

Research Article

Soybean tolerance to sulfentrazone and diclosulam in sandy soil

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HIGHLIGHTS

- Sulfentrazone and diclosulam at doses of up to 200 and 35.3 g ha⁻¹, respectively, cause slight injury in soybean.
- Sulfentrazone, despite causing injury, did not affected soybean grain yield at labeled dose.
- In a rainy season, diclosulam impacted on soybean yield at the labeled dose in the evaluated cultivar.

ABSTRACT

Background: The use of pre-emergent herbicides is an important tool to control weeds, however the tolerance of soybean to these herbicides can vary according to the type of soil.

Objective: The aim of this study was to evaluate sulfentrazone and diclosulam soybean selectivity in a sandy soil, in order to establish these herbicides as supporting tools in weed control.

Methods: The treatments consisted of six doses of sulfentrazone (150 to 400 g ha⁻¹) and diclosulam (25.2 to 75.6 g ha⁻¹), and an untreated control. A field study was repeated in two growing seasons (2013/14 and 2014/15) in a sandy soil.

Results: The occurrence of injury depended on the growing season. The first season presented lower rainfall rates during the crop cycle. The recommended dose of sulfentrazone (200 g ha⁻¹) caused 26% and 10% of plant injury at 15 days after the treatment for the first and second season, respectively. For diclosulam, the recommended dose of 35.3 g ha⁻¹ caused 20% and 8% of plant injury, respectively, for the first and second season. However, at the recommended doses, only for diclosulam and in the rainy season there was a reduction in soybean productivity.

Conclusions: The herbicide sulfentrazone, although causing visual soybean plant injury, present satisfactory selectivity at recommended doses and can be used as an important tool on weed control on sandy soils. Similar response was observed for diclosulam in 2013/14. However, in a rainfall season diclosulam impacted on soybean yield at the recommended dose for sandy soils, with selectivity depending of the growth season.

1 INTRODUCTION

Herbicides are the main tool for weed control in soybean (*Glycine max* L. Merrill) in most areas of the world. With the advent and widespread adoption of

herbicide-tolerant (HT) crops, especially Roundup Ready® (RR) technology, weed control has been largely carried out with glyphosate, increasing the selection pressure of this herbicide in weed populations (Nandula, 2019). Herbicide tolerant

soybean is cultivated in several countries around the world, totaling an area of 69.7 million hectares (ISAAA, 2017). In Brazil, 33.7 million hectares were cultivated with HT soybean in 2017, representing 97% of the area cultivated with this crop (ISAAA, 2017). The rapid increase in the adoption of HT crops has occurred mainly because this technology simplifies weed control and decreases costs compared to other weed control alternatives (Zhou et al., 2016).

Glyphosate inhibits EPSPS (5-enolpyruvylshikimate-3-phosphate synthase). This herbicide is non-selective and systemic, mainly used in pre-sowing management in no-tillage systems and post-emergence of the RR crops (Fernández et al., 2015). Although glyphosate resistance evolution was considered unlikely (Bradshaw et al., 1997), the use of this herbicide as the primary weed control method resulted in the selection of resistant weeds, a few years after the beginning of glyphosate use in a large-scale and intensity (Baucom, 2016; Heap, 2020). Currently, there are 50 weed species resistant to glyphosate around the world, and nine glyphosate-resistant weed species in Brazil (Heap, 2020).

The strategies to prevent and manage herbicide resistance include crop rotation, integration of control methods, and mixture or sequential use of herbicides with different mechanisms of action in order to reduce the selection pressure exerted by a single herbicide (Baucom, 2016). Alternative pre-emergent soybean herbicides, which were widely used in the past, are considered an important weed control tool for areas with glyphosate resistance (Osipe et al., 2014; Nunes et al., 2018). The use of these chemistries was significantly reduced after the introduction of RR soybeans varieties.

In response to resistance, farmers have returned to using pre-emergent herbicides. In 2015, sulfentrazone was used in 17% of a total of 33.47 million hectares of soybean in the United States. In 2017, the use increased to 22% (USDA, 2019). In addition, pre-emergent herbicides such as metribuzin, S-metolachlor, and diclosulam have also become important alternatives for managing glyphosate-resistant weed species in soybean (Braz et al., 2017; Schryver et al., 2017). The use of pre-emergent herbicides increases weed control spectrum, reduces the size of weeds to be controlled in post-emergence, and improve weed control during the critical period of competition (Dan et al., 2010).

Herbicides such as sulfentrazone (protoporphyrinogen oxidase inhibitor, PPO) and diclosulam (acetolactate

synthase inhibitor, ALS) are alternatives for the management and prevention of glyphosate-resistant weeds (Constantin et al., 2018; Lopez-Ovejero et al., 2019). However, their adoption is hindered due to higher costs and potential crop injury, which is variable depending on the environment, mainly according to soil characteristics (Li et al., 2019). The potential crop injury is related to the prolonged soil persistence of these herbicides, which depends on factors such as dose, soil texture, organic matter content, soil moisture, temperature, microbial activity, pH, among others (Blanco and Velini, 2005; Monquero et al., 2013). However, the efficacy of these herbicides is also related to their behavior and residual activity in the soil (Santos et al., 2019), requiring adjustment of the used dose in order to obtain the desired weed control and to minimize crop injury.

The rapid and intense adoption of RR technology has allowed soybean-breeding programs to focus on high grain yield and other purposes, but tolerance to pre-emergence herbicides has not been considered. The actual need for pre-emergence herbicides requires the evaluation on different environments and cultivars in order to provide information not only about weed control, but also the effect of crop injury on soybean grain yield. This work had the hypothesis that although herbicides cause plant injury on soybean, it does not reflect in yield losses when the recommended dose is used for sandy soils. In this context, the aim of this study was to evaluate soybean tolerance to sulfentrazone and diclosulam in a sandy soil, in order to establish these herbicides as supporting tools in weed control.

