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Article

Efficacy of Control of Glyphosate-Tolerant Species of the Rubiaceae Family Through Double-Knockdown Applications

Eficácia do Controle de Espécies Tolerantes ao Glifosato da Família Rubiaceae Através de Aplicações em Golpe Duplo

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ABSTRACT - *Spermacoce latifolia*, *S. verticillata,* and *Richardia brasiliensis* (family Rubiaceae, tribe Spermacoceae) are glyphosate-tolerant weeds in the soybean producing areas of Brazil. The weed shifts to glyphosate-tolerant weeds across soybean-producing areas has shown the need for adoption of practices that conserve the efficacy of glyphosate. This study evaluated the effect of single- and double-knockdown herbicide applications on the control of *S. latifolia*, *S. verticillata,* and *R. brasiliensis* prior to soybean sowing. Trials were designed as a randomized block and treatments were arranged as a factorial. Factor A was three systemic herbicide treatments 10 days before sowing (DBS), while Factor B was three contact herbicide treatments applied 0 DBS ("sow and apply"). The single- and double-knockdown applications were followed by post-emergence applications of glyphosate or 2,4-D + glyphosate when the crop reached three leaves. The efficacy of control of each weed species was visually evaluated 14 days after sowing (DAS), as well as 0 and 28 days after post-emergence application (DAA). The double-knockdown applications generally provided higher levels of control for the target weeds of the Rubiaceae family than the single-knockdown applications. Glyphosate + 2,4-D, glyphosate + 2,4-D + diclosulam or glyphosate + [halauxifen-methyl + diclosulam] followed by glufosinate or paraquat achieved at least 90% of control of *S. latifolia* and *R. brasiliensis*, but did not control *S. verticillata* (<80%). Glufosinate at 457 g a.i. ha^{-1} was equivalent to paraquat at 400 g a.i. ha^{-1} as a contact herbicide in the double-knockdown applications, especially on the first assessment dates.

Keywords: 2,4-dichlorophenoxyacetic acid, ammonium glufosinate, *Spermacoce latifolia*, *Spermacoce verticillata*, *Richardia brasiliensis*.

*RESUMO - **Spermacoce latifolia, S. verticillata** e **Richardia brasiliensis** (família Rubiaceae, tribo Spermacoceae) são plantas daninhas tolerantes ao glyphosate nas áreas de soja do Brasil. Mudanças na flora daninha para espécies tolerantes ao glyphosate nas áreas de soja evidenciam a necessidade de adoção de práticas que conservem a eficácia desse herbicida. Este estudo avaliou o efeito de aplicações de herbicidas em golpe simples e duplo no controle de **S. latifolia, S. verticillata** e **R. brasiliensis** antes da semeadura da soja. O delineamento foi o de blocos casualizados, com tratamentos em esquema fatorial. O fator A constou de três tratamentos de herbicidas sistêmicos aos 10 dias antes do plantio (DBS), enquanto o fator B constou de três tratamentos de herbicidas de contato aplicado aos 0 DBS ("plante e aplique"). As aplicações em golpe simples e duplo foram seguidas por aplicações em pós-emergência de glyphosate ou 2,4-D + glyphosate quando a cultura da soja atingiu três folhas expandidas. A eficácia do controle de cada*

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espécie-alvo foi avaliada visualmente aos 14 dias após a semeadura (DAS), bem como aos 0 e 28 dias após a aplicação em pós-emergência (DAA) da cultura. As aplicações de golpe duplo geralmente proporcionaram maiores níveis de eficácia de controle para as plantas daninhas alvo da família Rubiaceae do que as aplicações em golpe simples. Glyphosate + 2,4-D, glyphosate + 2,4-D + diclosulam ou glyphosate + [halauxifen-metílico + diclosulam] seguido de glufosinato ou paraquat atingiram, pelo menos, 90% de controle de ***S. latifolia*** *e* ***R. brasiliensis****, mas não controlaram* ***S. verticillata*** *(<80%). O glufosinato a 457 g i.a. ha*$^{-1}$ *foi equivalente ao paraquat a 400 g i.a. ha*$^{-1}$ *como herbicida de contato nas aplicações em golpe duplo, especialmente nas primeiras avaliações.*

Palavras-chave: 2,4-diclorofenoxiacético, glufosinato de amônio, *Spermacoce latifolia*, *Spermacoce verticillata*, *Richardia brasiliensis*.

