Journal of Agricultural Science; Vol. 6, No. 2; 2014
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Diversity, Composition and Population Dynamics of Arthropods in the Genetically Modified Soybeans Roundup Ready® RR1 (GT 40-3-2) and Intacta RR2 PRO® (MON87701 x MON89788)

Wagner Justiniano[1], Marcos Gino Fernandes[1] & Cácia Leila Tigre Pereira Viana[1]

[1] Universidade Federal da Grande Dourados (UFGD), Faculdade de Ciências Biológicas e Ambientais (FCBA), Programa de Pós-Graduação em Entomologia e Conservação da Biodiversidade, Dourados/MS, Brasil

Correspondence: Wagner Justiniano, Universidade Federal da Grande Dourados (UFGD), Faculdade de Ciências Biológicas e Ambientais (FCBA), Programa de Pós-Graduação em Entomologia e Conservação da Biodiversidade, Dourados/MS, Brasil. E-mail: wagner.justiniano@monsanto.com

Received: November 7, 2013 Accepted: December 18, 2013 Online Published: January 15, 2014

doi:10.5539/jas.v6n2p33 URL: http://dx.doi.org/10.5539/jas.v6n2p33

Abstract

Knowledge of insect diversity is essential for ecological studies and pest management. The aim of this study was to study the occurrence, abundance of target and non-target pests in genetically modified insect resistant (Bt) and glyphosate-tolerant soybeans (RR1 and RR2), with and without the application of insecticides. Experiments were carried out in the agricultural year of 2011/2012 in four municipalities in the State of Mato Grosso do Sul. The treatments were: 1 - Roundup Ready® RR1 soybeans without insecticide application; 2 - Roundup Ready® RR1 soybeans with application whenever the control level was reached; 3 - Intacta RR2 PRO® soybeans without insecticide application; and 4 - Intacta RR2 PRO® soybeans with application whenever the control level was reached. The evaluations were initiated soon after emergence of the plants at weekly intervals. In order to obtain representative gradients of species composition, we used the method of ordering by non-metric multidimensional scaling (NMDS) and the Bray-Curtis dissimilarity index. Insects of the order Lepidoptera presented 7547 specimens, composing more than 70% of the insect community. The orders Coleoptera and Hemiptera also stood out, consisting of 2066 and 331 insects, respectively. Most of the samples were recorded in stages V8 to R2 for defoliating caterpillars and between R5.2 and R6 for the phytophagous stink bug complex. The Bt technology significantly reduced the target insect pests and favored populations of natural enemies. The treatments with insecticide application resulted in reduction of arthropods collected and changes in population outbreaks when compared to areas without spraying.

Keywords: *Bacillus thuringiensis*, *Glycine max*, insect bioecology, integrated pest management

1. Introduction

The soybean [*Glycine max* (L.) Merrill (Fabaceae: Phaseoleae)] stands out as the main agricultural commodity Brazil, where in the crop year 2011/2012 about 25 million hectares were planted, producing 66,383,000 tons. In 2012/2013, the acreage increased by about 8.8% to 27.241 million acres and the expected harvest is about 82.627 million tons, a 24.5% increase in national production of this crop (Conab, 2012).

Soybean productivity is threatened by abiotic factors including climatic and biotic factors such as attack of insect pests, which can be controlled by natural enemies such as predators, parasitoids and pathogens. However, their populations often reach levels of economic damage to the crop. High population levels of these pests can be a result of favorable environmental conditions and/or inadequate management, including overuse of pesticides (Embrapa, 2011).

There has been a long search for specific technologies with low persistence in the environment, including Integrated Pest Management "IPM" based on plant resistance to insects. With the advent of genetic transformation based on recombinant DNA technology, it has become possible to insert foreign genes into the genomes of plants, thus conferring insect resistance (Bennet, 1994). Genes from bacteria, such as *Bacillus thuringiensis* (Bt) and *Bacillus sphaericus* have been the main organisms used to confer plant resistance to insects on the commercial scale (Sharma et al., 2000).

Recent advances in agricultural biotechnology have resulted in transgenic plants which are efficient alternatives and have less environmental impact for controlling lepidopteran pests in areas where these are of agronomic concern (Williams et al., 1998).

In Brazil, the genetically modified soybeans resistant to insects (MON 87701) and glyphosate-tolerant (MON 89788) were released in August 2010. The event MON 87701 possesses the Cry1Ac gene, derived from *B. thuringiensis* and event MON 89788 has the cp4 epsps gene. These events are distinct, expressed in different cell organelles (Ctnbio, 2010). The technology commercially referred to as Intacta RR2 PRO® containing event MON 87701 confers resistance to major defoliating caterpillars such as the soybean caterpillar *Anticarsia gemmatalis* [Hübner, 1818], soybean loopers *Chrysodeixis includens* [Walker, 1857] (Bernardi et al., 2012) and *Rachiplusia nu* [Guenée, 1852] and bud borer *Crocidosema aporema* [Walsingham, 1914], target pests of Bt technology (Monsanto, 2012).

Knowledge of diversity and arthropods associated with agriculture is essential for ecological and IPM studies, considering that in agroecosystems the arthropod communities change according to the management exercised (Milane et al., 2012). Some arthropod species can cause damage to plants, can sometimes lead to death, however some beneficial species can control these pest species through predation or parasitism (Garcia et al., 2004).

Population dynamic studies provide useful information for developing models involving pest management (Gilbert et al., 1976), considering the possibility of obtaining population distribution data over a given period of time (Odum, 1988). Therefore, such studies can be successfully employed in IPM programs (Silveira Neto et al., 1976).

The objective of this work was to evaluate the abundance, diversity, population dynamics and influence of phenological growth stages on the composition of insect pests and beneficial arthropods in genetically modified soybeans resistance to insects Intacta RR2 PRO® (Bt) and glyphosate-tolerant RR1 (non-Bt).

2. Materials and Methods

Local installation of the experiment - The study was conducted during the agricultural year of 2011/2012 under field conditions using four treatments and four replications in randomized blocks. Treatments: 1 - Roundup Ready® RR1 soybeans without application of insecticides, 2 - Roundup Ready® RR1 soybeans with application of insecticides, both treatments used to cultivar Brasmax Potência RR1 (event GT 40-3-2), 3 - Intacta RR2 PRO® soybeans without insecticide application and 4 - Intacta RR2 PRO® soybean with insecticide application; for treatments 3 and 4 the experimental strain used was Monsoy L6910 (events MON 87701 and MON 89788). The cultivars tested showed similar cycle, growth habit and phenology.

