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Article

Glyphosate Impact on Arthropods Associated to Roundup Ready and Conventional Soybean (*Glycine max* L.)

*Impacto do glyphosate sobre artrópodes associados com soja RR e convencional (**Glycine max** L.)*

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ABSTRACT - This study aims to evaluate the impact of glyphosate-resistant soybean and its management with glyphosate on the canopy arthropod community. We study the direct impact of the insertion of the resistance gene and the indirect impact of management practices, specifically herbicide application. To do this, we use the following treatments: non-GM and GM soybean with mechanical weed control and GM soybean with one and three applications of glyphosate. Arthropods in the crop canopy were sampled over time in all treatments. The incorporation of the glyphosate resistance gene did not affect the richness and abundance of arthropods in the plant canopy. Glyphosate application reduced the richness of predators and chewing and sucking phytophagous arthropods in treatments with three herbicide applications. In the second season, total arthropod density was the lowest in transgenic soybean with three glyphosate applications. The density of *Cerotoma arcuatus*, a chewing phytophagous arthropod, followed similar trends, while both glyphosate treatments (one or three applications) reduced the densities of the predator *Solenopsis* sp. and the detritivore *Hypogastrura* sp. Meanwhile, the densities of the sucking phytophagous arthropods *Bemisia tabaci*, *Caliothrips brasiliensis*, and *Tetranychus* sp. were the highest in transgenic soybean with three glyphosate applications.

Keywords: *Glycine max*, transgenics, phytophagous, predators, parasitoids, detritivores.

RESUMO - O uso da soja transgênica resistente ao glyphosate pode causar impactos diretos ou indiretos nos componentes do agroecossistema. O impacto direto é provocado pela inserção do gene de resistência, e o indireto está relacionado às alterações nas práticas de manejo. Dessa forma, este trabalho objetivou avaliar o impacto da soja resistente ao glyphosate e seu manejo com esse herbicida sobre a comunidade de artrópodes do dossel das plantas. Os tratamentos foram: soja transgênica e não transgênica com capina mecânica das plantas daninhas; e soja transgênica com uma e três aplicações de glyphosate. As populações de artrópodes no dossel das plantas foram amostradas ao longo de dois cultivos. A incorporação do gene de resistência ao glyphosate não afetou a riqueza e a abundância de artrópodes no dossel das plantas. Já a aplicação do glyphosate reduziu a riqueza de predadores e de fitófagos mastigadores e sugadores nos tratamentos com três aplicações desse herbicida. No segundo ano de cultivo a densidade total de artrópodes foi menor na soja transgênica com três aplicações de glyphosate em relação aos demais tratamentos. O mesmo ocorreu com a densidade do fitófago mastigador ***Cerotoma arcuatus*** *nos dois anos de cultivo. Já as densidades dos*

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fitófagos succionadores ***Bemisia tabaci, Caliothrips brasiliensis*** *e* ***Tetranychus*** *sp. foram maiores na soja transgênica com três aplicações de glyphosate do que nos demais tratamentos. A aplicação de glyphosate (uma ou três) reduziu densidades do predador* ***Solenopsis*** *sp. e do detritívoro* ***Hypogastrura*** *sp.*

Palavras-chave: *Glycine max*, transgenia, fitófagos, predadores, parasitoides, detritívoros.

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is the world's most important oilseed crop, with direct applications for human food, oil production, and animal feed. Genetically modified, herbicide-resistant soybean was introduced in Brazil in 2003 (Hungria et al., 2014). This heralded a profound change in the country's weed control systems, since several products or product combinations were replaced by a single active ingredient: glyphosate (Egan et al., 2014).

Arthropods are one of the most important components in any agroecosystem, with populations of detritivorous, parasitoid, phytophagous, and predatory species (Médiène et al., 2011). Arthropod communities can be directly or indirectly affected by the use of GM soy. The direct impact is caused by the molecular alteration in soybean plants resulting from the insertion of the CP4 (cp4 epsps) gene, which originated in the *Agrobacterium* sp. strain CP4 and promotes glyphosate resistance. The indirect effects are related to changes in soybean management practices, mainly due to post-emergence glyphosate application.

The impact of glyphosate on the arthropod community extends to the toxicity of the compound as well as changes it causes to the weed community (Egan et al., 2014). Interactions between weeds and arthropods include consumption of weeds by phytophagous and detritivorous insects, as well as by natural enemies of weed plants (Bàrberi et al., 2010). Herbicide application can, therefore, bring about a reduction in the arthropod population by removing food sources. This causes an imbalance in the ecosystem and may affect the food webs and the trophic relationships within them, which can result in significant increases in the pest insect population (Dahlin and Ninkovic, 2013).

