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Residual of the mixture of glyphosate and 2,4-d herbicides on yellow red latosol with soybean cultivation

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The objective of this study was to evaluate the residual effect of glyphosate and 2.4-D herbicides in the initial development of the soy crop in a Yellow Red Latosol. The experiment was conducted in randomized design, consisting of 4 different doses of herbicides (0, 1, 2 and 5 times the recommended dose of the glyphosate and 2.4-D mixture), applied at intervals of 0, 3, 7 and 10 days before sowing, with 4 repetitions on a soybean culture (Monsoy 8372rr cultivar) performed up to 28 days after sowing, at 7 days intervals. The variables analyzed were plant height, leaf area, phytotoxicity, aerial dry biomass, root dry biomass and chlorophylls a and b, the latter being performed from the second evaluation after sowing. For the conditions evaluated, plant height, leaf area and aerial dry biomass were lower in the plants that received the highest doses of the glyphosate mixture and 2.4-D. The higher doses of glyphosate and 2.4-D resulted in higher symptoms of phytotoxicity in transgenic soy plants. The periods of application of the glyphosate herbicide mixture plus 2.4-D closer to the date of sowing of the soybean were the ones that most harmed the development of the crop.

Key words: Transgenic soy, phytotoxicity, initial development.

INTRODUCTION

The soy crop (*Glycine max*), has great importance in the economy of Mato Grosso, being the largest producing state of this crop in Brazil (CONAB, 2018). Due to its adaptation in several regions, the cultivation of this oleaginous has been occupying large areas in the country and promoting the creation of new agricultural

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borders. To achieve high levels of soybean productivity, the use of agrochemicals throughout the crop cycle is an indispensable practice, for the initial phase the control of weeds is a determining factor to ensure good plant growth. Weeds compete for space and nutrients and interfere in the growth and development of cultivated plants (Lamego et al., 2015).

The mixing of herbicides in the spray tank is not allowed by Brazilian legislation, however, it is a common practice for the control of weeds that are usually associated with soy culture. Research by Gazziero (2015), who interviewed farmers and professionals in the area, concluded that for 97% of respondents tank mix is a common practice. The use of the glyphosate molecule with the 2.4-D mixture in the spray tank has been used for spraying in pre-planting, being common among farmers.

The residual effect of herbicides applied in pre-planting or in predecessor crops, has been verified by several farmers, causing crop losses and harming productivity (Mancuso et al., 2011). Gomes et al. (2017) observed that the residual effect of the glyphosate and 2.4-D mixture harmed the initial development of corn plants cultivated in Red Yellow Latosol and Quartz NeoSol. Even transgenic soybean plants have negative effects on their initial development due to the presence of the residual glyphosate in soils (Casonatto et al., 2014).

On the other hand, in studies on the residual effect of herbicides, Minozzi et al. (2014) found that the least effective treatments were those without the use of residual herbicide and with residual herbicides, but without post-emergence application of glyphosate. These authors further state that the addition of the residual herbicides diclosulam or sulfentrazone to desiccation made a single post-emergence application of glyphosate in the V4 stage of soya sufficient for weed control.

With the advance of resistance of some monocultures to glyphosate, as in the case of soy and corn, the selection pressure has caused the increase of individuals of some species of weeds to herbicides widely used in soy cultivation, thus producers are driven to increase the number of applications, use higher doses of herbicides, and even use the mixture of active principles to improve weed control. The mix of herbicides becomes promising in weed control, since it can demonstrate an increase in the number of species controlled within the floristic weed complex (Vieira Júnior et al., 2015).

Considering the decision of the Ministry of Agriculture that authorized the mixing of agrochemicals in tank through the Normative Instruction no. 40, published on October 11, 2018 and that it is eventually common to find soy plants with symptoms of phytotoxicity caused by herbicides used in desiccation of weeds, this work aimed to evaluate the residual effects of the mixture of glyphosate and 2.4-D herbicides, on the initial development of the soy crop cultivated in Red Yellow Latosol.