2 MATERIALS AND METHODS

The study was carried out in Agronomic Experimental Station of the Universidade Federal do Rio Grande do Sul, Eldorado do Sul, Brazil, in two growing seasons, in 2013/14 and 2014/15. The soil of the experimental area was classified as a typical Dystrophic Red Argisol (Ultisol) (Streck et al., 2008), with 17% clay, 2.2% organic matter, pH 5.1, 36 mg dm⁻³ phosphorus, and 113 mg dm⁻³ potassium. The climate of the region is subtropical humid (Cfa) with warm summers, as defined by Köppen's climate classification. The main weather conditions of the two growing seasons are presented in Figure 1. Meteorological data were obtained from weather station located inside of the Agronomic Experimental Station.

In both years, the experiments were installed in areas cultivated with ryegrass during the winter. The

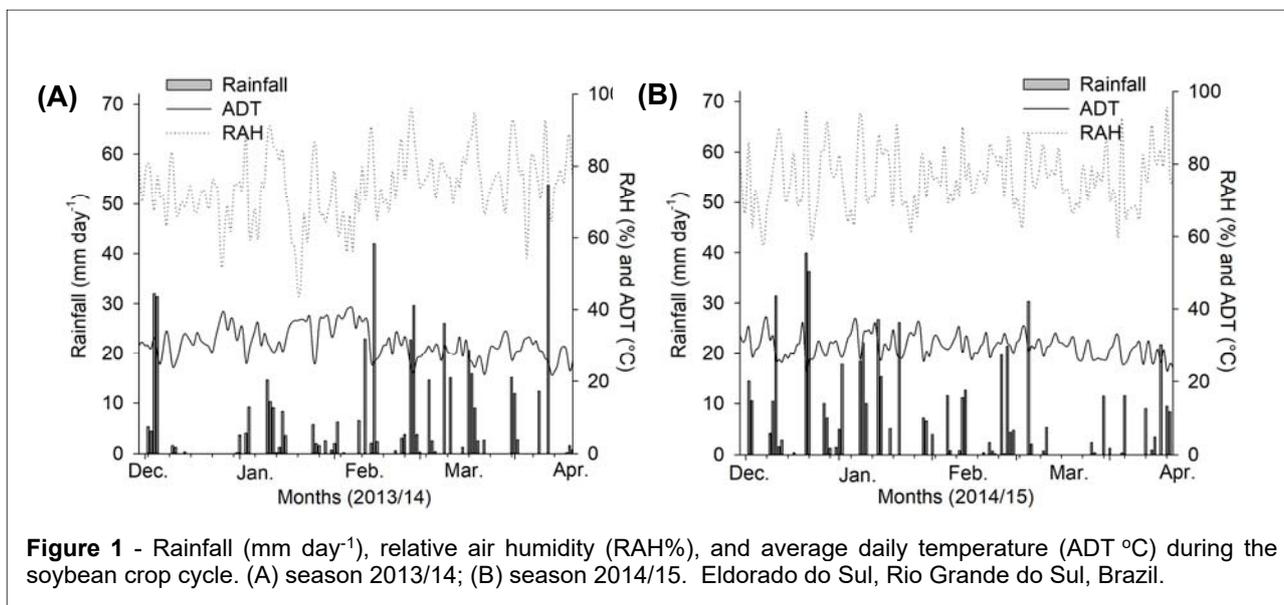


Figure 1 - Rainfall (mm day⁻¹), relative air humidity (RAH%), and average daily temperature (ADT °C) during the soybean crop cycle. (A) season 2013/14; (B) season 2014/15. Eldorado do Sul, Rio Grande do Sul, Brazil.

experiments were carried out within the same field, but in different areas each year. 20 days before sowing, the area was desiccated with glyphosate (1,080 g a.e. ha⁻¹). The experiments were performed using a randomized complete block design with four replications. The experimental units consisted of plots with five rows of soybean, with a spacing of 45 cm and a length of 6 m. In both seasons, sowing was performed in early December, and seedling emergence occurred five days later. The soybean cultivar used was SYN 1059 RR, at a seeding rate of 300,000 seeds ha⁻¹. The fertilizer used was based on the soil analysis and consisted of 300 kg ha⁻¹ of 05-20-20 (N-P₂O₅-K₂O). Seeds were treated with a combination of fungicide (triadimenol 40.5 g 100 kg⁻¹ of seeds) and insecticide (imidacloprid 100 g 100 kg⁻¹ of seeds). In addition, the seeds were inoculated with *Bradyrhizobium japonicum* (Nitragin Cell Tech HC, 300 mL 100 kg⁻¹ of seeds), applied at the time of seeding. Insect control was performed with *Bacillus thuringiensis* (17 g ha⁻¹) and lambda-cyhalothrin (10 g ha⁻¹). The other phytosanitary managements were carried out following monitoring and technical recommendations for the crop.

The treatments consisted of sulfentrazone (Boral 500 SC, 500 g L⁻¹, FMC Química do Brasil) at 150, 200, 250, 300, 350, and 400 g ha⁻¹, diclosulam (Spider 840 WG, 840 g L⁻¹, Dow AgroSciences Industrial Ltda.) at 25.2, 35.3, 45.4, 55.4, 65.5, and 75.6 g ha⁻¹, and a non-treated control. In all plots, including non-treated control, emerged weeds were periodically removed manually, resulting in a completely weed-free experiment. The treatments were applied one day after sowing with a CO₂-pressurized backpack sprayer equipped with a spray boom with four flat-fan nozzles (DG 110.015),

with constant pressure of 220 kPa and a speed of 1.0 m s⁻¹, which resulted in a spray volume of 200 L ha⁻¹.

Evaluations of plant injury were performed at 15, 30, 45, and 60 days after treatments (DAT), with visual assessment using a percentage scale, in which 0 (zero) means no plant injury and 100% represents plant death. Grain yield was determined from the 5 m harvest of the three central lines (6.75 m²). In the second year of the study, were included evaluations of shoot height (SH) from soil level to the last productive node at 15 and 30 days after seeding (DAS), and shoot dry mass (SDM) at the full bloom (R2) stage in 20 plants per plot. Yield components related to number of pods plant⁻¹ (NPP), and mass of grains pod⁻¹ (MGP) were evaluated based on plants sampled in 2 m of the central row.

