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Efficacy and Interaction of Haloxyfop-Clethodim Tank Mixtures to Post Emergence Control of Sourgrass in Brazil

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Abstract **– After detection of glyphosate-resistant biotypes of sourgrass (*Digitaria insularis*) in Brazil, ACCase inhibiting herbicides have become an excellent choice for post-emergence control of this species. Therefore, this work was developed with the objective of evaluating the efficacy and interaction of haloxyfop-clethodim tank mixtures to sourgrass control. The experiment was carried out in a greenhouse of IFSULDEMINAS, Campus Machado, in 2017, with randomized block design and six replications. Plots consisted of 1L-plastic pots filled with a mixture of sieved clay soil and commercial substrate, properly fertilized. A 4x4 factorial scheme was adopted, in which four were the doses of clethodim (0, 27, 54 and 108 g ha^{-1}), and four were the doses of haloxyfop (0, 15.6, 31.2 and 62,4 g ha^{-1}). At the time of application, full tillering stage was registered for the plants. Percentage control was evaluated at 21 and 28 DAA, and residual mass of dry matter at 28 DAA. Haloxyfop was more effective than clethodim to control sourgrass, reaching 96.7% of control at 28 DAA, at the dose of 62.4 g ha^{-1}. The contribution of haloxyfop in the mixtures was more significant than clethodim for controlling sourgrass, ensuring the effectiveness of the combinations. All mixtures evaluated were considered additive, suggesting this FOP-DIM combination does not promote efficacy problems. The mixtures of clethodim and haloxyfop were effective to control sourgrass.**

Keywords – Digitaria insularis**, Chemical Control, Post Emergence, Additive Effect.**

I. Introduction

Sourgrass (*Digitaria insularis* (L.) Fedde) is a very important weed commonly found in Brazilian farming areas, pastures, coffee plantations, orchards, roadsides and vacant lands. Years ago, this weed used to be less common in cultivated soils, however currently it has been frequently classified as one of the most important weed also in no-tillage areas of Brazilian cerrado and in southern cropping areas of the country [1].

These plants usually have slow initial growth up to 50 days after emergence (DAE), but with great potential to form dense perennial clumps that, under favorable conditions, may accumulate a large amount of dry matter. Species have typical ecophysiology of tropical plants, influenced by seasons and photoperiod conditions. When germinated in spring, with increasing photoperiod, they have long vegetative cycle, slow development and late flowering (80-100 DAE), with large accumulation of dry matter. When germinated in late summer, in decreasing photoperiod condition, plants have reduced vegetative cycle, less dry mass accumulation and early flowering [2], [3], [4]. Notably, this species frequently blooms between spring and summer; however, flowering plants commonly are visible throughout the year.

Sourgrass performs photosynthesis by C4 cycle, with Kranz structure and simple sheath, which is extremely favorable to its development in hot environments with less water availability. This species is considered a hard-to-kill weed in Brazil, and often requires the application of glyphosate doses higher than those recommended for appropriate control of other Poaceae family species [3].

The first world case of a glyphosate-resistant biotype of sourgrass was reported in Paraguay, in 2005. In Brazil, the first cases were reported in 2008 and 2011, in soybean and maize fields and in citrus orchards [1], [5], [6]. These glyphosate-resistant biotypes became sourgrass chemical control even more difficult.

In areas where herbicide-resistant weed biotypes are identified, the most common change adopted by farmers is the inclusion of alternative herbicides in growing system, applied alone or mixed with those herbicides for which resistance was detected [7]. In Brazil, glyphosate-resistant sourgrass biotypes have been frequently controlled in post emergence by Acetyl-CoA carboxylase (ACCase) inhibiting herbicides [8], [9].

