

# Interaction of clethodim with glyphosate and/or 2,4-d at different doses and spray volumes in the control of glyphosate-resistant ryegrass

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**Abstract: Background:** Ryegrass is considered an important weed in crops in the state of Rio Grande do Sul, Brazil, for being resistant to the herbicide glyphosate.

**Objective:** This study evaluates ryegrass control by applying different spray volumes and doses of clethodim alone or in combination with glyphosate and/or 2,4-D.

**Methods:** Two field experiments were conducted in a 5 x 3 factorial design. In experiment I, factor A consisted of clethodim (96 g a.i. ha<sup>-1</sup>); clethodim (96 g a.i. ha<sup>-1</sup>) + glyphosate (1,080 g a.e. ha<sup>-1</sup>); clethodim (96 g a.i. ha<sup>-1</sup>) + 2,4-D (1,047 g a.e. ha<sup>-1</sup>); clethodim (96 g a.i. ha<sup>-1</sup>) + glyphosate (1,080 g a.e. ha<sup>-1</sup>) + 2,4-D (1,047 g a.e. ha<sup>-1</sup>); and control without treatment. Factor B consisted of three spray volumes (40, 80, and 120 L ha<sup>-1</sup>). In the second experiment, factor A consisted of the same herbicides

**Keywords:** *Lolium multiflorum*, interaction, antagonism, application technology.

and their associations aforementioned and factor B consisted of three increasing doses of the herbicide clethodim (96, 192 and 288 g a.i. ha<sup>-1</sup>). The herbicides were applied at the milky stage of ryegrass grains. Ryegrass control at 10, 20, and 30 days after application (DAA) of herbicides and dry matter were evaluated.

**Results:** Spray volume reduction did not interfere with ryegrass control by clethodim and glyphosate or clethodim, glyphosate, and 2,4-D at 30 DAA, but it did interfere with the application of clethodim alone or associated only with 2,4-D. Doses above 192 g a.i. ha<sup>-1</sup> of clethodim alone or in mixture were efficient for ryegrass control.

**Conclusions:** Greater clethodim doses and spray volume increased control of ryegrass at the milky grain stage and decreased the antagonism in the associations with 2,4-D.

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## 1. Introduction

Ryegrass (*Lolium multiflorum*) is an annual poaceae adapted to the low temperatures of the southern region of Brazil, being developed in winter and spring. It can be sown as a winter cover through direct seeding, as agricultural crop for forage use, or even to provide mulch in orchards (Melo et al., 2012).

In direct sowing or in orchards, ryegrass control at different phenological stages is usually performed with application of non-selective herbicides and glyphosate is the most used (Vargas et al., 2006). Glyphosate is used in large-scale for its efficiency combined with the relatively low cost of the product. However, continuous glyphosate use and biocological characteristics of ryegrass resulted in the selection of biotypes resistant to this herbicide (Melo et al., 2012; Heap, 2020). As a management alternative in situations where ryegrass biotypes are resistant to glyphosate, herbicides that inhibit the ACCase enzyme (Acetyl Coenzyme A carboxylase) are a good control option.

ACCase inhibitor herbicides are used mainly in dicotyledonous crops, as they control only poaceae species (Bianchi et al., 2020), and the selectivity of these herbicides occurs through rapid metabolization or insensitivity of the enzyme. In poaceae tolerant to these herbicides, such as wheat to diclofop and clodinafop and rice to cyhalofop and profoxydim, it occurs due to rapid metabolization, although dicotyledons usually have tolerance due to insensitivity of the enzyme ACCase (Trezzi et al., 2007; Han et al., 2013).

ACCase inhibitor herbicides are divided into chemical groups aryloxyphenoxypropionate, cyclohexanedione, and phenylpyrazoles, acting to inhibit the formation of lipids, which make up 5 to 10% of the plant dry matter (Powles, Yu, 2010). The herbicide clethodim is an important inhibitor of the enzyme ACCase, belonging to the chemical group cyclohexanedione and used to control annual and perennial grasses in post-emergence, not causing phytotoxicity to dicotyledonous crops (Bianchi et al., 2020).

Association between herbicides is required as weeds grow heterogeneously in the soil, increasing the spectrum of control, besides serving as an alternative for the management of resistant biotypes (Jhala et al., 2013). In situations in which the species complex includes glyphosate-resistant of *Conyza* spp, *Lolium multiflorum*, *Eleusine indica*, and other glyphosate tolerant weeds, such as *Commelina* spp., *Ipomoea* spp.,

*Spermacoce latifolia*, and *Richardia brasiliensis*, combinations with glyphosate may include ACCase inhibitor and 2,4-D herbicides in order to broaden the spectrum of action (Maciel et al., 2013).

