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Full Length Article

Selectivity of Pre-Emergence Herbicides Applied at Sowing or Early Post-Emergence of Soybean

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Abstract

Post-emergence application of imazethapyr/flumioxazin, sulfentrazone/diuron, clomazone or s-metolachlor is believed to cause injury to soybeans. The objective was to evaluate the selectivity of these herbicides applied at sowing, at 7 or 14 days after sowing (DAS) of soybean. The trial was conducted at two locations in Jesuítas and at one location in Ubiratã, state of Paraná (PR), Brazil. The treatments were arranged in a 5 × 3 factorial scheme, the herbicides imazethapyr/flumioxazin, sulfentrazone/diuron, clomazone, s-metolachlor and non-treated control were used, which were applied at three periods (sowing, 7 and 14 DAS). Soybean injury symptoms, plant height and soybean yield were evaluated. The highest phytotoxic potential (up to 56.2%) was observed for the application of imazethapyr/flumioxazin and sulfentrazone/diuron at 14 DAS. The application of clomazone and s-metolachlor were selective in soybean, at pre-emergence, at 7 and 14 DAS. Imazethapyr/flumioxazin or sulfentrazone/diuron at 14 DAS caused significant injury to soybean including reducing plant height and, for sulfentrazone/diuron, a reduction in yield was found for application at 14 DAS compared to 7 DAS. The application of the herbicides in pre-sowing, at 7 and 14 DAS was selective for soybean except sulfentrazone/diuron at 14 DAS, which reduced soybean yield.

Keywords: Injury; Imazethapyr/flumioxazin; Sulfentrazone/diuron; Clomazone; S-metolachlor; Agronomic performance

Introduction

Herbicides with pre-emergence effects are very important in the management of weeds that are difficult to control, and it is important that the residual effect lasts until the vegetative development of the soybean. In this sense, their application has been investigated, even in soybean post-emergence to obtain a greater residual effect within the soybean growing cycle (Oliveira *et al.* 2017; Chahal *et al.* 2018; Sarangi and Jhala 2019).

Mixtures of herbicides with different mechanisms of action are often recommended as an effective tool for weed management especially in relation to herbicide resistance in weeds (Kniss *et al.* 2022). With the aim of improving efficacy and expanding the spectrum of action, some companies have introduced pre-emergence mixtures on the market. For a pre-emergence application, it is necessary to consider some factors such as environmental conditions and soil properties to define a safe dose (Barroso *et al.* 2021).

Pre-emergence herbicides must be selective for soybean plants, selectivity is the differentiated response the crop presents to the application of a given herbicide, which may or may not suffer injuries. The level of injuries can be changed according to the application conditions, physiological status, plant morphology and plant recovery capacity after herbicide application through inactivation/ metabolization of the molecule (Carvalho *et al.* 2009; Nandula *et al.* 2019).

Studies report the selectivity of pre-emergence herbicides in soybean (Belfry *et al.* 2015; Belfry *et al.* 2016; Fornazza *et al.* 2018; Hay *et al.* 2019). However, few studies addressed the selectivity of these herbicides in the early post-emergence of soybeans, especially in Brazil. Neto *et al.* (2009) observed that the application of glyphosate in a mixture with the pre-emergence flumioxazin or s-metolachlor at the V_1 stage (phenological stage: first trifoliolate: one set of unfolded trifoliolate leaves) of soybean caused injury to the crop with growth delay and inter-row

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closure, however, it did not interfere with soybean yield.

Many products have been recommended for application in pre-sowing soybean (Dalazen *et al*. 2020; Albrecht *et al*. 2021, 2022). In this sense, the acetolactate synthase (ALS) inhibitor herbicides, such as imazethapyr can be highlighted. Protoporphyrinogen oxidase (PPO) inhibitor herbicides are also important, such as flumioxazin or sulfentrazone, also herbicides that inhibit the biosynthesis of very-long-chain fatty acids (VLCFA), such as s-metolachlor. Another important herbicide is clomazone, which is an inhibitor of carotenoid biosynthesis.

However, there is a need for studies that evaluate the selectivity of these herbicides in soybean post-sowing. Early post-emergence application of imazethapyr/flumioxazin, sulfentrazone/diuron, clomazone or s-metolachlor is believed to cause injury to soybeans. Therefore, the objective was to evaluate the selectivity of these herbicides applied at sowing, at 7 or 14 days after soybean sowing (DAS).

