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# Selectivity of post-emergence herbicides and foliar fertilizer in soybean crop

## Seletividade de herbicidas pós-emergentes isolados ou associados a fertilizante foliar na cultura da soja

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#### ABSTRACT

Herbicides and foliar fertilizers are commonly associated in soybean crops. However, these mixtures can cause different effects on the plants, therefore requiring further studies. Herein, we assess the selectivity of post-emergence herbicides associated with foliar fertilizer (FF) in soybean crops (Roundup Ready). The experiment was carried out in a randomized block design with four replications. The treatments applied were: chlorimuron (17.5 g a.i. ha<sup>-1</sup>), chlorimuron + FF (17.5 + 693 g a.i. ha<sup>-1</sup>), cloransulam (40 g a.i. ha<sup>-1</sup>), cloransulam + FF (40 + 693 g a.i. ha<sup>-1</sup>), fomesafen (225 g a.i. ha<sup>-1</sup>), fomesafen + FF (225 + 693 g a.i. ha<sup>-1</sup>), glyphosate (1,280 g a.i. ha<sup>-1</sup>), glyphosate + FF (1,280 + 693 g a.i. ha<sup>-1</sup>), and two controls, being one with manual weeding and the other without weed control. Visual assessments of phytotoxicity and chlorophyll index were carried out weekly from seven to thirty-five days after applying the treatments (DAA). In these periods, chlorophyll levels were also assessed. In addition, the yield, 1000-grain weight, and moisture data were assessed at the end of the crop cycle. Phytotoxicity was higher than 30% when cloransulam and chlorimuron were applied, while phytotoxicity was close to 5% for fomesafen and glyphosate, either in the absence or presence of foliar fertilizer. There was no significant difference in grain yield, 1000-grain weight, and moisture. The association of herbicides with zinc-based foliar fertilizer did not influence the selectivity of post-emergence herbicides applied to the RR soybean.

KEYWORDS: phytotoxicity; tank mix; foliar fertilization.

#### RESUMO

A associação de herbicidas com fertilizantes foliares é comumente adotada na cultura da soja. Entretanto, essas associações podem ocasionar diferentes efeitos quando aplicadas na soja, necessitando portanto de maiores estudos. Assim, objetivou-se avaliar a seletividade de herbicidas pós-emergentes associados com fertilizante foliar (FF) à base de zinco na cultura da soja RR (resistente ao glifosato). O experimento foi conduzido em delineamento experimental de blocos casualizados, com quatro repetições. Os tratamentos aplicados foram chlorimuron (17,5 g i.a. ha<sup>-1</sup>); chlorimuron + FF (17,5 + 693 g i.a. ha<sup>-1</sup>); cloransulam (40 g i.a. ha<sup>-1</sup>); cloransulam + FF (40 + 693 g i.a. ha<sup>-1</sup>); fomesafem (225 g i.a. ha<sup>-1</sup>); fomesafem + FF (225 + 693 g i.a. ha<sup>-1</sup>); glyphosate (1.280 g i.a. ha<sup>-1</sup>); glyphosate + FF (1.280 + 693 g i.a. ha<sup>-1</sup>) e duas testemunhas sendo uma capinada e outra infestada. Foram realizadas avaliações visuais de fitotoxicidade aos 7, 14, 21, 28 e 35 dias após a aplicação dos tratamentos (DAT), e também nessas épocas os teores de clorofila. Ao final do ciclo da cultura foram determinados os dados referentes a produtividade e o peso de 1000 grãos. A fitotoxicidade foi superior a 30% quando se aplicou o cloransulam e o chlorimuron, enquanto que para o fomesafem e o glyphosate a fitotoxicidade foram próximas a 5%, na ausência ou presença do fertilizante foliar. Não ocorreu diferença significativa para a produtividade de grãos, peso de 1000 grãos e umidade. A associação de herbicidas com fertilizante foliar a base de zinco não influenciou na seletividade dos herbicidas aplicados em pós-emergência na cultura da soja RR.

PALAVRAS-CHAVE: fitotoxicidade; mistura em tanque; adubação foliar.

