SCAP
SOCIEDADE DE CIÊNCIAS AGRÁRIAS DE PORTUGAL

Techniques for mitigating the symptoms of injury of glyphosate in RR and RR2 soybean

Técnicas de mitigação de sintomas de injúrias de glyphosate em soja RR e RR2

Luisa Carolina Baccin[1,*], Fábio Henrique Krenchinski[2], Leandro Paiola Albrecht[3], Gabriela Gayoso da Cruz[3], Alfredo Júnior Paiola Albrecht[3], Mateus Daupubel Mattiuzzi[4], Carine Cantú[5] e Aline Pertuzati[6]

[1] Departamento de Produção Vegetal, ESALQ/USP. Piracicaba, SP, Brazil
[2] Departamento de Produção e Melhoramento Vegetal, UNESP. Botucatu, SP, Brazil
[3] Departamento de Ciências Agronômicas, UFPR. Palotina, PR, Brazil
[4] Departamento de Agronomia, UEM. Maringá, PR, Brazil
[5] Centro de Ciências Agrarias, Unioeste. Marechal Cândido Rondon, PR, Brazil
[6] Departamento de Agronomia, Unicentro. Guarapuava, PR, Brazil
*(*E-mail: luisabaccin@usp.br)*

https://doi.org/10.19084/rca.18326
Received/recebido: 2019.03.25
Accepted/aceite: 2019.10.23

A B S T R A C T

The use of high rates of glyphosate, even if tolerant (RR) technology, can cause leaf insults, such as the yellow flashing effect, which is characterized by a yellowing of the leaves, which can lead to loss of yield. The present study aimed to increase doses of glyphosate in tolerant soybean. Tests were conducted in greenhouse 2015/2016 and 2016/2017, in Palotina, western region of Paraná in a completely randomized arrangement. The first assay was conducted in a 3x8 factorial set (products x cultivars) with four replicates mixed with glyphosate with growth regulator, one compound of amino acids and one source of manganese, and in the second a factorial scheme 6x3 (products x cultivars) using glyphosate, a growth regulator, a source of manganese and the glyphosate associated to the other products, besides a control without application. A phytotoxicity, chlorophyll index, shoot dry mass and root mass were evaluated visually. A variation of response to cultivar was observed due to characteristics of each genotype. The differences between the products were not observed. A cultivar 'M6210' presented greater symptom of crop injury while cultivating 'BMX Ponta', presenting greater tolerance. No phytotoxic effect was observed for the test.

Keywords: *Glycine max* (L.) Merr., herbicides; yellow flashing, transgenic crops.

R E S U M O

A utilização de altas doses de glyphosate, mesmo na tecnologia tolerante (RR) pode causar injúrias nas plantas, conhecido como efeito "Yellow flashing", que é caracterizado por um amarelecimento das folhas apicais, podendo levar à perda de produtividade. O presente estudo objetivou avaliar técnicas visando a atenuação do efeito de doses de glyphosate em cultivares tolerantes de soja (RR e RR2). Os ensaios foram conduzidos em estufa nos anos 2015/2016 e 2016/2017, em Palotina, região oeste do Paraná em um arranjo inteiramente casualizado. O primeiro ensaio foi conduzido em arranjo fatorial 3x8 (produtos x cultivares) com quatro repetições utilizando misturas de glyphosate com regulador de crescimento, um composto de aminoácidos e uma fonte de manganês, e no segundo um esquema fatorial 6x3 (produtos x cultivares) utilizando glyphosate, um regulador de crescimento, uma fonte de manganês isolados e o glyphosate associado aos demais produtos, além de um tratamento controle sem aplicação. Foram avaliados visualmente a fitotoxicidade, índice de clorofila, massa seca de parte aérea e de raiz. Observou-se uma diferença de resposta para as cultivares devido a características de cada genótipo. Não foram observadas diferenças entre os produtos. A cultivar 'M6210' apresentou-se maior sintoma de fitointoxicação enquanto a cultivar 'BMX Ponta' apresentou maior tolerância. Para o segundo ensaio não foi observado efeito fitotóxico.

Palavras-chave: *Glycine max* (L.) Merr., herbicidas, *yellow flashing*, transgénicos.

INTRODUCTION

The Roundup Ready® (RR) technology introduced genes (*cp4epsps*) from *Agrobacterium* sp. to soybean plants, which codes enzyme EPSP synthase with a high catalytic activity in the presence of glyphosate and maintains aromatic amino acid levels in tolerant plants (Reddy, 2001).

Previous studies have shown that the effects of glyphosate application to RR soybean causes a reduction of chlorophyll content and increased phytotoxicity, possibly because of AMPA (aminomethylphosphonic acid) accumulation, which is the first phytotoxic metabolite of glyphosate. Some cultivars of RR soybean exhibit small visible injuries while other cultivars present more pronounced symptoms.

Lower volume of plant biomass, less nodulation and, consequently, reduced biological nitrogen fixation, as well as reduced uptake levels of macro- and micro-nutrients, in addition to low yields and low seeds quality are common effects (Zobiole *et al*., 2009a,b; Albrecht *et al*., 2011, 2014a,b; Krenchinski *et al.*, 2017).

With the increasing utilization of glyphosate-resistant technology, farmers have noticed that some RR cultivars show visible injuries after post-emergence application of the herbicide. A typical symptom that can be seen in the field is called "yellow flashing", which consists of yellowing of the upper leaves of the plant (Zobiole *et al*., 2011).

Glyphosate application is performed at various crop stages, depending on the level of weed infestation, but Albrecht *et al.* (2014a) demonstrated that glyphosate application at the R1 stage causes a reduction of plants height and an increase of phytotoxicity. Reddy and Zablotowicz (2003) observed that application of glyphosate reduced nodule mass.

Studies conducted with herbicides indicate that an exogenous application of amino acids may be a tool to reduce inhibition of plants growth. In the case of glyphosate, which inhibits enzyme 5-enol–pyruvyl-3-shikimate-phosphate (EPSP) synthase, some studies indicate that exogenous applications of amino acid mixtures succeeded in preventing growth inhibition (Zobiole *et al.*, 2010, 2011).

