



## Article

CESCO, V.J.S.<sup>1\*</sup>  
KRENCHINSKI, F.H.<sup>1</sup>  
RODRIGUES, D.M.<sup>1</sup>  
NARDI, R.<sup>2</sup>  
ALBRECHT, A.J.P.<sup>2</sup>  
ALBRECHT, L.P.<sup>2</sup>

\* Corresponding author:  
<[victorcesco@hotmail.com](mailto:victorcesco@hotmail.com)>

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## AGRONOMIC PERFORMANCE OF INTACTA RR2 SOYBEAN SUBMITTED TO DOSES OF GLYPHOSATE

*Desempenho Agronômico da Soja Intacta RR2 Submetida a Doses de Glyphosate*

**ABSTRACT** - This study aimed at evaluating the agronomic performance of Intacta RR2 soybean submitted to the application of glyphosate doses in post-emergence. For this, two experiments were conducted in two locations (Palotina and Marechal Cândido Rondon - Paraná state). Applications were carried out with increasing rates of glyphosate (0, 720, 1,440, 2,160, 2,880, 3,600, 4,320 g a.e. ha<sup>-1</sup>), constituting seven treatments with an experimental design in randomized blocks with four replications, totaling 28 plots for each location. The cultivar used for both experiments was Monsoy 6210 Intacta RR2 IPRO™. The analyzed variables were phytotoxicity at 3, 7, 14, 21 and 28 DAA, A-B and total chlorophyll index, final height, number of pods, 100-seed weight, and yield. A regression analysis was used. There was a positive behavior of the linear regression for phytotoxicity and a negative one for final height, number of pods, A-B and total chlorophyll index, and yield in the city of Palotina. In Marechal Cândido Rondon, there were adjustments of the positive linear regressions for phytotoxicity, and negative linear regressions for chlorophyll index, final height, number of pods and yield. Results show that the glyphosate increase from the dose 1,440 g a.e. ha<sup>-1</sup> in the studied cultivar damaged the agronomic performance of the culture.

**Keywords:** *Glycine max*, yield, phytotoxicity, herbicides, transgenic soybean.

**RESUMO** - O presente trabalho objetivou avaliar o desempenho agronômico da soja Intacta RR2 submetida à aplicação de doses de glyphosate em pós-emergência. Para isso, foram realizados dois experimentos em duas localidades (Palotina-PR e Marechal Cândido Rondon-PR). Foram feitas aplicações de doses crescentes de glyphosate (0, 720, 1.440, 2.160, 2.880, 3.600 e 4.320 g e.a. ha<sup>-1</sup>), constituindo sete tratamentos em delineamento em blocos ao acaso com quatro repetições, totalizando 28 parcelas para cada local. O cultivar utilizado em ambos os experimentos foi o Monsoy 6210 Intacta RR2 IPRO™. As variáveis analisadas foram fitotoxicidade aos 3, 7, 14, 21 e 28 DAA, índice de clorofila A, B e total, altura final, número de vagens, massa de 100 sementes e produtividade. Foi empregada a análise de regressão. Houve comportamento positivo da regressão linear para a fitotoxicidade e negativo para altura final, número de vagens, índice de clorofila A, B e total e, ainda, produtividade no município de Palotina. Em Marechal Cândido Rondon, houve ajustes de regressões lineares positivas para a fitotoxicidade e negativas para índice de clorofila total, altura final, número de vagens e produtividade. A partir dos resultados, verificou-se que o aumento das doses de glyphosate a partir de 1.440 g e.a. ha<sup>-1</sup> no cultivar estudado prejudicou o desempenho agronômico da cultura.

**Palavras-chave:** *Glycine max*, produtividade, fitotoxicidade, herbicidas, soja transgênica.

<sup>1</sup> UNESP, Botucatu-SP, Brasil; <sup>2</sup> Universidade Federal do Paraná, Palotina-PR, Brasil.

## INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is a very significant culture in the Brazilian production scenario. In Paraná, the yield of the 2013/2014 harvest was around 2,950 kg ha<sup>-1</sup> with a production of about 14,780,700 tons (Conab, 2015). The yield of genetically modified soybeans adopted in the state of Paraná in the same harvest was estimated at 3,080 kg ha<sup>-1</sup> with a production of 14.990 ton (CÉLERES, 2013).

In Brazil and in the world, the increased use of transgenic soybean cultivars is due to the many benefits of this technology in weed management. According to Zablotowicz and Reddy (2007), the use of certain doses of glyphosate can affect the development of soybean crops.

Producers who use this technology have come observed, in some cultivars, the presence of injuries after the application of glyphosate (Santos et al., 2007; Zablotowicz and Reddy, 2007). According to Zobiolo et al. (2010a), the increase in glyphosate doses on RR soybean promotes a reduction in water absorption and other deleterious responses in the culture.

The phytotoxicity effect can cause a decrease in the photosynthetic rate and a reduction in chlorophyll content, nutrient concentration and dry biomass of shoot and root. In certain situations, it causes leaf yellowing on RR soybean, leading many producers to use foliar fertilizers to reduce this phytointoxication without necessarily obtaining the recovery of the culture (Zobiolo et al., 2010a).

After the application of the herbicide, the visual symptom may be the yellowing of higher leaves, which can be attributed to the immobilization of bivalent cations (Bott et al., 2008; Zobiolo et al., 2010a). According to Zobiolo et al. (2010b), factors such as the applied dose and the phenological stage at the time of application influence the intensity of the effects the herbicide may cause. Effects such as accumulation of nutrients and dry matter production of the shoot, even in soybean with the RR technology, changed according to the application of glyphosate.