The repetitions were conducted at the following locations, area 1 - Pingo de Ouro Farm (-21.6724 S, -54.6392 W, 370 meters altitude), Area 2 - Boa Sorte Farm (-22.0175 S, -54.5358 W, 304 meters altitude), area 3 Rincão Porã Farm (-22.2376 S, -54.7106 W, 399 meters altitude) and Area 4 - Irmãos Biazzi Farm (-22.4890 S, -54.7561 W, 405 meters altitude) in the municipalities of Rio Brilhante, Douradina, Dourados and Caarapó, all located in the Brazilian state of Mato Grosso do Sul. Planting of the areas occurred on days 22, 26, 28 and 29 of October 2011, respectively, and each repetition was represented by an area of 450 m^2.

Fertilization was performed as recommended by soil analysis, respecting the estimate of productivity and technological level for each area of each farm. Thus, area 1 was fertilized at pre-planting with 300 $kg \cdot ha^{-1}$ of the formula 02-16-28 (NPK) and at planting with 150 $kg \cdot ha^{-1}$ of 02-23-23 (NPK); area 2 at planting with 160 $Kg.ha^{-1}$ of 11-54-00 and coverage with 80 $kg \cdot h^{-1}$ potassium chloride (KCl); area 3 at planting with 300 $Kg \cdot ha^{-1}$ of 02-18-18; and area 4 at planting with 300 $Kg.ha^{-1}$ 00-23-23.

Sowing was performed in areas free of weeds, using a John Deere model 1109 vacuum mechanized planter, with 9 rows and 45cm row spacing; seed density was between 13 and 14 seeds per linear meter.

Installation and management of the experiments - For weed control an electric backpack sprayer was used with application rate of 120 $L \cdot ha^{-1}$ and AI 110 015 spray nozzles, utilizing the commercial herbicide Roundup Ready® 2.5 $L \cdot ha^{-1}$ (active ingredient glyphosate) in stages V4 to V5 of the culture. For disease management, preventive spraying was performed using an electric backpack sprayer equipped with TT 110 015 spray nozzles and application rate of 120 $L.ha^{-1}$. The products used were: 1st application between stages V6 and V8 with Derosal 0.5 $L \cdot ha^{-1}$ (carbendazim), 2nd application, stage R1 with Derosal 0.5 $L \cdot ha^{-1}$ (carbendazim), 3rd application, stage R1 with Priori Xtra 0.3 $L \cdot ha^{-1}$ (Azoxystrobin + Cyproconazole) + Nimbus 0.5% v/v of the mixture (paraffinic mineral oil, adjuvant), 4th application, in stage R3 using Priori Xtra 0.4 $L \cdot ha^{-1}$ (Azoxystrobin + Cyproconazole) + Nimbus 0.5% v/v of the mixture (paraffinic mineral oil, adjuvant).

For insect management in treatments RR1 and Intacta RR2 PRO® with insecticide application, the control levels adopted in the study were maintained: defoliating caterpillars in the vegetative stage ≥ 30% defoliation or 20 large larvae (≥ 1.5 cm), and in the reproductive stage ≤ 15% defoliation or 20 large larvae (≥ 1.5 cm) per linear meter of soybeans. Sucking insects in the reproductive stage ≥ 1 and stink bug ≥ 0.5 cm per meter (level adopted for seed production) (Embrapa, 2011).

Using control levels recommended by Embrapa (2011) were performed 3.75 insecticide sprayings, on average, in the four sites studied considering the treatment RR1 (with insecticide spraying) for management of defoliating caterpillars, had no spraying in the treatment Intacta RR2 PRO® (with spraying). For management of phytophagous stink bugs were sprayed on average 2.5 and 2.75 times, in the four locations, in soybean Intacta RR2 PRO® and RR1 (both treatments with insecticide), respectively.

The products used in management of Lepidoptera were Lannate 0.6 $L{\cdot}ha^{-1}$ (methomyl) and Belt 0.06 $L{\cdot}ha^{-1}$ (flubendiamide) and for the stinkbugs Connect 0.75 $L{\cdot}ha^{-1}$ (imidacloprid + beta- cyfluthrin) and Engeo Pleno 0.2 $L{\cdot}ha^{-1}$ (lambda-cyhalothrin + thiamethoxam).

Sampling - Evaluations were performed immediately after plant emergence and periodically using intervals between 7 to 10 days, identifying the phenological stages proposed by Fehr and Caviness (1977) and Farias et al. (2007), from plants emergence to physiological maturity of the crop.

This proposed design for classifying the stages of soybean development considers the vegetative and reproductive stages. Vegetative stages are described by the letter V and reproductive stages by the letter R. Excluding VE (emergence) and VC (cotyledon) stage, stages V and R are followed by numerical indices that indicate the actual stage of plant development (e.g., V1, V2, V3, V5, V6… V*n*), where the *n* is the number of nodes with fully developed leaves above the cotyledons, and stage R, from 1 to 8, which describe the flowering period maturation, comprising the plant flowering (R1 and R2), the pod development (R3 and R4), developing seeds (R5 and R6), and physiological maturity (R7 and R8).

The total area used for each plot measured 18 meters wide by 25 meters long (450 m^2), where 10 collection points were used per repetition in the respective culture phenological stages.

For sampling of the complex of insect pests and beneficial arthropods a ground cloth was used, consisting of two wood sticks connected by a white cloth, with a length of 1m and a width of 1.4 m (large enough to cover soybean line adjacent sampled). For sampling, one end of cloth was placed between the soybean rows, adjusted to the base of one row and other extended over the plants of the adjacent row. The plants in one row (area=0.45 m^2) were shaken vigorously so as to cause the insect pests to fall on the cloth (Stürmer et al., 2012).

Statistical analysis - The faunistic analysis was performed according to Silveira Neto et al. (1976), calculating the indices of frequency, constancy, abundance and dominance. Frequency was defined as the percentage of individuals of a species in relation to the total. The constant is defined as the percentage of samples in which a determined species was present. Having obtained this constant, the species were rated as: constant (w) when present in over 50% of weekly samples; accessory (y) when present in 25 to 50% of the samples; and accidental (z) when present in less than 25% of the samples.

Abundance, which is the number of individuals of a particular species found in a given area (unit area or volume), may vary across space and time (Southwood, 1995). To estimate the abundance classes, limits established by the confidence intervals (CI) of 5% and 1% probability were used to determine the following classes: rare (r), the number of individuals of the species below the lower threshold of the CI at 1% probability; dispersed (d), number of individuals among the lower limits of the CI at 1% and 5% probability; common (c), number of individuals within the CI at 5%; abundant (a), number of individuals between the upper limits of the CI at 1% and 5% probability, and very abundant (va), number of individuals greater than the upper limit of the CI at 1% probability.

