Australian Journal of Crop Science

AJCS

AJCS 13(08):1251-1257 (2019) ISSN:1835-2707
doi: 10.21475/ajcs.19.13.08.p1527

Effects of glyphosate and foliar fertilizers on the glyphosate resistant (GR) soybean

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Abstract

Currently the soybean crop is responsible for a great share of the protein consumed by humans and animals, it is also a source of oil and renewable materials for the industry. Due to the importance of soybeans worldwide, especially genetically modified soybeans (resistant to glyphosate - GR), the improvement in yield indexes became the targets of promising research. The objective of this work was to evaluate the responses of the "GR" soybean crop as a function of glyphosate herbicide and foliar fertilizer, under soil without nutrient deficiency. The experiment was conducted in a randomized block design with four replications, consisted of two factors. Factor A was consisted by glyphosate (1080 and 2160 g a.e ha^{-1}) and factor B by foliar fertilizers (Fertilizer A – Mg= 5%, Mn= 0,5%, Mo= 0.5%, L-Glutamic acid= 5% and glycine betaine 3%; Fertilizer B - Zn (5%), Mn (3%), Cu (0,5%), B (0.5%) and S (4%); Fertilizer C - Mo and L-Glutamic acid). The treatments were applied isolated and in mixtures, plus the control treatment, totalizing 21 treatments. The variables evaluated were phytotoxicity at 14 and 21 DAT, a thousand-grain weight and grain yield. The analysis of variance was performed and, when significant, the comparison of groups of treatments using orthogonal contrasts was applied to all variables. Soybean yield was also compared to the means of the treatments by the Scott-Knott test ($p \leq 0.05$). Phytotoxicity was detected in the soybean crop at 14 DAT with the application of glyphosate, foliar fertilizers and the association of the two products. However, only the highest dose of glyphosate, with or without foliar fertilizers, generally reduced crop grain yield. In addition, the application of foliar fertilizers isolated and associated with glyphosate, in soils with adequate levels of nutrients, does not increase thousand-grain weight or grain. It is concluded that the glyphosate dose for soybeans should be respected and foliar fertilizers should be carefully studied before recommendation.

Keywords: foliar fertilizers; orthogonal contrasts; soybean grain yield; soybean herbicide injury.
Abbreviations: GR_ Glyphosate resistant, BNF_ Biological nitrogen fixation, DAT_ Days after treatment, a.e_ acid equivalent, a.i_ active ingredient, Mo_ molybdenum, N_ Nitrogen, Mn_ Manganese, Cu_ Cupper, Zn_ Zinc, Fe_ Iron.

Introduction

Globally, soybeans cover an area of 126 million hectares, yielding nearly 350 million tons of grains. United States is the largest producer and Brazil appears in the second position, with estimated production of 108 million tons, corresponding to approximately 35 million hectares (USDA, 2018). Oil and meal are the major byproducts of soybeans, more recently research has raised soybeans as a renewable material source for the industry and will become the main source of protein for humans and animals diet (Liu, 2016; Miransari, 2016). However, a great challenge to obtain high yields are to deal with abiotic stresses like pests, diseases and weed plants.

To control weeds in soybean, the main herbicide used is glyphosate. This herbicide is systemic, non-selective, low phytotoxic to glyphosate resistant crop (GR) at the recommended doses, besides presenting a broad spectrum of action, allowing efficient control of grasses and broadleaf plants (Rodrigues and Almeida, 2011; Merotto et al., 2015). However, the increase use of this herbicide during the soybean cycle can negatively affect plant nutrition, especially N, Mn, Cu, Zn and Fe, biological nitrogen fixation (BNF), and yet in the chlorophyll content (Serra et al., 2011, Zobiole et al., 2011, Fan et al., 2017).
The damage diagnosis of soybean after application of glyphosate called *yellow flashing* is common in soybean crop

(Zobiole et al., 2011). In previous studies, Zobiole et al. (2010) found that some early cultivars were more susceptible to glyphosate, and showed a reduction in chlorophyll content, photosynthetic rate, nutrient concentration, shoot dry matter and root dry matter when submitted to this treatment. Other changes are related to photosynthetic and fluorescence parameters of plant chlorophyll (Barbagallo et al., 2003; Krenchinski et al., 2017).

