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Sourgrass phenological stage and efficacy of ACCase-inhibiting herbicides

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Highlights

- Herbicides are more efficient when applied before the sourgrass tillering or flowering.
- The greater the developmental stage of sourgrass plants, the more difficult the control.
- Herbicide mixtures or sequential applications may be needed for the control of sourgrass.

Abstract

Background: Sourgrass (*Digitaria insularis*) is a highly disseminated weed in Brazil. It is a perennial grass weed that has high infesting capacity in agricultural areas and presents glyphosate-resistant biotypes. An effective post-emergence control of sourgrass plants depends on their phenological stage at the time of herbicide application.
Objective: This work was developed with the objective of evaluating the effect of the sourgrass phenological stage at the time of herbicide application on the efficacy of ACCase-inhibiting herbicides.
Methods: Two independent experiments were conducted under greenhouse conditions to evaluate two ACCase-inhibiting herbicides: clethodim, and haloxyfop. The treatments were applied using a completely randomized block design, with an 8×4 factorial arrangement consisted of 8 herbicide rates (4, 2, 1, 1/2, 1/4, 1/8, and 1/16 times the recommended rate, and a control plot without herbicide application) and four phenological stages of sourgrass.
Results: The development of sourgrass plants after tillering decreased the efficacy of the herbicide molecules; however, clethodim and haloxyfop were efficient to control sourgrass at earlier developmental stages. Morphological, anatomical, and physiological changes in older plants may explain the lower susceptibility to herbicides.
Conclusions: Other control techniques should be considered for sourgrass plants at later developmental stages, such as sequential application or herbicide mixtures, to increase the efficacy of ACCase herbicides.

1 Introduction

Brazil is the country that has the highest diversity of species of the *Digitaria* genus, with 26 native and 12 exotic species (Canto-Dorow and Longhi-Wagner, 2001). One of these species that have wide geographical distribution is the sourgrass (*Digitaria insularis* (L.) Fedde), which occurs in most environments favorable to agriculture (López-Ovejero et al., 2017). *D. insularis* 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). They can form rhizomes that are short, but evident, forming pronounced clumps. These plants have a fast growth, production of large number of seeds, and high seed dissemination capacity during the whole summer (Gemelli et al., 2012; Ferreira et al., 2018).

Glyphosate applications control susceptible young weeds grown from seeds; however, the control is difficult when the weeds are grown from rhizomes, (Andrade et al., 2019). Thus, the best time for controlling *D. insularis* plants is up to 45 days after emergence, when the rhizomes are not yet formed (Gazola et al., 2016). The importance of sourgrass infestations in agricultural areas can be great for soybean and maize crops and citrus orchards because of glyphosate-resistant biotypes (Carvalho et al., 2011; Heap, 2019). Thus, the species, which previously had low agronomic importance due to its easy control, became one of the most competitive and important weeds in the country (Andrade et al., 2019). Lacerda and Victoria Filho (2004) found that only 128.5 g ha^{-1} of glyphosate were enough to control 50% (DL50) of these plants. However, few years later, after a selection process of resistant biotypes, the required rates of glyphosate to obtain DL50 were higher than 2,880 g ha^{-1} (López-Ovejero et al., 2017; Costa et al., 2018).

Considering the resistance cases, the first changes in the management system should be the substitution of active ingredients or the combination of herbicides with different mechanisms of action. The best results of post-emergence control of glyphosate-resistant sourgrass in Brazil have been obtained with the use of ACCase-inhibitor herbicides, applied alone or in combination with glyphosate (Melo et al., 2012; Barroso et al., 2014; Zobiole et al., 2016; Carvalho et al., 2019).

Barroso et al. (2014) found that the herbicides quizalofop, haloxyfop, sethoxydim, and clethodim are good options for the control of sourgrass plants at initial developmental stages, showing efficacies higher than 90% at 28 days after application. An efficient control of weeds by using post-emergence herbicides depends mainly on the developmental stage of the target-plants at the time of application (Melo et al., 2012; Barroso et al., 2014; Andrade et al., 2019). However, little scientific information is available about the efficacy of ACCase-inhibitor herbicides regarding rates to be used, and about the effects of the plant phenology at the time of application. Thus, this work was developed with the objective of evaluating the effect of the sourgrass phenological stage at the time of herbicide application on the efficacy of ACCase-inhibiting herbicides.

