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RESEARCH ARTICLE

Sourgrass phenological stage and efficacy of glufosinate-ammonium

Estádio fenológico do capim-amargoso e eficácia do herbicida glufosinato de amônio

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Abstract: **Background**: Recently, biotypes of sourgrass (*Digitaria insularis*) were identified in Brazil with multiple resistance to EPSPs-ACCase inhibiting herbicides, which demands the recommendation of herbicides with alternative mode of action, such as glufosinate-ammonium.
Objective: Therefore, this work was developed with the objective of modelling the efficacy of glufosinate-ammonium on sourgrass regarding to five different phenological stages.
Methods: Treatments consisted of eight rates of glufosinate-ammonium (1,600, 800, 400, 200, 100, 50 and 25 g ha^{-1}, and check plots without herbicide application) and five phenological stages of sourgrass. Percentage control was evaluated at 14, 21 and 28 days after application (DAA), as well as shoot dry weight at 28 DAA.
Results: The more developed sourgrass plants were, the higher doses of glufosinate-ammonium were needed to achieve satisfactory control. Plants with three leaves were easily controlled by glufosinate-ammonium, once adequate control was reached with rates lower than 100 g ha^{-1}. If tillering stage is reached, higher doses were necessary to promote adequate control, of about 200 g ha^{-1}. Doses above 400 g ha^{-1} were necessary to reach adequate control of pre-flowering plant.
Conclusions: Glufosinate-ammonium is an excellent alternative for controlling sourgrass, however a second herbicide may be necessary for applications after tillering. Strong relation between sourgrass phenological development and increasing necessary rates of glufosinate-ammonium was identified.

Keywords: *Digitaria insularis*, glutamine synthetase, growth, modelling.

Resumo: **Introdução**: Recentemente, biótipos de capim-amargoso (*Digitaria insularis*) foram identificados no Brasil com resistência múltipla aos inibidores da EPSPs-ACCase, o que exige recomendações agrícolas fundamentadas em herbicidas com mecanismos de ação alternativos, tal como o glufosinato de amônio.
Objetivo: Este trabalho foi desenvolvido com o objetivo de modelar a eficácia do glufosinato de amônio em cinco estádios fenológicos do capim-amargoso.
Métodos: Os tratamentos constaram de oito doses do herbicida (1.600, 800, 400, 200, 100, 50 e 25 g ha^{-1}, incluindo testemunhas sem aplicação) e cinco estádios fenológicos do capim-amargoso. O controle percentual das plantas foi avaliado aos 14, 21 e 28 dias após aplicação (DAA), bem como a massa de matéria seca também aos 28 DAA.
Resultados: Quanto mais desenvolvidas estavam as plantas de capim-amargoso no momento da aplicação, maiores doses foram necessárias para a obtenção de controle satisfatório. Plantas com apenas três folhas foram facilmente controladas pelo glufosinato de amônio, uma vez que adequado controle foi alcançado com doses menores que 100 g ha^{-1}. Caso o estádio de perfilhamento seja alcançado, maiores doses foram necessárias para promover controle adequado, da ordem de 200 g ha^{-1}. Doses superiores a 400 g ha^{-1} foram necessárias para alcançar controle satisfatório do capim-amargoso em estádio de pré-florescimento.
Conclusões: O glufosinato de amônio é uma excelente alternativa para controle do capim-amargoso, porém um segundo herbicida pode ser necessário no caso de aplicações após perfilhamento. Foi identificada correlação entre o desenvolvimento fenológico do capim-amargoso e doses crescentes de glufosinato de amônio visando manutenção dos mesmos níveis de controle.

Palavras-chave: *Digitaria insularis*, glutamina sintetase, crescimento, modelagem.

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1. Introduction

Plants of sourgrass (*Digitaria insularis*) are commonly found in Brazilian agricultural areas. This species grows fast, produces large number of seeds, and has high seed dissemination capacity during the whole summer. Plants can produce rhizomes that are short, but evident, forming pronounced clumps (Gemelli et al., 2012; Ferreira et al., 2018). Sourgrass plants have aggressive growth and may survive in environments with different types and intensities of limitation for plant growth and development (Licorini et al., 2015).

