

Subdoses of Dicamba Herbicide on Yield Componentes in Function of the Soybean Growth Stage

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Abstract

Soybean cultivars without tolerance can suffer injury from exposure to tank herbicide residues. This study aimed at assessing the potential effects of the broad spectrum herbicide dicamba on sensitive soybean during its vegetative and reproductive stages. The trial was performed in a randomized complete block design with four replications. Six rates (1.4, 2.8, 5.6, 11.2, 16.8 and 28.0 g ae ha⁻¹) of dicamba were applied at two soybean growth stages (V3 and R2). The soybeans were planted in Passo Fundo, Rio Grande do Sul state in 2015/16 agricultural year. The dicamba induced symptoms of soybean phytotoxicity showed a steady rise at all the assessment times. The soybean decreased in height as the subdose of the herbicide increased. Dicamba was observed to negatively affect the number of nodes alone, during the V3 and R2 stages. The grain yield was adversely affected at the 28.0 g ae ha⁻¹ subdose, in the V3 application stage, and in response to the 16.8 and 28.0 g ae ha⁻¹ subdoses in the R2 stage.

Keywords: *Glycine max*, tank residues, drift, soybean injury, auxin mimic herbicide

1. Introduction

Over the recent past various crop cultivars have been developed by the breeding agencies, they possess the herbicide resistant trait to be able to avert the presence of herbicide-resistant weeds (Vink et al., 2012). When multi resistant cultivars are made available, farmers have the option of incorporating several new weed-control management techniques, including those against glyphosate-resistant or glyphosate-tolerant weeds, while conserving the glyphosate-resistant crops (Green & Castle, 2010; Vink et al., 2012). Roundup Ready 2 Xtend® (glyphosate- and dicamba-resistant) and Enlist™ (with resistance to glyphosate and 2,4-D) have already been made available for commercial cultivation in the United States, and are predicted for release in other countries like Brazil.

The herbicide dicamba is an auxin mimic, utilized globally for over 40 years in weed control of several dicots (Behrens et al., 2007). This herbicide disrupts cell wall plasticity and nucleic acid metabolism, stimulates the auxin response genes, causing ethylene overproduction and therefore increased abscisic acid levels (Kelley et al., 2004; Grossmann, 2010; Shaner, 2014). The abscisic acid rise results in stomatal closure and restricted CO₂ assimilation, thus inhibiting plant growth (Grossmann, 2010). The commonest symptoms of the presence of auxin-mimicking herbicides in soybean are stem and leaf epithelia, stem swelling and cracking, as well as chlorosis and necrosis (Andersen et al., 2004; Kelley et al., 2005).

The introduction of dicamba-resistant soybean cultivars has unavoidably and foreseeably necessitated herbicidal spraying, which leaves behind some residues in tanks, especially near cultivars which are nonresistant to them, resulting in insults and a lowered grain yield (Olszyk et al., 2015). An 18% drop in the grain yield was reported, caused by the residues from 1% of the commercial dose of dicamba applied to the soybean crop (Griffin et al., 2013). Under very severe circumstances, 85% yield decrease was reported in response to dicamba application in a dose of 41 g ai ha⁻¹ (Johnson et al., 2012). On the contrary, soybean shows higher susceptibility to dicamba during the reproductive phase, revealing 2.5 times greater sensitivity when the application is done in the R1 than during the V3 (Griffin et al., 2013).

Data regarding the soybean sensitivity to dicamba tank residues in the vegetative and reproductive stages under the environmental conditions of Brazil are quite scarce. Furthermore, analyses of the yield components may prove beneficial in the quantification of these effects. Exhaustive research demonstrates that the number of nodes, number of pods and grain weight are the main factors supporting high grain yields. Therefore, it becomes essential to evaluate the components influencing grain yield to clearly appreciate the way soybean exposure to auxin-mimicking herbicides impact the grain yield during the various developmental stages. A clear establishment of the effects of the dicamba residues applied on spray tanks for non-resistant soybean cultivars will enable the soy producers and pesticide applicators to understand the potential risk of injuring the soybean crop and thus reducing the soybean yield.

