EFFECT OF SULFONYLUREAS APPLICATION ON RR/STS SOYBEAN

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ABSTRACT

The aim of this study was to evaluate the selectivity of the herbicides chlorimuron-ethyl, metsulfuron-methyl and nicosulfuron, applied in RR/STS soybean. The experiment I consisted of the application of rates (0, 15, 30, 45 and 60 g a. i ha^{-1}) of chlorimuron-ethyl. For the experiment II, were applied rates (0; 1.8; 3.6; 5.4 and 7.2 g a. i ha^{-1}) of metsulfuron-methyl. In experiment III, were used rates (0, 50, 100, 150 and 200 g a. i ha^{-1}) of nicosulfuron. This was a randomized block design with four replications. We evaluated plant height, number of pods per plant, chlorophyll A, B and total, conductivity, vigor and seed germination, yield and thousand seed mass. Data were subjected to regression analysis. In general, the variables analyzed were not affected by herbicide application. The CD 250 RR/STS soybean cultivar has proven to be highly tolerant to the herbicides chlorimuron-ethyl, metsulfuron-methyl and nicosulfuron.

Keywords: ALS inhibitors, *Glycine max*, herbicide-tolerant crops, selectivity

EFEITO DA APLICAÇÃO DE SULFONILURÉIAS SOBRE SOJA RR/STS

RESUMO

O objetivo do trabalho foi avaliar a seletividade dos herbicidas chlorimuron-ethyl, metsulfuron-methyl e nicosulfuron, aplicados em soja RR/STS. O experimento I constituiu-se da aplicação de doses (0, 15, 30, 45 e 60 g i. a. ha^{-1}) de chlorimuron-ethyl. Para o experimento II foram aplicadas doses (0; 1,8; 3,6; 5,4 e 7,2 g i. a. ha^{-1}) de metsulfuron-methyl. Para o experimento III foram aplicadas doses (0, 50, 100, 150 e 200 g i. a. ha^{-1}) de nicosulfuron. Foi empregado delineamento experimental em blocos casualizados, com quatro repetições. Foram avaliadas: altura de planta, número de vagens por planta, clorofila A, B e total, condutividade, vigor e germinação das sementes, produtividade e massa de mil sementes. Os dados foram submetidos à análise de regressão. De maneira geral, as variáveis analisadas não sofreram influência pela aplicação dos herbicidas. O cultivar de soja CD 250 RR/STS apresentou-se tolerante aos herbicidas chlorimuron-ethyl, metsulfuron-methyl e nicosulfuron.

Palavras-chave: Culturas tolerantes a herbicidas, *Glycine max*, inibidores da ALS, seletividade

INTRODUCTION

It is remarkable the growth of areas occupied by soybean crops in Brazil; in the 2015/2016 growing season, it reached 32.18 million hectares (COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB, 2016). Another important aspect is that RR (Roundup Ready) soybean crops corresponded to 93.5% of the total soybean area in Brazil in the 2014/2015 season (CÉLERES, 2015).

Considering the role of the soybean complex in the world scenario, the researches aimed at increasing the quality and quantity produced of this grain are intense. This has required the constant reformulation and adaptation of technologies and management, as a more adequate positioning of the herbicide glyphosate, in addition to the emergence of new technologies.

Sulfonylurea-tolerant soybean (STS) is not a transgenic crop as it has been developed through the seed mutagenesis technique using the ethyl methanesulfonate (EMS) alkylating agent, which is an agent that does not cause mutation by insertion into the DNA, but by the modification of the already present base, by introducing an alkyl radical. In this case an ethyl, often in the nitrogenous base Guanine (ROGOZIN et al., 2001).

Mutant seeds of the ‘Williams 82’ soybean cultivar were selected according to tolerance to chlorsulfuron sulfonylurea. Thus, the soybean cultivar W20 was developed, which presented a high degree of tolerance, in post and pre-emergence, for some sulfonylureas. Research has shown that this characteristic is determined by a semi-dominant allele that has been designated Als1. Studies such as these are of fundamental importance to identify other mutations, and consequently to increase the tolerance of these soybean cultivars to sulfonylureas and other ALS-inhibiting herbicide families (SEBASTIAN et al., 1989; GHIO et al., 2013; WALTER et al., 2014). STS cultivars tolerate up to four times higher rates of chlorimuron-ethyl than those recommended for non-tolerant cultivars, without significant damage (COODETEC, 2011; ROSO & VIDAL, 2011).