The obtained data were analyzed in terms of normality (Shapiro-Wilk test) and, subsequently, submitted to analysis of variance (ANOVA) (p≤0.05) through SAS software version 8.0. In case of statistical significance, a non-linear regression was used to adjust the data to a sigmoidal model, using SigmaPlot software version 10.0 (Systat Software Inc 2006). The association between plant injury and grain yield was determined by regression analysis, and the degree of correlation (r) was analyzed by the t test.

3 RESULTS AND DISCUSSION

The ANOVA indicated significant effect (p≤0.05) for the doses of herbicides and growing seasons. The plant injury caused by sulfentrazone increased with increasing doses, ranging from 3 to 75% in the first season (2013/14) (Figure 2). At 15 and 30 DAT, plant injury of 42.5 and 36.6%, respectively, was

observed for the rate of 300 g ha⁻¹. The application of sulfentrazone at doses greater than recommended, which ranges from 200 to 300 g ha⁻¹, intensified the plant injury. Sulfentrazone at 350 and 400 g ha⁻¹ resulted in an increase in injuries to 63% and 76%, respectively, at 30 DAT (Figure 2B).

However, in 2014/15 season, plant injury caused by sulfentrazone did not reach 10%, regardless the dose used (Figure 2). This fact can be explained by the different rainfall conditions during the crop development in the two evaluated seasons (Figures 1A and 1B). In the first season (2013/14), rain precipitation was lower and less frequent compared to the second season (2014/15) (Figure 1). In a similar study performed with recommended doses of 200 and 300 g ha⁻¹ of sulfentrazone, only slight chlorosis was observed in the leaves at 7 and 15 DAT (Osipe et al., 2014). However, in addition to the difference in rainfall pattern, this study was conducted

in soil with 65% of clay, which results in higher adsorption to the soil colloids and in a smaller amount of herbicide in the soil solution compared to the soil of the present study, which had 17% clay.

Plant injury caused by diclosulam was lower in comparison to that caused by sulfentrazone, not exceeding 47% in the highest evaluated dose (Figures 2 and 3). In general, plant injury was more severe during the 2013/14 season, reaching values higher than 15% at 30 DAT in response to 35.3 g ha⁻¹ (Figure 3B). Considering that same dose at 45 and 60 DAT plant injuries decreased to 13% and 10%, respectively (Figures 3C and 3D). The recommended dose of diclosulam ranges from 25.2 to 35.3 g ha⁻¹.

In the 2014/15 season, plant injury was 45% at the highest herbicide dose (75.6 g ha⁻¹) at 30 DAT. However, at 45 and 60 DAT, no significant plant injury was observed, unlike in the 2013/14 season, regardless of the doses used (Figures 3C and 3D).

