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Interaction of dicamba or 2,4-D with acetyl-CoA carboxylase inhibiting herbicides to control fleabane and sourgrass

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ABSTRACT

The presence of monocotyledonous and dicotyledonous weeds resistant to glyphosate in the same area makes tank mixtures of herbicides necessary. However, mixtures containing latifolicides and graminicides may result in less effectiveness. This study aimed to evaluate the interactions of auxin-mimic herbicides with acetyl-CoA carboxylase (ACCase) inhibitors. Experiments were conducted in two consecutive years in an area with a high infestation of fleabane and sourgrass, with history of resistance to glyphosate. The average heights of fleabane and sourgrass plants were, respectively: 30 and 70 cm in the first year; 80 and 120 cm in the second year. Herbicides dicamba, 2,4-D, clethodim, quizalofop and glyphosate were applied alone or in combinations with each other, in a completely randomized design, with thirty treatments and four replications. The results obtained in the control evaluation at 35 days after application were analyzed by Colby's method. For fleabane control, in the two years evaluated, there were no problems of antagonism of auxin mimics with ACCase inhibitors. However, mixtures of ACCase inhibitors with 2,4-D were more antagonistic than mixtures with dicamba in sourgrass control, since for double mixtures between auxin mimics and ACCase inhibitors, interactions with 2,4-D were antagonistic in 62.5% cases, while for dicamba was 12.5%. In addition, antagonism effects were more pronounced in larger plants of sourgrass, and in mixtures with quizalofop, compared with clethodim. Tank mixtures of glyphosate and clethodim or quizalofop were synergistic for the control of sourgrass.

Introduction

From the end of the 1990s, due to the use of Roundup Ready® technology, glyphosate applications increased significantly, which contributed significantly to the selection of resistant

weed biotypes (Peterson et al. 2018; Heap, 2020). In Brazil, reports of resistance to glyphosate, as well as reports of control failures to this herbicide have been increasingly frequent, constituting one of the main challenges for agricultural sustainability of grain production systems (Lúcio et al. 2019).

Currently, nine weed species with biotypes resistant to glyphosate have already been reported, while those with greater dissemination throughout Brazil are fleabane (*Conyza* spp.) and sourgrass (*Digitaria insularis*) (Ovejero et al. 2017; Mendes et al. 2020; Heap, 2020). The occurrence of these two species of weeds is more pronounced in areas where there is no establishment of cover crops in the off-season (Oliveira Neto et al. 2013; Petter et al. 2015). Still related to this aspect, it has already been indicated that the integration of cover crops with the application of herbicides in mixture or in rotation, consists of an efficient strategy for the control of glyphosate-resistant fleabane and sourgrass (Marochi et al. 2018). For sourgrass, there are not many options for mechanisms of action of herbicides that ensure adequate control in post-emergence, especially in the case of plants at advanced development stage (Zobiole et al. 2016). The use of contact herbicides, such as ammonium-glufosinate or paraquat, is limited due to the dense clumps and the high capacity for regrowth due to the presence of rhizomes (Gemelli et al. 2012). Among the systemic herbicides, in addition to glyphosate, the best options are acetyl-CoA carboxylase (ACCase) inhibiting herbicides (Barroso et al. 2014). Regarding fleabane, there are more options of herbicides for post-emergence control, and combinations of products containing saflufenacil, 2,4-D, ammonium glufosinate, paraquat and glyphosate are commonly used for its management (Constantin et al. 2013). Moreover, research has shown dicamba is also effective in the control of fleabane (Kruger et al. 2010; Osipe et al. 2017).

In several regions of the country, the occurrence of simultaneous infestation of sourgrass and fleabane plants is frequent, both resistant to glyphosate (Ovejero et al. 2017; Mendes et al. 2020). In this way, mixtures of herbicides become a tool frequently used for weed management. The main advantages of these combinations are the improvement in the performance of control, expansion of the spectrum of action and mitigation of resistance to herbicides (Beckie and Reboud, 2009). Despite this, for some weeds, the combination of herbicides can be antagonistic. This occurs when the combined effect of two herbicides is less than the effect expected by the sum of these herbicides applied alone (Green, 1989).

For areas with simultaneous infestation of fleabane and sourgrass, reports of antagonism have been common among combinations of graminicides and latifolicides, especially in relation to the control of monocotyledonous species with ACCase-inhibiting herbicides mixed with 2,4-D (Pereira et al. 2018; Andreoti et al. 2019; Leal et al. 2020). From this context, the present study aimed to

evaluate the interaction of auxin-mimic herbicides (dicamba and 2,4-D), ACCase inhibitors and glyphosate alone or in combinations in the control of glyphosate-resistant fleabane and sourgrass.

Materials and Methods

This study was repeated in two years, during the 2012 and 2013 growing seasons, in Maringá city, state of Paraná, Brazil (23°28'22,32" S; 52°00'07,30" W) at 541 meters altitude. The experimental area was previously cultivated in no-till system and prior to the implementation of experiments, soil analysis presented the following physical-chemical properties: pH of 5.4; 21.2 g dm⁻³ C; 485 g kg⁻¹ clay, 154 g kg⁻¹ silt, 539 g kg⁻¹ sand. The experimental area had a high infestation of fleabane (*Conyza* spp.) and sourgrass (*Digitaria insularis*) with history of resistance to glyphosate. Resistance was confirmed by applying 960 g a.e. ha⁻¹ glyphosate to plants grown in a greenhouse from seeds collected in the experimental area (Ovejero et al. 2017; Mendes et al. 2020).

The climate of the municipality where the experimental area is located is Cfa - mesothermal humid, with hot summers and infrequent frosts, a tendency of rainfall concentration in the summer months, with no defined dry season, according to the Köppen classification. The averages of hot months are above 22°C, and of the coldest months, below 13°C. The average annual rainfall is between 1,600 and 1,800 mm (IAPAR, 2020). Figure 1 illustrates rainfall data observed during the experiments.