With the intensive use of glyphosate for weed control due to its convenience has been the reason to select resistant weeds. Ateh and Harvery (1999) observed that some weeds may require high doses of glyphosate in sequential applications or, in some cases, herbicide associations for a better management of these plants. One of the alternatives to these combinations is the use of glyphosate + chlorimuron-ethyl, providing an increased spectrum or potentially better control over these resistant weeds (Vangessel et al., 2001; Vidrine et al., 2002; Norsworthy and Grey, 2004; Procópio et al., 2007).

The purpose of this study was to evaluate the agronomic performance of Intacta RR2 soybean subjected to increasing doses of glyphosate.

## MATERIAL AND METHODS

This study was conducted during the 2013/2014 crop, using the Monsoy 6210 RR2 IPRO cultivar, and was carried out in two production environments: one located in the city of Palotina - Paraná state (Area 1) (24°20'41.0"S and 53°51'36.7"W), and another in the city of Marechal Cândido Rondon - Paraná state (Area 2) (24°41'49.4" S and 54°06'44.5" W).

The experiment consisted of seven treatments, which were composed of increasing doses of glyphosate at 0; 720; 1,440; 2,160; 2,880; 3,600 and 4,320 g a.e. (grams of acid equivalent) with a randomized block design and four replications, totaling 28 plots. Both experiments were sown on September 25<sup>th</sup>, 2013 and harvested on January 15<sup>th</sup>, 2014.

The soil analysis of both towns is displayed in Table 1. The area where the experiments were conducted in Palotina had its soil classified as typical eutroferric Red Latosol (Embrapa, 2006) and the climate, according to the Köppen's classification, was CFA - mesothermal humid subtropical. In the Marechal Cândido Rondon area, the soil was characterized as Eutric Kandiuudalf (Embrapa, 2006) with CFA climate.

Treatment application was performed at the V4 phenological stage of soybean development. For the application, a CO<sub>2</sub>-pressurized backpack sprayer with 2 BAR constant pressure was used,

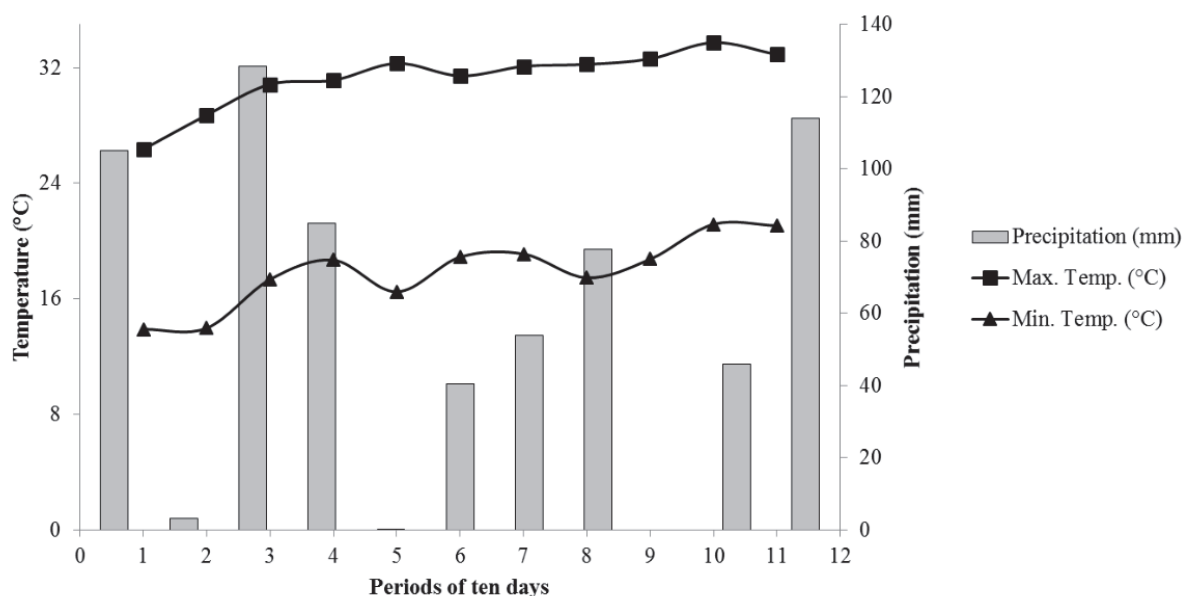
**Table 1** - Chemical analysis of soil from Palotina – Paraná state and Marechal Cândido Rondon –2013/2014 crop

Soil analysis		
Element	Palotina	Marechal Cândido Rondon
pH (CaCl <sub>2</sub> )	5.5	5.6
Aluminum saturation (%)	0	0
Base saturation (%)	58.16	76
Phosphor (Mehlich, mg dm <sup>-3</sup> )	44.27	31.61
Sulfur (mg dm <sup>-3</sup> )	5.17	7.52
H+Al (cmol <sub>c</sub> dm <sup>-3</sup> )	2.95	3.02
Calcium (cmol <sub>c</sub> dm <sup>-3</sup> )	3.14	9.44
Magnesium (cmol <sub>c</sub> dm <sup>-3</sup> )	0.8	3.09
Potassium (cmol <sub>c</sub> dm <sup>-3</sup> )	0.16	1.9
Cation exchange capacity (cmol <sub>c</sub> dm <sup>-3</sup> )	7.05	17.97