The chemical group of sulfonylureas was discovered and initially developed by Du Pont de Nemours & Company in 1975 and the first commercial product was chlorsulfuron in 1981

(OLIVEIRA JR, 2011). Herbicides of this group act via inhibition of acetolactate synthase (ALS), irreversibly. ALS is the initial biosynthesis enzyme of the branched-chain amino acids valine, leucine and isoleucine, catalyzing two parallel reactions, pyruvate condensation, to form acetolactate; and condensation of pyruvate with 2-oxybutyrate, to form acetohydroxybutyrate (EBERLEIN et al., 1997). These amino acids, valine, leucine and isoleucine, are required for the production of new cells and also for essential components in proteins (VIDAL, 1997; ZHOU et al., 2007).

Sulfonylureas mainly control dicotyledonous weeds but some molecules demonstrate good action against grasses. Thus, they are widely used to control weeds in wheat, rice, soybean, barley, cotton, potato and corn crops (BROWN, 1990; ZHOU et al., 2007).

In this way, the goal of the present work was to evaluate the selectivity of three herbicides of the sulfonylurea group: chlorimuron-ethyl, metsulfuron-methyl and nicosulfuron applied in post-emergence (V4) of the CD 250 RR/STS soybean cultivar.

MATERIAL AND METHODS

Three experiments were carried out in production area, in the municipality of Marialva (north central region of the state of Paraná), in the 2011/12 growing season. According to Köppen climate classification, the predominant climatic type is Cfa – mesothermal humid subtropical. This type of climate is characterized by the predominance of hot summers, low frequency of severe frosts and a tendency of rainfall concentration in the summer (INSTITUTO AGRONÔMICO DO PARANÁ – IAPAR, 1987).

Fertilization practices, crop planting and phytosanitary management were carried out according to the recommendations of Empresa Brasileira de Pesquisa Agropecuária – EMBRAPA (2011). Fertilization was carried out to amend the soil, considering the extraction of the crop. And all plots were kept free of weed interference, by manual weeding.

The cultivar CD 250 RR/STS was used, with indeterminate growth habit, relative maturity group 5.5, resistance to frogeye leaf spot, powdery mildew and stem canker. It is adapted to the southern region of Brazil, Paraguay and regions of the states of Mato Grosso do Sul and São Paulo, with recommended sowing from September 20th to December 10th and plant population of 200 to 300 thousand plants ha^{-1} (COODETEC, 2012).

Experiment I consisted of the application of increasing rates (0, 15, 30, 45 and 60 g a. i. ha^{-1}) of the herbicide chlorimuron-ethyl. For the experiment II, rates of metsulfuron-methyl (0, 1.8, 3.6, 5.4 and 7.2 g a. i. ha^{-1}) were applied. While for experiment III, were used the application of five increasing rates (0, 50, 100, 150 and 200 g a. i. ha^{-1}) of the herbicide nicosulfuron. For the three experiments the sowing was performed in the first week of November 2011, and the harvest in the last week of February 2012.

The experimental design used in the three experiments was the randomized blocks design, with four replications. The experimental units consisted of 5 m long plots and six soybean rows, and the four central rows were considered the working area, disregarding the first and last meter of the plot.

Herbicides were applied with a CO_2 pressurized backpack sprayer with bar equipped with four spray nozzles, at a constant pressure of 2 Bar, a flow rate of 0.65 L $min.^{-1}$, working at a height of 50 cm from the target, and at a speed of 1 m s^{-1}, reaching an applied strip of 50 cm wide per spray nozzle, and providing a spray volume of 200 L ha^{-1}.

Crop injury was evaluated by means of visual evaluations which assigned percentage scores ranging from 0 to 100% to each experimental unit (where 0 is the absence of injuries and 100%, death of plants), considering, in this case, symptoms significantly visible in plants, according to their development (SOCIEDADE BRASILEIRA DA CIÊNCIA DAS PLANTAS DANINHAS - SBCPD, 1995). This evaluation was performed at 7, 14, 21 and 28 days after application (DAA).

Variables related to agronomic performance (plant height, number of pods per plant, yield, and 100-seed mass) were evaluated.

The height was analyzed when the plants reached the R7 stage. For the determination of this variable, 10 plants were randomly selected in the working area of the plots, and measured using a millimeter ruler of wood, with the results expressed in centimeters. The number of pods per plant was evaluated at full maturity (stage R8) by manually counting the number of pods also in 10 plants randomly chosen in the working area of each plot.

