

GLYPHOSATE-RESISTANT SOYBEANS YIELD IN FUNCTION OF GLYPHOSATE SALTS, DOSES AND STADIUM PHENOLOGICAL

PRODUTIVIDADE DE CULTIVARES DE SOJA EM FUNÇÃO DOS SAIS DE GLYPHOSATE, DOSES E ÉPOCAS DE APLICAÇÃO

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ABSTRACT: Given the importance of soybean to the global economy and as a food source, improving crop management techniques is integral to obtaining higher yields. As such, this study aimed to assess the yield of soybean cultivars as a function of different glyphosate salt formulations, doses and application times. Two field experiments were conducted simultaneously using a randomized block design and 3x4x2 factorial scheme, with three repetitions. Factor A consisted of three glyphosate formulations: isopropylamine salt (Roundup Original[®]), ammonium salt (Roundup WG[®]), and potassium salt (Zapp Qi[®]), factor B four application times: 0, 14, 28, 42 days after planting (DAP), and factor C two doses: the recommended dose of 720 grams of acid equivalent per hectare and twice that (1440 grams of acid equivalent per hectare). There was no difference between the glyphosate salts for the BMX Ativa RR[®] cultivar, but yields of BMX Apolo RR[®] submitted to ammonium salt were 28% higher than those recorded for the other salts studied. Glyphosate potassium salt resulted in lower soybean yields. Potassium salt application between 14 and 28 DAP caused greater toxicity in both the soybean cultivars analyzed.

KEYWORDS: Isopropylamine salt. Potassium salt. Ammonium salt. Growth habit. *Glycine max*.

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is the main commodity in Brazilian agribusiness (CONAB 2018), the primary source of vegetable oil and a major protein source. In Brazil, the planted area was 35.1 million hectares in the 2017/2018 growing season, with 118 million metric tons produced and an average yield per hectare of 3,333 kg, a 9.3% increase in relation to the previous year (CONAB, 2018).

Given its importance, improving crop management techniques is vital to the global food supply (ZADINELLO et al., 2012). This includes weed management, which ensures crops achieve their full yield potential. Chemical weed control in the form of pre- and postemergent herbicides is the most common method in soybean crops.

Glyphosate is the main active ingredient used worldwide (GAUPP-BERGHAUSEN et al., 2015). This broad-spectrum herbicide is widely applied as a postemergence weed killer in glyphosate-resistant crops, with more than 150 commercial brands sold around the world in over 160 countries, registered for more than 100 crops (VELINI et al., 2009). It acts by inhibiting the biosynthesis of aromatic amino acids via the

precursor enzyme EPSPs (5-enolpyruvylshikimate-3-phosphate synthase), preventing shikimate transformation into chorismate (SENSEMAN, 2007).

Resistant cultivars such as RR soybean contain the gene *cp4-epsps*, which encodes a glyphosate-tolerant enzyme that prevents glyphosate binding, making plants insensitive to the herbicide (BRADSHAW et al., 1997; DUKE; POWLES, 2008). Glyphosate formulations contain several different types of salts, including isopropylamine, potassium and ammonium salt (BRADBERRY; PROUDFOOT; VALE, 2004).

Glyphosate salts can negatively affect plants that carry the glyphosate-resistant gene (ALBRECHT et al., 2011; LONDO et al., 2014; PINTO et al., 2016), and their application times can also influence soybean development. For example, RR soybean and maize crop yields were lower when glyphosate was applied with plants in full bloom as opposed to later stages (ZADINELLO et al., 2012; THOMAS et al., 2017).

Knowing the factors that trigger the negative effects of glyphosate in soybean crops is important in order to establish application guidelines that ensure maximum herbicide performance. This study aimed to assess the yield of soybean cultivars

as a function of different glyphosate salt formulations, doses and application times.

MATERIAL AND METHODS

Two experiments were conducted simultaneously in red nitisol, in the municipality of Sertão, Rio Grande do Sul (RS) state, Brazil

(28°02'33"S, 52°16'03"W), at an altitude of 705 m. Soil pH, clay and organic matter content were 5.05, 65% and 2.9%, respectively. The climate in the region is humid subtropical (Cfa) according to Köppen's classification. Rainfall amount and average air temperature by each ten days period (decennial) are shown in Figure 1.