The use of bioregulators may assist the plant in the recovery from these undesirable effects, as a form to increase crop growth and yields. Substances analogous to plant hormones, called plant regulators or bioregulators, have been largely applied in several crops, and studies have reported its effectiveness in soybean crops by improving the plants agronomic performance and seeds production components (Albrecht *et al.,* 2011; Zobiole *et al.,* 2011).

The use of manganese has also been studied to prevent damages caused by the herbicide due to the reduced chlorophyll content in the plants. This occurs in response to a manganese-induced deficiency after application of glyphosate caused by a low efficiency of nutrient accumulation due to the action of the herbicide in the same metabolic pathway. It was observed that even with the application of low doses of glyphosate the absorption and the translocation of manganese in the plants was reduced (Rosolem *et al.,* 2010; Zobiole *et al.*, 2010).

Regarding the mitigation of glyphosate application symptoms in RR soybean, supplementation provided to the plant, with application of products associated with the herbicide is a technically viable option for the producer, so the aim of this study was to assess the efficiency of products used for reversal of phytotoxicity caused by glyphosate herbicides in Roundup Ready® soybean cultivars.

MATERIALS AND METHODS

Plant material and growing conditions

Two experiments were carried out from November 2015 to January 2016 (experiment I) and March to May 2017 (experiment II). The experiments were conducted in a greenhouse under controlled ambient conditions, temperature between 20-25ºC, 60% of mean relative humidity, 5 mm/day of mean precipitation, and a photoperiod of nearly 12 hours, in the municipality of Palotina, in the western of state of Paraná, Brazil. Both experiments were carried out in a controlled ambient condition free of pests and diseases.

From the literature review, the phytotoxic effect of the application of high doses of glyphosate on RR and RR2 soybean was observed, causing productivity reduction. An initial screening was carried out to identify cultivars that presented greater and lesser injuries.

For the experiment I, eight RR soybean cultivars were used: 'TMG 7062', 'MONSOY 6210', 'BMX Ponta', 'CD 2720' and RR2: 'TMG 7262', 'BRS 359', 'BRS 388' and 'CD 2737'. The cultivars 'TMG 7062' and 'TMG 7262' have a semi-determined growth habit, while the other cultivars used in the experiment have an undetermined growth habit.

For the second experiment, the cultivars 'MONSOY 6210', 'TMG 7262' and 'BMX Ponta' were utilized. These cultivars present an undetermined growth habit and the effects of manganese and bioregulator on the reversal of glyphosate phytotoxicity were evaluated.

In both experiments, 5 L plastic pots filled with eutrophic Red Latosol were used and two plants were sown per pot.

Experimental design

Experiment 1 was conducted on a 3x8 factorial arrangement (products x cultivars) with four replicates, each pot containing two plants which were considered one replicate, totalizing 96 pots.

The second experiment was conducted on a 3x6 factorial arrangement and six treatments, containing four replicates, and each pot with two plants was considered a replicate, totalizing 72 pots, both in a completely randomized design (CRD) with factorial arrangement.

Treatments

In the first experiment the treatments consisted of combinations of Roundup Ready® glyphosate (2880 g a.e. ha^{-1}) with plant growth regulator (Stimulate®) (250 mL ha^{-1}), with chelated manganese (125g ha^{-1}) and with amino acids compound (Protemax®) (1 L ha^{-1}). The products and doses are described in Table 1.

The treatments of the second experiment consisted of one untreated control, Roundup Ready glyphosate (2880 g a.e. ha^{-1}), Stimulate bioregulator (250 mL ha^{-1}), and manganese (184.8 g ha^{-1}) alone and combined with the Stimulate herbicide and with manganese. The treatments and doses used are described in Table 2.

Table 1 - Products used in the management of the reversal effects of glyphosate in soybean. Experiment I, 2015/2016

Product	Rate
Glyphosate (Roundup Ready®) + Aminoacid (Protemax®)	2880 g a.e. ha^{-1} + 1 L ha^{-1}
Glyphosate (Roundup Ready®) + Manganese (Biometal®)	2880 g a.e. ha^{-1} + 1 L ha^{-1}
Glyphosate (Roundup Ready®) + Growth regulator (Stimulate®)	2880 g a.e. ha^{-1} + 0,25 L ha^{-1}

Table 2 - Products used in the management of the reversal effects of glyphosate in soybean. Experiment II, 2017

Treatments	Rate (g a.e. or L ha^{-1})
Nontreated	--
Glyphosate (Roundup Ready®)	2880 g a.e. ha^{-1}
Growth regulator (Stimulate®)	0,25 L ha^{-1}
Manganese (Platinum®)	2 L ha^{-1}
Glyphosate (Roundup Ready®) + Growth regulator (Stimulate®)	2880 g a.e. ha^{-1} + 0,25 L ha^{-1}
Glyphosate (Roundup Ready®) + Manganese (Platinum®)	2880 g a.e. ha^{-1} + 2 L ha^{-1}

Herbicide sprayed conditions

In both experiments, application was conducted at the V4 growth stage using a CO_2 pressurized backpack sprayer, at constant pressure, providing 150 L ha^{-1} of fluid volume.

Phytotoxicity rates

A score for visible phytotoxicity was given at 7, 14, 21, 28 and 35 days after application (DAA), ranging from 0 to 100%, where score zero is attributed to asymptomatic plants and 100% represents plant death from herbicide effect. The scores for visible damage were assessed according to the scoring proposed by the Brazilian Society of Weed Science (SBCPD, 1995) and chlorophyll content was also measured with the aid of a chlorophyll meter at 7, 14, 21, 28 and 35 DAA.

At 35 DAA, the plants were removed from the pots and the roots were separated from the shoots. Measurements of shoot dry matter were performed. The roots were washed and the nodules were carefully removed. The number and weight of nodules were also assessed as well as the root dry matter.

For the second experiment, visible phytotoxicity damages were assessed with scores at 3, 7, 14 DAA, also following the scores scale proposed by the Brazilian Society of Weed Science (SBCPD, 1995) ranging from 0 to 100%, where score zero is attributed to asymptomatic plants and 100% represents plant death from herbicide effect.

