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Effect of glyphosate management, formulations and rates on the agronomic performance of maize with the *cp4epsps* gene[1]

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ABSTRACT - Second-generation glyphosate-tolerant maize (Roundup Ready 2, RR2) is characterised by the expression of the *cp4epsps* gene. Despite some studies suggesting possible undesirable effects from the application of glyphosate in RR2 maize, other reports have demonstrated glyphosate selectivity for RR2 plants, with minor damage symptoms, if any, and with no negative effects on the chlorophyll indices. The aim of this study was to evaluate the effect of different types of glyphosate management, formulations and rates of application on chlorophyll indices and the agronomic performance of an RR2 maize genotype expressing the *cp4epsps* gene. Five experiments were conducted in a full factorial design (2 x 5 x 5) to evaluate two types of management, two formulations and five rates of glyphosate. Damage symptoms, chlorophyll indices and variables related to agronomic performance (plant height, stem diameter, yield and 100-grain weight) were evaluated. Similar crop responses were found for each of the glyphosate formulations (potassium salt and isopropylamine salt) and types of management (single application and sequential application) under test, indicating that the use of glyphosate-based commercial products affords flexibility in terms of formulation and frequency of application. Glyphosate formulated as potassium salt or isopropylamine salt and applied to RR2 maize either in a single application (full rate) or in two sequential applications (each at half rate) has a similar effect and may therefore be used without distinction. However, it is important to consider the rate of application since high rates of glyphosate (especially greater than 1,440 g ae ha^{-1}) may affect the development and production of RR2 maize.

Key words: EPSPs inhibitors. Glyphosate-tolerant maize. Herbicide selectivity. *Zea mays* L. Maize yield.

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INTRODUCTION

Second-generation glyphosate-tolerant maize is represented by the NK603 event (Roundup Ready™ 2 - RR2), approved in the United States and Brazil in 2000 and 2008, respectively, and the MON87427 event (Roundup Ready™), first approved in the United States (2013) and later (2016) in Brazil (INTERNATIONAL SERVICE FOR THE ACQUISITION OF AGRI-BIOTECH APPLICATIONS, 2022). Glyphosate tolerance is conferred by expression of the *cp4epsps* gene, derived from the CP4 strain of *Agrobacterium tumefaciens*. This gene encodes a 5-enolpyruvylshikimate-3-phosphate synthase (EPSPs), not sensitive to glyphosate (HECK *et al*., 2005). Glyphosate is mainly used as a post-emergent herbicide, targeting the EPSPs enzyme.

With the example of soybean expressing the *cp4epsps* gene, more research was carried out into the crop. It has been suggested that glyphosate may have a negative effect on several soybean parameters (ALBRECHT *et al*., 2018; SILVA *et al*., 2018). In various studies into RR2 maize, undesirable effects following glyphosate application, alone or in mixtures, were also detected: these included changes in the nutrient content of the leaves (OSÓRIO *et al*., 2015), plant height and reduced growth (ALBRECHT *et al*., 2017). A reduction in grain yield (CORREIA; SANTOS, 2013), visible crop damage (ARAÚJO *et al*., 2021; SILVA *et al*., 2020) and reduced biomass accumulation (CARVALHO *et al*., 2015) were also documented, as have disruptions in nutrient availability in the rhizosphere (JENKINS *et al*., 2017) and the rhizobacterial community (BARRIUSSO; MELLADO, 2012).

However, other studies have demonstrated glyphosate selectivity for RR2 maize plants, which resulted in minor damage symptoms, if any, with no negative effects on chlorophyll indices (GIOVANELLI *et al*., 2018; KRENCHINSKI *et al*., 2018; SILVA *et al*., 2017). In addition, Reddy, Bellaloui and Zablotowicz (2010) saw no damage symptoms, changes in RR2 maize yield or seed composition following glyphosate application; however, they did find a reduction in nitrate reductase activity.

It should also be noted that due to differences in other product components (e.g. adjuvants or salts), each glyphosate formulation is expected to influence the rate of uptake and translocation directly, and consequently, the activity of the herbicide (REIS *et al*., 2014). In this respect, while some reports showed that at least one glyphosate formulation was involved in the occurrence of undesirable effects in RR soybean, particularly visible damage (MAHONEY *et al*., 2014), other studies revealed that the type of glyphosate formulation was unrelated to the occurrence of adverse any effects in the crop (ALBRECHT *et al*., 2018) or in RR2 maize (MAHONEY *et al*., 2014). For these studies, damage was more related to increases in the rate of glyphosate than the type of formulation.

Regarding the undesirable effects of glyphosate formulations and rates of application on RR2 maize, it is believed that the rates may affect the agronomic performance of maize plants.

The aim of the present study was to evaluate the effect of glyphosate management, formulation and rate of application on chlorophyll indices and the agronomic performance of RR2 maize (with the *cp4epsps* gene).