Plants were harvested from the three central rows, discarding the first and last meter of the plot, totaling a harvested area of 4.05 m². The plants were at the R8 stage, that is, 95% of the pods had the typical mature pod color (FEHR et al., 1971). Pods were then threshed in an experimental thresher, cleaned with sieves and packed in paper bags for further analysis and evaluation. Grains produced in each plot had their mass measured and the moisture corrected to

13%, from these data we calculated the yield. For the 100-seed mass, the mass of two sub-samples were determined per plot and the moisture corrected to 13%.

The content of chlorophyll A, B and total chlorophyll was also evaluated in the two main experiments when the soybean was at the R2 development stage using an electronic chlorophyll content meter (ClorofiLOG). This device measures the absorption of light by the leaf at specific wavelength, the Falker chlorophyll content is determined through the absorption ratios at the different frequencies; this value is highly correlated with laboratory measurements and considers the presence of chlorophyll A and B (FALKER, 2009).

Germination and vigor of seeds were evaluated according to Brasil (2009) and electric conductivity of seeds according to Loeffler et al. (1988). The germination test was performed using four sub-samples of 50 seeds per field repetition of each treatment, placed to germinate between three sheets of filter paper, moistened with demineralized water, in the proportion of three times the weight of the dry paper. Rolls were made and taken to a germinator chamber set to maintain a constant temperature of 25°C. The evaluation was performed eight days after assembling the test, computing the percentage of normal seedlings obtained. The seed vigor test was analyzed in conjunction with the germination test, computing the percentage of normal seedlings obtained on the fifth day after assembling the test.

The electrical conductivity test was performed using four replicates of 50 seeds per field repetition of each treatment. The sub-samples were weighed on a precision scale, accurate to three decimal places. Then, they were soaked in a container with 75 mL distilled water and then kept in a germinator set at 25°C for 24 hours. After the period of the test, the electrical conductivity of the soaking solution was measured in a conductivity bridge (conductivity meter) with a constant electrode sensor 1.0. The result obtained in the conductivity meter was divided by the mass of each sub-sample, and the final result was expressed in dS m^{-1}. After met the basic assumptions for the analysis of variance, data were subjected to regression analysis ($p < 0.05$), according to Pimentel-Gomes and Garcia (2002).

RESULTS AND DISCUSSION

For the variables analyzed in experiment I, it was not possible to set a linear regression model for any of these variables according to the observed criteria (biological explanation, significant regression, non-significant regression deviations, coefficient of determination and residual analysis).

These results demonstrate the tolerance of the CD 250 RR/STS soybean cultivar to chlorimuron-ethyl, applied in post-emergence (V4) up to a dose of 60 g a. i. ha^{-1} (Table 1). Figure 1 shows the rainfall and temperature distribution throughout the period of the experiment in the field.

These results corroborate partially those found by Merotto Jr. et al. (2000), who verified a certain tolerance of soybean cultivars to the application of some sulfonylureas even before the STS technology. Reddy & Whiting (2000) observed that the yield of the DP 3571 S soybean cultivar (sulfonylurea tolerant) was not negatively influenced by the application of chlorimuron-ethyl (22 g a. i. ha^{-1}), in sequential application in early and late post-emergence.

The same trend was found for the other variables analyzed in experiment I; none of them showed a significant difference at 5% probability ($p < 0.05$), which allowed the adequate fit of a regression model.

Table 1. Response of the variables to the application of chlorimuron-ethyl (experiment I). Marialva, State of Paraná, 2011/12 growing season.

Rates (g a. i. ha^{-1})*	H	NPP	Yield	SM	CloA	CloB	CloT	EC	VIG	GER
0	64.50	18.79	1302.29	11.33	30.30	8.52	38.83	163.30	54.00	60.00
15	66.34	20.25	1249.40	11.47	30.68	7.77	38.43	146.76	64.25	70.00
30	66.34	21.54	1526.76	11.27	31.04	8.03	39.05	153.90	54.75	64.00
45	66.42	22.46	1459.65	11.64	31.33	8.28	39.60	183.18	49.00	56.25
60	66.79	21.29	1467.45	11.77	29.89	7.47	37.33	166.17	50.00	59.75
Mean	66.08	20.87	1401.11	11.50	30.65	8.01	38.65	162.66	54.40	62.00
C.V. (%)	6.10	11.90	16.33	5.01	4.35	6.23	4.17	30.58	39.02	30.39

*Grams of active ingredient per hectare.
H: plant height (cm), NPP: number of pods per $plant^{-1}$, yield (kg ha^{-1}), SM: 100-seed mass (g), CloA: chlorophyll A, CloB: chlorophyll B, CloT: total chlorophyll, EC: electrical conductivity (dS m^{-1}), VIG: vigor (%) and GER: seed germination (%).