Materials and Methods

Trials description and experimental design

The experiment was carried out in Jesuítas (trials 1 and 2, 24°27'59"S 53°25'32"W) and Ubiratã (trial 3, 24°16'55.7"S 53°30'47.9"W), state of Paraná (PR), Brazil. The three trials were carried out during 2020–2021 growing season. Trials 1 and 2 were located in clayey soil (18.75% sand, 17.5% silt, 63.75% clay), with organic matter (OM) of 24.7 g dm^{-3}, sum of basis (SB) of 9.1 cmolc dm^{-3}, cation exchange capacity (CEC) of 15.8 cmolc dm^{-3} and basis saturation (V) of 57.6%. Trial 3 was carried out in very clayey soil (17.5% sand, 17.5% silt, 65% clay), with OM of 29.5 g dm^{-3}, SB of 7.9 cmolc dm^{-3}, CEC of 14.1 cmolc dm^{-3} and V of 56%. According to the Köeppen classification, the climate of the region is subtropical (Cfa), and the meteorological conditions during the trials are illustrated in Fig. 1.

This was a randomized block design with four replications with factorial arrangement (5 × 3). Five treatments were used for the herbicide factor: imazethapyr/flumioxazin (100/50 g acid equivalent [ae]/active ingredient [a.i.] ha^{-1}, Zethamaxx®), sulfentrazone/diuron (245/490 g a.i. ha^{-1}, Stone®), clomazone (900 g a.i. ha^{-1}, Reactor® 360 CS), s-metolachlor (1,440 g a.i. ha^{-1}, Dual Gold®) and the untreated control. Herbicides were applied in three periods: at sowing, at 7 and 14 DAS. All plots were kept free of weed interference with the use of manual weeding carried out at 7 and 14 DAS before the application of herbicides and were no longer necessary until the soybean harvest. This was done to eliminate competition between weeds and soybeans, so that any effects of treatments on soybeans would not be due to the level of effectiveness in controlling weeds.

The experimental units consisted of plots of 6 soybean rows (2.7 m × 5 m long), under no-till system, with spacing between rows of 0.45 m and 13 seeds m^{-1}. Soybean cultivar BMX 64i61RSF was used in trial 1 and cultivar BS 2606 in trial 2 with sowing date of October 24, 2020, the areas were fallow after the maize harvest. In trial 3, the cultivar CZ 58B28 was sown on September 25, 2020 in a field previously grown with oats.

Herbicide application

Herbicides were applied using a CO_2 pressurized backpack sprayer at a constant pressure of 2 bar, equipped with a bar with 6 fan nozzles (XR 110.02, Teejet®) at a height of 50 cm from the target with a spray volume of 150 L ha^{-1}. Weather conditions at the time of application are listed in Table 1. In trials 1 and 2, at the application period at 7 DAS, soybean was at the V_E stage (emergence – cotyledons have been pulled through the soil surface) and at the application period at 14 DAS, at the V_C stage (unrolled unifoliolate leaves – unfolding of the unifoliolate leaves). In trial 3, at 7 DAS, soybean was at the V_C stage and at 14 DAS, at the V_2 stage (second trifoliolate - two sets of unfolded trifoliolate leaves).

Assessments

Crop injury symptoms were evaluated in soybean plants at V_4 (fourth trifoliolate), V_6–V_7 (sixth-seventh trifoliate) and R_1 (beginning of flowering – plants have at least one flower

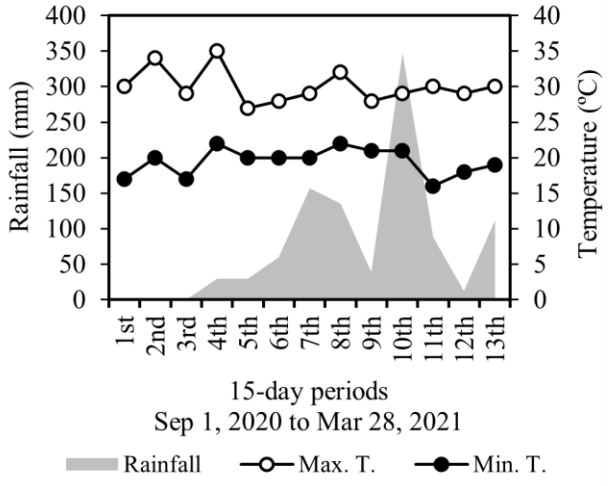

Fig. 1: Rainfall, maximum and minimum temperature at experimental. PR, Brazil, 2020-2021 crop season

Table 1: Application dates and weather conditions at the time of experiment applications

