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EFFICIENCY OF ISOLATED AND ASSOCIATED HERBICIDES TO CONTROL GLYPHOSATE-RESISTANT SOURGRASS

Eficiência de Herbicidas Isolados e Associados no Controle de Capim-Amargoso Resistente ao Glyphosate

ABSTRACT - Sourgrass is one of the weeds of great economic importance in Brazil due to its difficulty of control and conditions that allow its emergence and development throughout the year. Thus, this study aimed to evaluate the effectiveness of clethodim and haloxyfop applied alone or mixed with glyphosate and other herbicides to control glyphosate-resistant sourgrass at different stages of development. For this, three experiments were conducted in the field: in experiment 1, the herbicides were applied in sourgrass plants with 6 to 8 tillers; and in experiments 2 and 3, when plants were at full flowering, with up to 18 tillers. After treatment application, visual evaluations were performed at 14, 21, 28, 35, 42, 49, and 56 days after application (DAA) in experiment 1 and at 7, 14, 21, 28, and 35 DAA in experiments 2 and 3. Also in experiment 3, the shoot of remaining plants was collected in the last control evaluation to measure the dry matter. In experiment 1, the treatment glyphosate + clethodim presented a satisfactory control close to 90%, but after 35 DAA, sourgrass plants started showing significant resprouts, decreasing the control. In experiments 2 and 3, treatments showed no control above 90% and the herbicides clethodim and haloxyfop had similar final control when in mixture with glyphosate. Therefore, the isolated herbicide application at the tested doses was not sufficient for efficient control of sourgrass at more advanced stages of development.

Keywords: *Digitaria insularis*, grass control, clethodim, haloxyfop.

RESUMO - O capim-amargoso é uma das plantas daninhas de grande importância econômica no Brasil devido a sua dificuldade de controle, pelas condições que permitem emergência e desenvolvimento o ano todo. Diante disso, objetivou-se neste trabalho avaliar a eficácia dos herbicidas clethodim e haloxyfop, aplicados isoladamente ou em associação com glyphosate e outros herbicidas, no controle do capim-amargoso resistente ao glyphosate em diferentes estádios de desenvolvimento. Para isso, foram conduzidos três experimentos em campo: no experimento 1, os herbicidas foram aplicados em plantas de capim-amargoso com 6 a 8 perfilhos; e nos experimentos 2 e 3, quando as plantas estavam em pleno florescimento, com até 18 perfilhos. Após a aplicação dos tratamentos, foram realizadas avaliações visuais de controle aos 14, 21, 28, 35, 42, 49 e 56 dias após a aplicação (DAA), no experimento 1, e aos 7, 14, 21, 28 e 35 DAA, nos experimentos 2 e 3. Ainda no experimento 3, na última avaliação de controle coletou-se a parte aérea das plantas remanescentes, para mensuração da massa seca. No experimento 1, o tratamento glyphosate + clethodim apresentou controle satisfatório, próximo de 90%, porém após 35 DAA as plantas de capim-amargoso começaram a mostrar rebrotas significativas, diminuindo o controle. Nos experimentos 2 e 3, os tratamentos não apresentaram controle acima de 90%, e os

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herbicidas clethodim e haloxyfop obtiveram controle final similar nas associações com glyphosate. Conclui-se que a aplicação única dos herbicidas utilizados, nas doses testadas, não foi suficiente para um controle eficiente de capim-amargoso em estádios de desenvolvimento mais avançados.

Palavras-chave: *Digitaria insularis*, controle de gramíneas, clethodim, haloxyfop.

INTRODUCTION

The agro-technological management in the soybean crop is of great importance, especially among weeds. When performed correctly, it enables crop to express its full production potential. Thus, weeds are responsible for large losses in grain production since they affect crop development by intense competition for vital elements such as water, light, nutrients, and space (Lorenzi, 2014; Melo et al., 2015). The competition with crops and the presence of herbicide-resistant weeds lead to the need of applying alternative herbicides to glyphosate in order to obtain a better control, thus increasing production costs (Adegas et al., 2017).

Sourgrass (*Digitaria insularis*) is a perennial plant belonging to the Poaceae family that, under Brazilian conditions, can emerge and develop over the year (Martins et al., 2017). After its development and formation of rhizome, the difficulty of its control increases (Gemelli et al., 2012). According to Machado et al. (2008), plants with developed rhizomes have a lower translocation of glyphosate, which may lead to shoot resprouts due to starch reserve, thus hindering their control.