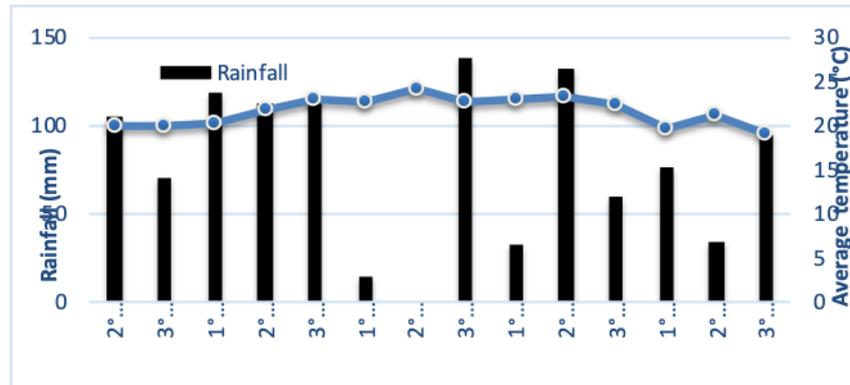


Figure 1. Rainfall amount and average air temperature by each ten days period (decennial) in 2015/16 soybean crop season.

Experiments 1 and 2 were performed with the BMX Apolo RR[®] and BMX Ativa RR[®] cultivars, respectively. A randomized block design was used for both experiments, with a 3x4x2 factorial scheme and three repetitions. Factor A consisted of three glyphosate formulations: isopropylamine salt (Roundup Original[®]), ammonium salt (Roundup WG[®]), and potassium salt (Zapp Qi[®]), factor B four application times: 0, 14, 28, 42 days after planting (DAP), and factor C two doses: the recommended dose of 720 grams of acid equivalent per hectare and twice that (1440 grams of acid equivalent per hectare).

Planting was performed on 11/19/2015 using a broadcast spreader (Semeato[®] SHM 13/17), equipped for seven rows spaced 0.45 m apart. Fertilization was carried out based on soil analysis and crop treatments were established in line with technical recommendations for soybean crops. The plots were kept weed-free. Glyphosate was applied using a CO₂-pressurized sprayer, equipped with a boom held 0.5 m away from the target, containing four ceramic spray nozzles (Micron 110.02/Air) spaced 0.5 m apart, with a flow rate of 200 L ha⁻¹. At the time of glyphosate application at 0, 14, 28 and 42 DAP the average temperature was 23.3; 21.2; 26.5 and 22.1 °C, respectively. Relative humidity was 68, 78, 57 and 82%, respectively. Wind speed was 4.4; 3.3; 5.0 and 2.8 m s⁻¹, respectively.

Manual harvesting of the study area (4.7 m²) of the plot was performed on 03/29/16, when the crop had reached maturity. The harvested plants were threshed in a stationary thresher and the samples weighed, with water content corrected to 13% and grain yield expressed in kg ha⁻¹.

The variables were submitted to analysis of variance (ANOVA) using ASSISTAT software (SILVA and AZEVEDO, 2002). When necessary, the data were transformed to square root plus one to stabilize variances for analysis. Cultivar was considered a random variable, and the other main effects of treatments were tested for cultivar-associated error based on the interaction of other treatments. Data were pooled across cultivars when no significant cultivar-treatment interaction occurred. Treatment means were compared using Tukey's test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Potassium salt glyphosate spraying as a function of application times altered crop yield (Table 1). The lowest yields were observed for application at 14 days after planting (DAP) in the BMX Ativa RR cultivar, and 28 DAP for BMX Apolo RR (Table 1). A comparison of the cultivars between the two experiments indicated different behavior in relation to potassium salt application (Table 1). BMX Apolo RR was less affected by this formulation at 14 DAP when compared to BMX

Ativa RR; however, the opposite was observed at 28 DAP. The isopropylamine and ammonium salt formulations showed no effect on soybean yield as a function of application times in either experiment. The average yield of the experiments was lower compared to the soybean grain yield of soybean to previous years. This was due to the lack of precipitation in the first three weeks of January (Figure 1).

Similar result happened when isopropylamine salt application at stages V₆ and R₂ of the CD 219 RR[®] cultivar did not affect yield, but spraying at stage R₂ resulted in a 14% decline in the V-TOP[®] cultivar in relation to treatments applied at R₄ and R₅ (ZADINELLO et al., 2012). Applying 720 g a.e ha⁻¹ of isopropylamine, potassium and ammonium salt at 20, 35 and 50 DAP resulted in different yields in BRS 244 RR[®] and M-SOY 7979RR[®], with a 20% decrease in the former (AGOSTINETTO et al., 2009).

Table 1. Yield (kg ha⁻¹) of the soybean cultivars BMX Ativa RR and BMX Apolo RR as a function of glyphosate isopropylamine, potassium and ammonium salt application at 0, 14, 28 and 42 days after planting.