Controlling glyphosate-resistant biotypes of sourgrass with glyphosate-ACCase mixtures is well understood in literature; however, mixtures of different groups of herbicides with the same mode of action were not evaluated. ACCase inhibiting herbicides are classified in three chemical groups: aryloxyphenoxypropionates (FOPs), cyclohexadiones (DIMs) and phenylpyrazolines (DENs). Frequently, FOPs and DIMs have different efficacy on the same species of weeds [9], [10]; what may suggest they do not bind exactly in the same site of enzyme or maybe they have specificities not completely understood; so, why not applying them together? This could be an interesting alternative to increase efficacy of chemical control and to reduce the possibilities of selecting biotypes with multiple resistance. Therefore, this work was developed with the objective of evaluating efficacy and interaction of different mixtures of haloxyfop (FOP) and clethodim (DIM) to post emergence control of sourgrass.

II. Material and Methods

The experiment was developed in greenhouse at the Federal Institute of Education, Science and Technology of the South of Minas Gerais, Campus Machado - MG (21º 40 'S, 45º 55' W, 850 m of altitude), in the municipality of Machado - MG, Brazil, between September and December, 2017. Due to the difficulty of chemical control, sourgrass (*Digitaria insularis* (L.) Fedde) was adopted as bioindicator of herbicide efficacy. Seeds were field collected and properly stored in paper bags.

Seeds of bioindicator were germinated in 2 L-plastic boxes, fulfilled with commercial substrate (*Pinus* bark + turf + vermiculite). At the phenological stage of one completely expanded leaf, seedlings were transplanted to plastic pots, where they were cultivated up to the end of the trial, in medium population of five plants per pot.

The experiment was installed according to a randomized block experimental design, where each plot was constituted by 1,0 L-plastic pot filled with a mixture of commercial substrate, disaggregated sieved clay soil and vermiculite (6:3:1 v/v), respectively. All the plots were properly fertilized and daily irrigated, without hydric deficit (Figure 1).

Treatments were organized according to a 4x4-factorial scheme, adopting four rates of haloxyfop (0, 15.6, 31.2 and 62.4 g ha^{-1}; Verdict®) and four rates of clethodim (0, 27, 54 and 108 g ha^{-1}; Select®), both ACCase inhibiting herbicides. This factorial scheme resulted in 16 treatments (tank mixtures) and 96 plots (six replicates). For all the treatments, mineral oil was added to spray solution, 0.4% v/v, and deionized water was used to prepare the solutions, for avoiding contamination.

Fig. 1. Perspective of experimental plots, with medium density of five plants of sourgrass at tillering phenological stage. Machado – MG, Brazil, 2017.

Herbicide applications were performed when plants reached phenological stage of tillering. For that, a CO_2-pressurized backpack sprayer was adopted, coupled to a single TeeJet XR 110.02 nozzle, placed at 0.50 m over the targets, reaching spray volume proportional to 200 L ha^{-1}. At 21 and 28 days after application (DAA), percent control was evaluated, as well as, mass of dry matter at 28 DAA. For percent evaluations, an arbitrary scale was adopted, varying between zero and 100%, where zero was attributed to plants with no symptoms and 100% for dead plants [11]. Mass of dry matter was reached harvesting all plant material remaining in the plots at 28 DAA; this material was dried in oven with air-forced circulation, at 70°C, by 72 hours, and furtherly weighted.

Data was analyzed by applying F-test on variance analysis, followed by Scott-Knott's test [12], both with 5% of significance (Software Sisvar v.5.0). If just one level of treatments is considered, quantitative treatments are observed, however to allow antagonism-synergism mixture analysis, regressions were not performed.

Analysis of antagonism-synergism was based on model elaborated by Colby (1967) [13]:

$$E = 100 - \frac{(100 - X) * (100 - Y)}{100}$$

Where: X is the percent control reached by haloxyfop at the rate of x; Y is the percent control reached by clethodim at the rate of y; and E is the *expected* percent control of herbicide mixtures in the same rates ($x + y$) [14], [15].