To define dominance, which is the action exerted by the dominant organisms in a community, we used the method of Laroca and Mielke (1975). The species is considered dominant when presenting relative frequency greater than 1/S x 100, where S is the total number of species found in the sampling period.

In order to measure the ecological dominance, that is the degree to which a taxon is more numerous than its competitors in an ecological community, we used the method of Laroca and Mielke (1975). In this method, species is considered dominant when presenting relative frequency greater than 1/S x 100, where S is the total number of species found in the sampling period.

To measure the diversity of the insect community, the following indices were calculated: Margalef richness index (α), that is the ratio between the number of species and number of individuals of a community; Shannon-Wiener diversity index (H") is defined by: H=- (∑ *pi*) x log *pi* with *pi*=*ni*/N, where *ni* indicates the density of each group and N means the density of all groups. Finally, Equitability index (E) is defined by: E= H/log*S*, where H corresponds to the Shannon index and *S* is the total number of groups in the community that estimates the distribution of individuals in the sample, verifying homogeneity in the number of species occurrence. All these ecological indices were determined using the ANAFAU software (Moraes & Haddad, 2003).

To determine species fluctuation all arthropods collected were used and the number of subjects was plotted within each stage of culture development.

For representative gradients in species composition, we used the ordering method by non-metric multidimensional scaling (NMDS) (Faith et al., 1987), with a Bray-Curtis dissimilarity index (Bray & Curtis, 1957) as a measure of the possible association between sample pairs, considering the relative abundance of each species. The number of dimensions that best represented the ordering was determined by comparing the fit (r^2) obtained in the linear regression between the values of Bray-Curtis distance matrices and the values derived for ordering of one, two, or three dimensions.

Effect of the treatments on species composition (NMDS gradient) was evaluated in a multivariate analysis (MANOVA), considering the Pillai Trace statistic.

3. Results and Discussion

Arthropods associated with RR1 soybeans presented the greatest population and specimen numbers compared to the Intacta RR2 PRO® soybean. However the greatest number of species collected, favoring diversity, was encountered in Intacta RR2 PRO® (Table 1).

The total number of arthropods collected in the four treatments was represented by 5 orders, 13 families, 24 species and 10,438 specimens. The absolute number of individuals collected in RR1 soybeans with and without insecticide treatments and Intacta RR2 PRO® with and without application were 2931, 4921, 1519 and 1067, respectively (Table 1).

Table 1. Species abundance obtained using ground cloth in RR1 and Intacta RR2 PRO® soybeans with and without insecticide application at four locations in the state of Mato Grosso do Sul, Brazil, 2013

Order / Family	Rio Brilhante				Douradina				Dourados				Caarapó				Total
Species	RRc	RRs	IntactaC	IntactaS	RRc	RRs	IntactaC	IntactaS	RRc	RRs	IntactaC	IntactaS	RRc	RRs	IntactaC	IntactaS	
Lepidoptera: Nuctuidae																	
Anticarsia gemmatalis	480	837	110	86	441	883	53	54	193	608	35	39	165	487	34	20	4525
Chrysodeixis includens	325	290	13	24	90	128	4	5	348	291	3	4	292	313	7	7	2144
Heliothis virescens	14	8	1	1	12	17	0	1	7	15	0	0	1	6	0	0	83
Spodoptera frugiperda	2	13	7	3	9	24	16	8	4	11	1	5	3	5	0	6	117
Spodoptera spp.	2	19	107	80	22	122	47	59	12	16	73	74	5	12	11	12	673
Mocis latipes	0	0	0	0	0	1	0	1	0	0	0	0	0	1	1	1	5
Coleptera: Lagriidae																	
Lagria villosa	2	2	2	3	0	0	1	5	0	1	2	1	2	0	2	0	23
Coleoptera: Chrysomelidae																	
Colaspis spp.	11	6	10	29	4	2	7	3	0	0	0	0	7	2	3	7	91
Diabrotica speciosa	8	3	2	3	0	1	1	4	2	3	4	2	21	25	15	18	112
Myochrous armatus	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	3
Coleoptera: Curculionidae																	
Sternechus subsignatus	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	2
Coleoptera: Coccinelidae																	
Cycloneda sanguine	0	0	2	0	0	0	0	10	0	0	1	0	3	1	3	6	26
Coleoptera: Carabidae																	
Lebia concinna	6	3	2	3	0	0	0	6	1	2	1	5	10	7	8	20	74
Hemiptera: Pentatomidae																	
Euschistus heros	44	108	64	138	27	19	21	36	144	317	99	275	48	39	48	114	1541
Edessa meditabunda	3	11	2	10	1	1	1	0	2	5	2	3	0	8	0	23	72
Nezara viridula	0	0	0	0	0	0	0	1	1	0	0	1	1	0	1	0	5
Chinavia sp.	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	2
Dichelops spp.	14	6	0	2	13	7	6	6	3	32	0	3	18	50	26	68	254
Hemiptera: Cicadellidae																	
Dalbulus maidis	0	1	0	0	0	1	0	2	0	0	5	5	5	9	12	11	51
Hemiptera: Nabidae																	
Nabis spp.	5	6	2	3	3	0	1	3	2	2	1	0	2	4	0	1	35
Hemiptera: Geocoridae																	
Geocoris spp.	1	2	1	5	3	2	5	6	1	0	3	4	8	24	8	30	103
Hemiptera: Reduviidae																	
Zellus spp.	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	3
Dermaptera: Forficulidae																	
Doru spp.	0	0	1	2	0	0	0	0	0	0	0	1	1	0	0	1	6
Neuroptera: Chrysopidae																	
Chrysoperla spp.	0	0	4	4	4	0	14	7	2	1	2	2	10	10	14	14	88
Outros																	
Araneae	23	31	45	33	24	22	31	31	4	8	22	24	10	27	35	30	400
TOTAL	940	1347	376	431	653	1230	209	249	726	1312	254	450	612	1032	228	389	10438
Number of species	15	17	18	18	13	14	15	20	15	14	15	17	19	19	16	18	25

RRc= RR1 soyebean with application; RRs=RR1 soybean without application; IntactaC= Intacta RR2 PRO® soybean with application and IntactaS=Intacta RR2 PRO® soybean without insecticide application.

Insects of the order Lepidoptera were the most abundant and presented 7,547 specimens, or slightly more than 70% of the total collected; most of these are considered insect pests and are of great economic importance (Table 1). Next are the orders Hemiptera, Coleoptera, Neuroptera and Dermaptera, which respectively accounted for 2066, 331, 88 and 6 individuals. These results were expected, since these orders include a large number of pests and natural enemies of soybean (Chiaradia et al., 2011; Hoffmann-Campo et al., 2000).

