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Effect of 2,4-D sub-dose on the initial development of common bean crop[1]

Efeito da subdose de 2,4-D no desenvolvimento inicial da cultura do feijoeiro

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ABSTRACT - The adoption of cultures tolerant to synthetic auxins can increase the occurrence of drift of these herbicides, interfering in the development of naturally sensitive cultures, such as beans. The aim of this study was to evaluate the germination and initial development of bean seedlings, using sub-doses of the herbicide 2,4-D. The design of experiment I was completely randomized, with eight treatments and four replications, except for germination, with eight replications. The seeds were sowed in 100 mL of water with concentrations of 0.0; 1.12; 2.14; 4.18; 8.37; 16.75; 33.50 and 67.00 g a.e. L^{-1} of 2,4-D. Germination, length, seedling fresh and dry mass and electrical conductivity were evaluated. The design of experiment II was completely randomized with five treatments and four replications. The beans were sowed in sand and then sprayed at concentrations of 0.0; 8.37; 16.75; 33.50 and 67.00 g e.a. L^{-1} of 2,4-D. Seed emergence, injury, fresh and dry shoot mass were evaluated. According to the regression analysis, there was a reduction in germination, regardless of the concentration used, as well as the seedling length. Seeds sown in this solution presented impaired root system and development, tending to mortality. Electrical conductivity increased as herbicide concentrations increased, indicating lower physiological potential of seeds. In experiment II, increasing 2,4-D concentrations reduced emergence and increased injury to bean plants. It is concluded that the sub-doses of the 2,4-D herbicide negatively affect the germination and initial development of bean seedlings.

Key words: Drift. Herbicides. synthetic auxins. Physiological potential. *Phaseolus vulgaris* L.

RESUMO - A adoção de culturas tolerantes as auxinas sintéticas pode aumentar a ocorrência de deriva desses herbicidas interferindo no desenvolvimento de culturas naturalmente sensíveis, como o feijão. Objetivou-se avaliar a germinação e o desenvolvimento inicial de plântulas de feijão, a partir de subdoses do herbicida 2,4-D. O delineamento do experimento I foi inteiramente casualizado, com oito tratamentos e quatro repetições, exceto para a germinação, com oito repetições. As sementes foram embebidas em 100 mL de água com concentrações de 0,0; 1,12; 2,14; 4,18; 8,37; 16,75; 33,50 e 67 g e.a. L^{-1} de 2,4-D. Foram avaliadas a germinação, comprimento, massa fresca e seca de plântulas e condutividade elétrica. O delineamento do experimento II foi inteiramente casualizado, com cinco tratamentos e quatro repetições. O feijão foi semeado em areia e em seguida foram pulverizadas concentrações de 0,0; 1,12; 2,14; 4,18; 8,37; 16,75; 33,50 e 67 g e.a L^{-1} de 2,4-D. Foram avaliadas emergência das sementes, injúria, massa fresca e seca da parte aérea. De acordo com a análise de regressão, houve redução na germinação, independente da concentração utilizada, assim como no comprimento de plântulas. Sementes embebidas nessa solução apresentaram o sistema radicular e desenvolvimento prejudicados. Houve aumento da condutividade elétrica conforme o acréscimo das concentrações do herbicida, indicando menor potencial fisiológico das sementes. No experimento II, o aumento das concentrações do 2,4-D reduziu a emergência e aumentou a injúria nas plantas. Conclui-se que as subdoses do herbicida 2,4-D interferem negativamente na germinação e no desenvolvimento inicial de plântulas de feijão.

Palavras-chave: Deriva. Herbicidas. Auxinas sintéticas. Potencial fisiológico. *Phaseolus vulgaris* L.

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INTRODUCTION

Bean (*Phaseolus vulgaris L.*) are one of the most widely cultivated and consumed legumes in Brazil, which is among its largest producers worldwide, with an average annual production of 3.5 million tons (COMPANHIA NACIONAL DE ABASTECIMENTO, 2017). Its consumption is due to the fact that it is an excellent source of proteins, carbohydrates and iron (OLIVEIRA *et al*., 2013).

At the beginning of its development, bean plants present slow growth, which exposes this crop to greater competition with weeds (MANABE *et al*., 2015), mainly for essential resources (PITELLI, 2015). This interspecific competition can reduce grain yield by 36 to 49% (BORCHARTT *et al*., 2011; TEIXEIRA *et al*., 2009), which highlights the need to adopt measures to reduce the weed community in crop systems. Herbicides are the most often used control method to this end.

