

## Subdoses of 2,4-D Choline Salt on Yield Components in Function of the Soybean Growth Stage

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### Abstract

Residues of herbicide tanks may cause damage to sensitive crops. The objective of this study is to evaluate the potential effects of subdoses of the herbicide 2,4-D choline salt on the vegetative and reproductive stages of soybean. The experiment was randomized blocks with a subdivided plot design and four replications. The application times were at V3 and R2 in the main plot. The herbicide 2,4-D choline salt was applied at the subdoses 0, 2.28, 4.56, 9.12, 18.24, 27.36 and 45.60 g a.e. ha<sup>-1</sup> in the subplots. Phytotoxicity was evaluated at 3, 7 and 14 days after application (DAT) of treatments, soybean height at 14 and 28 DAT and at ripening, yield components at ripening, and grain yield. The phytotoxicity evaluation of 2,4-D choline salt evidenced that symptoms were only observed up to 14 days after application (DAT) of treatments. The 2,4-D did not affect any of the variables tested. Grain yield was not influenced by application times or subdoses.

**Keywords:** *Glycine max*, residues, synthetic auxins, phytotoxicity

### 1. Introduction

In recent years, genetic improvement companies have developed several crops with resistance to herbicides aiming to fight the increase in the occurrence of herbicide resistant weeds (Vink et al., 2012). The availability of multi-resistant cultivars allows farmers to integrate new management strategies to control a wider range of weed species, even resistant biotypes, preserving resistant crops. Resistance to glyphosate is an example (Green & Castle, 2010; Vink et al. 2012).

Among the new technologies of resistance to herbicides, soybean and corn crops genetically modified to resist applications of 2,4-dichlorophenoxyacetic (2,4-D) have been tested, and should be available in the Brazilian market in the coming years. The 2,4-D herbicide is an auxin mimic herbicide that has been used worldwide to control several dicotyledonous weeds for over forty years (Behrens et al., 2007).

Such herbicides affect cell wall plasticity and nucleic acid metabolism and activate auxin response genes, leading to the overproduction of ethylene and subsequently abscisic acid (Kelley et al., 2004; Grossmann, 2010; Shaner, 2014). The increase in abscisic acid leads to stomatal closure, limiting the assimilation of CO<sub>2</sub>, followed by inhibition of growth (Grossmann, 2010). The most common symptoms of soybean auxin mimic herbicide subdoses are stem and leaf epinasty, swollen and cracked stems, chlorosis, and necrosis (Andersen et al., 2004; Kelley et al., 2005).

With the introduction of 2,4-D-tolerant soybean cultivars, the occurrence of a spraying containing residues of these herbicides in a tank is inevitable and predictable. Such symptoms occur with cultivars without genes tolerant to active ingredients based on 2,4-D, causing injuries and reductions in grain yield (Olszyk et al., 2015).

Soybean is a plant sensitive to 2,4-D-based herbicides, especially when exposed at the reproductive stage (Slife, 1956). The literature reports that the application of 140 g a.e. ha<sup>-1</sup> does not affect grain yield of soybeans when applied at the V3 stage. However, there is an 11% decrease in yield due to the application of 70 g a.e. ha<sup>-1</sup> at

soybean flowering (Wax et al., 1969). However, in recent literature, there is little information on its behavior for current cultivars in function of sensitivity to 2,4-D.

Information related to sensitivity of soybean to 2,4-D tank residues during vegetative and reproductive stages is little known in Brazilian environmental conditions. Analyses of yield components may be useful to quantify such effects. Extensive studies have shown that the number of nodes, the number of pods, and the weight of grains are the main contributors to high yields.

Thus, the evaluation of yield components is necessary to assist in determining the relationship between soybean exposure to auxin mimic herbicides and grain yield at different stages of development. The determination of the effects of the application of 2,4-D residues in spraying tanks on non-tolerant soybean cultivars helps to provide soy producers and pesticide applicators with information on the potential risk of injuries and on the reduction of soybean grain yield.