The chlorophyll content was measured at 3, 7, and 14 DAA, and at 14 DAA one plant was removed from each pot for measurement of shoot fresh matter and shoot dry matter.

Statistical analysis

The data were submitted to analysis of variance (ANOVA), the necessary splits were performed in the factorial interaction, and the means tested by Tukey at 5% probability ($p \leq 0.05$) and, when necessary, the data transformations were performed (Ferreira, 2011).

RESULTS

It was observed in two experiments that there was a significant interaction and the factors were dependent. There was a difference among the products due to the characteristics of each cultivar.

Experiment I

Visible phytotoxicity symptoms were more pronounced at 7 and 14 days after application (DAA) (Table 3). At 7 DAA, both the growth regulator and amino acid showed varying effects on the studied cultivars. For the 'BRS 359' cultivar, the amino acid had a higher effect between both products, presenting a lower score of visible phytotoxicity. At 21 and 28 DAA, there was no difference between the products for each cultivar studied, but there was a distinct response for the cultivars (Table 4).

At 35 DAA (Table 5) the visible injuries caused by application of the herbicide decreased considerably, and only cultivar 'Monsoy 6210' exhibited a slight mild toxicity symptom when treated with bioregulator and amino acid, showing in this case the manganese efficiency.

With respect to shoot dry matter, the association with manganese was more effective for cultivars 'BMX Ponta' and 'BRS 388', where the amino acid was more effective for cultivars 'BRS 388' and 'CD 2737', in which a positive effect of Stimulate® was observed (Table 6).

Considering the number of nodules (Table 7), 'BMX Ponta' and 'Monsoy 6210' showed a greater number of nodules when treated with manganese. Regarding the nodules weight, no difference was found for the different product combinations with glyphosate.

At 7 DAA, a decrease in chlorophyll content was observed, but there was a recovery by 35 DAA. Cultivar 'M6210' showed a better response to the association of glyphosate with amino acid, but cultivar 'BMX Ponta' exhibited a different behavior: at 28 DAA, this cultivar was more responsive to the application of glyphosate associated with manganese (Figure 1).

Table 3 - Visual note of phytotoxicity at 7 and 14 days after application (DAA) in soybean submitted to application of glyphosate and technologies in the management of phytotoxicity reversal. Experiment I, 2015/2016

Cultivar	Phytointoxication 7 DAA			Phytointoxication 14 DAA		
	Gly + S[1]	Gly + Mn[2]	Gly + Aa[3]	Gly + S[1]	Gly + Mn[2]	Gly + Aa[3]
TMG 7062	14.50 Aab	18.75 Aa	15.50 Aab	15.25 Aab	15.25 Aab	16.25 Aa
M6210	21.25 Ab	19.50 Aa	22.00 Ab	18.75 Ab	18.25 Ab	19.50 Aa
BMX Ponta	14.00 Aab	17.50 Aa	14.00 Aa	11.50 Aa	13.50 Aab	14.25 Aa
CD2720	15.25 Aab	14.50 Aa	15.25 Aab	16.00 Aab	15.00 Aab	14.50 Aa
TMG7262	12.25 Aa	13.75 Aa	11.00 Aa	10.50 Aa	11.75 Aa	14.25 Aa
BRS359	14.00 ABab	18.75 Ba	13.00 Aa	15.50 Aab	17.50 Ab	14.75 Aa
BRS388	15.25 Aab	18.50 Aa	14.25 Aa	17.25 Ab	15.00 Aab	14.25 Aa
CD2737	12.50 Aa	15.50 Aa	13.75 Aa	16.00 Aab	18.75 Ab	16.00 Aa
Mean		15.6			15.39	
CV%		21.41			16.81	

Means followed by capital letters in the row and lowercase letters in the column did not differ significantly ($p < 0.05$) by the Tukey test. Gly + S: Glyphosate (2880 g a.e. ha^{-1}) + Stimulate® (250 ml ha^{-1}); [2]Gly + Mn: Glyphosate (2880 g a.e. ha^{-1}) + Manganese Biometal® (1L ha^{-1}); [3]Gly + Aa: Glyphosate (2880 g a.e. ha^{-1}) + Protemax® (1L ha^{-1}).

Table 4 - Visual note of phytotoxicity at 21 and 28 days after application (DAA) in soybean submitted to application of glyphosate and technologies in the management of phytotoxicity reversal. Experiment I, Palotina – PR, 2015/2016

Cultivar	Phytointoxication 21 DAA			Phytointoxication 28 DAA*		
	Gly + S[1]	Gly + Mn[2]	Gly + Aa[3]	Gly + S[1]	Gly + Mn[2]	Gly + Aa[3]
TMG 7062	12.75 Aab	12.75 Aab	14,00 Aab	4,00 Aa	4,75 Aabc	4,00 Aa
M6210	17.25 Ab	16.25 Ab	17,75 Ab	9,50 Ab	8,20 Aac	11,20 Ab
BMX Ponta	9.00 Aa	11.50 Aab	12,00 Aab	4,75 Aa	3,50 Aab	5,75 Aa
CD2720	13.50 Aab	12.00 Aab	11,75 Aab	2,50 Aa	4,75 Aabc	3,25 Aa
TMG7262	8.00 Aa	9.50 Aa	12,00 Aab	1,75 Aa	2,00 Aa	4,75 Aa
BRS359	12.75 Aab	15.00 Aab	12,25 Aab	4,00 Aa	6,25 Aabc	4,00 Aa
BRS388	15.25 Ab	12.50 Aab	11,00 Aa	5,75 Aab	3,75 Aab	1,75 Aa
CD2737	13.50 Aab	16.75 Ab	13,75 Aab	3,75 Aa	7,25 Aab	5,25 Aa
Mean		13.03			4.85	
CV%		21.5			18.44*	

Means followed by capital letters in the row and lowercase letters in the column did not differ significantly ($p < 0.05$) by the Tukey test. Gly + S: Glyphosate (2880 g a.e. ha^{-1}) + Stimulate® (250 ml ha^{-1}); [2]Gly + Mn: Glyphosate (2880 g a.e. ha^{-1}) + Manganese Biometal® (1L ha^{-1}); [3]Gly + Aa: Glyphosate (2880 g a.e. ha^{-1}) + Protemax® (1L ha^{-1}). * Data transformation: Square root of Y + 1.0 - SQRT (Y + 1.0).