Herbicides based on synthetic auxin, such as 2,4-D (2,4-dichlorophenoxyacetic acid), are widely used in weed control. Despite being a growth regulator directly linked to plant growth and development, such as division, elongation, cell differentiation and apical dominance, in high concentrations this regulator can be lethal to plants, especially in eudicots, as is the case of beans (TAIZ *et al.*, 2017). Interaction with endogenous hormones, such as ethylene, gibberellic acid, abscisic acid and even auxins at low concentrations, can break seed dormancy and stimulate germination (BRADY; McCOURT, 2003). However, high concentrations cause the opposite effect, slowing or even inhibiting germination (USUI, 2001). The sowing period after the application of 2,4-D can also interfere in the initial development of the crops, even if this herbicide has limited soil residual (BAUMGARTNER *et al.*, 2017).

Recently, genetically modified organisms for tolerance to 2,4-D and dicamba, such as cotton and soybeans, have been approved for cultivation in Brazil and the United States (ALVES *et al.*, 2017). The adoption of tolerant cultivars will allow the application of these herbicides post-emergence of crops, which can intensify their use (PETERSON *et al.*, 2015). Thus, it is expected an increase in cases of phytotoxicity in cultures that have genes sensitive to these molecules, due to drift from spraying of adjacent areas (GODINHO JUNIOR *et al*., 2017).

However, depending on the concentration and the herbicide to which the seeds are exposed, there may be no damage to initial development. Some researchers have reported a stimulating effect on tree species (PEREIRA *et al*., 2015) and on bean crops (SILVA *et al*., 2016). However, it is necessary to assess the impact of 2,4-D sub-doses on bean plants and to identify the concentrations that stimulates initial development and/or do not harm the plants. Given the above, the present study aimed to evaluate the effect of sub-doses of the herbicide 2,4-D on the germination and initial development of bean seedlings.

MATERIAL AND METHODS

Two experiments were conducted between May and December 2018, at the State University of Londrina, city of Londrina, state of Paraná, Brazil, one in the laboratory and the other one in a greenhouse.

Experiment I - Effect of 2,4-D sub-doses on bean seedling germination and development

A completely randomized design was used, with eight treatments and four repetitions, except for the germination test, for which eight repetitions were performed. The treatments were composed by the concentrations of the herbicide 2,4-D dimethylamine salt (commercially available, DMA® 806 BR, 670 g a.e. L^{-1}) presented on Table 1. Seedling germination, length, fresh weight, dry weight and electrical conductivity were assessed.

Fifty bean seeds of the cultivar IPR Curió were placed in 0.15 L plastic cups and 0.10 L of the solution containing the herbicide concentrations were added; seeds were then immersed for three hours. Subsequently, seeds were placed on paper towels (Germitest®) moistened with distilled water, in the proportion of 2.5 times the weight of the dry substrate, and kept in a germinator at 25 °C in order to determine the number of germinated seeds. Seedlings were counted on the ninth day, considering the percentage of normal seedlings, following the criteria established in the Rules for Seed Testing - RST (BRASIL, 2009).

The length, fresh mass and dry mass of the seedlings were evaluated according to the procedure adopted for the germination test; however, 20 seeds

Table 1 - 2,4-D concentrations used for soaking the bean seeds (*Phaseolus vulgaris* L.)

Treatments	Concentration (g a.e. L^{-1})[1]	Dose (mL ha^{-1})
Control	0.00	0.00
2,4-D	1.12	1.66
2,4-D	2.14	3.12
2,4-D	4.18	6.25
2,4-D	8.37	12.50
2,4-D	16.75	25.00
2,4-D	33.50	50.00
2,4-D	67.00	100.00

[1](g a.e. L^{-1}) = gram of acid equivalent per liter

were used with four repetitions, allocated in the upper third part of the paper sheet (Germitest®). The length was measured with the aid of a millimeter ruler and the results were expressed in centimeters. The bean seedlings were weighed on a 0.0001 g precision scale to determine the fresh mass. Subsequently, the samples were packed in paper bags and dried in an oven with forced air circulation at 65 ºC until they reached constant mass, in order to determine the dry mass of the seedlings.