The experiment was a completely randomized design, evaluating 30 treatments with four replications. Treatments consisted of the application of herbicides dicamba, 2,4-D amine, clethodim, quizalofop-P-tefuryl and glyphosate alone and in double combination (synthetic auxin + ACCase inhibitor) or triple combination (synthetic auxin + ACCase inhibitor + glyphosate), in addition to a control, with no application (Table 1). The commercial products used were DMA 806 BR (670 g a.e. L⁻¹, SL, Dow AgroSciences), Roundup Original (356 g a.e. L⁻¹, SL, Monsanto), Select (240 g a.i. L⁻¹, EC, Arysta LifeScience) and Panther (120 g a.i. L⁻¹, EC, Arysta LifeScience) for the active ingredients 2,4-D amine, clethodim, quizalofop-P-tefuryl and glyphosate, respectively. As there is no commercial formulation of dicamba available for use in Brazil at the time the experiments were conducted, for the composition of treatments containing the said active ingredient, a sample based on diglycolamine salt (480 g a.e. L⁻¹, SC). The experimental unit was 4.5 meters wide and 5 meters long. The useful area considered for the evaluations was the central four meters of the plot (width), disregarding 0.5 m from each end in length, totaling 16 m².

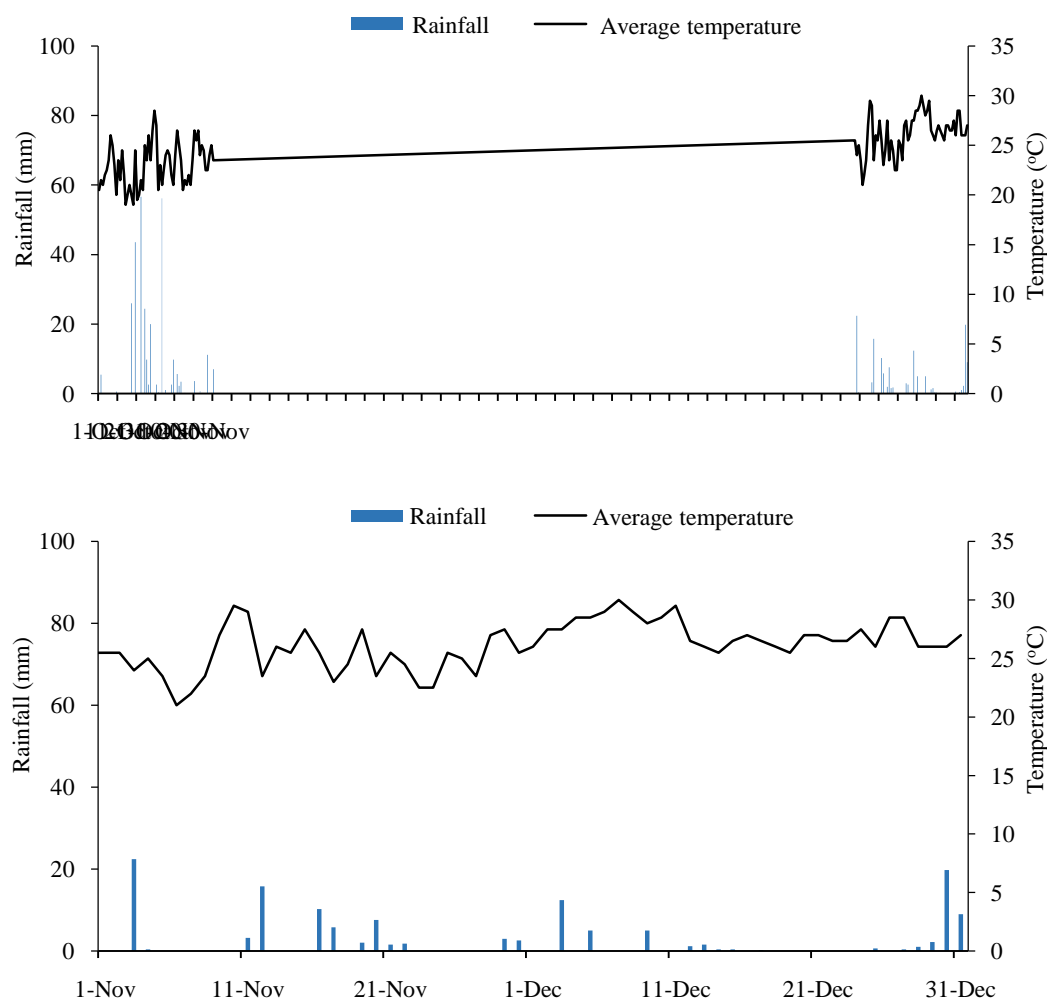


Figure 1. Rainfall and average temperature during the experiments. Maringá (Brazil), 2012 and 2013.

Herbicides were applied in post-emergence of weeds. The main species that made up the flora of the infesting community in the experimental area were fleabane and sourgrass. Infestation density and average height of fleabane and sourgrass plants were, respectively, 9 plants m^{-2} and 30 cm, and 11 plants m^{-2} and 70 cm (full bloom) for 2012, and 5 plants m^{-2} and 80 cm, and 9 plants m^{-2} and 120 cm (full bloom) in 2013.

Herbicides were applied with a CO_2 constant pressure backpack sprayer, equipped with bar with four XR-110.02 fan nozzles under pressure of 2.0 kgf cm^{-2} . These application conditions provided an application rate of 200 L ha^{-1} . Applications were carried out on October 16 in 2012 and November 14 in 2013, and the climatic conditions on these occasions were: air temperature between 23° and 26°C, relative humidity between 65 and 75% and winds of up to 4.2 km h^{-1} .