Table 5 - Visual note of phytotoxicity at 35 days after application (DAA) in RR and RR2 soybean submitted to application of glyphosate and technologies in the management of phytotoxicity reversal. Experiment I, 2015/2016

Cultivar	Phytointoxication 35 DAA*		
	Gly + S[1]	Gly + Mn[2]	Gly + Aa[3]
TMG 7062	1.25 Aa	1,25 Aa	1,50 Aa
M6210	5.50 Bb	2,50 Aa	4,75 Bb
BMX Ponta	2.00 Aa	1,75 Aa	1,75 Aa
CD2720	0.00 Aa	0,50 Aa	0,75 Aa
TMG7262	0.25 Aa	0,50 Aa	1,00 Aa
BRS359	2.00 Aa	1,75 Aa	1,50 Aa
BRS388	2.50 Aa	1,50 Aa	0,50 Aa
CD2737	1.25 Aa	2,50 Aa	0,50 Aa
Mean		1.63	
CV%		24.49*	

Means followed by capital letters in the row and lowercase letters in the column did not differ significantly ($p < 0.05$) by the Tukey test. Gly + S: Glyphosate (2880 g a.e. ha^{-1}) + Stimulate® (250 ml ha^{-1}); [2]Gly + Mn: Glyphosate (2880 g a.e. ha^{-1}) + Manganese Biometal® (1L ha^{-1}); [3]Gly + Aa: Glyphosate (2880 g a.e. ha^{-1}) + Protemax® (1L ha^{-1}). * Data transformation: Square root of Y + 1.0 - SQRT (Y + 1.0).

Table 6 - Dry matter (g) per plant of aerial part (AP) and root (R) soybean submitted to the application of glyphosate and technologies in the management of phytotoxicity reversal. Experiment I, 2015/2016

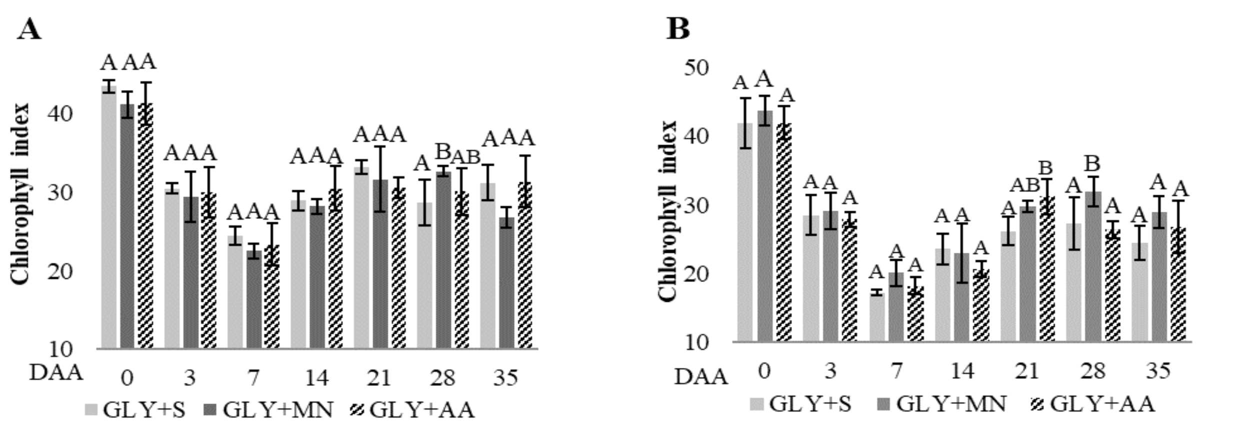
Cultivar	Dry matter AP			Dry matter R*		
	Gly + S[1]	Gly + Mn[2]	Gly + Aa[3]	Gly + S[1]	Gly + Mn[2]	Gly + Aa[3]
TMG 7062	19.0 Ab	14.62 Aab	20.25 Ac	3.71Aab	2.20 Aa	1.88 Aab
M6210	9.5 Aa	13.12 Aab	11.87 Aab	1.27 Aa	2.35 Aa	0.96 Aa
BMX Ponta	15.5 ABab	19.25 Bb	13.25 Aabc	2.62 Aab	2.14 Aa	0.98 Aa
CD2720	16.75 Aab	16.5 Aab	15.5 Aabc	3.36 Aab	2.28 Aa	3.15 Aab
TMG7262	13.25 Aab	15.5 Aab	11.0 Aa	1.26 Aa	2.77 Aa	1.09 Aa
BRS359	18.0 Ab	16.25 Aab	18.5 Abc	4.60 Bb	1.79 Aa	3.08 ABab
BRS388	12.75 Aab	20.25 Bb	18.62 Bbc	1.33 Aa	2.88 Aba	4.45 Bb
CD2737	17.5 Bb	10.75 Aa	16.5 Babc	3.17 Bab	1.01 Aa	2.39 ABab
Mean	15.58			2.36		
CV%	21.54			17.83*		

Means followed by capital letters in the row and lowercase letters in the column did not differ significantly ($p < 0.05$) by the Tukey test. Gly + S: Glyphosate (2880 g a.e. ha^{-1}) + Stimulate® (250 ml ha^{-1}); [2]Gly + Mn: Glyphosate (2880 g a.e. ha^{-1}) + Manganese Biometal® (1L ha^{-1}); [3]Gly + Aa: Glyphosate (2880 g a.e. ha^{-1}) + Protemax® (1L ha^{-1}). * Data transformation: Square root of Y + 1.0 - SQRT (Y + 1.0).

