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Phytotoxicity in transgenic soybean treated with glyphosate doses

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Abstract. RR soy was designed to reduce costs and favour weed management using only one type of herbicide, the glyphosate. Although, there are just some few studies that actually evaluates the effect of this product on the physiology and final production of soybeans in northern Mato Grosso. The aim of this work was to verify the effects of the herbicide glyphosate molecule, on vegetative characteristics and productivity in transgenic soybean. The experiment was conducted at the Farm Água Viva, Sinop-MT. The experimental design was a randomized block design with five replications, with five treatments, three glyphosate concentrations (480 g ia.ha^{-1}, 960 g ia.ha^{-1}, 1920 g ia.ha^{-1}) another treatment with mechanical control (hoe) and the control (no weed control). Was evaluated the following variables: phytotoxicity, plant height, chlorophyll content, fresh weight, dry weight and productivity. As for efficient control by glyphosate application at doses of 960 g ia.ha^{-1} and 1920 g ia.ha^{-1} significantly reduced weed populations up to 21 DAP. Phytotoxicity was found near the time of application, especially at the maximum concentration applied. The effect of the herbicide on the variable plant height was more pronounced at 21 DAA and the recommended concentration (960 g ia.ha^{-1}) did not differentiate using the hoe. For fresh pasta, this was reduced with the application of herbicide and dry weight was not different between treatments. Productivity was higher in treatments with hoe, minimum and medium concentrations of glyphosate herbicide. The maximum concentration of the herbicide damaged the productivity as well as treatment unchecked, the less productive. For chlorophyll values glyphosate application promoted a significant reduction, which can be explained by the decrease in manganese uptake by the plant.
Key words: Transgenic soybeans, Phytotoxicity.Glyphosate.

Introduction

The increasingly globalized world economy has been the main driver of the increase in soybean production in Brazil. With the increase in consumption in countries like China, demand for oilseed has been increasing. According to the United States Department of Agriculture (USDA), world production of soybeans in the 2013/14 crop year was 283.54 million tons, Brazil is the second largest producer with 88 million tones and the largest exporter grain, with 42.5 million tons (Conab, 2014).

According to EMBRAPA (2011), several transgenic soybean cultivars are currently being developed, the most known and commercially planted is the RR, which has received by biotechnology techniques, an extracted gene from a soil bacterium *Agrobacterium tumefasciens*, patented by a private company with the CP4-EPSPS name. When inserted into the soybean genome, made the plant resistant to the application of glyphosate herbicide.

Despite the genetic transformation, there are some symptoms in plants due to the application of glyphosate, but this is not considered by some authors as accentuated because there is a continuity of the vegetative growth of affected plants, symptoms of phytotoxicity occurring are only punctual, not interfering with variables such as height of aerial part, root length and growth of the main stem growing new side branches (Reis et al., 2010). Regardless of the concentration of glyphosate applied to soybean puts forward phytotoxic effect, either in a single application as sequential applications (Foloni et al., 2005), but it is known that just as happened with the cultivar BRS-244 RR soybeans no interference occurs in the productivity of crop grains. (Guimarães et al, 2008; Agostinetto et al, 2009b.).

The glyphosate is a product commonly used by farmers to control weeds and cleaning areas before planting a crop. The herbicide of this mechanism of action is the blocking of biosynthesis of aromatic amino acids by inhibiting the activity of 5-enol pyruvyl-shikimate-3-fosfatosintase - EPSPS (Foloni et al., 2005). Soybean resist to glyphosate due to the tolerance of the plant to the herbicide, by inserting a gene (AroA) derived from the genome of Agrobacterium sp. Strain CP4, which encodes an EPSPS variant, making glyphosate tolerant plant. In this manner RR soybean continue to produce essential material for their development and growth is not affected by the effects of herbicide (Foloni et al., 2005).

Although not have found reductions in productivity, the emergence of tolerant weeds has led producers to increase the dosages and herbicide application frequency, due to that, new studies on phytotoxic effects of glyphosate on soy are required. Given the above, the objective of this study was to investigate the effects of glyphosate on the vegetative characteristics and productivity of transgenic soybeans.

