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Identification, mapping, and chemical control of fleabane resistant to glyphosate, chlorimuron, paraquat, and 2,4-D

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Abstract

Monitoring herbicide-resistant weeds makes it possible to study the evolution and spread of resistance, which provides important information for their management. The objective of this study was to map fleabane accessions in the states of Paraná (PR) and Mato Grosso do Sul (MS), Brazil, to identify herbicide-resistant accessions and their response to soybean preplant chemical burndown management strategies. Fleabane seeds were collected in agricultural areas in PR and MS in 2018, 2019, and 2020. Initial screening was performed for glyphosate, chlorimuron, paraquat, 2,4-D, saflufenacil, and glufosinate efficacy. Subsequently, dose-response experiments were conducted. Field experiments were carried out in three locations, where accessions of multiple herbicide–resistant Sumatran fleabane were identified. Herbicides were used in single or sequential applications at three plant heights (<5 cm, 5 to 10 cm, and >10 cm). After preliminary screening, accessions were classified as putative resistant (<80% control for all four replicates), segregated (<80% control for one to three replicates), or susceptible (>80% control for all four replicates). There was no evidence of resistance to glufosinate or saflufenacil in any of the 461 accessions, while 65 demonstrated possible resistance or segregation to glyphosate only, 235 to glyphosate + chlorimuron, 79 to glyphosate + chlorimuron + paraquat, 59 to glyphosate + chlorimuron + 2,4-D, and 23 with four-way resistance (glyphosate, chlorimuron, paraquat, and 2,4-D). Of these 23 accessions, seven were analyzed using dose-response curves (F_2 generation), all from PR, confirming four-way resistance to glyphosate, chlorimuron, paraquat, and 2,4-D. To control resistant Sumatran fleabane, an application should prioritize smaller plants. Despite resistance to 2,4-D, double mixtures containing this herbicide were among the most effective treatments in plants <5 cm in height. If a sequential application is needed for plants >5 cm in height, we recommend glyphosate + synthetic auxin followed by glufosinate or glyphosate + saflufenacil.

Introduction

The genus *Erigeron* (syn. *Conyza*) belongs to the family Asteraceae and contains 150 species worldwide (Flann 2016). Hairy fleabane (*Erigeron bonariensis* L.), Sumatran fleabane, and horseweed (*E. canadensis* L.) stand out as weeds, with the Americas as their center of origin. Horseweed is originally from North America, while hairy and Sumatran fleabane are native to South America (Bajwa et al. 2016).

Erigeron spp. (fleabane) is spread exclusively by seeds, with each plant of hairy fleabane having the potential to produce around 110,000 seeds and horseweed producing up to 200,000 seeds (Bhowmik and Bekech 1993; Dauer et al. 2007; Wu et al. 2007). The seeds are extremely light and have a morphological modification known as a pappus, which facilitates wind dispersal (Liu et al. 2022), allowing them to travel great distances (Shields et al. 2006). However, Dauer et al. (2007) found that 99% of seeds were found within a 100-m radius, with smaller amounts reaching up to 500 m.

Worldwide, 108 cases of herbicide resistance for the three *Conyza* species combined have been reported. In Brazil, the first cases of glyphosate-resistant hairy fleabane and horseweed were recorded in 2005, while the first case of glyphosate-resistant Sumatran fleabane was recorded in 2010 (Heap 2023). There is a higher prevalence of Sumatran fleabane in Brazil, although hairy fleabane also occurs, especially in southern Brazil (Marochio et al. 2017; Ruiz et al. 2022). Cases have been reported of Sumatran fleabane with resistance to paraquat (Zobiole et al. 2019); 2,4-D (Queiroz et al. 2020); glyphosate, chlorimuron, and saflufenacil (Heap 2023);

and multiple resistance to chlorimuron and glyphosate (Santos et al. 2014a); chlorimuron, glyphosate, and paraquat (Albrecht et al. 2020a); and 2,4-D, diuron, glyphosate, paraquat, and saflufenacil (Pinho et al. 2019). Herbicide resistance makes fleabane difficult to manage and can increase production costs (Baccin et al. 2022).

Fleabane can cause major yield reduction in grain crops (Agostinetto et al. 2017; Bajwa et al. 2016; Trezzi et al. 2015). As such, monitoring herbicide-resistant populations is of paramount importance for early detection and establishing recommendations to mitigate their expansion. Mitigation strategies include rotating the herbicide sites of action and incorporating nonchemical weed control techniques into the production system (Hanson et al. 2009; Schultz et al. 2015).

In areas with high infestation and/or herbicide resistance, two or more applications of a herbicide are required to effectively control fleabane prior to soybean planting. Glyphosate and synthetic auxin mixtures are commonly used in the first application (Albrecht et al. 2022a; Cantu et al. 2021; Quinn et al. 2020), and burndown herbicides such as diquat, glufosinate, or glufosinate + saflufenacil in the second (Albrecht et al. 2022b,c; Dilliott et al. 2022).

The ecophysiological characteristics of fleabane associated with management, cultural treatments, no-till system, and the dependence and continuous use of herbicides for control have favored the selection of resistant accessions and dominance of this weed in agriculture (Bajwa et al. 2016). In this context, monitoring herbicide-resistant weed accessions allows scientists to study the evolution and spread of resistance, providing important information for management recommendations. Thus, the present study aimed to identify herbicide resistance in fleabane accessions in the states of Paraná (PR) and Mato Grosso do Sul (MS), Brazil, and their response to soybean preplant chemical burndown management strategies.