Arthropods of the order Araneae totaled 400 specimens, thus forming an important group of predators, since they feed on insects and generally show high species richness in natural and agricultural areas, sometimes being very abundant (Triplehorn & Johnson, 2005).

In RR1 soybeans with insecticide application 2870 specimens were collected, divided in 19 orders, where two species were classified as super-dominant, 11 as dominant, three super abundant, four plentiful, six rare, three super frequent, four very frequent and eight species common. In the RR1 treatment without insecticide application 4833 specimens were collected, also distributed in 19 species, of which three were super dominant, 12 dominant, two super abundant, two very abundant, four rare, three super frequent, two very frequent and eight species were classified as constant (Table 2).

Table 2. Faunal analysis for cultivation of RR1 soybeans using the ground cloth at four locations in the state of Mato Grosso do Sul, Brazil, 2013

	With insecticide application					**No insecticide application**				
Táxon	N	Dom.	**Abu.**	**Freq.**	**Cons.**	**N**	**Dom.**	**Abu.**	**Freq.**	**Cons.**
Anticarsia gemmatalis	1279	SD	sa	SF	W	2815	SD	sa	SF	W
Chrysodeixis includens	1055	SD	sa	SF	W	1022	SD	sa	SF	W
Heliothis virescens	34	D	ma	MF	W	46	D	c	F	Y
Spodoptera frugiperda	18	D	c	F	W	53	D	c	F	W
Spodoptera spp.	41	D	ma	MF	Y	169	D	ma	MF	W
Mocis latipes	0					2	ND	r	PF	Z
Lagria villosa	4	ND	r	PF	Z	3	ND	r	PF	Z
Colaspis spp.	22	D	c	F	W	10	D	d	PF	Y
Diabrotica speciosa	31	D	ma	MF	W	32	D	c	F	W
Myochrous armatus	0					0				
Sternechus subsignatus	0					0				
Cycloneda sanguinea	3	ND	r	PF	Z	1	ND	r	PF	Z
Lebia concinna	17	D	c	F	Y	12	D	c	F	Y
Euschistus heros	263	SD	sa	SF	W	483	SD	sa	SF	W
Edessa meditabunda	6	D	r	PF	Y	25	D	c	F	Y
Nezara viridula	2	ND	r	PF	Z	0				
Chinavia sp.	0					0				
Dichelops spp.	48	D	ma	MF	W	95	D	ma	MF	W
Dalbulus maidis	5	ND	r	PF	Z	11	D	c	F	Y
Nabis spp.	12	D	c	F	Y	12	D	c	F	W
Geocoris spp.	13	D	c	F	Y	28	D	c	F	Y
Zellus spp.	0					3	ND	r	PF	Z
Doru spp.	1	ND	r	PF	Z	0				
Chrysoperla spp.	16	D	c	F	Y	11	D	c	F	Y
TOTAL	2870					4833				

(N) number of insects captured; Dom=Dominance: Method Laroca & Mielke, 1975 (SD) Super Dominant (D) dominant (ND) not dominant; Abu=Abundance: (sa) Super Abundant, (ma) Very Abundant, (a) Abundant, (r) Rare, (c) Common, (d) Sparse; Freq.=Frequency: (SF) Super Frequent, (MF) Very Common, (F) frequent and (PF) Little Common; Cons.=Consistancy: (W) Constant (Y) Ancillary and (Z) Accidental.

The orders Lepidoptera and Hemiptera stand out by presenting greater frequency of insects sampled and are also the major pest species of this crop. Two of the three super-dominant species are contained in RR1 soybeans, *A. gemmatalis* and *C. includens*, key pests of the culture and targets of Bt technology.

In the Intacta RR2 PRO® soybean with insecticide application, 934 specimens were collected, distributed in 22 species, three species being super dominant, 11 dominant, three super abundant, six very abundant, eight rare, three super frequent, six very frequent and seven species constants, respectively. In the treatment utilizing Intacta RR2 PRO® without insecticide application 1401 specimens were collected, belonging to 23 species, one super dominant, 15 dominant, one super abundant, three very abundant, seven rare, one super frequent, three very frequent and nine constants, respectively (Table 3).

The number of taxa collected and analyzed was slightly higher for the Intacta RR2 PRO® soybean, presenting 22 and 23 species compared with the RR1 soybean with only 19 species. The lower number of specimens collected, mainly from target pests, may have promoted the development and establishment of rare species populations such as *M. latipes*, *S. subsignatus*, *M. armatus* and *Chinavia* spp., non-target insects of Bt technology.

Table 3. Faunal analysis for the soybean cultivar Intacta RR2 PRO® using the ground cloth at four locations in the state of Mato Grosso do Sul, Brazil, 2013

	With insecticide application					No insecticide application				
Táxon	N	Dom.	Abu.	Freq.	Cons.	N	Dom.	Abu.	Freq.	Cons.
Anticarsia gemmatalis	232	SD	sa	SF	W	199	D	ma	MF	W
Chrysodeixis includens	27	D	ma	MF	Y	40	D	c	F	Y
Heliothis virescens	1	ND	r	PF	W	2	ND	r	PF	W
Spodoptera frugiperda	24	D	ma	MF	W	22	D	c	F	W
Spodoptera spp.	238	SD	sa	SF	Y	225	D	ma	MF	W
Mocis latipes	1	ND	r	PF	Z	2	ND	r	PF	Z
Lagria villosa	7	D	d	PF	Y	9	D	d	F	Y
Colaspis spp.	20	D	ma	MF	W	39	D	c	F	W
Diabrotica speciosa	22	D	ma	MF	W	27	D	c	F	W
Myochrous armatus	1	ND	r	PF	Z	2	ND	r	PF	Z
Sternechus subsignatus	1	ND	r	PF	Z	1	ND	r	PF	Z
Cycloneda sanguinea	6	D	d	PF	Y	16	D	c	F	Y
Lebia concinna	11	D	c	F	Y	34	D	c	F	W
Euschistus heros	232	SD	sa	SF	W	563	SD	sa	SF	W
Edessa meditabunda	5	ND	r	PF	Y	36	D	c	F	Y
Nezara viridula	1	ND	r	PF	Z	2	ND	r	PF	Z
Chinavia sp.	0					2	ND	r	PF	Z
Dichelops spp.	32	D	ma	MF	W	79	D	ma	MF	Y
Dalbulus maidis	17	D	c	F	Y	18	D	c	F	Y
Nabis spp.	4	ND	r	PF	Y	7	D	d	PF	Y
Geocoris spp.	17	D	c	F	Y	45	D	c	F	W
Zellus spp.	0					0				
Doru spp.	1	ND	r	PF	Z	4	ND	r	PF	Z
Chrysoperla spp.	34	D	ma	MF	Y	27	D	c	F	Y
Total	934					1401				

(N) number of insects captured; Dom=Dominance: Method Laroca & Mielke, 1975 (SD) Super Dominant (D) dominant (ND) not dominant; Abu=Abundance: (sa) Super Abundant, (ma) Very Abundant, (a) Abundant, (r) Rare, (c) Comm, (d) Sparse; Freq.=Frequency: (SF) Super Frequent, (MF) Very Common, (F) and frequent, (PF) Little Common; Cons.=Constancy: (W) Constant, (Y) Ancillary and (Z) Accidental.