INTRODUCTION

The Rubiaceae is the fourth-largest family of the angiosperms by number of species, containing about 611 genera and approximately 13,100 species distributed throughout most of the world (Govaerts et al., 2007). In Brazil, 112 genera and 1,347 species of this family were found in surveys of natural vegetation, of which about 14 genera and 694 species are endemic to the country (Barbosa et al., 2015). Herbaceous species of Rubiaceae are also widely distributed in the agricultural areas of Brazil, especially those from the *Spermacoce* tribe (Ikeda et al., 2008; Marques et al., 2011). These species are generally tolerant of a broad array of environmental conditions such as acid soil, drought, and even soils highly contaminated by arsenic and iron ore (Campos et al., 2014). As a result, species of Spermacoce have been reported as important weeds in both annual and perennial crops due their adaptation to the climate and soil conditions of agroecosystems.

Soybean, *Glycine max* (L.) Merrill, has considerable economic, cultural and social importance in Brazil, where it was grown in over 34 million hectares in the 2016-2017 growing season (USDA, 2017). In Brazil, weed communities have shifted to species with tolerance to glyphosate due to the widespread adoption of glyphosate-tolerant soybeans (Galon et al., 2013; Takano et al., 2013). Among the glyphosate-tolerant weeds that occur in the soybean areas, *Spermacoce latifolia* (Aubl.) and *S. verticillata* (L.) are some of the most common species of the Spermacoce tribe. Another common hard-to-kill weed species of this tribe is *Richardia brasiliensis* (Gomes), which is widely distributed in soybean areas across southern, southeastern, and midwestern Brazil. As glyphosate does not effectively control these tolerant weeds, other herbicide modes of action must be mixed with glyphosate (Vidal et al., 2010).

The weeds' tolerance to herbicides generally may be explained by differential herbicide uptake, translocation, metabolization, compartmentalization, and affinity with the specific site of action (Galon et al., 2009). Also, the level of insensibility can be influenced by many factors such as weed growth stage and weather conditions, among others, which interact with the weeds' tolerance mechanisms. In *S. latifolia*, the use of ^{14}C-glyphosate showed that about 89% of the herbicide remained in the treated leaf and only 2% reached the plant roots by 72 hours after treatment (Galon et al., 2013). Thus, it was concluded that *S. latifolia* showed lower glyphosate translocation into the plant, illustrating at least one of the mechanisms that provide greater tolerance to this herbicide. The same tolerance mechanism to glyphosate was found in *Ipomoea grandifolia* (Dammer), while *Commelina benghalensis* (L.) had differential metabolization (Monquero et al., 2004).

The rapidly weed shifting to glyphosate-tolerant weed species throughout the soybean-producing areas of Brazil has shown the need for adoption of practices that would conserve the glyphosate efficacy. "Double-knockdown" applications have been a strategy for the control of glyphosate-resistant weeds as well as for the delay their herbicide resistance (Neve et al., 2003; Weersink et al., 2005). This technique involves a burndown application of systemic herbicide (e.g., glyphosate) followed by a subsequent herbicide treatment of a non selective contact herbicide (e.g., paraquat). Glyphosate followed by paraquat + diquat with 2-10 days interval between sprays provided 98 100% control of glyphosate-resistant *Lolium rigidum* (Gaud.) (Borger and Hashem, 2007). In addition to consistently improving pre-planting weed control over a single application, the double-knockdown technique can decrease the evolution of glyphosate resistance (Neve et al., 2003).

The objective of this study was to evaluate the effect of single- and double-knockdown herbicide applications prior to soybean sowing on the control of *S. latifolia*, *S. verticillata,* and *R. brasiliensis*.

MATERIALS AND METHODS

Five trials were conducted under field conditions during the 2016-2017 summer rainy season at different locations distributed across the southern, southeastern, and midwestern regions of Brazil (Table 1). The experimental areas were selected based on the commercial importance of soybean production and showed natural infestations of at least one of the weed species of the Rubiaceae family. Glyphosate-tolerant varieties indicated to each location were sown from October 27, 2016, to December 19, 2016, in rows spaced from 45 to 50 cm apart and seeded from 32 to 36 seeds m^{-2}. Agronomic practices and general inputs were those recommended to each region, and soil fertilization was planned based on soil analysis performed prior to the soybean sowing. The experimental areas always relied on natural rainfall coming from the summer rainy season, and the total rainfall amount was collected by local automated remote weather stations.