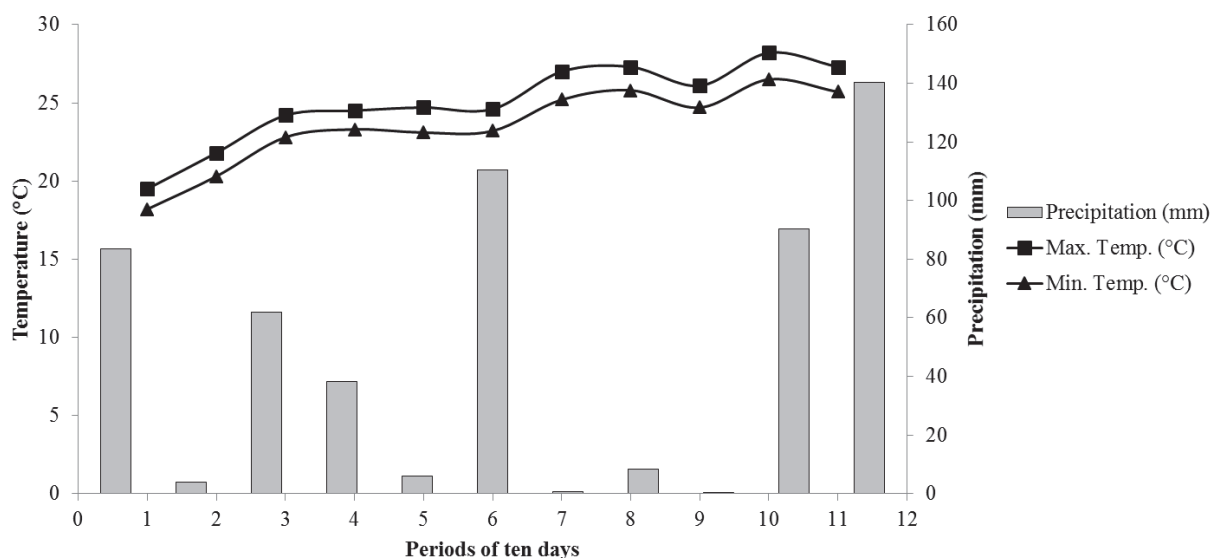
at the flow rate of 0.65 min L<sup>-1</sup>, equipped with a bar containing 6 Teejet series XR type 110.02 fan nozzles, generating a spraying volume of 200 L ha<sup>-1</sup>.

Weather conditions at the time of the application of the two experiments in the Palotina area were: average temperature of 28.6 degrees Celsius, RH%: 62.2% and wind speed of 1.4 km hour<sup>-1</sup>. In Marechal Cândido Rondon, these conditions were 27.3 degrees Celsius, RH%: 70.2% and wind speed of 2.16 km hour<sup>-1</sup>. The weather conditions at the locations are represented accordingly in Figures 1 and 2.

The following variables were analyzed: phytotoxicity at 3, 7, 14 and 21 days after application (DAA), A, B and Total chlorophyll index, total height, number of pods per plant, yield and 100 seed weight. For the phytotoxicity assessment, a percentage scale was used, from 0 to 100%, where zero (0%) represents no plant injury, and 100% total plant phytotoxicity (SBCPD, 1995). The variable chlorophyll content was measured in the third fully expanded trifoliolate at the R2 stage, counting from the apex to the base of the plant, obtaining the Falker index using the Clorofilog® device. The other evaluations were performed at the end of the crop cycle. Each plot had an area



**Figure 1** - Meteorological conditions (Maximum Temperature, Minimum Temperature (°C) and Precipitation (mm)) represented in each ten-day period in the soybean Intacta RR2 cycle in Palotina – Paraná state, 2013/2014 crop.



**Figure 2** - Meteorological conditions (Maximum Temperature, Minimum Temperature (°C) and Precipitation (mm)) represented in each ten-day period in the soybean Intacta RR2 cycle in Marechal Cândido Rondon – Paraná state, 2013/2014 crop.

of 11.25 m<sup>2</sup> and yield was assessed at the harvest, at 2 m from the two central rows of each plot; then, the moisture of the product was corrected to 13%. The used spacing was 45 cm. For each plot, 5 plants were used in the evaluations during the crop cycle.

The statistical assumptions were met according to Pimentel-Gomes and Garcia (2002), and an analysis of variance was carried out. For the experiment, the regression analysis was used ( $p < 0.01$  and  $p > 0.05$ ). The software used for the statistical analysis of data was SISVAR.