Then, E might be considered as the expected toxicity of the mixtures. If the observed response is higher than the expected, the mixture is considered synergistic; if the observed response is lower than the expected, the mixture is antagonistic; if the observed and expected response are equal, than the mixture is additive. For comparing expected and observed responses, LSD test was adopted, also with 5% of significance.

III. Results and Discussion

Efficacy of haloxyfop-clethodim mixtures are presented in Table 1. If herbicides are considered lonely, haloxyfop was the most efficient to control this biotype of sourgrass. The rate of 62.4 g ha^{-1} promoted 81.3% of control at 21 DAA and 96.7% at 28 DAA. On the other hand, the higher rate of clethodim reached 52 and 64.2% of control, at 21 and 28 DAA, respectively (Table 1). Phloem mobility of haloxyfop is higher than clethodim's, so haloxyfop may reach meristematic tissues easily and acts more efficiently on the rhizomes, what may contribute to understand these results [9].

If mixtures are considered, at 21 and 28 DAA, including haloxyfop in tank solutions promoted higher control of sourgrass than clethodim alone, even for the highest rate of clethodim. However, when mixtures included the highest rate of haloxyfop, percent control was not different to haloxyfop applied alone, newly confirming high efficacy of this molecule on sourgrass biotype. In the same way, dry matter of sourgrass was reduced by herbicides, with highlights to haloxyfop efficacy (Table 1).

Table 1. Percent control[1] and mass of dry matter of sourgrass (*Digitaria insularis*) after spraying different rates of haloxyfop and clethodim, isolated or in mixture, evaluated at 21 and 28 days after application (DAA). Machado - MG, Brazil, 2017

Haloxyfop (g ha^{-1})	Clethodim (g ha^{-1})			
	0	27	54	108
Evaluation of Percent Control at 21 DAA				
0	0.0 C c	34.7 C b	39.2 C b	52.0 B a
15.6	54.2 B b	65.5 B a	70.8 B a	73.3 A a
31.2	60.8 B b	78.7 A a	82.5 A a	78.3 A a
62.4	81.3 A a	82.7 A a	83.8 A a	83.3 A a
CV = 13.38	F_{int} = 6.790*	F_{fop} = 168.743*	F_{dim} = 34.017*	
Evaluation of Percent Control at 28 DAA				
0	0.0 C c	47.2 B b	48.8 B b	64.2 B a
15.6	64.0 B b	88.5 A a	89.7 A a	89.0 A a
31.2	86.7 A a	94.8 A a	97.7 A a	93.7 A a
62.4	96.7 A a	96.5 A a	97.5 A a	96.0 A a
CV = 11.94	F_{int} = 10.923*	F_{fop} = 185.675*	F_{dim} = 33.741*	
Evaluation of Mass of Dry Matter at 28 DAA				
0	7.64 B b	3.41 B a	2.98 B a	2.66 A a
15.6	2.93 A a	2.51 A a	2.41 A a	2.47 A a
31.2	2.75 A a	2.55 A a	2.11 A a	2.51 A a
62.4	2.43 A a	2.06 A a	2.22 A a	2.35 A a
CV = 18.58	F_{int} = 25.002*	F_{fop} = 64.383*	F_{dim} = 42.832*	

[1]Means followed by the same letter, upper case in the columns and lower case in the rows, are not different according to Scott-Knott's test, with 5% of significance; *Significant at F test, with 1% of significance.

Application of mixtures are an important alternative to control hard-to-kill weeds and herbicide resistant biotypes. Spraying herbicide mixtures are very common in Brazilian agriculture; however, there are lacks of information about efficacy and interaction of these herbicides, which may result in synergistic, additive or antagonistic performances [16].

After sourgrass glyphosate-resistant biotypes were identified in Brazil [5], [6], post-emergence control of this species became a challenge. Herbicides and mixtures of different mode of action have been tested extensively in order to promote sourgrass high control, and Acetyl-CoA carboxylase (ACCase) inhibiting herbicides have been sprayed frequently for this porpoise [8], [9], [17]. Associating herbicides with different mode of actions to glyphosate is a very important strategy to control hard-to-kill weeds and also to delay the selection of new glyphosate-resistant biotypes [15], [18], [19].