Table 1. List of treatments in the experiment to evaluate the interaction of auxin-mimic herbicides, ACCase inhibitors and glyphosate for the control of fleabane and sourgrass. Maringá (Brazil), 2012 and 2013.

Treatments	Doses (g a.i. ha ⁻¹)
Dicamba	402
Dicamba	670
2,4-D	402
2,4-D	670
Clethodim ¹	168
Quizalofop ¹	120
Glyphosate ¹	1440
Clethodim + glyphosate ¹	168 + 1440
Quizalofop + glyphosate ¹	120 + 1440
Dicamba + glyphosate ¹	402 + 1440
Dicamba + clethodim ¹	402 + 168
Dicamba + quizalofop	402 + 120
Dicamba + clethodim + glyphosate ¹	402 + 168 + 1440
Dicamba + quizalofop + glyphosate ¹	402 + 120 + 1440
Dicamba + glyphosate ¹	670 + 1440
Dicamba + clethodim ¹	670 + 168
Dicamba + quizalofop ¹	670 + 120
Dicamba + clethodim + glyphosate ¹	670 + 168 + 1440
Dicamba + quizalofop + glyphosate ¹	670 + 120 + 1440
2,4-D amine + glyphosate ¹	402 + 1440
2,4-D amine + clethodim ¹	402 + 168
2,4-D amine + quizalofop ¹	402 + 120
2,4-D amine + clethodim + glyphosate ¹	402 + 168 + 1440
2,4-D amine + quizalofop + glyphosate ¹	402 + 120 + 1440
2,4-D amine + glyphosate ¹	670 + 1440
2,4-D amine + clethodim ¹	670 + 168
2,4-D amine + quizalofop ¹	670 + 120
2,4-D amine + clethodim + glyphosate ¹	670 + 168 + 1440
2,4-D amine + quizalofop + glyphosate ¹	670 + 120 + 1440
Control without herbicide	-

Legend: a.e. = acid equivalent; a.i. = active ingredient. ¹ Addition of mineral to the mixture for application (0.5% v/v).

For the control evaluations, weeds in the controls without herbicide application were used as reference. Variables evaluated were: percentage of control (visual scale, 0-100%, where 0% means no symptoms and 100% total weed control) at 10, 22 and 35 days after application (DAA) of treatments. Control data were tested by analysis of variance ($p < 0.05$), and compared to each other by the Scott-Knott test ($p < 0.05$). Data referring to the last control evaluation (35 DAA) were submitted to interaction analysis of herbicides by Colby's method (1967) for two (a) or three (b) herbicides:

$$\begin{aligned} \text{a)} \quad E &= (X + Y) - \frac{(XY)}{100} \\ \text{b)} \quad E &= (X + Y + Z) - \frac{(XY + XZ + YZ)}{100} + \frac{XYZ}{100^{n-1}} \end{aligned}$$

In these equations, X, Y and Z refer, respectively, to the control obtained with the herbicides X, Y and Z applied alone. Thus, in the formula, X + Y + Z refers to the sum of the control means of the herbicides X, Y and Z, just as XYZ refers to the product of the control means. The other mathematical operations of the formulas follow the same pattern. This implies saying, for example, that XY is not the result of controlling the combination of herbicide X with herbicide Y, but rather the multiplication of the mean of control of herbicide X by the mean of herbicide Y, both applied in isolation. Additionally, 'n' indicates the number of combined herbicides (Gemelli, 2012; Takano et al. 2013). As the Colby's method (1967) does not consider the interaction of double mixtures for the evaluation of interactions in triple mixtures (only the isolated effect of each herbicide used), the second equation mentioned above was not used for calculations. For triple mixtures, the present study intends to check whether the addition of a third herbicide, for example, the addition of 2,4-D or dicamba to glyphosate + clethodim, or glyphosate + quizalofop, affects the performance of the double mixture. Thus, for sourgrass control, the mixture glyphosate + clethodim or glyphosate + quizalofop was considered to be the product "X" and 2,4-D or dicamba, the product "Y". As for fleabane, as the intention is to analyze whether the addition of the ACCase inhibitor herbicide interferes with the application of glyphosate + auxin mimic, these mixtures were considered the product "X", and the clethodim or quizalofop, the product "Y". According to Colby (1967), if the control result obtained by mixing two herbicides is higher than the expected value ("E" in the formula), the interaction is considered synergistic. If it is lower, the interaction is antagonistic; and if they are the same, the interaction is additive. In this experiment, the observed and expected values were compared using the confidence intervals (95%) of the means, as suggested by Gemelli (2012). Thus, if the differences between the observed and expected values were less than the confidence interval, the interaction was considered additive.

Results and Discussion

Interaction of herbicide combinations for fleabane control

Table 2 lists the results of the evaluations of control of fleabane plants, in both years of the experiment. It is worth mentioning that the evaluations of the interactions between the double and triple combinations will be discussed next, through the results obtained with Colby's method (1967). In addition, the isolated effects of ACCase-inhibiting herbicides (clethodim and quizalofop) on fleabane plants were practically null, since dicotyledonous species have natural tolerance to herbicides with this

mechanism of action (Takano et al. 2020). In general, the control averages for both species was lower in 2013 because the plants were taller compared to the previous year. The description of the contrast in plant height between years serves as a subsidy to explain the lower percentages of control provided by the herbicides in the second year compared to the first (Table 2). In this way, data were represented and discussed separately between the two years of evaluation. In general, at 10 DAA, the levels of control on fleabane plants in both years in which the experiment was conducted were low, not exceeding 50% in any treatment with the application of herbicides in isolation.

Table 2. Mean percentages of control of fleabane after herbicide application. Maringá (Brazil), 2012 and 2013.