Table 7 - Number and weight (g) of nodules in RR and RR2 soybean submitted to the application of glyphosate and technologies in the management of phytotoxicity reversal. Experiment I, 2015/2016

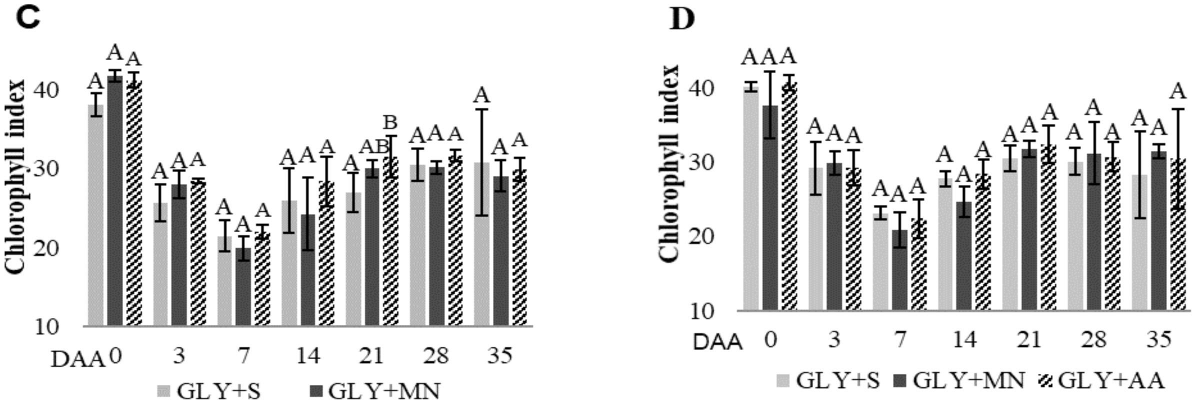
Cultivar	Number of nodules*			Weigh of nodules (g) [NS*]		
	Gly + S[1]	Gly + Mn[2]	Gly + Aa[3]	Gly + S[1]	Gly + Mn[2]	Gly + Aa[3]
TMG 7062	202.25 Aa	144.00 Aa	186.25 Aa	2.37	1.51	2.03
M6210	256.00 Aa	419.00 Bb	219.50 Aa	2.34	2.81	2.25
BMX Ponta	220.25 ABa	287.75 Bab	134.50 Aa	2.72	3.09	1.94
CD2720	204.00 Aa	157.25 Aa	139.25 Aa	2.96	2.11	2.27
TMG7262	135.00 Aa	181.00 Aa	151.50 Aa	1.74	2.35	2.23
BRS359	238.00 Aa	207.00 Aa	271.00 Aa	2.67	1.43	2.95
BRS388	194.50 Aa	247.50 Aab	265.25 Aa	2.43	2.52	2.92
CD2737	226.00 Aa	181.25 Aa	157.00 Aa	2.10	1.50	1.66
Mean	209.38			2.92		
CV%	21.99*			13.60*		

Means followed by capital letters in the row and lowercase letters in the column did not differ significantly ($p < 0.05$) by the Tukey test. Gly + S: Glyphosate (2880 g a.e. ha^{-1}) + Stimulate® (250 ml ha^{-1}); [2]Gly + Mn: Glyphosate (2880 g a.e. ha^{-1}) + Manganese Biometal® (1L ha^{-1}); [3]Gly + Aa: Glyphosate (2880 g a.e. ha^{-1}) + Protemax® (1L ha^{-1}). * Data transformation: Square root of Y + 1.0 - SQRT (Y + 1.0). [NS] Not significant.



Gly + S: Glyphosate (2880 g a.e. ha^{-1}) + Stimulate® (250 ml ha^{-1}); Gly + MN: Glyphosate (2880 g a.e. ha^{-1}) + Manganese Biometal® (1L ha^{-1}); Gly + AA: Glyphosate (2880 g a.e. ha^{-1}) + Protemax® (1L ha^{-1}).

Figure 1 - Chlorophyll index of the cultivars: BMX Ponta (A), MONSOY 6210 (B), submitted to glyphosate and phytotoxicity reversal technology. Experiment 1, 2015/2016.



Gly + S: Glyphosate (2880 g a.e. ha^{-1}) + Stimulate® (250 ml ha^{-1}); Gly + MN: Glyphosate (2880 g a.e. ha^{-1}) + Manganese Biometal® (1L ha^{-1}); Gly + AA: Glyphosate (2880 g a.e. ha^{-1}) + Protemax® (1L ha^{-1}).

Figure 2 - Chlorophyll index of the cultivars: BRS 388 (C) E CD 2737 (D), submitted to glyphosate and phytotoxicity reversal technology. Experiment I, 2015/2016.

Cultivars 'TMG 7262', 'BRS 359' and 'CD 2737' did not show differences with application of the products. For 'BRS 388' (Figure 2), it was found, at 21 DAA, that glyphosate application combined with amino acid provided a higher chlorophyll content, demonstrating the product efficiency.

Experiment II

In the second experiment, cultivar 'BMX Ponta' exhibited more tolerance to the application of the herbicide and 'Monsoy 6210' was more susceptible to phytotoxicity. For 'TMG 7262' (Figure 3), the application of reversing products resulted in higher

Table 8 - Dry mass (g) of aerial part (AP) of soybean submitted to glyphosate and phytotoxicity reversal technology. Experiment II, Palotina – PR, 2017