MATERIAL AND METHODS

The present study consisted of five field experiments conducted in the western part of the state of Paraná, Brazil. This region is well known for its high productive potential for cultivating maize as a first- or second-season crop among other economically important annual crops. The characteristics of the experimental sites are shown in Table 1.

In this region, the climate, according to the Köppen classification is type Cfa – subtropical humid mesothermal, with hot summers, a low frequency of severe frosts and a tendency for rain to concentrate during the summer. The soil at each site is of a very clayey texture. The meteorological conditions during the period of cultivation were close to the regional historical averages for the five

Table 1 - General aspects of the experiments

Experiment	Period	Locality	Coordinates	Altitude
Exp. I	Oct 2012 to Jan 2013	Palotina	24°34’S 53°85’W	329 m
Exp. II	Jan 2013 to Apr 2013		24°33’S 53°84’W	
Exp. III	Oct 2013 to Jan 2014		24°34’S 53°85’W	
Exp. IV	Jan 2014 to Apr 2014		24°35’S 53°86’W	
Exp. V	Oct 2012 to Jan 2013	Marechal Cândido Rondon	24°41’S 54°06’W	285 m

experiments, showing considerable rainfall and regular temperatures. The 30F53HR single hybrid was used in each of the experiments; this maize hybrid is tolerant to glyphosate due to the expression of the *cp4epsps* gene, and is also one of the most cultivated in Brazil. A no-till system was adopted, with plots comprising six rows, 5 m in length, with 0.5 m between rows. The data were collected from plants growing in the four central rows of each plot.

Each of the five field experiments was conducted in a randomised block design with four replications. The treatments were arranged in a 2 x 2 x 5 full factorial design (management x formulation x rate), totalling 80 plots for each field experiment. Management 1 consisted of a single application of glyphosate at stage V_4. Under Management 2, two sequential applications of glyphosate were made, the first at stage V_4 and the second close to stage V_6, 15 days after the first application.

The glyphosate formulations under test were isopropylamine salt (Roundup Ready®, 480 g ae L^{-1}, Monsanto do Brasil Ltda., Brazil) and potassium salt (Zapp® QI 620, 500 g ae L^{-1}, Syngenta Proteção de Cultivos Ltda., Brazil). The following total rates of glyphosate (rate factor) were evaluated: 0, 720, 1440, 2160 and 2880 g ae ha^{-1}. Under Management 1, the total rate was applied at stageV_4, while under Management 2, the total rate was divided into two applications (e.g. the rate of 720 g ae ha^{-1} was divided into two applications, each of 360 g ae ha^{-1}).

A CO_2 pressurised backpack sprayer equipped with a bar containing six fan nozzles (XR 110.02, Teejet® Technologies South America, Brazil) was used for applying the glyphosate. The herbicide was sprayed at a constant pressure of 2 bar and flow rate of 0.65 L min^{-1}. Sprayed at a height of 50 cm above the target and a speed of 1 m s^{-1}, the application covered an area 50 cm in width, providing a spray rate of 200 L ha^{-1}. The experimental areas were kept free of weeds by manual weeding.

Damage symptoms were visually evaluated 7, 14, 21 and 28 days after application (DAA), when each experimental unit was checked and a score assigned based on the observed damage intensity (0% for no damage up to 100% for plant death) (VELINI; OSIPE; GAZZIERO, 1995). The Falker chlorophyll indices for chlorophyll A, chlorophyll B and total chlorophyll were measured during stage R1 using an electronic chlorophyll meter (clorofiLOG - CFL1030, Falker Automação Agrícola Ltda., Brazil). Measurements were taken on the leaf opposite the ear, sampling 10 plants per plot.

The agronomic performance of the maize was evaluated by measuring the following parameters in 10 plants per plot: stem diameter (4.0 cm above the ground), height of the ear insertion, plant height (from the ground to the tassel insertion), yield and 100-grain weight. The measurements were taken shortly after physiological maturity (R_6).

To estimate yield, maize ears were harvested manually, threshed, cleaned with the aid of sieves and packed in paper bags. The 100-grain weight was then determined by weighing four 100-grain samples collected from each plot. The moisture content of the harvested material was corrected to 13% to express the yield and 100-grain weight.

The data from five field experiments were analysed separately. The basic assumptions for analysis of variance (ANOVA) were tested ($p \leq 0.05$). The data were submitted to ANOVA to evaluate the glyphosate management and formulations (qualitative factors); the F-test was used to compare the mean values ($p \leq 0.05$) using the Sisvar 5.6 software (FERREIRA, 2011).

Regression analysis was applied ($p \leq 0.05$) to evaluate the rates of glyphosate application (quantitative factor). The SigmaPlot® 13 software (Systat Software Inc.) was used to choose the best regression model considering the following fit criteria: biological explanation, significant regression, non-significant regression deviation and coefficient of determination. To prepare the figures, the Microsoft 365 Excel® (Microsoft Corp.) software was used.