In this work, performance of nine haloxyfop-clethodim mixtures was evaluated at 21 and 28 DAA and all of them were considered additive according to Colby's test (Table 2). Therefore, haloxyfop-clethodim mixtures do not reduce efficacy of chemical control and, for some combinations, mixture efficacy may be higher than herbicides applied lonely (Table 1). Several studies may be found evaluating control of sourgrass with ACCase inhibiting herbicides; however, none of them evaluated an aryloxyphenoxypropionate-cyclohexadione (FOP-DIM) mixture.

In field condition, a glyphosate-FOP-DIM triple mixture may also be considered for weed control, possibly resulting in higher efficacy and lower possibility of multiple resistance selection. In scientific literature, it is not completely clear if different FOP-DIM herbicides share exactly the same binding site of ACCase [20], [21], [22]. Efficacy differences between FOP-DIM molecules are commonly found in literature, suggesting herbicide interaction with binding sites is influenced by biotypes and molecules [10].

Table 2. Analysis of haloxyfop-clethodim mixtures interaction at 21 and 28 days after application (DAA) upon sourgrass (*Digitaria insularis*). Machado - MG, Brazil, 2017.

Haloxyfop ($g\ ha^{-1}$)	Clethodim ($g\ ha^{-1}$)								
	27			54			108		
	Obs.[1]	Exp.[2]	Int.[3]	Obs.[1]	Exp.[2]	Int.[3]	Obs.[1]	Exp.[2]	Int.[3]
Mixture analysis at 21 DAA - LSD_t = 9.82									
15.6	65.5	70.1	=	70.8	72.2	=	73.3	78.0	=
31.2	78.7	74.4	=	82.5	76.2	=	78.3	81.2	=
62.4	82.7	87.8	=	83.8	88.6	=	83.3	91.0	=
Mixture analysis at 28 DAA - LSD_t = 7.52									
15.6	88.5	81.0	=	89.7	81.6	=	89.0	87.5	=
31.2	94.8	93.0	=	97.5	93.2	=	93.7	95.4	=
62.4	96.5	98.3	=	97.7	98.3	=	96.0	98.9	=

[1]Observed values; [2]Expected values; [3]Interaction analysis, considering LSD test applied with 5% of significance, in which (+) means the mixture is synergistic, (=) means the mixture is additive and (-) means the mixture is antagonistic.

Earlier structural studies of Zhang et al. (2004) [21] showed that haloxyfop (FOP) is bound near the active site of the carboxyl transferase (CT) domain of ACCase, at the interface of its dimer, and a large conformational change in the dimer interface is required for haloxyfop binding. On the other side, Xiang et al. (2009) [22] observed that tepraloxydim (DIM) has a different mechanism of inhibiting the CT activity compared to haloxyfop. Tepraloxydim probes a different region of the dimer interface and requires only small but important conformational changes in the enzyme, in contrast to haloxyfop.

Eleven spontaneous mutations of acetyl-CoA carboxylase have been identified in many herbicide-resistant populations of 42 species of grassy weeds. A combination of partially overlapping binding sites of the three classes of herbicides (FOP, DIM and pinoxaden) and the structure of their variable parts explained cross-resistance among and between the classes of ACCase inhibitors, as well as differences in their specificity [23]. Therefore, once efficacy differences exist, mixing different classes of ACCase inhibiting herbicides may increase control (Table 1) and possibly reduces the selection pressure for new resistant biotypes, once this mixture is not antagonistic (Table 2).

IV. Conclusions

The contribution of haloxyfop in the mixtures was more significant than clethodim for controlling sourgrass, ensuring the effectiveness of the combinations. All mixtures evaluated were considered additive, suggesting this FOP-DIM combination does not promote efficacy problems. The mixtures of clethodim and haloxyfop were effective to control sourgrass.

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