Sfredo and Oliveira (2010) reported that applying micronutrients like molybdenum and cobalt, can increase the BNF and foliar applications of Mo resulted in increased Mo content in soybean seeds (Campo et al., 2009). When excessive, this element is stored mainly in the soluble fraction or bound to the cell wall fraction, preventing translocation to the leaves and maintaining the Mo homeostasis (Xu et al., 2018).

Micronutrients such as manganese (Mn) stand out more for their phytotoxicity due to their high concentration than deficiency problems, their high concentration can provide reduction in the photosynthetic rate and reduction in the dry matter of soybean plants (Santos et al., 2017). When applied by foliage in soybean, Mn is translocated to the seed and decreases the fungi incidence (*Cercospora kikuchii*, *Fusarium* spp. and *Aspergillus* spp.), but the element does not affect the lignin content in the tegument (Carvalho et al., 2015). The application of Mn by foliar is used, because the application of glyphosate in soybean can reduce, in some cultivars, 50 to 60% of the total Mn content in the leaves (Bott et al., 2008).

The experimental results are very controversial for the application of foliar fertilizers in the soybean crop, as the cost of these products are relatively low the producers have applied in their crops, with expectation of grain yield improvements (Ceretta et al., 2005). Usually this application is performed together with the weed control, associated with the herbicide glyphosate. Merotto et al. (2015), when working with increasing doses of glyphosate and foliar fertilizer verified that the application of foliar fertilizers does not alter the soybeans yield, otherwise the increase of the glyphosate dose, depending on the cultivar, may affect the yield.

As a result of the damage that this herbicide can cause to the physiology, nutrition and grain yield of the soybean crop (Bott et al., 2008, Zobiole et al., 2010, Merotto et al., 2015, Fan et al., 2017). In addition to the potential of foliar fertilizer (Cerreta et al., 2005), the present study compiles both subjects and their interaction, when applied to soybean crop.

In this way, the objective of the study was to evaluate the "GR" soybean crop responses under glyphosate herbicide and foliar fertilizers, associate or isolated, in a soil without nutrient depletion.

Results and Discussion

Analysis of variance of phytotoxicity, thousand-grain weight and soybean grain yield

Considering the data analysis variance results it can be emphasized that, there was significant effect ($p \leq 0.05$) on all variables analyzed. For the phytotoxicity and a thousand-grain weight, the orthogonal contrasts (Figure 1 and 2) were applied. For grain yield, it was applied either the orthogonal contrasts (Figure 3) and the Scott-Knott mean comparison test at $p \leq 0.05$ (Figure 4).

Phytotoxicity in GR soybean upon application of glyphosate and fertilizers

Phytotoxicity was observed in the soybean crop in the treatments of glyphosate, glyphosate + foliar fertilizers mixture and treatments that used only foliar fertilizers, compared to the control treatment (hoeing). This phytotoxicity is considered low but may favor crop yield loss (Figure 1). Even for soybean "GR" is acceptable and proven that the herbicide causes problems related to physiological parameters, the level of crop injury can be attributed to herbicide dose, genotype and application conditions (Zobiole et al., 2011; Merotto et al., 2015, Fan et al., 2017, Krenchinski et al., 2017). However, the nutrients phytotoxicity may be related to the concentration and demand actions that maintain the homeostasis of these nutrients in the plants, causing physiological changes (Santos et al., 2017; Xu et al., 2018).

In all the treatments contrasts in which glyphosate was applied in mixture with foliar fertilizers or alone (contrasts 5, 6, 7 and 8) there was a higher phytotoxicity when compared to the isolated application of foliar fertilizers (Figure 1). This increase was provided by the high dose of herbicide applied in the crop, as can be seen in contrast 9, in which the phytotoxicity was 4% higher when dose 2 (2.160 g a.i. ha^{-1}) was applied than dose 1 (1.80 g a.i. ha^{-1}). These results corroborate with those found by Merotto et al. (2015), in which the authors verified an increase in phytotoxicity and a yield loss, when spraying high doses of the herbicide.

When analyzing only the foliar fertilizers, no phytotoxicity on soybean was significant, so the three foliar fertilizers studied showed similar phytotoxicity symptoms, regardless of the dose applied (contrasts 10, 11, 12, 13, 14 and 15, Figure 1). Rare are the cases of soybean phytotoxicity by micronutrients excess, with the exception of Mn. When in excess, Mn can reduce the photosynthetic rate and soybean dry matter (Santos et al., 2017).

