; **c,** common; **va,** very abundant.

REFERENCES

Barwale RB, Gadwal RB, Zehr U, Zehr B (2004). Prospects for *Bt* cotton technology in India. AgBio. Forum. 7: 23-26.

Daly T, Buntin D (2005). Effect of *Bacillus thuringiensis* transgenic corn for lepidopteran control on nontarget arthropods. Environ. Entomol. 34: 1292-1301.

Degrande PE, Oliveira MA DE, Ribeiro JF, Barros R, Nogueira RF, Rodrigues ALL, Fernandes MG (2003). Avaliação de métodos para quantificar predadores de pragas do algodoeiro. Arq. Inst. Biol. 70: 291-294.

Degrande PE (2004). Níveis de controle das pragas do algodoeiro. Atualidades Agrícolas. São Paulo: Editora Basf, pp. 23-24.

Embrapa Agropecuária Oeste (2001). Algodão: tecnologia de produção. In: Embrapa Agropecuária Oeste, Dourados/MS, Brasil, p. 296.

Fitt GP (2008). Have Bt crops led to changes in insecticide use patterns and impacted IPM?. In: Romeis J, Shelton AM, Kennedy GG (Ed.), Integration of insect-resistant genetically modified crops within IPM programs, Springer Publishing, pp. 303-329.

Hagerty AM, Turnipseed SG, Sullivan MJ (2000). Impact of beneficial arthropod conservation in Bt and conventional cotton. In Dugger CP, Richer DA (Ed.), Proceedings, Beltwide Cotton Production Research Conferences, San Anto-nio, TX, National Cotton Council of America, Memphis, TN, pp. 976-978.

Head G, Moar W, Eubanks M, Freeman B, Ruberson J, Hagerty A, Turnipseed S (2005). A Multiyear, large-scale comparison of arthropod populations on commercially managed Bt and non-Bt cotton fields. Environ. Entomol. 34: 1257-1266.

Hilbeck A, Andow DA, Arpaia S, Birch ANE, Fontes EMG, Lövei GL, Sujii ER, Wheatley RE, Underwood E (2006). Methodology to support non-target and biodiversity risk assessment. In: Hilbeck A, Andow DA, Fontes EMG (Ed.), Environmental risk assessment of genetically modified organisms: Methodologies for assessing Bt cotton in Brazil. Cabi Publishing, pp. 108-132.

Hofs JL, Schoeman A, Mellet M, Vaissayre M (2005). Impact des cotonniers génétiquement modifiés sur la biodiversité de la faune entomologique: Le cas du coton Bt em Afrique du Sud. Int. J. Trop. Insect Sci. 25: 63-72.

Houssain F, Pray CE, Lu Y, Huang J, Hu R (2004). Genetically modified cotton and farmers health in China. Int. J. Occup. Environ. Health, 10: 296-303.

Huang J, Pray C, Scott R (2002). Enhancing the crops to feed the poor. Nature, 418: 678-684.

Li WD, Wu KM, Chen XX, Feng HQ, Guang X, Guo YY (2002). Effects of transgenic cotton carrying Cry1A+CpTI and Cry1Ac genes on diversity of arthropod communities in cotton fields in North China. Chin. J. Agric. Biotechnol. 1: 17-21.

Lövei GL, Andow DA, Arpaia S (2009). Transgenic insecticidal crops and natural enemies: A detailed review of laboratory studies. Environ. Entomol. 38: 293-306.

Men X, Ge F, Liu X, Yardim EN (2003). Diversity of arthropod communities in transgenic Bt cotton and nontransgenic cotton agroecosystems. Environ. Entomol., 32: 270-275.

Men X, Ge F, Edwards CA, Yardim EN (2004). Influence of Pesticide Applications on Pest and Predatory Arthropods Associated with Transgenic *Bt* Cotton and Nontransgenic Cotton Plants. Phytoparasitica, 32: 246-254.

Naranjo SE (2005). Long-term assessment of the effects of transgenic *Bt*-cotton on the abundance of nontarget arthropod natural enemies. Environ. Entomol. 34: 1193-1210.

Naranjo SE (2009). Impacts of Bt crops on non-target invertebrates and insecticide use patterns. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutr. Nat. Res. 4: 1-23.

Naranjo SE, Head G, Dively GP (2005). Field studies assessing arthropod nontarget effects in Bt transgenic crops: introduction. Environ. Entomol. 34: 1178-1180.

Perrett JJ, Higgins JJ (2006). A method for analyzing unreplicated agricultural experiments. Crop Sci. 46: 2482-2485.

Poole RW (1974). An introduction to quantitative ecology. McGraw-Hill, New York.

Quaim M, Subramarian A, Naik G, Zilberman D (2006). Adoption of Bt cotton and impact variability: Insights from Índia. Rev. Agric. Econ. 28: 48-58.

Romeis J, Meissle M, Bigler F (2006). Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. Nat. Biotechnol. 24: 63-70.

Romeis J, Bartsch D, Bigler F, Candolfi M, Gielkens MM, Hartley SE, Hellmich RL, Huesing JE, Jepson PC, Layton R, Quemada H, Raybould A, Rose RI, Shiemann J, Sears MK, Shelton AM, Sweet J, Vaituzis Z, Wolt JD (2008). Assessment of risk of insect-resistant transgenic crops to nontarget arthropods. Nat. Biotechnol. 26: 203-208.

Sanvido O, Stark M, Romeis J, Bigler F (2006). Ecological impacts of genetically modified crops: Experiences from ten years of experimental field research and commercial cultivation. Agroscope Reckenholz-Tänikon Research Station ART Reckenholzstrasse, p. 108.

Shelton AM, Zhao JZ, Roush RT (2002). Economic, ecological, food safety, and social consequences of the deployment of *Bt* transgenic plants. Annu. Rev. Entomol. 47: 845-881.
Silveira Neto S, Nakano O, Barbin D, Vila Nova NA (1976). Manual de ecologia dos insetos. São Paulo: Agronômica Ceres, p. 419.
Sisterson MS, Biggs RW, Olson C, Carriére Y, Dennehy TJ, Tabashnik BE (2004). Arthropod Abundance and Diversity in Bt and Non-Bt Cotton Fields. Environ. Entomol. 33: 921-929.
Southwood TRE (1995). Ecological methods: with particular reference to the study of insect populations. Chapman and Hall: London, p. 241.
Torres JB, Ruberson JR (2005). Canopy- and Ground-Dwelling Predatory Arthropods in Commercial *Bt* and non-*Bt* Cotton Fields: Patterns and Mechanisms. Environ. Entomol. 34: 1242-1256.
Uramoto K, Walder JMM, Zucchi RA (2005). Análise quantitativa e distribuição de populações de espécies de *Anastrepha* (Diptera: Tephritidae) no Campus Luiz de Queiroz, Piracicaba, SP. Neotrop. Entomol. 34: 33-39.
Wade MR, Scholz BCG, Lloyd RJ, Cleary AJ, Franzmann BA, Zalucki MP (2006). Temporal variation in arthropod sampling effectiveness: the case for using the beat sheet method in cotton. Entomol. Exp. Appl. 120: 139-153.
Whitehouse MEA, Wilson LJ, Fitt GP (2005). A comparison of arthropod communities in transgenic Bt and conventional cotton in Australia. Environ. Entomol. 34: 1224-1241.
Whitehouse MEA, Wilson LJ, Constable GA (2007). Target and non-target effects on the invertebrate community of Vip cotton, a new insecticidal transgenic. Aust. J. Agric. Res. 58: 273-285.