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Sequential Application Interval of Burndown Herbicides for Fluroxypyr in Horseweed (*Conyza* sp) Control

Intervalo de Aplicação Sequencial de Herbicidas de Contato Após Fluroxipir em Controle de Buva (*Conyza* sp.)

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Abstract
It aimed to evaluate the importance of double-knock applications in horseweed control, furthermore, to define the best interval and contact herbicide for the sequential application. Two experiments were

conducted, the first trial evaluated *Conyza* spp. Control with single application of fluroxypyr-methyl (240 g e.a. ha^{-1}) + glyphosate (1.200 g e.a. ha^{-1}), glufosinate (400 g i.a. ha^{-1}), ammonium glufosinate + saflufenacil (400 g i.a. ha^{-1}+ 35 g i.a. ha^{-1}), and diquat (400 g i.a. ha^{-1}). The second trial aimed to evaluate intervals and herbicides in sequencial application, carried out in a 4 X 3 + 1 factorial scheme, in which the factorial treatments had one application of fluroxypir-methyl + glyphosate, with the first factor referring to the intervals of three, six, 10, and 15 days between applications, and the second factor referring to the application of ammonium glufosinate, ammonium glufosinate + saflufenacil, and diquat. The doses of herbicides was the same as the previous experiment, and additional treatment corresponds to a control without application of herbicides. The control was visually analyzed at 14 and 28 days after sequencial application (DAA) for both sites, the means were compared by Tukey and Dunett ($P \geq 0,05$), and the intervals analyzed in regression. The unique application of herbicides was not effective in the horseweed control, when at 14 DAA occurred a positive relationship between increasing the interval period and control levels, with maximum control reached after 15 days. Among the products used in sequencial application, the mixture of glufosinate + saflufenacil stood out.

Keywords: Auxinic. Desiccation. Herbicides. Weeds. Pre-sowing. Soybean.

Resumo
Objetivou-se avaliar a importância de aplicação sequencial para o fluroxipir-metílico no controle de buva, além disso, definir o melhor intervalo e produto de contato a ser usado em sequencial. Dois experimentos foram conduzidos, o primeiro experimento avaliou o controle de *Conyza* spp. em aplicação única de fluroxipir-metílico (240 g e.a. ha^{-1} fluroxipir) + glifosato (1.200 g e.a. ha^{-1}), glufosinato de amônio (400 g i.a. ha^{-1}), glufosinato de amônio + saflufenacil (400 g i.a. ha^{-1}+ 35 g i.a. ha^{-1}), e diquate (400 g i.a. ha^{-1}). O segundo experimento avaliou intervalos e herbicidas em aplicação sequencial, conduzido em esquema fatorial 4X3 + 1, em que os tratamentos fatoriais tiveram uma aplicação de fluroxipir-metílico + glifosato, com primeiro fator referente aos intervalos de três, seis, 10 e 15 dias entre as aplicações, e segundo fator referente à aplicação de glufosinato de amônio, glufosinato de amônio + saflufenacil, e diquate. Os herbicidas foram usados em mesma dose do experimento anterior, e o tratamento adicional corresponde à uma testemunha não aplicada. O controle foi analisado aos 14 e 28 dias após aplicação sequencial (DAA), as médias foram comparadas por Tukey e Dunett ($p \geq 0,05$), e os intervalos analisados em regressão. A aplicação única de herbicidas não foi efetiva no controle de buva, em que aos 14 DAA houve relação positiva entre o aumento do período de intervalo e níveis de controle, com controle máximo atingido aos 15 dias. Dentre os produtos utilizados em aplicação sequencial, houve destaque para a mistura de glufosinato + saflufenacil.

Palavras-chave: Auxínico. Dessecação. Herbicidas. Plantas Daninhas. Pré-Plantio. Soja.