#### INTRODUCTION

Brazil stands out as the world's largest producer of soybeans with a volume of 273.8 million tons, which represents a growth of 6.5% or 16.8 million tons over the previous harvest (CONAB 2020). In establishing this productive level, micronutrients play an important role because, although they are required in small amounts, they are also essential elements for the plant to complete its vegetative cycle and, therefore, they cannot be lacking during the plant nutrition process. Among these micronutrients, we can mention zinc (Zn), Manganese (Mn), Boron (B), Cobalt (Co), and Molybdenum (Mo), which act as activators of several enzymes and influence the growth, development, and productivity of the soybean crop (HANSEL & OLIVEIRA 2016, OLIVEIRA et al. 2017).

The cuticle or stomata can absorb foliar fertilizers, and the absorption process can be influenced by several factors such as plant stage, root volume, and nutrient doses (FAGAN et al. 2016). However, nutrient translocation applied through leaves can be reduced, while the absorption of nutrients directly from the soil is greater because of the high volume of roots (BAZZO et al. 2021). Another factor that affects the agronomic performance of the soybean crop, as well as the yield and quality of the grains, is the presence of weeds, which compete with the crop and cause a decrease in the availability of resources such as light, water, space, and nutrients (FORTE et al. 2017).

The chemical control of weeds through the use of herbicides is the most common because of the efficiency and high operational yield, which allows flexibility in relation to the time of use and positioning in sowing lines (PEREIRA et al. 2018). With the advancement of transgenic plants and biotechnology in agriculture, one of the characteristics that increase the efficiency of chemical control is the selectivity of herbicides to crops (WESTWOOD et al. 2018), as in the case of glyphosate-resistant (RR) soybeans, which can metabolize the herbicide even before its action, in this way recovering from the poisoning effects without harming yield (GALON et al. 2021).

Tank mixing is a practice commonly used by producers, which consists of the association of pesticides and other inputs, such as foliar fertilizers, in the application tank, immediately before spraying. However, interactions between herbicides and foliar fertilizers can result in synergistic, additive, or antagonistic effects when compared to stand-alone applications (ALVARENGA et al. 2018). In the literature, many studies have described promising results regarding the association of herbicides and foliar fertilizers (FORTE et al. 2019, ANDRADE et al. 2020, GALON et al. 2021), which improve the effectiveness in terms of physiological stimuli, improve absorption, and change the pH of the solution (CARVALHO 2013).

According to GAZZIERO (2015), approximately 72% of rural producers are unaware of or do not consider enough information on tank mixing, and 99% show interest in receiving knowledge related to this practice. In this sense, considering that the mixture of active ingredients and foliar fertilizers in the spray solution can generate positive and negative impacts on the soybean crop, studies need to be carried out regarding the probable interactions between the phytosanitary products for their proper use. Thus, the objective of this study has been to assess the selectivity of post-emergence herbicides associated with zincbased foliar fertilizer on Roundup Ready (RR) soybeans.

#### MATERIAL AND METHODS

The experiment was established in the field, at the Experimental Farm of Agricultural Sciences (FAECA) of the Federal University of Grande Dourados (UFGD), in Dourados/MS/Brazil, in the geographic coordinates: 21°57' south latitude and 46°51' west longitude and altitude of 413 m. The characteristic Köppen climate classification is Cwa (humid mesothermal climate, hot summers, and dry winters) and average annual temperature is 22.7 °C (FIETZ & FISCH 2008).

Soil samples were taken from the experimental area prior to the establishment of the experiment to carry out the chemical and physical assessments, which were carried out by Laboratório Agro TecSolo Análises Agronômicas e Consultoria, described in Table 1. The experimental units consisted of 3 x 5 m plots (15 m<sup>2</sup>). The soil of the experimental area was classified as Distroferric Red Latosol (Oxisol) (SANTOS et al. 2018). The treatments consisted of zinc-based foliar fertilizer (Zintrac<sup>®</sup>, Yara, Porto Alegre, RS) associated or not with the herbicides glyphosate and cloransulam, as indicated in Table 2.

The soil for the experiment was prepared in a conventional manner consisting of plowing, harrowing, and leveling. Soybean seeds were treated before sowing with fungicide and insecticide, pyraclostrobin (Comet, Basf, São Paulo, SP, Brazil) 5 g a.i. + thiophanate-methyl (Tiofanato CCAB 500 SC, CCAB AGRO S.A., São Paulo, SP, Brazil) 45 g a.i. + fipronil (Nortox 800 WG, Nortox, Arapagongas, PR, Brazil) 50 g a.i. for 100 kg of soybean seeds. The soybean M 6210 IPRO, resistant to Roundup<sup>®</sup> (Glyphosate), was sown on

25/Oct/2020 in a mechanized manner using a vacuum seeder with seven rows spaced at 0.45 m, with 14 seeds per linear meter, resulting in a final population of approximately 311,000 plants ha<sup>-1</sup>. Base fertilization was carried out in the sowing line by applying 225 kg ha<sup>-1</sup> of the 02-08-18 formula.

