COMUNICACIONES
REPORTS
COMUNICAÇÕES

ARTHROPODS BIODIVERSITY INDEX IN BOLLGARD® COTTON (Cry1Ac) IN BRAZIL

Danielle Thomazoni, Miguel Ferreira Soria, Paulo Eduardo Degrande, Odival Faccenda and Pierre Jean Silvie

SUMMARY

Shannon-Wiener's diversity index (SWI) was used under untreated conditions of a cotton field during the 2006/2007 crop season in the Cerrado region, Brazil. Comparison was carried out between the transgenic NuOpal® (Bollgard®)(Cry1Ac) and the non-transgenic isogenic variety DeltaOpal®. SWI was calculated for target pests, non-target herbivores and predators groups. Two sampling methods were used: whole plant observation and beat sheet. As expected, the mean number of target pests, especially Pectinophora gossypiella *(Saund.) and* Alabama argillacea *(Hübner), was significantly smaller in Bt cotton. In the whole plant method sampling the SWI for non-target herbivores was significantly higher in Bt-cotton. The mean number of* Anthonomus grandis *(Boh.) and* Edessa meditabunda *(Fabr.) adults were significantly higher in NuOpal® with the whole plant sampling method. However, such differences were not observed with the beat sheet method. For the natural enemies, SWI and mean number of larvae and adults of the dominant predators did not show any significant difference between Bt and non-Bt cotton. These results confirm the conservation of some tritrophic interactions inside the Bt (untreated) cotton and contributes to a better sustainable management of non-target pests by enhancement of their natural biological control.*

Introduction

Biological communities have a degree of organization that is represented by their specific abundance distribution or relative frequency of the species present in the environment. The biological diversity in one biological community has two components: species richness (existing species number) and homogeneity, which depends on the larger or smaller uniformity of the distribution frequency of existing species (Hurlbert, 1971). The importance of the use of diversity indexes is their application in monitoring studies of biological communities dynamics and structural change detection, when the community environment is modified and the species have to adapt to the modifications, so as to contribute with the conservation of biodiversity in agroecosystems (Southwood, 1995).

Genetically modified (GM) cotton varieties expressing the

KEYWORDS / Bt-cotton / Cry1Ac / Diversity / Herbivores Non-Target / Predators / Shannon-Wiener's Index /

Received: 25/09/2012. Modified: 09/12/2013. Accepted: 12/12/2013.

Danielle Thomazoni. Biologist and Ph.D. in Sciences (Entomology), Universidade Federal de Grande Dourados (UFGD), Brazil. Research Entomologist, Instituto Mato-grossense do Algodão (IMAmt), Brazil. Address: Rodovia BR 070 Km 265, Campo Experimental, Zona Rural, Primavera do Leste, MT, Brazil. 78850-000. e-mail: daniellethomazoni@imamt.com.br

Miguel Ferreira Soria. Agronomist and Ph.D. in Vegetal Production, UFGD, Brazil. Research Entomologist, IMAmt, Brazil.

Paulo Eduardo Degrande. Agronomist and Ph.D. in Sciences (Zoology), Universidade de São Paulo, Brazil. Professor, UFGD, Brazil.

Odival Faccenda. Mathematician and Ph.D. in Agronomy (Energy in Agriculture), Universidade Estadual Paulista Júlio de Mesquita Filho, Brazil. Professor, Universidade Estadual de Mato Grosso do Sul, Brazil.

Pierre Jean Silvie. Biologist and Ph.D. in Entomology, Centre de Coopération Internationale en Recherche Agronomique Pour le Dévelopment (CIRAD), France. Research Entomologist, CIRAD, Montpellier, France.

ÍNDICE DE BIODIVERSIDAD DE ARTRÓPODOS EN ALGODÓN BOLLGARD® (Cry1Ac) CULTIVADO EN LA REGIÓN DE CERRADO EN BRASIL

Danielle Thomazoni, Miguel Ferreira Soria, Paulo Eduardo Degrande, Odival Faccenda y Pierre Jean Silvie

RESUMEN

El índice de biodiversidad de Shannon-Wiener's (ISW) fue utilizado en áreas dedicadas al cultivo de algodón no tratadas insecticidas. El trabajo se realizó en áreas algodoneras localizadas en la región de Cerrado, Brasil. Se comparó el algodón transgénico NuOpal® (Bollgard®)(Cry1Ac) con la isolínea no transgénica DeltaOpal®. El índice de biodiversidad fue calculado para todos los insectos presentes en el agroecosistema algodonero, incluyendo los insectos plagas de la variedad Bt, convencionales y enemigos naturales. Los métodos de muestreo utilizados fueron el uso del paño de sacudida y la planta entera. Como era previsible, el valor promedio calculado de las plagas, específicamente Pectinophora gossypiella *(Saund.) and* Alabama argillacea *(Hübner), fue significativamente menor en el algodón Bt. El ISW para los insectos plagas fue significativamente mayor en algodón Bt con el método de planta entera, en tanto que la media poblacional de adultos de* Anthonomus grandis *(Boh.) y* Edessa meditabunda *(Fabr.) fue mayor en NuOpal® usando el mismo método. No obstante, esta diferencia no fue observada con el método de paño de sacudida. Por otra parte, el ISW, para los enemigos naturales y el valor de la media poblacional de larvas y adultos de los predadores dominantes no presentaron diferencia significativa entre el algodón Bt y el convencional. Estos resultados corroboran la conservación de las interacciones tritróficas en el algodón Bt (no tratado con insecticidas) y aporta nuevos elementos técnicos para manejo integrado de insectos con énfasis en su control biológico natural.*

ÍNDICE DE BIODIVERSIDADE DE ARTRÓPODES EM ALGODÃO BOLLGARD® (Cry1Ac) NO BRASIL

Danielle Thomazoni, Miguel Ferreira Soria, Paulo Eduardo Degrande, Odival Faccenda e Pierre Jean Silvie