Detecting glyphosate-resistant biotypes of sourgrass (*Digitaria insularis*) has changed the perspective of chemical control of this species (Carvalho et al., 2011; Heap, 2021). Frequently, glyphosate used to be applied on sourgrass clumps with high efficacy, however, normal rates of this herbicide are not efficient anymore and including herbicides with alternative mode of action, like ACCase inhibitors, is a key step to reach success on chemical management (López-Ovejero et al., 2017; Costa et al., 2018). However, more recently, multiple resistance to EPSPs and ACCase inhibiting herbicides was also identified in sourgrass biotypes (Heap, 2021).

Considering sourgrass resistance to glyphosate and ACCase, the most important modification in the management system should be the substitution of these active ingredients or the combination with herbicides with different modes of action (Presoto et al., 2020). Besides that, it is important to detach that paraquat was commonly used to control sourgrass and the application of this molecule in Brazil is not allowed anymore. Thus, glufosinate-ammonium became an important alternative herbicide that may be applied to control sourgrass (Melo et al., 2012; Silva et al., 2017).

Glufosinate-ammonium has an exclusive mode of action, once it is the unique herbicide that acts on glutamine synthetase, which is a key enzyme on paths of nitrogen assimilation. Frequently, plant injuries after glufosinate application have been attributed to ammonia accumulation. However, recent studies indicated that the contact activity of glufosinate results from the accumulation of reactive oxygen species and subsequent lipid peroxidation. Glufosinate disrupts both photorespiration and the light reactions of photosynthesis, leading to photoreduction of molecular oxygen, which generates reactive species of oxygen (Takano et al., 2019; Takano and Dayan, 2020).

Besides glufosinate-ammonium may be recommended to manage multiple herbicide-resistant weeds, it does not translocate well in plants, and its efficacy on weeds is greatly affected by environmental conditions and plant size (Steckel et al., 1997; Coetzer et al., 2001; Sellers et al., 2003). This way, phenological stage of sourgrass at the moment of application is the main limitation for reaching high efficacy; the herbicide efficacy level decreases when applied on plants at later developmental stages, after tillering or with fully developed clumps (Melo et al., 2012; Andrade et al., 2019; Presoto et al., 2020).

Therefore, plant's phenological stage play an important role on glufosinate-ammonium efficacy and few information is available regarding this relationship. The right dose and moment of application may be well understood if the relation between sourgrass phenology and glufosinate-ammonium rate-efficacy would be stablished. Therefore, this work was developed with the objective of modelling efficacy of glufosinate-ammonium on sourgrass regarding to five different phenological stages.

2. Material and Methods

The experiment was carried out from January to April, 2020, at a greenhouse of the Federal Institute of Education, Science and Technology of the South of Minas Gerais, Machado Campus, Machado – MG (21°40'S, 45°55'W, and 850 m altitude). Sourgrass seeds were collected in an agricultural area of the Machado campus (Gonçalves Netto et al., 2015).

Sourgrass seeds (10 g) were germinated in 2-liter plastic boxes filled with commercial substrate (Pinus bark + turf + vermiculite) in order to select seedlings with adequate health; seeds were sown weekly to enable the joint application of herbicides on plants at different phenological stages. Furtherly, seedlings were weekly transplanted to 3-liter plastic pots when the stage of one fully expanded leaf was identified; each pot with a mean of eight plants represented one plot. Pots were filled with a mixture of commercial substrate, sieved clayey soil, and cattle manure at the proportion of 5:3:1, respectively. Mixture of all plots was properly fertilized and the experiment was irrigated daily.

Experiment was carried out in experimental design of completely randomized blocks, with an 8×5 factorial scheme and four replications (160 experimental plots). Treatments were organized with eight rates of glufosinate-ammonium (1,600, 800, 400, 200, 100, 50 and 25 g ha^{-1}, and check plots without herbicide application) and five phenological stages of sourgrass. Adjuvant Assist® (0.5% v/v) was added to each different herbicide concentration. At the time of herbicide application (March 22, 2020), four extra plots of each phenological stage were sampled to identify the exact phenological stage of the plants, based on the scale of Hess et al. (1997) (BBCH) (Table 1).

Table 1. Phenological stages of sourgrass plants at the time of application of glufosinate-ammonium. Machado, MG, Brazil, 2020.

