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RESEARCH ARTICLE

Susceptibility of non-tolerant soybean to low rates of dicamba

Suscetibilidade de soja não-tolerante à subdoses de dicamba

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Abstract: **Background**: The possibility of intoxicating non-tolerant soybean with dicamba is an important and currently problematic in agricultural fields.
Objective: Six experiments were carried out to evaluate the correlation between toxicity of low dicamba rates and yield of non-tolerant soybean cultivars.
Methods: Three areas were selected and two experiments were carried out in each area: one for soybean at V3 and another for R1/R2 stage. Five dicamba rates (five treatments) were applied in each experiment. Toxicity was evaluated at 7, 14, 21, and 28 days after application; the others variables evaluated were plant stand, pre-harvest plant height, production components, and crop yield. Additionally, the maximum toxicity was correlated to the relative yield for each plot.
Results: The dicamba rate of 1 g ha^{-1} was a critical threshold to distinguish rates that caused only phytotoxicity from those that decreased crop yield. Visual toxicity symptoms that did not cause meristematic changes (up to 20% in plants at vegetative stage and 10% in plants at reproductive stage) did not decrease soybean yield.
Conclusion: The correlation between dicamba toxicity and soybean crop yield is the most important factor to be considered under field conditions. Soybean plants sprayed at reproductive stage (R1/R2) had higher yield losses than at vegetative stage: each 10% increase in phytotoxicity in soybeans at R1/R2 would result, on average, in 12.2% yield loss.

Keywords: hormonal herbicides, drift, toxicity, yield.

Resumo: **Introdução**: A possibilidade de intoxicar a cultura da soja não-tolerante com o herbicida dicamba é uma realidade importante e atual nos campos agrícolas.
Objetivo: Seis experimentos foram desenvolvidos com o objetivo de correlacionar a intoxicação por subdoses de dicamba com a produtividade de cultivares de soja não-tolerantes ao herbicida.
Métodos: Foram selecionadas três áreas experimentais, em que dois experimentos foram instalados em cada área, um em estádio V3 e outro em R1/2. Em cada experimento foram aplicados cinco tratamentos (doses de dicamba). Avaliou-se a intoxicação da cultura aos 7, 14, 21 e 28 dias após aplicação, estande, altura pré-colheita, componentes de produção e produtividade. Ainda, realizou-se análise entre a intoxicação máxima de cada parcela e sua produção relativa.
Resultados: A dose de 1 g ha^{-1} foi um ponto crítico de separação entre doses apenas fitotóxicas e doses que efetivamente reduziram produtividade. Intoxicações visuais de até 20% em estádio vegetativo e 10% em estádio reprodutivo, sem alterações meristemáticas, não reduziram a produtividade da soja.
Conclusão: A relação entre intoxicação percentual por dicamba e produtividade é o fator mais importante a ser considerado em condição de campo. As perdas de rendimento em estádio reprodutivo são mais intensas que em estádio vegetativo; neste caso, a cada 10% de intoxicação da soja em R1/2 tem-se, em média, 12,2% de perda de rendimento.

Palavras-chave: herbicidas hormonais, deriva, intoxicação, produtividade.

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1. Introduction

Soybean (*Glycine max* L. Merrill) is a species native to East Asia that is highly adapted to Brazil, where it is mostly grown in the Center West, Southeast, and South regions of the country. Soybean production in Brazil has grown annually, not only because of favorable climate conditions, but also due to the use of new cultivars and improved agronomic techniques, which have contributed significantly to optimizing the production of these grains. Consequently, Brazil surpassed the United States as the world's largest soybean producing worldwide. According to CONAB (2022), the total soybean area grown in Brazil in the 2021-2022 crop season was 40.7 million hectares, which was 3.8% higher than that in the 2020-2021.

Weeds are among the biotic components that affect soybean production systems. These plants directly impair crop yield by competing for resources for plant growth and by affecting harvesting procedures and the quality of the harvested product, and indirectly by hosting pests, diseases, and nematodes (Gazziero, 2014; Piccinini et al., 2018). The weed infestation in soybean crops stands out when considering the several confirmed cases of herbicide-resistant biotypes of important species, mainly to glyphosate (Carvalho et al., 2015; Licorini et al., 2015; Braz et al., 2017; Dalazen et al., 2019; Heap, 2023).