Trial	Period	Date	T	Wind	RH
			ºC	km h^{-1}	%
1 and 2	Sowing	Oct 24, 2020	29.2	13	54
1 and 2	7 DAS	Oct 31, 2020	26.1	10	50
1 and 2	14 DAS	Nov 07, 2020	32.0	8	27
3	Sowing	Sep 25, 2020	25.9	9	48
3	7 DAS	Oct 02, 2020	24.0	9	64
3	14 DAS	Oct 09, 2020	26.0	9	62

DAS: days after sowing, T: temperature, RH: relative humidity

Table 2: Significance level for the variables according to ANOVA, by the F-test

		Crop injury			Plant height	Yield
		V_4 stage	V_6-V_7 stage	R_1 stage		
		%			cm	kg ha^{-1}
Trial 1	Herbicide	*	*	*	ns	ns
	Application period	*	*	*	*	*
	Interaction	*	*	*	*	*
	Mean	4.5	3.4	3.0	110.5	4,121
	CV (%)	27.4	25.4	41.6	4.7	7.6
Trial 2	Herbicide	*	*	*	*	ns
	Application period	*	*	*	*	ns
	Interaction	*	*	*	*	ns
	Mean	4.4	3.3	2.9	112.4	4,663
	CV (%)	26.5	37.0	39.4	4.0	10.8
Trial 3	Herbicide	*	*	*	ns	ns
	Application period	*	*	*	ns	ns
	Interaction	*	*	*	ns	ns
	Mean	11.3	7.0	5.1	112.9	4,998
	CV (%)	8.1	10.4	15.2	2.9	5.6

V_4: fourth trifoliolate. V_6-V_7: sixth-seventh trifoliate. R_1: beginning flowering - plants have at least one flower on any node
* Significant ($P \leq 0.05$), means differ each other by F-test. ^{ns} Non-significant ($P > 0.05$), means do not differ each other by F-test

on any node) phenological stages, in trials 1 and 2, at 35, 42 and 49 DAS, respectively, in trial 3, at 21, 28 and 35 DAS. These evaluations were carried out by visual analysis at each experimental unit (0 for no injuries, up to 100% for plant death) considering in this case significantly visible symptoms in the plants according to their development (Velini *et al*. 1995).

At the soybean physiological maturity, height was evaluated in 10 plants per plot. For yield, the three central rows of each 3 m long plot were harvested, the grains were measured for mass with moisture corrected to 13% and expressed in kg ha^{-1}.

Statistical analysis

Data were tested by analysis of variance (ANOVA) by F-test ($P \leq 0.05$) separately for each trial. For the levels of both factors, means were compared by Tukey (1949) test ($P \leq 0.05$). For the analysis, the software Sisvar 5.6 (Ferreira 2011) was used.

Results

F-test

For all crop injury assessments at the three trials, a significant effect ($P \leq 0.05$) was detected for both factors and for the interaction. A significant effect was observed on plant height in trial 1 for application period, interaction, but not for herbicide. In trial 2, a significant effect was observed for both the factors and for the interaction on plant height, while in trial 3, no significant effect was found ($P > 0.05$) (Table 2).

For soybean yield, a significant effect was found only for the application period and interaction in trial 1. For trials 2 and 3, no significant effect was detected for the factors and interaction (Table 2). The following sections present the outspread for the variables when a significant effect was found.

Crop injury

In the three trials, the highest phytotoxic potential was observed for the application of flumioxazin/imazethapyr and sulfentrazone/diuron at 14 DAS. Crop injury was up to 56.2%, which was observed at the V_4 stage, for the application of flumioxazin/imazethapyr at 14 DAS in the trial 3. No symptoms of injury were observed for all the trials and evaluations when the herbicides were applied at soybean sowing (Table 3, 4 and 5).

The application of s-metolachlor also caused injury to the crop at trial 3 with higher symptoms for the application at 14 DAS. All herbicides caused some injury in trial 3 when applied at 7 or 14 DAS. In trial 3 during the applications at 7 DAS, soybean was at the V_C stage and at 14 DAS at the V_2 stage, which helps to elucidate the pronounced symptoms of injury. Soybeans were at a phenological stage closer to the beginning of the evaluations at V_4 (Table 5).

Plant height

For plant height in trials 1 and 2, the action of flumioxazin/imazethapyr and sulfentrazone/diuron was influenced by the period of application. A reduction in plant height was observed when these herbicides were applied at 14 DAS. At trial 2, these herbicides also reduced plant height compared to s-metolachlor application and untreated control (Table 6).