Material and Methods

Screening

In 2018, 2019, and 2020, fleabane seeds were collected in agricultural areas from plants that survived after presowing, postemergence, or off-season herbicide application. Collections were obtained from different commercial farms based on information received from farmers and agronomists. A total of 461 accessions were collected in the two states (408 from Paraná, 53 from Mato Grosso do Sul) and stored in paper bags under refrigeration. Seed collection followed the methodology proposed by Burgos et al. (2013). Seeds were collected after herbicide application from one or more plants with similar characteristics, at specific control failure points. Some accessions were also taken from areas where little herbicide was used, based on information from farmers and technicians in the region, so as to find susceptible plants. Yet all collected accessions were classified as putative resistant to at least one herbicide.

A preliminary screening was performed in a greenhouse under a controlled temperature of 25 C, 5 mm d^{-1} irrigation, and a 12-h photoperiod. Seeds collected from each accession were sown in 0.8-L plastic pots filled with potting mix (Humusfértil®; Toledo, PR, Brazil), and once plants had one to two true leaves, they were transplanted into pots (0.8 L) at two plants per pot, showing no signs of transplant shock.

At the six-leaf stage, the following treatments were applied: glyphosate (Shadow® 480 SL, 720 g ae ha^{-1}), chlorimuron (Classic®, 20 g ai ha^{-1}) + mineral oil (Assist® EC, 0.5% v/v), paraquat (Paraquate Alta® 200 SL, 400 g ai ha^{-1}) + adhesive spreader based on soybean methyl ester (Mees™, 0.5% v/v), 2,4-D (DMA® 806 BR, 1,005 g ae ha^{-1}), saflufenacil (Heat®, 35 g ai ha^{-1}) + adhesive spreader based on soybean methyl ester (0.5% v/v) and glufosinate (Finale®, 500 g ai ha^{-1}) + adhesive spreader based on soybean methyl ester (0.5% v/v), and a control with no herbicide application. A completely randomized design with four replications was used.

The doses used for glyphosate, chlorimuron, and 2,4-D were those recommended on the commercial product labels: an intermediate dose was used for saflufenacil, and the highest dose was used for paraquat and the lowest for glufosinate (Rodrigues and Almeida 2018). The doses were chosen from within the range indicated on the commercial herbicide labels, based on what farmers in the region typically use. Herbicides were applied at 0.5 m above weed height, using a CO_2-pressurized backpack sprayer equipped with four TeeJet AIXR 110015 nozzles (Spraying Systems Co., Glendal Heights, IL) spaced 0.5 m apart, at a constant pressure of 196 kPa, and flow rate of 1 m s^{-1}, providing an application volume of 150 L ha^{-1}.

Fleabane control was assessed at 7, 14, 21, and 28 d after application (DAA) on a visual score scale from 0% to 100%, where 0% indicates no control and 100% indicates plant death (Velini et al. 1995). Control scores at 28 DAA were used to classify the accessions as putative resistant (<80% control for all four replicates), segregated (<80% control for one to three replicates), or susceptible (>80% control for all four replicates), based on an adaptation of the classifications proposed by Lopez-Ovejero et al. (2017) and Mendes et al. (2021).

Dose-Response Curve

Twenty-three accessions were identified as Sumatran fleabane with possible multiple resistance to glyphosate, chlorimuron, paraquat, and 2,4-D. Plants were grown in pots until seed production for use in the dose-response curve test. Of these, seven accessions were selected for the dose-response curve (F_2 generation). This is because germination and plant development issues resulted in insufficient numbers to proceed with the dose-response curve for the other accessions. Four accessions from Assis Chateaubriand, PR, were tested: SILV4-R (24.3161°S, 53.5069°W), TN1-R (24.2914°S, 53.5028°W), TN3-R (24.3253°S, 53.5208°W), and 514-R (24.2858°S, 53.5117°W); and three were tested from Palotina, PR: 480-R (24.3647°S, 53.8802°W), 521-R (24.2036°S, 53.7931°W), and 522-R (24.3553°S, 53.8856°W). The susceptible accession was collected in Palotina (24.2747°S, 53.6702°W). After seed collection, the sowing process, growing conditions, and growth stage for herbicide application were the same as those used in screening.

Saflufenacil and glufosinate were excluded because no plants survived the application of these herbicides during the preliminary screening. The doses adopted for each herbicide corresponded to 0, 1/8×, 1/4×, 1/2×, 1×, 2×, 4×, and 8× the dose used in the initial screening. The herbicides applied were glyphosate (0, 90, 180, 360, 720, 1,440, 2,880, and 5,760 g ae ha^{-1}), chlorimuron (0, 2.5, 5, 10, 20, 40, 80, and 160 g ai ha^{-1}), paraquat (0, 50, 100, 200, 400, 800, 1,600, and 3,200 g ai ha^{-1}), and 2,4-D (0, 125, 251, 502, 1,005, 2,010, 4,020, and 8,040 g ae ha^{-1}). The use of adjuvant oils was the same as that used in the initial screening.