RESULTS AND DISCUSSION

No signs of crop damage were found in any of the evaluations carried out across the five experiments; also, for each rate under test, there was no difference between the glyphosate formulations or types of management for the Falker chlorophyll indices in Experiments I and III. The same occurred for the chlorophyll A index in Experiments II and IV and for the chlorophyll B index in Experiment IV ($p > 0.05$) (data not shown). Although there were some differences for the other chlorophyll indices in Experiments II, IV and V (Table 2), no formulation or management could be associated with more-pronounced negative effects on these parameters. Similarly, the rate of application had no significant effect on any of the above physiological parameters ($p > 0.05$).

The agronomic parameters, height of the ear insertion (Experiments I and III), stem diameter (Experiments I, II and IV), yield (Experiment III) and 100-grain weight (Experiment IV) showed no differences when comparing the glyphosate formulations, types of management or rates of application; there was also no interaction between these factors ($p > 0.05$) (data not shown). Differences in plant height were detected across the five experiments (Table 3), while the height of the ear insertion varied significantly only in Experiments II, IV and V (Table 4). Although some differences were detected after certain interactions had been broken down, it was not possible to establish whether any management or formulation had affected these agronomic parameters. The same can be said for stem diameter when comparing Experiments III and V (Table 5). It is worth mentioning the fit of the linear model, showing a decreasing effect on plant height for an increase in the rates of glyphosate (isopropylamine salt under both managements) in Experiment III (Figure 1a) and for isopropylamine salt in Experiment V (Figure 1b).

Table 2 - Chlorophyll index (A, B and total) in RR2 maize plants under glyphosate application

Rate	Chlorophyll A (Exp. V)				Chlorophyll B (Exp II)			
	Isopropylamine salt		Potassium salt		Isopropylamine salt		Potassium salt	
	M 1	M 2	M 1	M 2	M 1	M 2	M 1	M 2
0	41.0 Aa	39.5 Ab	40.6 Aa	40.3 Aa	14.1 Aa	14.1 Aa	14.8 Aa	14.4 Aa
720	41.0 Aa	41.6 Aa	40.1 Aa	40.6 Aa	14.2 Aa	15.0 Aa	14.5 Aa	14.8 Aa
1.440	41.4 Aa	41.2 Aa	40.3 Aa	40.1 Aa	14.7 Aa	14.2 Aa	14.5 Aa	15.0 Aa
2.160	39.9 Ba	41.0 Aa	42.0 Aa	41.3 Aa	14.4 Ba	14.8 Aa	16.1 Aa	15.3 Aa
2.880	40.6 Aa	40.3 Aa	41.1 Aa	40.2 Aa	14.6 Aa	15.1 Aa	15.1 Aa	13.9 Bb
Rate	Chlorophyll B (Exp. V)				Total Chlorophyll (Exp. II)			
	Isopropylamine salt		Potassium salt		Isopropylamine salt		Potassium salt	
	M 1	M 2	M 1	M 2	M 1	M 2	M 1	M 2
0	13.4 Aa	11.9 Bb	13.3 Aa	13.1 Aa	53.9 Aa	53.8 Aa	55.2 Aa	54.5 Aa
720	13.6 Aa	14.2 Aa	12.8 Aa	13.2 Aa	53.9 Ab	55.8 Aa	54.6 Aa	54.8 Aa
1.440	13.6 Aa	13.6 Aa	12.5 Bb	13.9 Aa	54.9 Aa	53.7 Aa	53.9 Aa	55.2 Aa
2.160	12.8 Ba	13.2 Aa	14.7 Aa	14.0 Aa	54.5 Ba	55.4 Aa	57.3 Aa	56.0 Aa
2.880	13.2 Aa	13.4 Aa	13.1 Aa	12.7 Aa	54.8 Aa	54.8 Aa	55.9 Aa	53.9 Ab
Rate	Chlorophyll index (Exp. IV)				Chlorophyll index (Exp. V)			
	Isopropylamine salt		Potassium salt		Isopropylamine salt		Potassium salt	
	M 1	M 2	M 1	M 2	M 1	M 2	M 1	M 2
0	56.0 Aa	55.2 Aa	55.3 Aa	54.1 Aa	54.3 Aa	51.4 Ab	53.9 Aa	53.4 Aa
720	55.8 Aa	55.9 Aa	55.6 Aa	54.1 Aa	54.6 Aa	55.9 Aa	52.9 Aa	53.8 Aa
1.440	57.9 Aa	57.7 Aa	55.1 Ba	56.4 Aa	55.0 Aa	54.8 Aa	52.9 Aa	54.0 Aa
2.160	54.9 Aa	56.0 Aa	55.7 Aa	57.1 Aa	52.6 Ba	54.2 Aa	56.7 Aa	55.3 Aa
2.880	55.5 Aa	57.0 Aa	54.7 Aa	55.6 Aa	53.8 Aa	53.7 Aa	54.2 Aa	52.8 Aa

