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Glyphosate, isolated or in associations, at agronomic performance and seed quality of the RR®2 soybean

Glifosato, isolado ou em associações, no desempenho agronômico e qualidade de sementes de soja RR®2

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ABSTRACT: The "second generation" of glyphosate-tolerant soybean (RR®2 soybean) was developed through a different technique of insertion of the glyphosate-insensitive EPSPs gene. Information on the selectivity of glyphosate, alone or in combination, in RR2 soybean is lacking. This study evaluated the effects of glyphosate, isolated or in associations, applied at post-emergence (V4), at agronomic performance and seed quality of soybean cultivar NS 6700 IPRO (RR2). The experimental design was randomized block with four replications and seven treatments, conducted in the field for two growing seasons. The treatments consisted of glyphosate herbicide, alone or in combination with clethodim, cloransulam, chlorimuron, lactofen and fluazifop, besides the control without application. Analysis was performed for crop injury, Soil and Plant Analyzer Development (SPAD) index, as well as variables related to agronomic performance (height, number of pods per plant, yield and 1,000-seed weight) and seed quality (vigor, germination, abnormal seedlings and dead seeds). An additional test was conducted with the same cultivar and treatments in a greenhouse in a completely randomized design with four replications. The herbicides did not affect agronomic performance and seed quality of RR2 soybean. Thus, the soybean cultivar NS 6700 IPRO (RR2) was tolerant to glyphosate, alone or combined with other herbicides applied in post-emergence (V4).

KEYWORDS: crop injury; *Glycine max* (L.) Merrill; EPSPs inhibitors.

RESUMO: A "segunda geração" de soja tolerante ao glifosato (soja RR®2) foi desenvolvida por meio de uma diferente inserção do gene EPSPs insensível ao referido herbicida. Informações sobre a seletividade de glifosato, isolado ou em associação, são faltantes em soja RR2. Este estudo avaliou os efeitos do glifosato, isolado ou em associações, aplicado em pós-emergência (V4), no desempenho agronômico e na qualidade de sementes do cultivar de soja NS 6700 IPRO (RR2). Adotou-se o delineamento experimental de blocos ao acaso com quatro repetições e sete tratamentos, realizados no campo em duas safras. Os tratamentos consistiram no herbicida glifosato, isolado ou em associação com clethodim, cloransulam, chlorimuron, lactofen e fluazifop, além da testemunha sem aplicação. Foi realizada a avaliação dos sintomas de injúria e índice SPAD (*Soil and Plant Analyzer Development*), bem como de variáveis relacionadas ao desempenho agronômico (altura, número de vagens por planta, produtividade e massa de 1.000 sementes) e à qualidade das sementes (vigor, germinação, plântulas anormais e sementes mortas). Experimento complementar foi realizado com o mesmo cultivar e tratamentos em casa de vegetação em delineamento inteiramente casualizado com quatro repetições. Os herbicidas não afetaram o desempenho agronômico e a qualidade de sementes da soja RR2. Assim, o cultivar NS 6700 IPRO (RR2) foi tolerante ao glifosato, isolado ou em associação com outros herbicidas aplicados em pós-emergência (V4).

PALAVRAS-CHAVE: sintomas de injúria; *Glycine max* (L.) Merrill; inibidores do EPSPs.

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INTRODUCTION

The glyphosate-tolerant soybean (Roundup Ready® soybean - RR® soybean) was developed by introducing *cp4-EPSPs* gene, which encodes a glyphosate-insensitive EPSPs enzyme, present in the bacterium *Agrobacterium tumefaciens* strain CP4, into the plant genome so that glyphosate does not have the ability to block EPSPs (PADGETTE et al., 1995).

The "second generation" of glyphosate-tolerant soybean (RR2 soybean) was developed through a different technique of insertion of the *cp4-EPSPs* gene (in addition to the *cry1Ac* gene, from the bacterium *Bacillus thuringiensis* (Bt), which makes insects resistant), under the trademark Intacta® Roundup Ready® 2 Pro (MARTINELL et al., 2002; BERNARDI et al., 2012). ZOBIOLE et al. (2011) found no differences in the components of photosynthetic production, between RR and RR2 soybeans.