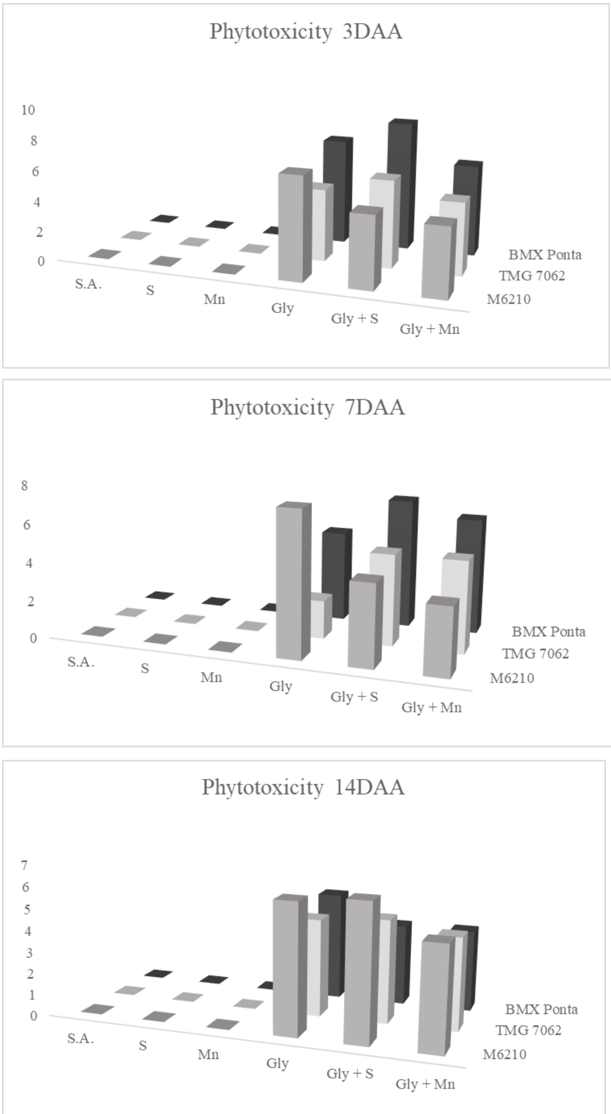
Treatment	Dry mass AP		
	M6210	TMG 7062	BMX Ponta
S.A.[1]	1.57 Aa	2.31 Aa	2.11 Aa
S[2]	1.42 Aa	2.08 Aa	2.28 Aa
Mn[3]	1.34 Aa	2.45 Ab	2.31 Ab
Gly[4]	1.22 Aa	2.70 Ab	2.48 Ab
Gly + S[5]	1.22 Aa	1.69 Aa	2.06 Aa
Gly + Mn[6]	1.37 Aa	1.75 Aab	2.31 Ab
Mean		1.94	
CV%		27.75	

Means followed by capital letters in the row and lowercase letters in the column did not differ significantly ($p < 0.05$) by the Tukey test. [1]S.A.: Nontreated; [2]S: Stimulate® (250 ml ha^{-1}); Mn: [3]Platinum Manganese® (2L ha^{-1}); [4]Gly : Glyphosate (2880 g a.e. ha^{-1}); [5]Gly+S: Glyphosate (2880 g a.e. ha^{-1}) + Stimulate® (250 ml ha^{-1}); [6]Gly + Mn: Glyphosate (2880 g a.e. ha^{-1}) + Platinum Manganese® (2L ha^{-1}).

Table 9 - Chlorophyll index in the day of application and 3, 7 and 14 days after application (DAA) in soybean submitted to glyphosate and phytotoxicity reversal technology. Experiment II, 2017

Treatment	0 DAA[NS]			3 DAA[NS]			7 DAA[NS]			14 DAA [NS]		
	M6210	TMG 7062	BMX Ponta	M6210	TMG 7062	BMX Ponta	M6210	TMG 7062	BMX Ponta	M6210	TMG 7062	BMX Ponta
S.A.[1]	32.08	33.32	32.25	31.30	32.32	33.81	31.24	33.31	35.56	31.22	32.21	31.75
S[2]	29.81	33.17	32.72	31.33	31.92	33.21	30.20	31.76	32.89	30.86	30.53	33.48
Mn[3]	31.43	32.42	34.15	29.85	33.06	33.33	30.75	32.17	33.46	30.08	29.28	34.32
Gly[4]	34.82	34.82	33.28	32.11	33.25	33.93	31.24	33.31	31.94	30.60	30.91	31.66
Gly + S[5]	32.92	32.92	32.82	29.00	31.26	32.10	29.67	31.39	33.55	30.00	27.77	31.76
Gly + Mn[6]	32.72	32.72	32.25	32.63	32.80	32.33	31.64	32.75	33.59	29.81	28.97	30.86
Mean		32.46			32.2			32.26			30.89	
CV%		5.75			7.35			5.86			8.46	

[1]S.A.: Nontreated; [2]S: Stimulate® (250 ml ha^{-1}); Mn: [3]Platinum Manganese® (2L ha^{-1}); [4]Gly : Glyphosate (2880 g a.e. ha^{-1}); [5]Gly+S: Glyphosate (2880 g a.e. ha^{-1}) + Stimulate® (250 ml ha^{-1}); [6]Gly + Mn: Glyphosate (2880 g a.e. ha^{-1}) + Platinum Manganese® (2L ha^{-1}). [NS] Not significant.



[1]S.A.: Nontreated; [2]S: Stimulate® (250 ml ha^{-1}); Mn: [3]Platinum Manganese® (2L ha^{-1}); [4]Gly : Glyphosate (2880 g a.e. ha^{-1}); [5]Gly+S: Glyphosate (2880 g a.e. ha^{-1}) + Stimulate® (250 ml ha^{-1}); [6]Gly + Mn: Glyphosate (2880 g a.e. ha^{-1}) + Platinum Manganese® (2L ha^{-1}).

Figure 3 - Visual note of phytotoxicity at 3, 7 and 14 days after application (DAA) in soybean submitted to application of glyphosate and technologies in the management of phytotoxicity reversal. Experiment II, Palotina – PR, 2017.

phytotoxicity scores compared to the application of the herbicide alone.

Regarding shoot dry weight (g), there were no differences between the products for each cultivar (Table 8), as well as for chlorophyll content (Table 9). It was found that the phytotoxicity level observed in the second experiment in general was less pronounced compared to the first experiment, making it more difficult to reach a conclusion about the technologies.

DISCUSSION

The visible symptoms may be caused by the immobilization of divalent cations such as iron and manganese, since glyphosate is a phosphonic acid that chelates cations, according to Merotto *et al.* (2015). Glyphosate is mobile by phloem and is rapidly translocated to younger tissues of root and tissue growth, accumulating at millimolar concentrations after foliar application, which may lead to a slight reduction in dry root mass but the accumulation can be overcome without causing effects on productivity (Feng *et al*., 1999; Hetherington *et al.*, 1999).

Duration of the effect is also related to the plant ability to absorb the elements immobilized by glyphosate. In the first experiment, the visible injury diminished considerably at 28 DAA, when application was conducted during the vegetative stage, corroborating data of Krenchinski *et al.* (2017) and Albrecht *et al.* (2014a).

The visible phytotoxicity symptom has a linear relation with the herbicide dosage. Krenchinski *et al*. (2017) mention that the phytotoxic effects found in their study were higher with the highest dose of glyphosate applied. As in the present study, the authors observed that the phytotoxicity symptoms had diminished significantly at 35 DAA due the plant recovery.