Methods

The experiment was conducted at the Farm Agua Viva, located in the municipality of Sinop - MT, Silvana road, at km 06, lot 90, (with latitude 11° 47 '857"S longitude 55° 26'429"W, altitude 367 m above sea level). The municipality of Sinop belongs to the Midwest, according to climatic classification of Köppen is (Aw) rainy tropical, warm and humid transition between humid equatorial climate of the Amazon and savanna (Cerrado). The region has an average annual rainfall of 2000 mm, with well-defined seasons, dry season (June to August), dry-wet (September to November), wet (December to February) and wet-dry (March to May).

The statistical design was a randomized block with 05 treatments (480 g ia.ha-1, 960 g ia.ha-1, 1920 ia.ha 1 g, hoe and free) and five repetitions. The plots consisted of six rows of five meters, totaling 15 m2 and borders were considered two side lines and 0.5 m at the ends of the plots, with a total floor area of 8.0 m².

The experiment was conducted in the 2011/2012 harvest, soybean production area (Glycine max (L.) Merrill) cvSoy 8867-RR and glyphosate herbicide used was Roundup Ready® containing 480g.L of isopropylamino salt [N (phosphonomethyl)] glycine glyphosate.

The area had conservationist system and was seeded on 07/11/2011, using the spacing of 0.45 m between rows and 13 seeds per meter (if seeding 288,000 seeds.ha-1) to 3cm depth. Glyphosate was applied at sowing to desiccation and basic fertilization at sowing was 360 kg ha-1 NPK formulation 02-20-20.

Before sowing, the seeds were inoculated with Bradyrhizobiumjaponicum and treated with Co and Mo micronutrients in the dosage of 05 g ha-1 and 25 g ha-1 sauce. The fungicide used in the seed treatment was a formulation comprising 5,6-dihydro-2-methyl-1,4-oxathi-ine-3-carboxanilide (200 gL-1) and Tetramethylthiuram Disulfide (200 gL-1), dosage 250 ml for each 100 kg of seed. The Insecticide used was the basis of FIPRONIL (RS - 5 - amino - 1 - (2,6 - dichloro - α, α, α - trifluoro - p - tolyl) - 4 trifluoromethylsulfinylpyrazole - 3 - carbonitrile) (250 g L-1) in 200 ml dosage of the product for every 100 kg of seed mixed in a concrete mixer, exactly equal to its planting in the field, and after 03 hours of his inoculating the seed was sown.

In conducting the experiment, it applied the fungicide (tebuconazole-1 L.ha-1) and insecticide (Acephate-0.75 L.ha-1) following the technical recommendations for the culture. For the application of the treatments, glyphosate herbicide was applied only once, when the weeds were with two pairs of leaves (about 30 days after sowing). For the application of the herbicide used was one rib hand sprayer with a pressure of 254 kPa, equipped with bar containing four spray nozzles, which permitted the application 150 L.ha of herbicide-1, at a pressure of approximately 30lb.pol2 .

The weed population was evaluated in all plots at 0; 7; 14 and 21 days after application of glyphosate. For this, we used the method Release of a wooden square with area of 0.25 m2. After the release of the wooden square, weeds within the same perimeter were visually identified and counted.

Following application of treatments, for a period of 7, 14 and 21 days was observed the occurrence of symptoms of phytotoxicity. As the literature there are no specific methods for grading the symptoms of phytotoxicity on soybean plants, we used a grading scale for foliar diseases final cycle developed by Reis et al. (2010).