Association between glyphosate and ACCase inhibitor herbicides is an option for burndown systems prior to direct seeding and for control in post-emergence after establishment of RR<sup>®</sup> soybean crops (Barroso et al., 2014). This practice has been adopted in situations in which RR<sup>®</sup> corn appears as volunteer plant and in areas where glyphosate-resistant weeds, such as ryegrass and sourgrass (*Digitaria insularis*) occur (Maciel et al., 2013).

Reports of incompatibility emerged due to applying combination of herbicides for broadleaf and grass weeds, which interferes with mechanisms of action, resulting in negative interaction. In general, antagonism occurs when the association between herbicides is less effective than the herbicides alone (Green, 1989). On the other hand, when the effect of the association is synergistic weed control increases, sometimes allowing the use of lower doses of a certain herbicide (Blouin et al., 2004). As a management alternative, farmers use increasing doses of herbicides, especially those with reports of incompatibility, in order to minimize problems caused by the antagonism of herbicides mixed. However, increasing herbicide doses increase production costs, selection pressure, and environmental impacts.

Selecting the appropriate application technology ensures better control at a lower cost. Reducing the spray volume of herbicide application is an alternative, as long as it does not negatively influence herbicide effectiveness or selectivity (Souza et al., 2012). In addition, the spray volume required for efficient control post-emergence depends on target weeds, their development stage, herbicide, and environmental conditions at application (King and Oliver, 1992). In certain situations, with proper management and ideal environmental conditions, reduced spray volumes of herbicide can be adopted to efficiently control weeds.

Reducing the spray volume may or may not change the effectiveness of the herbicide in controlling weeds, varying according to the characteristics of each product and target species. Studies that seek to understand the influence of spray volume reduction on weed control are of paramount importance as low-volume spray systems require less water than conventional systems and tend to increase operational capacity, besides reducing production costs (Souza et al., 2012), being an alternative for weed management in crops.

The hypothesis of this study is that reducing the application volume reduces ryegrass control due to the antagonistic effect of the 2,4-D herbicide on clethodim when combined in the spray. Furthermore, increasing the dose of clethodim and adding glyphosate to the mixture reduces the antagonistic effect of 2,4-D on clethodim. This study evaluates ryegrass control by applying different spray volumes and doses of clethodim alone or in combination with glyphosate and/or 2,4-D.

## 2. Materials and methods

Two experiments were conducted, both in the field, at the Terras Baixas Experimental Station (ETB), which belongs to Embrapa Temperate Climate, in the municipality of Capão do Leão, Rio Grande do Sul State, Brazil (31° 48' 49.8" S, 52° 28' 06.8" W, 18 m altitude). The soil is classified as Eutroferric Hydromorphic Solodic Planosol, belonging to the Pelotas mapping unit (EMBRAPA, 2013). The randomized blocks design was used with four replicates. Experimental units measured 12.5 m<sup>2</sup> (2.5 x 5.0 m).

The first experiment evaluated spray volume reduction. Treatments were arranged in a 5 x 3 factorial design with factor A consisting of four herbicide treatments, clethodim (Select<sup>®</sup> 240 EC, 240 g a.i. L<sup>-1</sup>, EC, Arysta Life science), clethodim plus glyphosate (Atanor glyphosate 48<sup>®</sup>, 356 g a.e. L<sup>-1</sup>, CS, Atanor) or 2,4-D (2,4-D Amine 72<sup>®</sup>, 698 g a.e. L<sup>-1</sup>, CS, Atanor) and clethodim plus glyphosate plus 2,4-D, with the recommended doses of 96 g a.i. ha<sup>-1</sup>, 1080 g a.e. ha<sup>-1</sup> and 1047 g a.e. ha<sup>-1</sup>, respectively, and the control without treatment (AGROFIT, 2016). Factor B consisted of three spray volumes (40, 80, and 120 L ha<sup>-1</sup>). In addition, Assist<sup>®</sup> mineral oil at 1.0% was added to the herbicide clethodim. Applications were conducted with a backpack sprayer using pressurized CO<sub>2</sub> and calibrated to provide the desired spray volume of herbicide solution, with fan-type spraying nozzles 110.015. For this it was necessary to increase the application speed. At application, average relative humidity, temperature, and wind speed were 64%, 20.6 °C, and 1.2 m s<sup>-1</sup>, respectively.