Glyphosate is a post-emergence herbicide, belonging to the chemical group of substituted glycines, selective only for RR crops. It inhibits the activity of the enzyme EPSPs, which is a catalyst for the synthesis of aromatic amino acids, which are essential to the plant (GALLI; MONTEZUMA, 2005).

The combination of glyphosate with other herbicides is a frequent practice among farmers and may be beneficial because it requires the same application time, lower cost compared to isolated applications of each herbicide, and provides a broader spectrum of weed control (NORRIS et al., 2001). WALSH et al. (2014), among others, studied the combination of glyphosate with other herbicides in RR soybean.

There are reports of probable negative interferences of glyphosate with the initial development of RR soybean plants, to which this product is recommended, therefore studies on the subject must still be elucidated in the scientific field (ZABLOTOWICZ; REDDY, 2007; ZOBIOLE et al., 2011). However, information about glyphosate effects, isolated or in associations, in RR2 soybean is lacking. This study evaluated the effects of glyphosate, isolated or in associations, applied at post-emergence (V4), at agronomic performance and seed quality of soybean cultivar NS 6700 IPRO (RR2).

MATERIALS AND METHODS

The experiment was conducted in the field in the 2013/14 and 2014/15 growing seasons (experiments I and II) and in a greenhouse (experiment III) in the city of Piracicaba, state of São Paulo, Brazil, 22°42'51.8"S, 47°37'17.4"W, altitude: 536 m. The soil chemical analysis of the experimental area is presented below (Table 1).

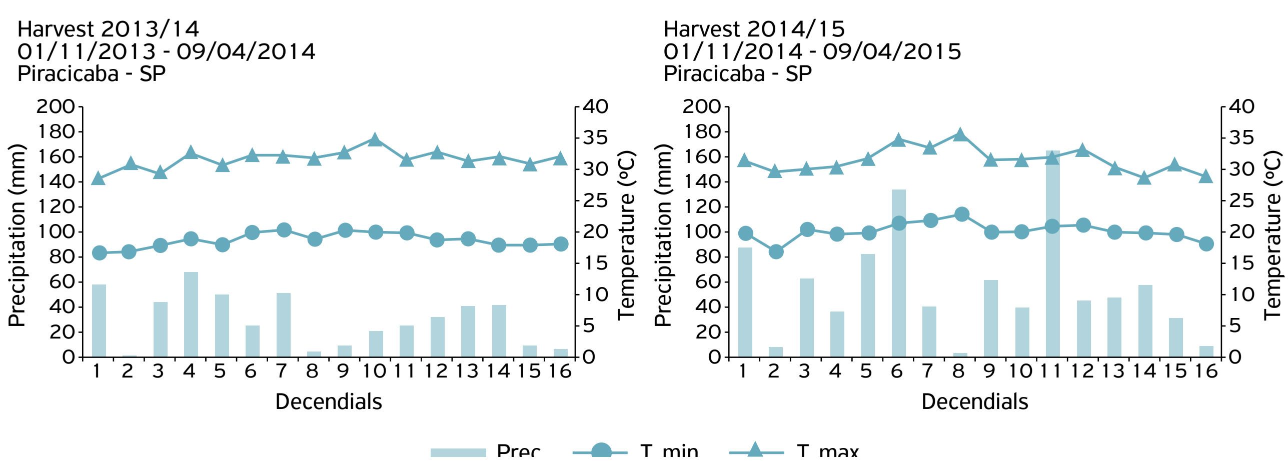
Fertilization practices, crop planting and phytosanitary management were carried out in accordance with the recommendations of EMBRAPA (2013). Fertilization was carried out to correct the soil, considering the extraction of the crop, and all plots were kept free of weed interference, by manual weeding.

The climate of the region is characterized as Cwa by the Köppen climate classification, that is, humid subtropical with dry winter. Next, Figure 1 illustrates the distribution of

Table 1. Soil chemical analysis at the 0 - 20 cm depth layer. Piracicaba, state of São Paulo.

pH ($CaCl_2$)	Al	H+Al	P (resin)	K	Ca	Mg	SB	CEC	V
5.3	< 1.0	25.0	10.0	2.8	26.0	13	41.8	66.8	63
				Clay	Silt	Sand			
				41.0	5.0	54.0			

Units: Al, H+Al, K, Ca, Mg, SB and CEC ($mmol_c\ dm^{-3}$); P (resin) ($mg\ dm^{-3}$); V, clay, silt, sand (%).