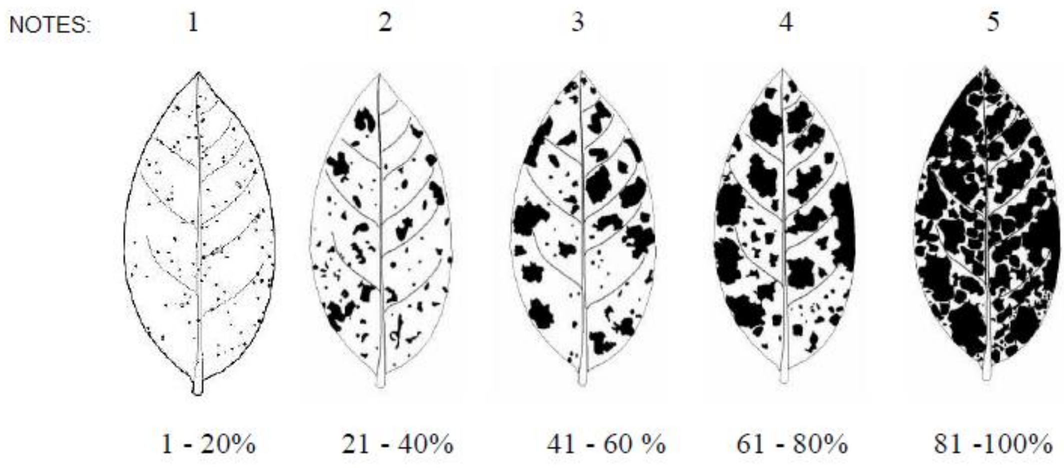

Figure 1: Diagram for the identification and quantification of the symptoms of phytotoxicity. Note 0 (zero) no symptoms in leaf area. Note 1 (one) from 1 to 20%, note two (2) between 21 and 40% note 3 (three) between 41 and 60%, note four (4) between 61 and 80% and score 5 over 81% of the leaf area.

The rating of injury was performed in all plants in the two center lines, thus obtaining the average value injury. Also evaluated the development of plant, using the variables: height, fresh and dry weight. The height was obtained at 7, 14 and 21 days after application and measure the soil level to the apical meristem with the aid of a measuring tape. Was evaluated 10 random plants from the center line (Foloni et al., 2005).

To obtain fresh and dry weight, the plants were cut close to the ground, at 14 and 21 days after the application and were immediately taken to the animal nutrition laboratory of UFMT. In the laboratory the plants were weighed on a digital balance accurate to obtain fresh weight, then were placed in a forced-air oven at 60 ° C ± 2 ° C until constant weight, weighed and immediately after, yielding the dried weight.

The chlorophyll levels were obtained at 14 and 21 days after herbicide applications, using a chlorophyll meter of clorofiLOG mark (CFL-1030 model). was evaluated 10 intact leaves, the middle region of the plant 10 plants randomly chosen in the plot.

Harvesting and threshing were done manually to 114 days after sowing, harvesting The plants only the useful portion. To determine the productivity values obtained were transformed into kg ha-1, these were corrected to 14% moisture, based on the recommendations of the Seed Analysis for Rules (Timossi & Durigan, 2002).

Statistical analysis was performed with the aid of statistical software SISVAR®, by analysis of variance and F test. When detected significance, as the treatments were qualitative, was held Tukey test at 5% significance.

Results and discussion

During the experiment was observed species of weeds that have occurred and the effect of treatments on their populations, these being: Biden spilosa (Figure 2); Brachiaria plantaginea (Figure 3); Ipomoea ssp (Corda de viola) (Figure 4).;Spermaco delatifolia (Erva quente) (Figure 5); Commelina benghalensis (Trapoeraba) (Figure 6).

It was observed that treatment with application of 480 ia.ha-1 Glyphosate g showed no effective control of the population of weeds, not differing treatment without control, except for the control of *Brachiaria plantaginea*, the dose of 480 g ai .ha-1 has had a significant effect, a result also reported by Martini et al. (2003), that evaluating the effect of glyphosate applications in three different weed species (*Brachiaria plantaginea, Digitaria horizontalis and Brachiaria decumbens*) observed that among the three major weeds present in the experimental area, *Brachiaria plantaginea* was the most susceptible, mainly due to higher susceptibility, faster absorption and lower demand dosages. The control using hoe was the most efficient, reducing to zero (0) the number of invasive plants within seven days after the control, note also that the application in dosages of 960 g ia.ha-1 and 1920 g ia.ha-1 significantly reduced the population of weeds until 21 days after application.

It is the occurrence of phytotoxicity of glyphosate herbicide on the plant soybean soy M-8867 RR. Other authors also found occurrence of phytotoxicity on RR soybean plant (Reis et al, 2010; Agostinetto et al, 2009ab.). The phytotoxic symptoms were observed most clearly in first evaluation 07 days after the application, where phytotoxicity was observed in all treatments using the herbicidal independent of dose (Table 1).