There were no visual phytotoxicity symptoms at 28 days after glyphosate application (data not shown), demonstrating that the soybean crop was able to metabolize the herbicide and foliar fertilizers. However, the soybean yield can be affected. Phytotoxicity effects are observed with increased glyphosate herbicide dose in intact RR2 soybean (second generation GR soybeans). However, as the crop grows the symptoms disappear or become smaller (Krenchinski et al., 2017).

It is important to point out that even though it is a genetically modified herbicide tolerant crop, it may have negative effects under high doses of glyphosate, with reduction of chlorophyll, decrease in BNF and problems in absorption of some essential nutrients (Zobiole et al. 2011, Fan et al., 2017).

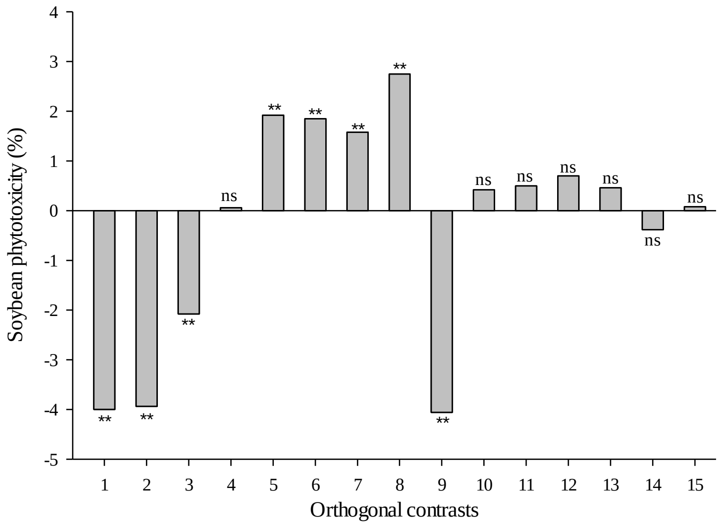
Thousand-grain weight

There was no effect observed in the thousand-grains weight in the treatments (Figure 2). Albrecht et al. (2011) found a reduction in this variable, especially when applying glyphosate at the vegetative stage of soybean development.

Table 1. Soil chemical characteristics in the study area. Federal University of Fronteira Sul, Erechim - Brazil, 2018.

pH	P	K	Ca^{2+}	Mg^{2+}	S	Zn	Mn	Cu	B	O.M
	mg dm^{-3}		$cmol_c$ dm^{-3}		mg dm^{-3}					%
6.1 [(1)]	8	37	7.4*	4.0*	6*	2.8*	15*	4.4*	0.4*	2.8**

[(1)] pH optimum for soybean crop * high levels in soil for soybean crop, ** medium content of organic matter (SBCS, 2016).



1 - Control (+) X glyphosate (-) p= 0.0000
2 - Control (+) X glyphosate + fertilizers (-) p= 0.0000
3 - Control (+) X fertilizers (-) p= 0.0020
4 - Glyphosate (+) X glyphosate + fertilizers (-) p= 0.8911
5 - Glyphosate (+) X fertilizers (-) p= 0.0002
6 - Glyphosate + fertilizers (+) X fertilizers (-) p= 0.0000
7 - Glyphosate + fertilizers dose 1 (+) X fertilizers dose 1 (-) p= 0.0019
8 - Glyphosate + fertilizers dose 2 (+) X fertilizers dose 2 (-) p= 0.0000
9 - Glyphosate dose 1 (+) X glyphosate dose 2 (-) p= 0.0000
10 - Fertilizer A dose 1 (+) X fertilizer A dose 2 (-) p= 0.3948
11 - Fertilizer B dose 1 (+) X fertilizer B dose 2 (-) p= 0.2740
12 - Fertilizer C dose 1 (+) X fertilizer C dose 2 (-) p= 0.1263
13 - Fertilizer A (+) X fertilizer C (-) p= 0.1874
14 - Fertilizer A (+) X fertilizer B (-) p= 0.2797
15 - Fertilizer C (+) X fertilizer B (-) p= 0.8093

Fig 1. Orthogonal contrasts for the soybean phytotoxicity at 14 days after application of the treatments, with application of different doses of glyphosate and foliar fertilizers. * and ** - significant contrast at $p \leq 0.05$ and, $p \leq 0.01$ levels, respectively and ns - nonsignificant contrast. Federal University of Fronteira Sul, Erechim - Brazil, 2018.