Table 1. Analys	is of chemica	l attributes a	and gra	nulometry	of the	e soil	carried	out a	at the	UFGD	experim	nental
farm (	FAECA), Dour	ados/MS/B	razil, 20	20.								

Chemical and granulometric attributes of the soil												
р	Н	OM	Р	Κ	Ca	Mg	Al	H + Al	SB	CEC	V%	Clay
CaCl	SMP	(g dm <sup>-3</sup> )	Mehlich (mg dm <sup>-3</sup> )		(cmol dm <sup>-3</sup> )					(%)	(g kg <sup>-1</sup> )	
5.21	6.47	22.18	10.62	0.4	6.31	2.35	0	3.37	9.06	12.43	73	460

Table 2. Herbicides	associated	or not	with folia	ar fertilizer	and t	their	respective	doses i	n the	post-em	ergence
application in	n soybean c	rop.									

Treatments	Products	Commercial name	Commercial product concentration	Dose (g a.i. ha <sup>-1</sup> )	
T1	Chlorimuron + Glyphosate*	Classic (Corteva, Barueri, SP, Brazil) + Glizmax (Corteva, São Paulo, SP, Brazil)	250 g kg <sup>-1</sup> + 648 g L <sup>-1</sup>	17.5 + 1,280	
Τ2	Chlorimuron + FF + Glyphosate*	Classic (Corteva, Barueri, SP, Brazil) + Zintrac (Yara, Porto Alegre, RS, Brazil) + Glizmax (Corteva, São Paulo, SP, Brazil)	250 g kg <sup>-1</sup> + 693 g L <sup>-1</sup> + 648 g L <sup>-1</sup>	17.5 + 693 + 1,280	
Т3	Cloransulam + Glyphosate*	Pacto (Corteva, Barueri, SP, Brazil) + Glizmax (Corteva, São Paulo, SP, Brazil)	840 g kg <sup>-1</sup> + 648 g L <sup>-1</sup>	40 + 1,280	
Τ4	Cloransulam + FF + Glyphosate*	Pacto (Corteva, Barueri, SP, Brazil) + Zintrac (Yara, Porto Alegre, RS, Brazil) + Glizmax (Corteva, São Paulo, SP, Brazil)	840 g kg <sup>-1</sup> + 693 g L <sup>-1</sup> + 648 g L <sup>-1</sup>	40 + 693 + 1,280	
Т5	Fomesafen	Flex (Syngenta, São Paulo, SP, Brazil)	250 g L <sup>-1</sup>	225	
Т6	Fomesafen + FF	Flex (Syngenta, São Paulo, SP, Brazil) + Zintrac (Yara, Porto Alegre, RS, Brazil)	250 g L <sup>-1</sup> + 693 g L <sup>-1</sup>	225 + 693	
Τ7	Glyphosate	Glizmax (Corteva, São Paulo, SP, Brazil)	648 g L <sup>-1</sup>	1,280	
Т8	Glyphosate + FF	Glizmax (Corteva, São Paulo, SP, Brazil) + Zintrac (Yara, Porto Alegre, RS, Brazil)	648 g L <sup>-1</sup> + 693 g L <sup>-1</sup>	1,280 + 693	
Т9	Weeding Control				
T10	Infested Control				

FF= foliar fertilizer; a.i. (active ingredient). A total of 3 mL of oil (Joint) was added to all herbicide treatments. \*Application of Glyphosate associated with Chlorimuron and Cloransulam.

The application of herbicides, associated or not with the foliar fertilizer, was carried out with a  $CO_2$ pressurized sprayer equipped with a rod containing six TTI 11002 nozzles, spaced 0.50 m apart, and the spray was applied 0.50 m from the target in a volume of 160 L ha<sup>-1</sup> and a working pressure of 250 kPa with a working speed of 1 m s<sup>-1</sup>. The application was carried out at the V4 stage of the soybean crop, where the environmental conditions related to humidity, temperature, and wind speed were: 58%, 29.1 °C, and 0.6 m s<sup>-1</sup>, respectively. The conditions of rainfall and maximum and minimum temperature during the experiment period are shown in Figure 1.