Treatments (g a.i. ha ⁻¹)	2012			2013		
	10 DAA	22 DAA	35 DAA	10 DAA	22 DAA	35 DAA
DIC (402)	32.5 e	77.5 b	94.0 b	41.3 b	48.0 d	52.5 c
DIC (670)	41.8 d	91.8 a	100.0 a	47.5 a	53.8 c	58.8 c
2,4-D (402)	28.8 e	56.3 d	60.8 g	37.0 b	28.8 f	33.8 d
2,4-D (670)	32.3 e	67.5 c	67.3 f	43.8 b	43.8 d	47.5 c
CLE (168)	0.0 f	0.0 e	0.0 h	0.0 d	0.0 g	0.0 f
QUI (120)	0.0 f	0.0 e	0.0 h	1.3 d	0.0 g	0.0 f
GLY (1440)	32.5 e	52.0 d	73.8 e	21.3 c	28.8 f	33.8 d
CLE+ GLY (168 + 1440)	28.0 e	52.5 d	70.8 f	15.0 c	30.0 f	35.0 d
QUI + GLY (120 + 1440)	40.0 d	55.0 d	68.8 f	10.5 c	26.3 f	27.5 e
DIC + GLY (402 + 1440)	70.0 a	95.0 a	99.5 a	47.5 a	63.0 b	68.8 b
DIC + CLE (402 + 168)	30.8 e	69.3 c	95.3 b	42.5 b	55.0 c	60.0 c
DIC + QUI (402 + 120)	32.5 e	73.8 c	94.5 b	43.8 b	50.0 c	52.5 c
DIC + CLE + GLY (402 + 168 + 1440)	66.8 a	90.5 a	99.5 a	52.5 a	73.5 a	78.8 a
DIC + QUI + GLY (402 + 120 + 1440)	75.0 a	95.3 a	100.0 a	56.8 a	71.8 a	79.3 a
DIC + GLY (670 + 1440)	70.8 a	95.5 a	100.0 a	55.0 a	75.5 a	85.0 a
DIC + CLE (670 + 168)	43.8 d	87.5 a	98.8 a	43.8 b	56.3 c	60.0 c
DIC + QUI (670 + 120)	38.8 d	83.8 b	97.8 a	42.5 b	53.8 c	57.5 c
DIC + CLE + GLY (670 + 168 + 1440)	65.5 b	96.0 a	100.0 a	50.0 a	76.5 a	82.5 a
DIC + QUI + GLY (670 + 120 + 1440)	64.5 b	96.5 a	99.5 a	56.3 a	72.5 a	80.0 a
2,4-D + GLY (402 + 1440)	60.8 b	78.0 b	80.5 d	48.8 a	47.5 d	50.0 c
2,4-D + CLE (402 + 168)	41.5 d	70.8 c	65.0 g	36.3 b	36.3 e	40.0 d
2,4-D + QUI (402 + 120)	30.8 e	55.0 d	63.8 g	17.5 c	26.3 f	26.3 e
2,4-D + CLE + GLY (402 + 168 + 1440)	55.5 c	88.5 a	77.5 d	45.0 b	37.5 e	39.5 d
2,4-D + QUI + GLY (402 + 120 + 1440)	62.5 b	82.5 b	85.5 c	46.3 b	37.5 e	36.3 d
2,4-D + GLY (670 + 1440)	67.3 a	94.8 a	92.5 b	52.5 a	51.3 c	55.0 c
2,4-D + CLE (670 + 168)	33.0 e	66.8 c	69.5 f	41.3 b	42.5 d	42.5 d
2,4-D + QUI (670 + 120)	30.8 e	65.5 c	72.5 e	40.0 b	40.0 e	36.3 d
2,4-D + CLE + GLY (670 + 168 + 1440)	70.8 a	95.0 a	94.8 b	51.3 a	48.8 d	51.3 c
2,4-D + QUI + GLY (670 + 120 + 1440)	69.8 a	92.5 a	92.0 b	55.0 a	52.5 c	55.0 c
Control without herbicide	0.0 f	0.0 e	0.0 h	0.0 d	0.0 g	0.0 f
CV (%)	9.84	9.32	5.25	14.94	13.19	14.88

DIC = dicamba; CLE = clethodim; GLY = glyphosate; QUI = quizalofop; 2,4-D = 2,4-D amine. *Mean values followed by different letters are significantly different by Scott-Knott test (p < 0.05).

In the evaluation of 22 DAA, symptoms of herbicides in plants were more evident in the first year of the experiment, with emphasis on the applications of dicamba at the dose of 670 g a.e. ha⁻¹, which even without the combination with glyphosate performed control greater than 90%. The isolated applications of 2,4-D provided control of up to 67%. On this occasion, with the addition of glyphosate to auxin mimics, these differences were less evident. When comparing the performance of auxin-mimic herbicides for the control of fleabane, it was demonstrated in the literature that dicamba based on diglycolamine salt has greater efficacy, followed by the same herbicide based on the dimethylamine salt, 2,4-D ester and 2,4-D amine (Kruger et al. 2010).

Also, in 2012, at 35 DAA, herbicide dicamba at doses of 402 and 670 g a.e. ha⁻¹ performed control of 94 and 100%, respectively, surpassing isolated applications of 2,4-D. The isolated application of glyphosate (1440 g a.e. ha⁻¹) did not show efficacy in the control of fleabane, demonstrating the need for mixing with another herbicide. Still in this evaluation, it is observed that some treatments containing the combination of dicamba and glyphosate reached 100% effectiveness in the control of fleabane, and when compared with 2,4-D + glyphosate, the highest value was 92%, with the first herbicide at the dose of 670 g a.e. ha⁻¹.