MATERIALS AND METHODS

Location and characterization of the experimental area

The experiment was conducted in an agricultural greenhouse at the Federal University of Mato Grosso (UFMT), Sinop campus, (11° 51' 50" S and 55° 29' 06" W, altitude 380 m) in the year 2017. The local climate is classified according to Koppen-Geiger as Am (tropical with dry winter), with two well-defined seasons, dry from May to September and rainy from October to April. The agricultural greenhouse used in the experiment was of the arch type, with 3.5 m of right foot, 6.4 m wide, 20 m long, cover and closed sides with transparent diffuser plastic film (75% transparency) and evaporative cooling system Pad&Fan that was programmed to maintain the temperature at 26°C, ±1°C and relative humidity at 65% during the entire period of the experiment.

Type of soil

The soil used in the experiment was classified as Red Dystrophic Yellow Latosol (RYLd) with a clay texture (Santos et al., 2013). Soil analysis was performed in the Laboratory of Plant Soil Analysis at the Federal University of Mato Grosso, Sinop, MT, Brazil. Soil pH was 5.4, P content was 0.8 mg dm^{-3}, K content was 44 mg dm^{-3}, Ca content 1.0 $cmol_c$ dm^{-3}, Mg content 0.4 $cmol_c$ dm^{-3}, H content 5.8 $cmol_c$ dm^{-3}, Al content 0.2 $cmol_c$ dm^{-3}, Organic Matter content 33 g dm^{-3}, Sand content 370 g dm^{-3}, Silt content 86 g dm^{-3} and Clay content 544 g dm^{-3}. After collecting the soil in the forest area, without the previous application of agrochemicals, the application of 99% RPTN (relative power of total neutralization) magnesian lime was carried out in the soil already distorted and sieved, and the period of 60 days was waited for the elevation of the saturation by bases to 60%, as recommended by the Bulletin of EMBRAPA (2004). After this period, the phosphate correction was performed using triple superphosphate. For the fertilization of planting was used the formulated NPK: 0-18-18, with dose of 300 kg ha^{-1}, because this dose is widely used in the culture of soy in the state of Mato Grosso. For the evaluation of the residual effect of glyphosate herbicides and 2.4-D in Yellow Red Latosol, the experimental design used was an entirely randomized (CRD), in a 4x4 factorial scheme with 4 repetitions. We used as experimental unit plastic vessels with capacity of 8 dm^{-3} that were filled with Yellow Red Latosol (LVAd). The treatments were represented by 4 different periods of application of the herbicide mixture before sowing (0, 3, 7 and 10 Days before Sowing) by 4 different recommended doses of the herbicide mixture (0, 3, 6 and 15 L ha^{-1} of recommended doses of the mixture, RDM). After the chemical corrections of the soil, the syrups composed by the different doses of the mixture of the glyphosate herbicides (commercial product Roundup Original®) and 2,4-D (commercial product 2,4-D AMINA 840 SL®) plus water were applied by means of a manual sprayer where the volume of syrup equivalent to 200 L ha^{-1} was adopted. Always after applying the herbicide mixture to the soil by spraying the syrup, the soil was turned so that there was homogenization with the mixture of herbicides.

The mixture of the herbicides to compose the recommended dose of the mixture (RDM), was composed of the dose of 2 L ha^{-1} of glyphosate (480 g L^{-1} of the active ingredient) plus 1 L ha^{-1} of 2.4-D (840 g L^{-1} of the active ingredient), thus forming 3 L ha^{-1} of RDM. After the preparation of the mixture, the doses equivalent to 0, 1, 2 and 5 times the RDM were used, which corresponded to 0, 3, 6 and 15 L ha^{-1} of the herbicide mixture?