Table 4. Diversity indices of Shannon-Wiener, Margalef and Equitability calculated for genetically modified soybeans, RR1 and Intacta RR2 PRO® at four locations in the state of Mato Grosso do Sul, Brazil, 2013

Treatments	Shannon-Wiener	Margalef	Equitability
RR1 with	1.4016	2.2607	0.4760
RR1 without	1.3366	2.1218	0.4539
Intacta with	2.0061	3.0704	0.6490
Intacta without	2.0474	3.0366	0.6530

RR1 with=RR1 soyebean with application; RR1 without RR1 soybean without application; Intacta without=Intacta RR2 PRO® soybean without application and Intacta with=Intacta RR2 PRO® soybean with insecticide application.

The calculated indices of richness, diversity and evenness (Table 4) show that despite the number of specimens and families sampled, in the area studied there occurred many rare and few abundant species (Tables 2 and 3). Thus, we can infer that the community studied shows similarity in structure compared with other agroecosystems and natural ecosystems. For example, Nunes et al. (2009) found values of up to 1.09 for the Shannon index, 10.75 for species richness and 0.32 for equitability in studies of soil fauna with different management.

The Margalef richness (α), Shannon-Wiener diversity (H") and equitability (E) indices presented similar values for the soybeans RR1 and Intacta RR2 PRO® (with and without insecticide application) (Table 4). These results represent equality in species richness within technologies RR1 and Intacta RR2 PRO®, indicating no difference in species composition when applying insecticides in the fields, where only the technologies differ for with respect to this component.

Values of the Equitability index (Table 4) can be considered low for the four treatments. This demonstrates that some species show a much larger number of individuals than others. This is further confirmed by the difference between the number of abundant and rare species, indicating that the distribution of species in the community is not uniform, since the more similar the representativeness of each species in the community, the higher this index (Peet, 1974).

Population fluctuation of the insect pest complex over time, considering the culture stages and the occurrence of the species, indicated that the total population of insect pests, regardless of treatment, increased from stage V6 to the full vegetative stage. The insect pest population grew significantly in treatments with RR1 soybeans (non-Bt) when compared with treatments with Intacta RR2 PRO® (Bt). However, only in cultivation of the RR1 soybean did population growth persist throughout the development of the culture (Figure 1).

During the soybean vegetative and reproductive stages, the lepidopterous targets of the Bt technology were significantly controlled and remained at levels below those of the RR1 treatments, even with application of insecticides. This is due to the toxic action of the Cry 1AC protein expressed in the genetically modified Bt cultivar, causing very high mortality of neonate caterpillars of the species *A. germmatalis* and *C. includens* (Macrae et al., 2005; Mcpherson & Macrae, 2009).

Greater fluctuations in arthropod populations were observed in the treatment using RR1 soybeans with insecticide application, caused by the reduction of insects due to use of chemical control among the reproductive stages R2 and R6 (Figure 1).

For non-target insect pests of Bt technology, especially the stink bug complex, in treatments without insecticide application an increase in population was observed in the reproductive stages R6 to R7.3, corroborating with. Salvadori et al., (2007) and Correa-Ferreira and Panizzi (1999). This is due to overlapping of generations which, when infesting the culture in the initial reproductive states R1 to R3, culminates in high populations in the later stages R6 and R7.3.

The population of insects in RR1 soybeans with insecticide application showed two outbreaks, both in the reproductive phase of the crop, the first between stages R1 and R3 and the second between R5.4 and R7.1. Decline in the insect population between outbreaks in R3 to R5.4 was observed occurred due to the application of insecticide and control of defoliating caterpillars (Figure 1A).

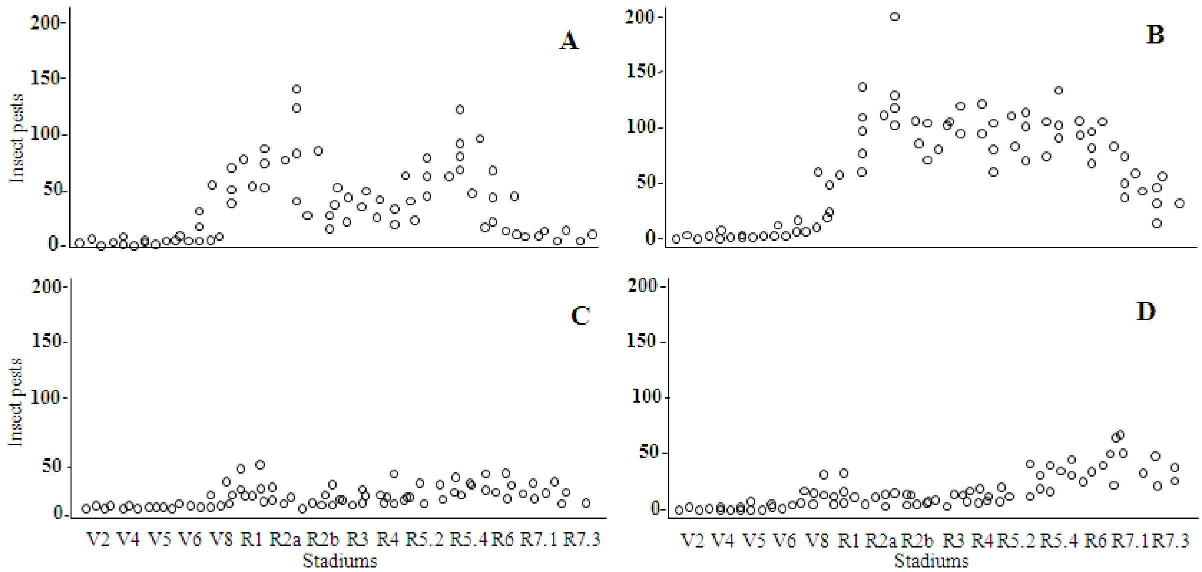

Figure 1. Population fluctuation of insect pests in the vegetative and reproductive stages of soybeans in the treatments, (A) RR1 with insecticide application, (B) RR1 without insecticide application, (C) Intacta RR2 PRO® with insecticide application, (D) Intacta RR2 PRO® without insecticide application at four locations in the state of Mato Grosso do Sul, Brazil, 2013

For the RR1 soybean without insecticide application high population levels were reached and maintained during the reproductive stage of the culture (stages R1 and R6) (Figure 1B).

