G.J.B.A.H.S., Vol.3(4):11-18 *(October-December, 2014)* ISSN: 2319 – 5584

GLOBAL JOURNAL OF BIOLOGY, AGRICULTURE & HEALTH SCIENCES
(*Published By: Global Institute for Research & Education*)

www.gifre.org

Intacta RR2 PRO® (MON87701 x MON89788) for Management of the Main Target and Non-Target Insects in Soybeans

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Abstract

The soybean agroecosystem consists of numerous insect pest species that when unmanaged can cause losses and reduction in productivity. The aim of this study was to evaluate the effect of the event MON 87701 x MON89788 on management of the target caterpillars *Anticarsia gemmatalis*, *Chrysodeixis includens* and the complex of phytophagous stinkbugs and predatory by the technology Intacta RR2 PRO® (Soy Bt). The experiment was conducted in the agricultural year of 2011/2012, with four treatments in the Brazilian state of Mato Grosso do Sul. *A. gemmatalis* and *C. includens*, both target of Bt technology, were effectively controlled in the field by Intacta RR2 PRO® soybeans expressing the toxin Cry 1AC, reducing number of insecticide applications needed for control. The use of Bt soybeans resulted in no statistical differences in the number and incidence of non-target pests, including the stink bug complex, and significantly favored the population of beneficial arthropods.

Keywords: *Glycine max*, Bt soybean, transgenic plants, induced resistance

1. Introduction

Soybean Integrated Pest Management (IPM-Soy) was established in Brazil in 1970 and has been constantly improved. MIP-Soy consists of decision making regarding the control of insect pests based on a set of information related to the bioecological integration of insects with the agricultural system. Therefore, the success of IPM in practice requires culture monitoring, based on the correct identification of pests and their natural enemies and knowledge of the developmental stage of the plant (Embrapa 2008).

Factors such as the time required for a measure of control to be efficient, accurate sampling, climate and decision-making for control contribute to the effectiveness of the tactics used to control and prevent reaching the economic injury level (EIL). Safety is one of the prerequisites for productivity, and it is fundamental to apply the Action Level (AL) in IPM-Soy, usually slightly below the EIL (Embrapa 2011).

In soybeans, the action level recommended for defoliation may vary according to the production region (Bueno et al 2011). In Brazil, the action level adopted is 30% for defoliation during the vegetative period, or 15% if the culture is in the stage of reproductive development (Embrapa 2011). The greater tolerance of the soybean plant to defoliation in the vegetative stage is because during this period, defoliation usually has little effect on production, mainly due to the large capacity of the plant to grow new leaves (Batistela 2010).

The search for specific technologies with low persistence in the environment, and efforts for Integrated Pest Management (IPM) based on plant resistance to insects is part of a long-term sustainable strategy. With the advent of genetic transformation based on recombinant DNA techniques, it has become possible to insert foreign genes into plant genomes, thus conferring insect resistance (Bennett 1994). Genes from bacteria like *Bacillus thuringiensis* (Bt) and *Bacillus sphaericus* have been the main group of organisms used to confer plant resistance to insects on a commercial scale (Sharma *et al* 2000, Bobrowski *et al* 2003).

The expression of Bt proteins in cultivated plants such as corn, rice, cotton, potato, tomato, tobacco, soybeans and canola has proven to be a highly effective method for the control of various insect pest species (Parrott *et al* 1994, Armstrong *et al* 1995, Stewart *et al* 1996). This resulted in enormous economic and environmental benefits such as increased productivity and a significant reduction in the use of chemical insecticides (Perlak *et al* 2001, Shelton *et al* 2002).

The effect of Bt toxins on natural enemies of insect pests such as parasitoids or predators was studied in the laboratory and field, indicating little or no effect on these organisms (Schuler *et al* 1999, Wraight *et al* 2000). Natural enemies are extremely important, since secondary pests can become a problem if the population of beneficial insects is reduced by the use of broad-spectrum chemical insecticides (Sharma *et al* 2000).

The first genetically modified soybean was approved in Brazil in 1998, called Roundup Ready® soybeans, containing the event GTS 40-30-2, resulting in a transgenic plant with the *cp4-epsps* gene (35S promoter, region of transit peptide to the chloroplast, coding region for the enzyme enolpyruvate 5-shikimate-3-phosphate synthase – EPSPS, 3' region of the nopaline synthase gene) which provides tolerance to the referred herbicide (CTNBio 1998, Bohm & Rombaldi 2010).

The event MON 87701 has the Cry1Ac gene, derived from *Bacillus thuringiensis* and event MON 89788 has the cp4 epsps gene, derived from *Agrobacterium* sp. These are separate events, expressed in different cellular organelles. The

MON 87701 soybean was produced by the *Agrobacterium tumefaciens* mediated transformation method using the plasmid PV-GMIR9, which is a binary vector. The T-DNA I contains the expression cassette of the cry1Ac gene and T-DNA II contains the expression cassette of the cp4 epsps gene, used only as a selection marker (CTNBio 2010).