The shoots were collected 28 DAA to determine dry biomass. The plant material was dried in a forced-air oven at 60 C until constant mass and then weighed on a precision scale. Data were submitted to regression analysis ($P < 0.05$) using a nonlinear logistic regression model (Streibig 1988) as follows:

Table 1. Geographic coordinates Sumatran fleabane accessions with multiple quadruple resistance and their respective GR_{50} values and RF for each location[a]

Location	Latitude	Longitude	Accession	Glyphosate		Chlorimuron		Paraquat		2,4-D	
				GR_{50}	RF	GR_{50}	RF	GR_{50}	RF	GR_{50}	RF
				g ae ha^{-1}		g ai ha^{-1}		g ai ha^{-1}		g ae ha^{-1}	
1	24.3647ºS	53.8802ºW	480-R	1,452	14	44	44	655	12	718	8
2	24.2036ºS	53.7931ºW	521-R	909	9	52	52	523	10	1,423	15
3	24.3553ºS	53.8856ºW	522-R	2,387	23	42	42	674	11	1,197	16

[a]Abbreviations: GR_{50}, dose required to reduce dry mass by 50%; RF, resistance factor.

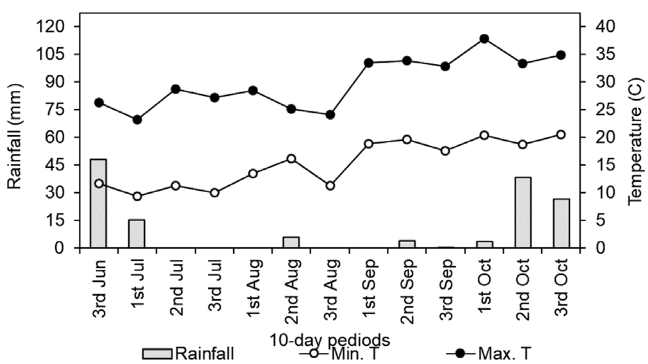

Figure 1. Rainfall and minimum and maximum temperatures during the experimental period. Source: weather station in Palotina, Paraná, Brazil (24.1790ºS, 53.8379ºW).

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

where y is the response variable; x is the herbicide dose; a is the amplitude between the maximum and minimum points; b is the dose that provides a 50% response by the variable, and c is the slope of the curve around b.

The nonlinear logistic model provides an estimate of the GR_{50} parameter (the dose required to reduce dry mass by 50%). Thus, it was chosen for mathematical calculation using the inverse equation of Streibig (1988), allowing the calculation of GR_{50}, as used in other studies (Albrecht et al. 2020a; Takano et al. 2017):

$$x = b\left(\left|\frac{a}{y} - 1\right|\right)^{\frac{1}{c}} \qquad [2]$$

For glyphosate, it was not possible to adjust to the model proposed by Streibig (1988). Thus, data were submitted to regression analysis ($P < 0.05$) using a four-parameter nonlinear logistic model (Seefeldt et al. 1995), as used in other studies (Wu et al. 2022; Yang et al. 2022):

$$y = minP\frac{a}{\left[1 + \left(\frac{x}{b}\right)^{c}\right]} \qquad [3]$$

where $minP$ is the minimum point of the curve; y is the response variable; x is the herbicide dose; a is the amplitude between the maximum and minimum points; b is the dose that provides a 50% response by the variable, and c is the slope of the curve.

The model for each herbicide was chosen according to the best fit according to Akaike Information Criteria values. SigmaPlot® 15 software (Systat Software Inc., San Jose, CA) was used for statistical analyses. Based on the GR_{50} values, the resistance factor (RF) was obtained, which is the result of the ratio between the resistant and susceptible accession (Albrecht et al. 2020b; Burgos 2015; Hall et al. 1998; Takano et al. 2017).

Chemical Control of Sumatran fleabane with Four-Way Resistance to Glyphosate, Chlorimuron, Paraquat, and 2,4-D

Field experiments were carried out with the aim of establishing Sumatran fleabane response to soybean preplant chemical burndown management strategies. Experiments were conducted between August and October 2020 at three locations at Palotina, PR, which contained accessions identified as Sumatran fleabane with four-way resistance to glyphosate, chlorimuron, paraquat, and 2,4-D, according to initial screening and the dose-response curve (Table 1). Climate in the region is classified as mesothermal subtropical humid. The weather conditions during the study period are shown in Figure 1.

In these locations, one of the most common management techniques for fleabane is the application, in the off-season, of glyphosate + synthetic auxin with glufosinate in sequence, in some cases with the application of diclosulam at soybean preemergence. At postemergence, an application of glyphosate alone or in a mixture with herbicides that inhibit acetolactate synthase (ALS) may be used. In maize crops at succession, it is common to use atrazine in a mixture with glyphosate.

The experiment was conducted as a randomized complete block design and double factorial arrangement (30 × 3), with 4- × 6-m plots and four replications. Thirty herbicide treatments were tested (Table 2), and a single application was carried out at three Sumatran fleabane plant heights (<5 cm, 5 to 10 cm, and >10 cm). In each of the plots, there were plants in approximately in the same proportion between the three heights. At the time of application, Locations 1, 2, and 3 contained 8, 26, and 7 Sumatran fleabane plants per meter, respectively. Flags with different colors were added for each of the three heights at the time of application at some points in each plot, to facilitate identification of heights in subsequent control evaluations. A CO_2-pressurized backpack sprayer equipped with four TeeJet AIXR110.015 nozzles spaced 0.5 m apart, at a constant pressure of 196 kPa and flow rate of 1 m s^{-1}, providing an application volume of 150 L ha^{-1}.

Sumatran fleabane control was evaluated at 28 DAA using a visual score scale from 0% to 100%, where 0% indicates no control and 100% indicates plant death (Velini et al. 1995). An average control score was assigned to each plot, according to each of the three plant heights. Group analysis was performed (Banzatto and Kronka 2013). To that end, data from each location were initially submitted individually for ANOVA using the F-test ($P < 0.05$) (Table 3). A ratio of 5.74 was obtained between the largest and smallest mean squared error (<7), thus enabling group analysis.