In this sense, horseweed (*Conyza* spp.) has caused significant agricultural crop losses due to selection of biotypes resistant to multiple modes of action (Braz et al., 2017; Heap, 2023). A viable strategy for managing these weeds is the use of soybean cultivars tolerant to different herbicides, mainly broadleaf herbicides, for example, the cultivar tolerant to dicamba (Spaunhorst et al., 2014) approved by the CTNBio (CTNBio, 2016) and released in the 2021-2022 crop season. Considering the several cases of horseweed resistance to glyphosate in Brazil (Mendes et al., 2021), large use of dicamba in agricultural areas is expected as an important alternative for the management of this weed (Osipe et al., 2017; Carvalho et al., 2022).

Dicamba is an herbicide from the chemical group of benzoic acids and class of synthetic auxin growth regulators and is also known as an auxin-mimicking herbicide. Epinasty of leaves and petioles is the first symptom observed in susceptible plants after dicamba application; but other metabolic functions are affected over time, resulting in other symptoms, such as deformations in leaf veins and consequently in leaf blade, growth halt, and root thickening. The death of susceptible plants is slow, usually between 3 and 5 weeks after dicamba application (Oliveira Júnior, 2011; Figueiredo et al., 2016; Zhou et al., 2016). Non-tolerant soybean cultivars are mainly sensitive to auxin herbicides, such as dicamba (Solomon and Bradley, 2014; Silva et al., 2018).

Furthermore, it is an herbicide with a higher vapor pressure, which consequently increases the possibility of drift during its application, thus reaching nontarget crops, such as adjacent crops, mainly those with soybean cultivars that are not tolerant to the molecule (Silva et al., 2018; Oliveira et al., 2019). Disregarding good agricultural practices can cause dicamba to drift to non-tolerant soybean crops, impacting their yield. Therefore, information about the effects of dicamba application, under different conditions, on crops that are not tolerant to the molecule is important (Carvalho et al., 2022). Thus, six experiments were carried out to correlate toxicity by low dicamba rates to yield of non-tolerant soybean cultivars.

2. Material and Methods

Six independent field experiments were carried out in three agricultural areas grown with soybean in the municipality of Machado, in the south of Minas Gerais, Brazil, between September 2019 and March 2020. The city's climate, according to Köppen and Geiger (1928), is classified as Cwa (hot summer and cold winter), with an average annual temperature of 20.6°C and an average annual rainfall of 1508 mm. The rainfall data recorded for the region during the experimental period are presented in Figure 1 (municipality of Paraguaçu), showing high-water availability in the 2019/2020 crop season.