The second experiment involved increasing doses of herbicide clethodim and was arranged similarly to the first experiment, with factor A consisting of the same herbicides and associations in the first experiment and factor B consisting of three increasing doses of the herbicide clethodim (96, 192 and 288 g a.i. ha<sup>-1</sup>). The adjuvant used and the dose were the same as in the first experiment. Applications were conducted with a backpack sprayer using pressurized CO<sub>2</sub> and calibrated to provide 120 L ha<sup>-1</sup> of herbicide spray, with fan-type spraying nozzles 110.015.

For both experiments, applications were made at the milky stage of ryegrass grains. The control, shoot dry matter (SDM) of ryegrass and the interaction between the associated herbicides were evaluated. Evaluations of ryegrass control were performed at 10, 20, and 30 days after application (DAA) using a percentage scale in which zero (0) and one hundred (100) corresponded to absence of injury and plant death, respectively. For the SDM variable, plants were cut at ground level at 30 DAA and the plant material was dried in a forced air oven at temperature of 60 °C until reaching constant mass (g ha<sup>-1</sup>).

Data were analyzed regarding homoscedasticity (Hartley's test) and normality (Shapiro-Wilk test). Using scripts from the R software (R Core Team, 2012) through the ExpDes.pt package, data were subjected to analysis of variance (p<0.05) and in case of statistical significance, the Tukey test was used to compare means (p<0.05).

### 3. Results and discussion

The analysis of variance indicated interaction between tested factors (Table 1). For the first experiment, corresponding to spray volume reduction, at 10 DAA treatments with clethodim alone or mixed with 2,4-D resulted in 30 to 38% ryegrass control regardless of spray volume (Table 2). Low control at 10 DAA can be expected due to the mechanism of action of herbicides, in which symptoms of necrosis at growth points can be observed from one week after clethodim application, leading to a greater number of days for plant death (Takano et al., 2020).

Ryegrass control at 10 and 20 DAA increased with the combination between clethodim and glyphosate for all spray volumes. However, it did not differ from the treatment where 2,4-D was added to the mixture in the volumes of 80 and 120 L ha<sup>-1</sup>. The spray volume of 40 L ha<sup>-1</sup> at 20 DAA resulted in ryegrass control less than other spray volumes evaluated. The highest percentages of control, in general, were observed for the spray volumes from 80 to 120, in which all treatments resulted in control great than 85% at 30 DAA (Table 2).

Combinations of clethodim and glyphosate or the three-way mixture reduced the SDM of ryegrass in relation to the mixture of clethodim and 2,4-D regardless of spray volume (Table 3). Mixing glyphosate and clethodim or other ACCase inhibitor herbicides reduced the biomass of glyphosate-resistant sourgrass (Barroso et al., 2014; Bianchi et al., 2020). Shoot dry matter data were similar to control data (Table 3).

At 30 DAA, reduced ryegrass control was observed with spray volume reduction (40 L ha<sup>-1</sup>) in treatments with clethodim alone or associated with 2,4-D, differing from volumes of 80 and 120 L ha<sup>-1</sup> (Table 2). Increasing the spray volume to 120 L ha<sup>-1</sup> caused a lower SDM when compared to the volumes of 40 and 80 L ha<sup>-1</sup> (Table 2).

At 30 DAA, for all spray volumes, association between clethodim and glyphosate and also the addition of 2,4-D to the mixture were more efficient for ryegrass control compared to the other treatments (Tables 2 and 3). In areas with glyphosate-resistant ryegrass, clethodim is an efficient alternative for the management of this weed (Vargas et al., 2006). Including glyphosate with clethodim increased ryegrass control and reduced SDM at most evaluation times and spray volumes, including treatments that also contained 2,4-D (Tables 2 and 3). On the other hand, the mixture between haloxyfop, glyphosate, and 2,4-D or dicamba showed antagonistic effect for sourgrass control, which was attributed to the decrease in the translocation of haloxyfop to the sites of action when associated with auxinic herbicides (Pereira et al., 2018).

Our results differ from previous literature that reports similar control of large crabgrass (*Digitaria sanguinalis*) when clethodim was applied at spray volumes between 26 and 140 L ha<sup>-1</sup> (Tredaway et al., 1998) and similar control of you need to state the weed when glyphosate was applied at spray volumes of 30, 60, and 150 L ha<sup>-1</sup> (Bueno et al., 2013). However, they are consistent with Almeida et al. (2014) who reported reduced control of *Urochloa ruziziensis* by glyphosate when spray volume was reduced from 200 to 50 L ha<sup>-1</sup>. Creech et al. (2015b) confirmed mixed results from other literature when they reported variable responses to spray volume when glyphosate and 2,4-D were applied to corn, soybean, velvetleaf, and amaranth. In a second paper, they reported that as spray volume increased, droplet diameter increased and the concentration of herbicide and surfactants in each droplet decreased, suggesting that the effect of spray volume on weed control may be associated with changes in droplet size (Creech et al., 2015a).

