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Article

ASSOCIATIONS BETWEEN HERBICIDES AND GLYPHOSATE IN AGRONOMIC PERFORMANCE OF RR2 INTACT SOYBEAN

Associações de Herbicidas com Glyphosate no Desempenho Agronômico da Soja Intacta RR2

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ABSTRACT - The Roundup Ready (RR) soybean was developed for weed control with the use of glyphosate. Some herbicide combinations with glyphosate has been used in weed control and some effects of this practice may be undesirable in the agronomic performance of RR soybean. The present study evaluated the agronomic performance of RR2 intact soybean applied with associations between herbicides and glyphosate at post-emergence, in two experiments conducted in two municipalities in the state of Paraná, Palotina and Marechal Cândido Rondon. This experiment consisted of seven treatments in a randomized block design with four replications, totaling 28 plots for each municipality. The cultivar used in both experiments was Monsoy 6210 Intact RR2 IPRO™. The variables analyzed were: phytotoxicity at 3, 7, 14, 21 and 28 days after application, chlorophyll A, B and total chlorophyll, final height, number of pods, 100 seed weight and yield. Data were analyzed by analysis of variance and Tukey's test at 5%. There were differences between treatments for phytotoxicity in both locations and differences in yield (Palotina) and final height (Marechal Cândido Rondon). From the data it is understood that some associations of certain herbicides such as lactofen, chlorimuron-ethyl in the glyphosate can potentiating effect cause phytotoxicity and even reduction in productivity.

Keywords: *Glycine max*, susceptibility, selectivity, phytotoxicity.

RESUMO - A soja Roundup Ready (RR) foi desenvolvida para controle de plantas daninhas com a utilização do herbicida glyphosate. Algumas associações de herbicidas com o glyphosate vêm sendo usadas no controle de plantas daninhas, e alguns efeitos dessa prática podem ser indesejados no desempenho agronômico da soja RR. O presente trabalho objetivou avaliar o desempenho agronômico da soja intacta RR2 submetida a associações de herbicidas com o glyphosate, em pós-emergência. Para isso, foram realizados dois experimentos em duas localidades (Palotina – PR e Marechal Cândido Rondon – PR). Esses experimentos contiveram sete tratamentos com delineamento em blocos ao acaso com quatro repetições. O cultivar utilizado em ambas as localidades foi o Monsoy 6210 Intacta RR2 IPRO™. As variáveis analisadas foram fitotoxicidade aos 3, 7, 14, 21 e 28 dias após aplicação, índice de clorofila A, B e total, altura final, número de vagens, massa de 100 sementes e produtividade. Foi feita a análise de variância e utilizado o teste de média de Tukey a 5%. Houve diferença entre os tratamentos para fitotoxicidade em ambas as localidades e diferença na produtividade (Palotina) e altura final (Marechal Cândido Rondon). A partir dos resultados, conclui-se que algumas associações de determinados herbicidas, como chlorimuron-ethyl e lactofen, na presença do glyphosate podem provocar efeito potencializador de fitotoxicidade e até mesmo na redução na produtividade.

Palavras-chave: *Glycine max*, suscetibilidade, seletividade, fitotoxicidade.

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INTRODUCTION

Soybean *Glycine max* is a very significant crop in the Brazilian production scenario. In the state of Paraná, its yield in the 2013/2014 growing season 2013/2014 was around 2,950 kg ha^{-1} (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^{-1} (Céleres, 2013). In the Brazilian scenario, production has related on the use of transgenic soybean cultivars due to the significant benefit of this technology to weed control. However, genetically modified soybean plants may suffer injuries from the use of glyphosate, in which Zablotowicz and Reddy (2007) reports that the use of certain doses of glyphosate may affect the soybean crop development.

Glyphosate is a broad-spectrum non-selective herbicide of the group of inhibitors of the aromatic chain amino acid synthesis presenting selectivity only for genetically modified crops that have the Roundup Ready technology (RR). It is systemic absorbed by the leaves and translocated via the phloem to meristematic tissues of the plant (Galli and Montezuma, 2005).