Soybean yield

An effect on soybean yield was observed only in trial 3. The sulfentrazone/diuron product was impacted by the application period with a reduction in yield for the application at 14 DAS compared to the application at 7 DAS but without differing from other herbicides. No differences were detected in the other outspreads (Table 7).

Table 3: Crop injury (%) at V_4, V_6-V_7 and R_1 soybean plants under herbicide application at sowing, 7 and 14 days after sowing (DAS). Trial 1

Application period	Sowing	7	14	Sowing	7	14	Sowing	7	14
		DAS			DAS			DAS	
Herbicide (g ha^{-1})[a]	Crop injury at V_4			Crop injury at V_6-V_7			Crop injury at R_1		
Imazethapyr/flumioxazin (100/50)	0.0 aA	0.0 aA	31.5 bB	0.0 aA	0.2 aA	21.5 bB	0.0 aA	0.0 aA	19.7 bB
Sulfentrazone/diuron (245/490)	0.0 aA	0.0 aA	34.5 cB	0.0 aA	0.2 aA	27.7 cB	0.0 aA	0.0 aA	24.7 cB
Clomazone (900)	0.0 aA	0.0 aA	0.2 aA	0.0 aA	0.0 aA	0.5 aA	0.0 aA	0.0 aA	0.0 aA
S-metolachlor (1.440)	0.0 aA	0.0 aA	0.7 aA	0.0 aA	0.0 aA	0.5 aA	0.0 aA	0.0 aA	0.2 aA
Non-treated control	0.0 aA	0.0 aA	0.0 aA	0.0 aA	0.0 aA	0.0 aA	0.0 aA	0.0 aA	0.0 aA

V_4: fourth trifoliolate. V_6-V_7: sixth-seventh trifoliate. R_1: beginning flowering - plants have at least one flower on any node
[a]Rates at acid equivalent (ae) for imazethapyr, and at active ingredient (a.i.) for other herbicides
Means followed by the same letter, lowercase for herbicide and uppercase for application period, do not differ from each other by Tukey's test at the 5% probability level

Table 4: Crop injury (%) at V_4, V_6-V_7, and R_1 soybean plants under herbicide application at sowing, 7 and 14 days after sowing (DAS). Trial 2

Application period	Sowing	7	14	Sowing	7	14	Sowing	7	14
		DAS			DAS			DAS	
Herbicide (g ha^{-1})[a]	Crop injury at V_4			Crop injury at V_6-V_7			Crop injury at R_1		
Imazethapyr/flumioxazin (100/50)	0.0 aA	0.0 aA	14.5 cB	0.0 aA	0.0 aA	11.0 bB	0.0 aA	0.0 aA	9.7 bB
Sulfentrazone/diuron (245/490)	0.0 aA	0.5 aA	47.2 dB	0.0 aA	0.0 aA	38.5 cB	0.0 aA	0.0 aA	33.5 cB
Clomazone (900)	0.0 aA	0.0 aA	0.7 aA	0.0 aA	0.0 aA	0.5 aA	0.0 aA	0.0 aA	0.0 aA
S-metolachlor (1.440)	0.0 aA	0.0 aA	3.2 bB	0.0 aA	0.0 aA	0.0 aA	0.0 aA	0.0 aA	0.0 aA
Non-treated control	0.0 aA	0.0 aA	0.0 aA	0.0 aA	0.0 aA	0.0 aA	0.0 aA	0.0 aA	0.0 aA

V_4: fourth trifoliolate. V_6-V_7: sixth-seventh trifoliate. R_1: beginning flowering - plants have at least one flower on any node
[a]Rates at acid equivalent (ae) for imazethapyr, and at active ingredient (a.i.) for other herbicides
Means followed by the same letter, lowercase for herbicide and uppercase for application period, do not differ from each other by Tukey's test at the 5% probability level

Table 5: Crop injury (%) at V_4, V_6-V_7, and R_1 soybean plants under herbicide application at sowing, 7 and 14 days after sowing (DAS). Trial 3