Arthropods are one of the most important components in any agroecosystem, and their interactions with the environment can lead to benefits or losses for the system as a whole. Several authors note that weed control measures can affect the diversity and abundance of arthropods (Shelton and Edwards, 1983; Norris and Kogan, 2004; Albajes et al., 2014).

Therefore, studies on the impacts of glyphosate-resistant soybean should focus on arthropod community functions since they are key elements in the ecosystem. Such agroecological studies are an essential component of efforts to increase crop productivity and agroecosystem sustainability.

In this context, this paper aims to identify and analyze the impacts of use of glyphosate and the variations in weed population diversity and density on aerial arthropod communities in glyphosate-resistant soybean crops in Brazil.

MATERIAL AND METHODS

The experiment was carried out at Coimbra, Minas Gerais, Brazil, during the 2007/2008 and 2008/2009 growing seasons. The soybean varieties used were BRS Favorita RR (Roundup Ready®), a transgenic soybean, and MG/BR-46 Conquista, a non-transgenic soybean. These varieties are similar, with an average growing cycle of 115 days, determinate growth habit, and the same pattern of disease resistance. Both are recommended for cultivation in the center-south region of Brazil. The soybean fields were planted in the first fortnight of December 2007 (first season) and in the second fortnight of November 2008 (second season).

Daily values of air temperature (maximum, average, and minimum), relative humidity, and rainfall during the experimental period were recorded by a meteorological station installed at the crop site (Figure 1).