2 Material and Methods

The experiment was conducted at a greenhouse of the Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas, in Machado, MG, Brazil (21º40'S, 45º55'W, and 850 m altitude). Sourgrass seeds were collected in an agricultural area of the Machado campus, in an area infested with plants susceptible to glyphosate herbicide (Gonçalves Netto et al., 2015).

The sourgrass seeds were germinated in 2-liter plastic boxes filled with a commercial substrate (Pinus bark + turf + vermiculite) to select seedlings with adequate health; they were sown at different times to enable the joint application of herbicides to plants at different phenological stages. The seedlings were transplanted to 1-liter plastic pots when they had one fully expanded leaf; each pot with a mean of three plants represented one plot. The pots were filled with a mixture of commercial substrate, sieved clayey soil, vermiculite, and cattle manure at the proportion of 5:3:1:1, respectively. The soil of all plots was properly fertilized once and irrigated daily.

Two similar and independent experiments were conducted from August 2017 to June 2018. The first experiment was conducted to evaluate the efficacy of herbicide clethodim, which is classified in the cyclohexanedione group; and the second experiment was conducted to evaluate the efficacy of haloxyfop, which is from the aryloxyphenoxypropionate group, both ACCase-inhibitor herbicides.

Each experiment was conducted in a completely randomized block design, with an 8×4 factorial arrangement and five replications (160 experimental plots in each experiment). The treatments of both experiments consisted of 8 herbicide rates (4, 2, 1, 1/2, 1/4, 1/8, and 1/16 times the recommended rate, and a control plot without herbicide application) and four phenological stages of sourgrass. The recommended rates used were 108 g ha^{-1} of the active ingredient for clethodim, and 60 g ha^{-1} of the acid equivalent for haloxyfop (Rodrigues and Almeida, 2018).

Four plots of each phenological stage were sampled to measure the exact phenological stage, based on the scale of Hess et al. (1997) (BBCH), at the time of application of the herbicides clethodim (November 15, 2017) and haloxyfop (May 4, 2018) (Table 1). The herbicides were applied using a CO_2-pressurized backpack sprayer equipped with a spray boom consisting of two nozzles (XR 110.02; Teejet®, Wheaton, USA) positioned at 0.50 m above the targets, using pressure of 2.5 bar and a solution volume of 200 L ha^{-1}. Deionized water was used for the preparation of the solutions in all treatments to avoid contamination.

Table 1 - Phenological stages of sourgrass plants at the time of application of the herbicides clethodim and haloxyfop. Machado, MG, Brazil, 2017-2018

Stage	Description	Days after emergence	BBCH scale
	Clethodim		
1	3 leaves	35	13
2	2 tillers	42	22
3	5 tillers + 4 visible internodes	55	34
4	5 internodes to pre-flowering	76	49
	Haloxyfop		
1	3 to 4 leaves	19	14
2	1 developed tiller	33	21
3	3 tillers + 2 visible internodes	47	32
4	full flowering	61	60

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

Weed control and residual plant shoot dry weight (SDW) were evaluated at 28 days after application (DAA), in all plots. The control was evaluated as percentages, with 0% representing absence of symptoms caused by the herbicides, and 100% representing the death of the plant. The SDW was obtained by taking 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 plants (considered as 100%).

The data of each experiment were subjected to analysis of variance 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):

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

where y = percent 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 GR_{50} and GR_{80} (GR = growth reduction), using the principle of inverse equation, as described by Carvalho et al. (2015). The successive values of GR_{50} and GR_{80} were then subjected to linear or polynomial regression models with high R^2, correlating the variables of plant development with tolerance to herbicides.

3 Results and Discussion

The susceptibility of sourgrass at different phenological stages to the herbicide clethodim is shown in Table 2, presenting the statistic parameters for the fit of weed control percentage and shoot dry weight (SDW) of sourgrass at 28 DAA. The maximum recommended rate of clethodim is 108 g ha^{-1} (Rodrigues and Almeida, 2018), which was enough to promote satisfactory control of sourgrass up to the two-tiller stage (BBCH 22). The later stages (BBCH 34 and 49) required rates higher than 150 g ha^{-1} for an 80% control, which is the minimum percentage required to base a recommendation for agricultural crops (Table 2).