A technology commercially referred to as Intacta RR2 PRO® containing the events MON89788, which confers tolerance to the herbicide glyphosate, and MON 87701, which confers resistance to the major defoliating caterpillars of the culture, such as the caterpillar (*Anticarsia gemmatalis*), soybean looper (*Chrysodeixis includens* and *Rachiplusia nu*) and bud borer (*Crocidosema aporema*), target pests of Bt technology, reducing insecticide application (Bernardi *et al* 2012).

Thus, considering the importance of this culture and the impact that the event Bt can have on target and non-target insect populations, as well as changes in pest management strategy, it is essential to conduct studies aimed at better understanding this technology so it can be used as an additional tool in IPM.

Therefore, the aim of this study was to evaluate the effect of the Intacta RR2 PRO® soybean (event MON 87701 x MON89788), which confers insect resistance and herbicide tolerance, compared to RR1 soybeans tolerant only to herbicide, on management of the major target and not target insects of Bt technology.

2. Materials and Methods

Location of the experimental installation - 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, 370m), Area 2 - Boa Sorte Farm (-22.0175 S, -54.5358 W, 304m), area 3 Rincão Porã Farm (-22.2376 S, -54.7106 W, 399m) and Area 4 - Irmãos Biazzi Farm (-22.4890 S, -54.7561 W, 405m) 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 450m^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 300kg.ha^{-1} of the formula 02-16-28 (NPK) and at planting with 150Kg.ha^{-1} of 02-23-23 (NPK); area 2 at planting with 160kg.ha^{-1} of 11-54-00 and coverage with 80Kg.h^{-1} potassium chloride (KCl); area 3 at planting with 300Kg.ha^{-1} of 02-18-18; and area 4 at planting with 300Kg.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.ha-1 and AI 110 015 spray nozzles, utilizing the commercial herbicide Roundup Ready® 2.5 L.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.ha^{-1} (carbendazim), 2nd application, stage R1 with Derosal 0.5 L.ha^{-1} (carbendazim), 3rd application, stage R1with Priori Xtra 0.3 L.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.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).

Chemical control adopted in repetitions cultivated in Rio Brilhante and Douradina consisted of four interventions for the control of defoliators in stages R1, R2a, R3 and 5.4, in Dourados three sprayings were performed at stages R3, R5.4 and R6, and in Caarapó four sprays in stages R1, R3, R5.2 and R5.4. In only one of the areas were more than 20 larvae per meter in the row obtained, the concentration recommended to start control. Thus, applications considered the recommended control levels.

For management of stinkbugs in repetitions in Rio Brilhante and Caarapó, 3 insecticide applications were performed in stages R5.2, R4 and R6 in treatments with both RR1 and Intacta RR2 PRO® with insecticide application in stages R5.2, R.4 and R7.1. For the repetition located in Douradina spraying was only performed in stage R5.4 for both treatments with application. In Dourados there was the greatest number of insecticide applications, totaling four in RR1 soybeans in stages R3, R5.4, R6 and R7.1 and in Intacta RR2 PRO® soybeans three applications in the same stages as the RR1 soybeans except for R3.

The products used in management of Lepidoptera were Lannate 0.6 L.ha^{-1} (methomyl) and Belt 0.06 L.ha^{-1} (flubendiamide) and for the stinkbugs Connect 0.75 L.ha^{-1} (imidacloprid + beta- cyfluthrin) and Engeo Pleno 0.2 L.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 & Caviness (1977) until physiological maturity of the crop. The total area used for each plot measured 18 meters wide by 25 meters long (450m^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 1.4m (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 = 0.45m^2) were shaken vigorously so as to cause the insect pests to fall on the cloth (Stürmer *et al* 2012).

For assessment of defoliation a visual scale was adapted (Beuerlein *et al* 2005), estimating the level of defoliation in the treatments based on leaf consumption caused by defoliators.

Statistical analysis - The number of species and individuals from all collection points sampled were used; and the average number per culture stage was used for fabrication of graphs representing population fluctuation during plant development. Data was subjected to analysis of variance and means were compared by the Tukey test at 5%.