Once the applications of the herbicides in the soil in the different periods prior to sowing were finished, the soy seeds of the Monsoy 8372rr cultivar, which is tolerant to the glyphosate herbicide, were manually sown in the pots. The experiment was conducted for 28

days after sowing (DAS) and irrigation was carried out through simulated rainfall in a controlled environment in the agricultural greenhouse. The frequency of irrigation was twice a day, once in the morning and once in the evening, applying a blade equivalent to 10 mm of rain per day.

Biometric and physiological evaluations

After soybeans were sown, four evaluations were performed between periods of 7 days each to determine the residual effect of herbicides on soybean plants. The following variables were analyzed: Height of plants (cm), which was performed by measuring the distance between the base of the plant and the last trifoil using a ruler; Aerial dry biomass and root dry biomass (g), where the results of these variables were obtained by weighing the material on a precision scale after drying all the material in a greenhouse at 75°C for 24 h; Foliar area (cm^2), where the values were obtained through the use of the bench area meter model LI-3100 (Li-Cor); Phytotoxicity in soybean plants where the symptoms were visually evaluated and assigned grades according to the scale (EWRC), ranging from 1 (no symptoms) to 9 (plant senescence) according to (EWRC, 1964); Chlorophyll a and b through the reading of the leaves which was performed using a chlorophyllometer model chlorophyllog 1.11 (Falker®), and this variable was evaluated only after the second evaluation after sowing (14 DAS).

Statistical analysis

The data were submitted to analysis of variance by the F test followed by the Tukey test ($p \leq 0.01$). Regression was performed on the growth variables and the model was chosen based on the significance of the regression coefficients, associated with the highest value of the determination coefficient (R^2). Statistical analyses were performed using the SISVAR software (Ferreira, 2011).

RESULTS AND DISCUSSION

Height of plants

At 28 days after sowing (DAS), the comparison between the recommended doses of the mixture (RDM) of glyphosate and 2,4-D with the average of all application periods in days before sowing (DBS) showed that there was a reduction in the height of soy plants for all doses compared to the control, but there was no difference between the doses of the mixture (Figure 1). Although not different from the other doses of the mixture applied, the concentration of 15 L ha^{-1} of the mixture showed that as the dose of herbicides is increased, the height of the soy plants is reduced indicating that this practice can cause damage to the subsequent crop even when the grace period recommended by the manufacturer of the product is respected. In a study made by Casonatto et al. (2014) in different classes of soils, a smaller size of the plants was observed with the increase of the glyphosate dose. When the doses of the mixture used were related to the average height of soybean plants of each period of application before sowing, only for the dose of 15 L ha^{-1} there was a difference between periods, and the lowest height of soybean plants were observed for 7, 3 and 0 days before sowing (DBS), whereas for the period of 10 DBS the soybean plants had higher growth (Figure 2).

This result shows that the grace period between the application of herbicides and sowing should be greater than 10 days, also indicating that the greater grace periods of herbicides allow the greater probability of adsorption of the molecules of the product to organic matter and clays present in the soil. These results corroborate with those observed by Silva et al. (2011) who also reported that for all the periods of application of the herbicide there was a reduction in the height of the plants in relation to the control and that the greatest periods of deficiency were those with the greatest performance.

Aerial dry biomass

The values observed for aerial dry biomass followed the same line of results obtained for plant height. All applied doses of the mixture caused reduction of aerial dry biomass compared to the control, even when adopted the dose of 3 L ha^{-1} of the mixture that corresponds to that recommended by the manufacturers, there was difference compared to the control. For the control, an air dry mass of 2.99 g was observed, for the dose of 3 L ha^{-1} the biomass observed was 2.67 g which corresponded to a reduction of 10.71% in relation to the control, thus occurring a difference between these values. The lowest value of aerial dry biomass was observed for the dose of 15 L ha^{-1} with 2.40 g which corresponded to a reduction of 19.74%. This reduction of aerial dry biomass is explained because glyphosate affects the synthesis of the amino acid tryptophan in the plant, precursor of auxin which is the hormone responsible for cell elongation (Casonatto et al., 2014). Silva et al. (2015) also observed that the highest applied doses of the glyphosate mixture and 2.4-D were those that caused the greatest reductions in aerial dry biomass in soybean plants. For comparison between periods for each dose of the mixture used, no differences were observed in aerial dry biomass.