Application period	Sowing	7	14	Sowing	7	14	Sowing	7	14
		DAS			DAS			DAS	
Herbicide (g ha^{-1})[a]	Crop injury at V_4			Crop injury at V_6-V_7			Crop injury at R_1		
Imazethapyr/flumioxazin (100/50)	0.0 aA	3.0 bB	56.2 eC	0.0 aA	2.0 bB	32.7 eC	0.0 aA	1.0 aA	25.7 dB
Sulfentrazone/diuron (245/490)	0.0 aA	15.5 cB	44.5 dC	0.0 aA	12.5 dB	28.2 dC	0.0 aA	3.7 bB	26.2 dC
Clomazone (900)	0.0 aA	2.5 bB	5.5 bC	0.0 aA	2.7 bB	2.0 bB	0.0 aA	1.0 aAB	2.2 bB
S-metolachlor (1.440)	0.0 aA	17.5 dB	25.5 cC	0.0 aA	9.7 cB	14.7 cC	0.0 aA	4.2 bB	12.2 cC
Non-treated control	0.0 aA	0.0 aA	0.0 aA	0.0 aA	0.0 aA	0.0 aA	0.0 aA	0.0 aA	0.0 aA

V_4: fourth trifoliolate. V_6-V_7: sixth-seventh trifoliate. R_1: beginning flowering - plants have at least one flower on any node
[a]Rates at acid equivalent (ae) for imazethapyr and at active ingredient (a.i.) for other herbicides
Means followed by the same letter, lowercase for herbicide and uppercase for application period, do not differ from each other by Tukey's test at the 5% probability level

Table 6: Plant height (cm) of soybean under herbicide (g ha^{-1})[a] applied at sowing, and 7 and 14 days after sowing (DAS)

Application period	Trial 1			Trial 2		
	Sowing	7 DAS	14 DAS	Sowing	7 DAS	14 DAS
Imazethapyr/flumioxazin (100/50)	114.4 A	115.2 A	91.9 B	112.8 aA	114.5 aA	104.8 bcB
Sulfentrazone/diuron (245/490)	114.2 A	114.9 A	93.2 B	111.4 aA	118.2 aA	97.2 cB
Clomazone (900)	113.7 A	110.7 A	113.5 A	114.9 aA	116.9 aA	112.2 abA
S-metolachlor (1.440)	114.7 A	111.5 A	110.0 A	115.0 aA	113.1 aA	113.8 aA
Non-treated control	114.1 A	113.9 A	112.2 A	112.4 aA	113.8 aA	115.0 aA

[a]Rates at acid equivalent (ae) for imazethapyr, and at active ingredient (a.i.) for other herbicides
Means followed by the same letter, lowercase for herbicide and uppercase for application period, do not differ from each other by Tukey's test at the 5% probability level

Table 7: Soybean yield (kg ha^{-1}) under herbicide (g ha^{-1})[a] applied at sowing, and 7 and 14 days after sowing (DAS). Trial 1

Application period	Sowing	7 DAS	14 DAS
Imazethapyr/flumioxazin (100/50)	4.173 A	4.101 A	3.869 A
Sulfentrazone/diuron (245/490)	4.042 AB	4.303 A	3.696 B
Clomazone (900)	4.332 A	4.174 A	4.048 A
S-metolachlor (1.440)	4.194 A	4.119 A	4.239 A
Non-treated control	4.239 A	4.122 A	4.159 A

[a]Rates at acid equivalent (ae) for imazethapyr, and at active ingredient (ai) for other herbicides
Means followed by the same letter, lowercase for herbicide and uppercase for application period, do not differ from each other by Tukey's test at 5% probability level

Discussion

The injury to soybean plants caused by herbicides is perceived by the changes they cause in the plant's physiology, which may eventually cause its death and yield reductions (Moscardi *et al*. 2012; Nandula *et al*. 2019). In the present study, injury was not observed when herbicides were applied during sowing. The highest phytotoxic potential was found for flumioxazin/imazethapyr and sulfentrazone/diuron. Injury symptoms after herbicide

application, in addition to foliar lesions, can also be observed as a reduction in plant height. Plants require light for growth and development and when the application of herbicides results in changes in their size, they may suffer consequences in their physiology, altering other parameters, such as yield (Arantes *et al*. 2015).

The application of sulfentrazone (200 or 300 g a.i. ha^{-1}) one day after soybean sowing caused mild leaf chlorosis, with a reduction in symptoms 15 days after crop emergence (Osipe *et al*. 2014). In another study, sulfentrazone (200 g a.i. ha^{-1}) applied one day after sowing soybeans caused injury to soybeans (up to 26%) 15 days after application, even so without impact on crop yield (Dalazen *et al*. 2020). Sulfentrazone is commonly applied in soybean pre-emergence either with an interval of days before sowing or upon sowing. Reiling *et al*. (2006) observed injury in soybeans due to the application of sulfentrazone 7 days before sowing, at sowing or at the V_E stage of soybeans. Symptoms were up to 17%, generally more intense for later applications or in soils with lower organic carbon content but without reductions in soybean yield. Our results corroborate these for the application of sulfentrazone in the case of a pre-mixture with diuron. The results regarding the application of diuron in soybean post-emergence are even scarcer, which reinforces the relevance of the present study.