Table 2. Treatments and respective doses of glyphosate and commercial products. Federal University of Fronteira Sul, Erechim - Brazil, 2018.

Treatments	g a.i. ha^{-1} of glyphosate	Foliar fertilizer (L ha^{-1})
Control (Hoeing)	...	...
Glyphosate	1.080 (dose 1)	...
Glyphosate	2.160 (dose 2)	...
Glyphosate + fertilizer A	1.080 (dose 1)	2.00 (dose 1)
Glyphosate + fertilizer A	2.160 (dose 2)	2.00 (dose 1)
Glyphosate + fertilizer B	1.080 (dose 1)	2.00 (dose 1)
Glyphosate + fertilizer B	2.160 (dose 2)	2.00 (dose 1)
Glyphosate + fertilizer C	1.080 (dose 1)	0.25 (dose 1)
Glyphosate + fertilizer C	2.160 (dose 2)	0.25 (dose 1)
Glyphosate + fertilizer A	1.080 (dose 1)	4.00 (dose 2)
Glyphosate + fertilizer A	2.160 (dose 2)	4.00 (dose 2)
Glyphosate + fertilizer B	1.080 (dose 1)	4.00 (dose 2)
Glyphosate + fertilizer B	2.160 (dose 2)	4.00 (dose 2)
Glyphosate + fertilizer C	1.080 (dose 1)	0.50 (dose 2)
Glyphosate + fertilizer C	2.160 (dose 2)	0.50 (dose 2)
Fertilizer A	...	2.00 (dose 1)
Fertilizer A	...	4.00 (dose 2)
Fertilizer B	...	2.00 (dose 1)
Fertilizer B	...	4.00 (dose 2)
Fertilizer C	...	0.25 (dose 1)
Fertilizer C	...	0.50 (dose 2)

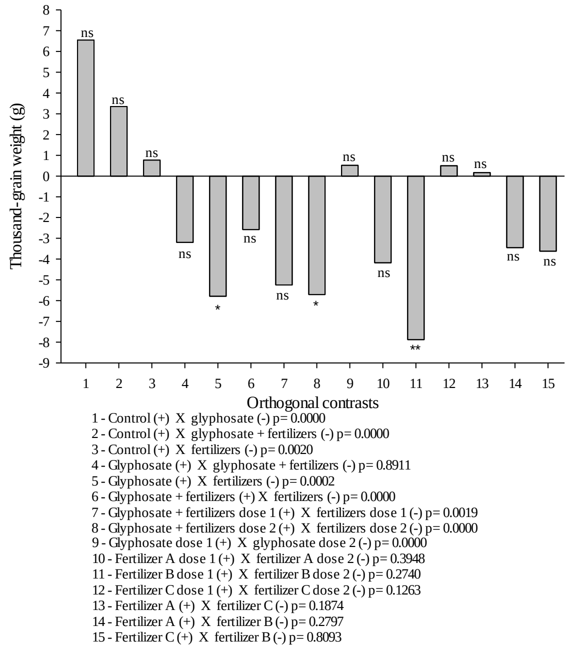


Fig 2. Orthogonal contrasts for a thousand-grain weight with application of different doses of glyphosate and foliar fertilizers. * and ** - significant contrast at p≤0.05 and, p≤0.01 levels, respectively and ns - nonsignificant contrast. Federal University of Fronteira Sul, Erechim - Brazil, 2018.

Table 3. Nutrient composition of foliar fertilizers applied isolated or mixed with glyphosate. Federal University of Fronteira Sul, Erechim - Brazil, 2018.

Foliar fertilizer A	Mg (5%), Mn (0.5%), Mo (0.5%), L-Glutamic acid (5%) and glycine betaine (3%).
Foliar fertilizer B	Zn (5%), Mn (3%), Cu (0.5%), B (0.5%) and S (4%)
Foliar fertilizer C	Mo and L-Glutamic acid