Source: Departamento de Engenharia de Biossistemas (LEB), Universidade de São Paulo (USP) / Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ).

Figure 1. Representation of precipitation, average minimum and average maximum temperatures for the period relative to soybean harvest 2013/14, and 2014/15, Piracicaba (SP, Brazil).

rainfall and temperature during the experimental period, in the two growing seasons.

The soybean cultivar used was the NS 6700 IPRO (RR2), which has a cycle of 120-150 days. Sowing was carried out in the first week of December, with season in the second week of April, for both growing seasons. The experimental design was randomized block for experiments I and II, and completely randomized for experiment III, always with four replications and seven treatments (Table 2).

For experiments I and II, the experimental units were composed of 5 m long plots and six soybean rows, 45 cm spaced apart. The four central rows were considered useful area, discarding the first and last meter of the plot.

For experiment III, they were composed of 7 L potsfilled with soil of medium texture. Six seeds were sown per pot and after emergence, thinning was done leaving 3 plants per pot, in the first week of October 2014 and completed in the second week of November of the same year.

Treatments were applied at the V4 phenological stage of soybean plants. For experiments I and II, via a CO_2 pressurized backpack sprayer, with a bar equipped with four spray nozzles, at a constant pressure of 2 bar, a flow rate of 0.65 L min.$^{-1}$, working at a height of 50 cm and at a rate of 1 m s^{-1}, reaching an applied band of 50 cm wide per spray nozzle, and providing a spray volume of 200 L ha^{-1}. For the experiment III, the application was via an automated spray chamber, with sprayer containing a fan tip nozzle, model XR 80.02, calibrated for a spray volume of 200 L ha^{-1}.

Crop injury was evaluated through visual assessments in which percentage scores were assigned to each experimental unit (0 for no injuries, up to 100% for plant death), considering in this case symptoms significantly visible in the plants, according to their development (VELINI et al., 1995). This evaluation was performed at 7, 14, 21 and 28 days after application (DAA) for experiments I and II and at 7, 14 and 21 DAA for experiment III.

The Soil and Plant Analyzer Development (SPAD) index was evaluated at 28 DAA for experiments I and II, and at 7, 14 and 21 DAA for experiment III. For this evaluation, we used the portable meter SPAD-502 of Konica Minolta. This instrument quantitatively evaluates leaf green intensity by measuring light transmissions, the equipment calculates an SPAD index that is generally highly correlated with leaf chlorophyll content (MARKWELL et al., 1995). Ten plants, randomly chosen in the useful area of the plots, were evaluated for experiments I and II, and for experiment III, three plants of each pot.

For the experiments I and II, it was also evaluated the variables related to the agronomic performance (plant height, number of pods per plant, yield and 1000-seed weight). For the determination of the height of plants, 10 plants, randomly chosen in the useful area of the plots, were evaluated using a millimeter woodenruler, with results expressed in centimeters. The number of pods per plant was evaluated at full maturity (R8 stage) by manually counting the number of pods present, also in 10 plants randomly chosen in the useful area of each plot.

Plants were seasoned manually at the R8 stage, when 95% of the pods had the typical mature pod color. Pods were then seasoned and threshed for experimentation, cleaned with sieves and packed in paper bags, for further analysis and evaluation.

Regarding the seeds, the physiological quality of seeds was analyzed by means of the first count germination (indicative of vigor), second count (germination), percentage of abnormal seedlings and dead seeds, according to BRASIL (2009), evaluations for experiments I and II.

The germination test was performed using four sub-samples of 50 seeds per field repetition of each treatment, placed to germinate between three sheets of filter paper, moistened with demineralized water, in the proportion of three times the weight of the dry paper. Rolls were made and taken to a germinator regulated to maintain a constant temperature of 25°C. The evaluation was performed eight days after assembling the test, computing the percentage of normal seedlings obtained. The seed vigor test was performed along with the germination test, computing the percentage of normal seedlings obtained on the fifth day after assembling the test. In the first and second counts, the percentage of dead seeds was also computed, while the percentage of abnormal seedlings was computed in the second count.