RESUMO

O índice de diversidade de Shannon-Wiener (ISW) foi utilizado em condições de cultivo de algodoeiro não tratado, sem aplicação de inseticidas durante a safra 2006/2007 no Cerrado, Brasil. Foi realizada comparação entre NuOpal® (Bollgard®)(Cry1Ac) e sua isolinha não transgênica. O índice foi calculado para pragas-alvo do algodão Bt, pragas não-alvo e inimigos naturais. Foram utilizados dois métodos de amostragem: planta inteira e pano de batida. Como esperado, o número médio de pragas-alvo, especialmente Pectinophora gossypiella *(Saund.) e* Alabama argillacea *(Hübner), foi significativamente menor em algodão Bt. Na amostragem por planta inteira, o ISW para herbívoros não-alvo foi significativamente maior em algodão Bt. O número médio de adultos de* Anthonomus grandis *(Boh.) e* Edessa meditabunda *(Fabr.) foi significativamente maior em NuOpal® utilizando o método de amostragem de planta inteira. Entretanto, esta mesma diferença não foi observada com o método do pano de batida. Para inimigos naturais, o ISW e o número médio de larvas e adultos de predadores dominantes não apresentou diferença signficativa entre algodão Bt e não-Bt. Estes resultados confirmam a conservação de algumas interações tritróficas dentro do sistema algodão Bt (não pulverizado) e contribui para um manejo de herbívoros não-alvo sustentável pelo incremento do seu controle biológico natural.*

Bacillus thuringiensis (Bt) Cry1Ac protein (NuOpal® and DP90B) were introduced commercially in Brazil during the 2006/2007 crop season. Knowledge about the non-target species (herbivores and natural enemies) present in the Bt-cotton in different field conditions is still incipient in Latin America, in spite of the economic importance of knowing the biological diversity and maintaining a biological control during the introduction of GM crops (Romeis *et al*., 2008; Lovei *et al*., 2009; Adenle, 2012).

Pest resistant GM varieties were initially grown in countries such as the USA, Argentina, Australia, China, Mexico and South Africa, allowing fewer insecticide applications, reduction in production costs and reduction of the risks to human health (Shelton *et al*., 2002; Naranjo, 2009). Another aspect is the promotion and the preservation of natural enemies, contributing to integrate pest management systems with a strong biological control component and assessment of risk of Bt-cotton to non-target arthropods, leading to a sustainable production and preserving the environment (Romeis *et al*., 2006; 2008; Sarvjeet, 2012).

There are few published studies conducted about the impact of the Bt-cotton varieties on the diversity of arthropods, especially with respect to the values found in diversity indexes, like the Shannon-Wiener's index. Using this index, no difference in arthropod biodiversity between Bt and non-Bt cotton was shown by Li *et al*. (2002), or an increase in the arthropod communities diversity and pest sub-communities (Men *et al*., 2003). In Brazil, Ramiro and Faria (2006) observed no significant differences in the total predator specimens collected from Bollgard® cotton as compared to treatments with Delta Pine Acala 90, with or without chemical control of caterpillars.

The objective of this research was to study the arthropod biodiversity associated with Bt-cotton (NuOpal®), as compared to the non-transgenic isogenic DeltaOpal® in the absence of insecticide sprays, promoting the knowledge of structural changes in arthropod communities in the Bt and non-Bt cotton, constituting a basis for the regulation of population dynamics of insect pests and the damage caused by such pests. This paper presents the first study of biodiversity of non-target herbivores and natural enemies (mainly predators) sampled with two methods: whole plant and beat sheet, in the Brazilian Cerrado Biome (Savannah) region, Mato Grosso do Sul State, Brazil. A faunistic analysis of the genera and species found on Bt-cotton compared with non Bt-cotton using the Shannon-Wiener's index is discussed.

Materials and Methods

This research was conducted at the Faculdade de Ciências Agrárias (FCA), Univer-

sidade Federal da Grande Dourados, in Dourados, Mato Grosso do Sul,, Brazil, between February and June 2007. An irrigation system was installed in order to facilitate crop development during the experiment. Basic and side dressing fertilizations were performed with 400kg·ha^{-1} NPK (8-20-20). Conventional planting system was adopted. Soil tillage was accomplished on February 14, 2007, before seeding by plowing and harrowing,. Seeds of the NuOpal® (Bt) and DeltaOpal® (non-Bt) varieties used in the experiment were provided by MDM–Seeds of Cotton©, and were pre-treated with the fungicides Euparen® (tolylfluanid) (200g/100kg seeds), Monceren® (pencycuron) (200g/100kg seeds), and Baytan® (triadimenol) (40ml/20Kg seeds), in order to control diseases that cause damping-off. Both varieties were manually seeded on February 15, 2007, at a density of 13 seeds/m and a row spacing of 0.90m. Emergence occurred 4 days later. Weed control was performed by handweeding during the entire cycle for both varieties. Blitz® baits (fipronil) were applied at the beginning of the crop cycle on the surroundings of the experimental area to control leafcutting ants of the genera *Atta* and *Acromyrmex*.

The total experimental area was 18×72m (0.12ha) with 32 sub-areas (plots) that were demarcated by random drawing, 16 for each treatment: DeltaOpal® and NuOpal®. Each Each sub-area comprised five rows of the treatment variety, measuring 4.5×9m. The three central rows were sampled; one row at each end of each subarea was the border of the sampling unit. In order to reduce the incidence of *Anthonomus grandis* (Boheman, 1843) during the experiment, 23 traps were installed containing grandlure pheromone + insecticide, to capture boll weevils in the vicinity of the experiment area.

The sampling and quantification of the Bollgard® technology target insects, non-target pests, and natural enemies found were performed every seven days during the entire evaluation period, from the crop's VE stage (emergence), on February 21, until June 13. Two visual sampling methods were used: 'beat sheet', recommended by (Degrande *et al*., 2003), and 'whole plant'. Seventeen whole plant samplings and 8 beat sheet samplings were made for each treatment, preparing 272 replicates for the whole plant method and 128 for the beat sheet method in each treatment over the experimental period.

In the beat sheet method, samplings were taken in the crop inter-row between the three central rows of each sub area, at a random point, totaling 16 points per treatment and observation date. The beat sheet was white to facilitate insect visualization; sheet width matched the crop row spacing (0.9m), had a 1m length, and was adjusted so as to cover the inter-rows. Then, both rows were vigorously shaken, causing the insects, either immature or adult, to fall onto the sheet, allowing them to be visualized, counted, and identified at the family and/or species level while still in the field. The presence of the parasitoid *Catolaccus grandis* (Burks, 1954) and the target lepidopteran *Pectinophora gossypiella* was quantified in this method by the number of damaged reproductive structures (bolls) fallen onto the beat sheet. The bolls were then opened to reveal individuals of those insects.