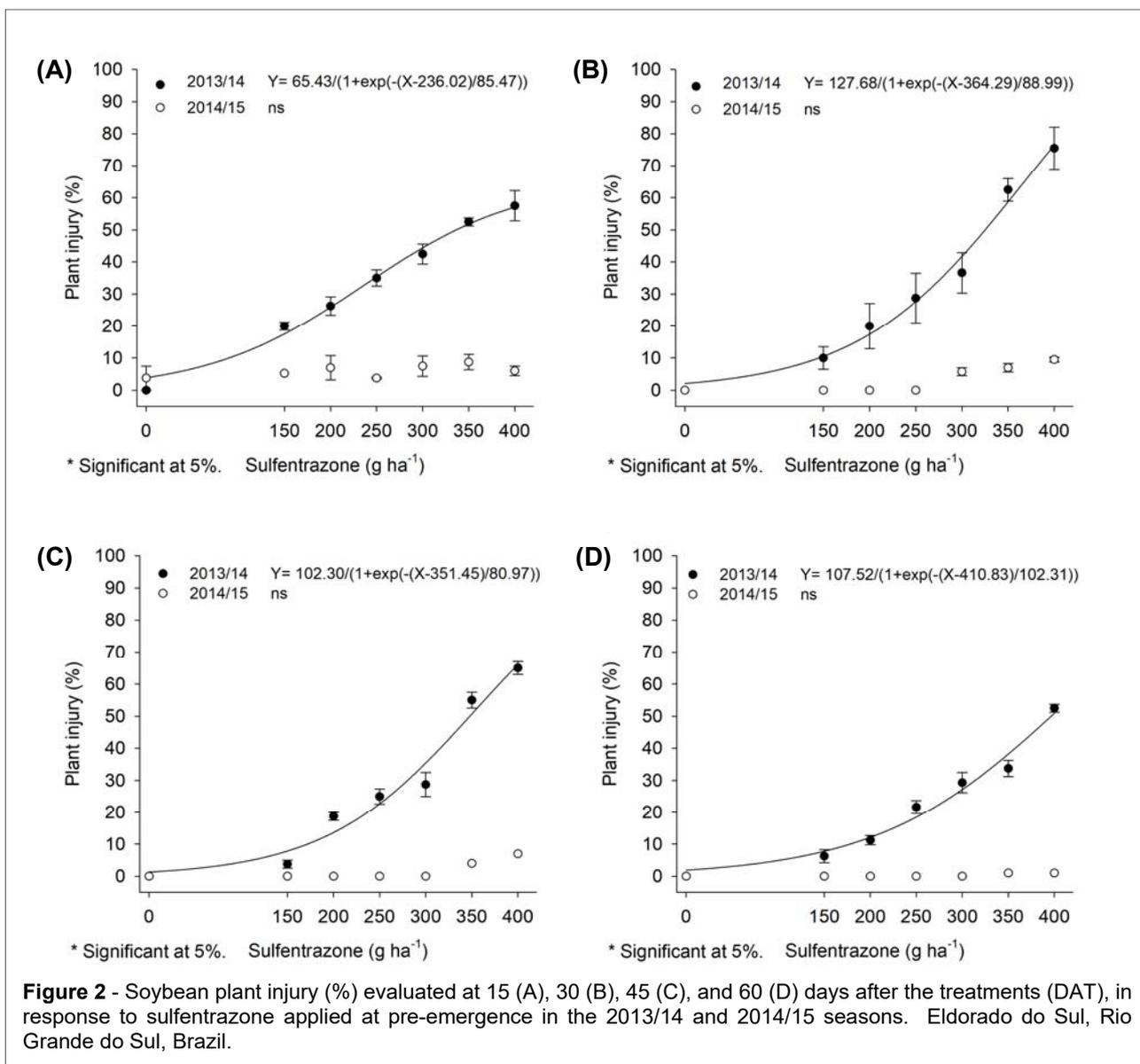


Figure 2 - Soybean plant injury (%) evaluated at 15 (A), 30 (B), 45 (C), and 60 (D) days after the treatments (DAT), in response to sulfentrazone applied at pre-emergence in the 2013/14 and 2014/15 seasons. Eldorado do Sul, Rio Grande do Sul, Brazil.

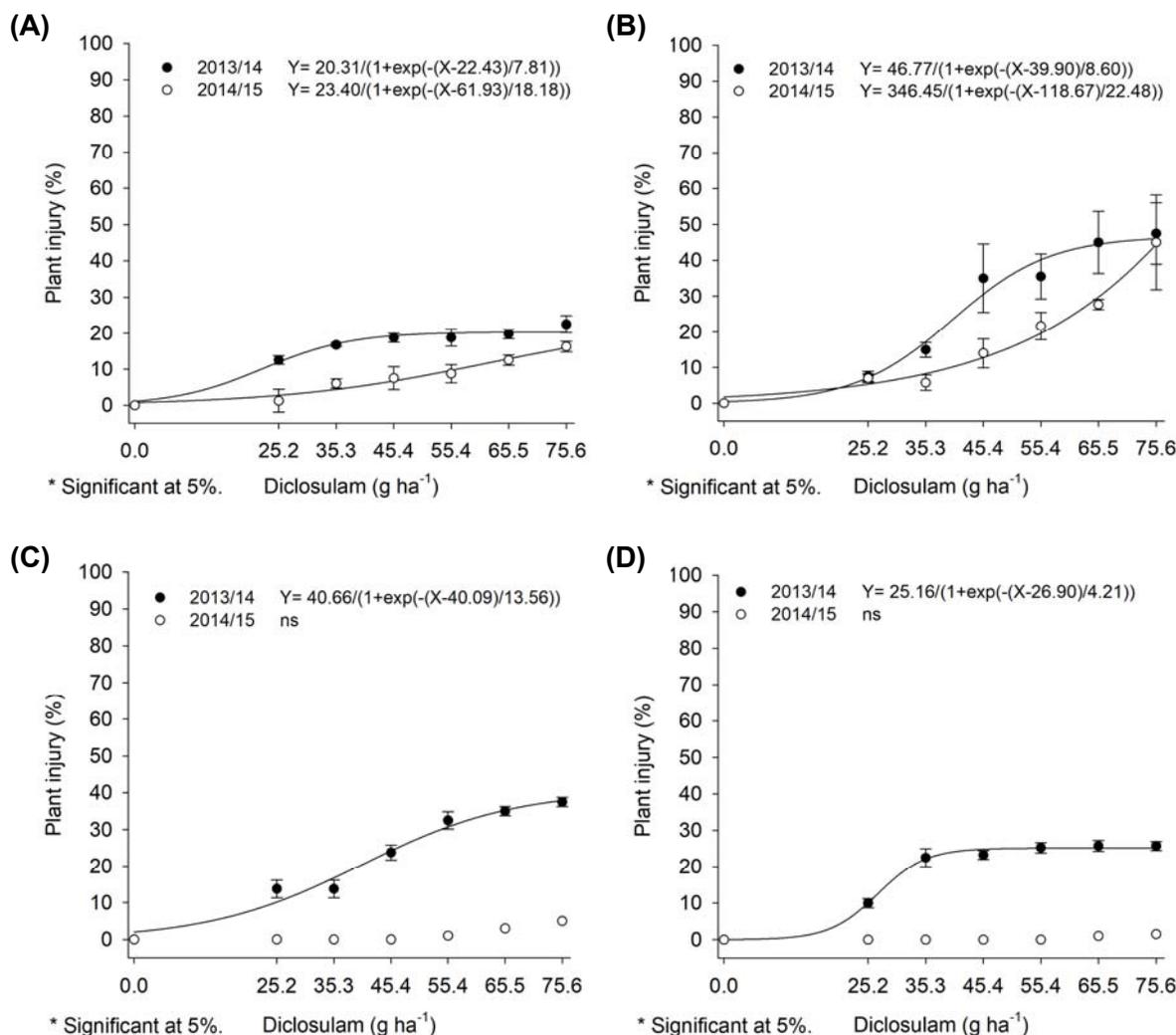


Figure 3 - Soybean plant injury (%) evaluated at 15 (A), 30 (B), 45 (C), and 60 (D) days after the treatments (DAT) in response to diclosulam applied at pre-emergence in the 2013/14 and 2014/15 seasons. Eldorado do Sul, Rio Grande do Sul, Brazil.

Similar to the observations for sulfentrazone, the highest rainfall in the season 2014/15, especially after the second evaluation (30 DAT), may have contributed to the lower availability of the herbicide in the soil and reduced plant injury in the evaluation at 45 DAT. These year-dependent results are similar to those found in studies conducted at five sites in the 2009/10 and 2010/11 seasons, which presented, respectively, 50 and 0% of plant injury at 14 DAT, using a rate of 25 g ha⁻¹ of diclosulam (Lopez-Ovejero et al., 2013).