3. Results and Discussion

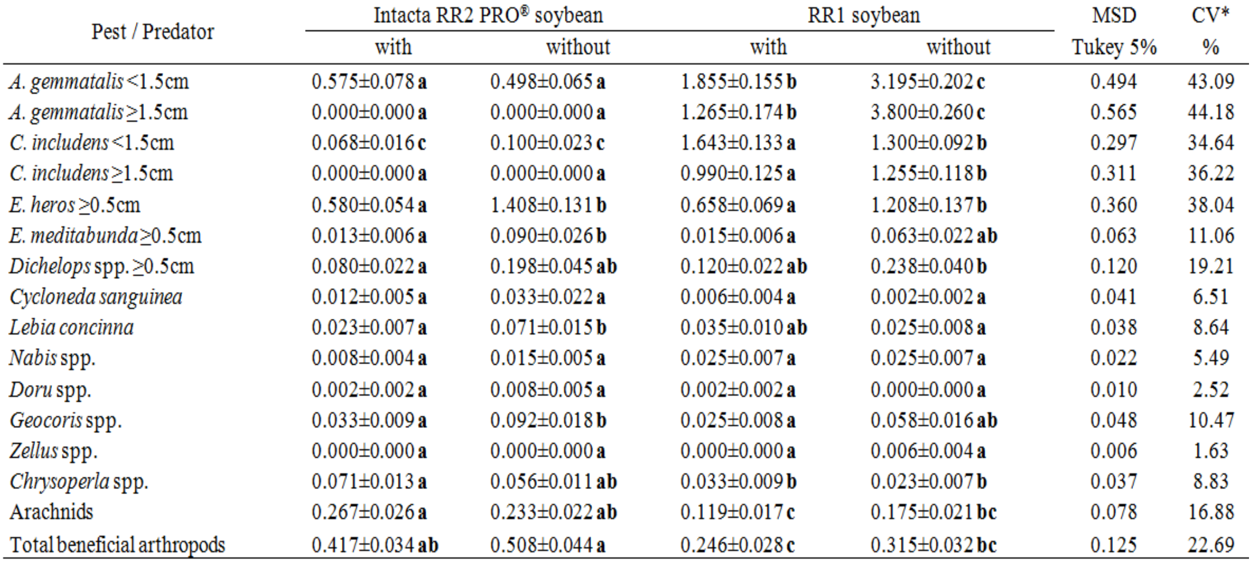
In the RR1 soybean treatments, with and without insecticide application, mean populations of 1.855 and 3.195 were found for small species of *A. gemmatalis* (<1.5 cm) and 1.265 and 3.8 for large larvae (≥1.5 cm), respectively, differing among themselves and also when compared to Intacta RR2 PRO® soybean treatments with and without insecticide application, presenting averages less than one small caterpillar and no large caterpillars (Table 1).

The species *C. includens* was less abundant when compared to the soybean caterpillar, where the average numbers of individuals in treatments using RR1 soybeans with and without application were 1.643 and 1.3 small caterpillars and 0.99 and 1.255 large caterpillars, respectively. For the treatments using Intacta RR2 PRO® soybeans with and without insecticide application, the average number of insects was near zero for small larvae and zero for large larvae, therefore it is possible to highlight the proven efficiency of the event for these target species as already reported in tests performed in the laboratory (Macrae *et al* 2005, Mcpherson & Macrae 2009) (Table 1).

For the complex of phytophagous pentatomids, greatest abundance was reported for the species *E. heros*, with no difference between treatments using RR1 and Intacta RR2 PRO® soybeans with insecticide application; the same was observed when comparing RR1 and Intacta RR2 PRO® soybeans without spraying. As for the *E. meditambunda*, the Intacta RR2 PRO® soybean with application presented smaller populations compared to the Intacta RR2 PRO® soybean without and RR1 soybean with chemical control. For the species *Dichelops* spp., a higher average population resulted from cultivation of RR1 soybeans without chemical application, differing from the treatment with Bt soybeans to which insecticide was applied (Table 1).

The population of beneficial arthropods differed significantly in only three species sampled, *Lebia concinna* occurred with greater abundance in the soybean Intacta RR2 PRO® without insecticide spraying when compared to Intacta RR2 PRO® and RR1 soybeans with and without application of the insecticide. As for *Geocoris* spp., a higher insect population was observed in the soybean Intacta RR2 PRO® without spraying, differing from the soybean Intacta RR2 PRO® with application and RR1 without application. Intacta RR2 PRO® with application resulted in two times more individuals of the species *Chrysoperla* spp. compared with RR1 soybeans with application, and three times more beneficial insects when compared to the RR1 soybean without insecticide application (Table 1).

Table 1. Average number of insect pests and beneficial arthropods (± standard error of mean) sampled by the ground cloth in soybean; pests n = 10, predators n = 12.