Leaf area

As it was observed for the height of plants, the unfolding of the DBS evaluated within each RDM used showed that for the leaf area also there was only difference between periods for the dose of 15 L ha^{-1}. In this result it was observed that for the periods of 3 and 0 DBS the smallest foliar areas occurred, the largest already occurred at 10 and 7 DBS (Figure 3). Comparing the periods of 10 with 0 DBS, it was observed that there was a reduction in foliar area of approximately 40%. These results indicate that with the reduction in leaf area, automatically occurs a

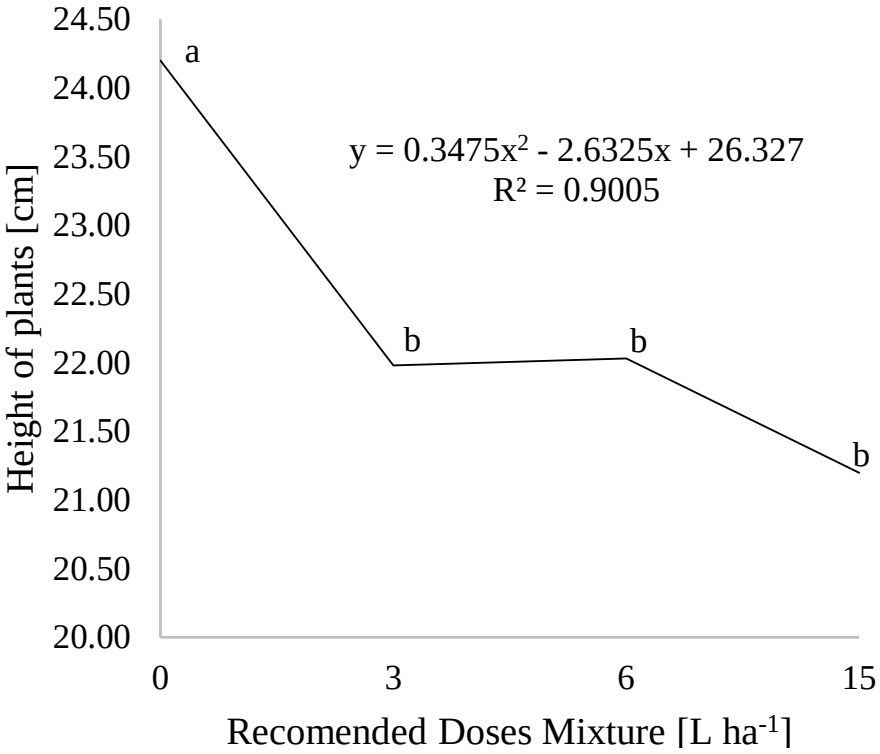

Figure 1. Height of soybean plants at 28 days after sowing in relation to recommended doses of the mixture (RDM) of glyphosate and 2.4-D. Averages followed by the same letter do not differ statistically by Tukey's test ($p < 0.01$) probability.