Figure 1. Rainfall index and maximum and minimum average temperature during the experiment period - 01/Oct/2020 to 10/Mar/2021. Dourados/MS/Brazil, UFGD, 2020-2021.

After the emergence of the crop, maintenance was carried out to ensure the full development of the plants by following the recommendations for each product (AGROFIT 2021). In the V2 stage, molluscicide applications (Metarex - 5% metaldehyde) were necessary. In the V8 stage, applications were carried out for Zeta-Cypermethrin (Mustang, FMC, Campinas, SP, Brazil) 40 g a.i. ha<sup>-1</sup> and Bifenthrin 100 EC (Nortox, Arapongas, PR, Brazil) 36 g a.i. ha-1, Trifloxystrobin (Nativo, Bayer, São Paulo, SP, Brazil) 60 g a.i. ha-1 and Prothioconazole (Blavity, BAS, São Paulo, SP, Brazil) 40 g a.i. ha-1, Flubendiamide (Belt, Bayer, São Paulo, SP, Brazil) 33.6 g a.i. ha-1, and soybean oil methyl ester 28.8 g a.i. In the R3 phenological stage, the following were used: Pyraclostrobin (Comet, BASF, São Paulo, SP, Brazil) 81 g a.i. ha-1, Epoxconazole (Rubric, FMC, Campinas, SP, Brazil) 50 g a.i. ha<sup>-1</sup>, Fluxapyroxad (Orkestra, BASF, São Paulo, SP, Brazil) 50 g a.i. ha<sup>-1</sup>, Sulfoxaflor (Verter, Corteva, Barueri, SP, Brazil) 30 g a.i. ha<sup>-1</sup>, Lambda-Cyhalothrin (Trinca, UPL, Ituverava, SP, Brazil) 45 g a.i. ha<sup>-1</sup>, Indoxacarb (Avatar, FMC, Campinas, SP, Brazil) 52.50 g a.i. ha<sup>-1</sup>, and soybean oil methyl ester 28.8 g a.i. In the R5.4 and R7 phenological stages, first Acetamiprid Nortox 200 SP (Nortox S/A, Arapongas, PR, Brazil) 45 g a.i. ha-1, Phenpropatrine (Danimen, Sumitomo, São Paulo, SP, Brazil) 67.5 g a.i. ha<sup>-1</sup> were applied, then Ethiprole (Curbix, Bayer, São Paulo, SP, Brazil) 160 g a.i. ha<sup>-1</sup>. Throughout the crop cycle, weeding was carried out according to the weed infestation in the plots referring to the weeded control treatment.

Visual phytotoxicity assessments were performed at 7, 14, 21, 28, and 35 days after the first application of treatments (DAT) following the scale proposed by the Brazilian Society of Weed Sciences (SBCPD 1995), in which 0% represents no damage and 61-100% means a drastic reduction in yield with the possibility of total destruction of the plants (death of the plant). In the same period, the chlorophyll content of the leaves of the upper third of three random plants per plot were collected with the aid of the device Clorofilog<sup>®</sup>/CFL 1030 (Falker, Porto Alegre, RS, Brazil).

The pre-harvest desiccation occurred on 25/Feb/2021, when the plants were in a R7.3 phenological stage and the herbicide used was glufosinate ammonium + flumioxazin (400 + 22.5 g a.i. ha<sup>-1</sup>). On the fifth day, after the plants showed more than 95% of leaf senescence, three central lines of the useful area of the plots were harvested, and 0.5 m from the edges of the borders were discarded. The total weight of the grains was verified with the aid of a balance and right after, with a portable grain moisture meter model AL-102 ECOR (Agrologic, Curitiba, PR, Brazil), the grain moisture was determined at 14%, being the standardization in this same percentage. Samples were taken from the total weight of the grains, which were taken to the laboratory for the separation of a thousand grains with an electronic counter model NV-C/01 (Névoa, Campinas, SP, Brazil) with subsequent weighing.