In the second year, it was observed that, among the herbicides applied alone, again, dicamba stood out the most, providing 59% control at 35 DAA in the dose of 670 g a.e. ha⁻¹, not differing statistically from the dose of 402 g a.e. ha⁻¹. However, none of the isolated treatments showed satisfactory control for fleabane, requiring the addition of another herbicide to obtain adequate control. The addition of glyphosate to dicamba considerably increased weed control, since this combination with dicamba at the dose of 670 g a.e. ha⁻¹, performed 85% control. For the mixtures of 2,4-D and glyphosate, the results were lower, with percentages close to 55%.

As already discussed, depending on the height of fleabane plants at the time of herbicide application, there was a decrease in the control levels of this weed. For taller fleabane plants (> 30 cm), many times a single punctual application is not enough to guarantee the control effectiveness (Braz et al. 2017). This has already been widely reported in the literature, showing that fleabane plants at more advanced stages of development offer greater difficulties to chemical control, and as a consequence, there is the occurrence of side shoots after herbicide applications (Vangessel, 2001; Moreira et al. 2010).

The results obtained by Colby's method (1967) with the double and triple combinations, for the years 2012 and 2013, are listed in Table 3. For the experiment in 2012, in which fleabane plants were 30 cm tall, it was found that none of the double mixtures was classified as antagonistic. For the treatment with dicamba at 402 g a.e. ha⁻¹ combined with glyphosate, the mixture was considered synergistic, with 99% control. Despite this, the difference between the observed and

expected values for this mixture was very small (1.1%), which in practice is often difficult to be noticed. The other mixtures evaluated were considered additive, therefore, there were no problems when the herbicides were combined.

For the 2013 experiment, the results were similar to the previous year. No double association with dicamba or 2,4-D was considered antagonistic for the control of fleabane. In this case, the mixture of dicamba (670 g ha⁻¹) + glyphosate stands out, classified as synergistic, which provided an observed control 12% higher than expected. In this context, the results indicate that the addition of glyphosate to auxin-mimics was beneficial for fleabane control, and in some situations, there may be synergism in these combinations. When synergism was not verified, the effects of these herbicides were added, which also validates their application in combination, since it is possible to optimize operational practices in the application of herbicides and to expand the control spectrum.

Table 3. Evaluation of the effects of mixtures involving dicamba and 2,4-D on the control of fleabane plants at 35 DAA. Maringá (Brazil), 2012 and 2013.

Treatments (g a.i. ha ⁻¹)	Double combinations					
	2012			2013		
	Obs. ¹	Exp. ²	Effect	Obs.	Exp.	Effect
DIC + GLY (402 + 1440)	99.5	98.4 ± 0.9	SIN	68.8	68.5 ± 13.6	ADI
DIC + CLE (402 + 168)	95.3	94.0 ± 6.0	ADI	60.0	52.5 ± 8.1	ADI
DIC + QUI (402 + 120)	94.5	94.0 ± 6.7	ADI	52.5	52.5 ± 10.3	ADI
DIC + GLY (670 + 1440)	100.0	100.0 ± 0.0	ADI	85.0	72.7 ± 6.5	SIN
DIC + CLE (670 + 168)	98.8	100.0 ± 1.5	ADI	60.0	58.8 ± 13.0	ADI
DIC + QUI (670 + 120)	97.8	100.0 ± 2.4	ADI	57.5	58.8 ± 15.2	ADI
2,4-D + GLY (402 + 1440)	80.5	89.7 ± 10.2	ADI	50.0	56.1 ± 23.4	ADI
2,4-D + CLE (402 + 168)	65.0	60.8 ± 9.2	ADI	40.0	33.8 ± 13.0	ADI
2,4-D + QUI (402 + 120)	63.8	60.8 ± 7.6	ADI	26.3	33.8 ± 7.6	ADI
2,4-D + GLY (670 + 1440)	92.5	91.4 ± 6.7	ADI	55.0	65.2 ± 11.2	ADI
2,4-D + CLE (670 + 168)	69.5	67.3 ± 6.7	ADI	42.5	47.5 ± 13.8	ADI
2,4-D + QUI (670 + 120)	72.5	67.3 ± 10.3	ADI	36.3	47.5 ± 11.8	ADI
Triple combinations						
DIC + CLE + GLY (402 + 168 + 1440)	99.5	99.5 ± 0.9	ADI	78.8	68.8 ± 7.6	SIN
DIC + QUI + GLY (402 + 120 + 1440)	100.0	99.5 ± 0.0	SIN	79.3	68.8 ± 6.9	SIN
DIC + CLE + GLY (670 + 168 + 1440)	100.0	100.0 ± 0.0	ADI	82.5	85.0 ± 4.6	ADI
DIC + QUI + GLY (670 + 120 + 1440)	99.5	100.0 ± 1.6	ADI	80.0	85.0 ± 10.4	ADI
2,4-D + CLE + GLY (402 + 168 + 1440)	77.5	77.5 ± 10.3	ADI	39.5	50.0 ± 12.7	ADI
2,4-D + QUI + GLY (402 + 120 + 1440)	80.5	77.5 ± 8.4	ADI	36.3	50.0 ± 16.4	ADI
2,4-D + CLE + GLY (670 + 168 + 1440)	94.8	92.5 ± 7.3	ADI	51.3	55.0 ± 13.6	ADI
2,4-D + QUI + GLY (670 + 120 + 1440)	92.0	92.5 ± 6.9	ADI	55.0	55.0 ± 11.2	ADI

DIC = dicamba; CLE = clethodim; GLY = glyphosate; QUI = quizalofop; 2,4-D = 2,4-D amine; SIN = Synergistic; ADI = Additive. ¹ obs = Mean of the control percentage values obtained at 35 DAA. ² exp = control percentage values considering Colby's formula (1967) and the 95% confidence interval.

As for ACCase inhibitors, in general, because they had no control over the species, they did not bring benefits to fleabane control. However, clethodim or quizalofop did not impair the action of 2,4-D and dicamba in the control of fleabane, indicating the possibility of their use without interfering with the latifolicide action of the mixture.