The same uppercase letters on a line between the formulations within each management and rate do not differ significantly ($p \leq 0.05$) by F-test. The same lowercase letters on a line between managements (single application - M1 and sequential application - M2) within each formulation and rate do not differ significantly ($p \leq 0.05$) by F-test

Table 3 - Height (cm) of RR2 maize plants under glyphosate application

Rate	Exp. I				Exp. II			
	Isopropylamine salt		Potassium salt		Isopropylamine salt		Potassium salt	
	M 1	M 2	M 1	M 2	M 1	M 2	M 1	M 2
0	177.0 Aa	175.1 Aa	177.8 Aa	176.1 Aa	185.1 Aa	182.6 Ba	183.3 Aa	187.0 Aa
720	180.5 Aa	176.3 Aa	177.3 Aa	178.4 Aa	185.6 Aa	184.1 Aa	182.7 Aa	180.5 Aa
1.440	177.5 Aa	174.9 Aa	182.1 Aa	175.5 Ab	183.8 Aa	184.0 Aa	184.2 Aa	186.6 Aa
2,160	178.3 Aa	178.2 Aa	175.3 Aa	175.4 Aa	181.1 Aa	183.3 Aa	184.1 Aa	178.2 Bb
2.880	176.9 Aa	179.3 Aa	178.8 Aa	180.0 Aa	183.3 Aa	182.3 Aa	185.1 Aa	181.2 Aa

Continuation Table 3

Rate	Exp. III				Exp. IV			
	Isopropylamine salt		Potassium salt		Isopropylamine salt		Potassium salt	
	M 1	M 2	M 1	M 2	M 1	M 2	M 1	M 2
0	186.1 Aa	183.4 Aa	183.2 Aa	187.0 Aa	206.6 Aa	204.6 Aa	198.1 Bb	207.6 Aa
720	182.5 Aa	184.3 Aa	178.2 Aa	177.2 Ba	205.5 Aa	207.7 Aa	204.1 Aa	204.0 Aa
1.440	176.9 Aa	180.3 Aa	180.0 Aa	176.6 Aa	203.3 Aa	200.2 Aa	204.1 Aa	200.8 Aa
2.160	179.9 Aa	178.7 Aa	182.1 Aa	182.9 Aa	201.9 Ba	204.8 Aa	210.6 Aa	209.7 Aa
2.880	178.3 Aa	176.2 Aa	180.9 Aa	178.9 Aa	206.9 Aa	199.2 Bb	209.7 Aa	207.5 Aa

Rate	Exp. V			
	Isopropylamine salt		Potassium salt	
	M 1	M 2	M 1	M 2
0	193.3 Aa	190.2 Aa	191.4 Aa	197.3 Aa
720	190.7 Aa	192.0 Aa	188.2 Aa	182.6 Ba
1.440	190.2 Aa	193.2 Aa	186.4 Ab	197.8 Aa
2.160	184.0 Ba	188.5 Aa	192.9 Aa	181.1 Ab
2.880	189.7 Aa	185.3 Aa	191.4 Aa	182.5 Ab

The same uppercase letters on a line between the formulations within each management and rate do not differ significantly ($p \leq 0.05$) by F-test. The same lowercase letters on a line between managements (single application - M1 and sequential application - M2) within each formulation and rate do not differ significantly ($p \leq 0.05$) by F-test

Table 4 – Height of the ear insertion (cm) in RR2 maize plants under glyphosate application

Rate	Exp. II				Exp. IV			
	Isopropylamine salt		Potassium salt		Isopropylamine salt		Potassium salt	
	M 1	M 2	M 1	M 2	M 1	M 2	M 1	M 2
0	100.3 Aa	99.2 Aa	99.6 Aa	99.1 Aa	110.7 Aa	111.9 Aa	104.9 Bb	110.5 Aa
720	99.1 Aa	101.8 Aa	99.5 Aa	98.5 Aa	109.5 Aa	111.7 Aa	111.7 Aa	110.0 Aa
1.440	100.2 Aa	99.9 Aa	100.3 Aa	103.7 Aa	106.1 Aa	110.9 Aa	108.9 Aa	106.1 Aa
2.160	97.3 Bb	102.9 Aa	102.2 Aa	98.4 Aa	105.5 Ba	110.7 Aa	113.7 Aa	112.4 Aa
2.880	99.0 Aa	97.2 Aa	100.3 Aa	97.8 Aa	110.8 Aa	108.4 Ba	110.0 Aa	114.5 Aa