For the electrical conductivity test, the seeds were separated into sets of 50 units and immersed in plastic cups containing 0.075 L of solution of each treatment, and the control was immersed in distilled water. The samples were placed in black plastic bags and placed in a Biochemical Oxygen Demand (BOD) germination chamber, at a temperature of 25 ºC and constant white fluorescent lights, for 24 hours. After this period, the solutions were read with the aid of a conductivity meter (DiST®5 HI-9831) and the results were expressed in $\mu S\ cm^{-1}$.

Experiment II - Simulated 2,4-D drift for the development of bean seeds

A completely randomized design was used, with five treatments and four replications. The treatments were composed of the concentrations of the herbicide 2,4-D dimethylamine salt (commercially available, DMA® 806 BR, 670 g a.e. L^{-1}) shown in Table 2.

The seeds of the cultivar IPR Curió were sown in plastic boxes containing sand, considered as experimental units. Fifty bean seeds were allocated per experimental unit, at an approximate depth of 0.04 m.

The treatments were applied after sowing, with the aid of a CO_2 pressurized backpack sprayer, equipped with flat spray nozzle with pre-orifice, model ADI 11002. The nozzle were spaced 0.5 m apart and positioned 0.5 m from the target surface. The working pressure used was 414 kPa, with a displacement speed of 3.6 km h^{-1}, resulting in an application rate equivalent to 150 L of the mixture per ha^{-1}. The sprayings were carried out under suitable meteorological conditions, with an air temperature below 30 ºC and relative humidity above 55%.

Fifteen days after treatment (DAT), in phenological stage V2-V3, seedling emergence, visual injury, fresh and dry shoot mass were assessed, as described in Experiment I. However, the visual assessment of injury was carried out on a percentage scale, in which zero and one hundred represent absence of injury and plant death, respectively (FRANS; CROWLEY, 1986).

The assumptions to validate the analysis of variance in a completely randomized design were verified and validated in both experiments. The decomposition of the degrees of freedom of the treatments in regression models was adopted as a procedure for the comparison between treatments (2,4-D g a.e. L^{-1} concentration). The regression models were chosen according to the analysis of variance criteria for the lack of adjustment, and the coefficient of determination (R^2) was adjusted.

The emergence rate of seedlings in the sandboxes (Experiment II) was assessed using the logistic model with *logit* link function, given by:

$$\log\left(\frac{\pi}{1-\pi}\right) = \beta_0 + \beta_1$$

Where π is the rate of germinated seeds and x is the 2,4-D g a.e. L^{-1} concentration.

In the analysis of variance, in which the effect of the treatments for the response variables was assessed, the p-degree polynomial model was adjusted as follows:

$$\gamma_i = \beta_0 + \beta_1 X + \beta_2 X^2 + \cdots + \beta_p X^p + \varepsilon_i$$

Where y is the dependent variable, β is the regression coefficient, x is the independent variable, ε is the random error and p is the degree of the adjusted polynomial. The control data without application of herbicide were disregarded from the statistical analyses in order to meet the assumptions of the analysis of variance. Pearson's correlation coefficient (r) ($p < 0.05$) between variables was assessed for Experiments I and II.

Table 2 - 2,4-D concentrations applied in pre-emergence of bean plants (*Phaseolus vulgaris* L.)

Treatments	Concentration (g a.e. L^{-1})[1]	Dose (mL ha^{-1})
Control	0.00	0.00
2,4-D	8.37	12.50
2,4-D	16.75	25.00
2,4-D	33.50	50.00
2,4-D	67.00	100.00

[1](g a.e. L^{-1}) = gram of acid equivalent per liter

RESULTS AND DISCUSSION

Experiment I - Effect of 2,4-D sub-doses on bean seedling germination and development

Table 3 shows the adjusted regression models and their respective adjusted determination coefficients (R^2), in order to explain the response of bean seedlings to being soaked in sub-doses of 2,4-D.