Table 2. Treatments applied post-emergence (V4) of RR2 soybeans in 2013/14 and 2014/15 seasons. Piracicaba, state of São Paulo.

Treatments	Commercial product	Rates
1. control	-	0
2. glyphosate	Roundup Ready®	1440
3. glyphosate + clethodim	Roundup Ready®+ Select® 240 EC	1440 + 108
4. glyphosate + cloransulam	Roundup Ready®+ Pacto®	1440 + 40
5. glyphosate + chlorimuron	Roundup Ready®+ Classic®	1440 + 17.5
6. glyphosate + lactofen	Roundup Ready®+ Cobra®	1440 + 180
7. glyphosate + fluazifop	Roundup Ready®+ Fusilade®250 EW	1440 + 187.5

*Rates in grams of acid equivalent per hectare (g a.e. ha^{-1}) for the herbicide glyphosate. Grams of active ingredient per hectare (g a.i. ha^{-1}) for other herbicides.

For experiment III, height of soybean plants at 7, 14 and 21 DAA was also evaluated. The three plants of each pot were measured with ruler and results were expressed in centimeters. When the majority of the plants reached the R2 growth stage, the aerial part and the root system of the plants of each pot were collected to measure the fresh and dry weight of shoot and the dry weight of the root system. For drying, a greenhouse with forced ventilation was used at 65ºC for 72h, and, to determine the weight, an analytical balance was used with precision of three decimal places.

Data were subjected to analysis of variance and F-test, and the means of the treatments were compared by the Tukey's test ($p < 0.05$), according to PIMENTEL-GOMES; GARCIA (2002).

RESULTS AND DISCUSSION

For the experiment I, crop injury symptoms were verified for the application of glyphosate (1440 g e.a. ha^{-1}) combined with chlorimuron (17.5 g i.a. ha^{-1}), lactofen (180 g i.a. ha^{-1}) and fluazifop (187.5 g i.a. ha^{-1}) at 7 and 14 DAA, whereas for glyphosate (1440 g e.a. ha^{-1}) + cloransulam (40 g i.a. ha^{-1}) only at 7 DAA. At 21 and 28 DAA, all treatments showed no symptoms. For the experiment II, at 7 DAA, no differences were detected between the treatments; however, at 14 and 21 DAA, the treatment glyphosate + lactofen presented higher symptoms of crop injury than the other treatments, and, at 28 DAA, the soybean plants were fully recovered, with 0.0% crop injuryfor all treatments (Table 3).

Results similar to those obtained in the field were verified in greenhouse (experiment III), for the evaluation of crop injury (Table 4). At 7 DAA, the application of glyphosate + lactofen caused greater injury to soybean plants than all other treatments, whereas the application of glyphosate + cloransulam and glyphosate + chlorimuron also caused crop injury when compared to the control. At 14 and 21 DAA,

Table 4. Crop injury (%) at 7, 14, 21 and 28 days after application of RR2 soybean subjected to application of glyphosate, alone or in combination, at post-emergence (V4). Piracicaba, state of São Paulo, 2014 (experiment III).

Treatments[1]	Crop injury (DAA)					
	7*		14*		21*	
1. CO	0.0	a	0.0	a	0.0	a
2. GLY	0.0	a	0.0	a	0.0	a
3. GLY + CLE	0.0	a	1.3	ab	1.3	a
4. GLY + CLO	2.8	b	1.3	ab	0.0	a
5. GLY+ CHL	5.0	b	1.3	ab	0.0	a
6. GLY + LAC	9.3	c	8.8	c	6.3	b
7. GLY+ FLU	4.0	b	4.0	bc	0.0	a
Mean	3.0		2.4		1.1	
LSD	3.0		5.1		2.9	
CV (%)	17.3		30.8		22.9	

[1]CO: control; GLY: glyphosate (1440 g e.a. ha^{-1}); CLE: clethodim (108 g i.a. ha^{-1}); CLO: cloransulam (40 g i.a. ha^{-1}); CHL: chlorimuron (17.5 g i.a. ha^{-1}); LAC: lactofen (180 g i.a. ha^{-1}); FLU: fluazifop (187.5 g i.a. ha^{-1}). DAA: days after application.
*Means in the column, followed by different letters, are significantly different from each other by Tukey's test at 5% probability.