1 Introduction

Horseweed (*Conyza* sp.), a species belonging to the Asteraceae family, can reduce soybean yield by 19% to 68%, depending on plant density per square meter (Coelho, 2019). In maize, yield losses caused by this species may reach up to 92%, according to the Herbicide Resistance Action Committee (HRAC, 2021), making it one of the most critical challenges in weed management across production systems. *Conyza* species are characterized by high seed production per plant and extensive seed dispersal capacity (Dauer; Mortensen; Vangessel, 2007). Furthermore, resistance cases have been reported in Brazil to several herbicide modes of action, including 5-enolpyruvylshikimate-3-

phosphate synthase (EPSPS) inhibitors, photosystem I and II inhibitors, acetolactate synthase (ALS) inhibitors, protoporphyrinogen oxidase (PROTOX) inhibitors, and synthetic auxin herbicides (HEAP, 2024).

Pre-sowing desiccation has become a key strategy in the weeds' integrated management in soybean cultivation. The most effective control of horseweed has been achieved using a combination of auxinic herbicides with glyphosate, followed by a sequential application of contact and pre-emergence herbicides (Wu; Walker; Robinson, 2008; Cantu *et al*., 2021). The rationale for sequential applications lies in enhancing and complementing the initial stress imposed by the first herbicide, particularly when managing hard-to-control weed species, thus minimizing regrowth.

However, the efficacy of herbicide combinations is influenced by the interval between applications, which varies depending on the herbicide chemistry. In the case of *C. bonariensis*, when glyphosate is used, the optimal interval for sequential application with 2,4-D - a systemic herbicide - is either one day or tank-mixed. Conversely, for non-systemic and fast-acting herbicides such as paraquat or paraquat + diquat, the recommended interval is five to seven days (Werth *et al*., 2010).

The overall effectiveness of the management strategy is also influenced by the choice of contact herbicide in the sequential treatment. Ammonium glufosinate has shown superior efficacy (Albrecht *et al*., 2020). This herbicide acts as a glutamine synthetase (GS) inhibitor, leading to feedback inhibition of photorespiration and the subsequent accumulation of reactive oxygen species (ROS) and lipid peroxidation (Takano *et al*., 2020b). A synergistic combination of ammonium glufosinate with PROTOX inhibitors has been proposed (Takano *et al*., 2020a), as this mode of action also induces ROS accumulation due to protoporphyrin IX buildup in the chloroplast. In addition, diquat remains a widely used herbicide due to its rapid action through electron diversion in photosystem I (PSI) and deactivation upon contact with soil colloids (Piasecki, 2024).

Due to the increasing occurrence of herbicide-resistant weed biotypes and the limited availability of herbicides with novel modes of action, there is an urgent need to optimize the efficacy of existing products and management strategies while minimizing environmental impact. Accordingly, the objective of this study was to evaluate the importance of sequential application of contact herbicides following the use of methyl fluroxypyr in horseweed control, to determine the optimal interval for sequential application, and to identify the most effective contact herbicide for this purpose.

2 Material and Methods

Two field experiments were conducted at different locations and times: Site 1 – the Experimental Farm of Universidade Estadual de Londrina (Londrina, PR), with applications carried out from October 23rd to November 7th, 2023; and Site 2 – Capão da Onça Experimental Farm (Ponta

Grossa, PR), with applications conducted from December 29th, 2023 to January 9th, 2024. Both sites consisted of plots measuring 3.0 × 5.0 m, containing between 1.0 and 1.5 horseweed plants per square meter, with an average height from 20 to 30 cm. The experimental design used was a randomized complete block design with four replications.