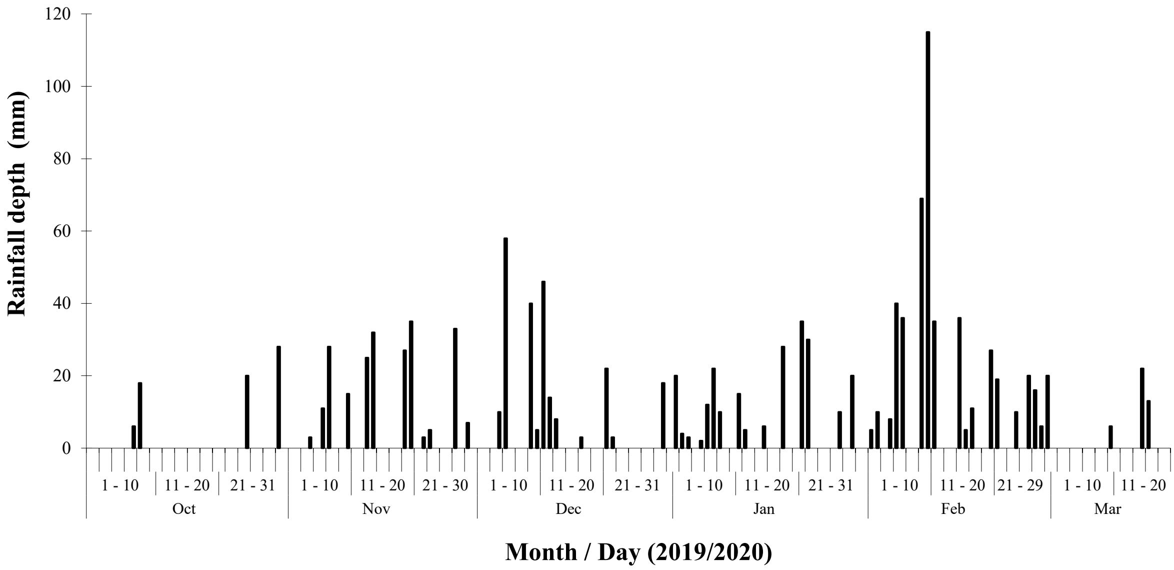

Figure 1. Mean rainfall depths recorded for the region during the development of the experiments. Paraguaçu, MG, Brazil, 2019/2020.

The first area (21°35'13"S, 45°52'53"W) was previously prepared using conventional tillage system, with irrigation by central pivot; seeds of the soybean cultivar Monsoy 5917 IPRO (indeterminate growth habit) were sown on September 30, 2019. The second area (21°46'17"S, 45°45'55"W) was cultivated under rainfed conditions and no-tillage system; seeds of the soybean cultivar Monsoy 6410 IPRO (indeterminate growth habit) were sown on October 30, 2019, on straw of previous oat crops. The third area (21°36'48"S, 45°51'51"W) was cultivated under a rainfed condition and no-tillage system; seeds of the soybean cultivar Pionner 97R50 IPRO (indeterminate growth habit) were sown on November 04, 2019, on straw of previous sorghum crops.

The soil chemical attributes of the experimental areas are described in Table 1. Each area was subjected to two independent experiments, one consisting of application of the treatments to soybean plants at the phenological stage V3 (two developed trifoliate leaves) and other consisting of application of treatments to soybean plants at R1/R2 stage (beginning of flowering). Soybean seeds were sown with spacing of 0.5 m between rows in all areas. The seeds of all soybean cultivars received industrial treatment with fungicides and insecticides, aiming to provide protection against the initial attack of pathogens and pests. Furthermore, inoculation was carried out via seed treatment to ensure an adequate supply of nitrogen to the plants through the process of biological nitrogen fixation.

Table 1. Chemical attributes of soils collected in the experimental areas. Machado, MG, Brazil, 2019/2020.

Area	pH H_2O	OM dag kg^{-1}	P mg dm^{-3}	K	Ca	Mg	H+Al	SB	CEC	BS %	AS %
				mg dm^{-3}	------------------cmol$_c$ dm^{-3}------------------						
1	6.2	3.6	8.0	144.0	3.9	1.0	3.0	5.3	8.3	63.8	0.0
2	5.9	3.0	4.0	172.0	2.9	1.5	3.2	4.8	8.0	60.0	0.0
3	6.1	3.8	20.2	126.0	4.8	1.9	2.1	7.1	9.2	77.3	0.0

Textural class: clayey soil.; OM = organic matter; SB = sum of bases; CEC = cation exchange capacity; BS = base saturation; AS = aluminum saturation.

All the experiments were carried out in commercial areas, maintained with an excellent level of crop nutrition and phytosanitary management. Fertilization practices were carried out according to soil analysis (Table 1). During soybean development, herbicides, insecticides, and fungicides were applied in accordance to agricultural recommendations, keeping the crop free of weeds, pests and diseases (Seixas et al., 2020).

Each experiment was installed in a randomized block design with four replications. Five treatments were used in all experiments, consisting of rates of dicamba herbicide (0, 0.1, 1.0, 10.0, and 100 g ha^{-1} of active ingredient; Atectra®, diglycolamine (DGA) salt). These rates were based on estimates of losses by drift conditions or procedures used for cleaning spraying tanks. The experimental plots consisted of six 5-m planting rows, totaling an area of 15 m^2. The evaluation area consisted of the two central rows of the plots, disregarding 1 m from the ends. All treatments were applied using a CO_2 pressurized backpack sprayer coupled to a spray boom with four TTI 110.02 tips, calibrated for 200 L ha^{-1}. The weather data recorded for each area on the days of herbicide application are described in Table 2.

Table 2. Weather conditions recorded for the experimental areas on the days and times of application of dicamba treatments. Machado, MG, Brazil, 2019/2020.