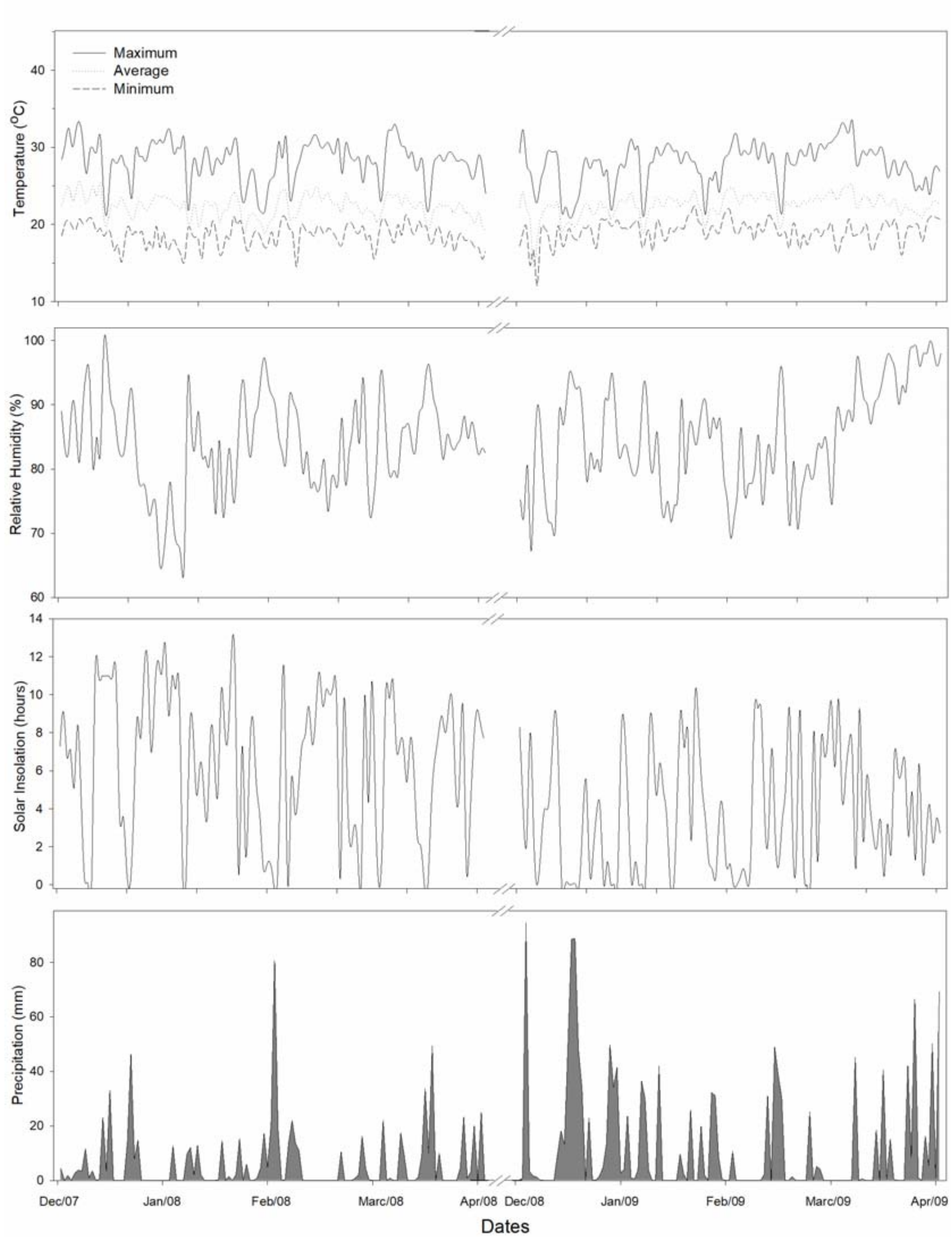


Figure 1 **-** Air temperatures (maximum, average, minimum), relative humidity, solar insolation, and rainfall during two growing seasons of soybean. Coimbra, Minas Gerais, Brazil, 2007-2009.

The experimental design was conducted in randomized blocks with five replicates. Each experimental plot consisted of an area of 10 x 10 m, row spacings of 0.5 m, and planting density of 18 seeds per meter. The treatments were: 1 - non-transgenic soybean with mechanical weeding; 2 - transgenic soybean with mechanical weeding; 3 - transgenic soybean with one application of glyphosate (1,080 g ha^{-1}) 15 days after plant emergence; and 4 - transgenic soybean with three applications of glyphosate (1,080 g ha^{-1}) 15, 30, and 45 days after plant emergence.

Sampling of the arthropod populations in the canopy of plants took place at 10, 15, 31, 37, 46, 57, 64, 94, and 108 days after the emergence of the plants in the first season. In the second season, these populations were evaluated at 10, 15, 31, 50, 69, 90, and 105 days after emergence.

In each experimental plot, the apical leaves of ûve (soybean) plants were beaten against a white plastic tray (35 x 30 x 5 cm) according to a sampling plan developed by Moura et al. (2007). The arthropods collected were placed in vials containing 70% ethanol and later separated according to morphospecies and sent to taxonomists for identiûcation.

The impact of the treatments on the arthropod assemblage was determined by comparing arthropod richness and relative abundance of each species. Total arthropod richness, as well as richness of each arthropod guild (detritivorous, herbivorous, and predators), was recorded in terms of the total number of species observed in each treatment and the total number from each guild.

These analyses employed two processes of species selection to model the treatment effects on arthropod abundance. In the first process, we limited our focus to species that occurred in all treatments with a frequency of over 10%. We recorded the total frequency with which each of these species occurred (Pereira et al., 2010). Subsequently, we entered the density data (arthropods per sample) for each species into the PROC STEPDISC procedure with STEPWISE selection in SAS to select the species that most explained the total variance observed (SAS, 2001; Pereira et al., 2010). We selected species based on two criteria: 1) the significance level of the F test of the covariance analysis, with the selected species coverable and the treatments as dependent variables, and 2) the partial square correlation, which predicts the effects of the treatments on the species (SAS, 2001; Pereira et al., 2010). These species were considered the most important because they represent the largest amount of observed variation between treatments. We conducted a canonical variate analysis (CVA) on the density data of the selected species. This indirect ordering technique reduces the size of the original dataset into a set of variables that may be used to graphically illustrate the relative positions and orientations of the mean responses of the arthropod community in each treatment (Kedwards et al., 1999). The significance of the difference (indicated by the ordering) between the treatments was determined by comparing the treatments two by two in the approximate F test ($p < 0.05$), using the Mahalanobis distance between the respective classes of canonical means. The analyses were performed using the SAS CANDISC procedure (SAS, 2001).

The abundance data of the main species that led to the emergence of differences between treatments were individually submitted to analysis of variance by time-repeated measurements (Hurlbert, 1984; Stewart-Oater et al., 1986; Green, 1993; Paine, 1996). These analyses were performed using the SAS ANOVA procedure with the PROFILE specification, as suggested by von Ende (2001). We carried out canonical correlations (PROC CANCOR; SAS Institute, 2001) and the simple correlations (PROC COR, SAS Institute, 2001) to verify the interrelation of the responses between herbivorous pests and predators in the different treatments. We tested the normality and homogeneity of the variances using the UNIVARIATE procedure (SAS Institute, 2001).

Finally, we calculated the mean densities (± standard error) of the most important species for each sampling date. The density data of these species for each treatment over time were represented using curves. The comparison between the treatments was performed by the intersection comparison of the standard errors (Pereira et al., 2010).