This study is based on the hypothesis that the soybean susceptibility to dicamba results in decreased grain yield. Therefore, this research aimed at evaluating the potential results of the dicamba herbicide on soybean, during the vegetative and reproductive growth stages.

2. Material and Methods

2.1 Plant Material and Experimental Conditions

The experiment was performed in the experimental field of the University of Passo Fundo (UPF), situated in the middle plateau region of the Rio Grande do Sul, Brazil. Located at 678 m altitude, with the geographical co-ordinates of 28°12'59"S and 52°23'37"W, the soil in this experimental area is of the typical dark red variety (Embrapa, 2006). This region experiences subtropical and humid climate with hot summers classified as Cfa, according to the Köppen classification.

Prior to installing the experiment, at 20 days before sowing, glyphosate was applied across a total area of 1080 g ai ha⁻¹ to the vegetation present there. Soybean, Syngenta IPRO, was planted in December 2015, maintaining 0.45 m line spacing with a population of 280,000 plants ha⁻¹.

The total rainfall received during the period of the experiment was 1365.2 mm, which is more than the climatological normal (which is 855.0 mm) for this period, (Figure 1). The air temperature during this period was, on average, 21 °C, falling within the climatological normal of 20.5 °C between December and April (Embrapa, 2016).

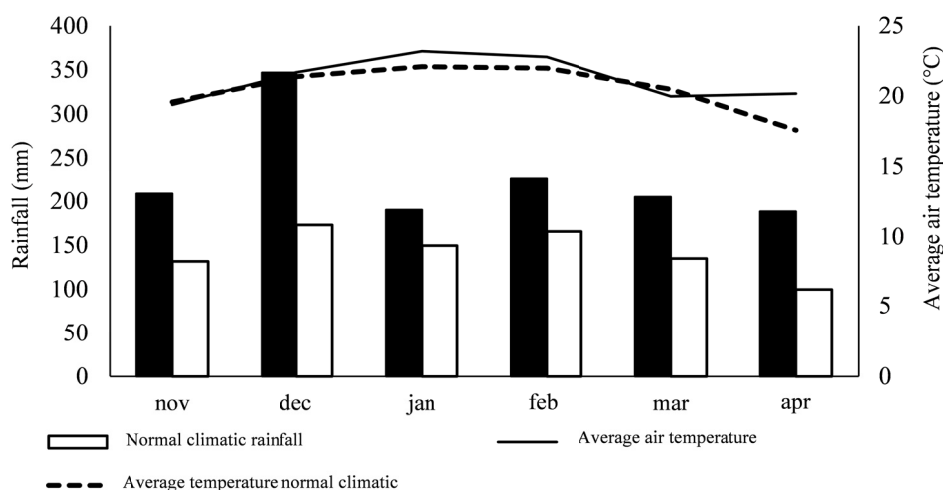


Figure 1. Rainfall monthly, normal rainfall climatic, average air temperature monthly and average air temperature normal climatic, in period of trial conduction. Passo Fundo-RS. 2016

2.2 Experimental Design and Treatments

The randomized block design was adopted for the experiment, arranged according to the split-plot scheme and included four replicates, each plot being 5 m in length and 3.5 m in width. The application times (stages V3 and R2 of soybean) were allocated for the main plot and for the subplots the dicamba (Atectra® 480 g ae L⁻¹ BASF Corporation) herbicide sub-doses were selected. The subdoses applied were: 0; 1.4; 2.8; 5.6; 11.2; 16.8 and 28.0 g ae ha⁻¹, respectively, representing 0; 0.25; 0.5; 1; 2; 3 and 5% of the commercial dose.

2.3 Procedures

Only when the soybean achieved the developmental stages specified (V3 and R2), the herbicide was added using a CO₂ pressurized spray with a 110.015 fan tip, calibrated to apply the herbicide syrup at a volume of 150 L ha⁻¹. At 3, 7, 14 and 28 days post the application of the treatments (DAT), the phytotoxicity was estimated on a percentage scale, in which zero indicated the absence of symptoms and plant death, as shown in Table 1 (Frans et al., 1986; Robinson et al., 2013). At 14 and 28 DAT, the heights of ten plants within the useful plot area were measured from the main stem base to the node of the last trefoil that had emerged. During all time of the trial conduction glyphosate was applied across a total area of 1080 g ai ha⁻¹ to aiming at keeping the crop free from the presence of weeds.