## RESULTS AND DISCUSSION

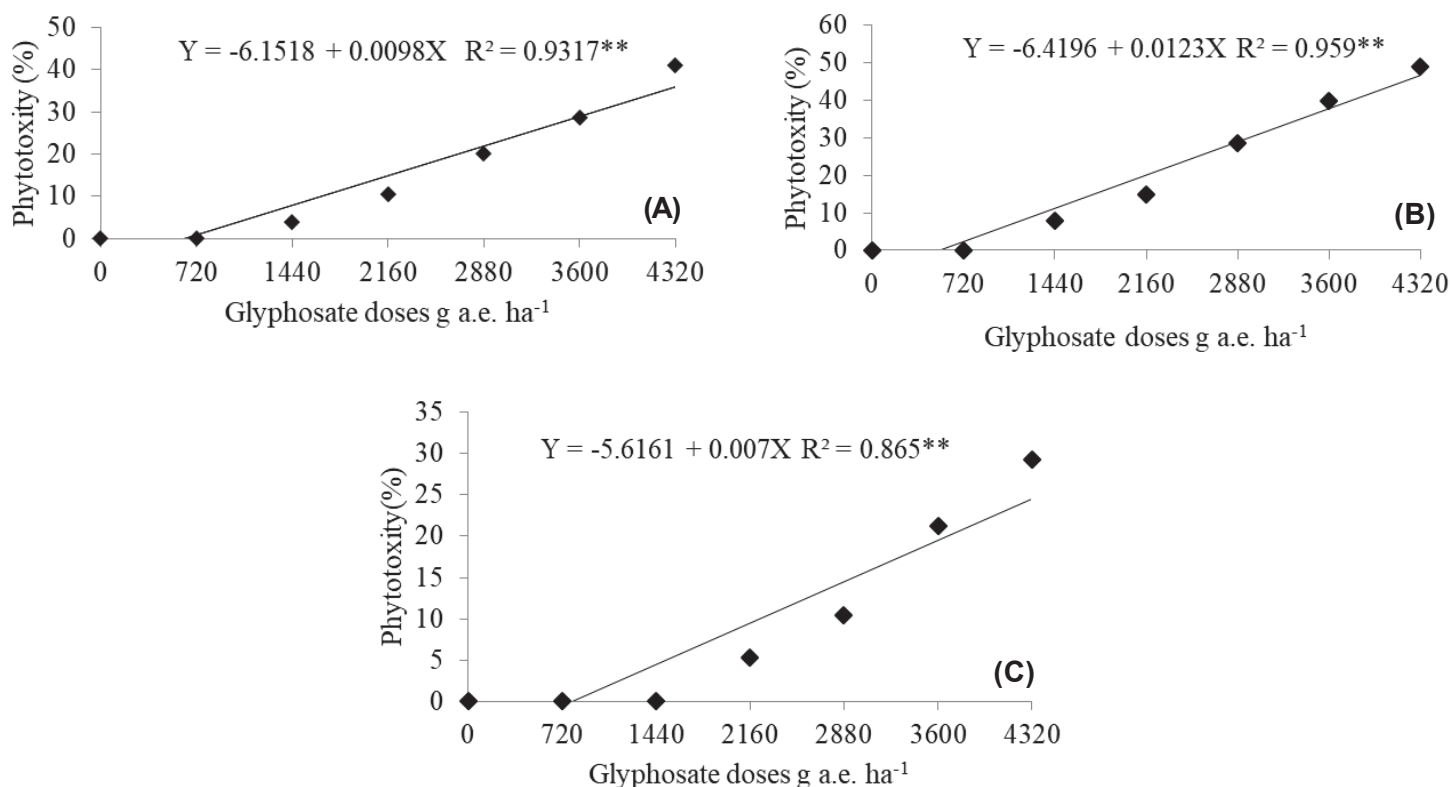
The regression analysis was performed for all variables that were adequate. In area 1, there was a linear regression for the variables: phytotoxicity at 3, 7 and 14 DAA, A-B and total chlorophyll; final height; number of pods and yield. There was no regression adjustment for the 100-seed weight in area 1 and 2, and for chlorophyll A and B only in area 2. Only the 100 seed weight corroborated the data by Bertram and Pedersen (2004), who used different doses of glyphosate at different growth stages of RR soybean, and observed that there was no weight reduction of the product, even with much higher doses than the recommended one.

### Area 1 - Palotina - PR

In Figure 3, it is possible to see that the adjustment of a linear model with increasing effect due to the increase of glyphosate doses was possible. For 14 days after the application, phytotoxicity decreased in all treatments.

For each gram of acid equivalent added at 3, 7 and 14 DAA, there was a phytotoxicity increase of 0.0098%, 0.0123% and 0.007%, respectively. According to Stefanello et al. (2011), the phytotoxicity presented by soybean might be related to the presence of inert ingredients in the commercial formulation of glyphosate, to which some soybean cultivars may be more susceptible. Krenchinski et al. (2017), using the same cultivar, observed the same phytotoxicity effects with increasing doses of glyphosate.

For A-B and Total chlorophyll (Figure 4), it was possible to adjust a linear regression model with decreasing effect, due to the increase in glyphosate doses. For A-B and total chlorophyll, there was an angular coefficient of -0.0004, -0.0003 and -0.0007, respectively, for each g a.e. ha<sup>-1</sup> of glyphosate. Zobiolo et al. (2010a) and Krenchinski et al. (2017) reported that there are significant decreases in photosynthetic parameters (chlorophyll, photosynthesis, stomatal