However, with the release of transgenic soybean and increased use of glyphosate, there are some plants that presented a certain degree of tolerance to glyphosate, for example, *Commelina benghalensis*, which requires proper management for more effective control as sequential applications of glyphosate or associations with other herbicides (Ferreira Neto et al., 2009).

In no-till system, weed control requires the use of two or more herbicides to control weeds. The most common practice is to spray, at one time, total and residual action herbicides (Vidal et al., 2003).

The use of associations of herbicides in weed control, combines different action mechanisms, further increasing the action spectrum (Norris et al., 2001). According to Hydrick et al. (1994), associations between glyphosate and chlorimuron promoted similar or better weed control.

In agreement with Colby (1967), the association is considered synergistic, when the combined effect is higher than when compared to isolated applications of each herbicide; the association is additive when the combined effect is similar to isolated effects; and antagonistic when the effect of association is weaker than the effect of herbicides applied alone. Thus, the possibility of antagonism depends on the association used for specific weeds. According to Shaw and Arnold (2002), associations between glyphosate and chlorimuron and between glyphosate and cloransulam-methyl promoted antagonism to control Ip*omoea lacunosa*. In the control of *Sorghum halepense*, these associations did not present this antagonism.

An association widely used by farmers is the use of glyphosate with clethodim to control *Digitaria insularis*. (DIGIN) at the beginning of the soybean development and also in the early development of this weed. According to Gemelli et al. (2013), the use of glyphosate combined with this herbicide and other different mechanisms of action has a greater chance of control, reducing the selection pressure caused by the use of a single mechanism of action. In some cases of an association of herbicides with glyphosate can cause deleterious effects to the RR soybean, for example your association with lactofen can cause chlorosis and leaf necrosis and when associated with clhorimuron-ethyl , may cause a slight chlorosis in leaves (Correia et al., 2008). However, there is still a gap in the research of associations between herbicides in RR2 soybean, related phytotoxicity, highlighting the importance of the study.

The general aim of this study was to evaluate the agronomic performance of RR2 intact soybean applied with different associations of herbicides.

MATERIAL AND METHODS

The study was conducted in the 2013/2014 growing season with the cultivar Monsoy 6210 RR2 IPRO with associations between glyphosate and other herbicides (grass and broadleaf herbicides) with seven treatments in a randomized complete block design with four replications, totaling 28 plots (Table 1). This experiment was performed in two environments: one located in the municipality of Palotina - Paraná State (24°20'41.0" S and 53°51'36.7" W), and another in the municipality of Marechal Cândido Rondon – Paraná State (24°41'49.4" S and 54°06'44.5" W) which was sown on September 25th, 2013 and harvested on January 15th, 2014.

Table 1 - Treatments and doses used in associations between herbicides and glyphosate

Treatment	Comercial doses product ha^{-1}
Control	0
Glyphosate*	1440
Glyphosate + Fluazifop-p-buthyl**	1440 + 187.5
Glyphosate + Cletodim**	1440 + 108
Glyphosate + Cloransulam methyl**	1440 + 40
Glyphosate + Chlorimuron ethyl**	1440 + 17.5
Glyphosate + Lactofen**	1440 + 180

* Dose of glyphosate in grams of acid equivalent (g.a.e). ** Dose of herbicides in grams of active ingredient (g a.i.). Only for Chletodim it was added emulsifiable mineral oil at 0.5% of the spray solution.

The soil of the experimental area in Palotina was classified as typical Red eutroferric latosol (Embrapa, 2006) and the climate was classified as mesothermal humid subtropical (Cfa), according to Köppen. In turn, the soil in Marechal Cândido Rondon was classified as Red clay soil eutrophic (Embrapa, 2006) and the climate was classified as Cfa. The soil analysis both cities are represented on Table 2.

The application of the treatments was performed at V4 soybean growth stage of soybean development, using a CO_2 propelled backpack sprayer with constant pressure of 2 BAR (or 29 PSI), a flow rate of 0.65 L min^{-1}, equipped with bar containing 6 spray tips of Teejet series XR 110.02 to produce spray volume of 200 L ha^{-1}.

Weather conditions at the time of application in Palotina were: mean temperature of 28.6 degrees Celsius; RH of 62.2% and wind speed of 1.4 km h^{-1}. In Marechal Cândido Rondon, these conditions were 27.3 degrees Celsius, RH of 70.2% and wind speed of 2.16 km h^{-1}. Weather conditions throughout the cycle in Palotina and Marechal Cândido Rondon are shown in Figures 1 and 2, respectively.