In the second experiment with increasing doses of clethodim, ryegrass control was less when clethodim was

**Table 1** - Analysis of variance (ANOVA) framework for the Control at 10, 20, and 30 days after application (DAA) and shoot dry matter (SDM) of the study spray volume and increasing doses of the herbicide clethodim

Factor	Control (DAA)						SDM	
	10		20		30		30 DAA	
	GL	p	GL	p	GL	p	GL	p
Herbicide	4	0,0001	4	0,0001	4	0,0001	4	0,0001
Spray	2	0,0001	2	0,0001	2	0,0001	2	0,0079
Herbicide x Spray	8	0,0135	8	0,0003	8	0,0003	8	0,0372
Block	3	0,4594	3	0,4579	3	0,3436	3	0,6302

  

Factor	Control (DAA)						SDM	
	10		20		30		30 DAA	
	GL	p	GL	p	GL	p	GL	p
Herbicide	4	0,0001	4	0,0001	4	0,0001	4	0,0001
Dose	2	0,0469	2	0,0006	2	0,0075	2	0,0438
Herbicide x Dose	8	0,0367	8	0,0009	8	0,0337	8	0,0429
Block	3	0,3712	3	0,4356	3	0,2134	3	0,4492

\* Significant at 5% probability. <sup>ns</sup> Not significant a 5% probability

**Table 2** - Ryegrass in the milky phase of grains control (%) at 10, 20, and 30 days after application (DAA) of clethodim alone and associated with glyphosate and 2,4-D as a function of spray volume

Treatments	Control (%)		
	40 L ha <sup>-1</sup>	80 L ha <sup>-1</sup>	120 L ha <sup>-1</sup>
<b>10 DAA</b>			
Clethodim*	30 cA	36 bA	38 bA
Clethodim + glyphosate	65 aB <sup>1</sup>	75 aA	81 aA
Clethodim + 2,4-D	30 cA	35 bA	35 bA
Clethodim + glyphosate + 2,4-D	55 bB	68 aA	74 aA
Control treatment	0 dA	0 cA	0 cA
CV (%)	11,41		
<b>20 DAA</b>			
Clethodim	55 cB	69 bA	70 bA
Clethodim + glyphosate	83 aB	94 aA	96 aA
Clethodim + 2,4-D	52 cC	65 bB	72 bA
Clethodim + glyphosate + 2,4-D	72 bB	88 aA	93 aA
Control treatment	0 dA	0 cA	0 cA
CV (%)	6,72		
<b>30 DAA</b>			
Clethodim	77 bB	90 bcA	95 abA
Clethodim + glyphosate	97 aA	99 aA	100 aA
Clethodim + 2,4-D	75 bC	86 cB	92 bA
Clethodim + glyphosate + 2,4-D	93 aA	98 abA	99 abA
Control treatment	0 cA	0 dA	0 cA
CV (%) <sup>2</sup>	4,98		

<sup>1</sup> Means followed by the same lowercase letter in the column and uppercase letter in the line, did not indicate significant difference by tukey test ( $p < 0.05$ ).

<sup>2</sup> Coefficient of variation. \*Clethodim doses (96 g a.i. ha<sup>-1</sup>); clethodim (96 g a.i. ha<sup>-1</sup>) + glyphosate (1,080 g a.e. ha<sup>-1</sup>); clethodim (96 g a.i. ha<sup>-1</sup>) + 2,4-D (1,047 g a.e. ha<sup>-1</sup>); clethodim (96 g a.i. ha<sup>-1</sup>) + glyphosate (1,080 g a.e. ha<sup>-1</sup>) + 2,4-D (1,047 g a.e. ha<sup>-1</sup>)

**Table 3** - Shoot dry matter (SDM) (g ha<sup>-1</sup>) of ryegrass at 30 days after application (DAA) of clethodim alone and associated with glyphosate and 2,4-D as a function of spray volume