Stage	Description	Days after seeding	BBCH Scale
1	3 leaves	26	13
2	4-5 leaves, beginning of tillering	33	21
3	Tillering, beginning of internode elongation	40	30
4	Tillering, 2 visible internodes	47	32
5	Pre-flowering, 3 visible internodes, flag leaf visible	54	45

The BBCH scale was used as described by Hess et al. (1997).

Herbicide treatments were applied using a CO_2-pressurized backpack sprayer equipped with a spray boom with two nozzles (XR 110.02; Teejet®, Wheaton, USA) positioned at 0.50 m above the targets, using pressure of 2.5 bar and a spray volume of 200 L ha^{-1}. Deionized water was used to prepare the solutions in all treatments to avoid contamination. Weed percentage control was evaluated at 14, 21 and 28 days after application (DAA), in all plots. Control was evaluated as percentages, with 0% representing absence of symptoms caused by the herbicides, and 100% representing the death of the plant. At 28 DAA, residual plant shoot dry weight (SDW) was also evaluated. The SDW was obtained by collecting the aerial part of remaining plants in the plots and drying it in an oven at 70 °C for 72 hours. The SDW was corrected to percentages by comparing the weight found in the treatments with the weight of the check plots (considered as 100%).

Data of each experiment were subjected to analysis of variance followed by the F test. The data of weed control and SDW percentages in each phenological stage were fitted to log-logistic non-linear regression models (Seefeldt et al., 1995; Presoto et al., 2020):

$$y = Pmin + \frac{a}{\left[1 + \left(\frac{x}{b}\right)^{c}\right]}$$

where y = control or residual dry weight; x = herbicide rate; and $Pmin$, a, b, and c = parameters of the curve: $Pmin$ is the lower limit of the curve (minimum point), a is the difference between the maximum and minimum points of the curve, b is the rate that provides 50% of the response of the variable, and c is the slope of the curve.

The analyses of each phenological stage were complemented with the mathematical calculation of the values LD_{50} and LD_{80} (lethal dose, 50 or 80%), using the principle of inverse equation, according to Carvalho et al. (2005). Successive values of LD_{50} and LD_{80} were then subjected to polynomial regression models with high R^2, correlating the variables of plant development with herbicide susceptibility (Presoto et al., 2020).

3. Results and Discussion

Sourgrass weekly sowing promoted adequate range of phenological stages, what allowed application of glufosinate-ammonium on plants from three leaves to pre-flowering stage (Table 1), i.e., from 13 to 45 at BBCH scale (Hess et al., 1997). Percentage of control and shoot dry weight (SDW) were perfectly fitted to non-linear regressions, and R^2 was always higher than 0,95 (Table 2). Log-logistic model is the most recommended adjust for relationship of herbicide rate and efficacy.

Table 2. Statistic parameters of logistic models and coefficient of determination for glufosinate-ammonium efficacy when applied to sourgrass plants at different phenological stages. Machado, MG, Brazil, 2020.

Variable	Stage	Parameters				R^2
		Pmin	*a*	*b*	*c*	
Control[1] 14 DAA[2]	13	---	96.06	36.73	-5.41	0.98
	21	---	98.77	48.69	-2.46	0.99
	30	---	100.57	90.98	-2.44	0.99
	32	---	98.68	128.58	-2.95	0.99
	45	---	95.35	190.29	-2.25	0.99
Control[1] 21 DAA[2]	13	---	94.98	35.16	-5.74	0.96
	21	---	100.42	54.43	-2.25	0.98
	30	---	101.35	95.38	-2.87	0.99
	32	---	99.97	99.87	-2.52	0.99
	45	---	99.96	222.45	-2.02	0.99
Control[1] 28 DAA[2]	13	---	93.24	38.21	-6.39	0.95
	21	---	100.99	63.74	-2.26	0.98
	30	---	101.23	107.00	-3.71	0.99
	32	---	101.25	144.38	-2.98	0.99
	45	---	100.74	223.57	-2.23	0.99
Shoot dry weight percentage[1]	13	0.91	99.09	33.30	8.20	0.99
	21	0.47	99.27	28.02	3.28	0.99
	30	2.70	99.89	52.48	3.51	0.99
	32	10.98	88.65	49.25	2.34	0.99
	45	21.35	78.77	121.56	1.64	0.99

[1] Model: $y = a+(b/(1+(x/c)^d)$. [2] DAA = days after application.