The variables analyzed were: phytotoxicity at 3, 7, 14 and 21 days after application (DAA), chlorophyll A, B and total chlorophyll, total height, number of pods per plant, yield and 100 seed weight. For the assessment of phytotoxicity, we used a percentage scale from 0 to 100%, where zero (0%) represents no phytointoxication, and 100%, total phytointoxication of plants (SBCPD, 1995). Chlorophyll content was measured in the third fully expanded trifoliate at the R2 stage, counting from the apex to the base of the plant to obtain Falker index using the Clorofilog® device. Other evaluations were performed at the end of the crop cycle. Each plot was 11.25 m²,

Table 2 - Soil analysis for Palotina and Marechal Cândido Rondon, state of Paraná

Soil Analisys		
Element	Palotina	Marechal Cândido Rondon
pH ($CaCl_2$)	5.5	5.6
Aliminum saturation (%)	0	0
Base saturation (%)	58.16	76
Phosphor (Mehlich, mg dm^{-3})	44.27	31.61
Sulfur (mg dm^{-3})	5.17	7.52
H+Al ($cmol_c$ dm^{-3})	2.95	3.02
Calcium ($cmol_c$ dm^{-3})	3.14	9.44
Magnesium ($cmol_c$ dm^{-3})	0.8	3.09
Potassium ($cmol_c$ dm^{-3})	0.16	1.9
Cation exchange capacity ($cmol_c$ dm^{-3})	7.05	17.97

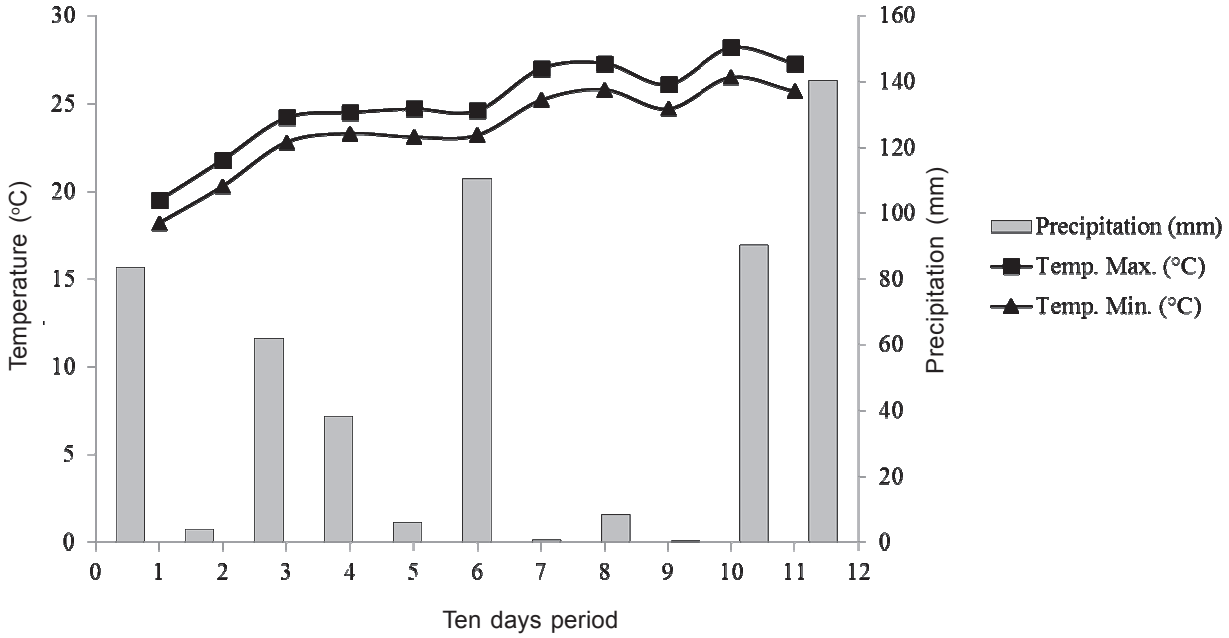

Figure 1 - Weather conditions (Maximum Temperature, Minimum Temperature (°C) and Rainfall (mm) in each ten day period in the cycle of RR2 intact soybean in Palotina, state of Paraná, 2013/2014 growing season.