For triple mixtures, the experiments of 2012 and 2013 (Table 3) revealed that the tested combinations are likely to be used for the control of fleabane. In the first experiment (2012), the results of most of the triple mixtures were classified as additives, being very close to 100% when dicamba was used. Only for treatment with dicamba, clethodim and glyphosate, the mixture was classified as synergistic. However, the differences observed between the observed and expected values were minimal (0.5%). In the 2013 experiment, there were greater difficulties in controlling fleabane, especially with 2,4-D. However, as in the 2012 experiment, no mixture was classified as antagonistic.

In short, for fleabane plants, regardless of the average height of the plants, applications involving two or three herbicides were not harmful, being considered additive, mostly, or synergistic, in some situations. In general, the levels of control provided by dicamba, alone or in combination, were higher than 2,4-D for the control of fleabane, especially for larger plants.

Interaction of herbicide combinations for sourgrass control

The results of sourgrass control, in the two years in which the experiment was conducted, are listed in Table 4. It is possible to observe in both experiments that, in the first evaluation, the control percentages exercised by the treatments were very low. The visual results of the action of systemic herbicides, such as ACCase inhibitors, can take 15-20 days to be noticed in sourgrass (Cassol et al. 2019).

The control percentages obtained with dicamba and 2,4-D applied alone to the plants of sourgrass were always zero. Generally, for grasses, auxin-mimic herbicides have no control effectiveness, mainly due to the metabolization rates of these herbicides (Christoffoleti et al. 2015).

For the 2013 experiment, levels of control obtained were lower than in the previous year. Again, just as discussed for fleabane, it is noteworthy that the stage of sourgrass plants at the time of application had a great influence on the control provided by herbicides. For the second year, differences were detected between clethodim and quizalofop, the first being more efficient than the second herbicide, either in isolated applications or in combination with glyphosate. In general, the differences in control levels between these herbicides were close to 15%, over the three evaluations.

Table 4. Mean percentage of control of sourgrass after herbicide application. Maringá (Brazil), 2012 and 2013.

Treatments (g i.a. ha ⁻¹)	2012			2013		
	10 DAA	22 DAA	35 DAA	10 DAA	22 DAA	35 DAA
DIC (402)	0.0 e	0.0 f	0.0 g	0.0 e	0.0 g	0.0 g
DIC (670)	0.0 e	0.0 f	0.0 g	1.3 e	0.0 g	0.0 g
2,4-D (402)	0.0 e	0.0 f	0.0 g	0.0 e	0.0 g	0.0 g
2,4-D (670)	0.0 e	0.0 f	0.0 g	2.5 e	0.0 g	0.0 g
CLE (168)	27.5 d	52.5 c	61.3 c	14.5 c	47.5 c	54.3 c
QUI (120)	28.8 d	50.0 c	58.8 c	8.8 d	31.3 e	32.5 e
GLY (1440)	27.5 d	23.8 e	27.5 f	8.0 d	22.5 f	19.3 f
CLE+ GLY (168 + 1440)	38.8 b	68.8 a	88.3 a	30.0 a	67.0 a	77.5 a
QUI + GLY (120 + 1440)	37.0 b	70.5 a	84.5 a	20.0 b	57.5 b	62.5 b
DIC + GLY (402 + 1440)	30.0 c	23.8 e	23.8 f	9.0 d	18.8 f	15.0 f
DIC + CLE (402 + 168)	23.0 d	45.0 d	56.3 c	17.0 b	50.0 c	56.3 c
DIC + QUI (402 + 120)	31.8 c	43.8 d	55.0 c	13.8 c	45.0 c	40.0 d
DIC + CLE + GLY (402 + 168 + 1440)	40.0 a	75.0 a	86.8 a	32.5 a	64.3 a	73.8 a
DIC + QUI + GLY (402 + 120 + 1440)	36.8 b	66.8 a	80.8 a	25.3 b	50.0 c	54.3 c
DIC + GLY (670 + 1440)	28.8 d	24.3 e	25.0 f	12.5 c	20.0 f	16.3 f
DIC + CLE (670 + 168)	33.8 c	45.0 d	59.5 c	22.5 b	51.3 c	58.8 c
DIC + QUI (670 + 120)	30.5 c	43.8 d	50.0 d	14.3 c	38.8 d	42.3 d
DIC + CLE + GLY (670 + 168 + 1440)	41.3 a	71.3 a	85.5 a	30.5 a	67.5 a	77.5 a
DIC + QUI + GLY (670 + 120 + 1440)	37.5 b	67.5 a	75.0 b	22.5 b	53.3 c	60.0 c
2,4-D + GLY (402 + 1440)	32.5 c	28.8 e	26.3 f	7.5 d	20.0 f	19.3 f
2,4-D + CLE (402 + 168)	27.5 d	46.3 d	61.3 c	18.8 b	52.5 c	57.5 c
2,4-D + QUI (402 + 120)	25.0 d	41.5 d	51.3 d	5.0 e	15.0 f	11.3 f
2,4-D + CLE + GLY (402 + 168 + 1440)	42.5 a	70.0 a	86.3 a	23.8 b	58.0 b	66.3 b
2,4-D + QUI + GLY (402 + 120 + 1440)	41.3 a	62.0 b	72.5 b	15.5 c	43.8 c	51.3 c
2,4-D + GLY (670 + 1440)	37.5 b	25.0 e	21.3 f	10.0 d	20.0 f	12.5 f
2,4-D + CLE (670 + 168)	32.5 c	42.5 d	51.3 d	11.3 d	35.0 d	38.8 d
2,4-D + QUI (670 + 120)	30.0 c	40.0 d	42.5 e	3.8 e	15.0 f	14.5 f
2,4-D + CLE + GLY (670 + 168 + 1440)	42.5 a	69.5 a	83.3 a	28.8 a	60.0 b	70.0 b
2,4-D + QUI + GLY (670 + 120 + 1440)	37.5 b	59.5 b	66.3 c	18.8 b	35.0 d	38.0 d
Control without herbicide	0.0 e	0.0 f	0.0 g	0.0 e	0.0 g	0.0 g
CV (%)	12.66	9.95	10.20	30.62	15.54	16.98

DIC = dicamba; CLE = clethodim; GLY = glyphosate; QUI = quizalofop; 2,4-D = 2,4-D amine. *Mean values followed by different letters are significantly different by Scott-Knott test ($p < 0.05$).