In the whole plant evaluation method, ten plants were evaluated separately on the three central rows of each sub area, i.e., 160 plants per treatment and observation date, by quantifying and identifying the insects sampled at the family and/or species level while still in the field.

In both methods, when necessary, those insects that could not be identified in the field were collected and placed in a recipient with 70% ethanol and taken and after taken to the laboratory for later identification. Samplings for *Heliothis virescens* and *Alabama argillacea* eggs were made with the whole plant method.

The diversity in Bt and non-Bt cotton with both sampling methods was based on calculations of frequency indices, constancy, abundance, and dominance (Silveira-Neto *et al*., 1976), considering the number of small (<1.0cm) and large caterpillars (>1.0cm), larvae, nymphs, and adults. Absolute frequency was defined as the total number of specimens observed in the various sampling conditions.

Constancy was defined as the percentage of samples in which a given species was present (Uramoto *et al*., 2005). After the constancy percentages over the sampling periods were obtained, the species were grouped into three categories: 'constant' (w), present in more than 50% of the weekly observations; 'accessory' (y), present in 25 to 50% of the observations; and 'accidental' (z), present in less than 25% of the observations.

Abundance is the number of individuals of a given species divided by the surface or volume unit, and may vary in space and time (Silveira-Neto *et al*., 1976). In order to estimate abundance, the limits established by the confidence interval (CI) at 5 and 1% probabilities were used, and the following five classes were determined: 'rare' (r) with a number of individuals in the species smaller than the lower CI limit at 1% probability; 'dispersed' (d) with a number of individuals between the lower limits of the confidence intervals at 1% and 5% probability; 'common' (c) within the confidence interval at 5%; 'abundant' (a), between the upper limits of the confidence intervals at 5% and 1% probability; and 'very abundant' (va) with a number of individuals greater than the upper CI limit at 1% probability.

An organism is considered dominant when it receives impact from the environment and becomes adapted to it (Silveira-Neto *et al*., 1976). In the present study, a species was considered 'dominant' when its relative frequency was >1/S, where S: total number of species found in the sampling period.

In order to compare the mean differences between groups of target pests, non-target pests, natural enemies and individuals of dominant species within each group, the development stage of the specimens was taken into consideration. The comparison between Bt and non-Bt treatments was calculated based on the mean of each treatment throughout the entire sampling period. The Student's t test was later used at a significance level α=5%. The original data were not normally distributed, and the test was applied to the data transformed to $\sqrt{X+0.5}$, thus meeting the assumptions associated with the model.

Target pest, non-target pest and natural enemy diversity in Bt and non-Bt-cotton environments were studied using Shannon-Wiener's index with a correction factor and natural logarithm (Poole 1974), by means of specimen frequency. This index measures the degree of uncertainty in predicting to which species will belong a randomly selected individual, from a sample with S species and N individuals (Silveira-Neto *et al*., 1976).

The smaller the Shannon-Wiener's index value, the smaller the degree of uncertainty, therefore reflecting the low diversity of a sample. Diversity tends to be higher for higher index values (Uramoto *et al*., 2005). Student's t test was used to check whether the species diversity difference between those environments was significant at α=5%. Data were analyzed using the statistical software package SPSS® (SPSS, 2006).

Results and Discussion

A total of 55 species were observed, distributed among 11 orders and 32 families, and were divided into three groups: target pests, non-target pests,

TABLE I
FAUNISTIC ANALYSIS OF GROUPS OF TARGET PESTS, NON-TARGET PESTS, AND NATURAL ENEMIES BY ORDER, FAMILY, AND SPECIES, SAMPLING METHOD, AND TYPE OF COTTON