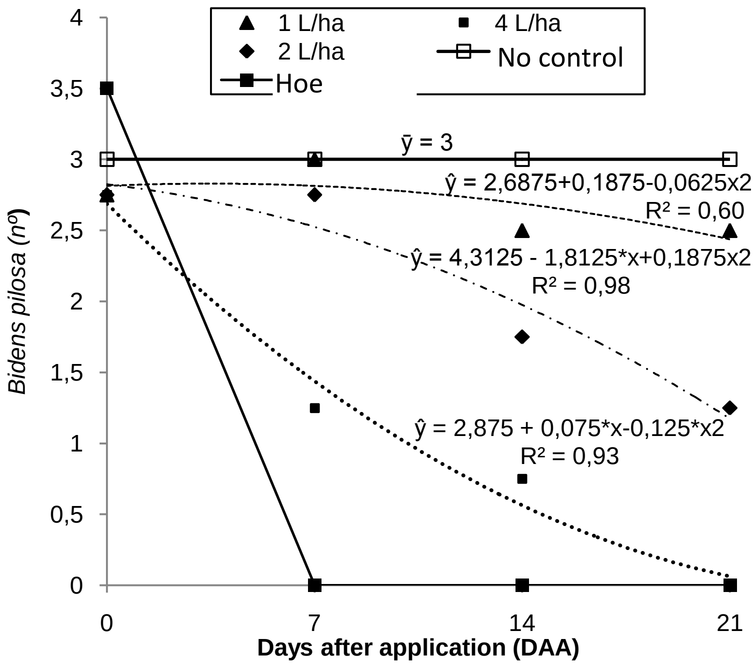

Figure 2. Average number of plants of the species Bidenspilosa (number of individuals m-1) in soybean growing 8867 RR under five weed management systems: without control; control with hoe; 1L / ha (480 g ia.ha-1); 2L / ha (960 g ia.ha-1); 4L / ha (1920 g ia.ha-1). Sinop-MT.

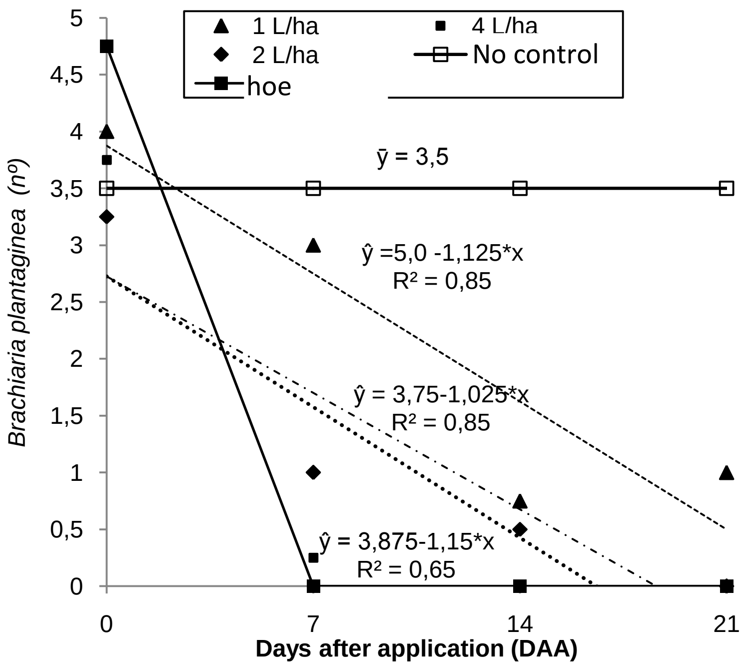

Figure 3. Average number of plant species Brachiaria plantaginea (number of individuals m-1) in soybean growing 8867 RR under five weed management systems: without control; control with hoe; 1L / ha (480 g ia.ha-1); 2L / ha (960 g ia.ha-1); 4L / ha (1920 g ia.ha-1). Sinop-MT.