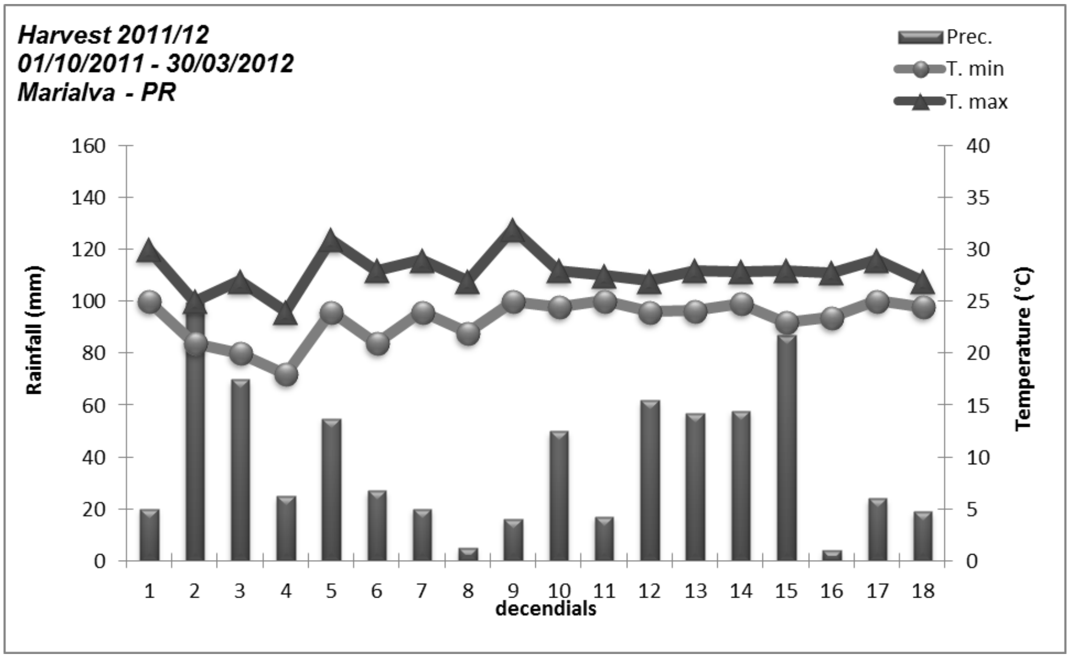

Figure 1. Rainfall, minimum average temperature and maximum average temperature for the period referring to the soybean crop cycle, in the municipality of Marialva, in the 2011/12 harvest. Source: INMET

For the application of metsulfuron-methyl rates, it was possible to fit a negative linear regression for the chlorophyll A, B and total, as shown in Figure 2, indicating minor injuries with increasing rates; these injuries were not perceived visually. However, the increase of rates of this herbicide did not significantly interfere with development and productivity, as well as with variables related to seed quality (Table 2).

With increasing rates of metsulfuron-methyl, there was a decline in chlorophyll in RR/STS soybean plants, demonstrating that high rates of this herbicide could cause injuries to the plant. Merotto Jr. et al. (2000) compared the tolerance of soybean cultivars to metsulfuron-methyl and found that even the cultivar tolerant to this herbicide showed yield at higher rates, which is related to the lower concentration of chlorophyll, causing chloroses.

Walker et al. (1994) analyzed soybean cultivars that showed tolerance to ALS inhibitors, and verified a decrease in initial plant growth at 10 days after application, but these plants recovered up at 21 and 30 DAA.

However, the low percentage of germination can be attributed to infestation of some fungi in seed storage, such as *Phomopsis* spp. and *Fusarium semitectum* (FRANÇA NETO & HENNING, 1992).