Variety	Days After Planting							
	0 Day		14 Days		28 Days		42 Days	
	Isopropylamine Salt							
BMX Ativa RR	1361.05	ns	1434.34		1624.79		1786.83	
BMX Apolo RR	1639.84		1332.85		1293.91		1932.66	
	Potassium Salt							
BMX Ativa RR	1499.43	aAB	1147.55	bB	1753.74	aA	2002.43	aA
BMX Apolo RR	1855.81	aA	1700.87	aAB	1184.49	bB	1925.35	aA
	Ammonium Salt							
BMX Ativa RR	1751.79	ns	1890.59		2381.06		1948.74	
BMX Apolo RR	1824.32		2571.49		2508.73		2087.20	

Means followed by the same lower case letter in the column do not differ according to Tukey's test at 5%.

There were no differences between the isopropylamine and ammonium salt formulations for interaction between dose and application times (Table 2). A difference in yield was observed for potassium salt as a function of dose (Table 2), with lower yields in BMX Ativa RR[®] at 28 DAP for a dose of 720 g a.e ha⁻¹ in relation to 1440 g a.e ha⁻¹. For the BMX Apolo RR[®] cultivar at 14 and 42 DAP, yield was lower when 720 g a.e ha⁻¹ was applied than 1440 g a.e ha⁻¹. However, at 0 DAP yield was higher at a dose of 720 g a.e ha⁻¹ (Table 2).

In an experiment with CD 214 RR[®], in which 720 and 1440 g a.e ha⁻¹ of isopropylamine salt was applied at 25 DAP, no differences in yield were observed (ALBRECHT et al., 2014). Similarly, a study with the M-SOY 8888-RR soybean cultivar found no differences between treatments of 720 and 1440 g a.e ha⁻¹ of isopropylamine salt applied 31 days after emergence (FOLONI et al., 2005). Moreover, no yield differences were reported in a study with the CD 219 RR[®] cultivar in which 1440 g a.e ha⁻¹ of Roundup Original[®] herbicide was applied at stages V₆ and R₂. However, for each additional gram of acid equivalent applied per hectare, yield declined by 0.4 kg ha⁻¹ (ALBRECHT et al., 2011).

A 720 g a.e ha⁻¹ dose of isopropylamine and potassium salt formulations at 14 and 28 DAP reduced soybean yield when compared to ammonium salt application. Probably, under these conditions, isopropylamine and potassium salts allow greater glyphosate absorption in the plant and consequently present higher toxicity. However no differences were observed between glyphosate formulations for 1440 g a.e ha⁻¹ (Table 3). In a greenhouse-based study with the M-SOY 8925 RR cultivar, 0; 500; 1000; 1500 and 2000 g a.e ha⁻¹ of isopropylamine and potassium salt were sprayed onto plants at 41 DAP, with no differences between the doses and salts applied (REIS et al., 2010). In the M-SOY 8867 RR cultivar, spraying 360 and 720 g a.e ha⁻¹ of glyphosate isopropylamine salt at planting showed crop selectivity, but 1440 g a.e ha⁻¹ reduced grain yield by 25% (PEREIRA et al., 2016).

Table 2. Yield (kg ha⁻¹) of the soybean cultivars BMX Ativa RR and BMX Apolo RR as a function of different doses of glyphosate acid equivalent applied at different times, using isopropylamine, potassium and ammonium salt formulations.

Days After Planting	Variety							
	BMX Ativa RR				BMX Apolo RR			
	Doses							
	720 g a.e ha ⁻¹		1440g a.e ha ⁻¹		720 g a.e ha ⁻¹		1440g a.e ha ⁻¹	
Isopropylamine Salt								
0 Day	1320.47	ns	1401.64		1454.24		1825.44	
14 Days	1524.31		1344.37		1383.14		1282.56	
28 Days	1371.61		1877.98		1310.66		1277.16	
42 Days	2128.46		1445.20		1743.00		2122.33	
Potassium Salt								
0 Day	1285.29	A	1713.58	A	2375.96	A	1335.67	B
14 Days	1119.92	A	1175.18	A	1331.57	B	2070.16	A
28 Days	1356.51	B	2150.97	A	1165.90	A	1203.09	A
42 Days	2057.79	A	1947.07	A	1414.56	B	2436.15	A
Ammonium Salt								
0 Day	1561.80	ns	1941.79		1687.49		1961.15	
14 Days	1517.39		2263.80		2484.49		2658.48	
28 Days	2811.05		1951.07		2670.86		2346.61	
42 Days	1277.83		2619.65		2166.49		2007.92	

Means followed by the same upper case letter in the row do not differ according to Tukey's test at 5%.