At a population of 4 to 8 plants per square meter, sourgrass may reduce soybean yield by up to 44%, reaching 75% in more severe cases (Adegas et al., 2017). According to Gonçalves et al. (2015), sourgrass is a host of diseases and pests that attack several crops and is used as a green bridge in the off-season.

The intensive use of herbicides in recent years has caused the selection of certain weed species because of the selection pressure caused by the herbicide, thus emerging resistant biotypes (Melo et al., 2015; Adegas et al., 2017). The first case of sourgrass resistance to the herbicide glyphosate was registered in Paraguay in 2005. In Brazil, sourgrass resistance to glyphosate was registered for the first time in 2008 in the State of Paraná (Lopez-Ovejero et al., 2017). Sourgrass resistance to ACCase-inhibitor herbicides, such as fenoxaprop and haloxyfop, was registered in the Midwest region of Brazil (Heap, 2018).

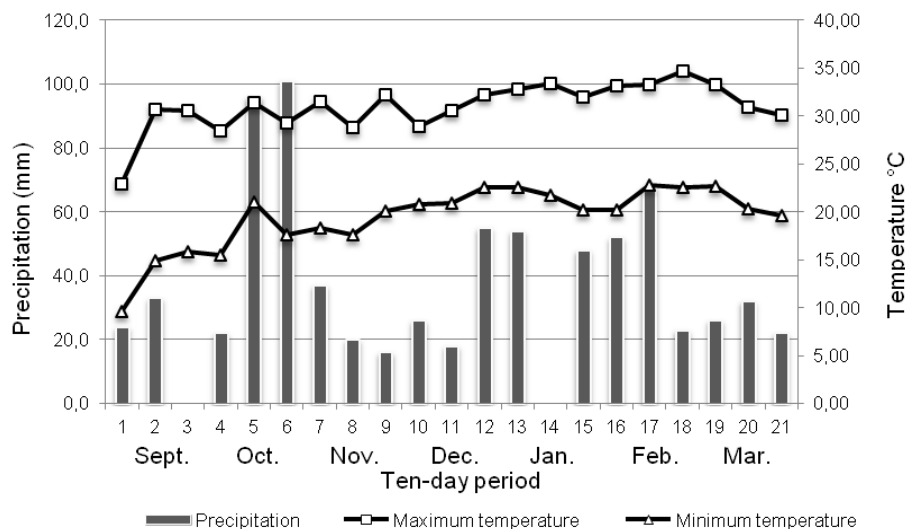
With resistance to glyphosate-based herbicides and low control efficiency, a widely used alternative is the application of graminicides in isolation or in sequence, which improve sourgrass control. According to Barroso et al. (2014), herbicides such as clethodim and haloxyfop show good efficiency in visual control at advanced stages of development. However, because this plant has the resprouting capacity, herbicides applied alone, even at high doses, are not sufficient for the effective control of perennial sourgrass, requiring sequential applications (Zobiolo et al., 2016).

Considering the importance of sourgrass and the difficulty of its control, this study aimed to evaluate the effectiveness of clethodim and haloxyfop, applied alone or mixed with glyphosate and other herbicides, to control this weed at different stages of development in areas with reporting of resistance to glyphosate.

MATERIAL AND METHODS

The experiments were conducted in the field, in the western region of Paraná, with climate classified according to Köppen as cfa (mesothermal humid subtropical) (Caviglione et al., 2000). The soil is classified as a eutroferric Red Latosol, being characteristic of the region (Embrapa, 2013). The meteorological data (maximum temperature, minimum temperature, and precipitation) collected during the experimental period are shown in Figure 1.

Three experiments were carried out, two of them in the same area of Maripá, PR, at the geographical coordinates 24°23'21.6" S and 53°45'47.3" W, with an altitude of 335 m. The other



Source: C.VALE – Cooperativa Agroindustrial.

Figure 1 - Precipitation and maximum and minimum temperature in the period from September 1, 2016, to March 31, 2017, for the western region of Paraná.

experiment was conducted in Palotina-PR, at the geographical coordinates 24°16'52.4" S and 53°51'45.4" W, with an altitude of 315 m.