Treatments	SDM (g ha <sup>-1</sup> )		
	40 L ha <sup>-1</sup>	80 L ha <sup>-1</sup>	120 L ha <sup>-1</sup>
Clethodim*	0,304 aB	0,318 aAB	0,376 bcA
Clethodim + glyphosate	0,302 aA	0,290 aA	0,266 aA
Clethodim + 2,4-D	0,394 bB	0,480 bB	0,318 bcA
Clethodim + glyphosate + 2,4-D	0,320 aA	0,282 aA	0,246 aA
Control treatment	0,485 bA	0,480 bA	0,476 cA
CV (%)	20,47		

<sup>1</sup> Means followed by the same lowercase letter in the column and uppercase letter in the line, did not indicate significant difference by tukey test ( $p < 0.05$ );<sup>2</sup>

Coefficient of variation. \*Clethodim doses (96 g a.i. ha<sup>-1</sup>); clethodim (96 g a.i. ha<sup>-1</sup>) + glyphosate (1,080 g a.e. ha<sup>-1</sup>); clethodim (96 g a.i. ha<sup>-1</sup>) + 2,4-D (1,047 g a.e. ha<sup>-1</sup>); clethodim (96 g a.i. ha<sup>-1</sup>) + glyphosate (1,080 g a.e. ha<sup>-1</sup>) + 2,4-D (1,047 g a.e. ha<sup>-1</sup>)

applied alone or associated with 2,4-D compared to the other treatments for all doses 10 DAA (Table 4). Addition of 2,4-D in the mixture between clethodim and glyphosate did not interfere with ryegrass control when compared to the treatment without 2,4-D at 10 and 20 DAA for the three doses evaluated (Table 4). Similarly, the use of doses above 105 g a.i. ha<sup>-1</sup> of clethodim mixed with glyphosate and 2,4-D was efficient to control volunteer glyphosate-resistant corn (Harre et al., 2020). In another study, sourgrass control was effective with application of a mixture between haloxyfop and glyphosate (124 g a.i. + 1,440 g a.e. ha<sup>-1</sup>). Sourgrass

control was reduced when 2,4-D (1,000 g a.i. ha<sup>-1</sup>) was added to the mixture (Pereira et al., 2018).

Clethodim applied at 192 or 288 g a.i. ha<sup>-1</sup> reduced the SDM of ryegrass when mixed with 2,4-D, although there was no difference regarding the dose of clethodim for the other mixtures (Table 5). Similar behavior was observed by increasing the dose of clodinafop from 48 to 96 g ha<sup>-1</sup> in mixture with 2,4-D, reducing the shoot green matter of ryegrass, while smaller doses showed antagonistic interaction (Trezzi et al., 2007). However, increasing the doses of clethodim mixed with 2,4-D increased the

**Table 4** - Ryegrass control (%) at 10, 20, and 30 days after application (DAA) of clethodim alone and associated with glyphosate and 2,4-D as a function of different doses of clethodim

Treatments	Control (%)		
	96 g a.i. ha <sup>-1</sup>	192 g a.i. ha <sup>-1</sup>	288 g a.i. ha <sup>-1</sup>
<b>10 DAA</b>			
Clethodim*	33 bA	34 bA	38 bA
Clethodim + glyphosate	70 aA	73 aA	71 aA
Clethodim + 2,4-D	40 bAB <sup>1</sup>	34 bB	43 bA
Clethodim + glyphosate + 2,4-D	62 aB	76 aA	72 aA
Control treatment	0 cA	0 cA	0 cA
CV (%)	12,15		
<b>20 DAA</b>			
Clethodim	83 cB	86 bA	87 bA
Clethodim + glyphosate	98 aA	98 aA	97 aA
Clethodim + 2,4-D	79 cB	79 cB	88 bA
Clethodim + glyphosate + 2,4-D	92 bB	97 aA	96 aA
Control treatment	0 dA	0 dA	0 cA
CV (%)	3,59		
<b>30 DAA</b>			
Clethodim	97 abA	97 abA	98 aA
Clethodim + glyphosate	100 aA	100 aA	100 aA
Clethodim + 2,4-D	89 bB	93 bB	98 aA
Clethodim + glyphosate + 2,4-D	98 aA	100 aA	99 aA
Control treatment	0 cA	0 cA	0 bA
CV (%)	2,64		