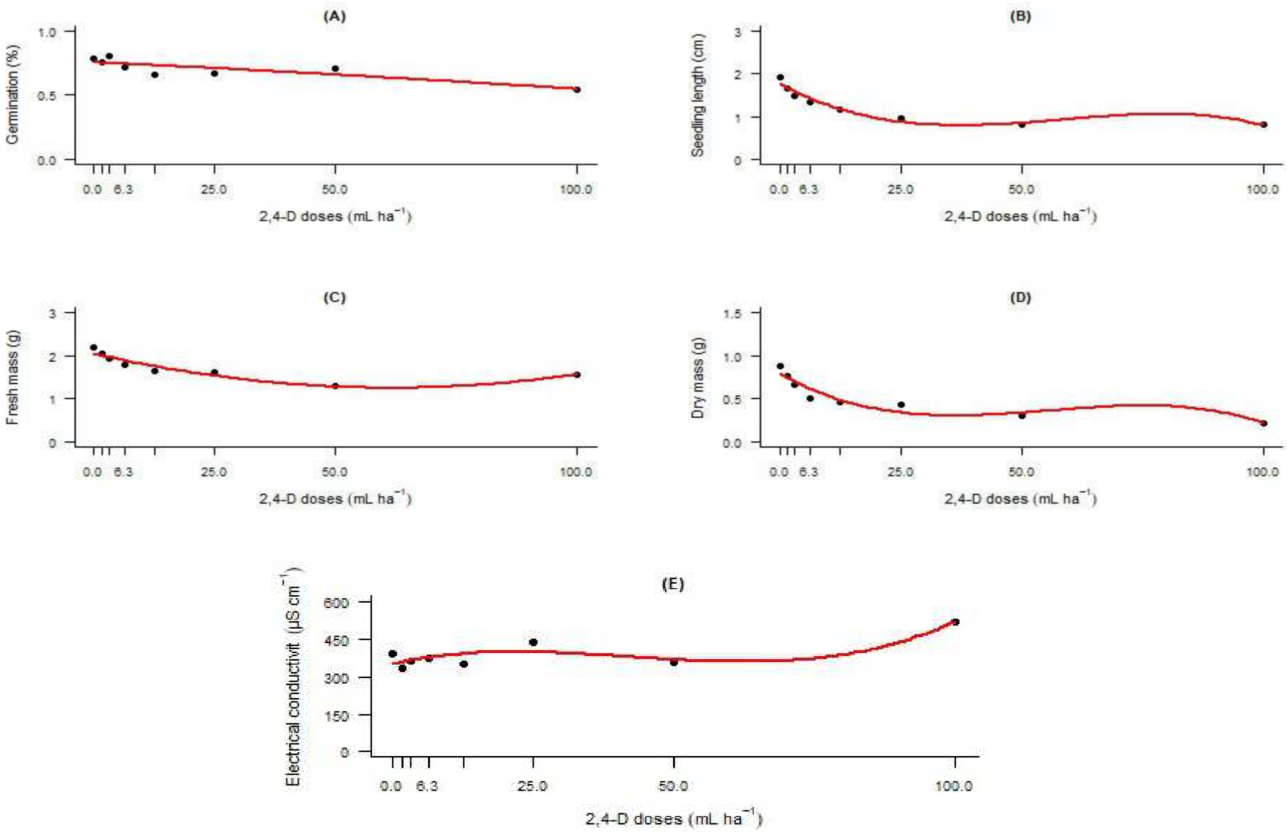
The linear regression model adjusted for the variable percentage of germination (Figure 1A; Figure 2) found a negative association, that is, as the concentrations increased, there was a reduction in the germination of bean seeds, suggesting an inhibitory effect of phytohormones. The action of synthetic auxin stimulates abscisic acid synthesis and ethylene production, which interferes with cell division and elongation, as well as the formation of reactive oxygen species (ROS) (GROSSMANN, 2010). This form of oxygen is more reactive than molecular oxygen (O_2) and alters the integrity of the membranes and causes oxidative stress in the seeds, which can lead to cell death (CHANG *et al.*, 2016).