Group	Order/Family	Species	Stage[1]	Sampling method			
				Whole plant		Beat sheet	
				NuOpal® FC(A)D[2]	DeltaOpal® FC(A)D[2]	NuOpal® FC(A)D[2]	DeltaOpal® FC(A)D[2]
Target pests	Lepidoptera/Noctuidae	*Alabama argillacea*	SC+LC	9 yn	55 w(c)s	0	31y(c)s
	Lepidoptera/Noctuidae	*Heliothis virescens*	SC+LC	0	1 z(c)n	0	1z(c)n
	Lepidoptera/Gelechiidae	*Pectinophora gossypiella*	Cat	0	39 y(c)s	0	35y(c)s
Total				9	95	0	67
Non-target pests	Coleoptera/Chrysomelidae	*Cerotoma arcuata*	Ad	5 z(d)n	2 z(d)n	0	0
	Coleoptera/Chrysomelidae	Chrysomelidae sp.1	Ad	2 z(d)n	2 z(d)n	0	1 z(d)n
	Coleoptera/Chrysomelidae	*Diabrotica speciosa*	Ad	52 w(c)n	45 w(c)n	35 w(c)s	26 w(c)n
	Coleoptera/Chrysomelidae	*Jansonius boggianii subaeneus*	Ad	37 w(c)n	38 w(c)n	13 y(c)n	21 y(c)n
	Coleoptera/Chrysomelidae	*Maecolaspis* sp.	Ad	19 y(d)n	10 y(d)n	0	0
	Coleoptera/Cicindellidae	*Megascelis* sp.	Ad	1 z(d)n	2 z(d)n	0	1 z(d)n
	Coleoptera/Curculionidae	*Anthonomus grandis*	L+Ad	235 y(ma)s	154 y(c)s	47w(a)s	45 w(c)s
	Coleoptera/Lagriidae	*Lagria villosa*	Ad	2 z(d)n	3 z(d)n	8 w(d)n	8 y(d)n
	Coleoptera/Melyridae	*Astylus variegatus*	Ad	26 y(c)n	10 z(d)n	19 w(c)n	13 y(c)n
	Hemiptera/Aleyroidade	*Bemisia tabaci*	Ad	903 w(ma)s	856 w(ma)s	0	0
	Hemiptera/Alydidae	*Neomegalotomus parvus*	Ad	0	3 z(d)n	1 w(r)n	1 z(d)n
	Hemiptera/Cicadellidae	*Agallia albidula*	Ad	315 w(ma)s	261 w(ma)s	11 w(c)n	12 z(c)n
	Hemiptera/Coreidae	*Hypselonotus* sp.	Ad	0	0	1 z(r)n	0
	Hemiptera/Lygaeidae	*Oxycarenus* sp.	Ad	5 z(d)n	2 z(d)n	3 y(r)n	2 y(d)n
	Hemiptera/Miridae	*Horciasinus signoreti*	Ad	1 z(d)n	1 z(d)n	7 y(d)n	7 y(d)n
	Hemiptera/Miridae	*Horcias nobilellus*	Ad	611 w(ma)s	549 w(ma)s	142w(ma)s	150 w(ma)s
	Hemiptera/Pentatomidae	*Chinavia* spp.	N+Ad	5 z(d)n	2 z(d)n	6 y(d)n	2 y(d)n
	Hemiptera/Pentatomidae	*Edessa meditabunda*	N+Ad	129 w(c)s	74 w(c)n	61 w(ma)s	75 w(ma)s
	Hemiptera/Pentatomidae	*Euschistus heros*	N+Ad	114 w(c)s	93 w(c)s	40 w(c)s	31 w(c)s
	Hemiptera/Pentatomidae	*Nezara viridula*	N+Ad	63 w(c)n	61 w(c)n	40 w(c)s	31 w(c)s
	Hemiptera/Pentatomidae	*Piezodorus guildini*	N+Ad	38 w(c)n	17 w(c)n	6 w(d)n	6 y(d)n
	Hemiptera/Pyrrhocoridae	*Dysdercus* sp.	N+Ad	217 w(a)s	219w(ma)s	99 w(ma)s	165 w(ma)s
	Lepidoptera/Noctuidae	*Spodoptera eridania*	SC+LC	5 z(d)n	9 y(d)n	9 w(d)n	11 y(c)n
	Lepidoptera/Noctuidae	*Spodoptera frugiperda*	SC+LC	4 y(d)n	6 y(d)n	0	4 z(d)n
	Lepidoptera/Noctuidae	*Pseudoplusia includes*	SC+LC	4 y(d)n	13 y(d)n	7 w(d)n	12 w(c)n
	Orthoptera/Gryllidae	*Gryllus* sp.	Ad	3 z(d)n	4 z(d)n	0	1 z(d)n
	Orthoptera/Tettigoniidae	Tettigoniidae sp.1	Ad	0	1 z(d)n	2 y(r)n	0
	Thysanoptera/Thripidae	*Frankliniella* sp.	Ad	50 w(c)n	43 w(c)n	0	0
Total				2846	2480	557	625
Natural enemies	Araneae	Araneae	Ad	152 w(va)s	185 w(va)s	58 w(va)s	72 w(va)s
	Coleoptera/Carabidae	*Callida* sp.	Ad	13 z(c)n	16 z(c)n	1 z(d)n	1 z(r)n
	Coleoptera/Carabidae	*Lebia concinna*	Ad	3 z(d)n	5 z(d)n	1 z(d)n	2 y(r)n
	Coleoptera/Coccinellidae	*Cycloneda sanguinea*	L+Ad	74 w(va)s	74 w(va)s	44 w(va)s	44 w(va)s
	Coleoptera/Coccinellidae	*Eriopsis connexa*	L+Ad	4 z(d)n	5 z(d)n	0	0
	Coleoptera/Coccinellidae	*Hyperaspis festiva*	Ad	9 z(c)n	7 y(d)n	1 z(d)n	0
	Coleoptera/Coccinellidae	*Olla v-nigrum*	Ad	3 z(d)n	5 z(d)n	2 z(d)n	1 z(r)n
	Coleoptera/Coccinellidae	*Scymnus* sp.	L+Ad	191 w(va)s	174 w(va)s	92 w(va)s	82 w(va)s
	Dermaptera/Forficulidae	*Doru luteipes*	Ad	7 y(c)n	12 y(c)n	4 y(d)n	7w(d)n
	Diptera/Dolichopodidae	*Condylostylus* sp.	Ad	1 z(d)n	1 z(d)n	0	0
	Diptera/Syrphidae	*Toxomerus* sp.	L+Ad	7 y(c)n	8 y(d)n	1 z(d)n	0
	Hemiptera/Anthocoridae	*Orius* sp.	Ad	27 y(c)n	45 y(c)s	23 y(c)s	41 y(a)s
	Hemiptera/Lygaeidae	*Geocoris* sp.	Ad	42 w(c)s	53 w(c)s	20 w(c)s	28 w(c)s
	Hemiptera/Nabidae	*Nabis* sp.	Ad	2 z(d)n	0	1 z(d)n	0
	Hemiptera/Pentatomidae	*Podisus* sp.	N+Ad	2 z(d)n	3 z(d)n	3 y(d)n	5 y(d)n
	Hemiptera/Reduviidae	*Repipta* sp.	Ad	1 z(d)n	2 z(d)n	0	0
	Hemiptera/Reduviidae	*Zelus armillatus*	Ad	1 z(d)n	3 z(d)n	0	0
	Hemiptera/Reduviidae	*Zelus longipes*	Ad	12 y(c)n	10 y(c)n	3 y(d)n	6 w(d)n
	Hymenoptera/Formicidae	*Solenopsis invicta*	Ad	25 y(c)n	17 y(c)n	1 z(d)n	1 z(r)n
	Hymenoptera/Pteromalidae	*Catolaccus grandis*	L	45 z(c)s	39 z(c)s	45 z(va)s	39 z(a)s
	Mantodea/Mantidae	Mantidae sp.1	Ad	2 z(d)n	1 z(d)n	0	1 z(r)n
	Neuroptera/Chrysopidae	*Chrysoperla* sp.	L	28 y(c)s	21 y(c)n	26 w(c)s	20 w(c)n
	Neuroptera/Hemerobiidae	*Nusulala* sp.	L	2 z(d)n	8 y(d)n	2 y(d)n	5 y(d)n
	Neuroptera/Mantispidae	Mantispidae sp.1	Ad	1 z(d)n	0	0	0
Total				654	694	328	355
Grand total				3509	3269	885	1047

[1] SC: small caterpillar, LC: large caterpillar, Cat: caterpillar, L: larva, N: nymph, Ad: adult. [2] F: total number observed in different sampling conditions; C (constancy): w: constant, y: accessory, z: accidental; A (abundance): va: very abundant, a: abundant, c: common, d: dispersed, r: rare; D (dominance): s: dominant, n: non-dominant.

and natural enemies (Table I). The very abundant (va) species of non target herbivores, both in NuOpal® and DeltaOpal® cotton with the whole plant and beat sheet methods, were *Horciasoides nobilellus* (Bergston, 1883) and *Dysdercus* sp. The species *Bemisia tabaci* (Gennadius, 1889) and *Agallia albidula* (Uhler, 1895) were very abundant in Bt and non-Bt cotton only with the whole plant method. The species *Edessa meditabunda* (Fabr., 1794), however, was very abundant in both varieties, but only with the beat sheet method. The non-target species *Anthonomus grandis* (Boheman, 1843) was found to be abundant in Bt cotton with both sampling methods.