The chlorophyll content was affected in both experiments. The association of glyphosate with manganese provided a satisfactory result, with an increase in the chlorophyll content. Glyphosate has a negative linear effect on the chlorophyll content because this herbicide can cause damages to chloroplasts. Other hypothesis is that the herbicide chelates the cationic ions such as iron and manganese, and the enzymes required for chlorophyll biosynthesis (catalase and peroxidase) are extremely sensitive to a deficiency of these micronutrients (Malavolta *et al.,* 1989; Reddy *et al.,* 2004).

The association of bioregulators with glyphosate provides modifications for having a direct physiological action on the plants, especially when the herbicide is applied on the leaves during the vegetative stage. Plants that are in hormonal balance exhibit an adequate growth of shoots and roots, with a good development of vegetative and reproductive structures (Albrecht *et al.*, 2010, 2012), as observed in the present study.

Foliar application of manganese increased the production of plants dry matter, as pointed out in studies by Oliveira Junior *et al*. (2000). Such association also provided a greater number of nodules per plant. Glyphosate applications may inhibit soybean-fixing bacterium (rhysobium) symbiosis. Manganese plays a co-factor role in the activation of various enzymes, and this micronutrient is responsible for the biosynthesis of amino acids and secondary products, such as flavonoids. Flavonoids, in turn, act in the root system stimulating nodulation, and, therefore, the manganese deficiency and the stress undergone by the plant signals to the symbiotic bacteria to discontinue the biological nitrogen fixation (Albrecht *et al*., 2010).

In a study containing manganese mixture with glyphosate, Freitas *et al*. (2018) observed the increase in productivity after treatment with manganese-containing the fertilizer in its formulation. This exogenous supply of the nutrient can overcome the temporary deficiency caused by the herbicide without any alteration in the agronomic performance of the crop.

Santos *et al*. (2015) conducted research with manganese application and was successful in increasing productivity assessment for this treatment. Bertolin *et al.* (2010) when evaluating the efficiency of plant regulators also observed increased productivity. This efficiency can be attributed due to the hormonal balance that is affected in the plant, promoting its growth.

However, when the phytotoxic effect of glyphosate is increased with the mixture with bioregulator it can be attributed to the low response of the cultivar to the technologies used. The application of the growth regulator may have caused a hormonal imbalance, promoting glyphosate phytotoxicity.

Zobiole *et al.* (2010) mentions that the response of each cultivar may be related to its maturity group. Early-cycle cultivars are more affected by phytotoxicity than long-cycle cultivars due to the longer detoxification period by glyphosate or AMPA, which is formed as a product of glyphosate degradation. Thus, it can be seen in the present study that the early-cycle M6210 cultivar presented more injuries after application of the herbicide.

The results of this study contribute to the understanding of these technologies, but more studies are necessary on these and other products and formulations due to the large number of products (fertilizers and growth regulators) available in the market. Studies with different formulations and with other cultivars and under field conditions may offer more results for the basis of the technical recommendations, since for the conditions of this experiment, in pots placed in a greenhouse, there was no difference, but under field conditions the answer may be different.

CONCLUSIONS

Differences were observed among cultivars due to genotype characteristics. For the products used in mixture no differences were observed and in the second experiment no phytotoxicity symptom was observed. Based on the results obtained in the present experiment the products did not demonstrate significant effect on the reversion of phytotoxicity caused by glyphosate.

ACKNOWLEDGEMENTS

The authors would like to thank CNPQ – Conselho nacional de Desenvolvimento Científico e Tecnológico, *Universidade Federal do Paraná* and *SUPRA Pesquisa*.

REFERENCES

Albrecht, L.P.; Braccini, A.L.; Scapim, C.A.; Ávila, M.R.; Albrecht, A.J.P. & Barbosa, M.C. (2010) -Qualidade das sementes de soja produzidas sob manejo com biorregulador. *Revista Brasileira de Sementes*, vol. 32, n. 4, p. 39-48. http://dx.doi.org/10.1590/S0101-31222010000400005

Albrecht, A.J.P.; Albrecht, L.P.; Krenchinski, F.H.; Placido, H.F.; Lorenzetti, J.B.; Victoria Filho, R. & Barroso, A.A.M. (2014a) - Behavior of RR soybeans subjected to different formulations and rates of glyphosate in the reproductive period. *Planta Daninha*, vol. 32, n. 4, p. 851-859. http://dx.doi.org/10.1590/S0100-83582014000400020

Albrecht, L.P.; Albrecht, A.J.P.; Braccini, A.L.; Oliveira, JR., R.S.; Zobiole, L.H.S. & Ávila, M.R. (2014b) - The role of glyphosate in RR soybean production and seed quality. *Planta Daninha*, vol. 32, n. 2, p. 401-407. http://dx.doi.org/10.1590/S0100-83582014000200018

Albrecht, L.P.; Braccini, A.L.; Scapim, C.A.; Ávila, M.R. & Albrecht, A.J.P. (2012) - Biorregulador na composição química na produtividade de grãos de soja. *Revista Ciência Agronômica,* vol. 43, n. 4, p. 774-782. http://dx.doi.org/10.1590/S1806-66902012000400020

Albrecht, L.P.; Braccini, A.L.; Scapim, C.A.; Ávila, M.R.; Albrecht, A.J.P. & Ricci, T.T. (2011) - Manejo de biorregulador nos componentes de produção e desempenho das plantas de soja. *Bioscience Journal*, vol. 27, n. 6, p. 865-876.