At the time of the harvest or maturation, 10 plants from within the useful area of each plot were recorded for plant height, number of nodes in the main stem, number of branches, total number of pods and thousand seed weight. To analyze the data, the mean values of the 10 plants evaluated were used. Also, the three central lines in the useful area of the plot were harvested using a mechanized plot harvester, and the grain yield was determined.

Table 1. Scale for visual estimate of soybean injury affected by synthetic auxin herbicides (Robinson et al., 2013)

Escala	
0	No injury, plant growth is normal.
10	Slight reduction in height or canopy volume, cupped or bubbled leaves on less than one equal to the upper 10% of the plant, bent petioles, and, chlorosis or necrosis.
20	Moderately crinkled leaflets (extended across less than or equal to the upper 20% of the plant), curled petioles, reduced height and canopy volume, cupped terminal leaflets.
30	Moderate to high reduction of height and canopy; compacted internodes and plants begin to have an abnormal appearance; malformation with drawstring, fiddleneck, or cupped effects on less than or equal to the upper 30% of the plant; many petioles curled and main stems may be bent.
40	Highly stunted plants (less than or equal to 40% of the plant), petioles curled and main stems bent or starting to curl, upper leaves exhibit severe malformation and expansion of new leaves suppressed, plant may have patches of necrotic tissue.
50	Very high reduction of plant height (less than or equal to 50% of the plant) with little likelihood of recovery from the apical meristem, new growth suppressed, formation of pods reduced or malformed, some leaf and stem tissue becomes necrotic, petioles and stems show severe twisting.
60	Severe height and canopy reduction, including any new growth from axillary buds; leaves severely cupped or fiddlenecked on less than or equal to 60% of the plant; petioles and stems twisted, swollen, and splitting; more extensive die-back of tissue.
70	Severe to very severe reduction of plants, new growth callused and inhibited, most leaves severely deformed and mostly necrotic, extensive petiole bending.
80	Very severe soybean injury, less than or equal to 80% of the plants mainly prostrate, petioles twisted with leaves drooping, leaves chlorotic or necrotic, stems severely twisted, swollen, and split.
90	Plant dying, less than or equal to 90% of the plants mainly prostrate, leaves and stems mostly chlorotic or necrotic, all petioles severely twisted, swollen, or split.
100	All plants dead.

2.3 Statistical Analysis

Data were first analyzed for normality (Shapiro Wilk test) and, later submitted to the analysis of variance ($p \leq 0.05$). For the variables of phytotoxicity at 3, 7, 14 and 28 DAT; stature at 14, 28 DAT and at maturation, where statistical significance was observed, the Tukey test was done at 5% probability of error. For the other variables, number of nodes in the main stem, number of branches, number of pods, thousand seed weight and grain yield, in the event of statistical significance, regression analysis was performed for the factors investigated.

The regression analysis was done utilizing the Sigma Plot 10.0 program (Sigmaplot, 2007), and the data were adjusted to the sigmoidal regression equation of the logistic type, as follows: $y = a/[1 + (x/x_0)^b]$.

3. Results and Discussion

3.1 Symptoms of Phytotoxicity

Phytotoxicity symptoms caused by the dicamba herbicide in soybean were evident in response to almost all the subdoses applied. In fact, the responses became more intense throughout the evaluation period (Table 2). Three days after application of the treatments (DAT), the strongest symptoms were seen induced by the 11.20 g ae ha⁻¹ subdose, achieving values of 71.2% and 15.0% for the highest subdose during the applications at V3 and R2, respectively. For 7, 14 and 28 DAT, the symptoms which were assessed were increased, becoming noticeable even at the 1.4 g ae ha⁻¹ subdose, during both the V3 and R2 stages. The dicamba herbicide induced

phytotoxicity observed in the glyphosate-resistant soybean was recorded as 21% of injury at the end of one week post application of the 3 g ae ha⁻¹ and as 80% after the 41 g ae ha⁻¹ applied to the soybean in its vegetative phase (Johnson et al., 2012).