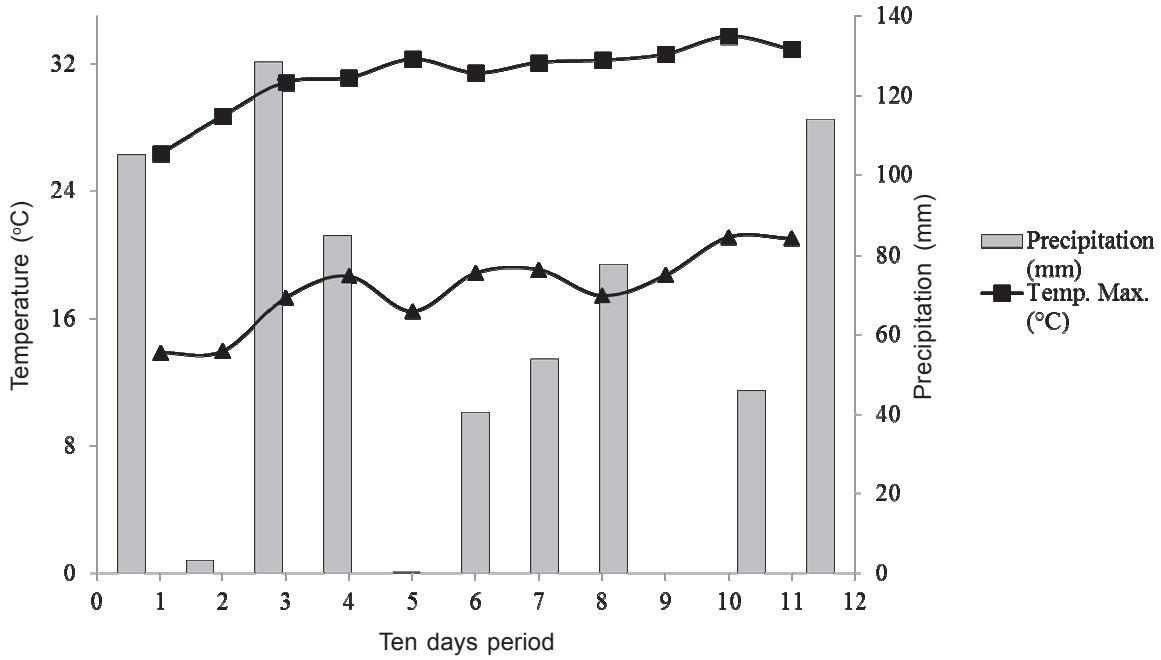

Figure 2 - Weather conditions (Maximum Temperature, Minimum Temperature (°C) and Rainfall (mm) in each ten day period in the cycle of RR2 intact soybean in Marechal Cândido Rondon, state of Paraná, 2013/2014 growing season.

yield was assessed by harvesting 2 meters of the two central rows of each plot and then the moisture of the product was corrected to 13% moisture. The row spacing used was 45 cm.

It was carried out based fertilization (250 kg ha^{-1} of NPK 0-20-20) as well as application of insecticides in the V8 stage with Methomyl, and fungicide Pyraclostrobin + Epoxiconazole the R1 stage. All plots were weeded so that there was competition between the plants. All plots were weeded manually to avoid competition with the crop, isolating this factor.

Statistical assumptions were met according to Pimentel-Gomes and Garcia (2002) and analysis of variance was run. For this experiment, we used the Tukey's test (pe^0.05). The program used for statistical analysis of the data was SISVAR.

RESULTS AND DISCUSSION

In relation to phytotoxicity, in Palotina and Marechal Cândido Rondon (Table 3), it was observed an increase on the third day after application. The treatments glyphosate + chlorimuron ethyl and glyphosate + lactofen showed the highest phytotoxicity in both locations. At 7 and 14 DAA, there were also significant differences and the same treatments exhibited the highest phytotoxicity, but at 14 DAA, there was a decrease in phytotoxicity for all the treatments in both locations. For the 21 and 28 DAA, there were no symptoms of phytotoxicity to all treatments.