Throughout all control evaluations conducted in both experiments, the isolated application of glyphosate exercised maximum control on sourgrass at the level of 27.5%. These results, added to the history of failures in the control of this weed, demonstrate the fact that the biotype of sourgrass present in the experimental area showed resistance to glyphosate. The results of the interactions of the double and triple combinations of herbicides are presented in Table 5. The double combinations of clethodim or quizalofop with glyphosate were synergistic in both experiments. In the first year, the expected control percentages were close to 70%, using the Colby's method (1967), and the observed values were 88.3 and 84.5%. In 2013, despite lower levels of control, the combinations were also synergistic, with gains of

15 to 20%. Even presenting resistance to glyphosate, several authors reinforce the importance of using glyphosate, in combination with ACCase inhibiting herbicides aiming at the successful control of sourgrass (Melo et al. 2012; Zobiole et al. 2016; Cassol et al. 2019; Gomes et al. 2020). Glyphosate is rapidly translocated from treated leaves to metabolic drains, especially for storage and meristem tissues, making it an excellent control option for perennial species (Bromilow et al. 1990). The combination of glyphosate with other herbicides can favor the absorption and translocation of these products and consequently improve the levels of effectiveness (Lym, 2000; Takano et al. 2013). For double associations of ACCase inhibitors with auxin mimics, it was possible to observe antagonistic interactions. This became more evident when auxin mimics were applied with the highest dose (670 g a.i. ha⁻¹). In 2012, problems were found related to the antagonism between the mixtures of dicamba 670 g a.i. ha⁻¹ + quizalofop, 2,4-D (670 g a.i. ha⁻¹) + quizalofop and 2,4-D (670 g a.i. ha⁻¹) + clethodim. However, antagonistic effects in mixtures involving 2,4-D were more evident for quizalofop than for clethodim. For the 2013 experiment, all mixtures with dicamba were classified as additives. For 2,4-D, antagonism with the two ACCase inhibiting herbicides was observed. The efficiency reductions for sourgrass control were up to 20%, as verified in the mixture with 2,4-D (402 g a.i. ha⁻¹) + quizalofop.

Table 5. Evaluation of the effects of mixtures involving clethodim and quizalofop on the control of sourgrass plants at 35 DAA. Maringá (Brazil), 2012 and 2013.

Treatments (g a.i. ha ⁻¹)	Double combinations					
	2012			2013		
	Obs. ¹	Exp. ²	Effect	Obs.	Exp.	Effect
CLE + GLY (168 + 1440)	88.3	71.9 ± 3.7	SIN	77.5	63.1 ± 4.6	SIN
QUI + GLY (120 + 1440)	84.5	70.1 ± 8.4	SIN	62.5	45.5 ± 10.3	SIN
DIC + CLE (402 + 168)	56.3	61.3 ± 10.0	ADI	56.3	54.3 ± 10.0	ADI
DIC + QUI (402 + 120)	55.0	58.8 ± 6.5	ADI	40.0	32.5 ± 11.3	ADI
DIC + CLE (670 + 168)	59.5	61.3 ± 5.4	ADI	58.8	54.3 ± 11.9	ADI
DIC + QUI (670 + 120)	50.0	58.8 ± 7.0	ANT	42.3	32.5 ± 14.6	ADI
2,4-D + CLE (402 + 168)	61.3	61.3 ± 7.6	ADI	57.5	54.3 ± 13.8	ADI
2,4-D + QUI (402 + 120)	51.3	58.8 ± 7.6	ADI	11.3	32.5 ± 4.0	ANT
2,4-D + CLE (670 + 168)	51.3	61.3 ± 8.8	ANT	38.8	54.3 ± 11.9	ANT
2,4-D + QUI (670 + 120)	42.5	58.8 ± 10.2	ANT	14.5	32.5 ± 7.8	ANT
Triple combinations						
DIC + CLE + GLY (402 + 168 + 1440)	86.8	88.3 ± 8.6	ADI	73.8	77.5 ± 7.6	ADI
DIC + QUI + GLY (402 + 120 + 1440)	80.8	84.5 ± 6.5	ADI	54.3	62.5 ± 6.9	ANT
DIC + CLE + GLY (670 + 168 + 1440)	85.5	88.3 ± 5.3	ADI	77.5	77.5 ± 4.6	ADI
DIC + QUI + GLY (670 + 120 + 1440)	75.0	84.5 ± 9.5	ADI	60.0	62.5 ± 14.5	ADI
2,4-D + CLE + GLY (402 + 168 + 1440)	86.3	88.3 ± 5.7	ADI	66.3	77.5 ± 7.6	ANT
2,4-D + QUI + GLY (402 + 120 + 1440)	72.5	84.5 ± 10.3	ANT	51.3	62.5 ± 7.6	ANT
2,4-D + CLE + GLY (670 + 168 + 1440)	83.3	88.3 ± 7.8	ADI	70.0	77.5 ± 6.5	ANT
2,4-D + QUI + GLY (670 + 120 + 1440)	66.3	84.5 ± 10.0	ANT	38.0	62.5 ± 9.1	ANT

DIC = dicamba; CLE = clethodim; GLY = glyphosate; QUI = quizalofop; 2,4-D = 2,4-D amine; SIN = Synergistic; ADI = Additive; ; ANT = Antagonistic. ¹ obs = Mean of the control percentage values obtained at 35 DAA. ² exp = control percentage values considering Colby's formula (1967) and the 95% confidence interval.