Diuron is a photosystem II inhibitor, its translocation is very low or null, causing symptoms of interveinal chlorosis and, depending on the dose, the plant may recover or be taken to death. It has a slower action compared to sulfentrazone (protoporphyrinogen oxidase – PPO inhibitor), characterized by leaf chlorosis with reduced growth (Jr *et al*. 2021). PPO-inhibiting herbicides applied directly to leaves may have low selectivity (Green and Owen 2011). However, some crops can recover from foliar symptoms (Silva *et al*. 2018; Jr *et al*. 2021).

Other studies evaluating post-emergence or pre-emergence soybean herbicides reported that flumioxazin and s-metolachlor can be applied on the day of soybean sowing, and sulfentrazone should be applied at least fourteen days before sowing to avoid damage to soybean plants (Gazola *et al*. 2021). While herbicides s-metolachlor and imazethapyr caused 10 to 15% of injury and fomesafen or fomesafen + imazethapyr caused 15 to 25% of injury in soybean for soybean post-emergence application (Oliveira *et al*. 2017).

The post-emergence application of s-metolachlor in different mixtures at the V_3–V_4 soybean stage caused injury of up to 12% but without negative impacts on the agronomic performance of the crop and with efficiency in controlling weeds (Sarangi and Jhala 2019). The selectivity demonstrated by s-metolachlor may be related to the plant metabolism rate, however, this is not fully confirmed as observed in the present study.

The selectivity presented by clomazone in the soybean crop, however, may be related to a set of secondary factors, such as hydroxylation, breakage of the chain at the -N-CH_2 radical, conjugation with metabolites, consequently a more accentuated metabolism and a reduction in the translocation to the trials of action (Jr *et al*. 2021). Recent studies evaluating the application of clomazone in soybean post-emergence are scarce, which highlights the importance of the present study.

The investigation of possible effects of pre-emergence herbicides on soybeans is very important given the few studies in this subject. Ribeiro *et al*. (2021) observed a reduction in the height of soybean plants (V_C stage), for the application of sulfentrazone in pre-emergence of soybeans, however, from V_2 and onwards, no further reductions were observed. In addition to sulfentrazone, herbicides imazethapyr, chlorimuron, cloransulam, metribuzin, flumioxazin, saflufenacil, acetochlor, s-metolachlor, dimethenamid-P and pyroxasulfone were also tested and found to have no effects on growth, development and biological nitrogen fixation of soybean plants.

Clomazone and s-metolachlor were selective for soybean as pre-emergence at 7 and 14 DAS. Imazethapyr/flumioxazin or sulfentrazone/diuron at 14 DAS caused significant injury to soybean including reduced plant height and for sulfentrazone/diuron a reduction in yield was found for application at 14 DAS compared to 7 DAS. The highest phytotoxic potential was observed for the application at 14 DAS of flumioxazin/imazethapyr and sulfentrazone/diuron with crop injury of up to 56.2%. Therefore, the recommendation for the application of these herbicides during the initial post-emergence of soybeans should be considered with caution. The use of pre-emergence herbicides is a great tool for weed management. Nevertheless, the best herbicide alternative and dose must be defined with criteria with the aim of not causing significant damage to soybeans especially if they are applied after sowing.

Conclusion

The application of clomazone and s-metolachlor were selective for soybean when applied as pre-emergence at 7 and 14 days after sowing (DAS). The application of imazethapyr/flumioxazin or sulfentrazone/diuron at 14 DAS caused significant injury to soybean. The application of sulfentrazone/diuron caused a reduction in yield for application at 14 DAS compared to 7 DAS. The highest phytotoxic potential was observed for the application of flumioxazin/imazethapyr and sulfentrazone/diuron at 14 DAS but without yield reduction. The application of herbicides as pre-sowing at 7 and 14 DAS was selective for soybean except sulfentrazone/diuron at 14 DAS which reduced soybean yield.

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Author Contributions

JRB, AJP and LPA planned the experiment. JRB, FCG, LGM, MF and ESP conducted the experiment in the field. JRB and AJP interpreted the results, JRB, AJPA and AFMS wrote the original version of the manuscript. AFMS statistically analyzed the data. All authors contributed to the final editing of the manuscript.

Conflict of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable in this manuscript.

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