Table 2. Phytotoxicity (%) to soybean at 3, 7, 14 and 28 days after treatments applications (DAT), Syngenta 13561 IPRO cultivar, in function of dicamba subdoses and growth stages of soybean. Passo Fundo-RS, 2016

Dose (g ae ha ⁻¹)	Growth Stage		Average
	V3	R2	
<i>Phytotoxicity at 3 DAT</i>			
0.0	D ¹ 0.0 a	BA 20.0 a	20.0 d
1.4	D 30.0 a	BA 20.0 b	20.0 d
2.8	D 32.5 a	BA 20.0 b	21.2 d
5.6	D 32.0 a	BA 21.2 a	21.6 d
11.2	C 14.5 a	AB 27.0 a	10.7 c
16.8	B 30.0 a	AB 27.0 a	19.8 b
28.0	A 71.2 a	AB 15.0 b	43.1 a
CV (%)			9.7
<i>Phytotoxicity at 7 DAT</i>			
0.0	20.0	20.0	20.0 ee
1.4	26.5	20.7	23.6 de
2.8	27.0	10.5	28.7 de
5.6	10.7	12.0	11.3 ce
11.2	17.5	23.2	20.3 ce
16.8	27.5	35.5	31.5 be
28.0	45.0	52.5	48.7 ae
CV (%)			33.6
<i>Phytotoxicity at 14 DAT</i>			
0.0	DF 20.0 a	EE 10.0 a	10.0 fc
1.4	DE 10.0 a	ED 16.2 a	15.0 ec
2.8	DE 10.5 b	EC 28.7 a	20.2 de
5.6	CD 14.7 b	EB 50.0 a	28.0 cd
11.2	BC 23.7 b	EB 55.7 a	35.6 bc
16.8	AB 32.5 b	AB 62.0 a	43.1 bc
28.0	DA 40.0 b	EA 69.5 a	52.5 ac
CV (%)			18.76
<i>Phytotoxicity at 28 DAT</i>			
0.0	D 10.0 a	AE 10.0 a	10.0 fc
1.4	C 16.2 a	AD 16.2 a	16.2 ec
2.8	C 23.7 a	AC 28.7 a	26.2 dc
5.6	B 37.2 b	AB 50.0 a	43.6 cc
11.2	B 45.7 a	B 55.7 a	50.7 bc
16.8	B 45.0 b	AB 62.0 a	53.5 bc
28.0	A 65.0 a	AA 69.5 a	67.2 ac
CV (%)			14.95

Note. ¹Averages followed by the same letter high case at column and lowercase at line, do not differ by Tukey's test at 5% of error probability.

Another study stated that 40% phytotoxicity was observed in the soybean one week after the dicamba was applied in a dose of 5.6 g ae ha⁻¹ and 80% with the application of the dose of 56 g ae ha⁻¹ during the V3 growth stage (Andersen et al., 2004). During the R1 stage of the glyphosate-resistant soybean, the herbicide caused 19 and 64% phytotoxicity one week after the application of the subdoses of 1.1 and 70 g ae ha⁻¹ respectively,

(Griffin et al., 2013). Usually, soybean showed susceptibility to the dicamba levels, revealing that at both the higher doses of 2,4-D at 7 and 14 DAT, the effects were dramatically more enhanced, and the soybean revealed no ability to recover until the final assessment at 28 DAT. However, when applied during the R2 stage, the symptoms became more intense, as the soybean was more vulnerable at this stage.

3.2 Plant Height

The dicamba herbicide dose applied induced a decrease in the stature of the soybean at all three evaluation times (14 and 28 DAT and maturation) (Table 3). Normally, the plant decreased in stature as the herbicide subdose was gradually increased. At 14 DAT, with the application being done in the V3 phase, all the subdoses induced stature reduction when compared with the control. During the R2 stage, the decrease was even more evident, especially in response to the subdoses of 5.60; 11.2; 16.80 and 28.0 g ae ha⁻¹, which reduced the stature by 8, 19, 18 and 21% compared with the control.