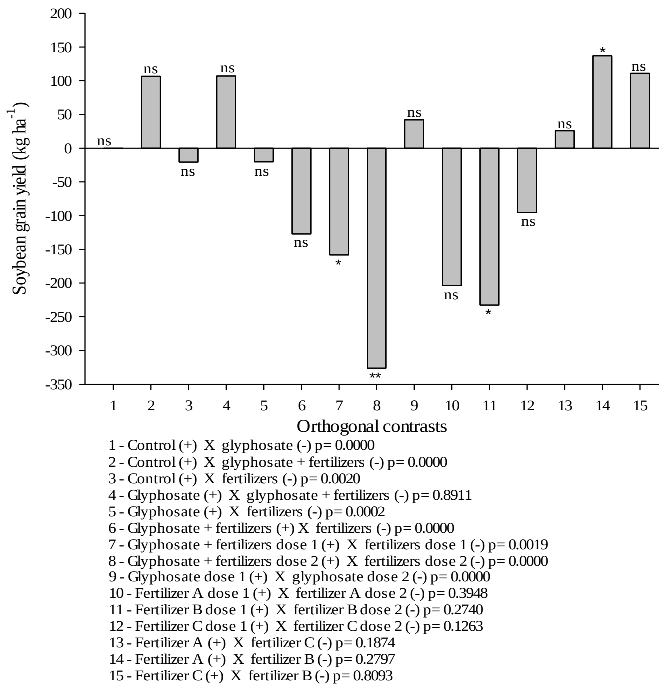


Fig 3. Orthogonal contrasts for the soybean grain yield, with application of different doses of glyphosate and foliar fertilizers. * and ** - significant contrast at p≤0.05 and, p≤0.01 levels, respectively and ns - nonsignificant contrast. Federal University of Fronteira Sul, Erechim - Brazil, 2018.

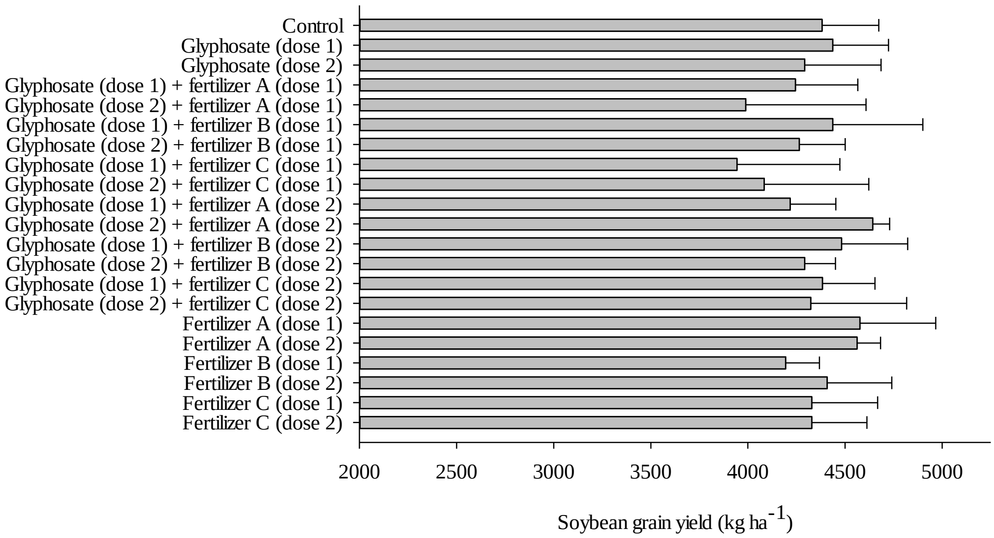


Fig 4. Soybean grain yield as a function of glyphosate doses and foliar fertilizers and their mixtures, through foliar application. Means followed by the same letter do not differ by Scott-Knott's test at p≤0.05. Federal University of Fronteira Sul, Erechim - Brazil, 2018.

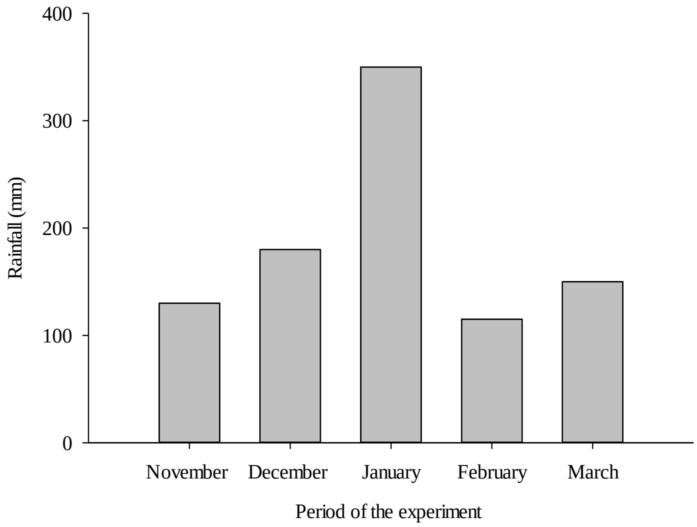


Fig 5. Rainfall in the study period, in the 2014/15 agricultural season. Federal University of Fronteira Sul, Erechim - Brazil, 2018.