Table 3. Crop injury (%) at 7, 14, 21 and 28 days after application of RR2 soybean subjected to application of glyphosate, alone or in combination, at post-emergence (V4). Piracicaba, state of São Paulo, 2013/14 season (experiment I), and 2014/15 season (experiment II).

Treatments[1]	Experiment I						Experiment II					
	Crop injury (DAA)											
	7*		14*		21**	28**	7**	14*		21*		28**
1. CO	0.0	a	0.0	a	0.0	0.0	0.0	0.0	a	0.0	a	0.0
2. GLY	0.0	a	0.0	a	0.0	0.0	0.0	0.0	a	0.0	a	0.0
3. GLY + CLE	0.0	a	0.0	a	0.0	0.0	0.8	0.3	a	0.8	a	0.0
4. GLY + CLO	2.5	ab	0.0	a	0.0	0.0	0.3	0.0	a	0.0	a	0.0
5. GLY+ CHL	12.5	c	8.8	b	0.0	0.0	0.0	1.0	a	0.8	a	0.0
6. GLY + LAC	13.8	c	11.3	b	0.0	0.0	1.5	5.3	b	4.5	b	0.0
7. GLY+ FLU	5.0	b	1.3	a	0.0	0.0	0.8	0.0	a	0.0	a	0.0
Mean	4.8		3.0		0.0	0.0	0.5	0.9		0.9		0.0
LSD	4.2		4.0		0.0	0.0	3.0	3.0		1.9		0.0
CV (%)	37.5		56.8		0.0	0.0	35.0	22.1		19.9		0.0

[1]CO: control; GLY: glyphosate (1440 g e.a. ha^{-1}); CLE: clethodim (108 g i.a. ha^{-1}); CLO: cloransulam (40 g i.a. ha^{-1}); CHL: chlorimuron (17.5 g i.a. ha^{-1}); LAC: lactofen (180 g i.a. ha^{-1}); FLU: fluazifop (187.5 g i.a. ha^{-1}). DAA: days after application.
*Means followed by different letters, in the same column, are significantly different by Tukey's test at 5% probability.
** Means are not significantly different from each other by Tukey's test at 5% probability.

crop injury symptoms are observed, superior to the control, only for application of glyphosate + lactofen.

Results corroborate with WALSH et al. (2014) observed symptoms of crop injury in RR soybean for glyphosate application (900 g e.a. ha^{-1}) + lactofen (240 g i.a. ha^{-1}), but at 28 DAA, the soybean plants were fully recovered, with 0.0% crop injury. ELLIS; GRIFFIN (2003) reported crop injury of 14 and 6% 28 DAA for tank mixes of glyphosate plus acifluorfen and fomesafen, respectively.

STEWART et al. (2010) did not observe visual symptoms of crop injury in RR soybean for application of glyphosate (900 g e.a. ha^{-1}), with single or sequential application. SILVA et al. (2016) also reported visual symptoms of crop injury for glyphosate application (960 g ea. ha^{-1}), alone or combined with chlorimuron (20 g i.a. ha^{-1}) or cloransulam (40 g i.a. ha^{-1}). BELFRY et al. (2015) verified that application of cloransulam (70 g i.a. ha^{-1}), at pre-emergence, caused no significant symptoms of crop injury, in evaluations of 2 and 4 weeks after soybean emergence, for cultivars S03W4, Madison, OAC Lakeview and S23T5. SOLTANI et al. (2006) found no symptoms of injury in RR soybean for the post-emergence application of glyphosate (960 g e.a. ha^{-1}) combined with clethodim (up to 30 g i.a. ha^{-1}) or fluazifop (up to 75 g i.a. ha^{-1}).

KRENCHINSKI et al. (2017) found injuries in RR2 soybean under application of glyphosate; however, RR2 soybeans recovered from visual intoxication injuries after glyphosate application. It was one of the few works to report effects of the application of glyphosate on soybean RR2.