Table 3. Plant height (cm) of soybean at 14, 28 days after treatments applications (DAT) and at maturation, Syngenta 13561 IPRO cultivar, in function of dicamba subdoses and growth stages of soybean. Passo Fundo-RS, 2016

Dose (g ae ha ⁻¹)	Growth Stage		Average
	V3	R2	
<i>Plant height at 14 DAT</i>			
0.0	A ¹ 30.6 ^{ns}	A 88.3 ^{ns}	59.5 ac
1.4	B 18.7	AB 88.7	53.7 bc
2.8	B 18.3	AB 84.4	51.3 bc
5.6	B 16.0	BB 81.3	48.7 cc
11.2	B 14.6	CB 71.4	43.0 dc
16.8	B 14.3	CB 72.1	43.2 dc
28.0	B 14.2	CB 69.6	41.9 dc
CV (%)			5.94
<i>Plant height at 28 DAT</i>			
0.0	AD 66.2 ^{ns}	A 103.4 ^{ns}	84.8 a
1.4	BD 46.6	A 198.0	72.3 b
2.8	BD 46.9	A 196.4	71.6 b
5.6	BC 39.9	B 185.4	62.6 c
11.2	CD 35.9	C 176.0	56.0 d
16.8	CD 36.6	C 172.7	54.6 d
28.0	DD 30.5	C 172.0	51.2 d
CV (%)			5.9
<i>Plant height at Maturation</i>			
0.0	A 97.4 a	AB 94.1 a	95.7 a
1.4	B 38.8 b	AB 93.6 a	66.2 b
2.8	B 40.4 b	AB 87.7 a	64.0 b
5.6	B 20.0 b	AB 78.7 a	49.3 b
11.2	B 37.1 a	BB 50.3 a	43.7 b
16.8	B 21.0 b	AB 70.1 a	45.5 b
28.0	B 22.3 b	AB 68.2 a	45.2 b
CV (%)			34.16

Note. ¹Averages followed by the same letter high case at column and lowercase at line, do not differ by Tukey's test at 5% of error probability. ^{ns}not significant at level of 5% of error probability.

At 28 DAT, the negative influences exerted by the dicamba on the soybean plant stature were more noticeable as the subdoses were increased. During the V3 stage, the soybean subdoses applied were 1.4; 2.8; 5.6; 11.2; 16.8 and 28.0 g ae ha⁻¹ induced plant height decrease by 29, 29, 40, 45, 44 and 53% relative to the control. In the R2 stage, soybean revealed higher tolerance during this evaluation period, where only from the 5.6 g ae ha⁻¹ subdose,

the reduction in plant height was evident; however, at the maximum subdose used (28.0 g ae ha⁻¹) the reduction was 30% compared with the control. On measuring the plant height at maturation, the herbicide applied during V3 confirmed its intense negative effect on plant stature. At the lowest subdose (1.4 g ae ha⁻¹) and the highest subdose (28.0 g ae ha⁻¹) the plants varied greatly from the control, demonstrating a reduced yield of 60 and 77%, respectively. During the R2, a difference was noted in which the subdoses exerted a lower effect in response to the application at V3. The highest subdose (28.0 g ae ha⁻¹) caused 27% decrease in stature, much lower than that recorded in the V3 stage. This finding is mostly due to the stature of the soybean plants at the time of application; in the R2, the plant growth was practically complete, in contrast to the V3 phase at which the soybean still needed to increase in stature.

3.3 Yield Components

The yield components determined included number of nodes, number of branches, thousand seed weight and number of pods. The dicamba herbicide doses affected the number of nodes and number of pods (Table 4, Figure 2). The other yield components showed no statistical difference from the control (without herbicide application).