This fact can be attributed to the increase of 720 g ha^{-1} of glyphosate when compared to the present study. The main result found was the increase of approximately 6 g in the thousand-grain weight when applied foliar fertilizers in comparison to glyphosate and glyphosate + foliar fertilizers (contrasts 5 and 8, Figure 2). This increase in accumulation of assimilates is often related to the physiological parameters that are reduced with glyphosate application (Zobiole et al., 2010; Zobiole et al., 2011; Merotto et al., 2015; Fan et al., 2017). At field experiments, Hungria et al. (2014), reported no changes in the attributes related to BNF and consequently the grain yield was not affected with applications of glyphosate in GR soybean. In the foliar fertilizers group comparison, it was not possible to verify differences in the thousand-grain weight. However, between doses, there was a 7.88 g increase in the variable with the application of dose 2 (foliar fertilizer B) when compared to dose 1 (contrast 11, Figure 2). This response may be related to the greater accumulation of foliar fertilizers present in the product formulation (Table 3). In a study conducted by Diesel et al. (2010), in soil with pH of 5.90 and with adequate level of nutrients, the authors did not find an increase in soybean thousand-grain weight applying Co and Mo by foliar. However, in the present study "foliar fertilizer B" presents other elements (Zn, Mn, Cu, B and S) and not Co and Mo, justifying in parts the divergent response.

Soybean grain yield

In Figure 3 it is possible to observe that foliar fertilizer did not show a positive effect on the soybean grain yield. The same result was observed for the mixture of the foliar fertilizers and glyphosate, in which a soybean crop cultivated in soils with adequate levels of these elements (Table 1) does not impact the grain yield. Some research results show an increase of soybean grain yield, however, the application of micronutrients occurred along soil fertilization in soils with low pH and lower micronutrients supply (Barbosa et al., 2016), different of the present study (Table 1).

It was observed that when applying twice the dose of glyphosate herbicide there was no negative effect on soybean grain yield when compared to the recommended dose (1080 g a.i. ha^{-1}) (contrast 9, Figure 3). However, when comparing the treatments means alone, the increase of the glyphosate dose (dose 2) reduced 90 and 180 kg ha^{-1} of grain yield comparing to the control treatment and the dose 1 of the herbicide, respectively (Figure 4).

It is known scientifically that the reduction in the chlorophyll content, photosynthetic rate, nutrient concentration and dry matter of shoot and root, as well as parameters related to soybean fluorescence of plants submitted to glyphosate application (Zobiole, et al., 2010; Barbagallo et al., 2003; Krenchinski et al., 2017). Fan et al. (2017), suggest that the application of glyphosate may cause stress on GR soybeans involving BNF and even if possible, high rates of glyphosate should be avoided.

Among the analyzed contrasts, the application of glyphosate + foliar fertilizers in the two doses compared with foliar fertilizers, resulted in the decrease of grain yield. It is important to highlight that the phytotoxicity in both contrasts (contrasts 7 and 8, Figure 1) was higher when associated with glyphosate and foliar fertilizers.

Foliar fertilizers on soil without a history of nutrient deficiency (Table 2) did not increase the yield of soybean grains (Figure 3 and 4). The association of these with the herbicide glyphosate, depending on the dose, can damage the crop in productive terms (Figure 4). Merotto et al. (2015), found a reduction in yield from the application of glyphosate at 1440 g a.i. ha^{-1}, depending on the cultivar.

By the fact that the present study was conducted in an area without nutrient depletion, studies in areas with deficiencies of these elements (mainly micronutrients) should be conducted to prove the effect of foliar fertilizers on the soybean yield.

Materials and Methods

Study area characterization

The study was conducted in a field, in the Quatro Irmãos (RS) city, during the growing season 2014/15. The geographic coordinates were 27º 44 'S and 52º 26' W, 680 m of altitude and Cfa climate (humid temperate with hot summer) according to the Köppen-Geiger classification (Peel et al., 2007). The soil of the experimental area is classified as Typic Dystrudepts (Soil Survey Staff, 2014). The study area showed consolidated no-till for more than 10 years and Table 1 shows the chemical characteristics of the soil in study area.