Table 3 - Phytotoxicity of RR2 intact soybean subjected to managements with glyphosate at the V4 growth stage, 2013/2014 growing season, in Palotina (area 1) and Rondon-PR (area 2), state of Paraná

Palotina -% Phytotoxicity					
Treatment	3 DAA	7 DAA	14 DAA	21 DAA	28 DAA
Control	0.00 A	0.00 A	0.00 A	0.00 A	0.00 A
Gly	0.00 A	12.75 B	4.50 B	0.00 A	0.00 A
Gly + Flu	2.95 B	11.75 B	4.00 B	0.00 A	0.00 A
Gly + Cle	0.00 A	13.00 B	5.25 B	0.00 A	0.00 A
Gly + Clor	5.25 B	12.50 B	5.00 B	0.00 A	0.00 A
Gly + Chlo	10.50 C	36.00 D	19.25 C	0.00 A	0.00 A
Gly + Lact	21.25 D	28.25 C	25.75 D	0.00 A	0.00 A
Mean	5.7	16.32	9.11	0	0
VC (%)	20.81	13.72	11.15	0	0
SMD	3.34	2.37	5.23	0	0
Marechal Cândido Rondon					
Control	0.00 A	0.00 A	0.00 A	0.00 A	0.00 A
Gly	3.00 B	10.00 B	0.00 A	0.00 A	0.00 A
Gly + Flu	3.00 B	10.00 B	0.00 A	0.00 A	0.00 A
Gly + Cle	3.00 B	10.00 B	0.00 A	0.00 A	0.00 A
Gly + Clor	3.00 B	12.50 B	0.00 A	0.00 A	0.00 A
Gly + Chlo	16.25 C	37.50 D	27.50 C	0.00 A	0.00 A
Gly + Lact	20.00 D	31.25 C	10.75 B	0.00 A	0.00 A
Mean	6.90	15.9	5.46	0	0
VC (%)	13.71	10.67	20.07	0	0
SMD	2.20	3.96	2.56	0	0

* Mean values followed by different letters in the same column are significantly different at 5% probability by Tukey's test. Legend: Gly: glyphosate alone; Flu: fluazifop-p-buthyl; Cle: cletodim; Clo: cloransulam-methyl; Chlo: chlorimuron-ethyl; Lact: lactofen.

Studies by Correia et al. (2008) have shown that association between glyphosate and lactofen in the spray mixture enhances the phytotoxic compared to isolated applications. The same authors report that any type of stress imposed to the plant, such as water deficit, can potentiate the effects. This potentiation reported by Correia et al. (2008) may be because the lactofen belongs to the class of diphenyl ethers, which inhibit protoporphyrinogen oxidase (PROTOX). The lactofen acts in the formation of free radicals in the cytoplasm that are highly reactive in the presence of light and oxygen . the first moments after the application of the herbicide, these radicals cause lipid peroxidation of membranes (Becerril and Duke, 1989), compromising the structure of chlorophyll and optimizing the process of the appearance of chlorotic symptoms. For crops such as soybeans which has selectivity to this herbicide, chlorosis symptoms are common but have a recovery being that the new leaves do not exhibit these symptoms. The lactofen be a contact herbicide and can cause serious internal and external morphological changes of the sheet and

the higher the dose, these symptoms are more significant in the soybean plant (Damião Filho et al., 1992). The same author reported that the degree of sensitivity varies depending on the cultivar.

On the association between glyphosate + chlorimuron ethyl, Maciel et al. (2009) also reported a high level of intoxication after 7 DAA and also a decrease of the same after 14 DAA, indicating the potential recovery of the cultivar, which agrees with the results obtained herein in both areas. However, these authors used 10 g a.i. ha^{-1} chlorimuron-ethyl and, in this study, the dose was 17.5 g a.i. ha^{-1}.

The treatments glyphosate + fluazifop-p-buthyl, glyphosate + clethodim and glyphosate + cloransulam-methyl, in the area 1, increased the phytotoxicity up to 14 DAA. In general, symptoms of phytotoxicity caused by treatments were only observed at 3, 7, and 14 DAA.