The hypothesis of this work is that soybean is susceptible to 2,4-D, causing a decrease in grain yield. Thus, the objective of this study is to evaluate the potential effects of subdoses of the herbicide 2,4-D choline salt on the vegetative and reproductive stages of soybean.

## 2. Material and Methods

### 2.1 Plant Material and Experimental Conditions

The experiment was conducted at the experimental field of the University of Passo Fundo (UPF), located in the middle plateau of Rio Grande do Sul, Brazil. The mean altitude of the experimental area is 678 m, and the geographical location is 28°12'59" S and 52°23'37" W. The soil of the experimental area is typical dark red (Embrapa, 2006), and the climate of the region is characterized as subtropical humid with a hot summer (Cfa) according to the climatic classification of Köppen. Before the installation of the experiments, twenty days before sowing, glyphosate was applied in the whole area (1,080 g a.e. ha<sup>-1</sup>) to eliminate weed vegetation in the area. The sowing of soybean (Syngenta IPRO) was carried out in December 2015. The line spacing was 0.45 m, and the population was 280.000 plants ha<sup>-1</sup>.

During the conduction of the experiments, rainfall totaled 1,365.2 mm, which is higher than the climatological normal for this period, which is 855.0 mm (Figure 1). The average air temperature in this period was 21.21 °C, which is within the climatological normal of 20.5 °C for these months (Embrapa, 2016).

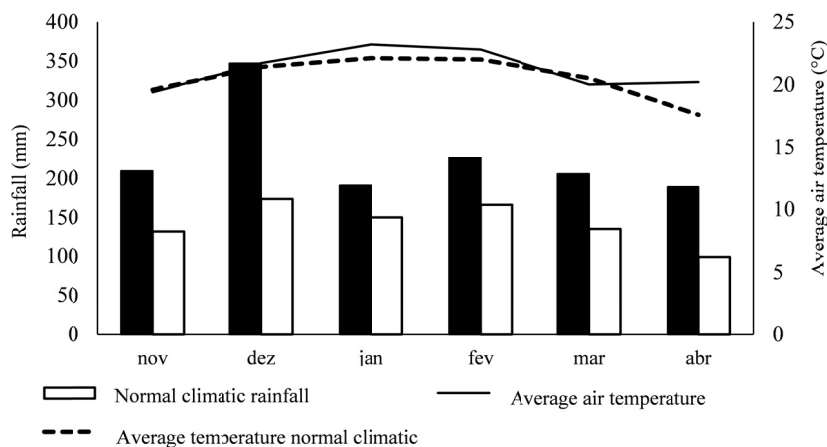


Figure 1. Rainfall monthly, normal climatological rainfall, average monthly air temperature, and average normal climatological temperature during the experimental period. Passo Fundo, RS, 2016

### 2.2 Experimental Design and Treatments

The experimental design was randomized blocks arranged in subdivided plots with four replications. The plots were five meters in length and 3.5 meters in width. The main plot represented the stages of application of soybean (V3, represented by the third node of the stem with developed leaves, and R2, represented by the flowers in the last four nodes of the stem with developed leaves). The subplots represented the subdoses of herbicides. The herbicide 2,4-D choline salt (GF-3073), at the subdoses 0, 2.28, 4.56, 9.12, 18.24, 27.36 and 45.60 g a.e. ha<sup>-1</sup>, represented 0, 0.25, 0.5, 1, 2, 3 and 5% of the commercial dose, respectively.

### 2.3 Procedures

The herbicides were applied when the soybean plants reached the desired stages of development (V3 and R2). For this, a backpack spray, pressurized with CO<sub>2</sub>, provided with fan-like tips AD 110.015, calibrated to provide an application volume of 150 L ha<sup>-1</sup> of herbicidal syrup, was used. Phytotoxicity was evaluated 3, 7, 14 and 28 days after the application of treatments (DAT) using a percentage scale, where zero represented absence of symptoms and one hundred the death of plants (Table 1) (Frans et al., 1986; Robinson et al., 2013). At 14 and 28 DAT, the height of ten plants within the useful area of the plot was measured from the base of the main stem to the node of the last trefoil emitted.