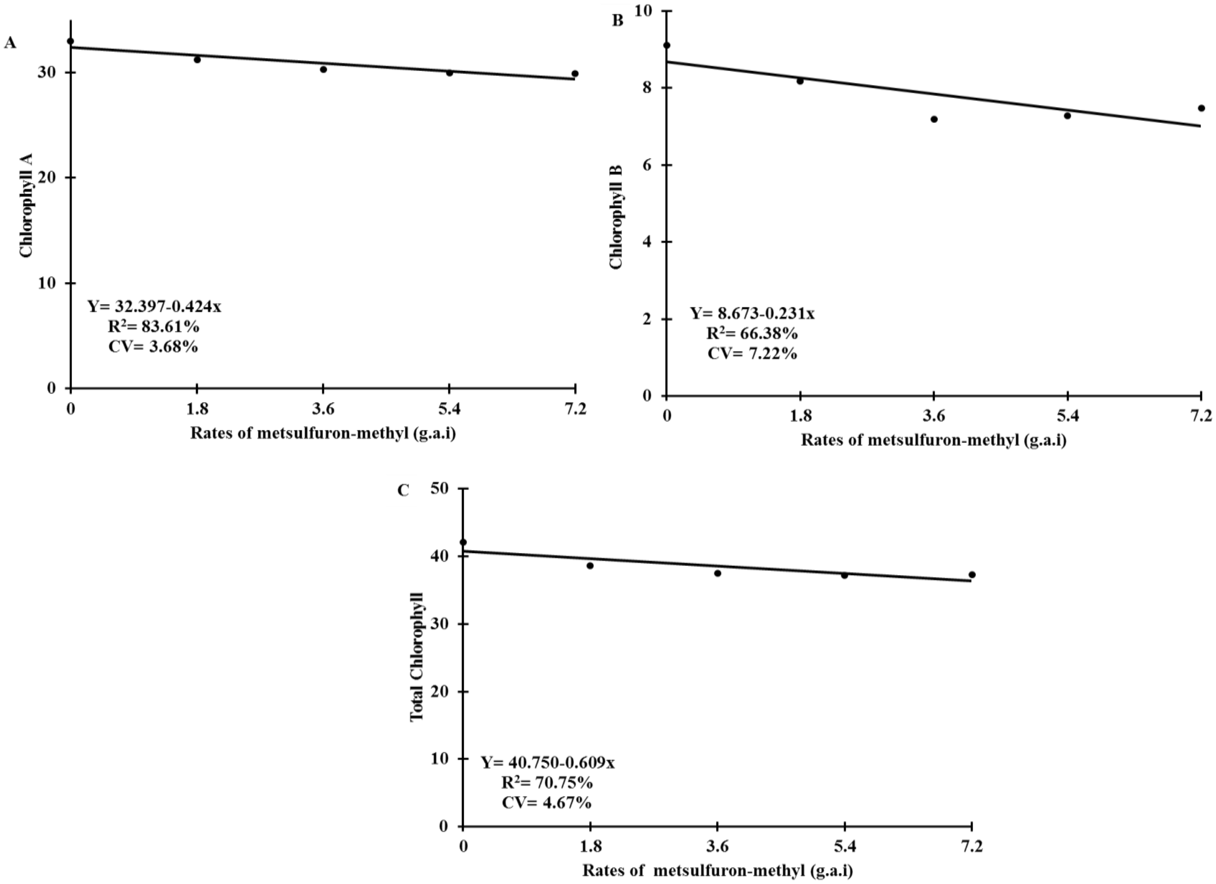

Figure 2. Variation in chlorophyll A (a), chlorophyll B (b) and total chlorophyll (c) of RR/STS soybean plants according to the application of rates of metsulfuron-methyl. Marialva, State of Paraná, 2011/12 growing season.

Table 2. Response of the variables to the application of metsulfuron-methyl (experiment II). Marialva, State of Paraná, 2011/12 growing season.

Rates (g a. i. ha^{-1})*	H	NPP	Yield	SM	CloA	CloB	CloT
0	68.58	23.59	1474.29	11.26	149.87	57.00	66.00
1.8	71.88	24.33	1368.29	10.51	187.06	49.50	57.25
3.6	68.38	24.34	1441.58	10.92	159.18	55.25	60.25
5.4	65.71	22.68	1197.38	10.81	167.64	56.75	63.00
7.2	67.38	22.67	1357.20	10.90	191.14	48.50	52.50
Mean	68.38	23.52	1367.75	11.08	170.98	53.40	59.80
C.V. (%)	4.32	12.94	15.22	6.31	21.44	33.85	28.48

*Grams of active ingredient per hectare.
H: plant height (cm), NPP: number of pods per $plant^{-1}$, yield (kg ha^{-1}), SM: 100-seed mass (g), CloA: chlorophyll A, CloB: chlorophyll B, CloT: total chlorophyll, EC: electrical conductivity (dS m^{-1}), VIG: vigor (%) and GER: seed germination (%).