Rate	Exp. V			
	Isopropylamine salt		Potassium salt	
	M 1	M 2	M 1	M 2
0	103.5 Aa	99.9 Aa	101.5 Aa	102.7 Aa
720	99.9 Aa	103.9 Aa	100.0 Aa	99.4 Aa
1440	103.8 Aa	101.5 Aa	101.3 Aa	106.0 Aa
2160	94.6 Bb	103.9 Aa	103.1 Aa	95.5 Bb
2880	99.1 Aa	98.1 Aa	100.9 Aa	96.5 Aa

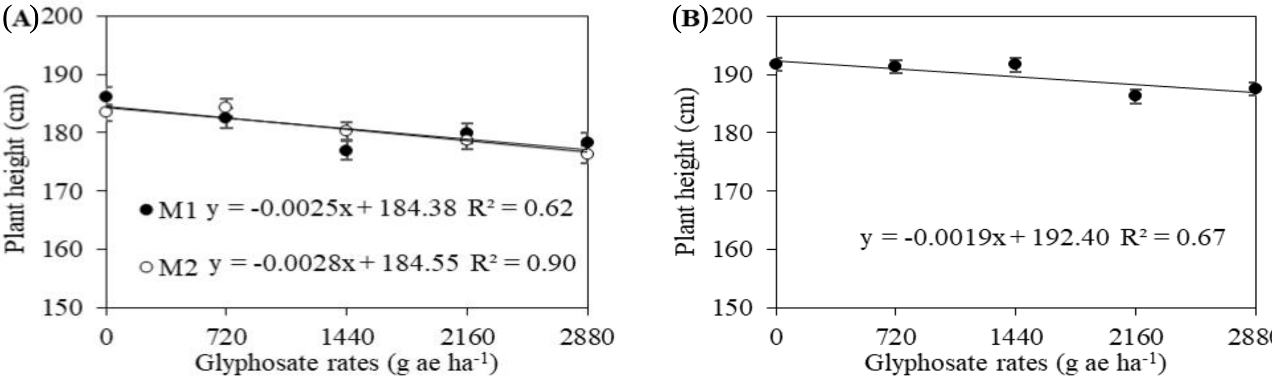
The same uppercase letters on a line between the formulations within each management and rate do not differ significantly ($p \leq 0.05$) by F-test. The same lowercase letters on a line between managements (single application - M1 and sequential application - M2) within each formulation and rate do not differ significantly ($p \leq 0.05$) by F-test

Table 5 - Stem diameter (mm) in RR2 maize plants under glyphosate application

Rate	Exp. III				Exp. V			
	Isopropylamine salt		Potassium salt		Isopropylamine salt		Potassium salt	
	M 1	M 2	M 1	M 2	M 1	M 2	M 1	M 2
0	24.8 Aa	23.6 Bb	23.8 Ba	24.6 Aa	23.6 Aa	23.7 Aa	23.2 Aa	24.7 Aa
720	24.2 Aa	24.2 Aa	24.0 Aa	24.6 Aa	23.6 Aa	23.6 Aa	23.4 Aa	22.4 Aa
1.440	24.5 Aa	24.2 Aa	23.9 Aa	24.5 Aa	23.8 Aa	23.2 Aa	24.0 Aa	23.3 Aa
2.160	24.5 Aa	24.4 Aa	24.1 Aa	24.5 Aa	23.5 Aa	22.2 Ba	23.6 Aa	23.9 Aa
2.880	23.9 Aa	24.3 Aa	24.5 Aa	24.2 Aa	24.1 Aa	23.6 Aa	24.5 Aa	23.4 Aa

The same uppercase letters on a line between the formulations within each management and rate do not differ significantly ($p \leq 0.05$) by F-test. The same lowercase letters on a line between managements (single application - M1 and sequential application - M2) within each formulation and rate do not differ significantly ($p \leq 0.05$) by F-test

Figure 1 - Plant height under rates of glyphosate-isopropylamine salt for Management 1 and Management 2 in Exp. III (A). Plant height under rates of glyphosate-isopropylamine salt in Exp. V (B). Vertical bars, at the mean values show the standard error



The regression analysis indicated a significant effect ($p \leq 0.05$) from the rates of glyphosate (isopropylamine salt) on maize yield in Experiment II that reduced as the rate increased (Figure 2a). There was also a reduction in maize yield with the increasing rates of potassium salt (Management 1) in Experiment IV (Figure 2b) as well as for the isopropylamine salt (Management 2) in Experiment V (Figure 2c). A reduction was also seen in the 100-grain weight with increasing rates of isopropylamine salt (Management 1) in Experiment II (Figure 2d). The rate of application had no significant effect on the other agronomic parameters under evaluation ($p > 0.05$).

Only in Experiments III and IV were there no differences found between treatments for yield (Table 6) or 100-grain weight (Table 7), respectively. Similar to the other variables, and despite some differences, it was not possible to establish whether any of the glyphosate managements or formulations led to changes in yield or in the 100-grain weight. However, a reduction in yield related to increases in the rate of glyphosate were seen for the isopropylamine salt (Experiment II) and the potassium salt (Experiment IV), while in Experiment V, the same pattern was seen for the isopropylamine salt under Management 2. Based on the above results, it can be said that, irrespective of the glyphosate formulation or management, the herbicide can hamper RR2 maize yield if applied at rates greater than 1,440 g ae ha^{-1}.