Table 3 - Estimations of the equations and coefficients of determination (R^2) of the adjusted models of the variables assessed in beans seeds (*Phaseolus vulgaris L.*) after soaking in sub-doses of the herbicide 2,4-D

Variables	Equation	R^2
Germination	$\hat{Y} = 37.54 – 0.10\ x$	0.70
Length	$\hat{Y} = 1.69 – 0.06\ x + 0.001\ x^2 - 6.10\text{-}6\ x^3$	0.87
Fresh mass	$\hat{Y} = 12.61 – 0.55\ x + 0.01\ x^2 - 6.10\text{-}5\ x^3$	0.78
Dry mass	$\hat{Y} = 0.8 – 0.03\ x + 0.0007\ x^2 - 3.95.10\text{-}6\ x^3$	0.78
Electric conductivity	$\hat{Y} = 352.8 + 4.73\ x – 0.14\ x^2 + 0.001\ x^3$	0.73

x = 2,4-D concentration (gram of acid equivalent per liter)

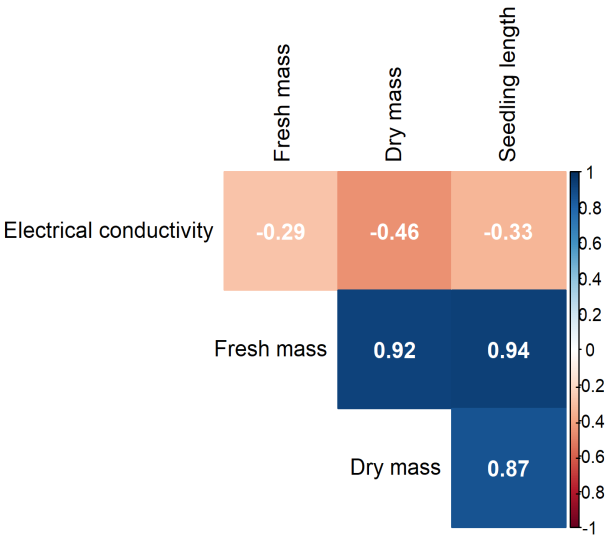
Figure 1 - Regression model of the parameters assessed for beans (*Phaseolus vulgaris L.*) after soaking in sub-doses of the herbicide 2,4-D. (A) Germination (%); (B) Seedling length (%); (C) Fresh mass (g); (D) Dry mass (g) and (E) Electrical conductivity (μS cm^{-1})



It was observed that increases in herbicide concentrations resulted in variation in seedling length (Figure 1B) and dry mass (Figure 1D), with greater losses at concentrations above 33.5 g and L^{-1}, with a reduction of more than 70% in seedling length when compared to the control. The symptoms were characterized by shrinking of the cotyledon leaves and inhibition of root development, resulting in direct interference in the dry mass of the seedlings (Figure 2). In studies with application of 2,4-D close to sowing, a reduction was also observed in the stand density of eudicots (SILVA *et al*., 2011).

The variables of fresh and dry mass and seedling length were reduced after the application of treatments with 2,4-D due to the sensitivity of the crop to the herbicide. The way the vascular bundles of eudicots are organized facilitates the translocation of these herbicides, due to the fact that their vascular tissues are ringed and exchange matters. In addition, when these plants metabolize synthetic auxins, this process generally occurs more slowly compared to endogenous auxin (SONG, 2014).

Figure 2 – Pearson's correlation coefficient (r) for the variables Electrical conductivity (µS cm^{-1}), Fresh mass (g), Dry mass (g) and Seedling length (cm) of beans (*Phaseolus vulgaris L.*) submitted to different sub-doses of the herbicide 2,4-D



From the concentration of 33.50 g a.e. L^{-1}, there was an increase in electrolytes leached by the seeds while soaked (Figure 1E). The release of electrolytes is caused by loss of integrity of the cell membrane, which can be estimated from the electrical conductivity test (ROSISCA *et al*., 2019). This test is an efficient method to assess the physiological potential of the seeds (TORRES *et al*., 2015), that is, the greater the number of electrolytes released, the lower the vigor and the germination index of the seeds. Thus, there was a negative correlation between electrical conductivity and the other variables analyzed (Figure 2).

Experiment II - Simulated 2,4-D drift for the development of bean seeds

Table 4 presents the adjusted regression models and their respective adjusted determination coefficients (R^2), to explain the response of the bean plants as a function of the application of 2,4-D sub-doses.

The adjusted models indicate that, according to the injury observed to quantify the magnitude of the damage resulting from the simulated drift of the herbicide 2,4-D in the bean plants, at 15 DAT, the injury rate increased as herbicide concentrations increased, and in the concentration of 67.00 g a.e. L^{-1} presented injury greater than 80%, considering a satisfactory control index from this value (Figure 3B). The injuries observed from sub-doses resulted in swelling of the tissues, hyponastic response of the petioles, chlorosis and necrosis of leaves and stems (GROSSMANN, 2010; OLIVEIRA *et al*., 2019), since synthetic auxin cause cellular disorganization of the mesophile (PAZMIÑO *et al*., 2011).