Intacta RR2 PRO® (Bt) with and without spraying presented population fluctuation of pests and similar fluctuation during vegetative and reproductive development, with total populations much lower than those observed in the two treatments using the RR1 technology (Figure 1C and D).

For Intacta RR2 PRO® soybeans without insecticide application an increase in the number of insect pests was observed in the final stages of the culture R6 and R7.1, probably due to non-use of insecticides for managing of the stinkbug complex, pests not targeted by the Bt technology (Figure 1D).

Populations of beneficial arthropods (natural enemies), represented by the orders Hemiptera, Dermaptera, Coleoptera, Neuroptera and Araneae occurred in all treatments, with the largest populations obtained in the later stages of culture (Figure 2), i.e., the presence of predators progressed during the vegetative and reproductive stages.

In treatments using Intacta RR2 PRO® with and without insecticide application, the population of natural enemies was relatively greater when compared with the treatments using RR1, especially with the application of insecticides (Table 1, Figure 2C and D). No use of insecticides for managing caterpillars may have favored the establishment and abundance of insect predators. The use of selective insecticides therefore allows for obtaining a greater number of beneficial insects in agricultural systems (Godoy et al., 2010; Carmo et al., 2009).

However, changes in arthropod populations may be functions of plant density, cultures involved, adaptability of the predator to the culture, density of prey specific to the predator, the availability of other food sources such as pollen, soil moisture and crop microclimate (French et al., 1998; Clark et al., 1997; Cárcamo & Spence, 1994). Thus, the need for studies that consider the complexity of the plant-insect interaction in genetically modified plants is evident to permit greater understanding of population dynamics. Several factors may explain the greater abundance of predators in the treatment using Intacta RR2 PRO® without insecticide spraying, differentiated from the RR1 treatment by: the absence of defoliation, greater shading and hence a different microclimate, milder humidity and temperatures, conditions that favor an increased number of natural enemies (Letourneau, 1987).

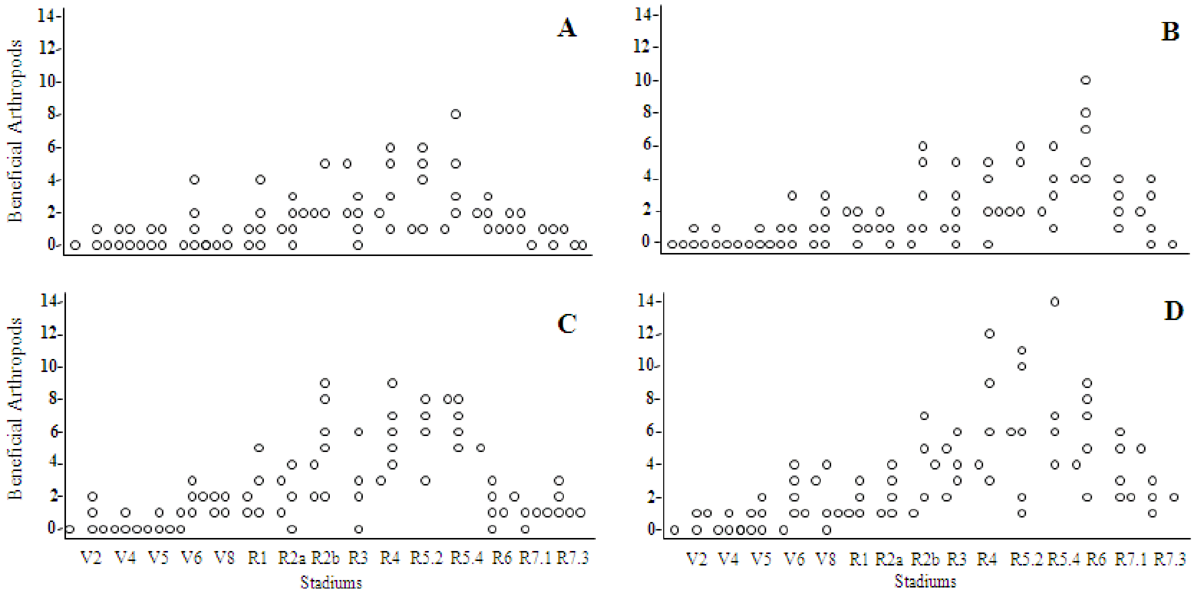

Figure 2. Population fluctuation of beneficial arthropods in the vegetative and reproductive stages of soybean treatments, (A) RR1 with insecticide application, (B) RR1 without insecticide application, (C) Intacta RR2 PRO® with insecticide application, (D) Intacta RR2 PRO® without insecticide application at four locations in the state of Mato Grosso do Sul, Brazil, 2013

The result of the score obtained by ranking the data (Figure 3), where each point in the figure is represented by one sample, shows the ordering of the points represents similarity similarity between the samples and consequently the species composition, i.e., the most common species are closest.