When applied increasing rates of nicosulfuron, a positive linear regression was fit for electrical conductivity (Figure 3a), as well as a negative linear regression for vigor and seed germination (Figures 3b, c). However, for other variables analyzed in this experiment, it was not possible to fit a linear regression model for any of these variables according to the observed criteria (biological explanation, significant regression, non-significant regression deviations, coefficient of determination and residual analysis) (Table 3). These results demonstrate the tolerance of the CD 250 RR/STS soybean cultivar to the herbicide nicosulfuron, applied in a post-emergence (V4) up to a dose of 200 g i. a. ha^{-1}.

Manley et al. (2001) verified injuries in the cultivars W20 STS, Asgrow 9122 STS, Asgrow 3200 STS, Asgrow 4045 STS, caused by the application of nicosulfuron (35 g i. a. ha^{-1}) in early post-emergence. The percentages of injury varied from 24 to 45, however in general no reductions in yield were observed during the four years of cultivation.

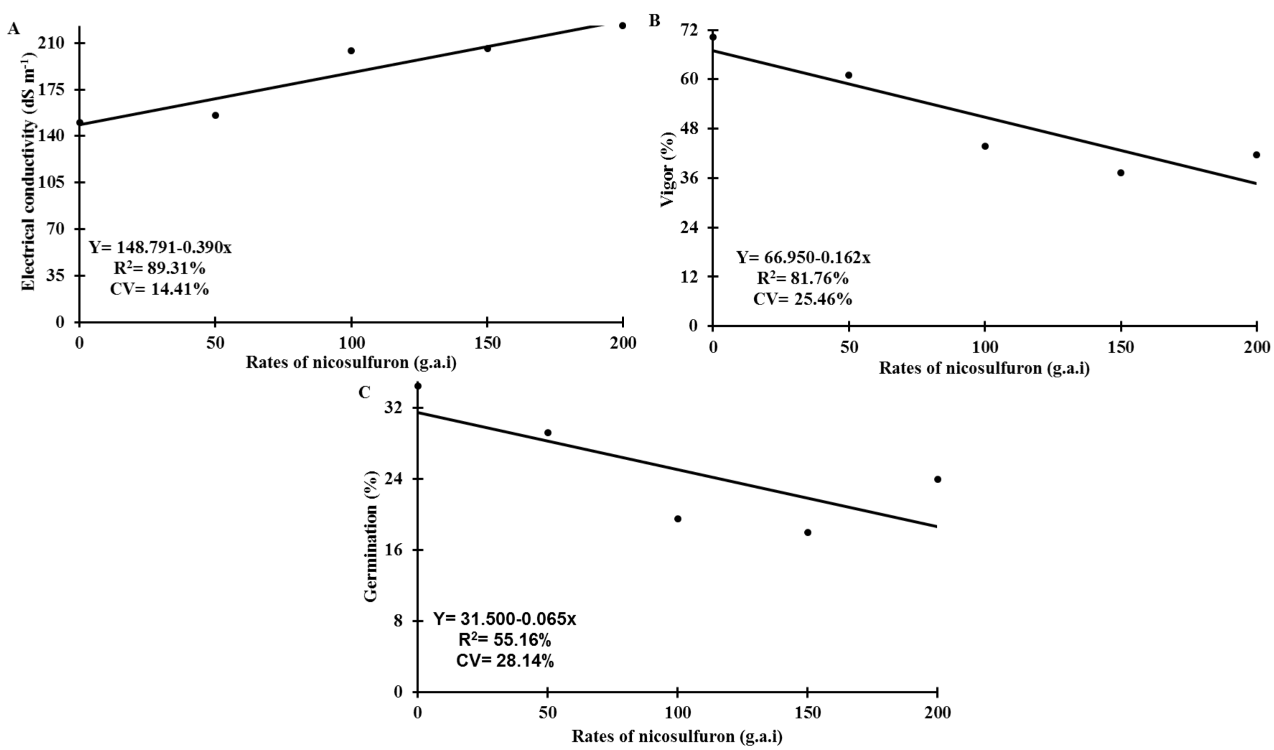

Figure 3. Variation in electrical conductivity (a), vigor (b) and germination (c) of seeds of RR/STS soybean plants according to the application of rates of nicosulfuron. Marialva, State of Paraná, 2011/12 growing season.