At harvest ripening, ten plants were collected within the useful area of each plot to measure height, number of nodes in the main stem, number of branches, total number of pods, and weight of one thousand seeds. The mean values of the ten evaluated plants were used for data analysis purposes. In addition, grain yield was determined by harvesting the three central lines in the useful area of the plot using a mechanized plot harvester.

Table 1. Scale of classification of visual injury in soybean plants affected by auxin mimic 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 or 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

The data were analyzed for normality (Shapiro Wilk test), and later were subjected to analysis of variance ( $p \leq 0.05$ ). For the variables phytotoxicity at 3, 7, 14 and 28 DAT and height at 14, 28 DAT and at ripening, the Tukey test was performed at 5% of error probability when a statistical significance was observed. For the variables number of nodes of the main stem, number of branches, number of pods, weight of one thousand seeds and yield, in case of statistical significance, a regression analysis was performed.

## 3. Results and Discussion

### 3.1 Symptoms of Phytotoxicity

The symptoms of soybean phytotoxicity caused by the 2,4-D herbicide were observed at the doses 27.36 and 45.60 g a.e. ha<sup>-1</sup> at the 3 and 7 DAT evaluations for application at V3 and R2 (Table 2). In evaluations at 14 DAT, the phytotoxicity was 8%, only perceptible at the highest subdose and when applied at the V3 stage. At 28 DAT, the symptoms of phytotoxicity were no longer visually perceptible in any of the herbicide subdoses and application times (data not shown).

Table 2. Phytotoxicity (%) of soybean at 3, 7 and 14 days after application of treatments (DAT), cultivar Syngenta 13561 IPRO, in function of the application of subdoses of the herbicide 2,4-D choline salt and growth stages of soybean. Passo Fundo, RS, 2016

Dose (g a.e. ha <sup>-1</sup> )	Growth Stage		Average
	V3	R2	
<i>Phytotoxicity at 3 DAT</i>			
0.0	0.0 c	0.0 c	0.0 c <sup>1</sup>
2.28	0.0 c	0.0 c	0.0 c
4.56	0.0 c	0.0 c	0.0 c
9.12	0.0 c	1.0 c	0.5 c
18.24	1.5 c	2.0 c	1.7 c
27.36	5.0 b	7.5 b	6.2 b
45.60	13.7 a	18.7 a	16.2 a
CV (%)			69.21
<i>Phytotoxicity at 7 DAT</i>			
0.0	0.0 b	0.0 c	0.0 c
2.28	0.0 b	0.7 c	0.3 c
4.56	0.0 b	1.7 c	0.8 c
9.12	0.0 b	2.7 c	1.3 bc
18.24	0.7 b	2.5 c	1.6 bc
27.36	1.2 b	5.5 b	3.3 b
45.60	7.2 a	10.0 a	8.6 a
CV (%)			65.24
<i>Phytotoxicity at 14 DAT</i>			
0.0	B 0.0 b	A 0 b	0.0 b
2.28	B 0.0 b	A 0 b	0.0 b
4.56	B 0.0 b	A 0 b	0.0 b
9.12	B 1.0 b	A 0 b	0.5 b
18.24	B 0.7 b	A 0 b	0.3 b
27.36	B 1.2 b	A 0 b	0.6 b
45.60	A 8.0 a	A 0 aa	4.0 a
CV (%)			142.18

Note. <sup>1</sup> Means followed vertically by the same uppercase letter in rows and lowercase letter in lines do not differ by Tukey test at 5% probability.