Group analysis indicated a significant effect ($P < 0.05$) for locations and interaction between the factors and locations (Table 3). As such, means were compared individually for each

Table 2. Herbicide treatments to control Sumatran fleabane[a,e]

First application		Sequential application		Days between applications
Herbicide	Dose[b]	Herbicide	Dose[b]	
	g ha^{-1}		g ha^{-1}	
Control (without application)				
Gly + 2,4-D	1,242 + 804			
Gly + dicamba[c]	1,242 + 288			
Gly + triclopyr[d]	1,242 + 576			
Gly + 2,4-D + saflufenacil[c]	1,242 + 804 + 35			
Gly + dicamba + saflufenacil[c]	1,242 + 288 + 35			
Gly + triclopyr + saflufenacil[c]	1,242 + 576 + 35			
Gly + 2,4-D + glufosinate[c]	1,242 + 804 + 500			
Gly + dicamba + glufosinate[c]	1,242 + 288 + 500			
Gly + triclopyr + glufosinate[c]	1,242 + 576 + 500			
Gly + saflufenacil[c]	1,242 +35			
Glufosinate[c]	500			
Gly + 2,4-D	1,242 + 804	Gly + saflufenacil[c]	1,242 + 35	7
Gly + dicamba[c]	1,242 + 288	Gly + saflufenacil[c]	1,242 + 35	7
Gly + triclopyr[b]	1,242 + 576	Gly + saflufenacil[c]	1,242 + 35	7
Gly + 2,4-D	1,242 + 804	Glufosinate[c]	500	7
Gly + dicamba[c]	1,242 + 288	Glufosinate[c]	500	7
Gly + triclopyr[d]	1,242 + 576	Glufosinate[c]	500	7
Gly + 2,4-D	1,242 + 804	Gly + saflufenacil[c]	1,242 + 35	14
Gly + dicamba[c]	1,242 + 288	Gly + saflufenacil[c]	1,242 + 35	14
Gly + triclopyr[d]	1,242 + 576	Gly + saflufenacil[c]	1,242 + 35	14
Gly + 2,4-D	1,242 + 804	Glufosinate[c]	500	14
Gly + dicamba[c]	1,242 + 288	Glufosinate[c]	500	14
Gly + triclopyr[d]	1,242 + 576	Glufosinate[c]	500	14
Gly + 2,4-D	1,242 + 804	Gly + saflufenacil[c]	1,242 + 35	21
Gly + dicamba[c]	1,242 + 288	Gly + saflufenacil[c]	1,242 + 35	21
Gly + triclopyr[d]	1,242 + 576	Gly + saflufenacil[c]	1,242 + 35	21
Gly + 2,4-D	1,242 + 804	Glufosinate[c]	500	21
Gly + dicamba[c]	1,242 + 288	Glufosinate[c]	500	21
Gly + triclopyr[d]	1,242 + 576	Glufosinate[c]	500	21

[a]Commercial products: glyphosate (Crucial®), 2,4-D (DMA® 806 BR), dicamba (Atectra®), triclopyr (Triclon®), saflufenacil (Heat®) and glufosinate (Finale®).
[b]Doses in g ai ha^{-1} for saflufenacil and glufosinate, in g ae ha^{-1} for the other herbicides.
[c]Addition of adhesive spreader based on soybean methyl ester (Mees™, 0.5% v/v).
[d]Addition of mineral oil (Lanzar®, 0.5% v/v).
[e]Abbreviation: Gly, glyphosate.

Table 3. Summary of individual and group ANOVA results for the three locations[a]

Individual	Location 1			Location 2			Location 3		
Source	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Herbicide	4,499.2	330.9	0.00	4,651.8	132.4	0.00	4,641.3	59.5	0.00
Plant height	5,463.5	401.8	0.00	10,467.8	297.9	0.00	18,001.3	230.8	0.00
H × PH	257.7	19.0	0.00	311.8	8.9	0.00	353.5	4.5	0.00
Block	117.9	8.7	0.00	27.7	0.8	0.50	311.1	4.0	0.01
Error	13.6			35.1			78.0		
Mean		89.2			87.5			83.3	
CV (%)		4.1			6.8			10.6	

Ratio of the largest to smallest MS error (78.0/13.6) = 5.7 (<7, group analysis is permitted)

Group		
Source	*F*	*P*
Location	66.4	0.00
Herbicide	265.5	0.00
Plant height	642.7	0.00
L × H	7.6	0.00
L × PH	18.6	0.00
H × PH	12.3	0.00
L × H × PH	3.5	0.00
Block	3.5	0.00
Mean	86.6	
CV (%)	8.1	

[a]Abbreviations: CV, coefficient of variation; F, variance ratio (F test); H, herbicide; L, location, MS, mean square; P, probability value; PH, plant height.