<sup>1</sup>Means followed by the same lowercase letter in the column and uppercase letter in the line, did not indicate significant difference by tukey test ( $p < 0.05$ ); <sup>2</sup> Coefficient of variation. \*Clethodim doses (96 g<sup>1</sup>, 192 g our 288 g a.i. ha<sup>-1</sup>); clethodim (96 g<sup>1</sup>, 192 g our 288 g a.i. ha<sup>-1</sup>) + glyphosate (1,080 g a.e. ha<sup>-1</sup>); clethodim (96 g<sup>1</sup>, 192 g our 288 g a.i. ha<sup>-1</sup>) + 2,4-D (1,047 g a.e. ha<sup>-1</sup>); clethodim (96 g<sup>1</sup>, 192 g our 288 g a.i. ha<sup>-1</sup>) + glyphosate (1,080 g a.e. ha<sup>-1</sup>) + 2,4-D (1,047 g a.e. ha<sup>-1</sup>)

antagonism in the mixture (Gomes et al., 2020), showing that increasing the dose of clethodim in some cases may not change the antagonistic effect caused by 2,4-D.

The three-way mixture resulted in lower control when clethodim was applied at 96 g a.i. ha<sup>-1</sup> compared to the other rates evaluated 20 DAA, but control 30 DAA was similar regardless of clethodim rate. Interactions evaluated at 10 and 20 DAA for all doses of clethodim were higher in the mixture between clethodim and glyphosate and in the mixture of clethodim, glyphosate, and 2,4-D, while the subsequent evaluation (30 DAA) did not differ

(Table 4). Applying clethodim and glyphosate is a viable alternative for ryegrass control in doses above 108 g ha<sup>-1</sup> of the graminicide (Melo et al., 2012). Synergism observed in the association between glyphosate and ACCase inhibitor herbicides is commonly attributed to the increase in the flow of photoassimilates provided by the action of glyphosate, improving absorption and translocation of ACCase inhibitor herbicides (Barroso et al., 2014; Bianchi et al., 2020).

Pretreatment of ryegrass plants (*Lolium rigidum*) with 2,4-D increased the expression of P-450 and improved

**Table 5** - Shoot dry matter (SDM) (g ha<sup>-1</sup>) of ryegrass at 30 days after application (DAA) of clethodim alone and associated with glyphosate and 2,4-D as a function of different doses of clethodim

Treatments	SDM (g ha <sup>-1</sup> )		
	96 g a.i. ha <sup>-1</sup>	192 g a.i. ha <sup>-1</sup>	288 g a.i. ha <sup>-1</sup>
Clethodim*	0,264 abB	0,236 aA	0,264 abB
Clethodim + glyphosate	0,217 aA	0,216 aA	0,212 aA
Clethodim + 2,4-D	0,338 bB	0,288 bA	0,291 bA
Clethodim + glyphosate + 2,4-D	0,248 abA	0,246 abA	0,240 abA
Control treatment	0,464 cA	0,485 cA	0,460 cA
CV (%)	21,93		

<sup>1</sup> Means followed by the same lowercase letter in the column and uppercase letter in the line, did not indicate significant difference by tukey test ( $p < 0.05$ ); <sup>2</sup> Coefficient of variation. \*Clethodim doses (96 g<sup>1</sup>, 192 g our 288 g a.i. ha<sup>-1</sup>); clethodim (96 g<sup>1</sup>, 192 g our 288 g a.i. ha<sup>-1</sup>) + glyphosate (1,080 g a.e. ha<sup>-1</sup>); clethodim (96 g<sup>1</sup>, 192 g our 288 g a.i. ha<sup>-1</sup>) + 2,4-D (1,047 g a.e. ha<sup>-1</sup>); clethodim (96 g<sup>1</sup>, 192 g our 288 g a.i. ha<sup>-1</sup>) + glyphosate (1,080 g a.e. ha<sup>-1</sup>) + 2,4-D (1,047 g a.e. ha<sup>-1</sup>)

plant metabolism when diclofop was applied, with survival of 71% of the plant population considered susceptible to the herbicide (Han et al., 2013). Therefore, it can be inferred that 2,4-D has some antagonistic effect on the effectiveness of clethodim when applied in mixture, and one of the possible causes is related to increasing metabolization and reduced translocation (Gomes et al., 2020). Another hypothesis to be highlighted is that 2,4-D increases the expression of P450 enzymes, and thus provides an increase in the metabolism of ACCase-inhibiting herbicides (Han et al., 2013; Polito et al., 2021).

At 30 DAA, all treatments were effective for ryegrass control. However, at doses of 96 and 192 g a.i. ha<sup>-1</sup>, the mixture between of clethodim and 2,4-D resulted in reduced control compared to other treatments. On the other hand, increasing the dose of clethodim to 288 g a.e. showed no difference between treatments, nullifying this possible negative effect on the interaction (Table 4).