Table 4. Number of branches (branches plant⁻¹), thousand seed weight (TSW) (g) e number of pods (pods plant⁻¹) of soybean, Syngenta 13561 IPRO cultivar, in function of dicamba subdoses and growth stages of soybean. Passo Fundo-RS, 2016

Dose (g ae ha ⁻¹)	Branches	TSW	Pods
0.0	1.51 ^{ns}	169.1 ^{ns}	44.5 ^{ns}
1.4	3.86 ^{ns}	176.2 ^{ns}	43.4 ^{ns}
2.8	2.42 ^{ns}	165.7 ^{ns}	46.5 ^{ns}
5.6	2.87 ^{ns}	168.0 ^{ns}	46.1 ^{ns}
11.2	3.38 ^{ns}	163.5 ^{ns}	45.0 ^{ns}
16.8	3.20 ^{ns}	160.0 ^{ns}	49.1 ^{ns}
28.0	2.61 ^{ns}	170.7 ^{ns}	44.6 ^{ns}
CV (%)	59.13 ^{ns}	7.33 ^{ns}	20.70 ^{ns}
Growth Stage	Branches	TSW	Pods
V3	3.4 ^{ns}	171.4 ^{ns}	51.5 a ¹
R2	2.1 ^{ns}	165.5 ^{ns}	39.7 b
CV (%)	77.54	14.89	22.46

Note. ¹Averages followed by the same letter high case at column and lowercase at line, do not differ by Tukey's test at 5% of error probability. ^{ns}not significant at level of 5% of error probability.

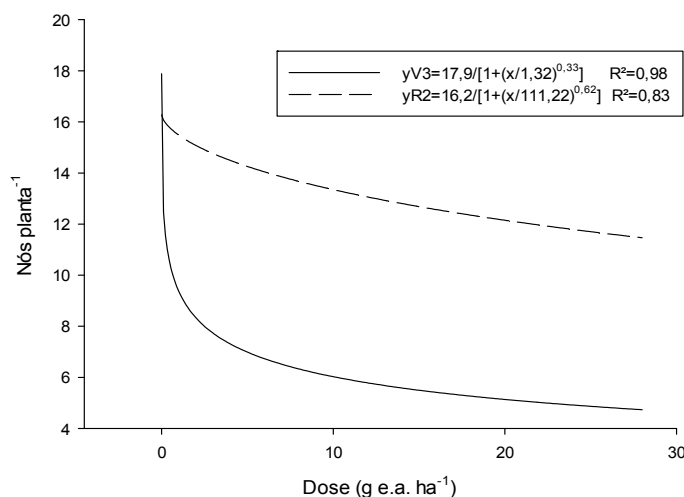


Figure 2. Number of nodes (nodes plant⁻¹), of soybean, Syngenta 13561 IPRO cultivar, in function of dicamba subdoses and growth stages of soybean. Passo Fundo-RS, 2016

The number of pods showed statistical significance only for the factor, stage of development. Thus, the dicamba dose applied during the R2 caused 23% decrease in the number of pods compared to its application during V3. In light of this, the soybean reveals higher susceptibility to the dicamba doses during the reproductive phase, largely because this factor strongly affects the grain yield. For the variable number of nodes, interactions were observed between the factors tested, with a fit to the logistic-type sigmoidal regression equation model in the two application stages. The values of the coefficient of determination (R^2) were 0.98 and 0.83, for the V3 and R2 stages, respectively (Figure 2). The factor, number of nodes, showed the greatest response in the V3 stage from the smallest subdose used (1.4 g ae ha^{-1}) and a 71% decrease in the number of nodes in response to the 28 g ae ha^{-1} subdose. During the R2 stage, the number of nodes was affected only from the $18.24 \text{ g ae ha}^{-1}$ subdose revealing a 34% decrease in the number of nodes. Corroborating to these results, the factor, number of pods showed less influence due to the added subdose of 5.9 g ae ha^{-1} of dicamba during the V3 and V7 stages than during the reproductive phase (Kelley et al., 2005).