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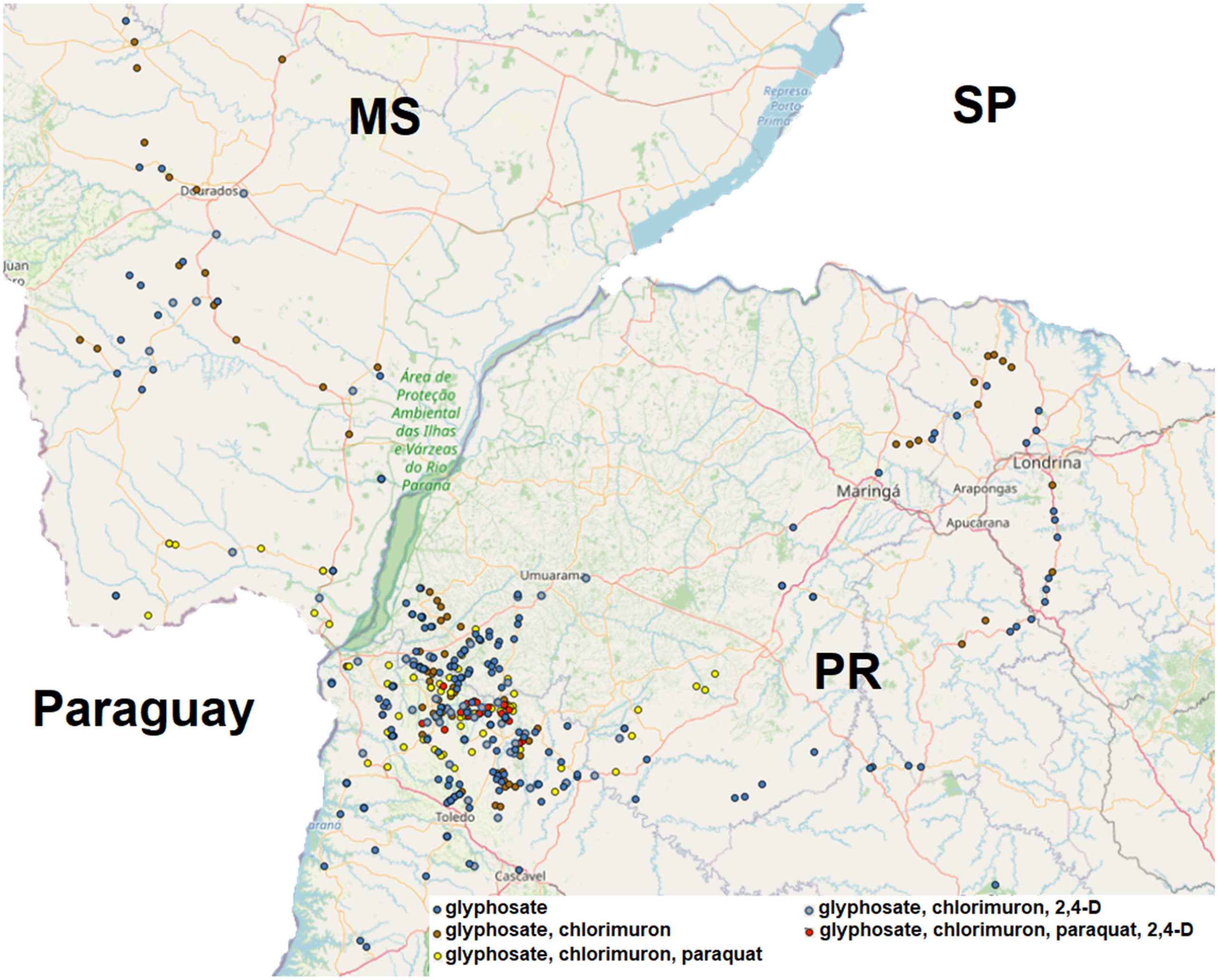

Figure 2. Location of the fleabane accessions with possible resistance or segregation for glyphosate, chlorimuron, paraquat, and 2,4-D in Paraná (PR) and Mato Grosso do Sul (MS). SP indicates São Paulo.

area using the Scott-Knott test ($P < 0.05$) for herbicide treatments and Tukey's test ($P < 0.05$) for plant height. Sisvar 5.6 software (Ferreira 2011) was used for the statistical analyses.

Results and Discussion

Fleabane Mapping in Paraná and Mato Grosso do Sul

Based on the 461 accessions analyzed, no fleabane plants with putative resistance to glufosinate and saflufenacil were identified, while all samples were putative resistant or segregated for resistance to glyphosate plus one or more of the herbicides tested (Figure 2).

Of the accessions analyzed, 65 showed putative resistance or segregation only to glyphosate, 235 to glyphosate + chlorimuron, 79 to glyphosate + chlorimuron + paraquat, 59 to glyphosate + chlorimuron + 2,4-D, and 23 exhibited four-way resistance (glyphosate, chlorimuron, paraquat, and 2,4-D). The accessions with possible multiple resistance were all resistant to glyphosate and chlorimuron, and of those that exhibited segregation only, four were segregated for glyphosate, 25 for chlorimuron, 17 to paraquat, and 18 for 2,4-D. Seven accessions displayed concomitant segregation for 2,4-D or paraquat (Table 4). These results indicate a response pattern like that verified by Albrecht et al (2020b) in Paraguay, which borders Paraná and Mato Grosso do Sul, except that in the present study, resistance to chlorimuron was not as widespread and no putative resistance to 2,4-D was found.

Of the 53 accessions from Mato Grosso do Sul, those identified as being putative resistant or segregated were from Caarapó, Amambai, Dourados, and Maracaju (in central and southern Mato Grosso do Sul), regions with large-scale grain cultivation operations. In Paraná, most accessions at risk for resistance or segregation for other herbicides were concentrated in the western region (Figure 3), an important grain-growing area with previous reports of herbicide-resistant fleabane (Albrecht et al. 2020a; Pinho et al. 2019; Queiroz et al. 2020; Santos et al. 2014a,b; Trezzi et al. 2011; Zobiole et al. 2019). Accessions with possible multiple resistance were concentrated in the municipalities of Palotina and Assis Chateaubriand of Paraná, with cases of possible resistance to 2,4-D or paraquat in adjacent regions. Accessions with possible multiple resistance to glyphosate, chlorimuron, paraquat, and 2,4-D were identified in Palotina, Maripá, and Assis Chateaubriand municipalities of Paraná. These accessions are spread throughout this microregion over a radius of about 20 km (Figure 3).