The fact of *B. tabaci* was observed as the most abundant species with similar population densities between Bt and non-Bt cotton in the whole plant method (Naranjo, 2005) can be attributed to the behavior of this insect. Its quick flight when the plant is touched, made it difficult to be sampled by beat sheet, demonstrating the importance of selecting the adequate sampling method to monitor non-target insects with an economic importance in transgenic varieties in the field (Naranjo *et al*., 2005; Wade *et al*., 2006). Another point that will be considered is the differences in 'leaf hair' between the cotton varieties and in the mode of action of Bt toxins inserted in these genetically modified cotton varieties, which can influence the abundance of insects, as observed in Australia (Whitehouse *et al*., 2007), with high numbers of whitefly in Bt cotton (VipCotton).

Dominance was found in the target pest group for the species *A. argillacea* and *P. gossypiella* in non-Bt cotton, with both sampling methods. The presence of the species *H. virescens* was also detected, but at a low frequency when compared with the other two target pests, i.e., one caterpillar in DeltaOpal® with both sampling methods. The non-target herbivores species, *A. grandis*, *H. nobilellus*, *Euschistus heros* (Fabr., 1798) and *Dysdercus* sp. were dominant in both varieties with both sampling methods, while *B. tabaci* and *A. albidula* were dominant in both NuOpal® and DeltaOpal® only with the whole plant method. For the pentatomid species *Nezara viridula* (L., 1758) and *E. meditabunda*, the dominance was observed in both varieties, but only with the beat sheet method. In the whole plant sampling, the sucking herbivore species *E. meditabunda* was dominant in NuOpal® only, while the chewing herbivore *Diabrotica speciosa* (Germar, 1824) was dominant only in the Bt variety, with the beat sheet sampling (Table I).

These dominance results showed the reduction in feeding competition between non-target herbivores and target insects controlled by the Bollgard® technology, as a reduction in competition for food resources with target caterpillars controlled by the Cry1Ac toxin and the sucking herbivores like the pentatomids and mirids. They show the importance of the knowledge of bioecology interaction between insects in an agroecosystem. Also, the sampling method used can influence the quantification of each insect species affecting the Bt and non-Bt varieties, considering the behavior of each species and their migration from soybean varieties to cotton (Lu *et al*., 2010), as in the case of some pentatomids, searching food resources in the cotton-soybean agroecosystem. The correct selection of the sampling method leads to real interpretations about the effect of the GM plant on the arthropod population, and consequently on the biological control, which may be potentiated with the adoption of Bt crops.

Considering the constancy throughout the sampling period, *A. argillacea* had constant incidence only in non-Bt cotton with the whole plant method. The constant non-target herbivore species in both NuOpal® and DeltaOpal® in both samplings were *D. speciosa*, *H. nobilellus*, *E. meditabunda*, *E. heros*, *N. viridula* (L., 1758) and *Dysdercus* sp., while *B. tabaci*, *A. albidula*, *Piezodorus guildini* (Westwood, 1837), *Jansonius boggianii subaeneus* and *Frankliniella* sp. were constant in both Bt and non-Bt cotton with the whole plant sampling, and *A. grandis* and *Pseudoplusia includens* (Walker, 1857) were constant in both types of cotton with beat sheet sampling. The non-target herbivores species *Lagria villosa* (Fabr., 1783), *Astylus variegatus* (Germar, 1824), *Neomegalotomus parvus* (Westwood, 1842), *A. albidula*, *Piezodorus guildini* (Westwood, 1837) and *Spodoptera eridania* (Cramer, 1782) were constant only in Bt cotton with the whole plant method (Table I).

With regard to non-target herbivores diversity, the Shannon-Wiener's index with the whole plant method for Bt cotton showed statistically significant differences, being higher than non-Bt, thus demonstrating that the NuOpal® variety showed higher diversity of non-target herbivores than the DeltaOpal® variety with the whole plant sampling. However, in the beat sheet method, the index did not show significant difference (Table II). This result in the diversity index can be explained by fact that the mean number of the non-target herbivores *A. grandis*

TABLE II
SHANNON-WIENER'S DIVERSITY INDEX, (VARIANCE), AND NUMBER OF NON-TARGET PEST SPECIES AND NATURAL ENEMIES PRESENT IN THE BT- AND NON-BT COTTON ENVIRONMENTS

Whole plant	Bt cotton [1] (n=272)	Non-Bt cotton [1] (n=272)	t-Student	P
Non-target pests	2.11(0.004)(25) a	2.04(0.005)(27) b	1.98	0.047
Natural enemies	2.17(0.002)(24)	2.21(0.001)(22)	-0.558	0.576
Beat sheet	Bt cotton [1] (n=128)	Non-Bt cotton [1] (n=128)	t-Student	P
Non-target pests	2.32(0.001)(20)	2.23(0.001)(22)	1.643	0.100
Natural enemies	2.04(0.002)(18)	2.12(0.002)(16)	-1.053	0.292

[1] Different letters in a row represent non-significant values at 5%, assuming equal variances by Levene's test.