In these areas, soybean and corn were grown in succession under a no-tillage system, which presented problems to control sourgrass with the herbicide glyphosate. The experimental design was a randomized block design with four replications and plots with a size of 5 × 3 m. Treatments were applied (Tables 1, 2, and 3) with a CO₂-pressurized backpack sprayer with a constant pressure of 2 kgf cm⁻² and equipped with a boom containing six Teejet XR 110.015 tips spaced at 50 cm from each other, providing an application volume of 150 L ha⁻¹.

Experiment 1 was carried out in the off-season, before soybean crop set-up, with treatments as shown in Table 1. At application time, sourgrass plants had an average of 6 to 8 tillers, height of 45 cm, and infestation of 4 plants per m². Moreover, environmental conditions were as follows: air temperature of 27.8 °C, relative air humidity of 48.6%, and wind speed of 2.2 km h⁻¹. A visual evaluation of the effectiveness in the control of sourgrass plants was carried out at 14, 21, 28, 35, 42, 49, and 56 days after treatment application (DAA) by assigning zero for the absence of symptoms and 100 for total weed control (SBCPD, 1995).

Experiment 2 was set-up after soybean implantation. Treatment application (Table 2) was performed when the soybean crop was at the V₄ stage. Sourgrass plants were at full flowering, with an average of 8 to 10 tillers, 80 cm of height, and infestation of 6 plants per m². The conditions at the application time were air temperature of 26 °C, relative air humidity of 50%, and wind speed of 2.0 km h⁻¹. The percentage of sourgrass control (SBCPD, 1995) was visually evaluated at 7, 14, 21, 28, and 35 DAA.

Experiment 3 was similar to experiment 2 and was set-up after soybean crop establishment, but with a higher infestation and the presence of perennial sourgrass plants due to resprouts. The used treatments are shown in Table 3. On the day of application, soybean was at the V₄ stage, and sourgrass plants were at full flowering, with an average of 16 to 18 tillers, 90 cm of height, and infestation of 10 plants per m². Environmental conditions at application time were air temperature of 25.6 °C, relative air humidity of 62%, and wind speed of 4.2 km h⁻¹.

The percentage of control (SBCPD, 1995) was visually evaluated at 7, 14, 21, 28, and 35 DAA. The remaining sourgrass plants were collected at 35 DAA in order to obtain the dry matter. The collection was carried out in a useful area on the plot of 1 m². After collection, the plants were packed into kraft paper bags and dried in a forced air ventilation oven at 50 °C for 72 hours. These samples were weighed on a precision scale to determine the dry matter, with values expressed in grams (g).

Table 1 - Herbicides applied to control sourgrass (*D. insularis*) at the phenological stage of 6 to 8 tillers in the off-season. Experiment 1, Maripá, PR. 2017

Treatment	Commercial name	Doses (g a.i. or a.e. ha ⁻¹)
Control without application	–	–
Paraquat - diuron + imazethapyr	Gramocil + Pivot 100 SL	200-100 + 100
Glyphosate + clethodim ⁽¹⁾	Glizmax + Select 240 EC	1,200 + 192
Glyphosate + haloxyfop-methyl ⁽²⁾	Glizmax + Verdict R	1,200 + 120
Glyphosate + flumioxazin-imazethapyr	Glizmax + Zethamaxx	1,200 + 60-120
Oxyfluorfen + haloxyfop-methyl ⁽²⁾	Goal BR + Verdict R	360 + 120

⁽¹⁾ Treatment applied mixed with Lanza 0.5% v/v. ⁽²⁾ Treatment applied mixed with Joint 0.5 v/v.

Table 2 - Herbicides applied to control sourgrass (*D. insularis*) at the phenological stage of 8 to 10 tillers in the middle of the soybean crop. Experiment 2, Maripá, PR. 2017

Treatment	Commercial name	Doses (g a.i. or a.e. ha ⁻¹)
Control without application	–	–
Glyphosate + clethodim ⁽¹⁾	Glizmax + Select 240 EC	1,200 + 192
Glyphosate + haloxyfop-methyl ⁽²⁾	Glizmax + Verdict R	1,200 + 120
Clethodim ⁽¹⁾	Select 240 EC	192
Haloxyfop-methyl ⁽²⁾	Verdict R	120
Oxyfluorfen + haloxyfop-methyl ⁽²⁾	Goal BR+ Verdict R	360 + 120

⁽¹⁾ Treatment applied mixed with Lanza 0.5% v/v. ⁽²⁾ Treatment applied mixed with Joint 0.5 v/v.