Table 1 - Area coordinates, soil characteristics, rainfall amount, soybean sowing and variety, application timing, and target weeds

Parameter	Location[(1)]				
	JAG	COR	PDL	ITA	SIN
Area coordinates					
Latitude	S 23º03’59”	S 13º23’50”	S 15º34’65”	S 12º10’55”	S 11º52’05”
Longitude	W 51º34’58”	W 46º06’24”	W 54º25’02”	W 56º35’17”	W 55º16’52”
Soil characteristics					
Texture	Sandy clay	Sandy loam	Clay	Sandy clay	Sandy clay
Organic matter	7.3%	1.0%	2.6%	1.8%	1.4%
Collected rainfall amount[(2)]					
30 to 0 DBS	71 mm	30 mm	88 mm	60 mm	77 mm
0 to 30 DAS	185 mm	102 mm	224 mm	392 mm	387 mm
Soybean sowing and variety					
Sowing date	Dec 19, 16	Oct 28, 16	Dec 03, 16	Oct 27, 16	Oct 27, 16
Variety	CD 218	P98N82	TMG132 RR	M8372 IPRO	M8372 IPRO
Herbicide application timing[(3)]					
10 DBS	Dec 7, 16	Oct 17, 16	Nov 22, 16	Oct 17, 16	Oct 14, 16
0 DBS	Dec 19, 16	Oct 28, 16	Dec 03, 16	Oct 27, 16	Oct 27, 16
0DAA	Jan 26, 17	Nov 23, 16	Dec 26, 16	Nov 22, 16	Nov 14, 16
Target weeds of Rubiaceae family					
Specie[(4)]	RCHBR	BOIVE	BOILF	BOILF; BOIVE	BOIVE
Plant density	45 m^{-2}	22 m^{-2}	64 m^{-2}	3 m^{-2}; 8 m^{-2}	6 m^{-2}
Growth stage[(5)]	28 to 51	19 to 61	14 to 19	39; 51	10 to 51

[(1)] Jaguapitã-PR (JAG), Correntina-BA (COR), Primavera do Leste-MT (PDL), Itanhangá-MT (ITA) and Sinop-MT (SIN). [(2)] Collected rainfall amount from 30 to 0 days before sowing (DBS), and from 0 to 30 days after sowing (DAS). [(3)] Application timing at 10 and 0 days before sowing (DBS), and 0 days after post-emergence application (DAA) of the soybean. [(4)] *Spermacoce latifolia* (Aubl.) (BOILF), *Spermacoce verticillata* (L.) (BOIVE) and *Richardia brasiliensis* (Gomes) (RCHBR). [(5)] Weed growth stage according to the BBCH scale (Hess et al., 1997).

The experimental design was a completely randomized block arranged as a factorial, with four replications. Factor A was three systemic herbicide treatments applied 10 days before sowing (DBS), while Factor B was three contact herbicide treatments applied 0 DBS (“sow and spray”) (Table 2). Therefore, the full combination of Factor A and Factor B provided three single- (treatments 1, 4, and 7) and six double-knockdown herbicide applications (treatments 2, 3, 5, 6,

Table 2 - Treatments and respective doses and application timings prior to soybean sowing