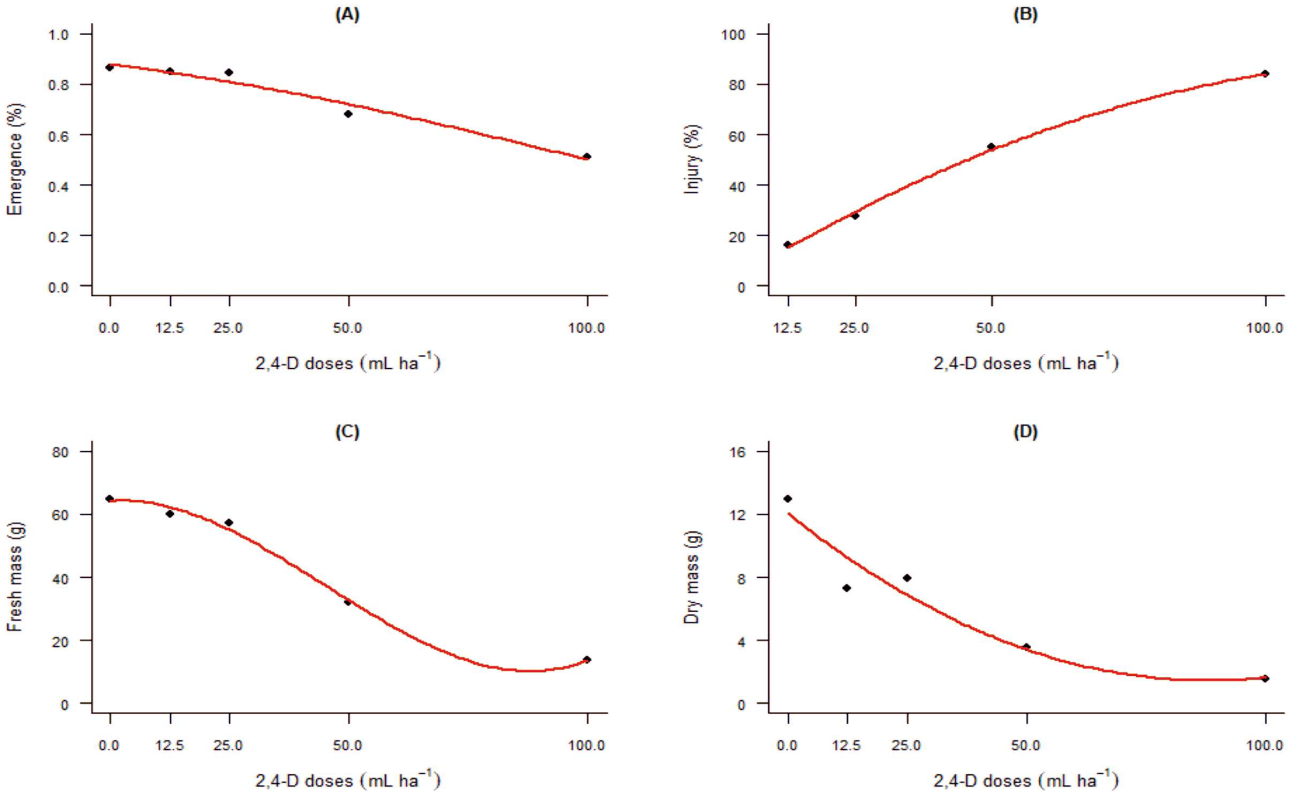
Regardless of the concentrations, there was a reduction in the emergence of bean seedlings (Figure 3A). In practice, bean seeds are subject to absorbing low concentrations of 2,4-D, both from drift from adjacent areas and from residuals in the soil. 2,4-D has limited residual activity in the soil, varying its half-life (persistence) according to

Table 4 - Estimations of the equations and determination coefficient (R^2) of the adjusted models of the variables assessed in bean seedlings (*Phaseolus vulgaris L.*) at 15 days after treatment with herbicide 2,4-D