The reduction of crop injury in the second season (2014/15) can be related with the increase of herbicide degradation and percolation in the soil profile due to higher rainfall (Figure 1). The microbiological activity related with the degradation of these herbicides is potentialized under conditions of higher water availability (Reddy and Locke, 1998). In addition, sulfentrazone presented greater percolation in the

soil profile with increased rainfall, reaching a depth of 40 cm for sandy soils (Monquero et al., 2010). Therefore, the reduction of herbicide availability in the soil surface may be the cause for lower plant injury in the second season. On the other hand, under lower water availability, percolation is less pronounced, leading to a higher concentration of herbicides in the soil surface where the initial development of the plants occurs, resulting in greater plant injury. Although the plant injury for diclosulam was lower in the second season, increased injury may occur under higher water availability, since this herbicide is more available in the soil solution and presents less percolation than sulfentrazone, which is reflected in other variables of crop development, such as plant height and dry matter (Monquero et al., 2010, 2013).

Absence of effect on soybean shoot height at 15 DAT was observed by diclosulam and sulfentrazone. However, shoot height at 30 DAT was reduced for both

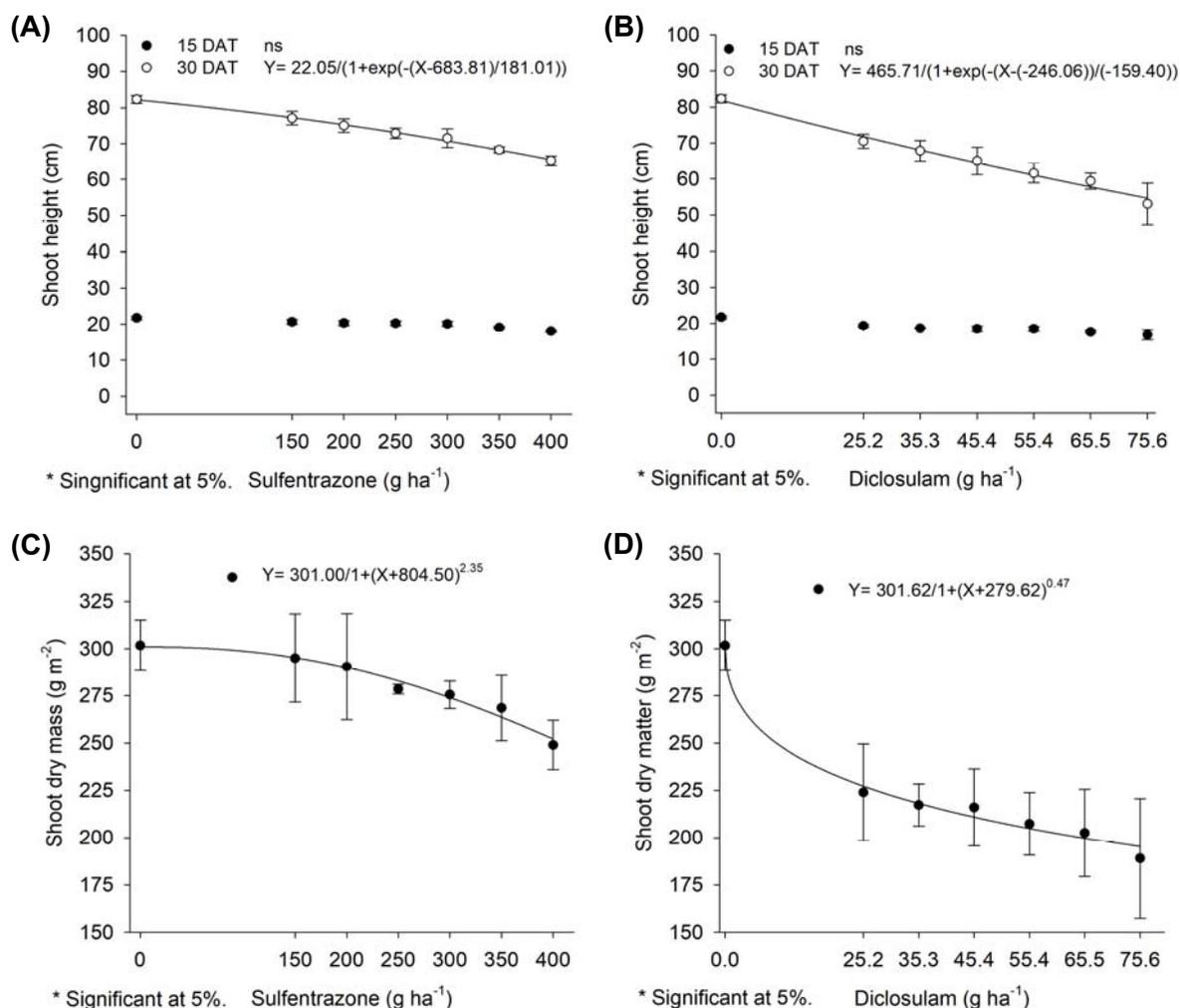


Figure 4 - Soybeans shoot height (SH) evaluated at 15 and 30 days after the treatments (DAT) and shoot dry mass (SDM) at the full bloom (R2) in response to sulfentrazone (A and C) and diclosulam (B and D) applied at pre-emergence in the 2014/15 season. Eldorado do Sul, Rio Grande do Sul, Brazil.

herbicides. The reduction caused by sulfentrazone was around 21% (-17.1 cm) for the highest dose of the herbicide (400 g ha⁻¹) when compared to the non-treated plots (Figure 3A). The application of the highest dose of the herbicide diclosulam (75.6 g ha⁻¹) reduced shoot height by approximately 36% (-29 cm) at 30 DAT, demonstrating the potential of damage on crop development (Figure 3B). However, the recommended doses for sulfentrazone (300 g ha⁻¹) and diclosulam (25.2 g ha⁻¹) caused less impact on soybean plant height, with reductions of approximately 13% (-10.6 cm) for both herbicides.