TABLE III
MEAN NUMBER OF ARTHROPOD SPECIMENS (SD) PER TYPE OF COTTON AND SAMPLING METHOD

Whole plant	Bt cotton [1] (n=272)	Non-Bt cotton [1] (n=272)	t-Student [2]	p
Target pests	0.03 (0.19) a	0.35 (0.76) b	7.029 [3]	0.000
Non-target pests	10.45 (6.94) a	9.11 (6.68) b	2.626	0.009
Natural enemies	2.36 (2.67)	2.50 (2.78)	0.817	0.414
Beat sheet	Bt cotton [1] (n=128)	Non-Bt cotton [1] (n=128)	t-Student[2]	p
Target pests	0.00 (0.00) a	0.52 (0.96) b	6.777 [3]	0.000
Non-target pests	4.35 (3.09)	4.87 (4.28)	0.498 [3]	0.619
Natural enemies	2.50 (2.38)	2.71 (2.21)	1.209	0.228

[1] Different letters in a row represent non-significant values at 5%, assuming equal variances by Levene's test.
[2] Original data transformed to $\sqrt{X+0.5}$ for statistical analysis purposes. [3] Different variances.

TABLE IV
MEAN NUMBER (SD) OF INDIVIDUALS FROM DOMINANT NON-TARGET PEST SPECIES PER TYPE OF COTTON AND SAMPLING METHOD

Whole plant	Stage [2]	Bt-cotton [3] (n=272)	Non-Bt cotton [3] (n=272)	t-Student [1]	p
Horciasoides nobilellus	Ad	2.25 (3.28)	2.02 (3.29)	0.848	0.397
Anthonomus grandis	Ad	0.86 (1.62) a	0.57 (1.19) b	2.267	0.024
Agallia albidula	Ad	1.16 (1.47)	0.96 (1.33)	1.703	0.089
Nezara viridula	N	0.06 (0.25)	0.03 (0.17)	1.345	0.179
Nezara viridula	Ad	0.17 (0.59)	0.19 (0.56)	0.594	0.553
Euschistus heros	N	0.04 (0.23)	0.03 (0.19)	0.787	0.431
Euschistus heros	Ad	0.38 (0.84)	0.31 (0.95)	1.017	0.309
Edessa meditabunda	N	0.04 (0.38)	0.03 (0.20)	0.319	0.750
Edessa meditabunda	Ad	0.43 (1.08) a	0.24 (0.73) b	2.451	0.015
Dysdercus sp.	N	0.06 (0.27)	0.07 (0.38)	0.316	0.752
Dysdercus sp.	Ad	0.74 (1.41)	0.73 (1.33)	0.121	0.903
Diabrotica speciosa	Ad	0.19 (0.47)	0.17 (0.45)	0.679	0.498
Beat sheet	**Stage [2]**	**Bt-cotton [3] (n=128)**	**Non-Bt cotton [3] (n=128)**	**t-Student [1]**	**p**
Horciasoides nobilellus	Ad	1.11 (1.43)	1.17(1.52)	0.250	0.803
Anthonomus grandis	Ad	0.37 (0.79)	0.35(0.78)	0.209	0.834
Agallia albidula	Ad	0.09 (0.28)	0.09(0.50)	0.219	0.827
Nezara viridula	N	0.08 (0.29)	0.06(0.27)	0.463	0.644
Nezara viridula	Ad	0.23 (0.55)	0.18(0.46)	0.812	0.418
Euschistus heros	N	0.00 (0.00)	0.02(0.12)	1.420	0.157
Euschistus heros	Ad	0.31 (0.64)	0.23(0.53)	1.111	0.268
Edessa meditabunda	N	0.01 (0.08)	0.00(0.00)	1.000	0.318
Edessa meditabunda	Ad	0.47 (1.37)	0.59(1.97)	0.241	0.810
Dysdercus sp.	N	0.02 (0.15)	0.06(0.62)	0.438	0.662
Dysdercus sp.	Ad	0.75 (1.23)	1.23(2.03)	1.821	0.070
Diabrotica speciosa	Ad	0.27 (0.64)	0.20(0.49)	0.859	0.391

[1] Original data transformed to $\sqrt{X+0.5}$ for statistical analysis purposes. [2] N: nymph, Ad: adult. [3] Different letters in a row represent non-significant values at 5%, assuming equal variances by Levene's test.

TABLE V
MEAN NUMBER OF SPECIMENS (SD) OF INDIVIDUALS FROM DOMINANT NATURAL ENEMY SPECIES PER TYPE OF COTTON AND SAMPLING METHOD

Whole plant	Stage [2]	Bt-cotton (n=272)	Non-Bt cotton (n=272)	t-Student [1]	P
Cycloneda sanguinea	L	0.20 (0.58)	0.21 (0.82)	0.231	0.817
Cycloneda sanguinea	Ad	0.07 (0.31)	0.07 (0.29)	0.235	0.815
Scymnus sp.	L	0.56 (1.29)	0.46 (1.15)	0.771	0.441
Scymnus sp.	Ad	0.15 (0.41)	0.18 (0.58)	0.425	0.671
Chrysoperla sp.	L	0.10 (0.51)	0.08 (0.28)	0.332	0.740
Geocoris sp.	Ad	0.15 (0.44)	0.19 (0.53)	0.951	0.342
Orius sp.	Ad	0.10 (0.45)	0.17 (0.62)	1.326	0.185
Araneae	Ad	0.56 (0.93)	0.68 (1.00)	1.629	0.104
Catolaccus grandis	L	0.14 (0.62)	0.12 (0.64)	0.533	0.594
Beat sheet	**Stage [2]**	**Bt-cotton (n=128)**	**Non-Bt cotton (n=128)**	**t-Student [1]**	**P**
Cycloneda sanguinea	L	0.27 (0.70)	0.27 (1.09)	0.577	0.564
Cycloneda sanguinea	Ad	0.07 (0.31)	0.08 (0.26)	0.367	0.714
Scymnus sp.	L	0.61 (1.13)	0.56 (0.91)	0.063	0.950
Scymnus sp.	Ad	0.11 (0.36)	0.08 (0.26)	0.694	0.489
Chrysoperla sp.	L	0.20 (0.71)	0.16 (0.38)	0.258	0.797
Geocoris sp.	Ad	0.16 (0.40)	0.22 (0.46)	1.160	0.247
Orius sp.	Ad	0.18 (0.63)	0.32 (0.84)	1.518	0.130
Araneae	Ad	0.45 (0.85)	0.56 (0.81)	1.343	0.180
Catolaccus grandis	L	0.29 (0.88)	0.25 (0.93)	0.546	0.585

[1] Original data transformed to $\sqrt{X+0.5}$ for statistical analysis purposes. [2] L: larva, Ad: adult.

and *E. meditabunda* adults in the whole plant method was significantly different between Bt and non-Bt cotton, being higher in Bt cotton than non-Bt (Table III). Yet, such difference was not observed with the beat sheet method (Table IV). The mean number of target pest individuals observed with both sampling methods was significantly smaller in NuOpal® than in DeltaOpal®.