The occurrence of resistant fleabane in a large region comprising two states can be explained by the easy wind dispersal of the species' seeds, due to their lightness and the presence of

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Table 4. Fleabane accessions with possible herbicide resistance or segregation in Paraná and Mato Grosso do Sul[ab]

State	No. of accessions	Glyphosate only	Glyphosate and chlorimuron	Glyphosate, chlorimuron, and paraquat	Glyphosate, chlorimuron, and 2,4-D	Glyphosate, chlorimuron, paraquat, and 2,4-D
PR	408	49	217	69	50	23
MS	53	16	18	10	9	0
Total	461	65 (14%)	235 (51%)	79 (17%)	59 (13%)	23 (5%)

[a]Abbreviations: MS, Mato Grosso do Sul; PR, Paraná.
[b]Rates used: glyphosate, 720 g ae ha^{-1}; chlorimuron, 20 g ai ha^{-1}; paraquat, 400 g ai ha^{-1}; 2,4-D, 1,005 g ae ha^{-1}.

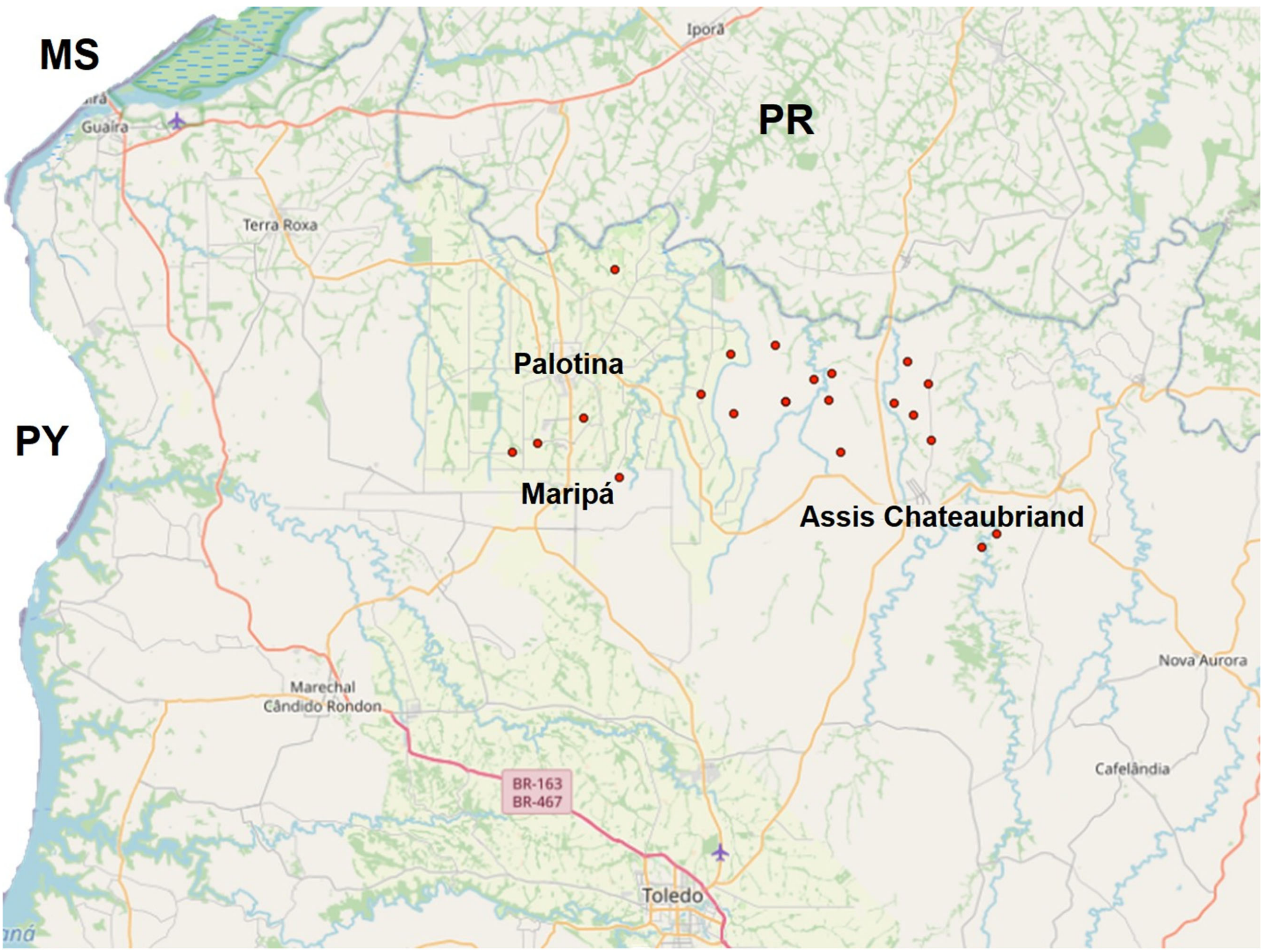

Figure 3. Location of Sumatran fleabane with possible multiple resistance or segregation for glyphosate, chlorimuron, paraquat, and 2,4-D in the regions of Palotina and Assis Chateaubriand, Paraná. Abbreviations: MS, Mato Grosso do Sul; PR, Paraná; PY, Paraguay.

pappi (Liu et al. 2022), but they also can be dispersed over short distances by agricultural machinery. In severe infestations, fleabane seeds can travel long distances from the source (Dauer et al. 2007). The collection of seeds aloft in the atmosphere suggests that under specific wind conditions and times, seeds can travel more than 550 km through the planetary boundary layer (Shields et al. 2006). That is, in a single dispersal event, seeds from Palotina or Assis Chateaubriand (both in Paraná) could reach Dourados in Mato Grosso do Sul, 250 km away, where accessions with possible resistance to 2,4-D or paraquat were found.