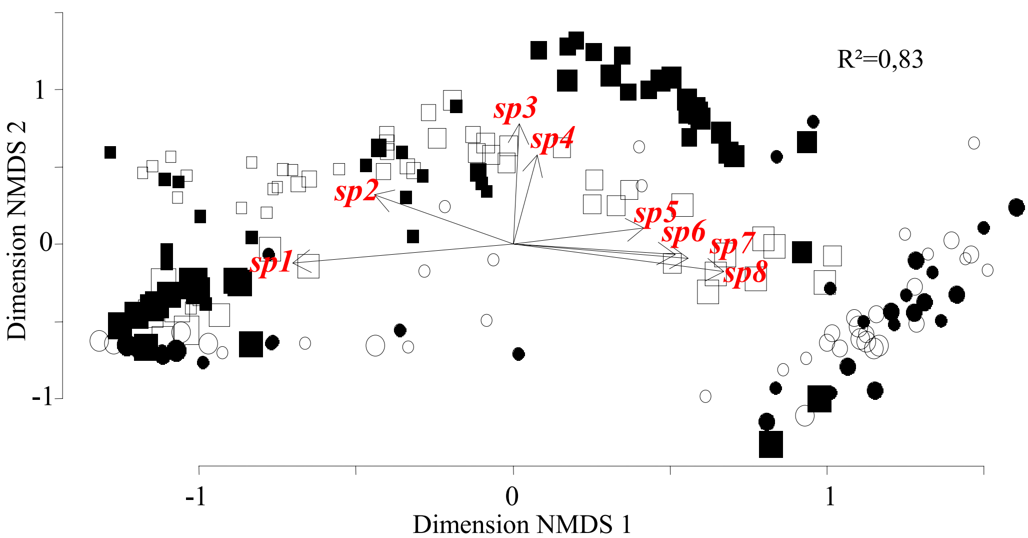

Figure 3. Ranking by non-metric multidimensional scaling (NMDS) in two dimensions (stress=0.145) of the stages □ RR1 without application, ■ RR1 with application, ○ Intacta RR2 PRO® without application, ● Intacta RR2 PRO® with application. Size of the filled or hollow circles or squares represents the culture stages, where the larger sizes indicate more advanced stage of culture for this sample. The vectors indicate how each species contributed to the separation of treatments in terms of ordering (r> 0.4): *sp1* - *Anticarsia gemmatalis* (<1.5 cm); *sp2* - *A. gemmatalis* (≥ 1.5 cm); *sp3* - *Chrysodeixis includens* (<1.5 cm); *sp4* - *C. includens* (≥ 1.5 cm); *sp5* - *Spodoptera* spp. (<1.5 cm); *sp6* - *Spodoptera* spp. (≥ 1.5 cm); *sp7* - *Euschistus heros* (nymph); *sp8* - *E. heros* (adult), at four locations in the state of Mato Grosso do Sul, Brazil, 2013

Composition of the insect pests studied showed positive interaction for the factors of stage (Pillai-Trace=1.41, F=37.86, df=22 and 350, p <0.001), treatment (Pillai-Trace=1.41; F=37.86; dof=22 and 350; p<0,001), application (Pillai-Trace=0.86; F=8.16; dof=2 and 174; p<0.001) and treatment versus application (Pillai-Trace=0.16; F=16.85; dof=2 and 174; p<0.001) (Figure 3), there was no significant interaction of these factors when considering beneficial arthropods, due to the low number of individuals collected.

The statistical difference observed for phenological interaction among stages, population distribution and arrangement of insects is explained by overlapping generations and species of arthropods occurring in the culture throughout plant development. The RR1 and Intacta RR2 PRO® soybeans technologies significantly affected pest composition when taking into account the target pests of Bt technology. However use of the insecticide applied whenever the action level was exceeded caused a significant reduction in the number of specimens when compared with the treatments without insecticides.

The species that contributed most to differentiate samples, in ascending order, were *Dichelops* spp. (nymph), *Euschistus heros* (nymph) sp7, *E. heros* (adult) sp8, *Mocis latipes*, *Lagria villosa*, *Diabrotica speciosa*, *Spodoptera frugiperda* (<1.5 cm) sp5, *S. frugiperda* (≥ 1.5 cm) sp6, *Nezara viridula* (adult), *Edessa meditabunda* (nymph), *Colaspis* spp., *Myochrous armatus*, *Anticarsia gemmatalis* (<1.5 cm) sp1, *Dichelops* spp. (adult), *Spodoptera* spp. (≥ 1.5 cm) sp5, *Dalbulus maidis*, *A. gemmatalis* (≥ 1.5 cm) sp2, *Heliothis virescens* (<1.5 cm), *H. virescens* (≥ 1.5 cm), *Chrysodeixis includens* (≥ 1.5 cm) sp3 and *C. includens* (<1.5 cm) sp4.

Some species contributed significantly to the difference between treatments evaluated. Arrows indicate the importance of each species within the arrangement (Figure 3), where the species *E. heros* and *Spodoptera* spp. contributed most to the ordering of the arrangement obtained because these were common in Intacta RR2 PRO® soybeans with and without insecticide application. This is justified because these species are not targeted insect pests of Bt technology. As for the species *A. gemmatalis* and *C. includens*, susceptible to the Cry 1Ac protein (Bernardi et al., 2012), the samples located at the left and top (Figure 3) are similar for the RR1 soybean with or without application, independent of the culture stage.

4. Conclusions

The Intacta RR2 PRO® soybean provides greater diversity of species compared to RR1 soybeans.

The arthropod population suffered a gradual increase during the development of soybean, where the greatest abundance occurs between stages V8 and R6.

The beneficial arthropods in Intacta RR2 PRO® soybeans are more abundant compared to RR1 soybean.

The main target insect pests and non-targets of Bt technology significantly contribute to the differentiation between pest populations in RR1 and Intacta RR2 PRO® soybeans, regardless of the use of chemical control.

References

Bennett, J. (1994). DNA-based techniques for control of rice insect and disease: Transformation, gene tagging and DNA fingerprinting. In *Rice pest science and management*. (1 ed., pp. 147-172). Philippines: International Rice Research Institute.

Bernardi, O., Malvestiti, G., Dourado, P. M., Oliveira, W. S., Martinelli, S., Berger, G. U., ... Omoto, C. (2012). Assessment of the high-dose concept and level of control provided by MON 87701 x MON 89788 soybean against *Anticarsia gemmatalis* and *Pseudoplusia includens* (Lepidoptera: Noctuidae) in Brazil. *Pest Management Science, 68*(1), 1083-1091. http://dx.doi.org/10.1002/ps.3271

Bray, J. R., & Curtis, J. T. (1957). An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs, 27*(1), 325-349. http://dx.doi.org/10.2307/1942268

Cárcamo, H. A., & Spence, J. R. (1994). Crop type effects on the activity and distribution of ground beetle (Coleoptera:Carabidae). *Environmental Entomology, 23*(3), 684-692,

Carmo, E. L., Carvalho, G. A., Carvalho, B. F., & Lasmar, O. (2009). Seletividade de diferentes agrotóxicos usados na cultura da soja ao parasitoide de ovos *Telenomus remus. Ciência Rural, 39*(8), 2293-2300. http://dx.doi.org/10.1590/S0103-84782009005000188

Chiaradia, L. A., Rebonatto, A., Smaniotto, M. A., Davila, M. R. F., & Nesi, C. N. (2011). Artropodofauna associada às lavouras de soja. *Revista de Ciências Agroveterinárias, 10*(1), 29-36.

Clark, M. S., Gage, S. H., & Spence, J. R. (1997). Habitats and management associated with common ground beetles (Coleoptera: Carabidae) in a Michigan agricultural landscape. *Environmental Entomology, 26*(1), 519-527.

Conab. (2012). *Acompanhamento de safra brasileira: Grãos, Terceiro levantamento, dezembro 2012/ Companhia Nacional de Abastecimento*. (1 ed., p. 30). Brasília: Conab.

Corrêa-Ferreira, B. S. & Panizzi, A. R. (1999). *Percevejos da soja e seu manejo* (1 ed., p. 45). Londrina: EMBRAPA-CNPSo.

Ctnbio. *Comissão Técnica Nacional De Biossegurança*. (2010). Liberação comercial de soja MON 87701 x MON 89788, que confere resistência a insetos e tolerância a herbicida, bem como todas as progênies dela provinientes, concluiu pelo DEFERIMENTO, nos termos deste parecer técnico n. 2.542/2010. Brasília.