This denotes the effect of the sourgrass phenological stage on the efficiency of clethodim. The

Table 2 - Statistic parameters of logistic models, coefficient of determination, GR_{50} and GR_{80} for efficacy of the herbicides clethodim applied to sourgrass plants at different phenological stages. Machado, MG, Brazil, 2017

Variable	Stage	Parameters				R^2	(3)GR_{50}	(3)GR_{80}
		Pmin	*a*	*b*	*c*			
Control(1) 28 DAA(2)	13	X	99.94	13.28	-3.75	0.998	13.28	19.228
	22	X	102.10	12.24	-1.65	0.993	11.94	26.736
	34	X	102.16	38.35	-0.93	0.985	36.65	152.267
	49	X	112.27	120.96	-0.57	0.983	82.52	588.189
Shoot dry weight percentage(1)	13	3.231	96.86	10.39	6.59	0.997	10.29	13.174
	22	2.512	98.26	7.29	0.60	0.955	6.87	93.423
	34	1.808	97.96	101.49	0.51	0.961	93.45	1894.910
	49	-1.291	97.61	168.38	0.47	0.950	151.61	2575.836

(1) Model: y = a+(b/(1+(x/c)d). (2) DAA = days after application. (3) GR = growth reduction.

increasing evolution of GR_{50} and GR_{80}, considering the control percentage and SDW at 28 DAA, respectively, is shown in Figures 1 and 2. The herbicide rates required to obtain these weed control levels increased significantly after the stage 20 of the BBCH scale (beginning of tillering) (Figure 1).

Researches have frequently sought to control glyphosate-resistant sourgrass biotypes by using post-emergence application of ACCase-inhibitor herbicides (Barroso et al., 2014; Carvalho et al., 2019). However, the main limitation for this practice is the phenological stage of the plants at the time of application; the herbicide efficacy level decreases when applied on plants at later developmental stages, with fully developed clumps (Melo et al., 2012; Adegas and Gazziero, 2014). Gemelli et al. (2012) reported that ACCase-inhibitor herbicides 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 with chlorosis. Thus, the leaves remain erect, i.e., the herbicide application does not decrease significantly the plant leaf area, which is still able to intercept light and, potentially, affect the development of crops. 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%. Zobiole et al. (2016) found that single application of graminicide herbicides was not enough to control sourgrass plants at full flowering stage, and they indicated a sequential application system (two applications) for weed controls above 80%. In both cases, the combination of clethodim with other herbicides was needed to increase the weed control efficiency.

The results found with application of the herbicide haloxyfop on sourgrass plants at different phenological stages were similar to those found with application of clethodim. The recommended rate of haloxyfop is 60 g ha^{-1}; this rate was efficient to control plants up to the tillering stage (BBCH 22). The fit of the SDW data for BBCH 60 was not possible because the maximum rate did not reach 50% decrease in SDW, which is required by the model (Table 3). The increasing evolution of GR_{50} and GR_{80}, considering the control percentage and SDW at 28 DAA, is shown in Figures 3 and 4, respectively. The decreases in the efficacy of haloxyfop were even more significant than those found for clethodim, thus requiring applications to young plants.

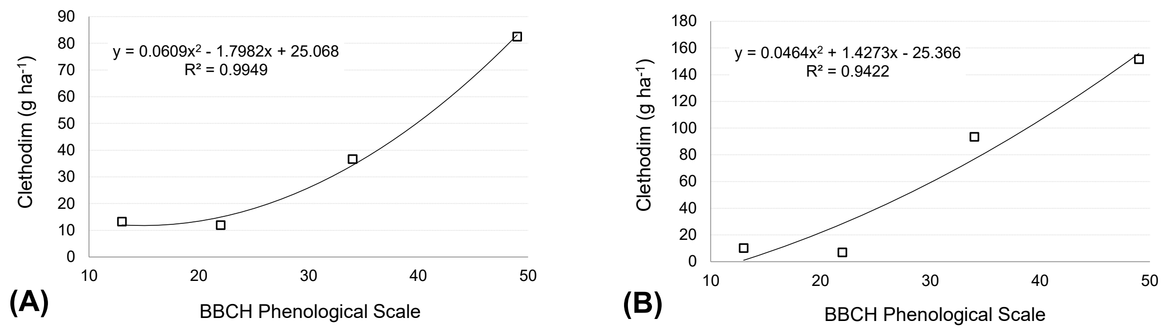

Figure 1 - Evolution of GR_{50} of sourgrass plants, estimated by the control percentage (A) and residual shoot dry weight (B) at 28 days after application, affected by the phenological stage at the time of applications of the herbicide clethodim. Machado, MG, Brazil, 2017.