Pest / Predator	Intacta RR2 PRO® soybean		RR1 soybean		MSD	CV*
	with	without	with	without	Tukey 5%	%
A. gemmatalis <1.5cm	0.575±0.078 **a**	0.498±0.065 **a**	1.855±0.155 **b**	3.195±0.202 **c**	0.494	43.09
A. gemmatalis ≥1.5cm	0.000±0.000 **a**	0.000±0.000 **a**	1.265±0.174 **b**	3.800±0.260 **c**	0.565	44.18
C. includens <1.5cm	0.068±0.016 **c**	0.100±0.023 **c**	1.643±0.133 **a**	1.300±0.092 **b**	0.297	34.64
C. includens ≥1.5cm	0.000±0.000 **a**	0.000±0.000 **a**	0.990±0.125 **a**	1.255±0.118 **b**	0.311	36.22
E. heros ≥0.5cm	0.580±0.054 **a**	1.408±0.131 **b**	0.658±0.069 **a**	1.208±0.137 **b**	0.360	38.04
E. meditabunda ≥0.5cm	0.013±0.006 **a**	0.090±0.026 **b**	0.015±0.006 **a**	0.063±0.022 **ab**	0.063	11.06
Dichelops spp. ≥0.5cm	0.080±0.022 **a**	0.198±0.045 **ab**	0.120±0.022 **ab**	0.238±0.040 **b**	0.120	19.21
Cycloneda sanguinea	0.012±0.005 **a**	0.033±0.022 **a**	0.006±0.004 **a**	0.002±0.002 **a**	0.041	6.51
Lebia concinna	0.023±0.007 **a**	0.071±0.015 **b**	0.035±0.010 **ab**	0.025±0.008 **a**	0.038	8.64
Nabis spp.	0.008±0.004 **a**	0.015±0.005 **a**	0.025±0.007 **a**	0.025±0.007 **a**	0.022	5.49
Doru spp.	0.002±0.002 **a**	0.008±0.005 **a**	0.002±0.002 **a**	0.000±0.000 **a**	0.010	2.52
Geocoris spp.	0.033±0.009 **a**	0.092±0.018 **b**	0.025±0.008 **a**	0.058±0.016 **ab**	0.048	10.47
Zellus spp.	0.000±0.000 **a**	0.000±0.000 **a**	0.000±0.000 **a**	0.006±0.004 **a**	0.006	1.63
Chrysoperla spp.	0.071±0.013 **a**	0.056±0.011 **ab**	0.033±0.009 **b**	0.023±0.007 **b**	0.037	8.83
Arachnids	0.267±0.026 **a**	0.233±0.022 **ab**	0.119±0.017 **c**	0.175±0.021 **bc**	0.078	16.88
Total beneficial arthropods	0.417±0.034 **ab**	0.508±0.044 **a**	0.246±0.028 **c**	0.315±0.032 **bc**	0.125	22.69

Stage utilized for caterpillars n = 10 (V6, V8, R1, R2a, R2b, R3, R4, R5.2, R5.4 and R6);
Stage utilized for stink bugs n = 10 (R1, R2a, R2b, R3, R4, R5.2, R5.4, R6, R7.1 and R7.3);
Stage utilized for predators n = 12 (V6, V8, R1, R2a, R2b, R3, R4, R5.2, R5.4, R6, R7.1 and R7.3);
Averages in the same row followed by the same letter do not differ by the Tukey test at 5%.
MSD = Minimum Significant Difference.
Average containing actual data
CV * = Data transformed in $\sqrt{x+1}$

Arachnids were also favored by cultivation of the soybean Intacta RR2 PRO®, even with insecticide application, and differed significantly from the RR1 soybean with and without spraying (Table 1). In general, beneficial arthropods were more abundant in Intacta RR2 PRO® without application and statistically differed from the treatment using RR1 soybeans without spraying. Similar results were observed in China when using Bt-cotton, resulting in a reduction of the use of chemical insecticides and a 24% increase in the population of natural enemies when compared with cotton fields planted with the genetically unmodified species and subjected to conventional chemical control (Xia *et al* 1999).

Small soybean caterpillars *A. gemmatalis* showed widespread occurrence in all cultivated areas, with some variations in the population during culture development. In all locations, the population outbreak of *A. gemmatalis* occurred between the end of the vegetative stage (V8) and the early stages of the reproductive phase (R2), as reported in studies assessing the occurrence and behavior of the soybean caterpillar (Didonet *et al* 2003, Corrêa - Ferreira *et al* 2010). For RR1 soybeans with and without application of insecticides the average insect populations in the V6 stage were 1.45 and 1.0, in stage V8 5.3 and 5.27, and in R1 were 6.5 and 10.0, respectively (Figure 1A). In the treatments with Bt soybeans without insecticide application insect population reductions were observed in stage V6, 0.5 and 0.28, stage V8, 3.15 and 2.43, and stage R1, 1.02 and 1.07, respectively.

The population fluctuation of large larvae accompanied the trend observed for small larvae. However, population outbreaks were concentrated in the initial reproductive stages R1 and R2. The highest numbers of individuals observed were in R1, 2.5 and 2.25 and R2a, 11.8 and 7.9, for treatments using the RR1 soybean with and without spraying, respectively (Figure 1B). On the other hand, in Intacta RR2 PRO® soybeans with and without insecticide application no individuals were collected, since this species is targeted by Bt technology. Laboratory studies verified efficiency of the Cy1A protein as 100% mortality for neonates of this species (Macrae *et al* 2005, Mcpherson & Macrae 2009).