Bertolin, D.C.; Sá, M.E.; Arf, O.; Junior, E.F.; Colombo, A.S. & Carvalho, F.L.B.M. (2010) - Aumento da produtividade de soja com a aplicação de bioestimulantes. *Bragantia,* vol. 69, n. 2, p. 339-347. http://dx.doi.org/10.1590/S0006-87052010000200011

Feng, P.C.C.; Ryerse, J.S.; Jones, C.R. & Sammons, R.D. (1999) -Analysis of surfactant leaf damage using microscopy and its relation to glyphosate or deuterium oxide uptake in velvetleaf (*Abutilon theophrasti*). *Pesticide Science,* vol. 55, n. 3, p. 385-386. https://doi.org/10.1002/(SICI)1096-9063(199903)55:3%3C385::AID-PS921%3E3.0.CO;2-9

Ferreira, D.F. (2011) - Sisvar: a computer statistical analysis system. *Ciência e Agrotecnologia*, vol. 35, n. 6, p. 1039-1042. http://dx.doi.org/10.1590/S1413-70542011000600001

Freitas, G.A.; Weber, F.; Santos, A.C.M.; Silva Carneiro, J.S. & Silva, R.R. (2018) - Fertiactyl® pós na redução da fitotoxidez do herbicida Roundup Ready® na cultura da soja. *Revista em Agronegócio e Meio Ambiente*, vol. 11, n. 1, p. 99-116. http://dx.doi.org/10.17765/2176-9168.2018v11n1p99-116

Hetherington, P.R.; Reynolds, T.L.; Marshall, G. & Kirkwood, R.C. (1999) - The absorption, translocation and distribution of the herbicide glyphosate in maize expressing the CP-4 transgene. *Journal of Experimental Botany,* vol. 50, n. 339, p. 1567–1576. https://doi.org/10.1093/jxb/50.339.1567

Krenchinski, F.H.; Albrecht, L.P.; Albrecht, A.J.P.; Cesco, V.J.S.; Rodrigues, D.M.; Portz, R.L. & Zobiole, L.H.S. (2017) - Glyphosate affects chlorophyll, photosynthesis and water use of four Intacta RR2 soybean cultivars. *Acta Physiologiae Plantarum*, vol. 39, art. 63. http://dx.doi.org/10.1007/s11738-017-2358-0

Malavolta, E.; Vitti, G.C. & Oliveira, S.A. (1989) - *Avaliação do estado nutricional das plantas: princípios e aplicações.* Associação Brasileira para Pesquisa da Potassa e do Fosfato. 201p.

Merotto, A. JR.; Wagner, J. & Meneguzzi, C. (2015) - Efeitos do herbicida glifosato e da aplicação foliar de micronutrientes em soja transgênica. *Bioscience Journal,* vol, 31, n. 2, p. 499-508. https://doi.org/10.14393/BJ-v31n2a2015-22307

Oliveira JR, J. A.; Malavolta, E.; Cabral, C. P. (2000) Efeitos do manganês sobre a soja cultivada em solo de cerrado do Triângulo Mineiro. *Pesquisa Agropecuária Brasileira,* vol. 35, n. 8, p. 1629-1636. http://dx.doi.org/10.1590/S0100-204X2000000800016

Reddy, K.N. (2001) - Glyphosate-resistant soybean as a weed management tool: Opportunities and challenges. *Weed Biology and Management*, vol. 1, n. 4, p. 193–202. https://doi.org/10.1046/j.1445-6664.2001.00032.x

Reddy, K.N. & Zablotowicz, R.M. (2003) - Glyphosate-resistant soybean response to various salts of glyphosate and glyphosate accumulation in soybean nodules. *Weed Science*, vol. 51, n. 4, p. 496-502. https://doi.org/10.1614/0043-1745(2003)051[0496:GSRTVS]2.0.CO;2

Reddy, K.N., Rimando, A.M., Duke, S.O. (2004) - Aminomethylphosphonic acid, a metabolite of glyphosate, causes injury in glyphosate-treated, glyphosateresistant soybean. *Journal of Agriculture and Food Chemistry,* vol. 52, n. 16, p. 5139–5143. https://doi.org/10.1021/jf049605v

Rosolem, C.A.; Andrade, G.J.M.D.; Lisboa, I.P. & Zoca, S.M. (2010) - Manganese uptake and redistribution in soybean as affected by glyphosate. *Revista Brasileira de Ciência do Solo*, vol. 34, n. 6, p. 1915-1922. http://dx.doi.org/10.1590/S0100-06832010000600016

Santos, A.A.M.; Souza, M.A.S.; Freitas, G.A.; Silva, P.S.S. & Silva, R.R. (2015) - Substância húmica na redução da fitotoxidade dos herbicidas Roundup Ready + Lactofen na cultura da soja. *Revista Tecnologia & Ciência Agropecuária,* vol. 9, n. 3, p. 35-41.

SBCPD (1995) - *Procedimentos para instalação, avaliação e análise de experimentos com herbicidas.* Londrina - PR: Sociedade Brasileira da Ciência das Plantas Daninhas, 42 p.

Zobiole, L.H.S.; Oliveira, JR., R.S.; Kremer, R.J.; Constantin, J.; Bonato, C.M. & Muniz, A.S. (2010) - Water use efficiency and photosynthesis of glyphosate-resistant soybean as affected by glyphosate. *Pesticide Biochemistry and Physiology*, vol. 97, n. 3, p. 182–193. http://dx.doi.org/10.1016/j.pestbp.2010.01.004

Zobiole, L.H.S.; Oliveira JR., R.S.; Constantin, J. & Biffe, D.F. (2011) - Prevenção de injúrias causadas por glyphosate em soja RR por meio do uso de aminoácido. *Planta Daninha*, vol. 29, n. 1, p. 195-205. http://dx.doi.org/10.1590/S0100-83582011000100022

Zobiole, L.H.S.; Oliveira, JR., S.O.; Huber, D.M.; Constantin, J.; Casto, C.; Oliveira, F.A. & Olivia, A.O.J. (2009a) - Glyphosate reduces shoot concentrations of mineral nutrients in glyphosate-resistant soybeans. *Plant and Soil*, vol. 328,n. 1-2, p. 57-69. https://doi.org/10.1007/s11104-009-0081-3

Zobiole, L.H.S.; Oliveira, JR, R.S.; Kremer, R.J.; Constantin, J.; Yamada, T.; Castro, C.: Oliveira, F.A. & Oliveira, JR., A. (2009b) - Effect of glyphosate on symbiotic N_2 fixation and nickel concentration in glyphosate-resistant soybeans. *Applied Soil Ecology*, vol. 44, n. 2, p. 176-180. https://doi.org/10.1016/j.apsoil.2009.12.003