	Treatment[1] and doses (g a.e. or a.i. ha^{-1})	
	10 days before sowing	0 days before sowing
T1	glyphosate + 2,4-D (1,440 + 780)	-
T2	glyphosate + 2,4-D (1,440 + 780)	ammonium glufosinate[2] (457)
T3	glyphosate + 2,4-D (1,440 + 780)	Paraquat[2] (400)
T4	glyphosate + 2,4-D + diclosulam (1,440 + 780 + 25.2)	-
T5	glyphosate + 2,4-D + diclosulam (1,440 + 780 + 25.2)	ammonium glufosinate[2] (457)
T6	glyphosate + 2,4-D + diclosulam (1,440 + 780 + 25.2)	Paraquat[2] (400)
T7	glyphosate + [halauxifen-methyl + diclosulam][2] (1,440 + [5.4 + 25.2])	-
T8	glyphosate + [halauxifen-methyl + diclosulam] [2] (1,440 + [5.4 + 25.2])	ammonium glufosinate[2] (457)
T9	glyphosate + [halauxifen-methyl + diclosulam] [2] (1,440 + [5.4 + 25.2])	paraquat[2] (400)
T10	ammonium glufosinate[/2] + 2,4-D (912 + 457)	-
T11	glyphosate + chlorimuron-ethyl (1,440 + 25)	-
T12	glyphosate + 2,4-D + saflufenacil (1,440 + 780 + 49)	-
T13	untreated	-

[1] Glyphosate dimethylamine salt (Glizmax® Prime, 480 g a.e. L^{-1}, SL, Dow AgroSciences), 2,4 D dimethylamine salt (DMA® 806 BR, 669 g a.e. L^{-1}, SL, Dow AgroSciences), diclosulam (Spider®, 840 g a.i. kg^{-1}, WG, Dow AgroSciences), halauxifen-methyl + diclosulam (GF-3022, 695 g a.i. kg^{-1}, WG, Dow AgroSciences), ammonium glufosinate (Finale®, 200 g a.e. L^{-1}, SL, SL, Bayer Crop Sciences), chlorimuron-ethyl (Classic®, 250 g a.i. kg^{-1}, WG, Du Pont), saflufenacil (Heat®, 700 g a.i. kg^{-1}, WG, Basf), and paraquat (Gramoxone, 200 g a.e. L^{-1}, SL, Syngenta). [2] Added adjuvant according to the manufacturer's recommendation.

8, and 9). In addition, three single-knockdown applications (treatments 10, 11, and 12) as well as an untreated check-plot (treatment 13) were also included as standard treatments for comparison. The experimental units consisted of field plots measuring 3 m wide and 5 m long (15 m^2). Applications of the treatments were always performed in 100 L ha^{-1} using CO_2 backpack sprayers at 276 kPa equipped with a 3 m wide boom and AIXR 110015 flat-fan nozzles. The application conditions were: average air temperature ranging from 23 °C to 28 °C; average relative humidity of air ranging from 58 to 78%, average wind speed lower than 0.5 km h^{-1} and clear sky with few clouds.

To obtain and evaluate a complete weed control program as typically performed by soybean growers in Brazil, the treatments were followed by post-emergence applications when the crop reached three leaf stage. Treatments 1 through 10 were followed by a pre-mix of 2,4-D choline salt 195 g a.e. L^{-1} + glyphosate dimethylamine (DMA) salt 205 g a.e. L^{-1} at 780 + 820 g a.e. ha^{-1}, respectively (1,600 g a.e. ha^{-1}). This herbicide treatment will be an alternative for post emergence weed control in 2,4 D-tolerant soybean which are under development in Brazil and other countries. Treatments 11 and 12 were followed by glyphosate DMA salt 480 g a.e. L^{-1} at 1,440 g a.e. ha^{-1}, simulating a conventional utilization of this herbicide in glyphosate-tolerant soybeans. Post-emergence applications were performed as described for the burndown applications.

The efficacy of control of each target weed of the Rubiaceae family was assessed using a linear scale varying from 0 (no weed control) to 100% (complete weed control), according to

Camper (1986). In all trials, the assessments were visually performed 14 days after soybean sowing (DAS) as well as 0 and 28 days after application (DAA) of the post-emergence herbicides treatments. In these studies, the soybean was just established as a crop model on the experimental areas and consequently no visual assessments were performed to evaluate the treatments' effect on the crop.