The hypothesis to explain antagonism in the mixture of these herbicides in association is that ACCase inhibitor herbicides act to increase membrane depolarization with an anti-auxin mechanism in the proton efflux, while auxinic herbicides have the opposite effect (Shimabukuro and Hoffer, 1994; Liu et al., 2017). Thus, membrane polarization caused by auxinic herbicides tends to decrease the movement of ACCases herbicide, and together with the increase in the expression of P450 enzymes, they

increase the chance of metabolization by plants, adding an antagonistic effect to the mixture (Han et al., 2013; Polito et al., 2021).

#### 4. Conclusions

The use of clethodim alone or associated only with 2,4-D in the spray volume of 40 L ha<sup>-1</sup> reduces the efficiency for ryegrass control. The mixture between clethodim and glyphosate or glyphosate and 2,4-D allows spraying with low spray volume. The use of a greater volume of solution (120 L ha<sup>-1</sup>) allows controls greater than or equal to 92% and reduces a possible antagonistic effect in the mixture of clethodim with 2,4-D. The use of doses equal to or greater than 192 g a.i. of clethodim and the addition of glyphosate together with the spray solution, reduce the antagonistic effect of 2,4-D on clethodim in the control of ryegrass in the milky phase of grains.

#### Authors' contributions

The design and writing of the manuscript was carried out equally by all authors.

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#### References

- Almeida DP, Timossi PC, Lima SF, Silva UR, Reis EF. [Weather conditions and application volumes in the desiccation of *Urochloa ruziziensis* and spontaneous vegetation]. *Rev Bras Herbicida*. 2014;13(3):245-51. Portuguese. Available from: <https://doi.org/10.7824/rbh.v13i3.281>
- Barroso AAM, Albrecht AJP, Reis FC, Filho RV. [Accase and glyphosate different formulations herbicides association interactions on sourgrass control]. *Planta Daninha*, 2014;32(3):619-27. Portuguese. Available from: <https://doi.org/10.1590/S0100-83582014000300018>
- Bianchi L, Anunciato VM, Gazola T, Perissato SM, Dias RC, Tropaldi L et al. Effects of glyphosate and clethodim alone and in mixture in sourgrass (*Digitaria insularis*). *Crop Prot*. 2020;38:105322. Available from: <https://doi.org/10.1016/j.cropro.2020.105322>
- Blouin DC, Webster EP, Zhang W. Analysis of synergistic and antagonistic effects of herbicides using nonlinear mixed-model methodology. *Weed Technol*. 2004;18(2):464-72. Available from: <https://doi.org/10.1614/WT-03-047R1>
- Bueno MR, Alves GS, Paula ADM, Cunha JPAR. [Spray volume and adjuvant effects on weed control with glyphosate]. *Planta Daninha*. 2013;31(3):705-13. Portuguese. Available from: <https://doi.org/10.1590/S0100-83582013000300022>
- Creech CF, Henry RS, Fritz BK, Kruger GR. Influence of herbicide active ingredient, nozzle type, orifice size, spray pressure, and carrier volume rate on spray droplet size characteristics. *Weed Technol*. 2015a;29(2):298-310. Available from: <https://doi.org/10.1614/WT-D-14-00049.1>
- Creech CF, Henry RS, Werle R, Sandel ILD, Hewitt AJ, Kruger GR. Performance of postemergence herbicides applied at different carrier volume rates. *Weed Technol*. 2015b;29(3):611-24. Available from: <https://doi.org/10.1614/WT-D-14-00101.1>
- Gomes HLL, Sambatti VC, Dalazen G. Sourgrass control in response to the association of 2,4-d to accase inhibitor herbicides. *Biosci J*. 2020;36(4):1126-36. Available from: <https://doi.org/10.14393/BJ-v36n4a2020-47895>
- Green JM. Herbicide antagonism at the whole plant level. *Weed Technol*. 1989;3(2):217-26.
- Han H, Yu Q, Cawthray GR, Powles SB. Enhanced herbicide metabolism induced by 2,4-D in herbicide susceptible *Lolium rigidum* provides protection against diclofop-methyl. *Pest Manag Sci*. 2013;69(9):996-1000. Available from: <https://doi.org/10.1002/ps.3552>
- Harre NT, Young JM, Young BG. Influence of 2,4-D, dicamba, and glyphosate on clethodim efficacy of volunteer glyphosate-resistant corn. *Weed Technol*. 2020;34(3):394-401. Available from: <https://doi.org/10.1017/wet.2019.124>
- Heap I. Internacional survey of herbicide resistant weeds. *Weedscience*. 2020[access Sept 5, 2020]. Available from: [www.weedscience.org](http://www.weedscience.org)