For the analysis of the variables of Phytotoxicity and Chlorophyll, the experimental design was in randomized blocks with four blocks in a split plot. The Product and DAA factors had eight levels (Cloransulam + FF + Glyphosate\*, Cloransulam + Glyphosate\*, Chlorimuron + FF + Glyphosate\*, Chlorimuron + Glyphosate\*, Fomesafen, Fomesafen + FF, Glyphosate, and Glyphosate + FF) and

Phytotoxicity had five levels (7, 14, 21, 28, and 35), respectively. When the Chlorophyll variable was evaluated, there was no collection in the experiment at 35 days. The statistical model was given by Equation 1.

$$\gamma_{ijk} = \mu + P_i + B_j + e_{ij} + D_k + (P \times D)_{ik} + \varepsilon_{ijk}.$$
(1)

In Equation 1, the term  $y_{ijk}$  is the value of the response variable observed in the i-th level of the Product factor (P), k-th level of the DAA factor (D), and the j-th Block (B). The  $\mu$  component represents the overall mean in the experiment. At the plot level, there is the effect of the i-th level of the P factor (Pi), the effect of the j-th Block (Bj), and the residual at the plot level (e<sub>ij</sub>). In the subplot, there is the effect of the k-th level of the D factor (D<sub>k</sub>), the effect of the interaction of the i-th level of the P factor with the k-th level of the D factor ((P×D)<sub>ik</sub>), and the error at the subplot level ( $\epsilon_{ijk}$ ).

The experimental design in randomized blocks was used to analyze the variables of total weight, moisture, and 1000-grain weight. In this experimental design, we had a total of 32 observations. The statistical model was given by Equation 2.

$$y_{ij} = \mu + P_i + B_j + e_{ij}$$
 (2)

In Equation 2, the term  $y_{ij}$  is the value of the response variable observed at the i-th level of the Product factor (P) in the j-th Block (B). The  $\mu$  component represents the overall mean in the experiment. In this model, there is the effect of the i-th level of the P factor (P<sub>i</sub>), the effect of the j-th Block (B<sub>j</sub>), and the error (e<sub>ij</sub>).

The F-test was used at a 5% significance level to verify the main and interaction effects. After, the Tukey test was used at a 5% significance level to compare the mean. All statistical analyses were performed in the R software (R CORE TEAM 2021). For the analysis of variance, the library ExpDes.pt was used (FERREIRA et al. 2018). Graphic presentations were carried out by the library ggplot2 (WICKHAM 2016).

## **RESULTS AND DISCUSSION**

Table 3 shows that there was no effect of Product (p>0.05) in the analysis of variance when analyzing the variables of Total Weight, Moisture, and 1000-grain Weight.

Factor	DF	F	Р
Phytotoxicity			
Block	3	1.102	0.370
Product	7	95.619	< 0.01
DAA	4	281.635	< 0.01
Product x DAA	28	27.447	< 0.01
Chlorophyll			
Block	3	0.740	0.549
Product	7	1.164	0.364
DAA	3	29.843	< 0.01
Product x DAA	21	1.646	0.062
Total Weight			
Block	3	0.812	0.502
Product	7	1.587	0.194
Moisture			
Block	3	0.475	0.703
Product	7	0.454	0.857
1000-grain weight			
Block	3	1.328	0.292
Product	7	2.014	0.101

Table 3. Result of the analysis of variance for the variables of Phytotoxicity, Chlorophyll, Total Weight, Moisture (%), and 1000-grain Weight.

DF = degrees of freedom; F = F-statistic; P = p value associated with F-statistic.

In Table 4, when the variable of Phytotoxicity was evaluated, we could note that the Product versus DAA interaction was significant by the F-test (p<0.05) and therefore we had to carry out the breakdown of these factors.

Factor	DF	F	Р
Phytotoxicity			
Product: DAA = 7	7	167.234	< 0.01
Product: DAA = 14	7	15.035	< 0.01
Product: DAA = 21	7	3.033	< 0.01
Product: DAA = 28	7	0.192	0.987
Product: DAA = 35	7	0.028	0.999

 Table 4. Result of the analysis of variance for the breakdown of the Product factor within each level of the DAA factor when the variable of Phytotoxicity is evaluated.

DF = degrees of freedom; F = F-statistic; P = p value associated with F-statistic.

According to the results of Table 5, through the F-statistics, the results of the Tukey test were presented for the breakdown of the Product factor within days 7, 14, and 21 (p<0.05) when the variable of Phytotoxicity was evaluated. Table 5 presents the result of the breakdown of the Product factor within each level of the DAA factor when the variable of Phytotoxicity was evaluated. In the analysis of Table 5, we can note that the addition of zinc-based foliar fertilizer in the solution did not increase the phytotoxicity of the herbicides used.