The results of the faunistic analysis of non-target species of Cry1Ac (Bollgard® cotton) sampled between the NuOpal® and DeltaOpal® environments demonstrate that abundance, dominance and constancy of the species can be attributed to several factors, such as the lack of insecticidal activity of the transgenic variety (Cry1Ac) on non-target herbivores and predators in NuOpal®, which can affect directly the diversity and trophic interactions of these insects, promoting the knowledge of these tritrophic interactions and the integration of Bt cotton use and biological control (Li *et al*., 2002; Romeis *et al*., 2008).

Among the natural enemies sampled, predators were mainly present. The predators Araneae, *Cycloneda sanguinea* (L., 1763) and *Scymnus* sp. were very abundant both in NuOpal® and DeltaOpal® with both sampling methods. With the beat sheet method, the parasitoid species *C. grandis* was abundant both in NuOpal® and DeltaOpal®, and the predator bug *Orius* sp. was abundant in non Bt cotton only.

Dominant natural enemies both in Bt and non-Bt cotton, with both the whole plant and beat sheet methods, were Araneae, *C. sanguinea*, *Scymnus* sp., *Geocoris* sp. and *C. grandis*. On the other hand, the predator *Orius* sp. was dominant in both NuOpal® and DeltaOpal® with the beat sheet method. However, this bug was only dominant in DeltaOpal® with the whole plant sampling. The beneficial arthropods Araneae, *C. sanguinea*, *Scymnus* sp. and *Geocoris* sp. Did not vary in both Bt and non-Bt cotton with both sampling methods. However, in the beat sheet, the predators *Doru luteipes* (Scudder, 1876) and *Zelus longipes* (L., 1767) were constant in DeltaOpal® only, while *Chrysoperla* sp. was constant in both Bt and non-Bt cotton (Table I).

The faunistic analysis for the natural enemies is mainly correlated with the tritrophic interactions and the direct and indirect effects of Bt toxin plants on the preys and hosts of these benefical insects (Shelton *et al*., 2002). Another point to be considered is the traits adopted in the transgenic varieties (Wan *et al*., 2002; Yang *et al*., 2005), like the application or not of insecticides with regard to the control level to the non-target herbivores of Bt plants (Degrande, 2004; Thomazoni *et al*., 2010), the use of selective insecticides, and the sampling method adopted correlated with the behavior and bioecology of the natural enemy, which can be influence the diversity of these insects (Men *et al*., 2003), as demonstrated in this study, in the beat sheet method for *Orius* sp., which was dominant in both NuOpal® and DeltaOpal® and was only dominant in DeltaOpal® with the whole plant sampling.

With regard to natural enemies diversity in both sampling methods, the Shannon-Wiener's index and also the mean number of natural enemies was not significantly different between cotton varieties using both sampling methods (Table II). The mean number of larvae and adults in the dominant genera and species of the predators *C. sanguinea*, *Scymnus* sp., *Chrysoperla* sp., *Geocoris* sp., *Orius* sp., Araneae and *C. grandis* did not show significant differences between Bt and non-Bt cotton in any of the sampling methods (Table V).

The dominance of predator *Chrysoperla* sp. in Bt cotton with both sampling methods, may indicate that this insect possibly did not suffer a negative impact from the Bt toxin present in the transgenic variety (Hilbeck *et al*., 2006). In contrast to our results, a small difference in *Geocoris* sp. population density between Bt and non-Bt cotton, both without chemical control application, was observed by Naranjo (2005). In the case of *Orius* sp. populations, the lack of chemical control application during the entire development cycle of both varieties can influence predator abundance, and influenced this genus, which was abundant in both Bt and non-Bt cotton. This result was confirmed by the measure of the mean number of individuals of *Orius* sp., and the same result was observed with Araneae in both sampling methods. But other studies have shown a higher mean number of these predators in Bt cotton than in non Bt cotton (Wan *et al*., 2002; Hagerty *et al*., 2005).

The similarity in the mean number of individuals of the Chrysopidae family between Bt and non-Bt cotton was also observed in Australia (Whitehouse *et al*., 2005), and the presence of individuals of this family was also observed in Bt cotton (Sisterson *et al*., 2004). However, Hagerty *et al*. (2005) found that chrysopid populations belonging to the Chrysopidae and Hemerobiidae families were more abundant in Bollgard® cotton when compared with non-Bt cotton, showing a negative effect of Bt cotton on the diversity of these natural enemies.

A lack of significant difference in the mean number of sampled Coccinellidae was also observed in China (Yang *et al*., 2005). However, in other countries (Hagerty *et al*., 2005; Hofs *et al*., 2005) differences in the mean number of specimens from this family of predators have been observed. Such difference in results can be attributed to the number of prey sampled between Bt and non-Bt cotton, whose development was favored by the lack of action of insecticides (Marvier *et al*., 2007) that are otherwise commonly applied for their control, showing the importance of integrating pest management tactics (Romeis *et al*., 2006, 2008), in this case, Bt cotton and biological control.

This biodiversity study was conducted to better understand the biology and ecology of the predator/pest interactions in Bt and non-Bt cotton varieties without application of insecticides, in a savannah agroecosystem in Brazil, being relevant because the arthropods play an important part in the structure and operation of the ecosystems and the maintenance of the biological diversity (Tscharntke and Clough, 2007; Scherr and McNeely, 2008; Sarvjeet, 2012). With the crescent introduction of the Bt cotton varieties in Brazil, it is crucial to quantify the diversity of insect communities present in Bt and non-Bt cotton plots and to determine how these communities are influenced by environmental changes provoked by natural causes or by human activity, like agronomic practices such a insecticide control for non-herbivores insects, and how these affect the biodiversity by tritrophic interactions that can contribute with the reduction of target pests, as natural enemies are the main cause of insect mortality in agroecosystems (Parra, 2000; Peixoto *et al*., 2007). In this way, this research can show how to integrate natural control with the transgenic plants, promoting the conservation of the beneficial insects, using the different monitoring sampling and diversity indexes.