To validate the importance of sequential herbicide applications for horseweed control, an additional experiment was conducted under the same conditions and locations, using 3.0 × 5.0 m plots arranged in a randomized complete block design with four replications. Treatments included: single application of ammonium glufosinate at 400 g a.i. ha^{-1} (Finale® – BASF S.A.); ammonium glufosinate + saflufenacil (Heat® – BASF S.A.) at 400 + 35 g a.i. ha^{-1}; diquat at 400 g a.i. ha^{-1} (Reglone® – Syngenta S.A.); and a tank mix of [methyl fluroxypyr + clethodim] (Araddo® – Adama Brasil S/A, Londrina – PR) (345.6 g a.i. ha^{-1} of fluroxypyr and 240 g a.i. ha^{-1} of its acid equivalent (a.e.) + 168 g a.i. ha^{-1} of clethodim) + glyphosate (Roundup Original Mais® – Monsanto Company USA) at 240 g a.e. ha^{-1} of fluroxypyr + 1,200 g a.e. ha^{-1} of glyphosate. The aim was to validate the use of sequential herbicide application. All the treatments were applied using a CO_2-pressurized backpack sprayer calibrated to deliver 150 L ha^{-1}.

To evaluate the effect of application intervals in sequential treatments, a factorial experiment was conducted using a 4 × 3 + 1 scheme. The first factor was the interval between the initial and sequential application: 3, 6, 10, and 15 days. The second factor consisted of the herbicides used in the sequential application: ammonium glufosinate at 400 g a.i. ha^{-1}, ammonium glufosinate + saflufenacil at 400 + 35 g a.i. ha^{-1}, and diquat at 400 g a.i. ha^{-1}. Additionally, one control treatment without herbicide application was included. For this experiment, with the exception of the untreated control, all plots received an initial application of [methyl fluroxypyr + clethodim] + glyphosate at 240 g a.e. ha^{-1} of fluroxypyr and 1,200 g a.e. ha^{-1} of glyphosate.

To optimize the action of herbicides in the sequential applications, the treatments were applied at the most favorable time of day according to the specific requirements of each active ingredient. Treatments containing ammonium glufosinate were applied during peak solar radiation hours (Takano; Dayan, 2021), between 11:00 and 12:00 a.m., whereas treatments containing diquat were applied at the end of the day, after 6:00 p.m. (Oliveira *et al*., 2022). Assessments were conducted at 14 and 28 days after application (DAA). Weed control was evaluated visually on a 0 to 100% scale, where 0 indicates no visible symptoms and 100% indicates complete plant destruction, based on the injury and control scale proposed by Frans *et al*. (1986).

Data were subjected to analysis of variance at a 5% probability level ($P \geq 0.05$). When significant, means of qualitative factors were compared using Tukey's test ($P \geq 0.05$). For significant quantitative factors, regression analysis was performed for the evaluated periods. In the experiment involving only a single herbicide application, means were compared with the untreated control using

Dunnett's test ($P \geq 0.05$). All the analyses were performed using RStudio software, with the ExpDes.pt, AgroR, and asbio packages.

3 Results and Discussion

The evaluations showed differences in horseweed control among the herbicides when applied as a single application (Table 1). Additionally, at 14 DAA, there was a significant effect of the interval between the application of fluroxypyr + glyphosate and the sequential application, as well as differences among the contact herbicides used in this sequential treatment for all the periods and locations assessed, except for the evaluation conducted at 28 DAA in Ponta Grossa (Table 2). No significant interaction was observed between the factors of application intervals and herbicides used in the sequential application.

Table 1 - Summary of the analysis of variance for single application of ammonium glufosinate, ammonium glufosinate + saflufenacil, diquat at 400 g a.i. ha^{-1}, and [methyl fluroxypyr + clethodim] + glyphosate for horseweed control at two distinct locations

	Londrina				**Ponta Grossa**			
	14 DAA		28 DAA		14 DAA		28 DAA	
	MS	Pr (F)	MS	Pr (F)	MS	Pr (F)	MS	Pr (F)
Treatment	1768.23	0.00*	1835.42	0.007*	4026.73	$4.8\ 10^{-7}$*	4179.75	$6.79\ 10^{-7}$*
Block	9.89	0.94	260.42	0.40	80.06	$2.17\ 10^{-1}$	125.75	$1.24\ 10^{-1}$
Error	75.17		239.58		44.50		49.92	
CV (%)	22.2		91.72		10.77		12.37	

*Statistically significant by the F-test at 5% Probability of experimental error (F); CV: Coefficient of Variation; MS: Mean Square. DAA = Days After Application.
Source: reaearch data.