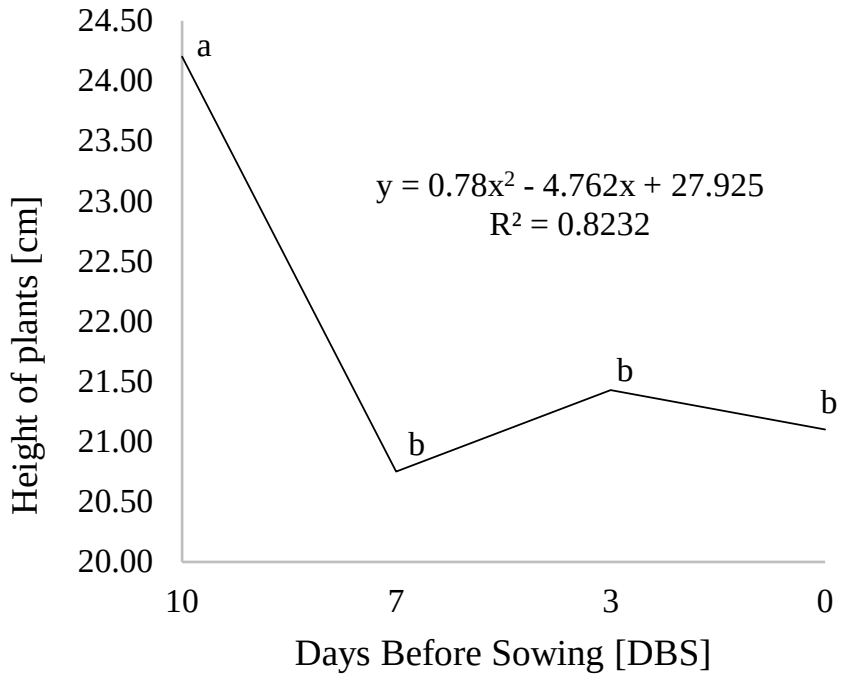

Figure 2. Height of soybean plants in relation to the days before sowing (DBS), for 15 L ha^{-1} of the recommended dose of the mixture (RDM) of glyphosate and 2.4-D herbicides. Averages followed by the same letter do not differ statistically by Tukey's test ($p < 0.01$) probability.

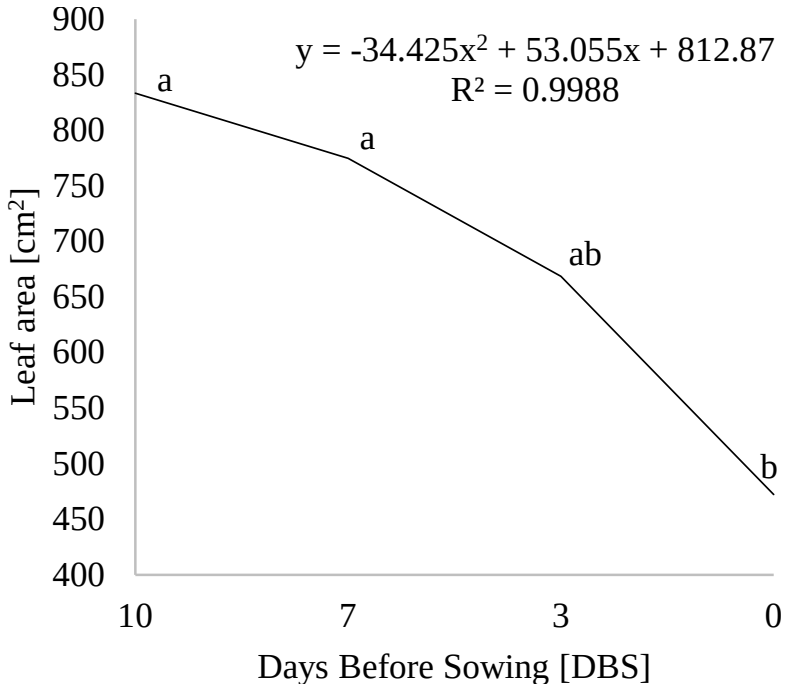

Figure 3. Foliar area of soybean plants for the recommended dose of the glyphosate mixture (RDM) and 2.4-D of 15 L ha^{-1}, for the days before sowing (DBS). Averages followed by the same letter do not differ statistically by Tukey's test ($p < 0.01$) probability.

decrease in the rate of photosynthesis in the plant and consequently the reduction of aerial dry biomass. It was also observed that the difference in leaf area values between periods was more expressive for the DRM of 15 L ha^{-1}, showing that with the increase in the dose used, the probability of causing damage to the crop increases. This result is corroborated by Reis et al. (2010) who evaluated several pre-seeding glyphosate dosages and found that the higher the dose, the smaller the leaf area of soy plants.