The accumulation of shoot dry matter (SDM), evaluated at the flowering stage, decreased by 17.5% and 37.4% for the higher doses of sulfentrazone and diclosulam, respectively (Figures 4C and 4D). The reduction of SDM by diclosulam was already pronounced at the lowest rate used (25.2 g ha⁻¹) compared with the untreated control, presenting a SDM accumulation of 225.0 and 301.75 g m⁻², respectively, which represents approximately 25% reduction

compared to the non-treated control. Sulfentrazone showed a smaller effect on SDM (Figure 4C). At the recommended doses of sulfentrazone of 200 and 300 g ha⁻¹ SDM decreased by 4.7 and 8.7%, respectively. At the highest dose evaluated (400 g ha⁻¹), the reduction in SDM was approximately 17%. In sensitive soybean cultivars, the application of 420 g ha⁻¹ of sulfentrazone caused a 56% reduction in SDM in soil with 76% silt, 16% clay, and 0.9% organic matter (Swantek et al., 1998).

The grain yield components number of pods per plant (NPP) and mass of grains pod⁻¹ (MGP) were also significantly reduced by the herbicides doses increment (Figure 5). The lower dose of sulfentrazone, which corresponded to 150 g ha⁻¹, did not cause a reduction in the NPP. However, the reduction was around 10 and 15 pods plant⁻¹ for treatments receiving 300 and 400 g ha⁻¹ of sulfentrazone, respectively (Figure 5A). The herbicide diclosulam showed a similar effect, with no significant NPP reduction for the lowest dose (25.2 g ha⁻¹) and a reduction of around 9

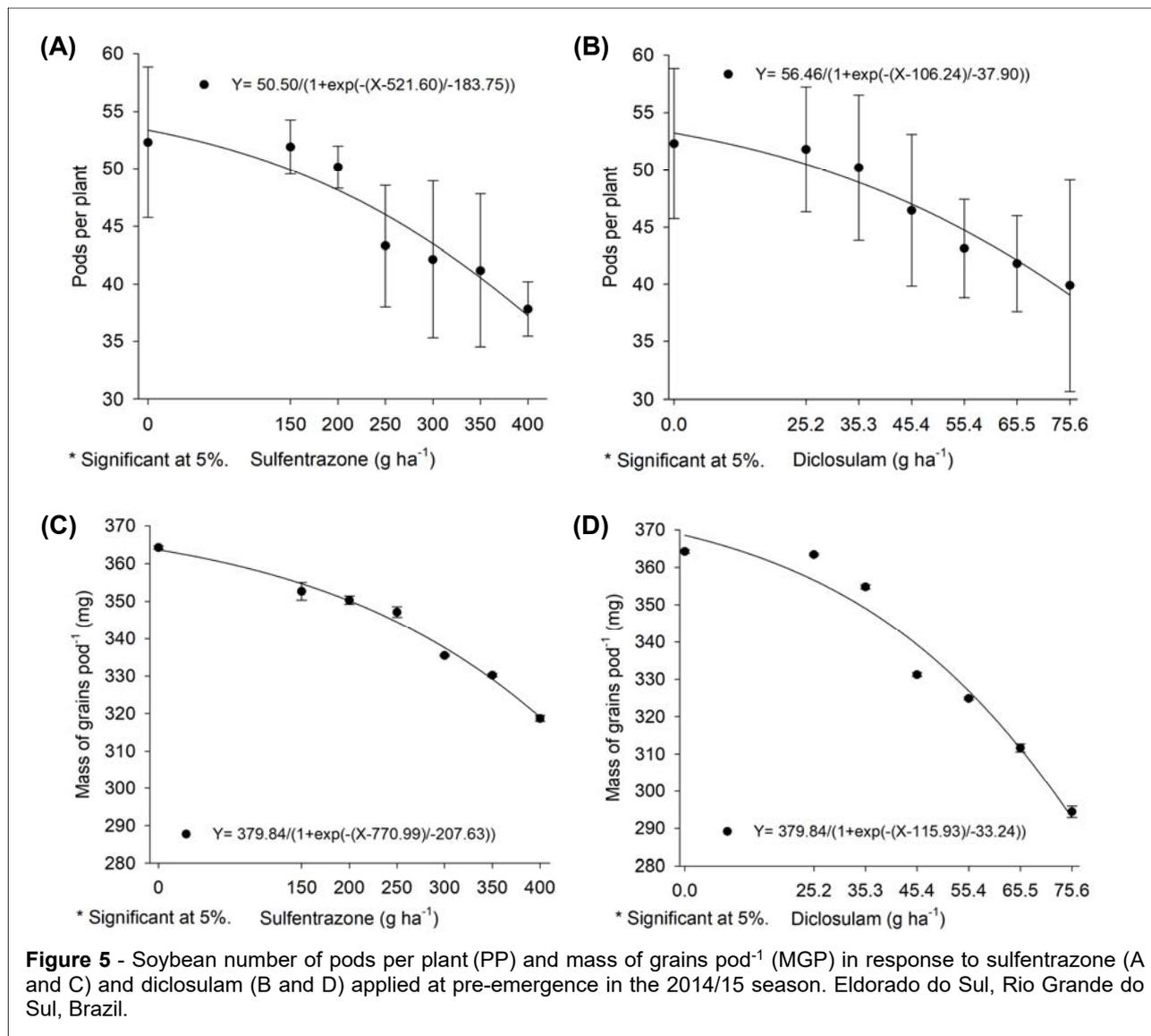


Figure 5 - Soybean number of pods per plant (PP) and mass of grains pod⁻¹ (MGP) in response to sulfentrazone (A and C) and diclosulam (B and D) applied at pre-emergence in the 2014/15 season. Eldorado do Sul, Rio Grande do Sul, Brazil.