The occurrence of small *C. includens* caterpillars occurred in all sites studied. However, the population outbreak of the occurred late, after *A. gemmatalis*, where there always occurred a decrease in the *A. gemmatalis* population soon followed by an increase in *C. includens*. This finding corroborates with information reported by Corrêa-Ferreira *et al* (2010) and Didonet *et al* (2003).

The infestation of *C. includens* occurred in or after the reproductive stage R2, extending in culture development until total filling of the grains in R6, where the population outbreak of this species was observed between R5.2 and R5.4 for both RR1 treatments with and without spraying (Figure 1C).

For the Intacta RR2 PRO® soybean (with and without the use of insecticide) a reduction in insects of the species *A. gemmatalis* and *C. includens* was verified, indicating the effectiveness of the technology to target pests, as reported by Mcpherson & Macrae (2009) and Macrae *et al* (2005) (Figure 1), not requiring the use of synthetic insecticides for these treatments.

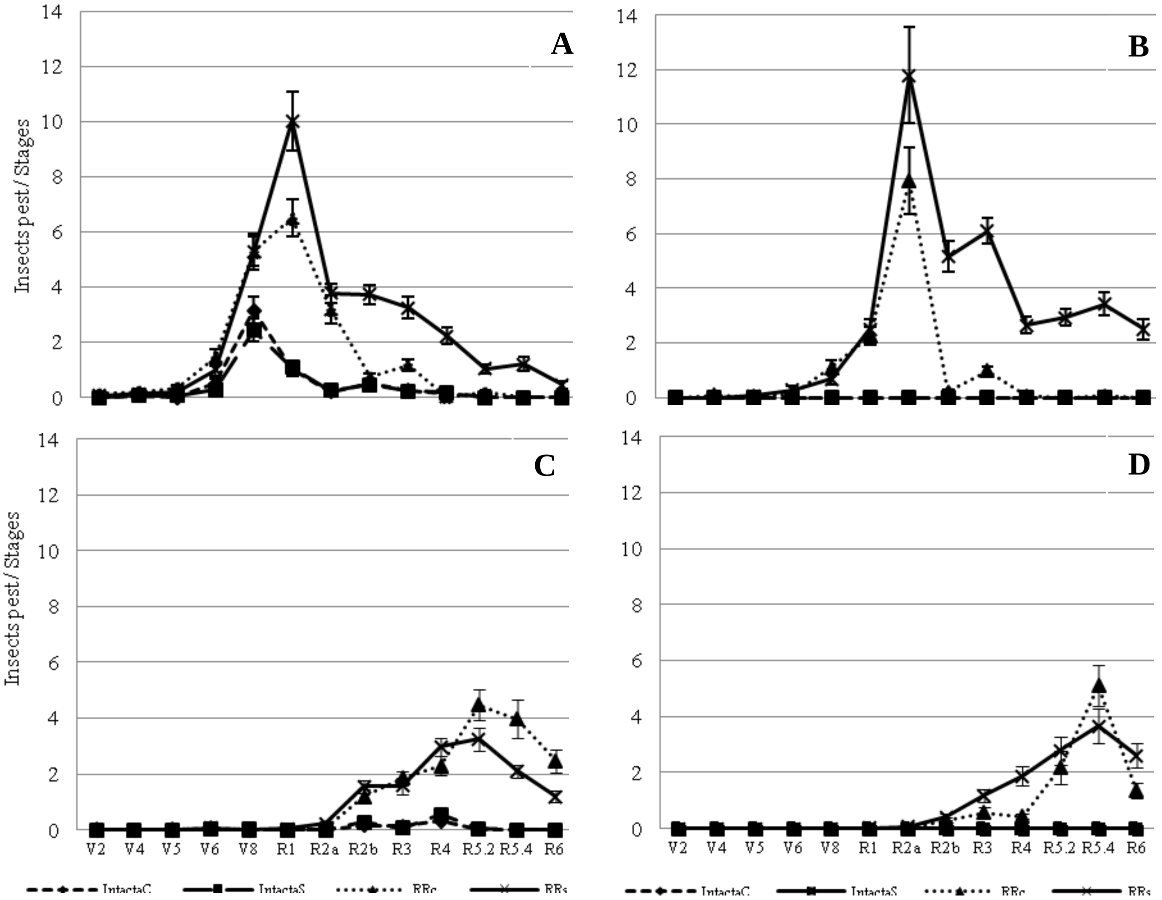


Figure 1. Population fluctuation of caterpillars (± standard error of the mean) (**A**) *Anticarsia gemmatalis* (<1.5 cm), (**B**) *A. gemmatalis* (≥ 1.5 cm), (**C**) *Chrysodeixis includens* (<1.5 cm), (**D**) *C. includens* (≥ 1.5 cm) per linear meter for soybean treatments (IntactaC = Intacta RR2 PRO® soybeans with insecticide application; IntactaS = Intacta RR2 PRO® soybeans without insecticide application; RRc = RR1 soybeans with insecticide application; RRs = RR1 soybeans without insecticide application.