There were no differences for the SPAD index for the three experiments (Table 5). SILVA et al. (2016) also registered no differences for SPAD index in RR soybean for glyphosate application (960 g e.a. ha^{-1}) alone or combined with (20 g i.a. ha^{-1}) or cloransulam (40 g e.a. ha^{-1}).

Table 6 lists the results for variables related to agronomic performance. For number of pods per plant, no differences were detected between the treatments for the two experiments. Only the application of glyphosate alone caused a reduction

Table 5. SPAD index of RR2 soybean subjected to application of glyphosate, alone or in combination, at post-emergence (V4). Piracicaba, state of São Paulo, 2013/14 season (experiment I), and 2014/15 season (experiment II) and greenhouse 2014 (experiment III).

Treatments[1]	Exp I		Exp II	Exp III	
	SPAD index (DAA)				
	28		7	14	21
1. CO	41.7	38.5	36.1	38.7	38.2
2. GLY	43.1	34.7	35.8	36.7	37.1
3. GLY + CLE	42.4	34.5	35.8	41.3	40.0
4. GLY + CLO	42.8	36.4	39.0	38.0	36.7
5. GLY+ CHL	44.6	38.1	36.4	37.4	36.8
6. GLY + LAC	45.1	37.6	35.3	37.3	35.4
7. GLY+ FLU	42.7	36.6	36.4	38.0	37.3
Mean	43.2	36.6	36.4	38.2	37.4
LSD	3.7	4.7	7.2	5.2	4.8
CV (%)	3.6	5.5	8.5	5.8	5.5

[1]CO: control; GLY: glyphosate (1440 g e.a. ha^{-1});
CLE: clethodim (108 g i.a. ha^{-1}); CLO: cloransulam (40 g i.a. ha^{-1});
CHL: chlorimuron (17.5 g i.a. ha^{-1}); LAC: lactofen (180 g i.a. ha^{-1});
FLU: fluazifop (187.5 g i.a. ha^{-1}).
*Means are not significantly different from each other by Tukey's test at 5% probability.

Table 6. Variables related to the agronomic performance[2] of RR2 soybean subjected to application of glyphosate, alone or in combination, at post-emergence (V4). Piracicaba, state of São Paulo, 2013/14 season (experiment I), and 2014/15 season (experiment II).

Treatments[1]	Experiment I						Experiment II				
	H*		NPP**	Y*		SW**	H**	NPP**	Y**	SW*	
1. CO	76.3	a	37.5	4084.5	ab	158.0	104.2	40.5	3937.9	182.6	a
2. GLY	63.7	b	33.0	3989.5	ab	166.7	96.2	41.1	3641.4	179.4	ab
3. GLY + CLE	68.9	ab	46.6	4125.2	a	166.2	96.9	40.9	3732.6	175.6	ab
4. GLY + CLO	68.2	ab	38.3	4210.0	a	172.2	97.8	41.4	4298.9	180.1	ab
5. GLY+ CHL	77.2	a	46.2	4003.0	ab	166.3	96.9	41.4	3791.4	183.6	a
6. GLY + LAC	75.9	a	45.8	3104.1	b	159.2	96.8	42.1	3620.4	173.2	b
7. GLY+ FLU	71.9	ab	39.2	3446.7	ab	170.6	102.9	41.7	4118.7	183.1	a
Mean	71.7		40.9	3851.8		165.6	98.81	41.3	3887.3	179.7	
LSD	10.6		15.1	1009.6		18.2	11.7	5.5	963.4	8.6	
CV (%)	6.3		15.7	11.2		4.7	5.1	5.7	10.6	2.0	

[1]CO: control; GLY: glyphosate (1440 g e.a. ha^{-1}); CLE: clethodim (108 g i.a. ha^{-1}); CLO: cloransulam (40 g i.a. ha^{-1}); CHL: chlorimuron (17.5 g i.a. ha^{-1}); LAC: lactofen (180 g i.a. ha^{-1}); FLU: fluazifop (187.5 g i.a. ha^{-1}). [2]H: plant height (cm), NPP: number of pods per plant, Y: yield (kg ha^{-1}), SW: 1000-seed weight (g).
*Means in the column, followed by different letters, are significantly different from each other by Tukey's test at 5% probability.
**Means are not significantly different from each other by Tukey's test at 5% probability.

in the plant height compared to the control, without application, for experiment I. For experiment II, for this variable, no differences between treatments were observed.