Plant phytotoxicity

According to the EWRC scale of phytotoxicity, the increase in RDM also promoted the progressive increase of phytotoxicity of herbicides in soy plants. For the plants that received the 3 L ha^{-1} dose of the herbicide mixture, small leaf changes such as discoloration and deformations in some plants were observed. For the 6 L ha^{-1} dose the symptoms of chlorosis and leaf plugging were observed in several plants. For the 15 L ha^{-1} dose, besides small deformations, discolorations and bushings, some epinasties of apical meristems were also observed. Very similar symptoms were also observed by Casonatto et al. (2014) in soybean plants.

Phytotoxicity assessments performed between 7 and 14 days after sowing (DAS) showed that symptoms increased as the RDM increased. When phytotoxicity symptom evaluations were performed at 21 and 28 DAS, stabilization of phytotoxicity incidence in plants was observed as the RDM increased. This result indicates that for the periods of 21 and 28 DAS the evaluations of the residual effect of these herbicides on the development of the plants are more inaccurate. The Figure 4 shows that in the phytotoxicity evaluations performed at 21 and 28 DAS with the averages of all the RDMs used, lower phytotoxicity indices were observed when compared to the evaluations performed at 7 and 14 DAS.

Thus, it can be observed that the phytotoxicity symptoms were reduced over time, showing that the plants presented capacity to recover from the damage caused by herbicides. These results corroborate those obtained by Gomes et al. (2017), who observed that the phytotoxicity symptoms caused by herbicides in corn plants were reduced as the crop cycle developed.

Only the RDM of 6 and 15 L ha^{-1} showed a difference when the phytotoxicity of soybean plants was evaluated among the different DAS (Figure 5). Through these results it can be observed that the first evaluations were those with the highest average phytotoxicity (7 and 14 DAS), for the doses of 6 and 15 L ha^{-1}, however, the plants were able to recover along their normal development path. Similar results were observed by Silva and Silva (2007) who verified that the symptoms of