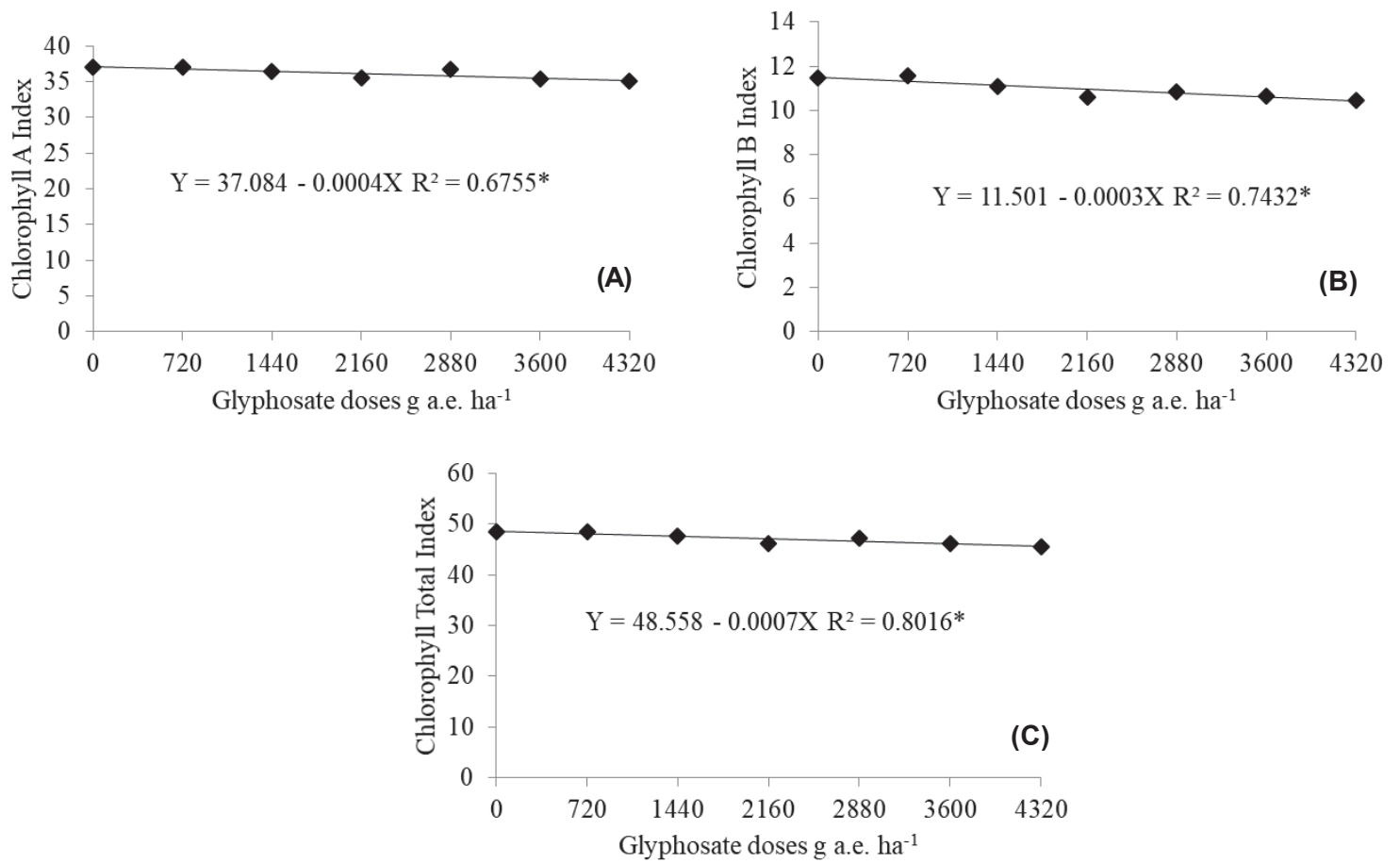
\*\* Significant at 1% probability.

**Figure 3** - Phytotoxicity 3 days (A), 7 days (B) and 14 days (C) after glyphosate application (g a.e. ha<sup>-1</sup>) at the phenological stage V4 of soybean Intacta RR2 in Palotina-Paraná state.

conductance and transpiration) when glyphosate is used in single or sequential applications. Albrecht and Ávila (2010) demonstrated that the reduction in chlorophyll levels may be caused by the degradation of glyphosate to AMPA. According to Reddy et al. (2004), an increase in glyphosate doses can increase the amount of AMPA phytotoxicity, which potentiates its effect.

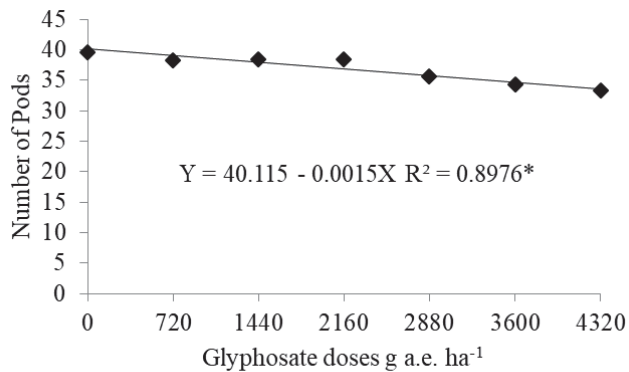
For the number of pods (Figure 5), it was also possible to adjust a linear regression model with decreasing effects, due to the increase in glyphosate doses, with an angular coefficient of -0.0015 for each g a.e. ha<sup>-1</sup> of glyphosate. The control sample was the one that obtained the highest number of pods compared to the other treatments. Zadinello et al. (2012) observed a decrease in the number of pods when the application of glyphosate was held at the R2 stage with doses around 1.44 L ha<sup>-1</sup> of glyphosate commercial product. These data corroborates Albrecht et al. (2011a), who also reported decreasing effects on the number of pods at the beginning of the reproductive development stage. However, in this study there was a decrease in the number of pods when it was applied at the growth stage V4. This effect may be related to nutritional problems (Zobiolo et al., 2010b), low water use and photosynthesis (Zobiolo et al., 2010c) due to increasing doses of glyphosate.

As for the final height variable (Figure 6), it was possible to adjust a linear regression model with decreasing effect due to the increase of glyphosate doses and with an angular coefficient of -0.0012 for each g a.e. ha<sup>-1</sup> of glyphosate, expressing the damage caused by using glyphosate at the tested doses. In studies by Albrecht et al. (2010), harmful effects on plant height after applying glyphosate were also reported. This behavior may be related to the phytotoxicity of glyphosate on the crop (Figure 3); even when applied at the V4 stage, the plant did not demonstrate to be overcoming the herbicide-induced injuries at the end of the cycle. Results were similar to those reported by Yamashita and Guimarães (2006), where two cotton cultivars were submitted to three doses of glyphosate (0, 180 and 360 g a.e. ha<sup>-1</sup>) at two stages, 4 and 10 leaves, and observed that there was a reduction of 60 and 40%, respectively, in relation to the control treatment and without the recovery of this variable at 28 DAA.