Based on the results, no one glyphosate formulation or management can be considered more harmful to RR2 maize than another. The observed differences did not follow any defined pattern and the regression analysis showed no defined patterns that could distinguish between the single and sequential applications, confirming the results of comparing the individual mean values. Previous studies into RR2 maize have shown no difference between single and sequential glyphosate applications (OSÓRIO *et al*., 2015).

Figure 2 - Maize yield under rates of glyphosate-isopropylamine salt in Exp. II (A). Maize yield under rates of glyphosate-potassium salt for Management 1 in Exp. IV (B). Maize yield under rates of glyphosate-isopropylamine salt under Management 2 in Exp. V (C). 100-grain weight under rates of glyphosate and isopropylamine salt for Management 1 in Exp. II (D). Vertical bars at the mean values show the standard error

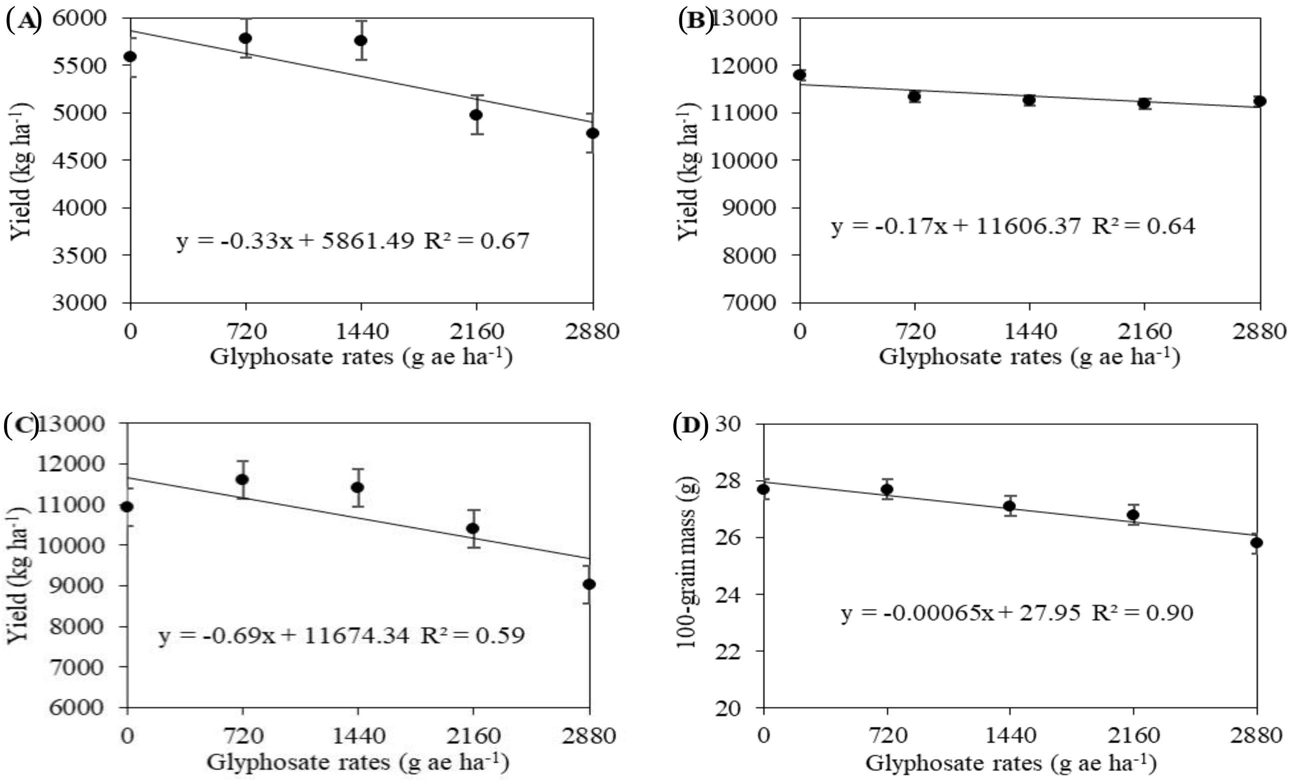


Table 6 - Yield (kg ha^{-1}) in RR2 maize plants under glyphosate application

Rate	Exp. I				Exp. II			
	Isopropylamine salt		Potassium salt		Isopropylamine salt		Potassium salt	
	M 1	M 2	M 1	M 2	M 1	M 2	M 1	M 2
0	10.689 Aa	11.078 Aa	10.330 Aa	9.340 Ba	5.974 Aa	5.193 Aa	5.647 Aa	5.401 Aa
720	11.389 Aa	11.683 Aa	11.178 Aa	10.388 Aa	6.228 Aa	5.347 Aa	5.938 Aa	4.300 Ab
1.440	11.762 Aa	10.667 Aa	10.335 Ba	10.121 Aa	5.492 Aa	6.035 Aa	5.547 Aa	4.714 Ba
2.160	11.869 Aa	11.015 Aa	10.883 Aa	11.459 Aa	4.494 Ba	5.463 Aa	5.873 Aa	5.314 Aa
2.880	11.236 Aa	9.022 Bb	11.255 Aa	11.591Aa	5.363 Aa	4.206 Aa	5.304 Aa	5.223 Aa
Rate	Exp. IV				Exp. V			
	Isopropylamine salt		Potassium salt		Isopropylamine salt		Potassium salt	
	M 1	M 2	M 1	M 2	M 1	M 2	M 1	M 2
0	11.785 Aa	11.671 Aa	11.788 Aa	11.405 Aa	10.612 Aa	10.927 Aa	11.797 Aa	10.477 Ab
720	11.160 Aa	11.090 Ba	11.324 Aa	11.931 Aa	10.777 Aa	11.609 Aa	11.450 Aa	9.897 Bb
1.440	11.138 Aa	11.230 Aa	11.254 Aa	11.584 Aa	10.826 Aa	11.403 Aa	10.189 Aa	11.198 Aa
2.160	11.644 Aa	11.227 Aa	11.195 Aa	11.254 Aa	11.536 Aa	10.403 Aa	10.704 Aa	9.849 Aa
2.880	11.236 Aa	11.494 Aa	11.234 Aa	11.536 Aa	11.037 Ba	9.031 Ab	12.386 Aa	9.143 Ab