For 1,000-seed weight, no differences were detected between treatments for experiment I. While for experiment II, application of glyphosate + lactofen reduced the 1,000-seed weight compared to the control, glyphosate + chlorimuron and glyphosate + fluazifop, however did not differ from the results from the application of glyphosate alone or combined with clethodim or cloransulam. For yield, in experiment II, no differences between treatments were registered. For experiment I, although differences between treatments were found, none of them reduced yield compared to control without application.

For experiment III (conducted in pots), no differences were detected between treatments, for the variables: plant height at 7, 14 and 21 DAA, and dry weight of shoot and roots (Table 7). These results followed the pattern of the results obtained in the field experiments.

ALBRECHT et al. (2011) for post-emergence (V6) application of glyphosate (up to 2880 g e.a. ha^{-1}) did not observe reduction in height of RR soybean plants. In turn, for 1,000 seed-weight, these authors verified a reduction for the rate of 2880 g e.a. ha^{-1}; for yield, the reduction was of the order of 0.40661 kg ha^{-1} to every g e.a. glyphosate. They also observed a reduction in the total number of pods per RR soybean plant for application of glyphosate (2880 g e.a. ha^{-1}) at the V6 stage and mainly at the R2 stage. Contrarily, MELHORANÇA FILHO et al. (2010) verified no reduction in height and yield of RR soybean, for application of glyphosate up to the rate 1,440 g e.a. ha^{-1}, but the dose of 1800 g e.a. ha^{-1} reduced both variables.

Moreover, ALONSO et al. (2013), for application of glyphosate, isolated or combined with cloransulam, chlorimuron or lactofen, verified a reduction in the height of RR soybean plants at 90 DAA, only for the application of glyphosate + lactofen and reduction in the 1,000-seed weight for glyphosate + lactofen or cloransulam. Nonetheless, none of these treatments reduced the number of pods per plant or yield.

In the same way, WALSH et al. (2014) found no reduction in RR soybean yield in response to the application of glyphosate alone or combined with lactofen, while application of glyphosate + chlorimuron (960 g a.e. ha^{-1} + 25 a.i. ha^{-1}) caused a yield reduction in RR soybean (ALBRECHT et al., 2012a) – results contrary to those observed in the present study. It should be emphasized that the maximum rate of chlorimuron for post-emergence application at soybean is 20 a.i. ha^{-1} (RODRIGUES; ALMEIDA, 2011), below the rate used in the cited study.

SOLTANI et al. (2006) reported no reductions in RR soybean yield for the post-emergence application of glyphosate associated with clethodim or fluazifop. This was also verified by SOLTANI et al. (2015) when applied clethodim (60 g i.a. ha^{-1}) and observed no reduction in soybean yield.

For the variables related to the physiological quality of seeds, for both growing seasons, no differences were noticed for the post-emergence (V4) application of the treatments used (Table 8).

Table 7. Height at 7, 14 and 21 days after application (cm), fresh and dry weight of shoot and roots[2] (g) of RR2 soybean subjected to application of glyphosate, alone or in combination, at post-emergence (V4). Piracicaba, state of São Paulo, greenhouse 2014 (experiment III).

Treatments[1]	Height			FWS	DWS	DWR
	7	14	21			
1. CO	42.4	45.2	50.8	139.2	26.7	17.7
2. GLY	42.8	47.0	51.7	146.1	23.5	19.9
3. GLY + CLE	44.7	48.8	55.4	122.7	24.9	14.4
4. GLY + CLO	39.3	42.2	47.0	172.2	20.6	18.3
5. GLY+ CHL	40.2	40.8	47.0	133.3	18.8	18.3
6. GLY + LAC	37.9	43.4	50.4	126.6	26.5	15.2
7. GLY+ FLU	40.4	46.2	51.7	132.8	29.5	20.8
Mean	41.2	44.9	50.6	139.0	24.3	17.5
LSD	7.4	11.8	15.9	49.6	16.9	11.2
CV (%)	7.7	11.3	13.4	15.3	29.6	27.3

[1]CO: control; GLY: glyphosate (1440 g e.a. ha^{-1}); CLE: clethodim (108 g i.a. ha^{-1}); CLO: cloransulam (40 g i.a. ha^{-1}); CHL: chlorimuron (17,5 g i.a. ha^{-1}); LAC: lactofen (180 g i.a. ha^{-1}); FLU: fluazifop (187.5 g i.a. ha^{-1}). [2] FWS: fresh weight of shoots, DWS: dry weight of shoots and DWR: dry weight of roots.
*Means are not significantly different from each other by Tukey's test at 5% probability.