Table 3. Average yield (kg ha⁻¹) of the BMX Ativa RR and BMX Apolo RR soybean cultivars as a function of different glyphosate formulations (isopropylamine, potassium and ammonium salt) and application at 0, 14, 28 and 42 DAP in different acid equivalent doses.

Salt	Days After Planting							
	0 Day		14 Days		28 Days		42 Days	
	720 g a.e ha ⁻¹							
Isopropylamine Salt	1387.35	aB	1453.72	bAB	1341.14	bB	1935.73	aA
Potassium Salt	1830.62	aA	1225.74	bC	1261.20	bBC	1736.17	aAB
Ammonium Salt	1624.64	aB	2000.94	aB	2740.95	aA	1722.16	aB
1440g a.e ha ⁻¹								
Isopropylamine Salt	1613.54	ns	1313.46		1577.57		1783.76	
Potassium Salt	1524.62		1622.67		1677.03		2191.61	
Ammonium Salt	1951.47		2461.14		2148.84		2313.78	

Means followed by the same lower case letter in the column and upper case letter in the column do not differ according to Tukey's test at 5%.

Spray application of glyphosate isopropylamine salt showed no toxicity to the crop as a function of application times (Figure 2A), but significant effects were observed for potassium and ammonium salts (Figures 2B and 2C, respectively). The highest selectivity was observed for potassium salt application at 0 and 42 DAP and ammonium salt at 14, 28 and 42 DAP. With respect to the individual effect of the dose for each type of glyphosate salt, differences were only observed for potassium salt (Figures 2D to 2F). Glyphosate

potassium salt at 720 g a.e ha⁻¹ reduced the soybean grain yield compared with 1440 g a.e ha⁻¹ dose. This kind of result could be expected in a scenario with high weed infestation. Because, the lower dose means less weed control and consequently greater interference. However, the experiments were performed in areas with low infestation and weeds were controlled weekly. Doses of 720 g a.e ha⁻¹ and 1440 g a.e ha⁻¹ of isopropylamine or potassium salt applied at 25 DAP did not differ in CD 214 RR cultivar (ALBRECHT et al., 2014).

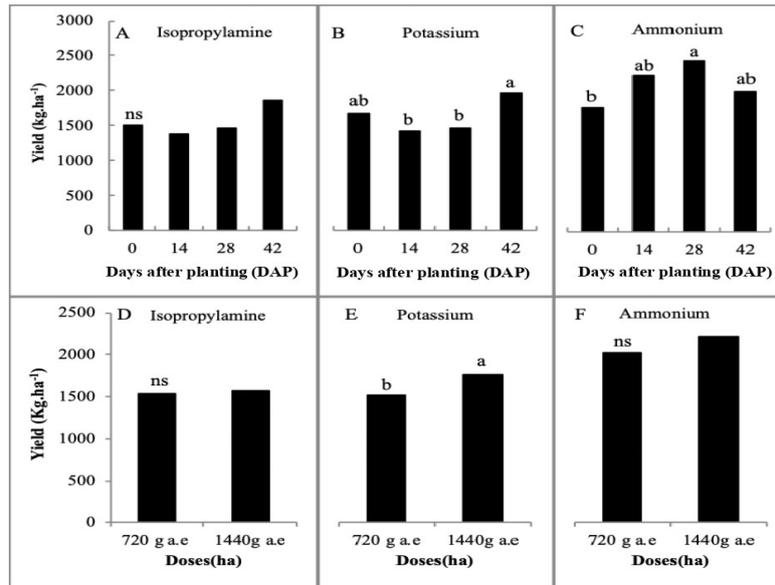


Figure 2. Yield (kg ha⁻¹) (average of two experiments) as a function of different application times and acid equivalent doses, using different glyphosate formulations (isopropylamine salt (A and D), potassium salt (B and E) and ammonium salt (C and F)).

BMX Ativa RR[®] exhibited no differences in glyphosate tolerance as a function of the type of salt applied (Figure 3A), while isopropylamine and potassium salt were the least selective for BMX Apolo RR[®] (Figure 3B). The effects of glyphosate isopropylamine salt differ between the M-SOY 8585 RR, P98R91 RR, Valiosa RR, CD 219 RR and TMG 108 RR cultivars (PETTER et al., 2007). Isopropylamine and ammonium salt formulations

(720 g a.e ha⁻¹) applied to TMG 125 RR at 25 DAP in two growing seasons showed no heterogeneity (REIS et al., 2014). Similarly, in an experiment with CD 214 RR involving the application of 720 g a.e ha⁻¹ and 1440 g a.e ha⁻¹ of isopropylamine and potassium salt at 25 DAP, no differences were observed between the salt types at 720 g a.e ha⁻¹ (ALBRECHT et al., 2014).