Table 3 - Herbicides applied to control perennial sourgrass (*D. insularis*) in the middle of the soybean crop. Experiment 3, Palotina, PR. 2017

Treatment	Commercial name	Doses (g a.i. or a.e. ha ⁻¹)
Control without application	–	–
Glyphosate + clethodim ⁽¹⁾	Glizmax + Select 240 EC	1,200 + 192
Glyphosate + haloxyfop-methyl ⁽²⁾	Glizmax + Verdict R	1,200 + 120
Glyphosate + clethodim ⁽¹⁾	Glizmax + Select 240 EC	1,200 + 384
Glyphosate + haloxyfop-methyl ⁽²⁾	Glizmax + Verdict R	1,200 + 240
Clethodim ⁽¹⁾	Select 240 EC	192
Haloxyfop-methyl ⁽²⁾	Verdict R	120
Oxyfluorfen + haloxyfop-methyl ⁽²⁾	Goal BR + Verdict R	360 + 120

⁽¹⁾ Treatment applied mixed with Lanza 0.5% v/v. ⁽²⁾ Treatment applied mixed with Joint 0.5 v/v.

The data were submitted to analysis of variance ($p < 0.05$) by using the statistical program Sisvar and the Tukey's test was used to compare the means ($p < 0.05$) (Ferreira, 2011).

RESULTS AND DISCUSSION

The results showed that sourgrass control with herbicides applied alone at the vegetative stage with 6 to 8 tillers plants (Table 4) could provide a control close to 90%. Melo et al. (2012) and Barroso et al. (2014) found similar results, with a control above 90% after the application of glyphosate + clethodim on sourgrass plants of 3 to 5 tillers. Sequential application, in some cases, may complement the action of other herbicides, facilitating the control of plants at advanced stages of development. In this same study, Melo et al. (2012) obtained a 100% sourgrass control with the sequential application of paraquat-diuron and ammonium-glufosinate after the first application of glyphosate + clethodim.

Treatments with a higher control (about 40%) at 14 days after application (DAA) were paraquat-diuron + imazethapyr, glyphosate + clethodim, and oxyfluorfen + haloxyfop (Table 4). However, the treatment paraquat-diuron + imazethapyr, despite having a fast control effect at 14 DAA, showed a high number of resprouts after 21 DAA, making it the worst treatment at the end of the evaluation period. This is due to the application at non-recommended stages and to the low herbicide translocation (Rodrigues and Almeida, 2011; Barroso et al., 2015).

Table 4 - Percentage of control of sourgrass (*D. insularis*) submitted to herbicide application in the off-season. Experiment 1, Maripá, PR. 2017

Treatment	Control (%)						
	14 DAA	21 DAA	28 DAA	35 DAA	42 DAA	49 DAA	56 DAA
Control	0.00 d	0.00 e	0.00 d	0.00 d	0.00 d	0.00 d	0.00 d
Paraq + imazetha ⁽¹⁾	43.75 a	55.25 c	22.00 c	13.00 c	8.25 d	2.50 d	1.25 d
Gly + cletho ⁽²⁾	42.50 a	78.25 a	94.25 a	97.25 a	95.00 a	90.75 a	86.00 a
Gly + haloxy ⁽³⁾	29.50 bc	65.25 bc	68.00 b	77.50 b	74.50 b	71.50 b	71.50 b
Gly + flumioz ⁽⁴⁾	23.75 c	31.00 d	17.50 d	9.25 c	6.75 d	5.00 d	2.00 d
Oxyfluo + haloxy ⁽⁵⁾	38.00 ab	69.00 ab	75.75 b	73.00 b	61.75 c	40.50 c	32.00 c
Mean	29.58	49.79	46.25	45.00	41.04	35.04	32.12
CV%	12.83	11.05	10.59	8.20	10.30	12.38	10.52
LSD	8.72	12.64	11.25	8.48	9.72	9.97	7.76

Means followed by the same letter in the same column did not differ significantly from each other by the Tukey's test ($p < 0.05$). ⁽¹⁾Paraquat-diuron + imazethapyr; ⁽²⁾Glyphosate + clethodim; ⁽³⁾Glyphosate + haloxyfop-methyl; ⁽⁴⁾Glyphosate + flumioxazin-imazethapyr; ⁽⁵⁾Oxyfluorfen + haloxyfop-methyl.