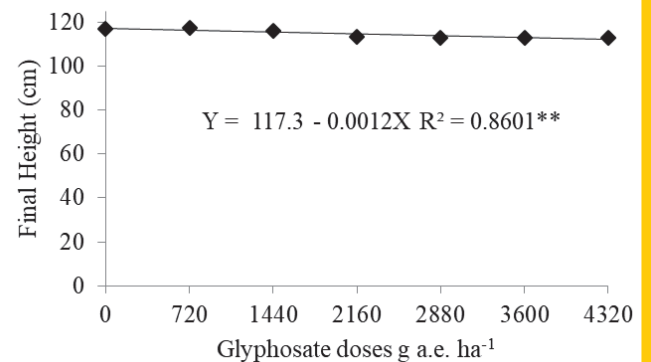
\* Significant at 5% probability.

**Figure 4** - A (A), B (B) and Total (C) Chlorophyll index at the phenological stage R2, according to increasing glyphosate doses (g a.e. ha<sup>-1</sup>) in soybean Intacta RR2. Palotina-Paraná state.



\* Significant at 5% probability.

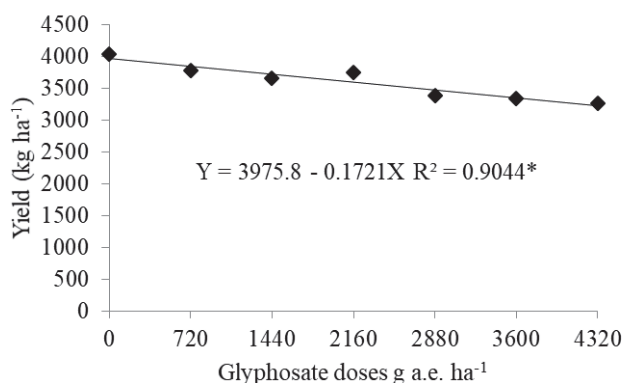
**Figure 5** - Number of pods according to increasing glyphosate doses (g a.e. ha<sup>-1</sup>) in soybean Intacta RR2, Palotina-Paraná state.



\*\* Significant at 1% probability.

**Figure 6** - Final height (cm) as according to increasing glyphosate doses (g a.e. ha<sup>-1</sup>) in soybean Intacta RR2, Palotina - Paraná state.

As for yield (Figure 7), there was also a linear regression with negative behavior for the treatments with a 0.172 kg grain yield decrease for each gram of acid equivalent, thus demonstrating the toxicity of glyphosate when applied at high concentrations. The 1,440 g a.e. dose of commercial glyphosate product (Treatment 3) also showed a reduction of around 247 kg ha<sup>-1</sup>



\* Significant at 5% probability.

**Figure 7** - Yield according to increasing glyphosate doses (g a.e. ha<sup>-1</sup>) in soybean Intacta RR2, Palotina-Paraná state.

in the yield, compared to the control treatment. Zadinello et al. (2012) report that there are possible consequences, such as direct changes in plant nutrition elements like Mn, and other nutrients like Ca, N, Mg, Fe and Cu, which can have their levels altered by the application of glyphosate. This statement also confirms Albrecht et al. (2011a), who state that plants with nutritional problems may have less biomass accumulation and may result in a lower yield. Elmore et al. (2001) found out that treatments with glyphosate doses higher than 960 g a.e. promoted a significant reduction in the final production. In this experiment, the recommended commercial dose of 1,440 g a.e. presented this yield decrease, which shows an increase in plant sensitivity with increased glyphosate doses.

## Area 2 - Marechal Cândido Rondon - PR

For phytotoxicity at 3, 7 and 14 DAA (Figure 8) in area 2, an adjustment of a linear regression model was possible with a diminishing effect, due to the increase in glyphosate doses for each g a.e. ha<sup>-1</sup> of glyphosate. At 7 DAA, there was a percentage phytotoxicity increase in RR soybean, but at 14 DAA a decrease in the injuries caused by glyphosate doses was seen. Each gram of glyphosate acid equivalent ha<sup>-1</sup> was responsible for promoting an increase in phytotoxicity (%) of 0.0092%, 0.0109% and 0.0062% at 3, 7 and 14 DAA, respectively.