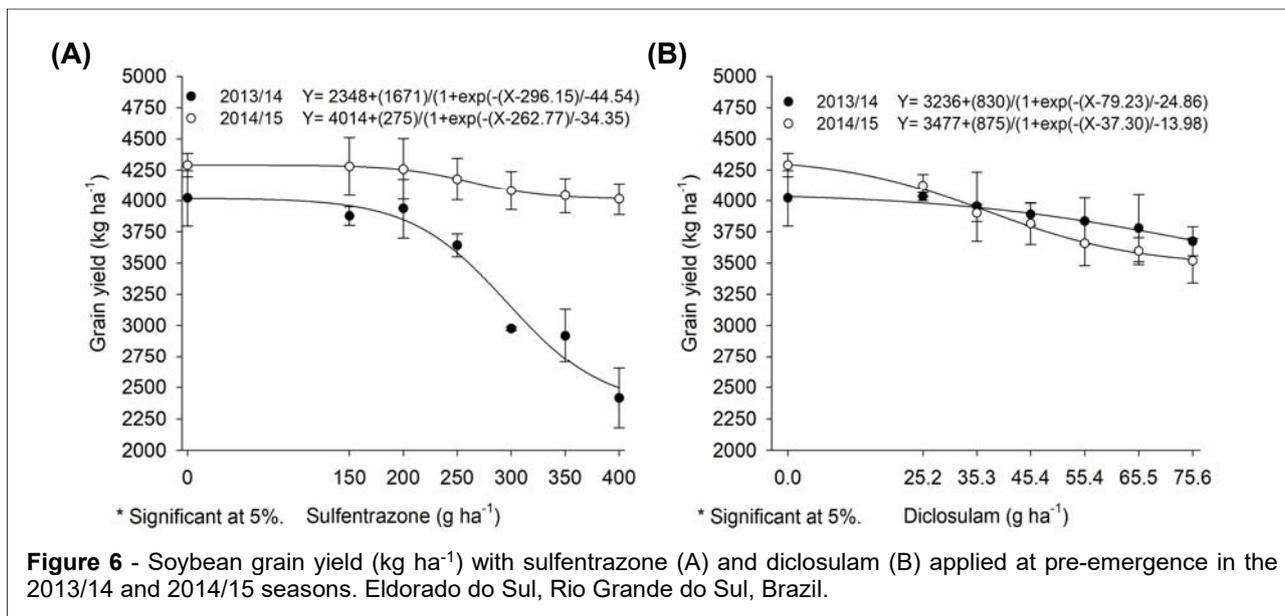
and 14 pods plant⁻¹ at the doses of 55.44 and 75.60 g ha⁻¹, respectively. The absence of effect on the number of pods per plant in soybean has also been reported for the use of 25.2 g ha⁻¹ of diclosulam in a study in two seasons in India (Singh et al., 2009).

Both herbicides interfered negatively on MGP (Figure 5C and 5D). For the herbicide sulfentrazone, the reduction was 28.75 and 45.56 mg pod⁻¹ for the doses of 300 and 400 g ha⁻¹, respectively, when compared to the untreated control (Figure 5C). The herbicide diclosulam showed a higher effect on this variable, causing a reduction of 39.37 and 59.91 mg pod⁻¹ at the doses 55.44 and 75.60 g ha⁻¹, respectively (Figure 5D).

Soybean grain yield was affected similarly to plant growth and grain yield components, with higher reductions for sulfentrazone in the first season (Figure 6A). In the absence of herbicides, soybean yield reached 4,020 and 4,290 kg ha⁻¹ in the 2013/14 and 2014/15 seasons, respectively. The grain yields

obtained in response to 400 g ha⁻¹ of sulfentrazone were 2,415 and 4,014 kg ha⁻¹ for the seasons 2013/14 and 2014/15, respectively. This corresponds to reductions of 40 and 6.5% in the grain yield for the first and second seasons, respectively. In the season 2013/14, doses from 250 g ha⁻¹ of sulfentrazone caused grain yields reductions statistically different of the control plots. However, for the season 2014/15, the doses capable of causing the reduction in grain were equal or higher than 350 g ha⁻¹ of sulfentrazone. The recommended doses for this herbicide, which range from 200 to 300 g ha⁻¹ for sandy and clayey soils, respectively.

The variability of the effects of sulfentrazone on grain yield was also identified in other studies. The absence of effects on soybean grain yield is reported for the use of 300 and 400 g ha⁻¹ of sulfentrazone (Lopez-Ovejero et al., 2013; Osipe et al., 2014). However, in both cited experiments clay content was greater than 65%, with organic matter contents greater than 2.2%. A series of studies in soils with



different textures (from clayey to sandy soils) showed that there was no significant effect of sulfentrazone on soybean grain yield, even at a dose of 840 g ha⁻¹, which is above the recommended dose (Mahoney et al., 2014). In this study, water availability was not evaluated, but in most areas, the organic matter content was high, reaching 6.5%, which may be related with the obtained results.