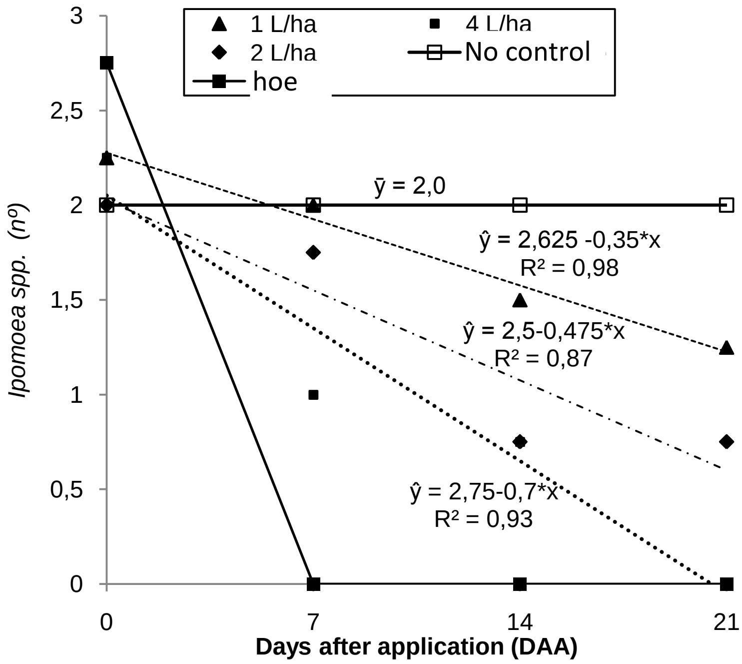

Figure 4. Average number of plant species *Ipomoea* ssp. (Number of individuals m-1) in soybean growing 8867 RR under five weed management systems: without control; control with hoe; 1L / ha (480 g ia.ha-1); 2L / ha (960 g ia.ha-1); 4L / ha (1920 g ia.ha-1). Sinop-MT.

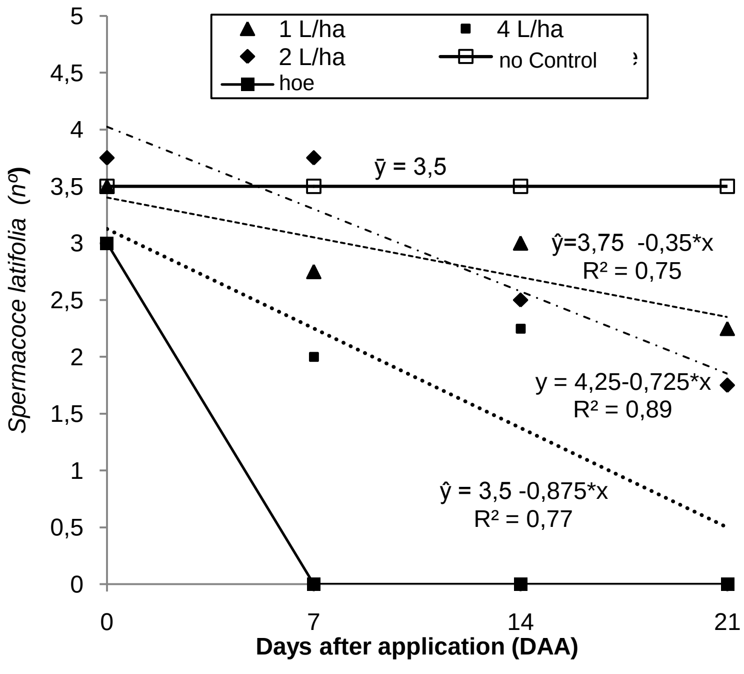

Figure 5. Average number of plant species Spermaco delatifolia (number of individuals m-1) in soybean growing 8867 RR under five weed management systems: without control; control with hoe; 1L / ha (480 g ia.ha-1); 2L / ha (960 g ia.ha-1); 4L / ha (1920 g ia.ha-1). Sinop-MT.