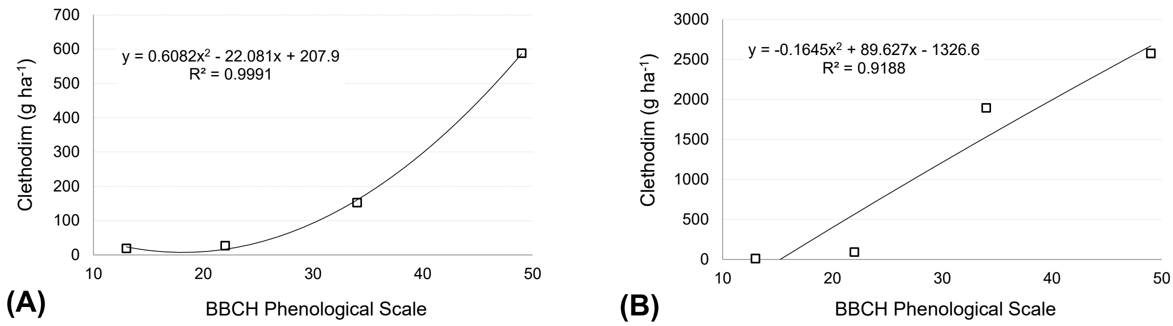

Figure 2 - Evolution of GR_{80} of sourgrass plants, estimated by the control percentage (A) and residual shoot dry weight (B) at 28 days after application, affected by the phenological stage at the time of applications of the herbicide clethodim. Machado, MG, Brazil, 2017.

Table 3 - Statistic parameters of logistic models, coefficient of determination, GR_{50} and GR_{80} for efficiency of the herbicide haloxyfop applied to sourgrass plants at different phenological stages. Machado, MG, Brazil, 2018

Variable	Stage	Parameters				R^2	(3)GR_{50}	(3)GR_{80}
		Pmin	*a*	*b*	*c*			
Control(1) 28 DAA(2)	14	X	101.92	3.98	-1.68	0.985	3.89	8.610
	21	X	101.77	3.79	-1.56	0.986	3.70	8.743
	32	X	120.29	85.15	-0.46	0.974	40.80	374.614
	60	X	126.48	179.87	-0.49	0.963	74.88	551.013
Shoot dry weight(1) percentage	14	3.139	96.34	3.08	1.87	0.996	2.95	7.052
	21	-8.318	108.84	13.88	0.31	0.911	23.59	417.696
	32	-17.485	113.14	191.20	0.39	0.935	347.78	1157.373
	60	-	-	-	-	-	> 240.00	-

(1) Model: y = a+(b/(1+(x/c)d). (2) DAA = days after application. (3) GR = growth reduction.

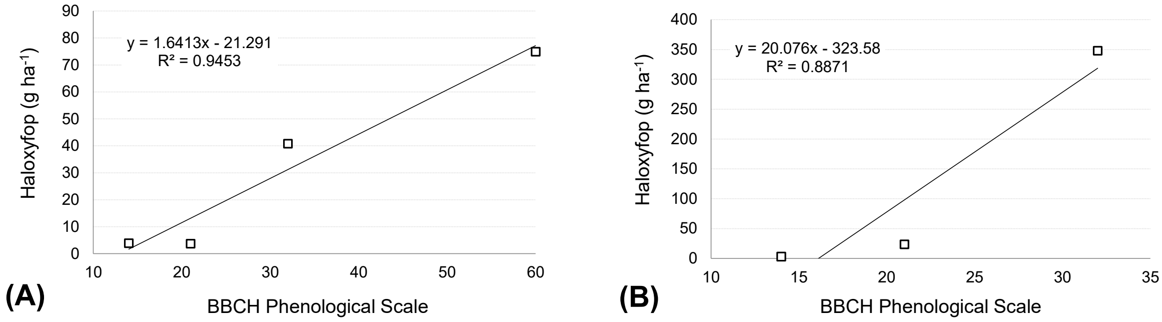

Figure 3 - Evolution of GR_{50} of sourgrass plants, estimated by the control percentage (A) and residual shoot dry weight (B) at 28 days after application, affected by the phenological stage at the time of applications of the herbicide haloxyfop. Machado, MG, Brazil, 2018.