Table 3. Response of the variables to the application of nicosulfuron (experiment III). Marialva, State of Paraná, 2011/12 growing season.

Rates (g a. i. ha^{-1})*	H	NPP	Yield	SM	CloA	CloB	CloT
0	68.25	21.54	1438.00	11.79	29.61	8.02	37.63
50	67.79	23.35	1410.13	11.29	29.42	7.05	36.43
100	68.09	26.04	1306.33	10.97	29.45	7.57	36.95
150	66.83	24.63	1140.32	11.36	30.55	7.99	38.53
200	67.29	23.54	1137.31	11.48	29.65	8.19	37.83
Mean	67.65	23.80	1286.41	11.38	29.73	7.76	37.47
C.V. (%)	4.53	12.81	18.47	5.06	5.32	9.83	5.71

*Grams of active ingredient per hectare.
H: plant height (cm), NPP: number of pods per $plant^{-1}$, yield (kg ha^{-1}), SM: 100-seed mass (g), CloA: chlorophyll A, CloB: chlorophyll B, CloT: total chlorophyll, EC: electrical conductivity (dS m^{-1}), VIG: vigor (%) and GER: seed germination (%).

Higher rates of this sulfonylurea cause damage to the physiological quality of soybean seeds (non-STS cultivars), and a decrease in germination and vigor can be observed, as well as an increase in salinity through the measurement of the electrical conductivity, impairing the quality of this seed. High rates of this herbicide can cause deleterious effects even to plants that are tolerant to it, as observed by Dan et al. (2010) in maize hybrids subjected to high rates of nicosulfuron.

In order to evaluate the effect of the herbicide on the quality of seeds, it is necessary to consider the phenological stage of the plant when dealing with pre-harvest desiccation (GUIMARÃES et al., 2012). In the present case that employed technology that confers tolerance to the group of sulfonylureas, the factor that interfered with the quality was high rates of nicosulfuron.

Crop injury was evaluated in the three experiments at 7, 14, 21 and 28 DAA. However, no percentage scores were recorded, since the soybean plants showed no symptoms of injury, results in accordance with those observed for most of the other variables.

It should also be noted that the low yields for the three experiments can be explained by the low rainfall in the month of December, during the end of the vegetative period and the beginning of the soybean reproductive period.

The high tolerance of the CD 250 RR/STS cultivar to the application of chlorimuron-ethyl is shown, up to the maximum rate used (60 g a. i. ha^{-1}). The maximum recommended rate of chlorimuron-ethyl for non-STS soybean cultivars is 20 g i. a. ha^{-1} (RODRIGUES & ALMEIDA, 2011), that is, three times lower than the maximum dose tested in this study. According to Roso & Vidal (2011), STS cultivars tolerate rates up to four times higher than the maximum recommended dose of chlorimuron-ethyl. The herbicides metsulfuron-methyl and nicosulfuron have no records for application in soybean (RODRIGUES & ALMEIDA, 2011), nor is there an exact decision on the use of these and other sulfonylureas in STS soybeans. In general, the results presented herein indicate tolerance of the CD 250 RR/STS cultivar to the application of metsulfuron-methyl and nicosulfuron, up to the rates of 7.2 and 200 g a. i. ha^{-1}. Studies like this are of great importance in decision making about the use of sulfonylureas in STS soybeans.

Nevertheless, it is necessary to continue the investigation on STS materials, with the use of different sulfonylureas, different genotypes and multiple soil and climate conditions, aiming to gather information that may support the safe positioning of this technology, as there are rare reports in the literature on this technology, which has great potential of use by farmers in the management of weeds.

CONCLUSIONS

The CD 250 RR/STS soybean cultivar was tolerant to the herbicides chlorimuron-ethyl, metsulfuron-methyl and nicosulfuron at the recommended rates. However, with the addition of nicosulfuron and metsulfuron-methyl, injuries may be observed in some variables under the study conditions.

ACKNOWLEDGEMENTS

We would like to thank the farmers Alfredo Albrecht and Eduardo de Pintor for collaboration and support during the setup and conduction of the experiments.

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Recebido em: 3/8/2016
Aceito para publicação em: 8/5/2017