Initially, the treatments designed as a factorial (treatments1 through 9) was analysed with the following mixed model (Equation 1):

$$\%Efficacy_{ijkl} = \mu + a_i + b_j + a \times b_{ij} + c_k + a \times c_{ik} + b \times c_{jk} + a \times b \times c_{ijk} + d_{l(k)} \quad \text{(eq. 1)}$$

where systemic herbicides (a), contact herbicides (b), locations (c) and their interactions were considered as fixed effects in the model, and block (d) was considered as random effect. Additionally, in order to compare the treatments into the factorial design with the standard treatments, data from all herbicide treatments (treatments 1 through 12) were analysed with the following mixed model (Equation 2):

$$\%Efficacy_{ijkl} = \mu + a_i + b_j + a \times b_{ij} + c_{l(k)} \quad \text{(eq. 2)}$$

where herbicide (a) (each herbicide program), locations (b) and their interactions were considered as fixed effects in the model, and block (c) was considered as random effect. The linear mixed models were estimated with the restricted maximum likelihood method (REML), and means were compared with Tukey's test (α=0.05).

RESULTS AND DISCUSSION

The efficacy of *Spermacoce latifolia* control has varied as a function of the interaction between systemic (applied 10 DBS) and contact (applied 0 DBS) herbicide treatments in the evaluation at 14 DAS (Table 3). The herbicide treatments 3 through 8 achieved the highest levels of *S. latifolia* control in this evaluation date, with average values of efficacy of control ranging from 87% to 99% (Table 4). These results persisted in the assessment performed when the crop reached three leaves (0 DAA), and higher levels of control were observed in Primavera do Leste/MT than Itanhangá-MT. *S. latifolia* control varied as a function of the systemic herbicide treatments at 28 DAA (Table 3), and treatments followed by 2,4-D and glyphosate were effective in its control (>95%) (Table 4). The effect of double-knockdown applications did not differ from the effect of single-knockdown applications at this evaluation date, regardless of the systemic herbicides, contact herbicides, and locations.

In the case of *S. verticillata*, the efficacy of control was also dependent on the interaction between both systemic and contact herbicide treatments in the evaluation performed at 14 DAS (Table 3). Generally, higher controls were obtained with the use of double than single-knockdown applications, and glufosinate was equivalent to paraquat as a second herbicide application (Table 5). At 0 DAA, double-knockdown applications were also the most effective practice to control *S. verticillata*, but differences in weed control levels were even more evident between the locations. The effect of most herbicide treatments was lower in Correntina-BA when compared to the effect of the same herbicide treatments in Itanhangá-MT and Sinop-MT. At 28 DAA, glyphosate + chlorimuron and glyphosate + 2,4-D + saflufenacil followed by glyphosate were the only treatments which achieved 80% of control, except in Corrrentina-BA.

The interaction of systemic herbicide treatments and contact herbicide treatments was significant for the efficacy of *Richardia brasiliensis* control in the visual assessment performed in Jaguapitã-PR at 14 DAS (Table 3). Although double-knockdown applications provided better levels of weed control than single applications, there were not always significant differences among their average values (Table 6). The superiority of double-knockdown applications persisted in the evaluation at 0 DAA and was significant when the first application was glyphosate + [halauxifen-methyl + diclosulam]. *R. brasiliensis* control did not vary as a function of the factors tested at 28 DAA (Table 3), and all herbicide treatments provided satisfactory control of this weed species (>94%) (Table 6). The effect of double-knockdown applications did not differ from the effect of single-knockdown applications at this assessment date, regardless of the systemic herbicides and contact herbicides.

Table 3 - P-values of the analysis of variance through the F-test for efficacy of control of *Spermacoce latifolia* (Aubl.), *S. verticillata* (L.) and *Richardia brasiliensis* (Gomes) in three assessment dates, in the 2016-2017 summer rainy season

Fixed effect	Evaluation date		
	14 DAS	0 DAA	28 DAA
Spermacoce latifolia			
Location	<0.0001	<0.0001	0.2905
Factor A[(1)]	<0.0001	0.0001	0.0041
Location x Factor A	0.5082	0.2222	0.1265
Factor B[(2)]	0.0406	0.0398	0.9939
Location x Factor B	0.4304	0.4955	0.9499
Factor A x Factor B	0.0001	<0.0001	0.9991
Location x Factor A x Factor B	0.1461	0.0796	0.9567
Spermacoce verticillata			
Location	<0.0001	<0.0001	<0.0001
Factor A[(1)]	<0.0001	<0.0001	<0.0001
Location x Factor A	<0.0001	<0.0001	0.0001
Factor B[(2)]	<0.0001	<0.0001	<0.0001
Location x Factor B	<0.0001	<0.0001	0.0126
Factor A x Factor B	0.0004	<0.0001	0.1744
Location x Factor A x Factor B	0.0738	0.2174	0.0507
Richardia brasiliensis			
Location	-	-	-
Factor A[(1)]	0.0011	0.0018	0.4433
Location x Factor A	-	-	-
Factor B[(2)]	<0.0001	0.0003	0.2350
Location x Factor B	---	---	-
Factor A x Factor B	0.0024	0.0023	0.7411
Location x Factor A x Factor B	-	-	-

/(1) Systemic herbicide treatments applied at 10 days before sowing. /(2) Contact herbicide treatments applied at 0 days before sowing.