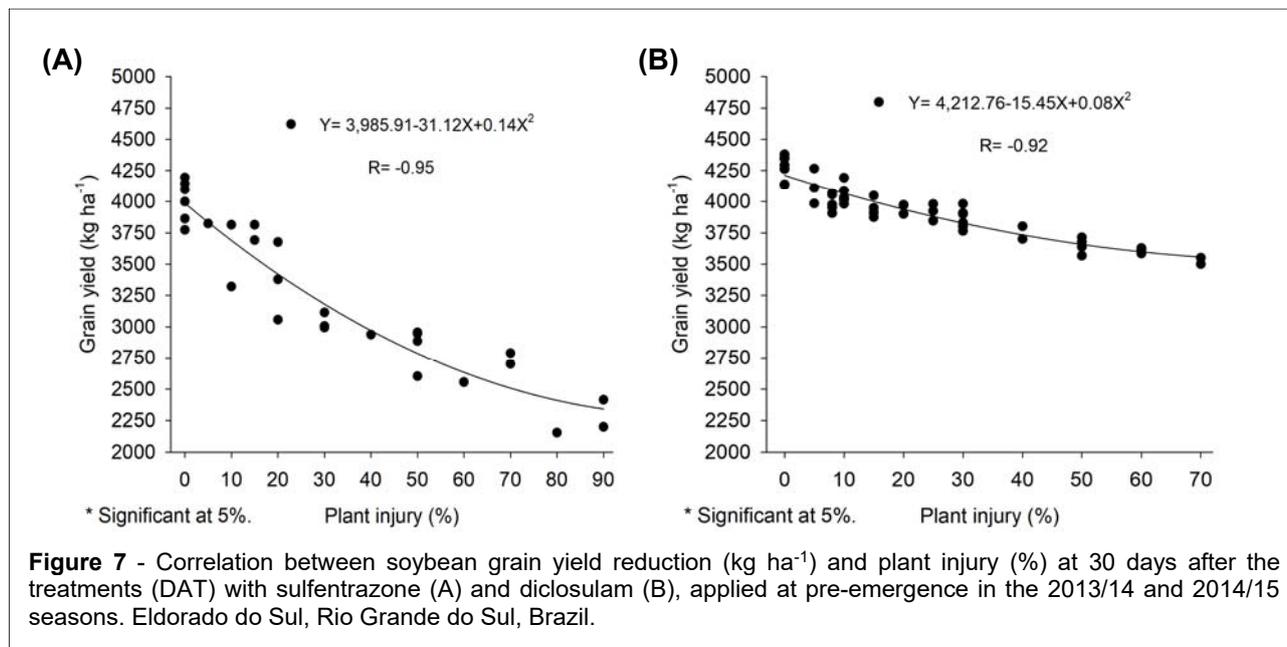
Another important factor to consider is the susceptibility of the cultivar to the herbicide sulfentrazone. In a study carried out with several soybean cultivars, there was a greater variation in plant injury in response to this herbicide (Swantek et al., 1998). In this study, plant survival was reduced in 17 and 35% for cultivars considered tolerant and susceptible, respectively, with the application of 420 g ha⁻¹ of sulfentrazone in pre-emergence. These results indicate the necessity of soybean genotype effect evaluations on the tolerance to sulfentrazone and other pre-emergent herbicides. In addition, it is also important to consider the pre-emergence herbicide tolerance in the current soybean breeding programs, similar to what occurred before the development of transgenic herbicide-resistant soybean.

Soybean grain yield was not affected by recommended doses (25.2 to 35.3 g ha⁻¹) of diclosulam at 2013/14 season (Figure 6B). This indicates that the soybean recovered from the injury observed during the initial growing period (Figure 3). However, at 2014/15 season doses from 35.3 g ha⁻¹ of diclosulam caused grain yields reductions compared to untreated control, for this dose the reduction was 388.21 kg ha⁻¹, corresponding to 9.05% (Figure 6B). The selectivity observed in the

first season are similar with other studies regarding the absence of effect on grain yield in response to diclosulam at 25.2 g ha⁻¹ (Lopez-Ovejero et al., 2013; Osipe et al., 2014). On the other hand, Fornazza et al. (2018) observed a reduction of 8 to 11% in soybean grain yield with the application of diclosulam (25.2 g ha⁻¹) and sulfentrazone (300 g ha⁻¹) in pre-emergence of early soybean cultivars in soil with clayey texture. Similarly, Constantin et al. (2018) observed a reduction in soybean grain yield in this same dose applied in pre-emergence of the crop.

Grain yield presented an inverse response to the herbicides sulfentrazone and diclosulam, but was variable depending of the growth season. In the 2013/14 season, with lower rainfall occurrence, the treatments that received sulfentrazone had lower grain yield in relation to the season 2014/15, which was rainier (Figures 1 and 6A). On the other hand, higher grain yield was obtained for diclosulam in the first year of the research (Figure 6B).

The plant injury of diclosulam in conditions of high-water availability was reported by Monquero et al. (2013), similar to what occurred in this study during the 2014/15 season. This fact can be related to the greater sorption of the herbicide diclosulam in relation to sulfentrazone (Koc diclosulam: 90 mL g⁻¹; Koc sulfentrazone: 43 mL g⁻¹) (Senseman, 2007). The lower adsorption of sulfentrazone favors leaching, causing less plant injury effects at rainy years. On the other hand, rainy periods favor the desorption of diclosulam, increasing its concentration in the soil solution. In fact, compared to untreated control, grain yield decreased in both years of the study in response to diclosulam. In the first year the reduction in grain yield occurred in doses from



75.6 g ha⁻¹, while in 2014/15 the reduction occurred from 35.3 g ha⁻¹ (Figures 1 and 6B).

Soybean grain yield and plant injury caused by the pre-emergent herbicides, evaluated at 30 DAT, were inversely correlated by a quadratic model, with correlation coefficient (R) of -0.95 and -0.92 to sulfentrazone and diclosulam, respectively (Figure 7). Great effect of plant injury on grain yield was observed for sulfentrazone (Figure 7A) in comparison with diclosulam (Figure 7B). Considering the correlation analysis between grain yield and injury at 30 DAT, it is observed that for sulfentrazone the injury of up to 7% did not result in a significant reduction (5%) in grain yield (Figure 7A). For diclosulam, the tolerable injury was greater, since injuries of up to 15% did not result in a significant decrease in grain yield (Figure 7B).

4 CONCLUSIONS

The herbicide sulfentrazone at doses up to 200 g ha⁻¹ cause slight plant injury in soybean SYN 1059 RR, with lower effects in growing season with the highest rainfall. For this herbicide the soybean grain yield was not limited when the recommended doses were applied. Thus, the herbicide sulfentrazone, although causing visual soybean plant injury, present satisfactory selectivity at recommended doses and can be used as an important tool on weed control on sandy soils, with similar conditions such as tested in this study. Similar response was observed for diclosulam in 2013/14. On the other hand, the herbicide diclosulam can impact on soybean yield in a rainfall season at the recommended dose for sandy soils, with selectivity depending of the growth season.

5 CONTRIBUTIONS

All authors participated equally in the study, from the conception, installation and execution of the experiment, as well as in the preparation of the manuscript.

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