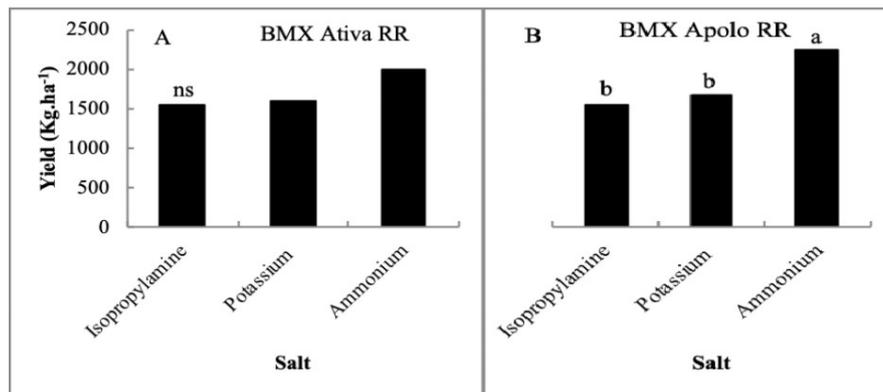


Figure 3. Yield of BMX Ativa RR (C) and BMX Apolo RR (D) as a function of different glyphosate salts.

Contrasting results have been observed for glyphosate selectivity to soybean cultivars. Glyphosate tolerance in soybean varies as a function of the cultivar used, dose, type of salt and application time. The divergent results in the literature may be related to the environment. Well-adapted species or cultivars under optimal nutritional conditions are better able to tolerate stress (TAIZ; ZEIGER, 2009). Greater phosphorus availability in the soil reduced phytointoxication by

glyphosate isopropylamine salt at doses of 900 and 1800 g a.e ha⁻¹ in the TMG 132 RR soybean cultivar (CASONATTO et al., 2014). In this respect, management practices that contribute to crop development and control weed populations tend to mitigate the unwanted effects of glyphosate herbicides. In addition to reducing weed populations and thereby lowering the glyphosate doses needed, the use of winter cover crops improves soil fertility

and helps retain water in the surface layers, contributing to better crop growth.

14 and 28 DAP caused greater toxicity in both the soybean cultivars analyzed. No differences were observed between 720 and 1440 g a.e ha⁻¹ doses for the isopropylamine and ammonium salts.

CONCLUSION

Glyphosate potassium salt resulted in lower soybean yields. Potassium salt application between

RESUMO: Diante da importância da cultura da soja tanto na economia mundial quanto para a alimentação, a melhoria das técnicas de cultivo torna-se um ponto fundamental na obtenção de maiores produtividades. Desta forma o objetivo do trabalho foi avaliar a produtividade de cultivares de soja sob a influência da aplicação de diferentes sais de glyphosate, doses e épocas de aplicação. Dois experimentos de campo foram realizados simultaneamente em delineamento de blocos casualizados com arranjo trifatorial (3x4x2) com três repetições. O fator A consistiu de três formulações de glyphosate: sal de isopropilamina (Roundup Original[®]), sal de amônio (Roundup WG[®]), sal de potássio (Zapp Qi[®]). O fator B consistiu de quatro épocas de aplicação: 0, 14, 28, 42 dias após a semeadura (DAS). O fator C consistiu em duas doses: a dose recomendada de 720 gramas de equivalente ácido por hectare e duas vezes a dose recomendada com 1440 gramas de equivalente ácido por hectare. Para a cultivar BMX Ativa RR[®] não se observou diferença quanto aos sais, porém a cultivar BMX Apolo RR[®] submetida a aplicação de sal de amônio demonstrou uma produtividade 28% superior em relação aos demais sais. O glyphosate na formulação sal de potássio apresentou redução da produtividade da cultura da soja. Aplicações de glyphosate na formulação sal de potássio realizadas entre os 14 e 28 dias após a semeadura causam maior toxidez em ambas cultivares de soja utilizadas.

PALAVRAS-CHAVE: Sal de isopropilamina. Sal de potássio. Sal de amônio. Hábito de crescimento. *Glycine max*.

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