Table 2 - Summary of the analysis of variance of the evaluation factors, where F1 corresponds to the intervals of three, six, 10, and 15 days between the first application and the sequential application; F2 corresponds to the contact products Glufosinate-ammonium, Glufosinate-ammonium + Saflufenacil, and Diquat used in the sequential application; and "Ad" refers to the additional treatment without herbicide application

	Londrina				**Ponta Grossa**			
	14 DAA		**28 DAA**		**14 DAA**		**28 DAA**	
	MS	**Pr (F)**	**MS**	**Pr (F)**	**MS**	**Pr (F)**	**MS**	**Pr (F)**
Block	269.23	0.05	336.38	0.006*	23.45	0.02*	182.46	0*
F1	309.72	0.03*	93.57	0.27	27.57	0.01*	32.61	0.15
F2	1356.25	0*	454.68	0.003*	136.64	0*	10.64	0.55
F1*F2	111.80	0.34	62.32	0.51	5.61	0.56	15.42	0.51
AdvsFatorial	18744.23	0*	26091.70	0*	32466.34	0*	31365.02	0*
Error	94.57		69.71		6.89		17.37	
CV (%)	14.79		10.76		3.03		4.9	

*Statistically significant by the F-test at 5% Probability of experimental error (F); CV: Coefficient of Variation; MS: Mean Square. DAA = Days After Application.
Source: reaearch data.

The use of a single application for horseweed control does not constitute a safe strategy for managing the species, even though it differed statistically from the additional untreated control (Table 1). Treatments consisting of a single application of [methyl-fluroxypyr + clethodim] + glyphosate resulted in a maximum injury of less than 15% at 14 DAA in Londrina, characterized by initial epinasty symptoms, typical of auxin-mimicking herbicides (Piasecki, 2024). When evaluated at 28 DAA, injury was below 5%, indicating plant recovery from the damage caused by the herbicide application (Table 3). Similar regrowth results have been observed for glyphosate mixed with 2,4-D, which can reach up to 100% regrowth depending on the plant's developmental stage at the time of application (Cesco *et al*., 2019).

Table 3 - Analysis of the percentage (%) of plant control at 14 and 28 DAA for the treatments Glufosinate-ammonium, Glufosinate-ammonium + Saflufenacil, Diquat at 400 g a.i. ha^{-1}, and [Methyl-fluroxypyr + Clethodim] + Glyphosate used in single applications for horseweed control at two distinct locations

	Londrina		**Ponta Grossa**	
Treatment	14 DAA	28 DAA	14 DAA	28 DAA
[Methyl-fluroxypyr + Clethodim] + Glyphosate	13.75c*	3.75b	15.0b*	10.0c
Glufosinate	36.25b*	5.00b	74.00a*	68.75ab*
Glufosinate + Saflufenacil	65.00a*	48.75a*	85.00a*	83.50a*
Diquat	41.25b*	10.00b	73.75a*	66.25b*

*Averages with the same lowercase letter in the column do not differ statistically by the Tukey test at 5% experimental error probability. Averages differ statistically from the control treatment without herbicide application by the Dunnett test at 5% experimental error probability.
Source: research data.

A single application of contact products showed initial necrosis symptoms, with glufosinate ammonium and diquat causing injuries of 36% and 41% in Londrina, and 74% and 73% in Ponta Grossa, respectively, at 14 DAA. However, when evaluated at 28 DAA, there was a reduction in the initial injury, indicating plant's regrowth. When evaluating the efficiency of the isolated application of glufosinate ammonium + saflufenacil, this treatment was superior to the others in all the evaluated periods in Londrina. On the other hand, in Ponta Grossa, its efficiency did not differ statistically from glufosinate and diquat at 14 DAA, nor from isolated glufosinate at 28 DAA.