Table 5. Result of the Tukey test for the breakdown of the Product factor within days 7, 14, and 21 when the variable of Phytotoxicity was evaluated.

	1								
Products	DAA								
	7	14	21	28	35	General mean			
Cloransulam + FF + Glyphosate*	35.75 A	13.25 A	7.00 AB	1.25	0.25	11.50			
Cloransulam + Glyphosate*	35.25 A	15.00 A	5.25 AB	1.25	0.25	11.40			
Chlorimuron + FF + Glyphosate*	34.00 A	12.00 A	9.00 A	1.75	0.00	11.35			
Chlorimuron + Glyphosate*	32.75 A	12.25 A	7.00 AB	0.75	0.50	10.65			
Fomesafen	5.00 B	6.00 B	2.25 B	0.50	0.25	2.80			
Fomesafen + FF	3.75 B	5.50 B	4.00 AB	1.00	0.50	2.95			
Glyphosate	3.50 B	2.75 B	5.50 AB	0.00	0.00	2.35			
Glyphosate+ FF	4.00 B	3.25 B	3.50 B	0.50	0.00	2.25			
General mean	19.25	8.75	5.44	0.88	0.22	6.91			

Means followed by equal capital letters in the columns, for Products, do not differ from each other by Tukey test (p>0.05).

Regarding the phytotoxic effects on soybean, we can observe that the herbicides chloransulan and chlorimuron, whose mechanism of action is the inhibition of the enzyme acetolactate synthase (ALS), presented the highest percentages of phytotoxicity at 7 and 14 DAA, differentiating from each other, regardless of the association or not with foliar fertilizer. Fomesafen and Glyphosate, which have the inhibition of the enzyme PROTOX and EPSPs as their mechanism of action, respectively, showed the lowest phytotoxicity were related to the treatment chlorimuron + FF + Glyphosate with 9%, and the lowest percentages were for the herbicides fomesafen and glyphosate associated with foliar fertilizer, with 4% and 3.5%, respectively. At 28 and 35 DAA, the treatments did not show significant differences in relation to the application (Table 5).

In Figure 2, we can observe the breakdown of each herbicide treatment as a function of the periods of assessment (DAA), in which all treatments showed a gradual decrease in phytotoxicity percentages during the assessment periods; that is, they started with higher percentages of phytotoxicity and throughout the DAA these symptoms were decreased until 35 DAA, when they became inexpressive. Treatments containing ALS-inhibiting herbicides, regardless of the association with foliar fertilizers, presented initial phytotoxicity scores above 30%, while at 35 DAA the values were close to zero, in which  $R^2$  data were greater than 0.8. The decrease in phytotoxicity percentages were smaller for the herbicides Glyphosate and fomasafen (starting from 6%), as the initial percentages were also lower. It is noteworthy that for both, the decrease in phytotoxicity was linear ( $R^2 = 0.83$ ).



Figure 2. Analysis of the breakdown of the DAA factor within each level of Product factor when Phytotoxicity is evaluated.

In Table 1, when the variable of chlorophyll was evaluated, we could note that only the DAA factor was statistically significant by the F-test (p<0.05). The regression adjustment of the chlorophyll variable as a function of DAA is presented below (Figure 3). In the correlation, a gradual increase in the chlorophyll index is observed with the assessment periods.

The absence of statistical differences for the variables of total weight, moisture, and 1000-grain weight shows that some herbicide treatments resulted in significant phytotoxic effects on the soybean crop, especially at 7 DAA, but this behavior did not result in losses inherent in the production and/or final quality of the product. Therefore, the herbicides used alone and/or associated with fertilizers did not result in yield losses when compared to controls without application of products. This means that the initial phytotoxic effects were mitigated and did not impact crop development and final yield. MEROTTO JÚNIOR et al. (2015) have also found no difference in yield and 1000-grain weight (g) in soybean cultivars assessed as a function of glyphosate and foliar fertilizer application. The same has occurred in the work of VIDRINE et al. (2002), where there was an increase in weed control with the use of glyphosate + chlorimuron-ethyl; however, no increase in yield was observed in the soybean crop.



Figure 3. Analysis of the breakdown of the DAA factor within each level of Product factor when Chlorophyll is evaluated.