Area	Phenology - Date	Time		RH (%)	Temp. (°C)	Wind (km ha^{-1})	Cloudiness
		Beginning	Ending				
1	V3 - 10/24/19	9:00 a.m.	9:30 a.m.	56.7	30.8	4.5	Partly cloudy
	R2 - 11/16/19	8:30 a.m.	9:00 a.m.	74.7	28.3	4.9	Open
2	V3 - 11/23/19	8:20 a.m.	8:40 a.m.	78.0	27.2	Absence	Cloudy
	R1 - 12/20/19	9:30 a.m.	9:50 a.m.	75.2	31.0	1.5	Cloudy
3	V3 - 12/02/19	8:20 a.m.	8:50 a.m.	74.4	23.9	7.0	Cloudy
	R1 - 12/26/19	8:10 a.m.	8:40 a.m.	81.2	29.1	3.8	Open

RH = relative humidity.

Toxicity in soybean plants was evaluated at 7, 14, 21, and 28 days after application (DAA) of herbicide rates. A scale of grades from 0% to 100% was used to evaluate the percentage of injuries, where zero denotes absence of symptoms (fully healthy plants) and 100% denotes the death of the plant (SBCPD, 1995). Final plant height (pre-harvest) was measured before the pre-harvest desiccation of plants in the areas, approximately one week before harvesting.

Soybean grain harvest was carried out on January 15, 2020 (Area 1), February 28, 2020 (Area 2), and March 20, 2020 (Area 3). Firstly, the number of plants (final plant stand) in each plot evaluation area was counted. The entire evaluation area of each plot was harvested in the areas 1 and 2. Ten consecutive plants in each of the two central rows of each plot were harvested in the area 3, totaling 20 plants per plot. All harvested plants were sent to a laboratory, where they were evaluated for number of pods per plant, and then threshed to obtain the number of grains per pod, yield per plant (g $plant^{-1}$), total yield (kg ha^{-1}), and 1,000-grain weight (g). All production data were adjusted to 13% moisture.

The data of all experiments were subjected to analysis of variance by the F test and, subsequently, to analysis by groups of experiments, according to test of homogeneity of variances (Pearson and Hartley, 1956). Significant means were compared by Tukey's test. All the tests were applied using $p<0.05$ as significance level. Then, a graphic analysis was carried out for correlating the relative yield percentage and maximum toxicity found for each plot, thus obtaining the correlation between toxicity and yield.

The test of Tukey was applied to all experimental data rather than regression analysis, considering that the objective was to compare the effect of treatments to the check plots (without herbicide application). The use of regression analysis would result in equations correlating herbicide rates and crop yields that would not differentiate the effect of the treatments from the control, therefore, with no practical field application.

3. Results and Discussion

The rate of the active ingredient that reaches the crop cannot be accurately established under actual conditions of drifting and/or toxicity to crops, since the product is not intentionally applied to the crop; however, symptoms of toxicity can be visually evaluated by using a scale of grades. Therefore, the correlation between toxicity and crop yield is more important than that between product rate and crop yield, mainly when considering that a same rate can cause different toxicity symptoms in different cultivars. However, when the correlation between toxicity and yield is linear, the estimation of potential production losses is easier.

In this sense, increasing dicamba rates sprayed on non -tolerant soybean plants caused increasing damages to the crop (Table 3). The herbicide rate of 1.0 g ha^{-1} was a critical point of toxicity, considering the groups of experiments with soybean plants subjected to herbicide spraying at V3 or R1/R2 phenological stage. Regarding the soybean plants at V3, the maximum toxicity found for the rate of 1.0 g ha^{-1} was less than 20%, not significantly impacting crop yield. However, plants at R1/R2 subjected to this same rate presented a maximum toxicity of 24.5% and decreases in crop yield (Table 3). Therefore, a small difference (in magnitude) was found for toxicity percentages in plants at different phenological stages; however, the occurrence of toxicity in plants treated at R1/R2 stage denotes a higher importance, since it had a more significant effect on crop yield.

Table 3. Toxicity percentage, plant stand, and crop yield (accumulated from three experiments) of soybean plants sprayed with different rates of herbicide dicamba at two phenological stages. Machado, MG, Brazil, 2019/2020.