There were no significant differences in values of chlorophyll A, B and Total chlorophyll in both areas. These results corroborate with Maciel et al. (2009), who used various associations of glyphosate with chlorimuron-ethyl and obtained no significant differences in chlorophyll.

The crop yield data (Table 4) shows that, in the area 1, treatments with glyphosate + chlorimuron-ethyl and glyphosate + lactofen did not allow the full recovery of plants to the application of these herbicides associated. Even thougth there were no significant differences, these two associations with glyphosate influenced the final yield with a reduction around 20% for both treatments.

In the area 2, there were only differences in height, evidencing the recovery of the plant without yield influence. Treatments with glyphosate + chlorimuron-ethyl and lactofen + glyphosate showed the lowest height compared to the control without herbicide. In studies by Ellis and Griffin (2003) associations containing glyphosate and chlorimuron (4.5 and 6.7 g a.i. ha^{-1}) and lactofen (112 and 168 g a.i. ha^{-1}) promoted leaves chlorosis and reduced plant height.

Table 4 - Height (cm), number of pods, 100 seed weight (g) and yield (kg ha-1) of RR2 intact soybean subjected to managements with glyphosate at the V4 growth stage, 2013/2014 growing season, in Palotina (area 1) and Rondon-PR (area 2), state of Paraná

Palotina				
Treatment	Height	Number of pods	100 seed weight	Yield
Control	113.81 A	42.65 A	12.74 A	3878.79 A
Gly	112.06 A	39.71 A	13.31 A	3714.39 A
Gly + Flu	113.87 A	43.81 A	13.45 A	3601.51 A
Gly + Cle	114.59 A	40.34 A	13.51 A	3734.09 A
Gly + Clor	113.81 A	40.40 A	13.49 A	3563.63 A
Gly + Chlo	112.06 A	39.25 A	13.47 A	3109.09 A
Gly + Lact	110.28 A	40.34 A	13.25 A	3159.09 A
Mean	112.93	41.16	13.32	3537.23
VC (%)	1.75	13.11	3.18	9.55
SMD	4.62	12.60	0.99	789.34
Marechal Cândido Rondon				
Control	121.12 A	51.18 A	12.94 A	3985.08 A
Gly	118.68 AB	48.96 A	12.60 A	3924.40 A
Gly + Flu	116.96 ABC	51.94 A	12.34 A	3960.83 A
Gly + Cle	113.87 BC	49.12 A	13.04 A	4122.55 A
Gly + Clor	117.00 ABC	49.06 A	12.99 A	4323.03 A
Gly + Chlo	111.12 C	49.84 A	13.09 A	3954.49 A
Gly + Lact	111.78 BC	55.59 A	12.94 A	3983.77 A
Mean	115.79	50.81	12.85	4036.31
VC (%)	2.57	14.48	3.55	12.11
SMD	6.95	17.20	1.065	1142.72

* Mean values followed by different letters in the same column are significantly different at 5% probability by Tukey's test. Legend: Gly: glyphosate alone; Flu: fluazifop-p-buthyl; Cle: cletodim; Clo: cloransulam-methyl; Chlo: chlorimuron-ethyl; Lact: lactofen.

The response of glyphosate + lactofen compared with glyphosate applied alone also corroborate Correia et al. (2008), given the potentiation of phytotoxic effects of lactofen when associated with glyphosate. Nevertheless, considering the harmful effects of glyphosate alone, it may lead to an increase in the potential for injuries caused in plants when combined with other herbicides (Albrecht and Ávila, 2010).

In addition, the 100 seed weight and the number of pods showed no differences in both areas where the experiments were conducted for this cultivar and under the study conditions.

According to our findings, the use of certain associations between herbicides and glyphosate can cause yield losses in RR2 intact soybean compared to glyphosate alone. In these cases, some associations may be more aggressive than others, like those associated with chlorimuron-ethyl and lactofen.

Negatively affected the agronomic performance of the cultivar Monsoy 6210 Intact RR2. Some associations may enhance the phytotoxic effect of glyphosate at post-emergence and reduce yield of the soybean Monsoy 6210 Intact RR2 IPRO. As expected, associations with grass herbicides, did not cause any type of damage when combined.

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