3.4 Grain Yield

The dicamba doses caused the soybean grain yield to drop, through the interaction among the factors investigated. Thus, the data were adjusted to the logistic-type logistic regression model at both the application times, and the coefficient of determination was found in the range of 0.98 to 0.99 for the V3 and R2 phases, respectively (Figure 3). The grain yield in general, decreased by 30% after the $28.0 \text{ g ae ha}^{-1}$ subdose was applied in the V3 stage, and by 21 and 56%, respectively, after the 16.8 and $28.0 \text{ g ae ha}^{-1}$ were applied during the R2 stage. When the dicamba was applied in the subdoses 4.4 and $17.5 \text{ g ae ha}^{-1}$ it induced the grain yield to drop by 4 and 10% when added during the V3 stage and by 15 and 36% when added at the R1 stage, respectively (Griffin et al., 2013). Similarly, the dose of 3 g ae ha^{-1} of dicamba induced a 20% of decline in grain yield, while the 41 g ae ha^{-1} subdose reduced it by 85% when the soybean was at a height of 20-30 cm (Johnson et al., 2012).

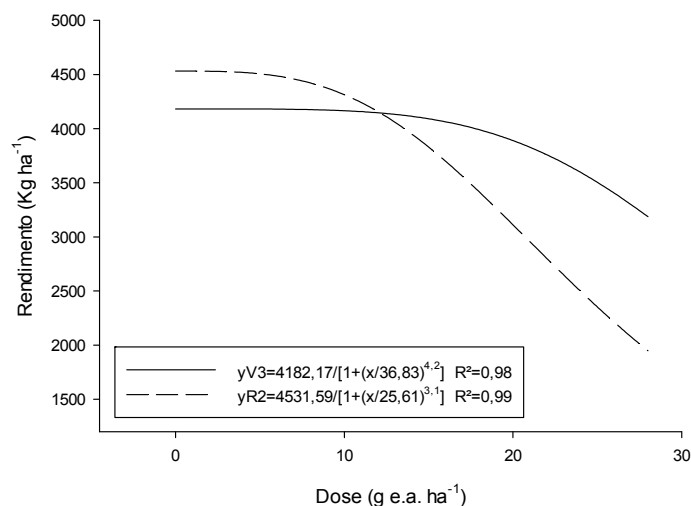


Figure 3. Yield (kg ha^{-1}), of soybean, Syngenta 13561 IPRO cultivar, in function of dicamba subdoses and growth stages of soybean. Passo Fundo-RS, 2016

Furthermore, when compared with the control, the yield decrease was noted to be in the order of 1, 5, 10, 20 and 50% with the application of 1.1; 5.8; 11.8; 25.2 and 60 g ae ha^{-1} , respectively; it was 1 when the dose was applied during the V3 phase and 0.75; 1.0; 2.0; 4.3 and $11.5 \text{ g ae ha}^{-1}$ when applied at the R1, respectively, with the grain yield becoming less in response to the increase in the dicamba subdose (Soltani et al., 2016).

This study helps to confirm that the dicamba subdose induced greater damage during the reproductive stage, when the ability of the plant to recover is very low. Tank residues present in the ratio of $16.8 \text{ g ae ha}^{-1}$, corresponding to 3% of the commercial dose used in this work (represented by 537 g ae ha^{-1}), caused injurious to the soybean cultivation, producing high phytotoxicity levels and affecting the plants by inducing a decrease in the plant height and number of the plant nodes, which justifies the lowered grain yield in the crop, particularly at the R2 stage. Another point of interest is the pluviometric index observed during the crop cycle (Figure 1). As the rainfall throughout the study period was above average, this was a positive indicator for the crop from the perspective of data recovery and herbicide metabolism (Shaner, 2014).

As the visual method of assessing phytotoxicity may be subjective to the extent of becoming a variable due to the differences in the human indices, this technique is often not very reliable. Thus, a more thorough evaluation, despite being rather slow, is preferable. Therefore, counting and evaluating the components of yield can raise the accuracy of the results and indicate the starting point at which the crop yield begins to respond to the herbicide and show decrease. Several other cultures show similar sensitivity to the drift or dicamba residues, like vines, smoke, tomato (Constantin et al., 2007; Oliveira Jr., 2007).

4. Conclusions

In conclusion, the dicamba residues from 5% of the commercial dose at stage V3 and, from 3% at stage R2, cause the soybean yield to decrease. Therefore, the spray tanks need to be thoroughly cleaned before applying these herbicides to soy products that do not tolerate them.

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