In any evaluation, the more developed the plants were, the higher doses of glufosinate-ammonium were needed to achieve satisfactory control (Figure 1). Plants with three leaves were easily controlled by glufosinate-ammonium, once adequate control was reached with rates lower than 100 g ha^{-1} of active ingredient. However, if tillering stage is reached, higher doses were necessary to promote adequate control, of about 200 g ha^{-1} of glufosinate-ammonium; and it is even more difficult if pre-flowering (BBCH 45) stage is identified (Figure 1; Table 2). In the more developed stage, control by 80% was reached using 409.56 g ha^{-1} of glufosinate-ammonium, however at the harvest moment, plant residual material was still present in the pots, in percentual rate higher than 20%, if compared to the check plots. Then, in the case of SDW, it was not possible to identify LD_{80} of glufosinate-ammonium for pre-flowering plants (Table 3).

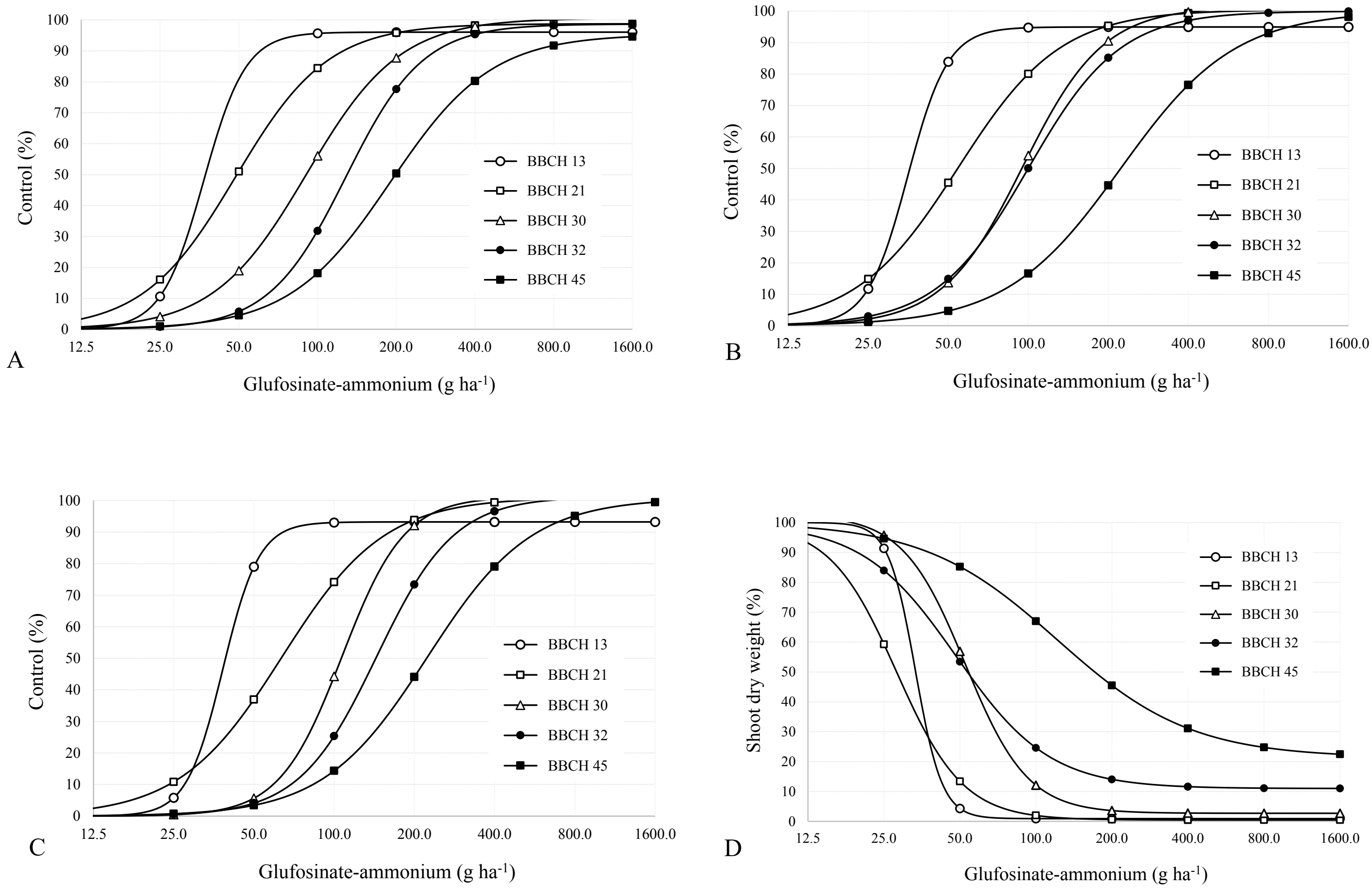

Figure 1. Control and shoot dry weight of sourgrass after application of different doses of glufosinate-ammonium. A – Control 14 DAA; B – Control 21 DAA; C – Control 28 DAA; D – Dry weight 28 DAA. DAA – Days after application. Machado, MG, Brazil, 2020.

Table 3. Values of LD_{50} and LD_{80} (g a.i. ha^{-1}) obtained for controlling sourgrass plants with glufosinate-ammonium with different periods of evaluation and phenological stages at the time of application. Machado, MG, Brazil, 2020.