The same uppercase letters on a line between the formulations within each management and rate do not differ significantly ($p \leq 0.05$) by F-test. The same lowercase letters on a line between managements (single application - M1 and sequential application - M2) within each formulation and rate do not differ significantly ($p \leq 0.05$) by F-test

Table 7 - 100-grain weight (g) in RR2 maize plants under glyphosate application

Rate	Exp. I				Exp. II			
	Isopropylamine salt		Potassium salt		Isopropylamine salt		Potassium salt	
	M 1	M 2	M 1	M 2	M 1	M 2	M 1	M 2
0	38.9 Aa	36.3 Aa	36.9 Aa	37.3 Aa	27.7 Aa	27.1 Aa	27.5 Aa	27.7 Aa
720	38.1 Aa	37.7 Aa	38.0 Aa	36.7 Aa	27.7 Aa	27.2 Aa	27.5 Aa	27.7 Aa
1.440	37.0 Aa	35.6 Aa	37.4 Aa	37.6 Aa	27.1 Aa	27.3 Ba	27.8 Aa	27.1 Aa
2.160	36.7 Aa	36.6 Aa	38.2 Aa	37.2 Aa	26.8 Aa	26.2 Ba	27.7 Aa	26.8 Aa
2.880	37.0 Ba	39.1 Aa	41.7 Aa	36.9 Ab	25.8 Ab	27.5 Aa	26.6 Aa	25.8 Ab
Rate	Exp. III	Exp. V						
	Isopropylamine salt		Potassium salt		Isopropylamine salt		Potassium salt	
	M 1	M 2	M 1	M 2	M 1	M 2	M 1	M 2
0	37.5 Aa	36.7 Aa	36.5 Ab	37.8 Aa	34.9 Aa	34.9 Aa	35.3 Aa	34.6 Aa
720	36.5 Aa	37.3 Aa	36.7 Aa	36.9 Aa	33.8 Aa	33.7 Aa	34.8 Aa	33.3 Aa
1.440	36.1 Bb	37.4 Aa	37.9 Aa	36.7 Ab	34.0 Aa	35.6 Aa	33.5 Ab	35.5 Aa
2.160	36.8 Aa	37.5 Aa	37.1 Aa	37.1 Aa	34.0 Aa	33.6 Aa	34.1 Aa	34.1 Aa
2.880	36.4 Aa	36.3 Aa	37.4 Aa	36.8 Aa	33.8 Ab	35.4 Aa	35.2 Aa	34.3 Aa

The same uppercase letters on a line between the formulations within each management and rate do not differ significantly ($p \leq 0.05$) by F-test. The same lowercase letters on a line between managements (single application - M1 and sequential application - M2) within each formulation and rate do not differ significantly ($p \leq 0.05$) by F-test

When comparing the glyphosate formulations, there was a marked contrast between the two salts under evaluation for the number of adjustments, with the isopropylamine salt affording the greater number. Nevertheless, it would be contentious to state that this formulation would cause greater damage to RR2 maize than would the potassium salt, as several regressions have also shown harmful effects on important variables, including grain yield, after applying the potassium salt. It was therefore not possible to determine which formulation was more harmful.