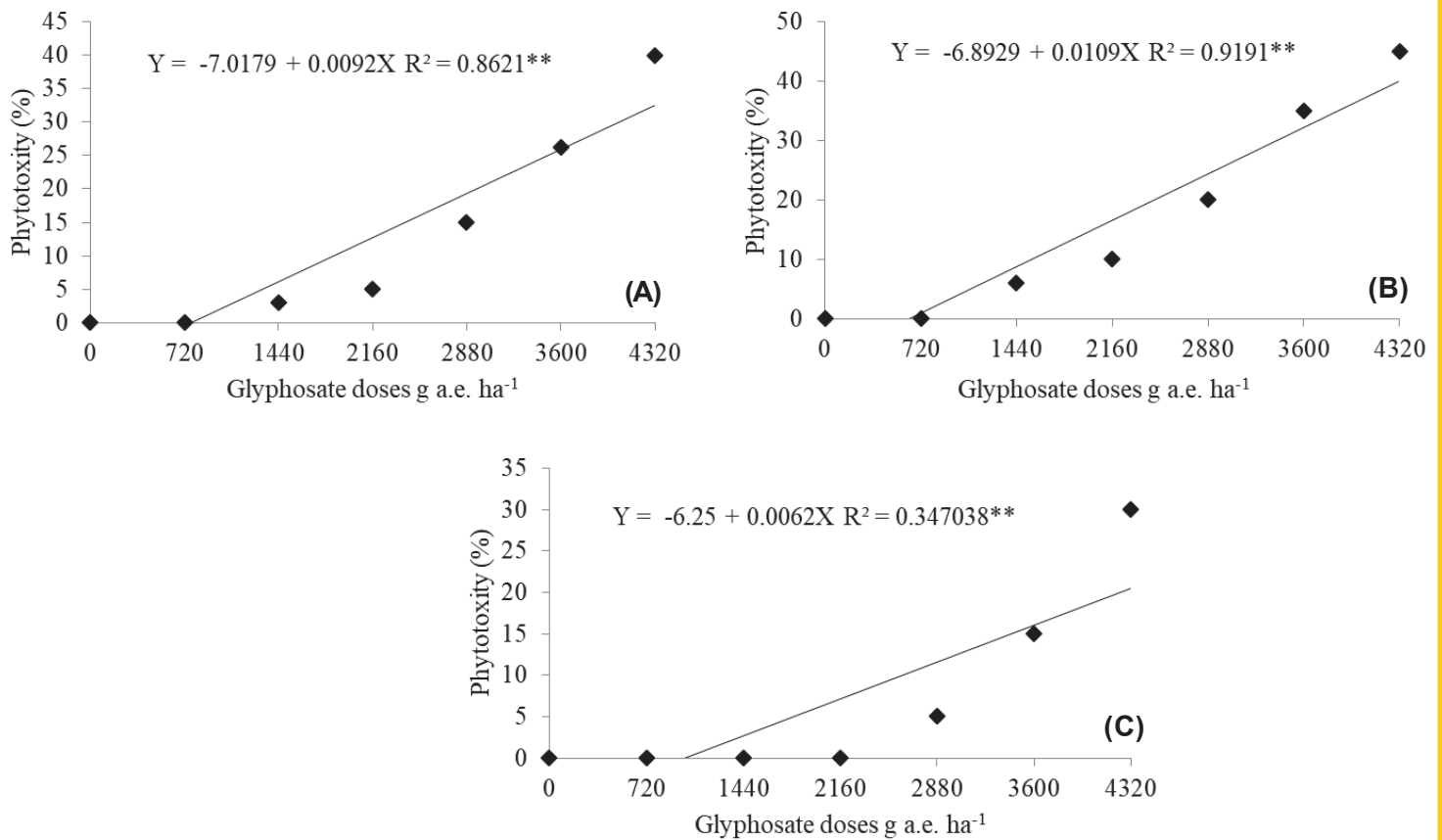
For Stefanello et al. (2011), phytotoxicity is also a problem due to the inert ingredients in glyphosate formulations, which are more susceptible in some RR soybean cultivars. Injuries appeared at 3 DAA, with the emergence of damages due to the increase in doses, and at 7 DAA a very intense chlorosis (yellowing) was seen during the vegetative stages V4-V5. However, plant recovery from the phytotoxic effect was already seen at 14 DAA, with a decrease in the percentage of phytotoxicity; at 21 DAA and 28 DAA, the injuries were no longer visible.

According to the results of the linear regression analysis on the total chlorophyll content (Figure 9), it was possible to adjust a linear regression model with decreasing effect, due to the increase of the glyphosate doses, with an angular coefficient of -0.0012 for each g a.e. ha<sup>-1</sup> of glyphosate.

In studies by Albrecht (2013), there was also a reduction in chlorophyll levels. Data from this study also corroborate the author where the relation between phytotoxicity and chlorophyll content was inversely proportional. This behavior is related to the results presented by Albrecht and Ávila (2010), saying that this can occur due to the accumulation of AMPA, which causes a reduction in chlorophyll levels and causes an increase in phytotoxicity. Zobiolo et al. (2011) also report that photosynthetic rate and chlorophyll show a decrease when there is an increase in glyphosate doses.

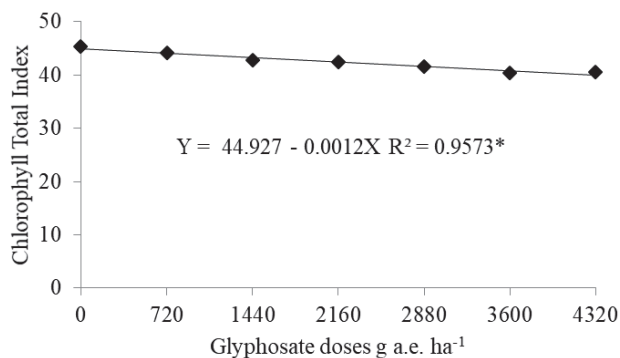
For the variable number of pods (Figure 10), it was possible to adjust a linear regression model with decreasing effect, due to the increase in glyphosate doses, with an angular coefficient of -0.001 for each g a.e. ha<sup>-1</sup>. These data corroborate studies carried out by Albrecht et al. (2011a), since there were linear regressions with negative trends for the number of pods with increasing doses of glyphosate.