RESULTS AND DISCUSSION

In the ûrst (first) growing season, we collected 105 arthropod species from the soybean canopy: 12 chewing phytophagous species, 33 sucking phytophagous, 43 predators, 11 detritivorous, and six parasitoids. Total arthropod richness ranged from 73 species in the non-transgenic soybean plot to 60 species in the plots with transgenic soybean and three glyphosate applications (Table 1). We observed 56 arthropod species during the second season, of which five were chewing phytophagous, 20 sucking phytophagous, three parasitoids, six detritivorous, and 22 predators. Total arthropod richness ranged from 43 species in the non-transgenic soybean plots to 36 species in transgenic soybean plots with three glyphosate applications (Table 1).

Arthropod species diversity for all guilds was similar across treatments ($p>0.05$). The lack of variation between treatments in species richness and density, along with minimal fluctuations in the populations of the selected arthropod species, indicates that the insertion of the glyphosate resistance gene did not affect arthropod populations in the soybean canopy. This is the first

Table 1 - Total species of arthropods and total species per guild on the canopy of transgenic soybeans (TS) and non-transgenic soybeans (NTS) with one and three applications of glyphosate (1Gly and 3Gly). Coimbra, Minas Gerais, Brazil. 2007-2009

Guild	Richness (number of species/treatment)				Number of species per guild
	NTS	TS	TS-1Gly	TS-3Gly	
2007/2008					
Chewing phytophagous	9	10	6	6	12
Sucking insects	26	26	22	20	33
Predators	32	28	24	20	43
Parasitoids	4	4	4	6	6
Detritivorous	8	7	10	8	11
Species by treatment	71	73	66	60	105
2008/2009					
Chewing phytophagous	5	4	4	4	5
Sucking insects	18	16	12	13	20
Predators	14	14	15	12	22
Parasitoids	3	3	3	3	3
Detritivorous	3	3	2	4	6
Species by treatment	43	39	36	36	56

study to report on the effect of glyphosate-resistant soy cultivation on arthropod diversity; studies to date have limited their focus to arthropod abundance (Evans et al., 2010; Svobodová et al., 2017). According to Funderburk et al. (1999), glyphosate-resistant soybean cultivation does not affect the seasonal variation of phytophagous arthropods and does not promote outbreaks of new pests in the crop.

The lower arthropod richness observed in the second growing season may be due to year-on-year variations. Wolda (1988) observed seasonal and annual variations in arthropod richness and abundance, while other authors have noted that arthropod populations respond to changes in air temperature, rainfall, photoperiod, relative humidity, organic matter decomposition, and vegetation cover (Denlinger, 1986; Semeão et al., 2012). During our study period, rainfall was considerably higher during the second growing season (Figure 1). Rainfall is the most important factor of arthropod mortality in tropical regions (Pereira et al., 2007; Leite et al., 2011), acting as a direct cause of mortality as well as increasing the density of entomopathogenic fungi and reducing soil organic matter, a source of food and refuge for many arthropods (Swamy and Proctor, 1994).

The higher richness of phytophagous arthropods compared to other guilds may be related to the fact that the crop was grown in a monoculture system. Phytophagous insects are better adapted to monocultures due to the accessible location of host plants and the lower presence of natural enemies in these agroecosystems (Letourneau et al., 2011).

Of the 105 and 56 arthropod species observed in the first and second experimental periods, respectively, 11 species had a frequency higher than 10%. These arthropods were the chewing phytophagous arthropod *Cerotoma arcuatus* (67% and 78% in the first and second growing season, respectively); the sucking phytophagous arthropods *Bemisia tabaci* (78% and 28%), *Caliothrips brasiliensis* (89% and 88%), and *Tetranychus* sp. (86% and 56%); the predators *Crematogaster* sp. (24% and 44%), *Doru luteipes* (10% and 13%), *Franklinothrips* spp. (81% and 86%), *Solenopsis* sp. (24% and 58%), and *Tapinoma* sp. (30% and 45%); the parasitoid *Trichogramma* sp. (48% and 12%), and the detritivorous *Hypogastrura* sp. (84% and 93%).

The phytophagous species *B. tabaci*, *C. brasiliensis*, *C. arcuatus*, and *Tetranychus* sp., the predators *D. luteipes* and *Solenopsis* sp., and the detritivorous *Hypogastrura* sp. were the species which best explained the maximum discrimination among the treatments (Table 2).