For all treatments and replications defoliation above the AL was not observed in the vegetative stages. However, only for the RR1 soybean with insecticide was control of defoliators conducted in the reproductive stages: stages R1, R2a, R3 and R5.4 in repetitions in Rio Brilhante and Douradina, R3, R5.4 and R6 in Dourados and R1, R3, R5.2 and R5.4 in Caarapó, totaling 3.75 insecticide applications when defoliation exceeded the AL.

In cultivation of the RR1 soybeans (without insecticide application) the defoliation percentages were greater than 15% in the initial stages of the reproductive phase, reaching a level equal to or greater than 70% at the end of the crop cycle (Figure 2). Ribeiro & Costa (2000) found that yield decreases with defoliation above 67%, disregarding developmental stages; defoliation levels above 50% substantially decrease the number of pods and seeds per plant when in the early stages of pod formation (R3) and the beginning of grain filling (R5); grain weight is dramatically reduced by defoliation greater than or equal to 67% in stages R3 and R6.

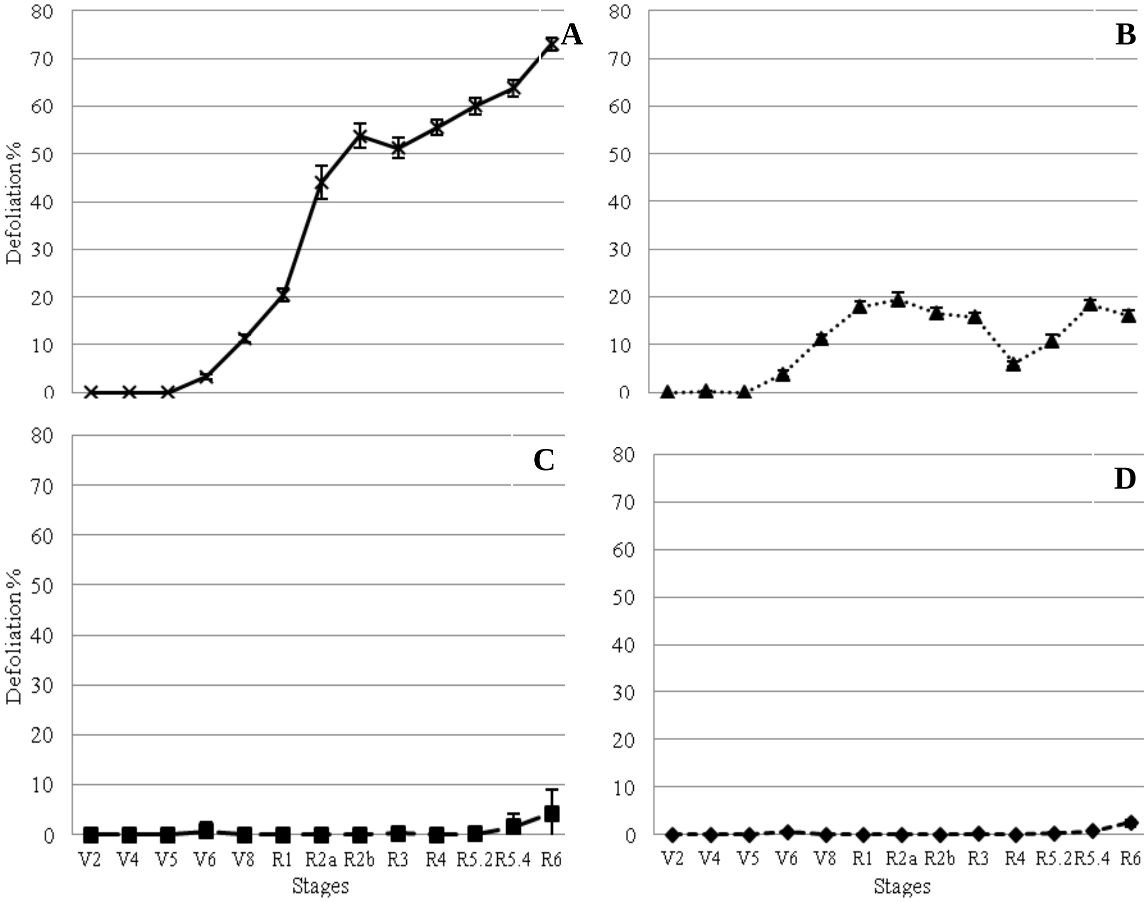


Figure 2. Percent defoliation caused by defoliating caterpillars (± standard error of the mean) *A. germmatalis* and *C. includens* (< and ≥ 1.5 cm) in treatments, (**A**) RR1 soybean without insecticide application; (**B**) RR1 soybean with insecticide application; (**C**) Intacta RR2 PRO® soybean without application insecticide; (**D**) Intacta RR2 PRO® soybean with insecticide application.