Dicamba (g ha^{-1})	Toxicity				Stand (plants m^{-1})	Yield kg ha^{-1}
	7 DAA	14 DAA	21 DAA	28 DAA		
Application at V3 stage						
Control	0.0 a	0.0 a	0.0 a	0.0 a	12.3 ab	4.333.5 ab
0.1	3.9 a	4.3 a	4.3 a	4.4 a	13.3 a	4.760.7 a
1	14.3 b	19.8 b	19.3 b	13.4 b	13.5 a	4.325.3 ab
10	24.6 c	37.3 c	37.3 c	30.8 c	13.5 a	3.628.4 b
100	56.1 d	69.4 d	74.4 d	76.0 d	9.5 b	1.566.7 c
$F_{treatment}$	152.16**	175.22**	208.06**	202.61**	4.66**	47.188**
$LSD_{5\%}$	7.32	8.59	8.43	8.73	3.12	455.93
CV (%)	31.85	28.29	26.85	30.17	21.63	17.23
Application at R1/R2 stage						
Control	0.0 a	0.0 a	0.0 a	0.0 a	12.9 ab	4.562.4 a
0.1	6.5 b	6.7 b	7.1 a	6.8 a	13.6 a	4.259.2 ab
1	17.3 c	23.8 c	24.5 b	23.2 b	13.0 a	3.890.0 b
10	28.9 d	39.3 d	41.5 c	40.4 c	14.5 a	2.848.8 c
100	64.6 e	70.0 e	72.4 d	75.2 d	9.6 b	71.60 d
$F_{treatment}$	590.10**	566.46**	270.82**	276.93**	4.94**	180.39**
$LSD_{5\%}$	4.22	4.74	7.11	7.29	3.33	547.00
CV (%)	15.50	14.61	21.04	21.55	22.55	15.06

CV = coefficient of variation; **F test significant at 1% probability; LSD – least significant difference, according to Tukey's test; Means followed by the same letters in the columns are not significantly different from each other by the Tukey's test at 5% probability.

The toxicity caused by applications of dicamba also caused changes in the crop production components. Considering the experiments subjected to herbicide spraying on soybean at V3, changes in production components were found for rates equal to or higher than 10 g ha^{-1}, presenting decreases 1,000-grain weight, and yield per plant (Table 4). Regarding the plants at R1/R2, the dicamba rate of 0.1 g ha^{-1} was enough to decrease the yield per plant; the rate of 1.0 g ha^{-1} affected negatively pre-harvest plant height; and all other variables were affected by dicamba rates equal to or higher than 10 g ha^{-1}, except 1,000-grain weight (Table 4). Spraying the herbicide rates on plants during reproductive stage caused greater damage to plant productive structures, affecting the pod development, thus resulting in fewer and lighter grains and, consequently, lower yield.

In general, the most common toxicity symptoms observed in soybean plants were: wrinkling on the edges and tips of leaflets, widespread cupping in leaflets, callus formation at the petiole insertion points on the stem, overgrowth of petioles, changes in meristematic regions, bud death, reduction of internodes, changes in the developmental cycle, flower abortion, and epinasty.

Regarding the toxicity symptoms observed in plants sprayed at V3 stage, similar results were found by Andersen et al. (2004), who applied dicamba rates between 5.6 and 112 g ha^{-1} and observed symptoms ranging from mild (<10%) to severe (96%), including changes in leaves, epinasty, and even death of apical buds. These applications resulted in yield losses ranging from 14% to 93% compared to plants not subjected to applications. Moreover, toxicity can also cause changes in seed germination and vigor (Costa et al., 2020a).

Table 4. Production components (pods per plant, grains per pod, and 1,000-grain weight), yield per plant, and pre-harvest plant height of soybean plants (accumulated from three experiments) sprayed with different dicamba rates at two phenological stages. Machado, MG, Brazil, 2019/2020.