Table 4 - Efficacy of control of *Spermacoce latifolia* (Aubl.) in three evaluation dates as a function of single- and double-knockdown herbicide applications observed in Primavera do Leste/MT (PDL) and Itanhangá/MT (ITA), in the 2016-17 summer rainy season

Treatment[(2)]	Assessment date[(1)]				
	14 DAS		0 DAA		28 DAA
	PDL	ITA	PDL	ITA	Mean
1. GLY+2,4-D	88 Aa[(3)]	72 Be	84 Aa	82 Acde	93 a
2. GLY+2,4-D / GLU	89 Aa	82 Bbcd	93 Aa	86 Babc	97 a
3. GLY+2,4-D / PAR	93 Aa	90 Aab	98 Aa	91 Bab	97 a
4. GLY+2,4-D+DIC	96 Aa	90 Bab	98 Aa	87 Babc	98 a
5. GLY+2,4-D+DIC / GLU	99 Aa	93 Ba	100 Aa	95 Aa	98 a
6. GLY+2,4-D+DIC / PAR	94 Aa	90 Aab	100 Aa	88 Babc	98 a
7. GLY+[HAL+DIC]	93 Aa	87 Babc	98 Aa	93 Aab	99 a
8. GLY+[HAL+DIC] / GLU	95 Aa	91 Aab	100 Aa	84 Bbcd	97 a
9. GLY+[HAL+DIC] / PAR	88 Aa	79 Bcde	92 Aa	73 Be	98 a
10. GLU+2,4-D	66 Bb	73 Ae	56 Bb	89 Aabc	88 ab
11. GLY+CHL	67 Bb	73 Ae	61 Bb	76 Ade	79 b
12. GLY+2,4-D+SAF	62 Bb	75 Ade	51 Bb	80 Acde	85 b
13. UNT	0	0	0	0	0
F value	3.4		10.0		2.0
P value	0.009		<0.0001		0.0383
Coeficient of variation	13.9		16.8		28.9

(1) Evaluation dates at 14 days after sowing (DAS), and 0 and 28 days after post-emergence application (DAA). (2) GLY: glyphosate dimethylamine salt; 2,4-D: 2,4-D dimethylamine salt; GLU: ammonium glufosinate; PAR: paraquat; DIC: diclosulam; [HAL+DIC]: halauxifen-methyl + diclosulam; CHL: chlorimuron-ethyl; SAL: saflufenacil; UNT: untreated. (3) Means followed by the same lowercase letter in the column and uppercase in the row not differ from each other by the Tukey's test (p<0.05).

Table 5 - Efficacy of control of *Spermacoce verticillata* (L.) in three evaluation dates as a function of single- and double-knockdown herbicide applications observed in Correntina-BA (COR), Itanhangá-MT (ITA) and Sinop/MT (SIN), in the 2016-17 summer rainy season