In cultivation of the Intacta RR2 PRO® soybeans (with and without insecticide application) no defoliation is reported as caused by the attack of caterpillars of the species *A. gemmatalis* and *C. includens.* Similar results were obtained by Bernardi *et al* (2012) with percentage defoliation above the action level when testing two non-Bt varieties compared to their Bt isolines which showed little or no defoliation (Figure 2).

Several species of phytophagous pentatomids associated with the soybean crop are considered primary or secondary pests, depending on population density, regional occurrence and phase in which the plant is attacked (Embrapa 2011).

The species *E. heros* was most abundant, occurring in the early reproductive stages, with its population outbreak between stages R6 and R7.1, i.e. in the grain filling and early physiological maturity stages of the crop, corroborating with data published by Roza- Gomes *et al* (2011) and Corrêa – Ferreira & Panizzi (1999).

In RR1 and Intacta RR2 PRO® soybeans without insecticide application the highest average numbers of stink bugs were obtained compared to treatments using insecticide application (Figure 3A).

The green belly stink bug (*Dichelops melacanthus*) manifests as a pest at the start of the crop cycle in wheat and corn. In soybeans, this species which has always occurred in low densities is gradually increasing its population levels and importance in the stink bug complex (Roza- Gomes *et al* 2011; Salvadori *et al* 2007). Similar results were observed for the population of *Dichelops* spp. in stages R7.1 and R7.3 prior to harvest, being more abundant in RR1 and Intacta RR2 PRO® soybeans, both without insecticide application (Figure 3B).

The average number of *Edessa meditabunda* insects was less than one per stage studied, thus highlighting its low occurrence (Figure 3C).

Population outbreaks for the stink bug complex occurred between stages R6 and R7.1, in those of grain filling and early physiological maturity, called the critical period. The population grows until the end of grain filling (R6), when it reaches a stage of greater population abundance, according to Corrêa-Ferreira & Panizzi (1999), studies which corroborate with the results obtained (Figure 3).

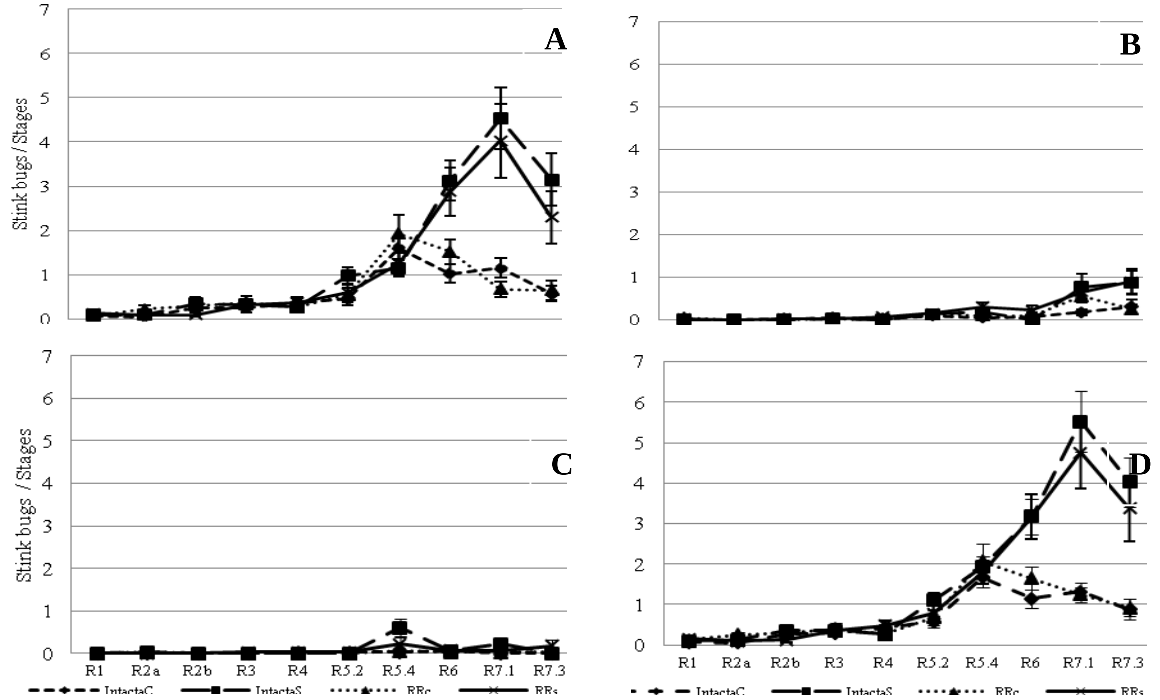


Figure 3. Population fluctuation of stink bugs (≥ 0.5 cm) (± standard error of the mean) (**A**) *Euschistus heros*, (**B**) *Dichelops* spp. (**C**) *Edessa meditabunda* and (**D**) average of the three species per linear meter of soybeans. IntactaC = Intacta RR2 PRO® soybeans with insecticide application; IntactaS = Intacta RR2 PRO® soybeans without insecticide application; RRc = RR1 soybeans with insecticide application; RRs = RR1 soybeans without insecticide application.