Based on the canonical coefficients, the species that most positively contributed to the divergence among the treatments on the canonical axes were *Solenopsis* sp. (axis 1 on both seasons), *C. arcuatus* (axis 1 on the second season), and *B. tabaci* (axis 2 on both seasons). The

Table 2 - Selection summary by STEPWISE with SAS STEPWISE STEPDISC procedure, aiming to select species to be included in the analysis of canonical variables, obtaining maximum discrimination between treatments. Coimbra, Minas Gerais, Brazil, 2007-2009

Variable	Partial R^2	Test F – the analysis of covariance		Square partial correlation	
		F	*p*	Average square canonical correlation	*p*
Phytophagous species					
Bemisia tabaci	0.0921	8.46	<0.0001	0.0127	<0.0001
Caliothrips brasiliensis	0.0421	3.65	0.0133	0.6331	<0.0001
Cerotoma arcuatus	0.1276	12.23	<0.0001	0.0976	<0.0001
Tetranychus sp.	0.0386	3.29	0.0212	0.1643	<0.0001
Predators					
Doru luteipes	0.0352	3.02	0.0306	0.1459	<0.0001
Solenopsis sp.	0.1655	16.66	<0.0001	0.0551	<0.0001
Detritivores					
Hypogastrura sp.	0.0312	2.65	0.0494	0.1543	<0.0001

species that most negatively contributed to the divergence among the treatments in the canonical axes were *C. brasiliensis* (axis 1 on the first season) and *C. arcuatus* (axis 2 on both seasons) (Table 3). Therefore, the species that most clearly indicated the change in treatment were the chewing phytophagous arthropod *C. arcuatus*, the sucking phytophagous arthropods *B. tabaci*, and *C. brasiliensis* and the predator *Solenopsis* sp.

The canonical analysis of variance indicated significant differences among the treatments in the first (Wilks' lambda = 0.1825 and F = 13.83 and df (numerator/denominator) = 21/359 and p<0.0001) and second seasons (Wilks' lambda = 0.5026 and F = 3.56 and df (numerator/denominator) = 21/276.21 and p<0.0001). Four canonical axes were calculated, two of which were significant in the first season (p<0.0001 and p<0.0001) and two in the second season (p<0.0001 and p = 0.025). The first and second canonical axes explained in 67% and 27% of the accumulated variance in the first season, respectively, and 69% and 17% in the second season (Table 3).

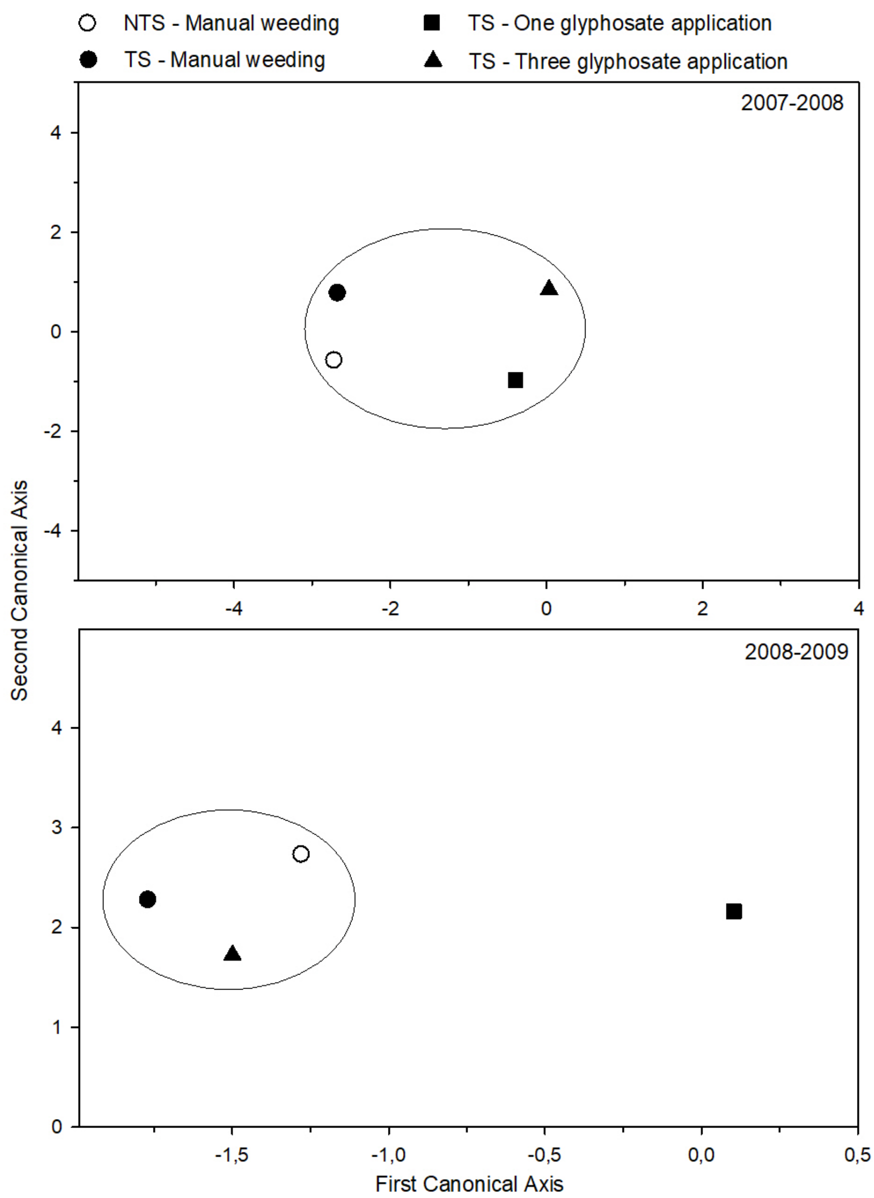
Table 3 - Canonical axes and their coefficients (among canonical structure) of the effect of non-transgenic soybeans (NTS) and transgenic soybeans (TS) with one or three applications of glyphosate (1Gly and 3Gly) on the group of species of phytophagous arthropods, predators, and detritivores, selected by STEPWISE with STEPDISC procedure of SAS STEPWISE. Coimbra, Minas Gerais, Brazil. 2007-2009