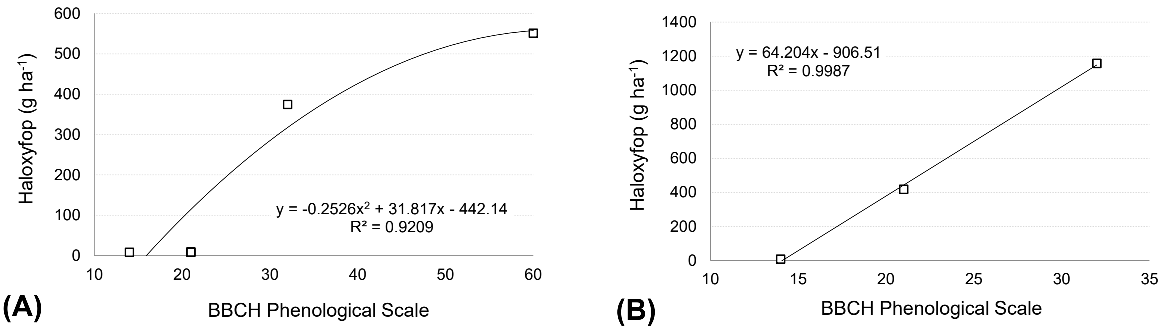

Figure 4 - Evolution of GR_{80} of sourgrass plants, estimated by the control percentage (A) and residual shoot dry weight (B) at 28 days after application, affected by the phenological stage at the time of applications of the herbicide haloxyfop. Machado, MG, Brazil, 2017.

These results agree with other studies that report that young plants developed from seeds are more easily controlled with the use of haloxyfop. 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 difficult. Thus, the best time for the control of *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, the plants are not yet at the flowering stage.

Anatomical characteristics of sourgrass leaves can affect the absorption and translocation of herbicides. These characteristics, mainly presence of trichomes and layers of wax on leaves, are found in later developmental stages of the plants (Carvalho et al., 2012; Barroso et al., 2015). A negative correlation between presence of trichomes and absorption of herbicides is found in most studies (Hess and Falk, 1990).

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 to the cuticle lipidic constitution and level of prevention of entry of solutes (Carvalho et al., 2012; Barroso et al., 2015). Machado et al. (2008) reported that the difficulty to control sourgrass plants 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. In addition, they found large quantity of starch in rhizomes, which may hinder the translocation of herbicides, including glyphosate, and generate a fast shoot regrowth of weed plants.

Therefore, the use of measures for controlling sourgrass plants at initial developmental stages are needed in agricultural areas. Sourgrass plants developed from seeds can be controlled by using only one application of a grass herbicide, and they are more easily controlled than plants from clumps. After the tillering and flowering stages, the control of sourgrass is difficult, often demanding herbicide mixtures or sequential applications. This denotes the importance of the control of plants at initial growth stages, mainly before the tillering and flowering stages.

4 Conclusions

The herbicides clethodim and haloxyfop were efficiency in the controling of sourgrass plants at initial developmental stages. Satisfactory control of sourgrass was possible up to the two-tiller stage (stage 22 in the BBCH scale), when using the recommended rate of both herbicides tested; however, the efficacy of the herbicides decreases as the plant grows after the tillering stage, when the control becomes increasingly ineffective. More developed plants require the use of other control technics, such as sequential applications or combination of herbicides, to increase the efficacy of ACCase-inhibitor herbicides.

5 Contributions

JCP: experiment conduction, data tabulation, and manuscript writing; JFA: experiment conduction and manuscript writing; Other authors: experiments conduction; SJPC: guidance, statistical analysis, paper writing and reviewing.

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