The population fluctuation of beneficial arthropods such as *Cycloneda sanguinea, Lebia concinna, Nabis* spp., *Doru* spp., *Geocoris* spp., *Zellus* spp., *Chrysoperla* spp., and arachnids occurred during all culture stages, where a slight increase in the population of predators may be observed in areas cultivated with Intacta RR2 PRO® soybeans (with and without insecticide application) when compared to RR1 soybeans (with and without insecticide application) (Figure 4).

Several factors may explain the greater abundance of predators in the Intacta RR2 PRO® soybeans. The microclimate in the planted area due to the lack of defoliation results in greater shading, humidity and cooler temperatures, conditions that favor the increase of natural enemies. Similar results were observed by Cividanes & Yamamoto (2002) when assessing natural enemies in soybean and corn grown in diverse systems. The lack of insecticide application to control caterpillars, target pests of Bt technology, may have also provided better conditions for the establishment of these biological control agents.

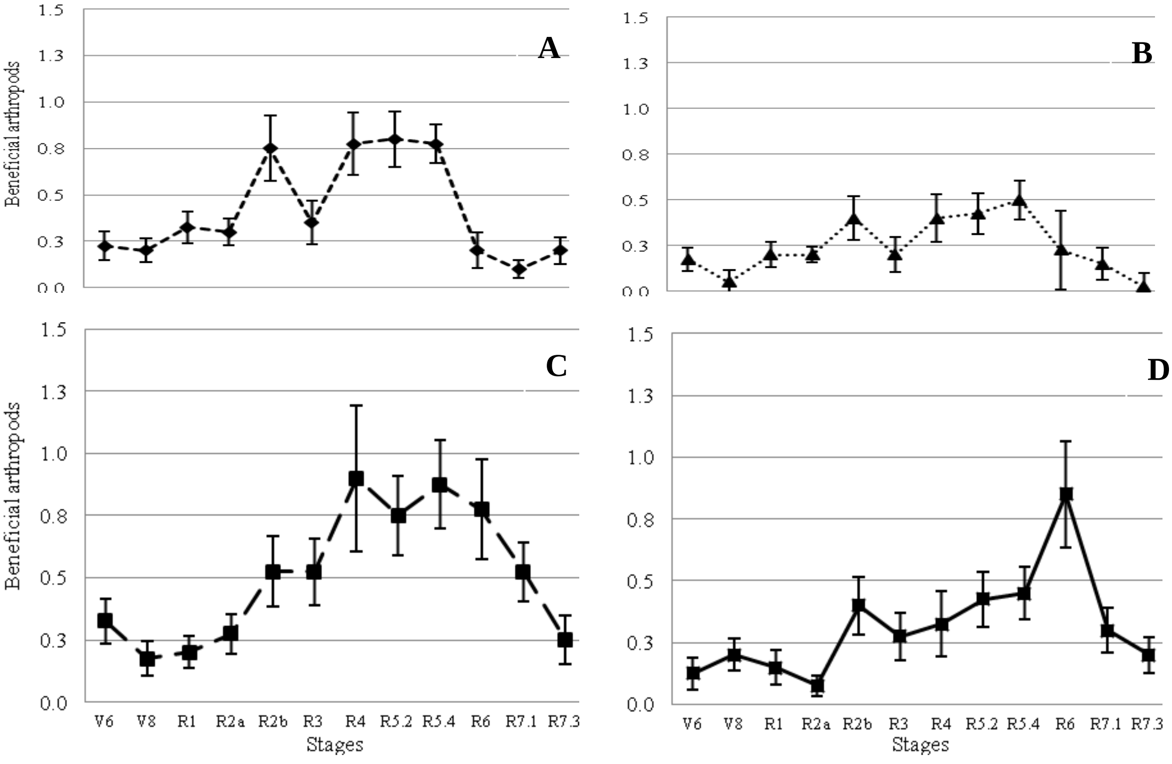


Figure 4. Population fluctuation of beneficial arthropods (± standard error of the mean) per linear meter of soybean, (**A**) Intacta RR2 PRO® soybeans with insecticide application; (**B**) RR1 soybeans with insecticide application; (**C**) Intacta RR2 PRO® soybeans without insecticide application, (**D**) RR1 soybeans without insecticide application.

4. Conclusions

It can be concluded that the Intacta RR2 PRO® soybean (Bt), expressing the toxin Cry 1Ac, efficiently controlled the target lepidoptera *A. gemmatalis* and *C. includens* in the field, reducing the number of insecticide applications necessary for management of these insect pests compared to RR1 soybeans. Regarding phytophagous stinkbug populations, non-targets of Bt technology, no significant difference was observed among the tested cultivars.

The Intacta RR2 PRO® soybean, with and without insecticide application, favored the beneficial arthropods compared with RR1 soybeans.

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