It is believed that selection began in a single location (probably Assis Chateaubriand), where paraquat and 2,4-D resistance were most frequent and spread via wind and agricultural machinery, but a genetic analysis would need to be performed to support this hypothesis. Other studies have reported agricultural machinery as a dispersal agent for herbicide-resistant weeds (Gazola et al. 2019; Mendes et al. 2021).

Confirmation of Sumatran Fleabane with Four-Way Resistance to Glyphosate, Chlorimuron, Paraquat, and 2,4-D

After initial screening, the seven accessions from Paraná (F_2 generation) of Sumatran fleabane were analyzed using dose-response curves. The four-way resistance to glyphosate (which

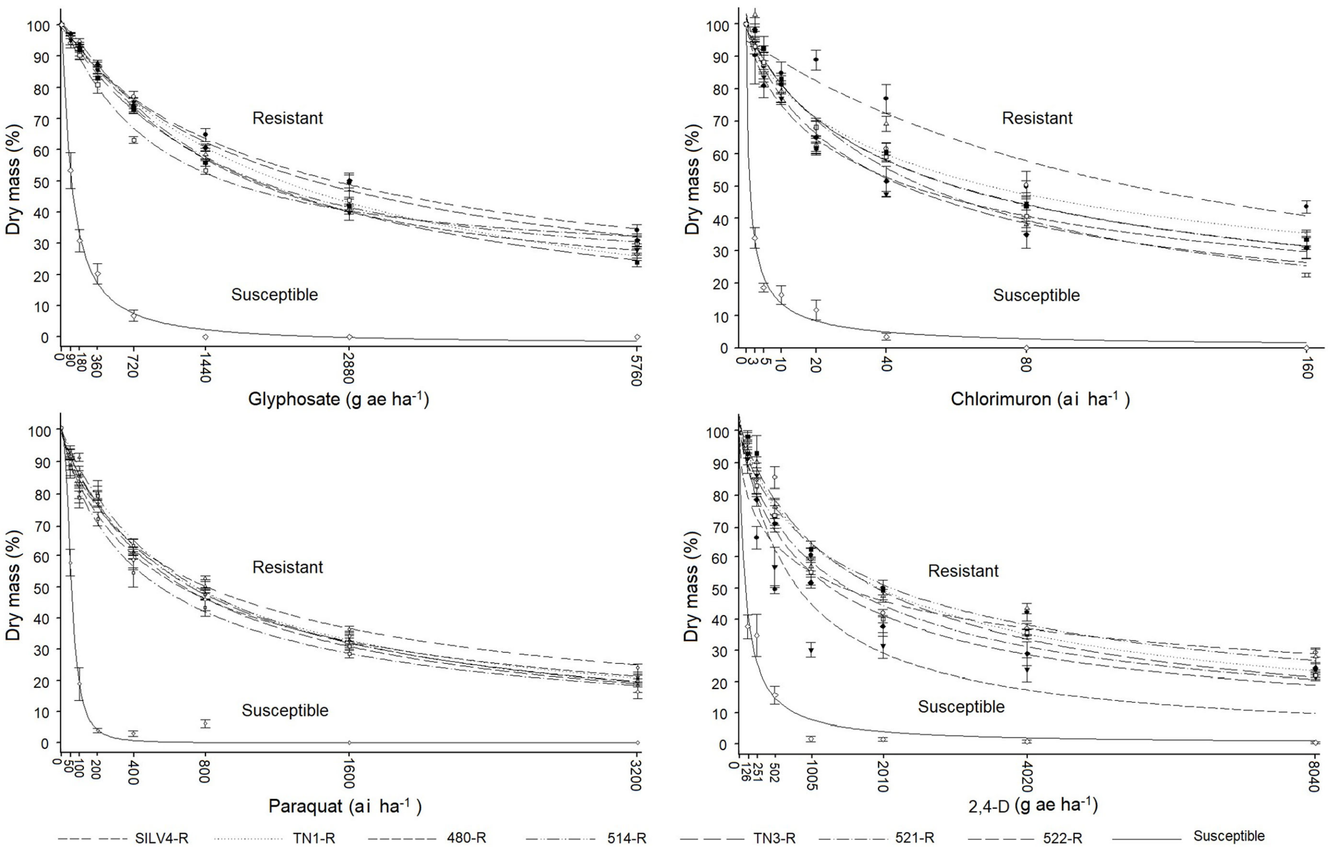

Figure 4. Dose-response curve for dry mass of Sumatran fleabane susceptible and resistant accessions (SILV4-R, TN1-R, 480-R, 514-R, TN3-R, 521-R, and 522-R) under glyphosate, chlorimuron, paraquat, and 2,4-D application. Data are from Palotina and Assis Chateaubriand, Paraná.