Table 8. Variables related to quality of RR2 soybean seeds[2] subjected to application of glyphosate, alone or in combination, at post-emergence (V4). Piracicaba, state of São Paulo, 2013/14 season (experiment I), and 2014/15 season (experiment II).

Treatments[1]	Experiment I				Experiment II			
	V	G	AS	DS	V	G	AS	DS
1. CO	94.3	94.8	3.5	1.8	86.5	91.3	6.3	2.5
2. GLY	92.8	93.3	5.3	1.5	83.0	88.3	8.3	3.5
3. GLY + CLE	92.5	93.5	3.8	2.8	88.5	90.8	7.0	2.3
4. GLY + CLO	95.8	96.0	3.0	1.0	86.5	92.3	5.8	2.0
5. GLY+ CHL	91.5	92.0	5.0	3.0	91.5	92.8	4.5	2.8
6. GLY + LAC	93.8	94.3	2.5	3.0	86.5	89.5	5.8	4.8
7. GLY+ FLU	93.5	94.0	3.5	3.3	85.3	87.3	8.5	4.3
Mean	93.4	94.0	3.7	2.3	86.8	90.3	6.5	3.1
LSD	5.9	5.9	4.7	4.1	9.9	11.2	9.6	3.6
CV (%)	2.7	2.7	23.0	27.6	4.9	5.3	27.2	19.6

[1]CO: control; GLY: glyphosate (1440 g e.a. ha^{-1}); CLE: clethodim (108 g i.a. ha^{-1}); CLO: cloransulam (40 g i.a. ha^{-1}); CHL: chlorimuron (17.5 g i.a. ha^{-1}); LAC: lactofen (180 g i.a. ha^{-1}); FLU: fluazifop (187.5 g i.a. ha^{-1}). [2]V: vigor (%), G: germination (%), AS: bnormal seedlings (%), DS: dead seeds (%).
Means are not significantly different from each other by Tukey's test at 5% probability.

However, ALBRECHT et al. (2012b) observed that the application of glyphosate (g e.a. ha^{-1}) may adversely affect the physiological quality of soybean seeds at the V6 and R2 stages. On the other hand, ALBRECHT et al. (2012c) observed that the post-emergence application of glyphosate (2880 g e.a. ha^{-1}) isolated or combined with fluazifop + fomesafen (312.5 + 312.5 g i.a. ha^{-1}) or chlorimuron (25 g i.a. ha^{-1}) did not affect the germination of RR soybean seeds in the two growing seasons. Nevertheless, they observed reductions in seed vigor, for combinations of glyphosate with these herbicides, in one of the seasons.

As previously mentioned, the glyphosate-insensitive EPSPs gene was inserted through another technique in RR2 soybean. It should be noted that, although there is information available about the selectivity of glyphosate in RR soybean, there is little information on the growth, development, agronomic performance, seed quality, or any other parameters of this herbicide in RR2 soybean. Therefore, the results of KRENCHINSKI et al. (2017) with rates of glyphosate, as well as the results found in the present study, with glyphosate isolated or in associations, are noteworthy and important in the positioning of glyphosate in RR2 soybean.

The combination and rotation of herbicides are important tools in weed management and in the prevention of selection of herbicide-resistant biotypes. Results obtained in the present work are of great importance in the positioning of glyphosate, alone or in combination with other herbicides, in the management of weeds in RR2 soybean.

CONCLUSION

The herbicides did not affect agronomic performance and seed quality of RR2 soybean. Thus, the soybean cultivar NS 6700 IPRO (RR2) was tolerant to glyphosate, isolated or in associations with other herbicides applied in post-emergence (V4).

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