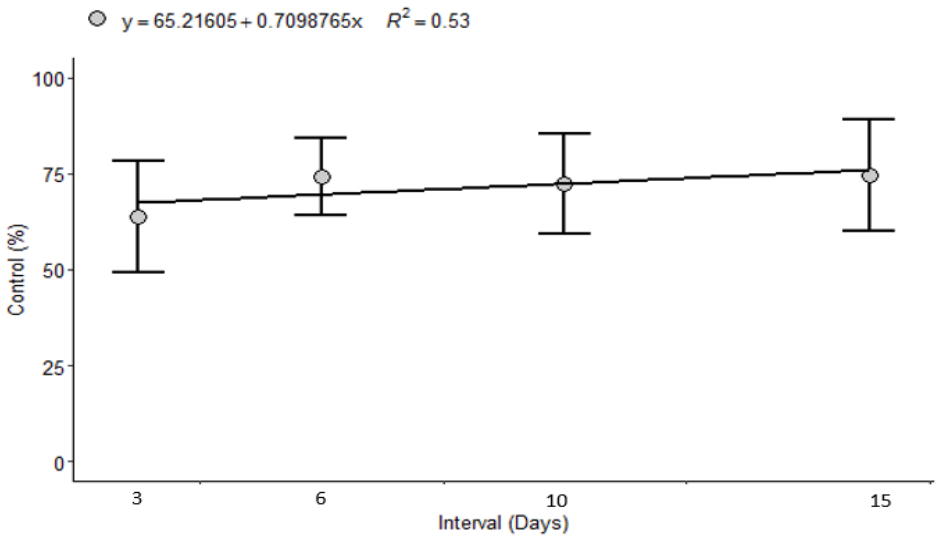
The efficiency of buva control using saflufenacil has already been reported in mixtures with glyphosate in single application (Dalazen *et al*., 2015; Cesco *et al*., 2019) or in sequential application (Cantu *et al*., 2021) in smaller plants, as well as reports of synergy from the mixture of saflufenacil with glufosinate in Amaranthus palmeri plants (Takano *et al*., 2020a). However, the single application of these herbicides depends on the plants' development stage and environmental conditions, as

observed in the present study, where there were distinct efficiency values for the two locations (48% in Londrina and 83% in Ponta Grossa), not characterizing it as an alternative safe control.

These data reinforce the importance of sequential application for buva control, as this management strategy can reduce the regrowth capacity even in conditions of plant stage uniformity (Cesco *et al*., 2019; Albrecht *et al*., 2020; Cantu *et al*., 2021). When evaluating different application intervals for post-application contact herbicides after fluroxipir+glyphosate in buva, no significant interaction between the factors was obtained, however, at 14 DAA, significance was observed for the number of days between this application and the sequential one.