For the herbicide 2,4-D, phytotoxicity symptoms of 20%, which appeared 14 days after application of herbicide, were caused by the application of 77.29 and 109 g a.e. ha<sup>-1</sup> at the stages V2, V5 and R2, respectively (Robinson et al., 2013). In a work using the maximum dose of 2,4-D (28 g a.e. ha<sup>-1</sup>), the maximum phytotoxicity caused two weeks after the application at the V3 stage was 5% and 0% at the R2 stage. Subsequent evaluations of symptoms were virtually absent (Solomon et al., 2014). In general, soybean was little affected by the 2,4-D. When 2,4-D is used at the highest doses at 7 and 14 DAT, the maximum phytotoxicity observed was 10% when applied at both V3 and R2. At 28 DAT, it was not possible to observe symptoms caused by the herbicide.

### 3.2 Soybean Height

The application of the herbicide 2,4-D caused a decrease in the height of soybean plants only when evaluated at 28 DAT (Table 3). At 14 DAT and at ripening, no decrease in height was observed at both application stages. At 28 DAT, the subdoses 18.24, 27.36 and 45.60 g a.e. ha<sup>-1</sup> negatively affected height by 7, 11 and 17%, respectively, in relation to the control. According to the evaluation at R2, there was no difference between the applied subdoses and the control. These results evidence a decreased soybean susceptibility to 2,4-D subdoses. In similar studies, decreases in the height of soybean plants were caused by the application of dicamba, mainly at the reproductive stages, and 2,4-D did not affect the vegetative or reproductive stages of soybean (Auch & Arnold 1978; Kelley et al. 2005; Robinson et al. 2013).

Table 3. Plant height (%) of soybean plants at 14 and 28 days after application of treatments (DAT) and at ripening, cultivar Syngenta 13561 IPRO, in function of the application of subdoses of the herbicide 2,4-D choline salt and growth stages of soybean. Passo Fundo, RS, 2016

Dose (g a.e. ha <sup>-1</sup> )	Growth Stage		Average
	V3	R2	
<i>Plant height at 14 DAT</i>			
0.0	30.7 <sup>ns</sup>	88.4 <sup>ns</sup>	59.6 <sup>ns</sup>
2.28	30.9	106.7	68.8
4.56	30.3	86.0	58.1
9.12	29.6	86.0	57.8
18.24	30.1	84.7	57.4
27.36	27.6	81.6	54.6
45.60	25.2	84.7	55.0
CV (%)			13.4
<i>Plant height at 28 DAT</i>			
0.0	<sup>1</sup> ABC 64.4	102.4 <sup>ns</sup>	83.4 abc
2.28	ABC 65.0	99.60	82.3 abc
4.56	ABC 63.7	99.80	81.7 abc
9.12	ABC 61.4	99.90	80.7 abc
18.24	BCD 59.9	98.20	79.0 bcd
27.36	CDE 57.2	98.70	78.0 cd
45.60	DCD 53.2	101.0	77.1 d
CV (%)			2.81 <sub>ss</sub>
<i>Plant height at Maturation</i>			
0.0	97.7 <sup>ns</sup>	98.8 <sup>ns</sup>	98.2 a
2.28	97.9	97.7	97.8 a
4.56	96.8	97.7	97.3 a
9.12	95.8	97.3	96.6 a
18.24	94.4	94.1	94.2 ab
27.36	91.1	97.7	94.4 ab
45.60	87.8	95.1	91.5 b
CV (%)			3.03

Note. <sup>1</sup> Means followed by the same uppercase letter in rows and lowercase letter in lines do not differ by Tukey test at 5% probability. <sup>ns</sup> not significant at 5% level of probability.

### 3.3 Yield Components

The following yield components were determined: number of nodes, number of branches, weight of one thousand seeds (WTS), and number of pods. The application of 2,4-D was not statistically significant for all variables of yield components (Table 4). Thus, all subdoses used at both stages of development did not exert negative effects on the number of nodes, number of branches, weight of one thousand seeds, and number of pods. The application of 2,4-D only had a negative effect on yield components when doses higher than 84 g a.e. ha<sup>-1</sup> were used. This dose was 84% higher than that used in this study (Robinson et al., 2013). Furthermore, the application of subdoses from 0 to 140 g a.e. ha<sup>-1</sup> of 2,4-D did not decrease the number of nodes, pods, and seed mass (Wax et al., 1969).