Dicamba ($g\ ha^{-1}$)	Number of pods per plant	Number of grains per pod	1,000-grain weight (g)	Yield per plant ($g\ plant^{-1}$)	Pre-harvest plant height (m)
Application at V3 stage					
Control	44.06 a	2.49 a	164.69 a	18.02 a	0.98 a
0.1	42.33 a	2.57 a	165.03 a	18.06 a	0.94 ab
1	40.24 a	2.47 a	162.99 a	16.23 ab	0.92 ab
10	38.87 a	2.31 ab	148.96 b	13.67 b	0.83 b
100	26.06 b	2.12 b	122.21 c	5.81 c	0.49 c
$F_{treatment}$	6.15**	6.06**	41.96**	41.74**	44.87**
$LSD_{5\%}$	11.57	0.29	11.39	3.18	0.12
CV (%)	26.01	10.55	6.42	19.06	12.39
Application at R1/R2 stage					
Control	40.63 a	2.63 a	169.23 a	17.95 a	0.94 a
0.1	37.38 a	2.57 a	165.76 a	15.80 b	0.95 a
1	38.34 a	2.39 a	168.26 a	15.13 b	0.77 b
10	30.94 b	1.90 b	174.03 a	10.01 c	0.54 c
100	3.19 c	1.06 c	116.04 b	0.53 d	0.39 d
$F_{treatment}$	167.70**	43.253**	21.20**	180.67**	154.30**
$LSD_{5\%}$	4.80	0.40	20.99	2.09	0.08
CV (%)	13.75	16.35	11.39	15.15	9.68

CV = coefficient of variation; **F test significant at 1% probability; LSD – least significant difference, according to Tukey's test; Means followed by the same letters in the columns are not significantly different from each other by the Tukey's test at 5% probability.

The soybean plants at V3 stage were more tolerant to dicamba toxicity, as toxicity percentages up to 20% had no impact on their production; however, higher toxicity percentages caused more symptoms in the meristematic region, altering the growth rate and plant development, thus affecting crop yield. Plants at reproductive stage were more sensitive to toxicity, as toxicity percentages from 15% to 20% were sufficient to negatively impact crop yield; the increases in toxicity in plants at this stage caused changes in flowering, resulting in flower abortion, poor formation of pods, and higher decrease in crop yield (Table 3).

Similarly, Solomon and Bradley (2014) evaluated the effect of several auxin herbicides on non-tolerant soybean crops and reported that toxicity percentages lower than 10% two weeks after application at the V3 stage did not decrease crop yield; however, more severe toxicity damages were found in plants treated at reproductive stages than in those at vegetative stage. Consequently, number of pods per plant and grains per pods decreased, mainly for toxicity in plants at R2. They reported changes in the cycle and delay in the maturity of the crop, which were also found in all field experiments of the present study, mainly after the plants had recovered from the injuries caused by herbicide spraying at vegetative stage.

Consistent information is found in the literature about higher decreases in soybean crop yield due to phytotoxicity during reproductive stages (Egan et al., 2014; Silva et al., 2018; Costa et al., 2020b). According to Griffin et al. (2013), soybean crops are 2.5-fold more susceptible to toxicity during the flowering stage than during vegetative stages. Thus, the developmental crop stage at which toxicity occurs is an important factor for the manifestation of injury symptoms and yield changes (Costa et al., 2020b; Carvalho et al., 2022). Regarding auxin herbicides, soybean plants have shown more susceptibility to dicamba than to 2,4-D (Egan et al., 2014; Solomon and Bradley, 2014; Silva et al., 2018; Costa et al., 2020b), which denotes the need for especial attention to this molecule.

Furthermore, Bish and Bradley (2017) reported that even low rates of the herbicide dicamba can cause damages to susceptible plants, which can be caused by drifting or even contamination of machinery and sprayers. Dicamba acts on plant metabolism by stimulating the synthesis of nucleic acids and proteins, triggering abnormalities in growth, as it affects cell division, enzyme activity, and respiration (USEPA, 2013).

The correlation between toxicity and crop yield in plants at V3 stage was best fitted to a parabolic model; despite the occurrence of decreases in yield, they were less pronounced compared to plants at the R1 stage, mainly under toxicity of up to 20% (Figure 2). Egan et al. (2014) reported that visuals symptoms of phytotoxicity are not fully adequate for forecasting yield losses, mainly for soybean plants at V3 stage. Despite the data dispersion in the present work, the regression fit was 91.1% for soybean plants at V3 and 90.8% for plants at R1/R2 stage, which is considered satisfactory. The linear regression analysis for plants at R1/R2 stage denoted that a 10% increase in visual toxicity would result in a 12.2% decrease in crop yield, i.e., more severe compared to plants at V3 stage (Figure 2).