As for final height (Figure 11), an adjustment was possible in the linear regression model with decreasing effect, due to the increase of the glyphosate doses with an angular coefficient of -0.002 for each g a.e. ha<sup>-1</sup>. As the injuries caused by glyphosate ("yellowing") are very characteristic features on the upper part of the plants, they may be responsible for reducing the final plant height. These data corroborate Albrecht and Ávila (2010), who point out in their studies



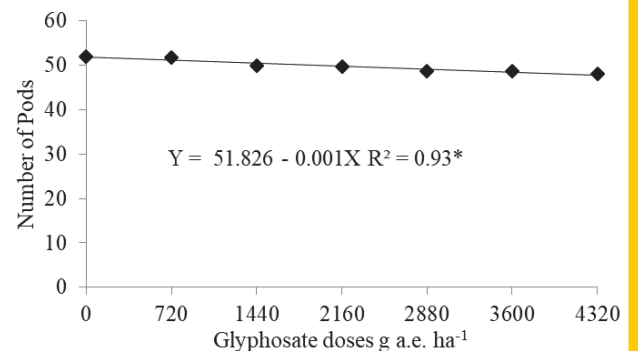
\*\* Significant at 1% probability.

**Figure 8** - Phytotoxicity 3 days (A), 7 days (B) and 14 days (C) after glyphosate application (g a.e. ha<sup>-1</sup>) at the phenological stage V4 of soybean Intacta RR2, in Marechal Cândido Rondon-Paraná state.



\* Significant at 5% probability.

**Figure 9** - Chlorophyll total index at the phenological stage R2, according to increasing glyphosate doses (g a.e. ha<sup>-1</sup>) in soybean Intacta RR2. Marechal Cândido Rondon-Paraná state.



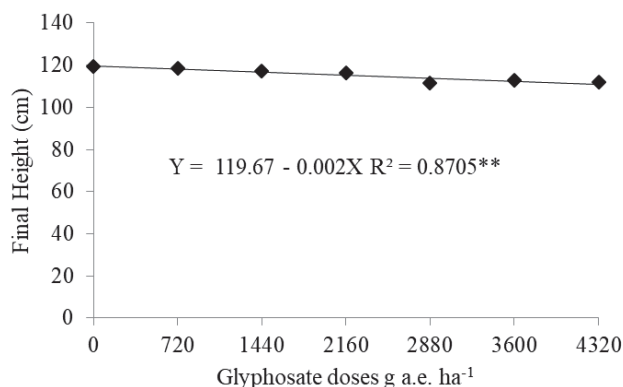
\* Significant at 5% probability.

**Figure 10** - Number of pods according to increasing glyphosate doses (g a.e. ha<sup>-1</sup>) in soybean Intacta RR2, Marechal Cândido Rondon-Paraná state.

that phytotoxicity with severe injuries can cause a decrease in plant growth. Albrecht et al. (2011a) also observed in their study a decrease in plant height.

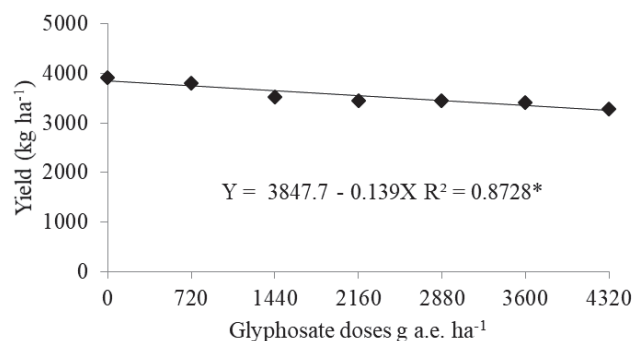
As for yield (Figure 12), there was a linear regression adjustment with a negative trend, due to the increase in doses of glyphosate and angular coefficient of -0.139. These data corroborate Albrecht (2013), who observed in his work the yield decrease with the increase in doses of





\*\* Significant at 1% probability.

**Figure 11** - Final height (cm) according to increasing glyphosate doses (g a.e. ha<sup>-1</sup>) in soybean Intacta RR2, Marechal Cândido Rondon-Paraná state.



\* Significant at 5% probability.

**Figure 12** - Yield according to increasing glyphosate doses (g a.e. ha<sup>-1</sup>) in soybean Intacta RR2, Marechal Cândido Rondon-Paraná state.

glyphosate on RR soybean, which resulted in negative linear regressions. Another possibility for this behavior on yield would be that glyphosate also changes the mutual symbiotic relationship of rhizobia with soybean (Zablotowicz and Reddy, 2004; María et al., 2006; Zobiolo et al., 2010a) that also feature the EPSPs enzyme (Zablotowicz and Reddy, 2004; Santos et al., 2004), and in the absence of this interaction, there is a reduction in nitrogen intake and, consequently, in the commitment to the production of amino acids and proteins for their productive development (Albrecht et al., 2011b). According to the results from this study and Albrecht (2014), it is possible to observe that Intacta RR2 soybean behaves in a similar manner in response to increasing levels of glyphosate; this shows its sensitivity to increasing doses of the herbicide.

Ultimately, this experiment shows that the application of glyphosate above the recommended doses at the V4 stage promotes significant phytotoxicity, may decrease the rate of photoassimilated compounds and, as a result, plants cannot recover from these injuries during their cycle, and this affects the final yield. Since it is a new technology in the market, there is a lack of information on the reaction of the varieties of Intacta RR2 soybean submitted to the use of glyphosate doses, so it should be handled with caution, because its effects can be detrimental to this technology.

The Monsoy 6210 Intacta RR2 cultivar was sensitive to glyphosate doses above 1,440 g a.e. ha<sup>-1</sup>, and, in response, deleterious effects on their development were observed, thus affecting its yield.

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