Table 4. Number of nodes (nodes plant<sup>-1</sup>), number of branches (branches plant<sup>-1</sup>), average length of branches - ALB (cm<sup>-1</sup>), weight of one thousand seeds (g), number of pods (pods plant<sup>-1</sup>) and yield (kg ha<sup>-1</sup>) of soybean, cultivar Syngenta 13561 IPRO, in function of the application of subdoses of the herbicide 2,4-D choline salt and growth stages of soybean. Passo Fundo, RS, 2016

Dose (g a.e. ha <sup>-1</sup> )	Nodes	Branches	ALB	Pods	Yield
0.0	17.5 <sup>ns</sup>	1.12 <sup>ns</sup>	160.9 <sup>ns</sup>	44.5 <sup>ns</sup>	4,709.2 <sup>ns</sup>
2.28	17.1	1.9	167.3	41.1	4,740.2
4.56	17.8	2.3	167.1	46.8	4,894.3
9.12	17.5	1.8	161.1	44.0	4,661.1
18.24	17.4	1.4	171.0	42.7	4,966.8
27.36	16.8	1.6	168.5	40.4	4,800.8
45.60	17.1	1.8	164.5	41.0	4,679.9
CV (%)	5.5	66.4	5.5	15.2	8.36
<hr/>					
<i>Growth Stage</i>					
V3	17.3 <sup>ns</sup>	1.8 <sup>ns</sup>	167.3 <sup>ns</sup>	43.2 <sup>ns</sup>	4,793.9 <sup>ns</sup>
R2	17.4	1.6	164.3	42.6	4,763.9 <sup>ns</sup>
CV(%)	9.74	38.71	4.36	25.14	8.36

Note. <sup>ns</sup> not significant at 5% error probability.

### 3.4 Grain Yield

Soybean grain yield was not negatively influenced by both application times and 2,4-D herbicide subdoses (Table 4). In a study conducted by Robinson et al. (2013), soybean grain yield decreased by 5% using the subdose 116 g a.e. ha<sup>-1</sup> and by 10% using the subdose 202 g a.e. ha<sup>-1</sup> at both V2 and R2. These results indicate that, by using the 2,4-D subdoses proposed by this work, the soybean grain yield does not decrease. The results of soybean yield of this work are different from those reported in the literature (Andersen et al., 2004; Slife, 1956; Wax et al., 1969). Such differences can be explained by the variation in tolerance to 2,4-D of different soybean cultivars (Friborg & Johnson, 1955).

With this work, the 2,4-D at the subdoses up to 45.60 g a.e. ha<sup>-1</sup>, representing 5% of the commercial dose, represented in this work by 912 g a.e. ha<sup>-1</sup>, is not enough to cause significant phytotoxicity or changes in yield components that could influence crop yield. Noteworthy is the rainfall index that occurred during the crop cycle (Figure 1). It is possible to observe that rainfall was higher than the average in all months, making a positive contribution to the crop from the point of view of damage recovery and metabolizing of herbicides (Shaner, 2014).

The visual assessment of phytotoxicity may be subjective to the point of becoming variable, making this method often less reliable. Thus, a thorough evaluation, although slow, and the evaluation of yield components can make the results of the work more accurate by indicating in which cases the cause of the decrease in crop yield occurs. Many other cultures are sensitive to 2,4-D and dicamba drifts or residues, such as vines, smoke, and tomato (Constantin et al., 2007; Oliveira Jr., 2007).

### 4. Conclusions

2,4-D tank residues up to 45.60 g a.e. ha<sup>-1</sup> equivalent to 5% of commercial rate, do not decrease soybean yield. Thus, care must be taken for a correct cleaning of spray tanks before applying products to soybean plants that are not tolerant to certain herbicides. Although sprays with 2,4-D residues in susceptible soybeans may occur, it is important to note that all necessary precautions should be taken in view of the difficulty of measuring the amount of residue present in the tank.

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