Embrapa. (2011). *Tecnologias de Produção de Soja - Região Central do Brasil 2012 e 2013*. (1 ed., p. 261) Londrina: Embrapa soja.

Faith, D. P., Minchin, P. R., & Belbin, L. (1987). Compositional dissimilarity as a robust measure of ecological distance. *Vegetatio, 69*(1), 57-68. http://dx.doi.org/10.1007/BF00038687

Farias, J. R. B., Nepomuceno, A. L., & Neumaier, N. (2007). *Ecofisiologia da soja*. (1 ed., p. 9) Londrina: Embrapa soja (Cicular Técnica 48).

Fehr, W. R., & Caviness, C. E. (1972). *Stages of soybean development*. (1 ed., p. 12). Ames: Iowa State University.

French, B. W., Elliott, N. C., & Berberet, R. C. (1998). Reverting conservation reserve program lands to wheat and livestock production: effects on ground beetles (Coleoptera: Carabidae) assemblages. *Environmental Entomology, 27*(1), 1323-1335.

Garcia, F. R. M., Nardi, N., Costa, M. K. M., & Brescovit, A. D. (2004). Ocorrência de artrópodes em lavoura de milho (*Zea mays*) no município de Arvoredo, SC. *Bioikos, 18*(1), 21-28.

Gilbert, N., Gutiérrez , A. P., Frazer, B. D., & Jones, R. E. (1976). *Ecological relationships*. (1 ed., p. 256). San Francisco: W. H. Freeman & Co.

Godoy, M. S., Carvalho, G. A., Carvalho, B. F., & Lasmar, O. (2010). Seletividade fisiológica de inseticidas em duas espécies de crisopídeos. *Pesquisa Agropecuária Brasileira, 45*(11), 1253-1258. http://dx.doi.org/10.1590/S0100-204X2010001100004

Hoffmann-Campo, C. B., Moscardi, F., Corrêa-Ferreira, B. S.. Oliveira, L. J., Sosa-Gomez, D. R., Panizzi, A. R., ... Oliveira, E. B. (2000). *Pragas da soja no Brasil e seu manejo integrado*. (1 ed., p. 70). Londrina: Embrapa Soja.

Laroca, S., & Mielke, U. H. H. (1975). Ensaio sobre ecologia de comunidade em Sphingidae na Serra do Mar, Paraná, Brasil (Lepidoptera). *Revista Brasileira de Biologia, 35*(1), 1-19.

Letourneau, D. K. (1987). The enemies hypothesis: tritrophic interactions and vegetational diversity in tropical agroecosystems. *Ecology, 68*(1), 1616- 1622. http://dx.doi.org/10.2307/1939853

Macrae, T. C., Baur, M. E., Boethel. D. J., Fitzpatrick, B. J., Gao, A., Gamundi, J. C., … Viegas, A. (2005). Laboratory and Field Evaluations of Transgenic Soybean Exhibiting High-Dose Expression of a Synthetic *Bacillus thuringiensis* cry1A Gene for Control of Lepidoptera. *Journal of Economic Entomology, 98*(2), 577-587. http://dx.doi.org/10.1603/0022-0493-98.2.577

Mcpherson, R. M., & Macrae T. C. (2009). Evaluation of Transgenic Soybean Exhibiting High Expression of a Synthetic *Bacillus thuringiensis* cry1A Transgene for Suppressing Lepidopteran Population Densities and Crop Injury. *Journal of Economic Entomology, 102*(4), 1640-1648. http://dx.doi.org/10.1603/029.102.0431

Milane, P., Harterreiten-Souza, É., Mencarini, L., Laumann, R., Carneiro, R., Sujii, E., & Pires, C. (2009). Estruturação da Comunidade de Insetos em Áreas de Produção de Hortaliças em Diferentes Fases de Transição Agroecológica na Região do Distrito Federal. *Revista Brasileira de Agroecologia, 4*(2), 1664-1667.

Monsanto. (2012). *Intacta RR2 PRO*. Retrieved from http://www.intactarr2pro.com.br/Default.aspx

Moraes, R. C. B., & Haddad, M. L. (2003). Software para análise faunística-ANAFAU. In: Simpósio de Controle Biológico, 8, 2003, Resumos. *Sociedade Entomológica do Brasil, 1*(1), 195.

Nunes, L. A. P. L., Araújo Filho, J. A., & Menezes, R. I. Q. (2009). Diversidade da fauna edáfica em solos submetidos a diferentes sistemas de manejo no semi-árido nordestino. *Scientia Agraria, 10*(1), 43-49.

Odum, E. P. (1988). *Ecologia* (1 ed., p. 434). Rio de Janeiro: Guanabara Koogan.

Peet, R. K. (1974). The measurement of species diversity. *Annual Review of Ecology and Systematics, 5*(1), 285-307. http://dx.doi.org/10.1146/annurev.es.05.110174.001441

Salvadori, J. R., Pereira, P. R. V. S., & Corrêa-Ferreira, B. S. (2007). *Pragas ocasionais em lavouras de soja no Rio Grande do Sul* (1 ed., p. 34). Passo Fundo: Embrapa Trigo.

Sharma, H. C., Sharma, K. K., Seetharama, N., & Ortiz, R. (2000). Prospects for using transgenic resistance to insects in crop improvement. *Electronic Journal of Biotechnology, 3*(2), 1-26. http://dx.doi.org/10.2225/vol3-issue2-fulltext-3

Silveira Neto, S., Nakano, O., Barbin, D., & Villa Nova, N. A. (1976). *Manual de Ecologia dos Insetos* (1 ed., p. 419). São Paulo: Agronômica Ceres.

Southwood, T. R. E. (1995). *Ecological methods: with particular reference to the study of insect populations*. (1 ed., p. 524). London: Chapman e Hall.

Stürmer, G. R., Cargnelutti Filho, A., Stefanelo, L. S., & Guedes, J. V. C. (2012). Eficiência de métodos de amostragem de lagartas e de percevejos na cultura de soja. *Ciência Rural, 42*(12). http://dx.doi.org/10.1590/S0103-84782012005000145

Triplehorn, C. A., & Johnson, N. F. (2005). *Borror and DeLong's*. Introduction to the Study of Insects (1 ed., p. 864). Belmont: Thomson Brooks/ Cole.

Williams, J. W., Summers, R. L., & Webb III, T. (1998). Applying plant functional types to construct biome maps from eastern North American pollen data: comparisons with model results. *Quaternary Science Review, 17*(1), 607-628. http://dx.doi.org/10.1016/S0277-3791(98)00014-6