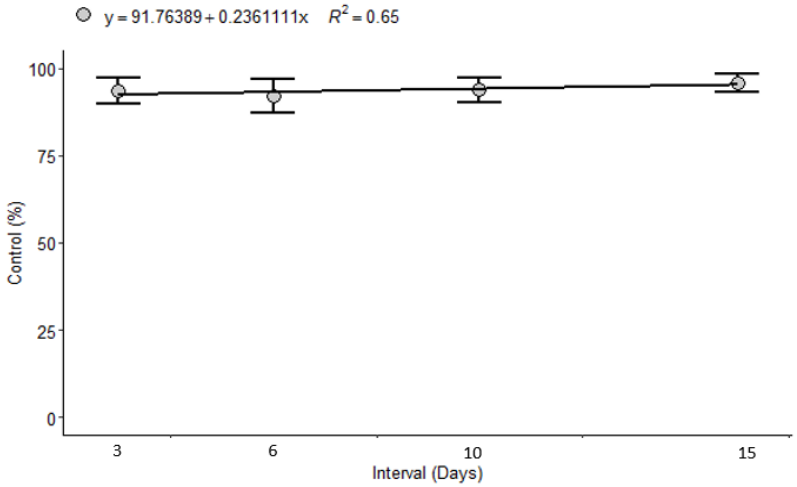
In both locations, a positive increasing relationship was observed between control and the increase in days between the first and second applications, with treatments applied 15 days after the [fluroxipir-methyl+cletodim]+glyphosate application showing the best control results, regardless of the contact herbicide used (Figures 1 and 2, Table 4). This result corroborates studies in the literature in which the best interval between diquat application after glyphosate application is five to seven days (Werth *et al*., 2010), which is the period necessary for the product to generate high stress on the plants, since the death of susceptible plant tissues occurs between seven and ten days (Piasecki, 2024).

Figure 1 - Regression analysis for different intervals between the application of [fluroxipir-methyl+cletodim]+glyphosate and contact products in sequential application for buva control in an experiment conducted in Londrina, Parana State



Source: research data.

Figure 2 - Regression analysis for different intervals between the application of [fluroxipir-methyl+cletodim]+glyphosate and contact products in sequential application for buva control in an experiment conducted in Ponta Grossa, Paraná State



Source: research data.

Table 4 - Summary of the variance analysis of the regression for intervals of three, six, 10, and 15 days between the first application and the sequential application of contact products when evaluating control percentages at 14 days after the sequential application

	Londrina		**Ponta Grossa**	
	MS	p-value	MS	p-value
Linear Effect	489.81	0.028	54.187	0.008
Deviations of regression	219.67	0.112ns	14.271	0.141ns
Residues	94.58		6.899	

ns: not significant by the F test at 5% experimental error probability; MS: Mean Square.
Source: research data.

Fluroxypyr is an auxin mimicker, a product that acts through the plant's hormonal imbalance, generating initial symptoms characteristic of epinasty. However, these symptoms occur gradually, and the plant's death may occur 20 to 30 days after application (Piasecki, 2024). Thus, it is believed that periods of up to 15 days after the application of auxinics may favor control by contact products in a sequential application, since the plant's tissues will be affected by the herbicide but still alive and capable of absorbing the applied product, which will contribute to oxidative stress until the plant's complete death.

The use of contact herbicides after the application of [fluroxypyr-methyl+cletodim] + glyphosate on weed (buva) showed satisfactory control results even at 28 days after application (DAA), with control above 75% for all locations and treatments (Table 5). The use of glufosinate

ammonium as a contact herbicide after the application of systemic herbicides achieved 78% and 91% control at 28 DAA for the experiments conducted in Londrina and Ponta Grossa, respectively. It is noteworthy that for Ponta Grossa, the result of this management already reached 90% control at 14 DAA. These results corroborate studies showing the effectiveness of glufosinate ammonium in the sequential application of the mixture of glyphosate with 2,4-D, another auxin mimicker (Albrecht *et al*., 2020; Cantu *et al*., 2021).

Table 5 - Evaluation of percentage (%) control of plants at 14 and 28 DAA for contact products Glufosinate ammonium, Glufosinate ammonium + Saflufenacil, Diquat used in sequential application of [fluroxypyr-methyl+cletodim] + glyphosate at different sequential application intervals

	Londrina		**Ponta Grosa**	
Sequential Applications	14 DAA	28 DAA	14 DAA	28 DAA
Glufosinate ammonium	61.25b	78.12b	90.5b	91.25ns
Glufosinate ammonium + Saflufenacil	73.12a	88.44a	96.12a	92.44ns
Diquat	79.37a	85.62a	94.69a	92.81ns

*DAA – Days after application on which the evaluation was conducted. Means followed by the same lowercase letter in the column do not differ statistically according to Tukey's test at 5% probability of experimental error. ns: not significant according to the F-test at 5% probability of experimental error.
Source: research data.