Experimental design

The study was conducted in a randomized block design, with 21 treatments and four replications. Two doses of glyphosate and foliar fertilizers were applied, isolated and in mixtures, as shown in Table 2. The nutrient composition of foliar fertilizers is described in Table 3. In summary, 3 doses of glyphosate (0 - control, 1080 and 2160 g a.i. ha^{-1}), 3 foliar fertilizers (fertilizers A, B and C) were tested by fractionating two doses (Dose 1 and 2, depending on the fertilizer), plus 2 treatments only with glyphosate herbicide doses, totaling 21 treatments. It was characterized as a single-factor experiment with all combinations (combinations of herbicides, fertilizers and doses).

Soil preparation and sowing

The soybean sowing was carried out under no-till system. The previous vegetation burndown was conducted 20 days before the sowing, applying herbicide glyphosate at 1080 g a.i. ha^{-1}. The pH correction and soil fertilization were performed according to the chemical analysis (Table 1). The fertilizers were applied along the sowing operation, constituted of 6 kg ha^{-1} of N_2, 90 kg ha^{-1} of P_2O_5 and 60 kg ha^{-1} of K_2O.

Each experimental unit was consisted by a plot of 11.75 m^2 (5 x 2.35 m), where the soybean cultivar BMX Ativa was sown, with a row planter of 0.47 m between lines and a plant density of 30 plants m^{-2}.

Treatments application

All 21 treatments are described in Table 2 as well as the a.e. doses of glyphosate used. Doses and concentrations of foliar fertilizers are shown in Table 3. The treatments application was done with a precision CO2 pressurized backpack sprayer equipped with four DG 110.02 spray nozzles under a constant pressure of 2.0 kgf cm^{-2} and 3.6 km h^{-1} speed, which allowed the flow of 130 L ha^{-1} of spray solution. The climatic conditions at the time of application were: high luminosity, air temperature of 23.5 °C, soil temperature of 25.6 °C, relative humidity of 75%, dry soil and winds of 0-2 km h^{-1}. The application was carried out when the crop had 3 fully developed leaves, starting at 18:00 h.

Crop management

The crop management was carried out according to the technical indications for the soybean crop, where weed control was carried out manually. The insects and diseases were managed when necessary with applications of insecticide and fungicide, so 4 applications of fungicide were done in the vegetative and reproductive stages so that the crop expressed the maximum yield. During the conduction of the experiment, there was no water deficit, as can be observed in Figure 5.

Variables analyzed

The phytotoxicity evaluation was performed visually at 14 and 28 days after application of the treatments (DAT). To evaluate the phytotoxicity of the treatments, zero marks (0%) were assigned to treatments with no phytotoxicity to the crop and 100% (100%) for complete plant death, according to the methodology proposed by SBCPD (1995). The harvest was carried out when the grains reached 15% water content, in a useful area of 3 m^2 per experimental unit, the grain threshing was done with a plotter thresher. After the harvest, the thousand-grain weight (g) was determined, counting 8 samples of 100 grains each and weighing them in an analytical scale. For analyzes, grain moisture was adjusted to 13% and yield data estimated to kg ha^{-1}.

Statistical analysis

The data were submitted to analysis of variance by the F test, when significant, the orthogonal contrasts analysis were performed for the variables phytotoxicity, thousand-grain weight and soybean grain yield, in addition to the comparison of means by the Scott-Knott test for the grain yield, at p≤0.05 level.

The contrasts values observed in Figures 1, 2 and 3 represent the estimated differences of one treatment group compared to the other. Negative contrasts values show that the second group was superior to the first.

Conclusion

Phytotoxicity occurred in the soybean crop with the glyphosate application of, but only the higher dose of glyphosate associated or not with foliar fertilizers reduced the crop yield. The application of foliar fertilizers isolated or associated to glyphosate in soils with adequate levels of nutrients does not increase the thousand-grain weight or soybean grain yield.

Acknowledgements

We thank the members of the research group Sustainable Management of Agricultural Systems at UFFS, the National Council for Scientific and Technological Development (CNPq) and the Foundation for Research Support of the State of Rio Grande do Sul (FAPERGS) for the financial assistance.

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