Phenological Stage	Control (%) 14 DAA[1]	21 DAA	28 DAA	Shoot Dry Weight (%)	Average[2]
		Lethal dose – 50% (g a.i. ha^{-1})			
13	37.29	35.81	39.09	33.37	36.39
21	49.19	54.23	63.19	28.06	48.66
30	90.56	94.50	106.30	54.09	86.36
32	129.75	99.89	143.19	54.58	106.85
45	198.73	222.54	222.10	170.96	203.58
		Lethal dose – 80% (g a.i. ha^{-1})			
13	49.42	47.08	50.63	39.66	46.70
21	87.78	99.86	115.22	43.03	86.47
30	158.74	151.13	153.00	81.92	136.20
32	210.53	173.22	225.27	124.92	183.49
45	396.35	442.30	409.56	> 1,600.00	416.07

[1]DAA – Days after application; [2]Average of all variables analyzed (control and dry weight).

Relationship of sourgrass phenological scale and lethal doses of glufosinate-ammonium was perfectly fitted to polynomial models (Figures 2 and 3). In this case, the more difficult control becomes evident according to plants' development. For management practices, it would be very important to perform applications of glufosinate-ammonium on plants at early stages of development, mainly before tillering. Once plants begin tillering, demanded rates are equal to or higher than 200 g ha^{-1} to reach 80% of control (Figure 3). In this case, an extra herbicide may be included in the tank mixture, in order to increase control and reduce pressure of selection on glufosinate-ammonium.