Treatment(2)	Assessment dates(1)								
	14 DAS			0 DAA			28 DAA		
	COR	ITA	SIN	COR	ITA	SIN	COR	ITA	SIN
1. GLY+2,4-D	14 Cd(3)	35 Bf	44 Ade	16 Cbc	36 Be	50 Ad	13 Ccd	40 Be	57 Ac
2. GLY+2,4-D / GLU	81 Aa	61 Ce	70 Bab	65 Aa	62 Abc	56 Bcd	24 Bbcd	53 Ade	57 Ac
3. GLY+2,4-D / PAR	56 Bbc	60 Be	75 Aab	39 Cabc	55 Bcd	76 Aab	23 Cbcd	50 Bde	72 Ab
4. GLY+2,4-D+DIC	15 Cd	40 Bf	45 Ade	13 Cc	43 Bde	58 Acd	14 Ccd	40 Be	66 Abc
5. GLY+2,4-D+DIC / GLU	85 Aa	66 Bde	63 Bbc	69 Aa	71 Aab	71 Aab	40 Cabc	54 Bde	73 Ab
6. GLY+2,4-D+DIC / PAR	56 Bbc	58 Be	78 Aa	31 Cabc	55 Bcd	83 Aa	25 Cbcd	62 Bcd	74 Ab
7. GLY+[HAL+DIC]	15 Cd	67 Acde	55 Bcd	14 Bbc	71 Aab	68 Abc	18 Cbcd	70 Bbc	76 Aab
8. GLY+[HAL+DIC] / GLU	74 Bab	81 Aab	69 Cabc	53 Cab	86 Aa	79 Bab	31 Cabcd	78 Aabc	72 Bb
9. GLY+[HAL+DIC] / PAR	54 Cc	74 Abcd	68 Babc	41 Babc	81 Aa	78 Aab	36 Babcd	72 Abc	73 Ab
10. GLU+2,4-D	14 Bd	35 Af	38 Ae	11 Cc	38 Be	45 Ad	11 Cd	20 Bf	64 Abc
11. GLY+CHL	16 Cd	89 Aa	70 Bab	16 Bbc	82 Aa	78 Aab	56 Ca	92 Aa	81 Ba
12. GLY+2,4-D+SAF	21 Bd	78 Aabc	75 Aab	15 Bbc	78 Aa	78 Aab	45 Cab	84 Aab	79 Ba
13. UNT	0	0	0	0	0	0	0	0	0
F value	28.9			9.5			4.4		
P value	<0.001			<0.001			<0.001		
Coeficient of variation	41.5			45.9			46.4		

(1) Evaluation dates at 14 days after sowing (DAS), and 0 and 28 days after post-emergence application (DAA). (2) GLY: glyphosate dimethylamine salt; 2,4-D: 2,4-D dimethylamine salt; GLU: ammonium glufosinate; PAR: paraquat; DIC: diclosulam; [HAL+DIC]: halauxifen-methyl + diclosulam; CHL: chlorimuron-ethyl; SAL: saflufenacil; UNT: untreated. (3) Means followed by the same lowercase letter in the column and uppercase in the row not differ from each other by the Tukey's test ($p<0.05$).

Table 6 - Efficacy of control of *Richardia brasiliensis* (Gomes) in three evaluation dates as a function of single- and double-knockdown herbicide applications observed in Jaguapitã-PR, in the 2016-16 summer rainy season

Treatment(2)	Assessment date(1)		
	14 DAS	0 DAA	28 DAA
1. GLY+2,4-D	94 abc(3)	90 abc	97 a
2. GLY+2,4-D / GLU	98 ab	92 ab	100 a
3. GLY+2,4-Df/ PAR	97 ab	91 abc	99 a
4. GLY+2,4-D+DIC	97 ab	92 ab	100 a
5. GLY+2,4-D+DIC / GLU	99 ab	94 ab	100 a
6. GLY+2,4-D+DIC / PAR	100 a	95 a	100 a
7. GLY+[HAL+DIC]	85 d	80 d	99 a
8. GLY+[HAL+DIC] / GLU	100 a	95 a	100 a
9. GLY+[HAL+DIC] / PAR	95 abc	90 abc	100 a
10. GLU+2,4-D	87 cd	82 cd	99 a
11. GLY+CHL	72 e	64 e	94 a
12. GLY+2,4-D+SAF	90 bcd	85 cd	100 a
13. UNT	0	0	0
F value	20.5	23.9	1.3
P value	<0.0001	<0.0001	0.2823
Coeficient of variation	9.0	10.4	3.4

(1) Evaluation dates at 14 days after sowing (DAS), and 0 and 28 days after post-emergence application (DAA). (2) GLY: glyphosate dimethylamine salt; 2,4-D: 2,4-D dimethylamine salt; GLU: ammonium glufosinate; PAR: paraquat; DIC: diclosulam; [HAL+DIC]: halauxifen-methyl + diclosulam; CHL: chlorimuron-ethyl; SAL: saflufenacil; UNT: untreated. (3) Means followed by the same lowercase letter in the column and uppercase in the row not differ from each other by the Tukey's test ($p<0.05$).