Variable	Canonic axes			
	1	2	1	2
	2007-2008		2008-2009	
Chewing phytophagous species				
Bemisia tabaci	-0.080	0.443	0.219	0.482
Caliothrips brasiliensis	-0.012	0.067	-0.128	0.405
Cerotoma arcuatus	0.029	-0.502	1.102	-0.046
Tetranychus sp.	-0.066	0.075	-0.117	0.080
Predators				
Doru luteipes	0.079	0.239	0.742	0.576
Solenopsis sp.	0.655	0.092	0.537	0.093
Detritivores				
Hypogastrura sp.	0.036	0.005	0.003	0.007
F	13.83	8.53	3.56	2.01
df (numerator/denominator)	21/359	12/252	21/276	12/194
P	<0.0001	<0.0001	<0.0001	0.025
Partial canonical correlation	0.67	0.27	0.69	0.17

There was no significant difference between any of the treatments in the first season (Figure 2), though the total abundance of arthropods was lower in plots of transgenic soybeans with three glyphosate applications in the second season (Figure 2).

No significant differences were detected in the densities of the main species of phytophagous, predatory, and detritivorous arthropods between the transgenic and non-transgenic soybean plots with mechanical weeding (Figures 3 and 4). These species are, therefore, unaffected by the incorporation of the glyphosate resistance gene.

However, the abundance of some of the main arthropod species did change due to glyphosate application. Plots of transgenic soybeans with three applications of glyphosate had higher densities of *B. tabaci* and *C. brasiliensis* in the first season and *Tetranychus* sp. in the second season. Meanwhile, the plots with three glyphosate applications had significantly lower densities of *C. arcuatus* in both growing seasons (Figure 3).



Treatments outside the same circle differ by the F test ($p<0.05$), based on the Mahalanobis distance between the means of the classes. Coimbra, Minas Gerais, Brazil. 2007-2009.

Figure 2 - Ordering diagram (CVA) of the arthropod community on the canopy of plants of the transgenic soybeans (TS) and non-transgenic soybeans (NTS) with one and three applications of glyphosate.