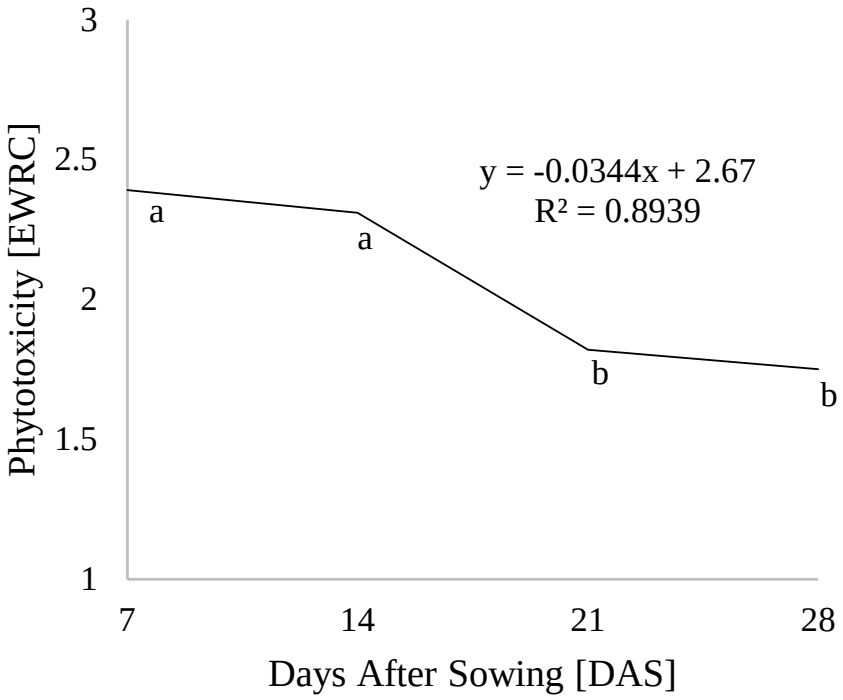

Figure 4. Average of the phytotoxicity notes of soy plants, for the periods of 7, 14, 21 and 28 days after sowing (DAS). Averages followed by the same letter do not differ statistically by Tukey's test ($p < 0.01$) probability.

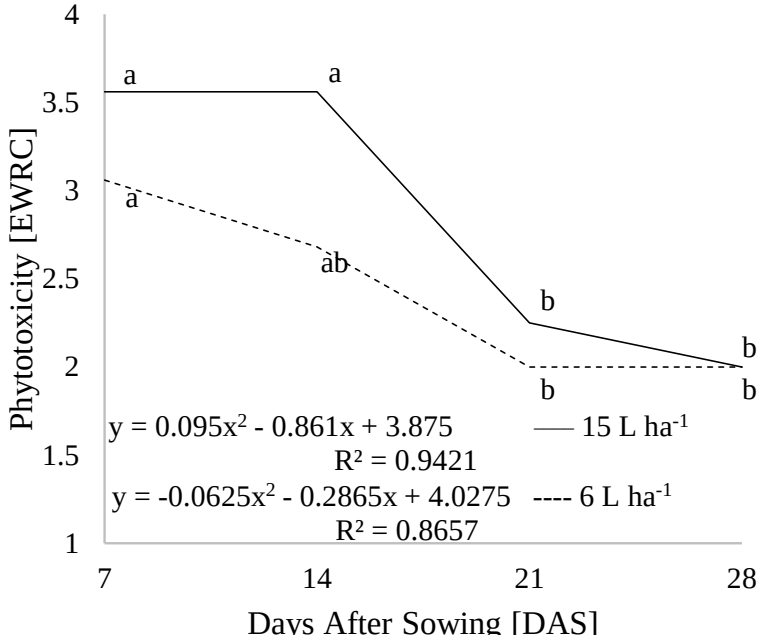

Figure 5. Phytotoxicity of soybean plants in relation to the days after sowing (DAS) for the doses of 6 and 15 L ha^{-1} of the recommended doses of the mixture (RDM). Averages followed by the same letter do not differ statistically by Tukey's test ($p < 0.01$) probability.

the residual activity of 2.4-D herbicide in the plant are more apparent in the first weeks after its application.

Dry root biomass and chlorophylls a and b

The data obtained from dry root biomass and chlorophylls a and b, when submitted to statistical analysis, showed no difference between treatments. The results obtained for chlorophylls a and b do not corroborate those observed by Casonatto et al. (2014) and Gomes et al. (2017) who found that chlorophylls a and b differed from the control when glyphosate RDM and 2.4-D 10 times more than recommended were used.

Conclusion

For the conditions evaluated, the height of plants, leaf area and aerial dry biomass were lower in the plants that received the highest doses of the glyphosate and 2.4-D mixture. The higher doses of glyphosate and 2.4-D resulted in higher symptoms of phytotoxicity in transgenic soy plants. The periods of application of the glyphosate herbicide mixture plus 2.4-D closer to the date of sowing of the soybean were the ones that most harmed the development of the crop.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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ABBREVIATION

UFMT, Federal University of Mato Grosso; **RYLd,** Red Dystrophic Yellow Latosol; **RPTN,** Relative Power of Total Neutralization; **NPK,** Nitrogen, Phosphorus, Potassium; **CRD,** Completely Randomized Design; **RDM,** Recommended Doses of the Herbicide Mixture; **DAS,** Days after Sowing; **DBS,** Days before Sowing;

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