However, for the evaluations in which differences between treatments were observed, ammonium glufosinate showed statistically lower control compared to the other treatments at 14 DAA in Londrina and Ponta Grossa, and at 28 DAA in Londrina (Table 5). The results obtained with sequential application of diquat also showed high control levels, above 85% at 28 DAA for both locations. Different results were reported by Cantu *et al*. (2021), where the application of diquat seven days after the mixture of glyphosate with auxin mimics reached a maximum control of 73% at 35 DAA in 16 cm-tall plants.

Diquat is a photosystem I inhibitor herbicide that generates ROS (reactive oxygen species) through electron diversion in the photosynthetic electron transport chain, causing lipid peroxidation, cell leakage, and consequent cell death. However, the rapid foliar absorption combined with high ROS formation may interfere with the herbicide's action, as its physicochemical characteristics allow for translocation in the plant, but the tissue rapid necrosis where the product is located may hinder its effectiveness (Piasecki, 2024).

Thus, it is believed that the higher efficiency of diquat treatment in the present study may be due to nighttime application of the herbicide. Literature reports indicate that dark periods after application may enhance translocation to meristematic regions and enable better action in other parts

of the plant upon later exposure to light (Oliveira *et al*., 2022), which is an important observation for improving the molecule's efficiency.

Furthermore, among the herbicides evaluated, the mixture of ammonium glufosinate + saflufenacil stood out, showing higher control than ammonium glufosinate alone at 14 and 28 DAA in Londrina and at 14 DAA in Ponta Grossa. This mixture did not differ from diquat in the same periods, achieving control above 88% at 28 DAA. Ammonium glufosinate, by inhibiting GS, leads to feedback inhibition of photorespiration, inactivation of RuBisCo activase, and disruption of the electron generation and consumption balance in the thylakoid membrane, resulting in ROS formation and lipid peroxidation (Takano *et al*., 2020b).

Saflufenacil, in turn, is a protoporphyrinogen oxidase (PROTOX) inhibitor herbicide. This enzyme participates in the chlorophyll biosynthesis pathway but also contributes to ROS formation through the accumulation of protoporphyrinogen IX in the chloroplast. This compound leaks into the cytosol and is non-enzymatically converted to protoporphyrin IX, which absorbs solar energy and generates free radicals (Piasecki, 2024). The control efficiency of the combined treatment using these two modes of action is greater than when applied separately.

The increased control results from GS inhibition by glufosinate causing a transient accumulation of glutamate, a precursor of chlorophyll, which enhances the action of PROTOX inhibitors. At the same time, PROTOX inhibition leads to ROS accumulation, which is further amplified in the presence of glufosinate. This excessive proton accumulation is responsible for cell death (Takano *et al*., 2020a). The synergism of saflufenacil has also been reported for glyphosate, as previously mentioned, highlighting the importance of combining these products, especially for reducing plant's regrowth (Dalazen *et al*., 2015).

These results reinforce the importance of multi-step horseweed management, which explores better results from already-used molecules through strategic herbicide placement in the field. Optimizing weed control in the context of herbicide resistance and reduced herbicide efficacy is necessary, as it is a broader study of other systemic and contact herbicides that may contribute to this management.

4 Conclusion

The standalone application of [fluroxypyr-methyl + clethodim] + glyphosate, ammonium glufosinate, ammonium glufosinate + saflufenacil, and diquat does not represent a reliable option for horseweed control. Ammonium glufosinate, ammonium glufosinate + saflufenacil, and diquat are effective when used in sequential application management with the fluroxypyr + glyphosate mixture, with particular emphasis on diquat application and the enhanced control provided by adding

saflufenacil to ammonium glufosinate. Regarding the intervals between applications, a 15-day gap resulted in increased control regardless of the contact herbicide used.

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