Variables	Equation	R^2
Emergence	$\log\left(\frac{\pi}{1-\pi}\right) = 1.94 - 0.0195 \times \log X$	
Injury	$\hat{Y} = -1.04 + 1.35\,x - 0.005\,x^2$	0.98
Fresh mass	$\hat{Y} = 64.13 + 0.12\,x - 0.024\,x^2 + 0.0002\,x^3$	0.96
Dry mass	$\hat{Y} = 12.05 - 0.24\,x + 0.0014\,x^2$	0.80

x = 2,4-D concentration (gram of acid equivalent per liter) π is the rate of germinated seeds

Figure 3 - Regression model of the parameters assessed in bean seedlings (*Phaseolus vulgaris L.*) at 15 days after treatment with sub-doses of the herbicide 2,4-D in sandboxes. (A) Emergence (%); (B) Injury (%); (C) Fresh mass (g) and (D) Dry mass (g)



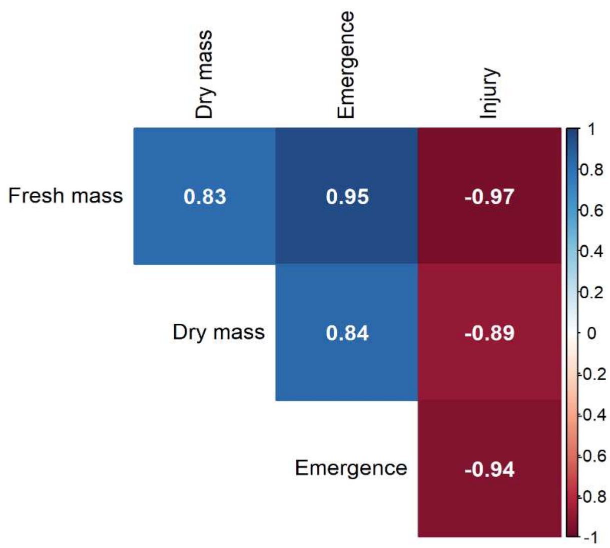
weather conditions and physical-chemical characteristics of the soil (BAUMGARTNER *et al.*, 2017). Increases in clay and organic matter content can minimize its availability in the soil (PROCÓPIO *et al*., 2009).

Sowing in sand possibly increased the damage to seedlings because this substrate has low adsorption and microbial degradation, resulting in increased availability of the herbicide in the soil. Thus, the herbicide was readily available to the seed, which directly affected the emergence of seedlings after absorption. Silva *et al.* (2011), in an area of sandy loam soil (76% sand and 20% clay), using 2,4-D at 1005 g a.e. ha^{-1} at the day of soybean sowing, observed a reduction in speed and percentage of germination. However, an experiment in an area with 55% sand, 38% clay and 2.4% organic matter, found no damage to crops when applied at a concentration of 670 g a.e. ha^{-1} right after sowing (PROCÓPIO *et al*., 2009).

As a result of the injury caused by the concentrations used in the study, there was direct interference in the fresh and dry mass of the shoot (Figure 3C and D), reducing the weights as concentrations increased (Figure 4).

Regardless of the concentration, there was a reduction in the proportion of seedlings that emerged, with a decrease of approximately 50% in emergence when subjected to the highest concentration (Figure 3A). Thus, it is essential

Figure 4 – Pearson's correlation coefficient (r) of the variables Fresh weight (g), Dry weight (g), Injury (%) and Emergence of bean plants (*Phaseolus vulgaris L.*) submitted to different sub-doses of the herbicide 2,4-D



to know the safety period for the application of the herbicide before sowing bean crops, in order to avoid damage to seedling emergence. Furthermore, according to the results obtained by the present study, it can be inferred that soil characteristics, such as texture, minerals, clay and organic matter, are determinants in the residual action of the herbicide.

The sub-doses of 2,4-D reduced the emergence and the development of bean seedlings. Thus, it is necessary to intensify care regarding the safe application of synthetic auxin herbicides, especially in areas close to sensitive crops, such as beans.

CONCLUSION

Regardless of whether the seeds were soaked or sprayed with sub-doses of 2,4-D, there was negative interference in the germination and initial development of bean seedlings. Thus, this herbicide does not have a stimulating effect on seedling emergence and development. Seeds soaked at a concentration of 33.50 g a.e. L^{-1} of 2,4-D had their mass indexes reduced by more than 70%. After the application of 67 g. a.e. L^{-1} of 2,4-D in the pre-emergence of bean plants, an injury rate of over 80% was found.

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