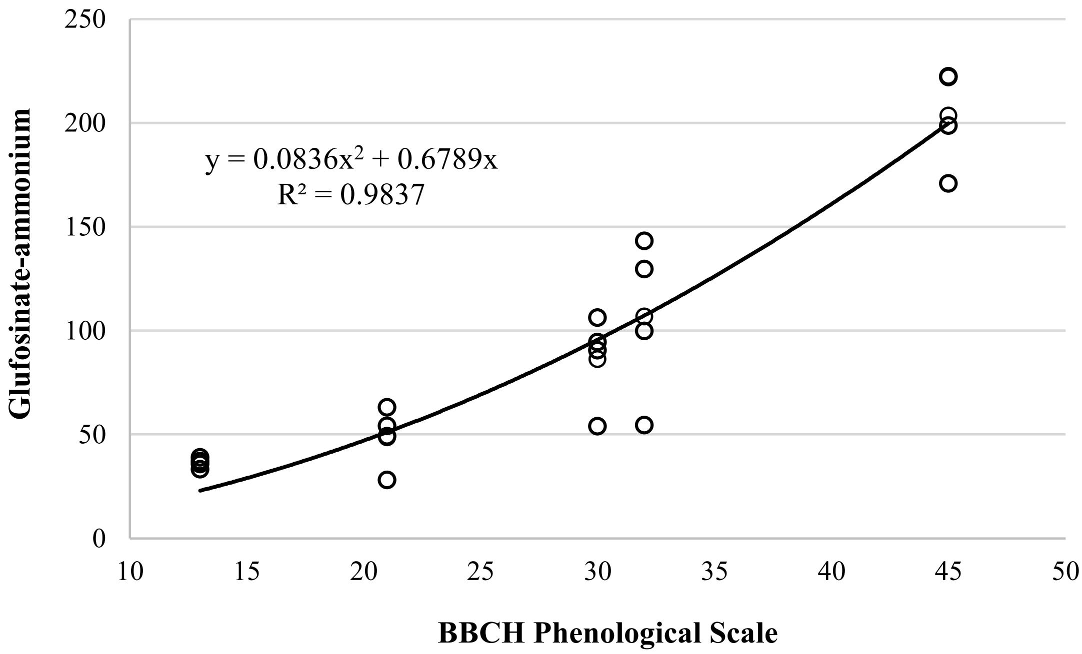

Figure 2. Evolution of LD_{50} (g ha^{-1} of glufosinate-ammonium) for sourgrass plants, estimated using evaluations of control percentage and residual shoot dry weight, affected by the plants' phenological stage at the time of applications of the herbicide glufosinate-ammonium. Machado, MG, Brazil, 2020.

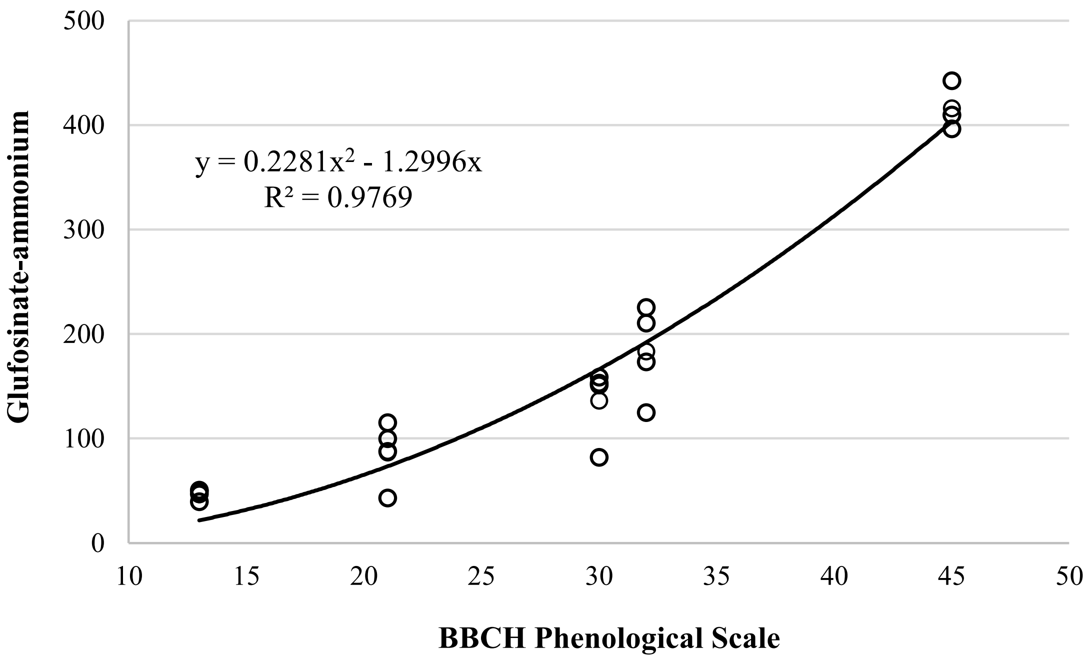

Figure 3. Evolution of LD_{80} (g ha^{-1} of glufosinate-ammonium) for sourgrass plants, estimated using evaluations of control percentage and residual shoot dry weight, affected by the plants' phenological stage at the time of applications of the herbicide glufosinate-ammonium. Machado, MG, Brazil, 2020.

Glyphosate-resistant sourgrass is a major problem in Brazilian agriculture; thus, farmers have adopted ACCase-inhibiting herbicides as the main post-emergence alternative herbicides (Barroso et al., 2014; Licorini et al., 2015; Carvalho et al., 2019). However, the limitation for this practice is also the phenological stage of the plants at the time of application; the herbicide efficacy level decreases when herbicides are applied on plants at later developmental stages, with fully developed clumps (Adegas and Gazziero, 2014; Presoto et al., 2020).