Conclusions

NuOpal® (Cry1Ac) is efficient in the control of target species (*P. gossypiella*, *H. virescens* and *A. argillacea*) under cultivation conditions of the Brazilian Cerrado biome (savannah) without insecticide sprays.

The whole plant sampling method, as detected by the diversity indexes, has a higher diversity of the non-target herbivores *A. grandis* and *E. meditabunda* in Bt cotton (Cry1Ac).

The natural enemies diversity on the non-sprayed Bt cotton (Cry1Ac) shows tritrophic interactions, and the conservation potential and benefits on that agroecossystem.

Moreover, this research demonstrated that the faunistic analysis and the diversity index of Shannon-Wiener can be used in studies of risk assessment of Bt varieties in non-target arthropod populations in Brazil.

ACKNOWLEDGEMENTS

The authors thank Sérgio Vanin (Universidade de São Paulo) for identifying the chrysomelid *Jansonius boggianii subaeneus*, Rogério Silvestre and Manoel Fernando Demétrio (Universidade Federal da Grande Dourados) for identifying the ants (*Solenopsis invicta* e *Atta* sp.), MDM-Sementes de Algodão© for the seeds of both varieties used in the experiment and CNPq for the grant awarded to the first author.

REFERENCES

Adenle AA (2012) Failure to achieve 2010 biodiversity's target in developing countries: How can conservation help? *Biodiv. Cons. 21*: 2435-2442.

Degrande PE, Oliveira MA, 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. *Atual. Agric. 1*: 22-23.

Hagerty AM, Kilpatrick AL, Turnipseed SG, Sullivan MJ (2005) Predaceous arthropods and lepidopteran pests on conventional, Bollgard, and Bollgard II cotton under untreated and disrupted conditions. *Env. Entomol. 34*(5):105-114.

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 (Eds.) *Environmental Risk Assessment of Genetically Modified Organisms: Methodologies for Assessing Bt Cotton in Brazil*. 1st ed. CABI. Cambridge, MA, USA. 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. Ins. Sci. 25*: 63-72.

Hurlbert SH (1971) The non-concept of species diversity: a critique and alternative parameters. *Ecology 52*: 577-586.

Li WD, Wu KM, Chen XX, Feng HQ, Guang X, Guo YY (2002) Effects of transgenic cotton car-

rying 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. *Env. Entomol. 38*: 293-306.

Lu Y, Wu K, Jiang Y, Xia B, Li P, Feng H, Wyckhuys KAG, Guo Y (2010) Mirid bug outbreaks in multiple crops correlated with wide-scale adoption of Bt cotton in China. *Science 328*: 1151-1154.

Marvier M, McCreedy C, Regetz J, Kareiva P (2007) A meta-analysis of effects of Bt cotton and maize on nontarget invertebrates. *Science 316*: 1475-1477.

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

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

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

Naranjo SE (2009) Impacts of Bt crops on non-target invertebrates and insecticide use patterns. CAB Reviews: *Persp. Agric. Vet. Sci. Nut. Nat. Res. 4*: 1-23.

Parra JRP (2000) O controle biológico e o manejo de pragas: passado, presente e futuro. In Guedes JC, Costa ID, Castiglioni E (Eds.) *Bases Técnicas do Manejo de Insetos*. UFSM. CCR.DFS. Santa Maria, Brazil. pp. 59-70.

Peixoto MS, Barros LC, Bassanezi RC (2007) Uma abordagem fuzzy para um modelo presa-predador acoplado ao parasitismo. *Tema - Tend. Mat. Aplic. Comput. 8*: 119-128.

Poole RW (1974) *An Introduction to Quantitative Ecology*. McGraw-Hill. New York, USA.

Ramiro ZA, Faria AM (2006) Levantamento de insetos predadores nos cultivares de algodão Bollgard®DP90 e convencional Delta Pine Acala 90. *Arq. Inst. Biol. 73*: 119-121.

Romeis J, Meissle M, Bigler F (2006) Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. *Nat. Biotechnol. 24*: 63-71.

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.

Sarvjeet K (2012) Risk assessment of Bt transgenic crops. In Sansinenea E (Ed,) Bacillus thuringiensis *Biotechnology*. Springer. New York, USA. pp 41-85.

Scherr SJ, McNeely JA (2008) Biodiversity conservation and agricultural sustainability: towards a new paradigm of 'eco-agriculture' landscapes. *Phil. Trans. Roy. Soc. Lond. B. 363*: 477-494.

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.

Southwood TRE. (1995) *Ecological Methods: With Particular Reference to the Study of Insect Populations*. Chapman & Hall. London, UK. 524 pp.

Silveira Neto S, Nakano O, Barbin D, Vila Nova NA (1976) *Manual de Ecologia dos Insetos*. Ceres. São Paulo, Brazil. 419 pp.

Sisterson MS, Biggs RW, Olson C, Carriére Y, Dennehy TJ, Tabashnik BE (2004) Arthropod abundance and diversity in Bt and Non-Bt cotton fields. *Env. Entomol. 33*: 921-929.

SSPS (2006) Statistical Package for Social Sciences 15.0. SPSS Inc. Chicago, Il, USA. 179 pp.

Tscharntke T, Bommarco R, Clough Y (2007) Conservation biological control and enemy diversity on a landscape scale. *Biol. Control 43*: 294-309.

Thomazoni D, Degrande PE, Silvie PJ, Faccenda O. (2010) Impact of Bollgard® genetically modified cotton on the biodiversity of arthropods under practical field conditions in Brazil. *Afr. J. Biotechnol. 9*(37): 6167-6176.

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.

Wan, FH, Liu WX, Guo JY (2002) Comparison analyses of the functional groups of natural enemy in transgenic Bt-cotton field and non-transgenic cotton fields with IPM and chemical control. *Acta Ecol. Sin. 22*: 935-942.

Whitehouse MEA, Wilson LJ, Fitt GP (2005) A comparison of arthropod communities in transgenic Bt and conventional cotton in Australia. *Env. 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. Agr. Res. 58*: 273-285.

Yang P, Li K, Shi S, Xia J, Guo R, Li S, Wang L (2005) Impacts of transgenic Bt cotton and integrated pest management education on smallholder cotton farmers. *Int. J. Pest Manag. 51*: 231-234.