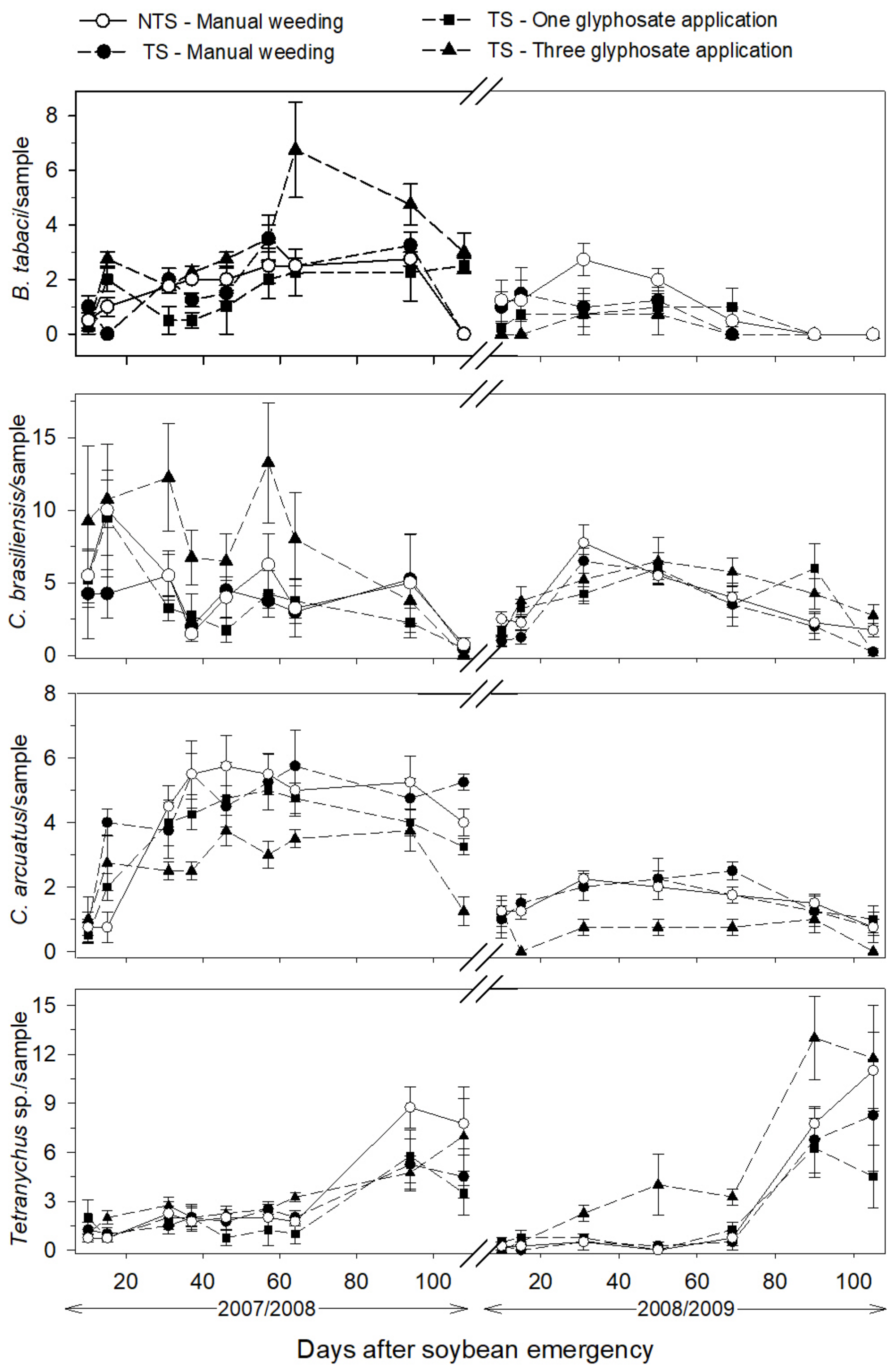


Figure 3 - Abundance (mean ± standard error) of phytophagous arthropods on the canopy of transgenic soybeans (TS) and non-transgenic soybeans (NTS) with one and three applications of glyphosate. Coimbra, Minas Gerais, Brazil. 2007-2009.

The density of some arthropod species decreased significantly with any amount of glyphosate addition. Predator ants (*Solenopsis* sp.) had significantly lower densities in both treatments with glyphosate application in both years. Densities of the detritivore *Hypogastrura* sp. were lower in both glyphosate treatments in the first year and the treatment with three glyphosate applications in the second year (Figure 4). Herbicide application can impact the arthropod community through the toxicity of the compound or changes to the weed community (Evans et al., 2010).