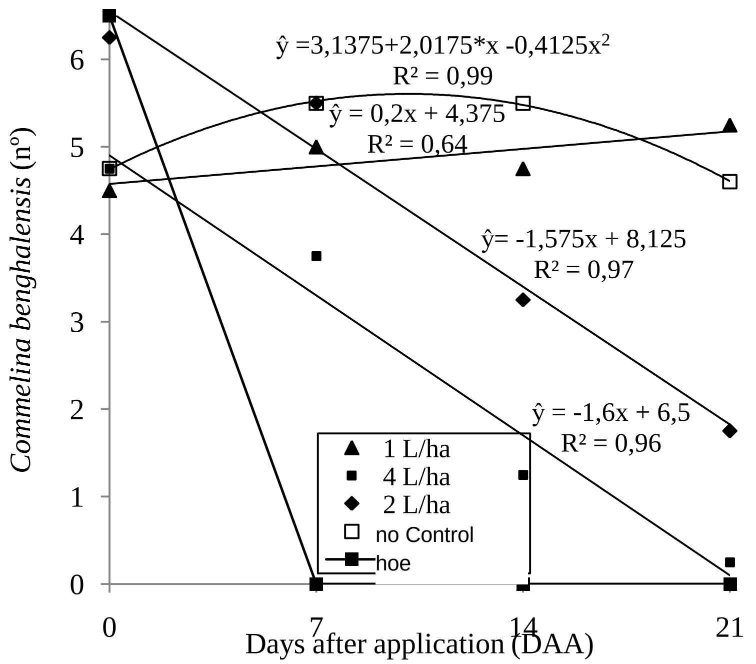

Figure 6. Average number of plant species Commelina benghalensis (number of individuals m-1) in soybean growing 8867 RR under five weed management systems: without control; control with hoe; 1L / ha (480 g ia.ha-1); 2L / ha (960 g ia.ha-1); 4L / ha (1920 g ia.ha-1). Sinop-MT.

Table 1. Different concentrations of glyphosate and weed control treatments on phytotoxicity in transgenic soybean plants in the 2011/2012 harvest, Sinop - MT.

Treatments	**Phytotoxicity (%)**		
	07 DAA	**14 DAA**	**21 DAA**
No control of weed	0 a	0 a	0 a
Contro lwith hoe	0 a	0 a	0 a
Minimum concentration of Glyphosate (480 g ia.ha-1)	24b	0 a	0 a
Recommended concentration of glyphosate (960 g ia.ha-1)	40 c	28 b	0 a
Maximum concentration of glyphosate (1920 g ia.ha-1)	60 d	36b	0 a
CV (%)		44, 46	

*The averages followed by the same letters do not differ at 5% probability.

It was initially yellowing and discoloration of the soybean leaves with a decrease these injuries over time and complete recovery plant at 21 days after application. This recovery was due to the characteristic of the RR soybean plants, which continue to produce essential compounds for their development and growth, not being affected by the effects of herbicide (Foloni et al., 2005).

Agostinetto et al. (2009) researching different glyphosate formulations to seven days after application at different times, found an increase in the phytotoxic effect in treatments applied to twenty days after soybean emergence. Other studies, using different formulations of glyphosate, found that the affected plants are recovered after several days regardless of the formulation used and that the grain productivity is similar to that with application of the isopropylamine salt formulation, which is registered for the crop (Foloni et al., 2005).

The height of the soybean plants was affected by the application of glyphosate in all evaluations being more pronounced DAA 14 and 21 DAA (Table 2). Treatment without control reached the highest, this is probably due to weed competition. Morphologically plants of a crop wither because of the competition with weeds, even though the competition has not yet reached critical levels. Community infesting plants reflect light at a certain wavelength, which is usually recognized by crop plants, which stimulate the growth in height even before the competition enforcement, in order to capture the most of the available radiation and shade the weeds (Radosevich et al., 1997).

Table 2.Different concentrations of glyphosate and weed control treatments on the height of transgenic soybean plants in the 2011/2012 harvest, Sinop - MT.

Treatments	Plant height (cm)		
	07 DAA	14 DAA	21 DAA
No control of weed	37,98 b	58,38 c	73,96 c
Control with hoe	33,28 a b	51,46 b	70,30 c
Minimum concentration of Glyphosate (480 g ia.ha^{-1})	32,14 a	45,42 a	62,58 b
Recommended concentration of Glyphosate(960 g ia.ha^{-1})	30,36 a	48,54 a b	68,70 c
Maximum concentration of Glyphosate (1920 g ia.ha^{-1})	31,08 a	47,18 a b	54,70 a
CV (%)	5,63		

*The averages followed by the same letters do not differ at 5% probability.

The effect of the herbicide on the variable plant height was more accentuated at 21 DAA, when the dose 1920 ia.ha 1 g reduced by 26% the height of plants in relation to the treatment without control, and 20% with respect to dose 960 g ia.ha-1. This result contrary to that found by Reis et al. (2010), where the height of the area showed no significant differences among treatments due to its tolerance to application of glyphosate, the RR soybean continued its vegetative growth even after higher dose.