Considering the two experiments, mixtures dicamba + ACCase inhibitor were antagonistic on one occasion, out of a total of eight tested. For 2,4-D + ACCase inhibitors, there was antagonism on five occasions, also out of a total of eight. Despite this, in practice, applications of this type hardly occur (ACCase + auxin mimics), since the presence of glyphosate is essential for the management applications of weeds.

In 2012, triple combinations containing clethodim were additive, regardless of the dose of dicamba and or 2,4-D applied, that is, the addition of an auxin mimic in the mixture of glyphosate + clethodim did not affect the results of control for sourgrass. When quizalofop was used, there was antagonism when it was mixed with 2,4-D. The increase in the dose of 2,4-D seems to have evidenced the antagonism of the mixture. In the dose of 402 g a.i. ha⁻¹ of 2,4-D, the reduction between expected and observed values was 12%, while for the dose of 670 g a.i. ha⁻¹, the difference was 18.2%. In the experiment conducted in 2013, all triple mixtures with 2,4-D were antagonistic for the control of sourgrass. In treatments with clethodim, the efficiency reductions were 7 - 8%, while for quizalofop, the differences between the expected and observed values were 10 - 24%. The last value was obtained for the treatment with the highest dose of 2,4-D (670 g a.i. ha⁻¹). For dicamba, as in the 2012 experiment, mixtures with clethodim and glyphosate were not antagonistic. With quizalofop, antagonism was found only when the dose of dicamba was 402 g a.i. ha⁻¹.

In general, dicamba was less antagonistic than 2,4-D in mixtures with ACCase inhibiting herbicides for sourgrass control. For triple mixtures, antagonism with 2,4-D was observed in 75% mixtures tested (6 out of 8), and with dicamba in 12.5% (1 out of 8). Among graminicides, clethodim had a lower number of antagonism cases than quizalofop.

In agreement with these results, clethodim was the ACCase inhibitor that showed less problems of antagonism with 2,4-D in the control of sourgrass in greenhouse (Gomes et al. 2020). In addition, 2,4-D showed higher levels of antagonism in mixtures with haloxyfop and phenoxaprop (both belonging to the chemical group of aryloxyphenoxypropionates or FOPs) than with the herbicide sethoxydim (chemical group of cyclohexanodiones or DIMs) (Mueller et al. 1989). Although FOPs and DIMs have the same mechanism of action, these herbicides differ in terms of the binding site in ACCase (Takano et al. 2020).

However, the physiological causes related to the more pronounced antagonistic effect of 2,4-D for FOPs herbicides are still unknown. The main hypotheses to explain such problems are: reduction of absorption and translocation of the herbicide with a control spectrum over monocots, increase in the rate of herbicide metabolism and decrease in the rate of hydrolysis from diclofop-methyl to diclofop acid, which is the active form of the herbicide (Taylor et al. 1983; Han et al. 2013). In other species such as Johnsongrass (*Sorghum halepense*), antagonism occurs due to the

lower translocation and activation of haloxyfop-methyl in the presence of 2,4-D (Mueller et al. 1990). Another possibility is the effect of 2,4-D on the activation of enzymes such as cytochrome P450 monooxygenases, which can increase the rate of metabolism of ACCase inhibitors such as diclofop (Han et al. 2013). For wild oats (*Avena fatua*), it has been reported that isolated applications of diclofop provided two types of symptoms to plants; chlorosis and necrosis in leaf tissues and the inhibition of cell division in meristematic regions (Todd and Stobbe, 1980). When the application of this herbicide occurred in combination with 2,4-D, although the symptoms of chlorosis and necrosis were observed, cell division in the meristematic areas was not inhibited, which suggests that 2,4-D can act physiologically conversely to diclofop, stimulating cell division or reducing levels of diclofop acid in meristem regions (Todd and Stobbe, 1980). Moreover, these authors did not find a reduction in the absorption of the graminicide, nor a decrease in the rate of metabolism of diclofop, when auxin mimic was added to the mixture. Despite the various hypotheses cited, the exact causes of antagonism between these herbicides are not fully understood and should be investigated in future research.

For the reduction of antagonism problems due to the combination of herbicides, some authors suggest increasing the dose of the ACCase inhibitor, sequential applications or the use of adjuvants in the spray solution (Mueller et al. 1989; Myers and Coble, 1992). Further, another possibility aimed at reducing antagonism problems refers to the interval of about ten days for the application of 2,4-D in relation to the date of use of the ACCase inhibitor (Leal et al. 2020). This that the antagonism between auxin mimics and ACCase inhibitors is not related to incompatibility of the mixture in the spray tank (Todd and Stobbe, 1980).

Conclusion

For the control of fleabane, regardless of the plant size, no antagonism was found between ACCase-inhibiting herbicides and auxin-mimic herbicides. For the control of sourgrass, 2,4-D presented more cases of antagonism than dicamba when combined with ACCase-inhibiting herbicides. In double mixtures between auxin mimics and ACCase inhibitors, interactions with 2,4-D were antagonistic in 62.5% cases, while for dicamba, the percentage of antagonism cases was 12.5%. Tank mixtures of glyphosate and clethodim or quizalofop were synergistic for the control of sourgrass. In triple mixtures with glyphosate, auxin mimics and ACCase inhibitors in sourgrass control, interactions with 2,4-D were antagonistic in 75% of cases, while for dicamba, the percentage of antagonism was 12.5%. There was no antagonism in tank mixtures of dicamba and clethodim for the control of sourgrass. For larger sourgrass plants, antagonism was more pronounced.

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Conflicts of Interest

All authors declare that they have no conflict of interest.

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