- Jhala A, Ramirez AHM, Knezevic SZ, Damme PV, Singh M. Herbicide tank mixtures for broad-spectrum weed control in florida citrus. *Weed Technol.* 2013;27(1):129-37. Available from: <https://doi.org/10.1614/WT-D-12-00105.1>
- King CA, Oliver LP. Application rate and timing of acifluorfen, bentazon, chlorimuron, and imazaquin. *Weed Technol.* 1992;6(3):526-34. Available from: <https://doi.org/10.1017/S0890037X00035740>
- Liu Z, Li P, Sun X, Zhou F, Yang C, Li L et al. Fluazifop-P-butyl induced ROS generation with IAA (indole-3-acetic acid) oxidation in *Acanthospermum hispidum* D.C. *Pestic Biochem Physiol.* 2017;143:312-18. Available from: <https://doi.org/10.1016/j.pestbp.2017.10.005>
- Maciel CDG, Zobiolo LHS, Souza, JI. [Efficacy of Haloxifop R (GR-142) herbicide isolated and associated to 2,4-D in maize hybrids RR<sup>®</sup> volunteer control]. *Rev Bras Herbic.* 2013;12(2):112-23. Portuguese. Available from: <https://doi.org/10.7824/rbh.v12i2.244>
- Melo MSC, Rosa LE, Brunharo CACG, Nicolai M, Christoffoleti PJ. [Chemical control alternatives for sourgrass (*Digitaria insularis*) resistant to glyphosate]. *Rev Bras Herbic.* 2012;11(2):195-203. Portuguese. Available from: <https://doi.org/10.7824/rbh.v11i2.145>
- Pereira GR, Zobiolo LHS, Rossi CVS. [Sourgrass response to mixtures of haloxifop and glyphosate with synthetic auxinic herbicides]. *Rev Bras Herbic.* 2018;17(2):1-7. Portuguese. Available from: <https://doi.org/10.7824/rbh.v17i2.606>
- Polito RA, Pasqualotto L, Dysarz R, Cinelli R, Heck T, Nunes AL. Interaction of acetyl-CoA carboxylase enzyme inhibiting herbicides with auxin herbicides on ryegrass. *Cienc Rural.* 2021;51(4):1-2. Available from: <https://doi.org/10.1590/0103-8478cr20200462>
- Powles SB, Yu Q. Evolution in action: plants resistant to herbicides. *Annu Rev Plant Biol.* 2010;61(7):317-47. Available from: <https://doi.org/10.1146/annurev-arplant-042809-112119>
- Shimabukuro RH, Hoffer BL. Effects of transmembrane proton gradient and lipid biosynthesis in the mode of action of diclofop-methyl. *Pestic Biochem Physiol.* 1994;48(2):85-97. Available from: <https://doi.org/10.1006/pest.1994.1010>
- Souza LA, Cunha JPAR, Pavanin LA. [Weed deposition of 2,4-D Amine herbicide applied with different spray volumes and nozzles]. *Rev Bras Cienc Agron.* 2012;43(1):78-85. Portuguese. Available from: <https://doi.org/10.1590/S1806-66902012000100010>
- Takano HK, Ovejero RFL, Belchior GG, Maymone GPL, Dayan FE. AC-Case-inhibiting herbicides: mechanism of action, resistance evolution and stewardship. *Sci Agric.* 2020;78(1):1-11. Available from: <https://doi.org/10.1590/1678-992x-2019-0102>
- Tredaway JA, Patterson MG, Wehtj GR. Interaction of clethodim with pyriothiac and bromoxynil applied in low volume. *Weed Technol.* 1998;12(1):185-89. Available from: <https://doi.org/10.1017/S0890037X00042779>
- Trezzi MM, Mattei D, Vidal RA, Kruse ND, Gustman MS, Viola R et al. [Antagonismo das associações de clodinafop-propargyl com metsulfuron-methyl e 2,4-d no controle de azevém (*Lolium multiflorum*)]. *Planta Daninha.* 2007;25(4):839-47. Portuguese. Available from: <https://doi.org/10.1590/S0100-83582007000400021>
- Vargas L, Roman ES, Rizzardi MA, Toledo REB. [Management of glyphosate-resistant ryegrass in apple orchards with herbicide select (clethodim)]. *Rev Bras Herbic.* 2006;5(1):30-6. Portuguese. Available from: <https://doi.org/10.7824/rbh.v5i1.42>