Foloni et al. (2005) evaluating plant height variable RR soy after application of the herbicide glyphosate was found that soy not adversely affected by the herbicide 15 DAA. In other reviews was no difference between plants without hoe control and different herbicide treatments, which always had plants with lower height. In their study on the prevention of injuries caused by glyphosate in RR soybeans, Zobiole et al (2011) found similar results on the influence of application of glyphosate in the variable plant height, this is due to the decrease in photosynthetic rate and chlorophyll production caused by glyphosate and probably the immobilization of nutrients in the leaves.

The chlorophyll levels differed significantly on all checks and treatments (Table 3). In the first (seven days after application), the treatments without control and hoe differed from treatments with herbicide use, at 14 days was observed that the recommended concentration differed about 7% of other treatments, and at 21 days there was no difference between the herbicide dosages applied, but control with a hoe reached the highest chlorophyll color values.

According Zobiole et al., (2011), chlorophyll levels are affected by application of glyphosate, already in the experiments of Serra et al. (2011) glyphosate application interfere negatively in N absorption efficiency, which indirectly reduces the chlorophyll levels and green color from the leaves.

The reduction in chlorophyll levels, indirectly verified by the reduction in the values obtained with the chlorophyll meter may have occurred due to reduced synthesis or greater degradation of chlorophyll by glyphosate, a fact verified by Kitchen et al. (1981) cited by Yamada and Castro (2007). Another coefficient would be the reduction in manganese absorption by the plant. Among the Mn functions is participation in the photolysis reaction of water and the evolution of O2 in the photosynthetic system in the formation of chlorophyll and function of chloroplasts molecule. It also serves on nitrogen metabolism and cyclic compounds such as precursor of amino acids, hormones, phenols and lignins (Malavolta, 2006).

In their study, Gordon (2007) reported that the gene added to transgenic soybeans may have changed other physiological processes in the plant and that the glyphosate herbicide may slow absorption and translocation of manganese in the plant or have an adverse effect on the population of soil microorganisms responsible for the reduction element in the form available to the plant.

Applying glyphosate in RR soybean, Huber (2007) noted that there was a reduction in the population of reducing organisms and increase the population of Mn oxidizing organisms in the rhizosphere of the plant, favoring the transformation of Mn 2+ (form absorbable by plants) in Mn4 + (not absorbable by the plant), causing as a consequence Mn deficiency in soybean RR.

From the 21 DAA there was no difference among treatments with herbicide use. Meschede et al. (2007) also had similar results, the authors studied the effect of maturators in sugarcane, observed that glyphosate reduced the chlorophyll levels **a** and chlorophyll **b** measures 15 days after application (DAA), but at 30 DAA the difference disappeared. The chlorophyll levels both **a** and **b** in the observation fall to levels close to those observed in treatment with glyphosate at 30 DAA.

The application of the herbicide glyphosate altered the fresh weight of aerea portion and reduced it when compared with the control. Among the dose differences were not observed, and this difference was highest in the higher dose (1920 g ia.ha^{-1})and control with hoe was 16,5 % and in relation to the treatment without control was 29,6% (Table 4).

Table 3. Chlorophyll levels in transgenic soybean plants in different concentrations of glyphosate and weed control treatments in the 2011/2012 harvest, Sinop - MT.

Treatments	**Chlorophylllevels**		
	07 DAA	14 DAA	21 DAA
No control of weed	36,88 a	40,92 b	45,67 bc
Control with hoe	37,40 a	40,19 a b	46,41 c
Minimum concentration of Glyphosate (480 g ia.ha^{-1})	36,90 b	38,56 a b	43,15 ab
Recommended concentration of Glyphosate (960 g ia.ha^{-1})	38,59 c	37,82 a	42,78 a
Maximum concentration of Glyphosate (1920 g ia.ha^{-1})	38,50 d	40,68 b	42,36 a

*The averages followed by the same letters do not differ at 5% probability.

Table 04. Different concentrations of glyphosate and weed control treatments on the fresh and dry weight of transgenic soybean plants in the 2011/2012 harvest, Sinop - MT.