These results are important, as they demonstrate that the application of foliar micronutrients in soybean crops does not necessarily result in increased yield. However, the association of foliar fertilizers

with herbicides have resulted in other beneficial effects to the production system, such as minimizing the impact of chlorophyll reduction in plants through the application of herbicides, increasing the photosynthetic rate and concentration of nutrients, increasing the production of shoot and root dry mass (ZOBIOLE et al. 2011), and reducing the incidence of pathogens, such as in the stage of fungal infection (CARVALHO et al. 2015). In the case of glyphosate, the application can cause the immobilization of nutrients such as Fe and Mn, in this way resulting in the yellowing of the leaves, the so-called "Yellow flashing", in which the application of foliar fertilizers can reduce these adverse effects (MEROTTO JÚNIOR et al. 2015). However, in our study, these other benefits have not been evidenced in the combination of herbicides and foliar fertilizers.

In addition, there were no statistical differences between the isolated products and their associations with the foliar fertilizer, regardless of the treatment and period after application. Therefore, for all the mechanisms of action of the herbicides used in our experiment (EPSPS, PROTOX, and ALS), the addition of the fertilizer did not result in a synergistic effect for phytotoxicity in soybean. The phytotoxic effects are related to the doses of both foliar fertilizers and herbicides used, and these associations must follow criteria that maintain the homeostasis of the applied nutrients, thus avoiding physiological changes and yield losses (SANTOS et al. 2017, XU et al. 2018). FORTE et al. (2019) have found greater phytotoxicity for the herbicide glyphosate associated with foliar fertilizers with increasing doses of the herbicide.

In addition to these aspects, the climatic condition can also influence the determination of a synergistic, antagonistic, or additive effect in the mixing of a herbicide with foliar fertilizer; in dry climate conditions and lower relative humidity, as observed in our experiment, the cuticles of soybean plants tend to become thicker and, thus, reduce the possibilities of synergism in the combination of herbicides and foliar fertilizers (MONTGOMERY et al. 2017, ALIVERDI et al. 2020). HECKMAN et al. (1999) have observed that the phytotoxicity in soybeans inherent in the application of post-emergence herbicides (acifluorfen, chlorimuron, imazethapyr, and bentazon) associated with manganese sulfate were not different from the herbicides applied without manganese sulfate.

The herbicides chloransulam and chlorimuron, belonging to the acetolactate synthase (ALS) inhibiting group, caused more severe initial symptoms (>30.00%) in soybean plants when compared to the symptoms found in the other treatments; however, at 35 DAA the values showed a significant decrease. Similar results have been observed by PROCÓPIO et al. (2007), who, at 13 DAA, have found that the addition of 100 g ha<sup>-1</sup> of imazethapyr to glyphosate, at any dose tested, and the combination of 10.0 g ha<sup>-1</sup> of chlorimuron-ethyl with 1,440 g ha<sup>-1</sup> of glyphosate promoted the highest levels of injuries to RR<sup>®</sup> soybean plants, with percentages higher than 30%; the authors have also verified a decrease in the intensity of symptoms caused by the application of herbicides at 25 DAA. Results found by CESCO et al. (2018) corroborate our work, as the association of chlorimuron with glyphosate in the development of RR Intact soybean caused phytotoxicity levels of 36% at seven days DAA. The actions of ALS-inhibiting herbicides reduce the levels of the amino acids valine, leucine, and isoleucine, which are directly linked to the production of cell growth proteins (GAZOLA et al. 2016).

However, even if there is recurrent reporting that ALS-inhibiting herbicides can cause significant phytotoxic effects in post-emergence soybeans, it is worth noting that adverse conditions, such as temperature and water deficit, tend to increase and/or prolong phytotoxicity symptoms in plants, which may lead to yield losses (DRANCA et al. 2018). In our experiment, soybeans were sown on 25/Oct, a period that, as can be seen in Figure 1, had high rainfall, close to 100 mm, which allowed the homogeneous emergence and establishment of the stand; however in the following month, November (when the post-emergence treatments were applied to the soybean crop), the total precipitation was close to 10 mm and it was associated with maximum average temperatures close to 35 °C; later, in December, there was an increase in rainfall, which reduced water stress and phytotoxicity percentages (Figure 1).