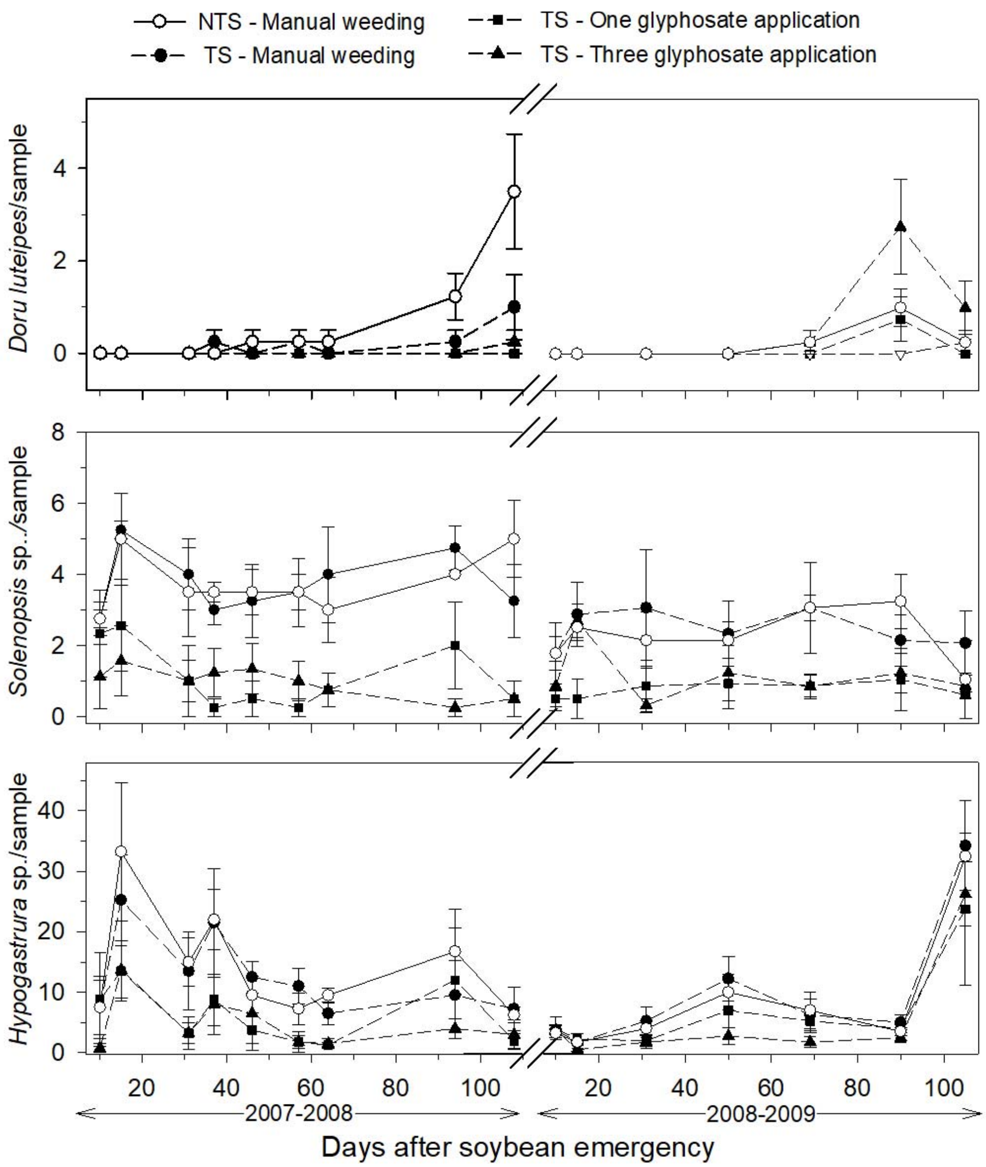


Figure 4 - Abundance (mean ± standard error) of predators and detritivores on the canopy of transgenic soybeans (TS) and non-transgenic soybeans (NTS) with one and three applications of glyphosate. Coimbra, Minas Gerais, Brazil. 2007-2009.

Most studies on the impact of glyphosate have shown that this effect is due to the habitat change where arthropods live and not necessarily to the toxicological effect of the compound itself (Taylor et al., 2006; Egan et al., 2014). Thus, the reduction in weed density due to glyphosate application may have contributed to the reduction in arthropod population density. Increased plant cover promotes greater soil protection and reduces organic matter loss, which is important in maintaining soil water and nutrient levels, these components are essential in the preservation of species of arthropods, allowing them to cohabit within the same environment.

This change can support the development of pests, especially polyphagous species, which can find an abundant food supply because of the reduction of agroecosystem diversity, facilitating the location of their food source - in this case, soybean plants. This increase is also associated with the reduction of the density of parasitoids and predators. Glyphosate application can also reduce the active nutrient pool in the soil due to reductions in the weed rhizosphere and, consequently, the density of microorganisms, affecting the fragmentation and degradation of organic tissues (Kremer et al., 2005; Arango et al., 2014).

However, we observed a higher abundance of the sucking phytophagous species *B. tabaci*, *C. brasiliensis*, and *Tetranychus* sp. in transgenic soybeans with three glyphosate applications than in transgenic soybeans with manual weeding. Spontaneous vegetation can affect the density of some pests. These species can take advantage of the abundant food supply and the reduction in agroecosystem diversity caused by sequential glyphosate applications.

The presence of the gene for glyphosate herbicide tolerance did not affect the richness, total arthropod density, or the seasonality of arthropod species in the canopy of soybean plants. In contrast, glyphosate application reduces the richness and densities of the predator *Solenopsis* sp. and the detritivore *Hypogastrura* sp. The use of three glyphosate applications on transgenic soybeans increases the densities of the sucking phytophagous arthropods *B. tabaci*, *C. brasiliensis*, and *Tetranychus* sp. and reduces the total arthropod density and the chewing phytophagous arthropod *C. arcuatus*. This study is fundamental to improving alternative management practices that guarantee the productivity and the sustainability of the soybean agroecosystem.

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