Treatments	**Freshweight (g)**	**Dryweight (g)**
No control of weed	862,46 b	130,36 a
Control with hoe	727,18 ab	111,56 a
Minimum concentration of Glyphosate (480 g ia.ha^{-1})	584,84 a	105,47 a
Recommended concentration of Glyphosate (960 g ia.ha^{-1})	626,81 a	93,22 a
Maximum concentration of Glyphosate (1920 g ia.ha^{-1})	607,06 a	105,46 a
CV (%)	16,46	13,18

*The averages followed by the same letters do not differ at 5% probability.

The dry mass variable there was no difference between treatments. Santos et al. (1999) checking the dry matter RR soybean plants treated with glyphosate herbicide with different doses (480, 960 and 1920 ia.ha-1) verified that there was a reduction in dry matter content from dosages of 480 g ai .ha-1 compared to control (control with hoes). Already Foloni et al. (2005) showed phytotoxicity problems, reduced productivity and dry mass RR soybean treated with glyphosate at different dosages and formulations.

The productivity of corn grain yield was affected by application of glyphosate. It can be seen that there was no difference between the 480 and 960 dose ia.ha-1 (Table 5). It was observed that the highest yield (4331.4 kg ha-1) was obtained in the treatment of prescribed concentration (960 ia.ha 1 g) and lower yields were obtained in treatment with no control (2689.2 kg ha -1) and at the dose of 1920 g ia.ha-1 (3221.4 kg ha-1) with 37.9% and 25.6% reduction in productivity respectively in relation to the recommended concentration.

These results on productivity can be explained by a "feed-back" where the compensatory photosynthesis consists of a higher rate of photosynthesis present in leaves which have undergone minor injury than plants with leaves intact (Richards, 1993). This process operates in together with the formation of a new photosynthetic apparatus multiplying their effects to the plant and ensuring rapid recovery after injury.

Table 5. Different glyphosate concentrations and control treatments of weeds in transgenic soybean yield in the 2011/2012 harvest, Sinop - MT.

Treatments	**Yield(kg.ha^{-1})**
No control of weed	2689,2 c
Control with hoe	4111,2ab
Minimum concentration of Glyphosate (480 g ia.ha^{-1})	4290,6 a
Recommended concentration of Glyphosate (960 g ia.ha^{-1})	4331,4 a
Maximum concentration of Glyphosate (1920 g ia.ha^{-1})	3221,4bc
CV (%)	19,74

*The averages followed by the same letters do not differ at 5% probability.

Studying various formulations of glyphosate in soybean RR Agostinetto et al. (2009a) found different results found in this study, because the productivity of crop grain was not affected by the herbicide glyphosate, regardless of the formulation or dose, compared with hoed control of weed. On average, treatments with application of glyphosate herbicide had higher grain yield in the order of 24%

relative to the infested check, mainly due to the decrease of interspecific competition.

On the other hand by Foloni et al. (2005) found that increased productivity in the treatment using control with hoe and reduced production in the treatment without this way of control.

High rates of glyphosate, independent of the formulation utilized, cause injuries in the leaves, which can be considered phytotoxic symptoms, but if the environmental and nutritional conditions are favorable, the plants grow normally, including launching other leaflets, which may not interfere significantly in the productivity at the end of its cycle (Reis et al., 2010). Albrecht & Avila (2009) obtained results that showed that high doses of glyphosate reflect in a significant decrease in the yield components and quality of soybean RR.

In his studies, Zobiole et al. 2011 observed in treatment with maximal concentration of glyphosate a reduction in productivity. Probably the negative effects on the physiology of the soybean RR plant as mineral nutrition, water use and photosynthetic efficiency, and even the accumulation of glyphosate degradation of the compounds in the plant, can be problematic in crop productivity.

Final considerations

After the study it can be concluded that: (i) the application in dosages of 960 g ia.ha-1 and 1920 g ia.ha-1 significantly reduced weed populations to 21 DAP; (Ii) There was glyphosate phytotoxicity for all doses, with a reduction of these injuries over time and fully recover of plants to 21DAA; (Iii) glyphosate reduced chlorophyll values in plants; (Iv) The concentrations (480.960 and 1920 g ia.ha-1) of glyphosate reduce the height of the plants, and the treatment without control gave higher values for the variable plant height; (V) the minimum or recommended glyphosate concentrations do not affect the final productivity being equal to those used hoe; (Vi) without the control of weeds reduction in productivity reaches 37.9% over the most productive treatment (recommended use).

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