Depending upon sourgrass phenological stage, a single application of grass herbicides may be not enough to control full flowering plants, so a system with sequential applications (two) might be recommended to reach weed control above 80% (Zobiole et al., 2016). Gemelli et al. (2012) reported that ACCase-inhibiting herbicides may affect sourgrass plants at later developmental stages, producing characteristic symptoms of necrosis in the plants' growth regions. However, despite they cause necrosis in the leaf ends, the other leaf parts remain only chlorotic. Thus, these leaves remain erect, i.e., the herbicide application does not significantly decrease plant leaf area, which is still able to intercept light and, potentially, affect the development of crops.

Most recently, a biotype of sourgrass was identified in Brazil with multiple resistance to glyphosate and ACCase-inhibiting herbicides (Heap, 2021), what makes glufosinate-ammonium an interesting option for including a third mode of action in the management system. For example, Melo et al. (2012) evaluated the application of different herbicides on sourgrass plants with 3-5 tillers and found that clethodim applied alone (108 g ha^{-1}) was not efficient, reaching a maximum control of 17.5%; however, if a triple mixture was adopted (glyphosate + clethodim + glufosinate-ammonium) the control was 100% at 28 and 35 DAA. Also evaluating efficacy of glufosinate-ammonium on sourgrass, Silva et al. (2017) found control of 100% after applying 300 or 450 g ha^{-1} on plants with 3-4 leaves or 3-4 tillers.

These results detach that sourgrass young plants grown from seeds are more easily controlled at early stages of development. Carvalho et al. (2019) found excellent control of sourgrass (higher than 90%) when applying 62.4 g ha^{-1} of haloxyfop to plants at tillering stage that grew from seeds. However, the control of adult plants with perennial clumps and presence of rhizomes is more difficult. Thus, the best moment for post-emergence controlling *D. insularis* plants is up to 45 days after emergence, when the rhizomes are not fully formed (Gemelli et al., 2012; Andrade et al., 2019) and, in general, plants are not yet at the flowering stage.

Andrade et al. (2019) and Presoto et al. (2020) registered increasing linear relation between sourgrass development and higher demanded rates of herbicides. There are some explanations about these observations. Mostly, more developed plants have also higher amount of energetic reserves and more efficient metabolic paths, which contribute for segregating or detoxifying herbicides. On the other side, application of contact herbicides is more difficult on bigger clumps, once spray should be able to cover all the leaves and the rate effectively applied may not correspond to the recommended rate.

In the case of sourgrass, anatomical characteristics of leaves may also affect the absorption and translocation of herbicides. Most studies have shown a negative correlation between presence of trichomes and the absorption of herbicides by leaves (Hess and Falk, 1990). The presence of trichomes and layers of wax on leaves are found more consistently in later developmental stages of the plants (Carvalho et al., 2012; Barroso et al., 2015). The cuticle, consisted of waxes, is an important barrier to the entry of microorganisms and agrochemicals; however, the herbicide absorption is not only related to the cuticle thickness, but also to the cuticle lipidic constitution and level of prevention of entry of solutes (Carvalho et al., 2012; Barroso et al., 2015).

Regarding to energetic reserves, large quantity of starch is found in sourgrass rhizomes (Machado et al., 2008), which may hinder the translocation of herbicides and generate a fast shoot regrowth of weed plants. It was also reported that the difficulty to control sourgrass plants grown from rhizomes can be related to the epidermal thickness of the leaf adaxial and abaxial surfaces and to the leaf blade thickness, which are greater when compared to plants from seeds.

4. Conclusions

The more developed sourgrass plants were, the higher doses of glufosinate-ammonium were needed to achieve satisfactory control. Plants with three leaves were easily controlled by glufosinate-ammonium, with rates lower than 100 g ha^{-1}. If tillering stage is reached, rates of about 200 g ha^{-1} necessary to promote adequate control. Doses above 400 g ha^{-1} were necessary to reach adequate control of pre-flowering plant. Glufosinate-ammonium is an excellent alternative for controlling sourgrass, however a second herbicide may be necessary for applications after tillering. Strong relation between sourgrass phenological development and increasing necessary rates of glufosinate-ammonium was identified.

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