Herbicides applied on the leaves only reach the roots or rhizomes when their translocation occurs via phloem (Barroso et al., 2015). The herbicide translocation pathway is related to its affinity for a lipophilic or hydrophilic environment, determined by an octanol-water partition coefficient (K_{ow}), and the water solubility on balance with the ionization potential (pKa) (Oliveira Jr et al., 2011).

Therefore, when diuron is applied on the plant shoot, it does not reach the rhizomes since its translocation is apoplastic, that is, its movement in the plant occurs mainly acropetally, by the xylem, and accumulates in the leaves, in which symptoms appear quickly (Dias et al., 2003). Similarly, paraquat does not translocate to the rhizome because of its fast damage to plant tissue (Oliveira Jr et al., 2011).

After 21 DAA, all treatments presented resprout, except for those containing graminicides (Table 4). The treatment oxyfluorfen + haloxyfop was initially superior to the treatment glyphosate + haloxyfop due to the fast effect of contact of oxyfluorfen, showing the destruction of the green part of plants. However, plants presented intense resprouts at 35 DAA.

The associations glyphosate + clethodim and glyphosate + haloxyfop were the best treatments. However, glyphosate + clethodim presented resprout at 56 DAA, whereas the treatment glyphosate + haloxyfop had no evidence of resprouting.

Clethodim has a faster action when compared to haloxyfop, but with less ability to move to the rhizome (Barroso et al., 2015). This fastest action is due to higher lipophilicity of clethodim when compared to haloxyfop ($\log K_{ow} = 4.17$ and 4.08 , respectively). According to Rodrigues and Almeida (2011), plants absorb quickly herbicides with a more lipophilic character. More lipophilic herbicides that have greater ease of absorption are more likely to keep a faster balance between the translocation via xylem and phloem and hence have limited mobility in the phloem (Oliveira Jr et al., 2011).

The herbicide haloxyfop has higher mobility in the phloem when compared to clethodim, reaching more easily the meristematic tissues and acting better on the rhizomes. On the other hand, clethodim has a higher effect on the plant shoot due to its higher lipophilicity, providing a faster phytotoxication effect (Rodrigues and Almeida, 2011).

In experiment 2, the application of the treatments glyphosate + clethodim and glyphosate + haloxyfop on sourgrass at full flowering (8 to 10 tillers), close to the soybean crop, provided no difference in weed control at the end of the evaluation period (Table 5). In experiment 1, on the other hand, glyphosate + clethodim application on sourgrass plants with 6 to 8 tillers was superior when compared to that observed with glyphosate + haloxyfop (Table 4).

This difference between the mentioned experiments occurred due to the accumulation of reserves (rhizomes). In this sense, Machado et al. (2008) observed that sourgrass plants, regardless of the stage of development, present rhizome formation, hindering translocation and accumulation of herbicides at the site of action due to starch accumulation.

Table 5 - Percentage of control of sourgrass (*D. insularis*) submitted to herbicide application in the middle of the soybean crop. Experiment 2, Maripá, PR. 2017

Treatment	Control (%)				
	7 DAA	14 DAA	21 DAA	28 DAA	35 DAA
Control	0.00 d	0.00 d	0.00 d	0.00 d	0.00 d
Glyphosate + clethodim	18.33 b	47.33 a	79.33 a	81.66 a	85.66 a
Glyphosate + haloxyfop	9.66 c	36.00 b	60.66 b	80.00 a	86.66 a
Clethodim	21.66 b	47.66 a	68.33 b	74.00 ab	75.00 b
Haloxyfop	10.66 c	22.66 c	46.33 c	68.00 b	69.66 b
Oxyfluorfen + haloxyfop	28.33 a	40.66 ab	62.33 b	57.33 c	51.66 c
Mean	17.77	32.38	52.88	60.16	61.44
CV%	12.27	10.27	6.13	5.77	4.16
LSD	5.14	9.43	9.20	9.83	7.24

Means followed by the same letter in the same column did not differ significantly from each other by the Tukey's test ($p < 0.05$).

The results found in experiment 2 were close to those observed by Zobiole et al. (2016) and Gemelli et al. (2013), in which the application of glyphosate + clethodim and glyphosate + haloxyfop in clump sourgrass plants under full flowering provided a control close to 80%.