The absence of any difference in the behaviour pattern of RR2 maize exposed to different glyphosate formulations has been suggested before. According to several reports, even when the glyphosate formulations varied in their chemical composition, including the type of salt, surfactants, inert ingredients and acid concentration, the response of the RR2 maize was generally similar (REIS *et al*., 2014; RODRIGUES; ALMEIDA, 2018; TRAVLOS; CHEIMONA; BILALIS, 2017).

Thus, these results show that the application of glyphosate-based herbicides in RR2 maize is flexible; nevertheless, it should be noted that the rate of glyphosate application was important and negative effects were seen when the rate exceeded 1,440 g ae ha^{-1}.

In the present study, glyphosate application in RR2 maize has rarely resulted in visible chlorosis or other damage symptoms, or such effects were temporary, with no consequences on the various chlorophyll indices. These findings are in line with those reported by Chahal and Jhala (2018), Giovanelli *et al.* (2018), Krenchinski *et al.* (2018), Langdon *et al*. (2020), Reddy, Bellaloui and Zablotowicz (2010), and Silva *et al*. (2017).

Other studies have shown that glyphosate is not harmful to RR2 maize. For example, Vieira Júnior *et al*. (2015), evaluating plant height and production components, found no adverse effects from the herbicide when applied at a rate of 1,296 g ae ha^{-1}, and Reddy Bellaloui and Zablotowicz (2010) reported no difference in yield (among other production components) or shikimate pathway levels for rates of up to 1,296 g ae ha^{-1}.

Other studies into RR2 maize have suggested possible undesirable effects from the application of glyphosate. Among these, changes in leaf nutrient concentrations (OSÓRIO *et al*., 2015), reduced plant height and impaired growth parameters when glyphosate was applied together with other herbicides (ALBRECHT *et al*., 2017). Reductions in biomass accumulation (CARVALHO *et al*., 2015) and grain yield (CORREIA; SANTOS, 2013), in addition to damage symptoms, were documented when glyphosate was applied mixed with other herbicides (ALBRECHT *et al*., 2017; SOLTANI; SHROPSHIRE; SIKKEMA, 2018).

The maximum rate recommended by the manufacturer (for the products used in this study) is 1,080 g ea ha^{-1} in a single application, or 1,680 g ae ha^{-1} divided into two applications, in post-emergent tolerant maize (RODRIGUES; ALMEIDA, 2018). As such, the present study indicates that even rates in line with the recommendations may have an undesirable effect on maize. However, it is important to point out that rates above the recommended rate, or even rates greater than 1,440 g ea ha^{-1}, albeit following the manufacturer's recommendations, are used under field conditions. This situation, found mainly in the management of resistant weeds that are difficult to control, is not ideal, and is sometimes ineffective even in the short term, increasing the problem of resistant weeds. In this respect, integrated weed-management is important, incorporating various control methods, such as pre-emergent and herbicide mixtures (WEHRMEISTER *et al*., 2022), herbicide crop rotation (SATORRE *et al*., 2020), cover crops (GOMES *et al*., 2022) and no-till systems (AKBARI *et al*., 2019).

There is a valuable research opportunity in this area, considering the success of this glyphosate-resistant genotype and the potential to induce glyphosate tolerance based on the introduction of other genes in addition to the *cp4epsps* gene. For instance, the *gdc-1* and *gdc-2* genes, which encode glyphosate-inactivating enzymes homologous to decarboxylases, have been suggested for this purpose (GREEN *et al*., 2008). Another example is the discovery of enzymes with a low affinity for the herbicide (glyphosate) and a high affinity for the substrate (phosphoenolpyruvate). According to Van de Berg *et al*. (2008), the *aroA1398* gene encodes an enzyme with increased tolerance to glyphosate (up to 800 times greater than the known $EPSP_S$). The introduction of this gene has already been tested in transgenic maize events, which have demonstrated complete tolerance, even when exposed to rates up to four times greater than the recommended rate. Among the transgenic events designed to confer tolerance to glyphosate and approved worldwide, six genes are responsible for the synthesis of glyphosate-insensitive enzymes: *cp4epsps*, *2mepsps*, *mepsps*, *epspsgrg23ace5*, *gat4621* and *gat4601* (INTERNATIONAL SERVICE FOR THE ACQUISITION OF AGRI-BIOTECH APPLICATIONS, 2022). The introduction of these new genes into maize and other crops may represent a milestone in the future of agriculture.

CONCLUSIONS

Glyphosate herbicide, formulated as either potassium salt or isopropylamine salt, has a similar effect when applied to RR2 maize, either in a single application (full rate) or in two sequential applications (each at half rate). However, it is important to be aware of the rate of application since high rates of glyphosate (especially greater than 1,440 g ae ha^{-1}) may affect the development and production of RR2 maize.

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