Therefore, these adverse climatic conditions resulted in stress in the development of soybean plants, as the need for water conservation leads to a decrease in photosynthesis and internal translocation (SUZUKI et al. 2014), which results in lower photosynthetic rate, lower stomatal conductance, and, finally, lower absorption and translocation of herbicides (ABBOTT & STERLING 2013). In addition, the stress condition of the soybean plants observed in this experiment may have caused changes in the secondary metabolism and in the hormonal levels of the plants, and through the application of herbicides we could observe that the sites of action of these products are also the formation pathways of compounds important for the development and survival of the species (ROCKENBACH et al. 2018). GONÇALVES et al. (2018) have verified that imazethapyr spraying resulted in decreased root dry matter in the V4 stage. PARSA et al. (2013) have also

reported decreased dry matter in the root system of different soybean cultivars subjected to imazethapyr spraying at the recommended dose.

There was no significant difference between the application of glyphosate associated or not with the foliar fertilizer, with values lower than 5% (very light) for the periods of 7 and 14 DAA. At 21 DAA, the level of phytotoxicity in isolated glyphosate increased to 6.5% (mild); however, the levels of association with foliar fertilizer remained below 5% for the same evaluated period. FORTE et al. (2019) have also observed low levels of phytotoxicity in the soybean crop in the treatments with glyphosate, glyphosate + FF, isolated treatments, and control (hoe); however, the authors reinforce that even with low percentages it is possible that there are crop yield losses.

ALONSO et al. (2013), when assessing the selectivity of isolated and mixed glyphosate for RR soybeans, have found that the herbicide applied alone at a dose of 720 g a.i. ha<sup>-1</sup> showed phytotoxicity rates of 20 and 25% at 3 and 7 DAA, respectively, and the phytotoxicity values dropped dramatically to 10% at 15 DAA in the evaluated work. Although glyphosate was applied to an herbicide-resistant soybean cultivar, some symptoms of damage may occur after application. This is possibly due to the accumulation of AMPA (aminomethylphosphonic acid), which is characterized as a phytotoxic metabolite that is formed in the degradation of Glyphosate (MEROTTO JÚNIOR et al. 2015).

For the treatments, the association or not with the foliar fertilizer did not influence the selectivity of the product. At seven DAA, the fomesafen treatment showed 5% phytotoxicity and fomesafen + FF showed 3.75%. The explanation for the greater tolerance to PROTOX-inhibiting herbicides is related to the ability of plants to metabolize peroxidative stress, potentially through antioxidant systems (CARBONARI et al. 2012).

In this sense, the greater recovery of soybeans with the application of fomesafen may be associated with a greater physiological efficiency of the plant. On the other hand, as it is a contact product, there is little translocation in the plant; that is, the recovery of leaves over the days after application is quick, regardless of the association with the foliar fertilizer.

ALS-inhibiting herbicides showed higher initial phytotoxicity values and, consequently, greater symptom reduction. On the other hand, fomasafen and Glyphosate had a lower initial phytotoxicity value. All treatments showed no visual damage at 35 DAA. Therefore, under the conditions of this experiment, the foliar fertilizers did not accelerate the reduction of the phytotoxic effects initially observed in the soybean crop. An acceleration of the recovery from the phytotoxic effects was expected in the association with foliar fertilizers, as these substances are applied with the aim of improving nutritional efficiency and tolerance of crops to abiotic stresses (DU JARDIN 2015), in addition to promoting an increase in the development of the soybean crop with possible mitigating effect after herbicide applications (MARQUES et al. 2014).

It is not possible to assess whether the foliar fertilizer contributed to the rapid recovery of chlorophyll levels after the initial phytotoxicity symptoms of the application. According to De PAULA (2019), the amino acids present in the foliar fertilizer collaborate with the rapid recovery from the stress situation in which the plant is inserted and help to enhance the photosynthetic capacity. Da CRUZ et al. (2019) have noticed an increase in chlorophyll levels after 35 days of glyphosate application, and the authors have related this to the partial recovery of the crop from the application stress. Such chlorophyll variations are common and may be associated with biotic and abiotic factors in the region, such as water availability, temperature, radiation, salinity, and direct competition with weeds (TAIZ et al. 2017).

### CONCLUSION

In the RR soybean cultivar studied, the use of foliar fertilizer did not influence the selectivity of herbicides, and there was no interference for grain yield, 1000-grain weight, and moisture. The ALS-inhibiting herbicides (chlorimuron and cloransulam) initially caused phytotoxic effects greater than 30%, but at the end of the assessments there was no statistical difference between the analyzed treatments.

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