Application of glyphosate mixed with clethodim and haloxyfop showed a higher sourgrass control in relation to graminicides applied alone, demonstrating an additive effect with the use of glyphosate. Melo et al. (2012) obtained similar results when using clethodim applied alone on sourgrass plants with 3 to 5 tillers, with a significantly lower control in relation to its application mixed with glyphosate. Zobiole et al. (2016) did not observe a significant increase in the control of sourgrass with the application of glyphosate mixed with clethodim and haloxyfop at 35 DAA, but numerically, it is observed an increased control with the mixtures.

In experiment 3, the application of glyphosate mixed with graminicides on sourgrass plants at full flowering (average of 16 to 18 tillers) provided a higher control in relation to graminicides applied alone. However, any treatment had an effective control (>80%) at 35 DAA (Table 6) since sourgrass plants were at a perennial stage and had rhizome formation.

The application of four times the recommended dose of clethodim and haloxyfop on sourgrass plants with 16 to 18 tillers did not provide an efficient control despite showing a higher control when compared to the application of twice the recommended dose (Table 6). Even though the phytointoxication that treatments caused in the soybean crop was not quantified, the treatment oxyfluorfen + haloxyfop-methyl caused serious injuries in soybean, but recoverable with crop development.

Applications of treatments containing graminicides mixed or not with glyphosate provided the lowest dry matter value in the remaining sourgrass plants, differing from the mean presented

Table 6 - Efficiency in the control of perennial sourgrass (*D. insularis*) submitted to herbicide application. Experiment 3, Palotina, PR. 2017

Treatment	Control (%)					Dry matter (g)
	7 DAA	14 DAA	21 DAA	28 DAA	35 DAA	
Control	0.00 d	0.00 e	0.00 d	0.00 e	0.00 e	665.33 c
Glyphosate+ clethodim	8.66 b	26.00 a	78.00 ab	76.33 bc	53.33 b	244.00 a
Glyphosate+ haloxyfop	7.66 b	16.66 cd	71.00 bc	74.00 c	51.00 b	225.33 a
Glyphosate+ clethodim ^{4x}	11.00 a	27.33 a	82.66 a	86.33 a	67.66 a	232.00 a
Glyphosate+ haloxyfop ^{4x}	9.00 b	22.33 abc	80.00 ab	84.33 ab	68.33 a	226.66 a
Clethodim	3.33 c	25.66 ab	66.66 c	62.66 d	37.00 c	398.66 ab
Haloxyfop	2.66 c	12.33 d	65.00 c	59.66 d	32.33 cd	385.66 ab
Oxyfluorfen+ haloxyfop	9.33 ab	19.66 bc	65.33 c	54.33 d	24.66 d	454.66 b
Mean	6.46	18.75	63.58	62.20	41.79	354.00
CV%	10.35	11.47	6.12	5.12	8.25	19.08
LSD	1.92	6.19	11.20	9.17	9.93	194.74

Means followed by the same letter in the same column did not differ significantly from each other by the Tukey's test ($p < 0.05$).

^{4x}Treatments applied with four times the dose of the package leaflet.

by the control treatment without weeding. Similarly, Zobiole et al. (2016) observed that the use of graminicides mixed or not also differed significantly from the control treatment because sourgrass plants were at an advanced stage, when dry matter production is very high when compared to the treatments with herbicides.

Due to the difficulty to control sourgrass plants in post-emergence, it is worth mentioning that pre-emergence herbicides are viable alternatives for the integrated management of this species. According to Drehmer et al. (2015), the herbicides S-metolachlor and flumioxazin-imazethapyr provide a sourgrass control above 90% at 60 DAA. In this sense, herbicide mixture is essential in the management of weeds of difficult control, such as sourgrass, as well as in the prevention of selection of herbicide-resistant biotypes (Heap and Duke, 2017; Kniss, 2018).

Based on the results, the herbicides clethodim and haloxyfop applied alone or mixed with glyphosate did not promote an efficient control, especially with perennial sourgrass plants, even when the graminicides were applied at high doses. However, graminicides mixed with glyphosate enhanced sourgrass control in relation to those applied alone. The problem is that sourgrass control is hampered when plants are at a perennial stage, with the need to carry out herbicide application at its early stages of development to obtain satisfactory control.

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