No previous field studies were found in the scientific literature evaluating the efficacy of control of weed species of the Rubiaceae family through the utilization of double-knockdown applications. In single-knockdown studies - one application timing- with *S. latifolia*, *S. verticillata,* and *R. brasiliensis*, several herbicide associations have been effective in killing these weed species. While glyphosate at 1,440 g a.e. ha^{-1} resulted in less than 85% control of *S. latifolia* and *R. brasiliensis*, mixing it with carfentrazone or flumioxazin provided control above 95% (Ferreira et al., 2006). In other cases, glyphosate at 1,080 g a.e. ha^{-1} achieved 84% control of *B. densiflora* and mixing it with 2,4-D (670 g a.e. ha^{-1}) provided complete control (Martins and Christoffoleti, 2014). Lactofen at 120 g a.i. ha^{-1}, paraquat at 400 g a.i. ha^{-1}, chlorimuron at 12 g a.i. ha^{-1}, and imazethapyr at 74 g a.i. ha^{-1} also delivered control of more than 90% of *B. densiflora* in the same study.

At least three explanations for the reduced effect of herbicide treatments between the present study and data from the literature may be examined, as well as differences among the locations of the present study. First, it is possible that the field identification of one or both species of *Spermacoce* genus is not correct, because they may possess morphological plasticity throughout soybean production areas. In fact, *S. latifolia* and *S. verticillata* are considered by many authors to be a distinct genus, *Borreria*, which shows that their identification itself is still not well-defined (Ferreira Jr. et al., 2012). Second, weed growth stages may have directly affected the herbicide control efficacy, since in the present study the target weeds were at advanced or even perennial growth stages (Table 1). Third, the environmental conditions at application may have also negatively influenced the levels of weed control in the locations with lower rainfall amounts, such as in Correntina-BA (Table 1).

The effect of glufosinate at 457 g a.i. ha^{-1} as a contact herbicide in the second spray was generally equivalent to paraquat at 400 g a.i. ha^{-1}, especially in the two first assessment dates (Tables 4, 5 and 6). This herbicide controls a great variety of weeds by irreversibly inhibiting glutamine synthetase, which is an enzyme that creates glutamine from glutamate and ammonium (Berlicki, 2008). Glufosinate at 400 g a.i. ha^{-1} was also reported as an alternative on the *Conyza* spp. control in burndown conditions, even with plants at the pre-flowering growth stage (Moreira et al., 2010). Thus, this herbicide can be used as an effective alternative to paraquat, which will be commercially banned in three years in Brazil by the 177 Collegiate Board Resolution (ANVISA, 2017).

Double-knockdown applications did not always result in a higher level of weed control than in single applications against weed species of the Rubiaceae family, especially in the case of *S. verticillata* (Table 6). However, the sequential application of systemic and contact herbicide treatments is likely to delay and even prevent the evolution of herbicide resistance to either chemical (Neve et al., 2003). For example, *Hedyotis verticillata* (syn. *S. verticillata*) was found to have high level of resistance to both glyphosate and paraquat in oil palm plantations from Malaysia (Chuah et al., 2005). Thus, the double-knockdown application is a valuable tool in integrated weed management strategies to decrease the risk of developing glyphosate resistance (Llewellyn et al., 2005). Additionally, this strategy could also reduce weed shifting to weed species with inherent tolerance to glyphosate such as *S. latifolia*, *S. verticillata,* and *R. brasiliensis*, among others.

In the present study, double-knockdown applications generally provided higher levels of control of *S. latifolia*, *S. verticillata,* and *R. brasiliensis* than the single-knockdown applications. Glyphosate + 2,4-D, glyphosate + 2,4-D + diclosulam, and glyphosate + [halauxifen-methyl + diclosulam] followed by glufosinate or paraquat achieved at least 90% of control of *S. latifolia* and *R. brasiliensis*, but did not achieve satisfactory level of control (> 80%) of *S. verticillata*. The effect of glufosinate at 457 g a.i. ha^{-1} as a contact herbicide in the double-knockdown applications was generally equivalent to paraquat at 400 g a.i. ha^{-1}, especially in the first assessment dates.

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