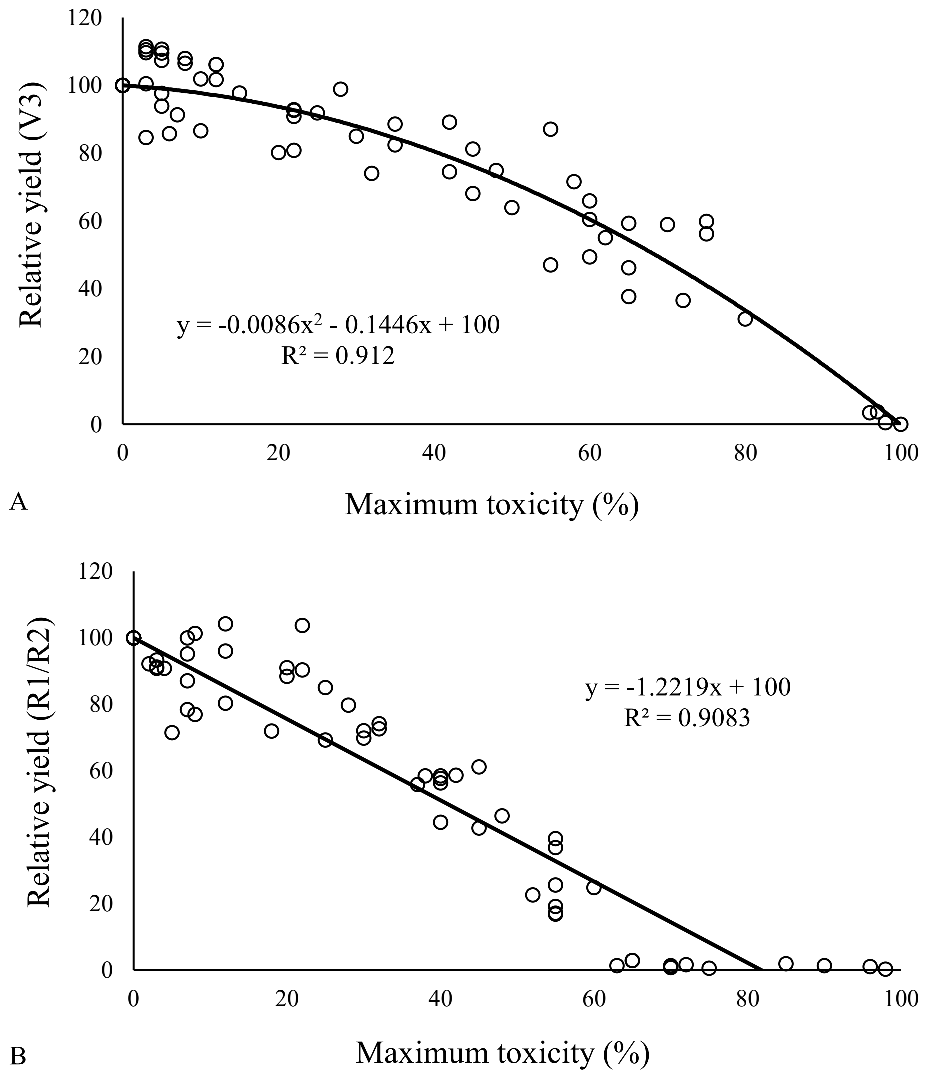

Figure 2. Relative soybean yield correlated to maximum toxicity (%) for each experimental plot after spraying dicamba on plants at V3 (A) or R1/R2 (B) phenological stages. Machado, MG, Brazil, 2019/2020.

4. Conclusions

Dicamba rate of 1 g ha^{-1} was a critical threshold to distinguish rates that caused only phytotoxicity from those that decreased soybean yield. Visual toxicity symptoms that did not cause meristematic changes (up to 20% in plants at vegetative stage and 10% in plants at reproductive stage) did not decrease crop yield.

The correlation between dicamba toxicity and soybean crop yield is the more important factor to be considered under field conditions. Yield losses for soybean plants sprayed at reproductive stage (R1/R2) were higher than those found for plants at vegetative stage: each 10% increase in phytotoxicity in soybeans at R1/R2 would result, on average, in 12.2% yield loss.

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