Table 5. GR_{50} and RF values of Sumatran fleabane accessions (F_2 generation) with multiple resistance to glyphosate, chlorimuron, paraquat, and 2,4-D[a]

	Glyphosate		Chlorimuron		Paraquat		2,4-D	
Accession	GR_{50}	RF	GR_{50}	RF	GR_{50}	RF	GR_{50}	RF
	g ae ha^{-1}		g ai ha^{-1}		g ai ha^{-1}		g ae ha^{-1}	
SILV4-R	2,414	24	122	122	786	14	1,366	15
TN1-R	2,550	25	67	67	737	13	1,801	20
480-R	1,452	14	44	44	655	12	718	8
514-R	1,319	13	55	55	736	13	1,932	21
TN3-R	2,054	20	54	54	731	13	1,763	19
521-R	909	9	52	52	523	10	1,423	15
522-R	2,387	23	42	42	674	12	1,197	13
Susceptible	102	–	1	–	55	–	92	–

[a]Abbreviations: GR_{50}, dose required to reduce dry mass by 50%; RF, resistance factor.

inhibits 5-enolpyruvylshikimate-3-phosphae synthase and is classified as a Group 9 herbicide by the Weed Science Society of America [WSSA] and Herbicide Resistance Action Committee [HRAC]), chlorimuron (an inhibitor of acetolactate synthase, categorized as a Group 2 herbicide by WSSA and HRAC), paraquat (a cell membrane disruptor; WSSA and HRAC Group 22), and 2,4-D (synthetic auxins; WSSA and HRAC Group 4) was confirmed. The susceptible accession showed high sensitivity to herbicides with GR_{50} values of 102, 1, 55, and 92 g ae ha^{-1} for glyphosate, chlorimuron, paraquat, and 2,4-D, respectively. Dose-response curves for multiple resistance confirmation are shown in Figure 4.

For glyphosate, the GR_{50} values varied from 909 to 2,550 g ae ha^{-1} in resistant accessions, with an RF value of 9 to 25. The GR_{50} values for chlorimuron ranged from 42 to 122 g ai ha^{-1} in resistant accessions; and from 523 to 786 g ai ha^{-1} for paraquat, with an RF between 10 and 14; whereas GR_{50} values for 2,4-D were 718 to 1,932 g ai ha^{-1}, and RF values varied from 8 to 21 (Table 5).

Glyphosate resistance has been reported for several years in Brazil, with a hairy fleabane accession from Rio Grande do Sul state showing 50% visual control (ED_{50}) at a dose of 5,760 g ae ha^{-1} (Vargas et al. 2007). Two other horseweed and hairy fleabane accessions obtained ED_{50} values of 705 and 677 g ae ha^{-1}, respectively (Lamego and Vidal 2008). In those studies, the possible resistance mechanisms involved were not elucidated.

Chlorimuron-resistant accessions exhibited GR_{50} values from 42 to 122 g ai ha^{-1}, with an RF of up to 122. In Sumatran fleabane,

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Table 6. Control of Sumatran fleabane resistant to glyphosate, chlorimuron, paraquat, and 2,4-D, at 28 d after application in plants <5 cm, 5–10 cm, and >10 cm in height[a–d]

			Sumatran fleabane control		
First application	Sequential application	Days between applications	<5 cm	5–10 cm	>10 cm
			%		
Control (without application)			0 dA	0 dA	0 eA
Gly + 2,4-D			92 bA	84 bA	61 dB
Gly + dicamba			88 bA	76 cB	60 dC
Gly + triclopyr			85 bA	75 cB	56 dC
Gly + 2,4-D + saflufenacil			97 aA	71 cB	55 dC
Gly + dicamba + saflufenacil			99 aA	85 bB	67 cC
Gly + triclopyr + saflufenacil			99 aA	87 bB	70 cC
Gly + 2,4-D + glufosinate			99 aA	99 aA	69 cB
Gly + dicamba + glufosinate			100 aA	97 aA	74 cB
Gly + triclopyr + glufosinate			100 aA	97 aA	74 cB
Gly + saflufenacil			92 bA	77 cB	56 dC
Glufosinate			100 aA	93 aA	58 dB
Gly + 2,4-D	Gly + saflufenacil	7	98 aA	97 aA	83 bB
Gly + dicamba	Gly + saflufenacil	7	100 aA	100 aA	99 aA
Gly + triclopyr	Gly + saflufenacil	7	100 aA	98 aA	96 aA
Gly + 2,4-D	Glufosinate	7	100 aA	100 aA	97 aA
Gly + dicamba	Glufosinate	7	99 aA	96 aAB	88 bB
Gly + triclopyr	Glufosinate	7	100 aA	100 aA	100 aA
Gly + 2,4-D	Gly + saflufenacil	14	100 aA	100 Aa	98 aA
Gly + dicamba	Gly + saflufenacil	14	100 aA	100 aA	98 aA
Gly + triclopyr	Gly + saflufenacil	14	100 aA	100 aA	100 aA
Gly + 2,4-D	Glufosinate	14	100 aA	100 aA	99 aA
Gly + dicamba	Glufosinate	14	100 aA	100 aA	99 aA
Gly + triclopyr	Glufosinate	14	100 aA	100 aA	100 aA
Gly + 2,4-D	Gly + saflufenacil	21	100 aA	100 aA	99 aA
Gly + dicamba	Gly + saflufenacil	21	100 aA	100 aA	99 aA
Gly + triclopyr	Gly + saflufenacil	21	100 aA	100 aA	100 aA
Gly + 2,4-D	Glufosinate	21	100 aA	100 aA	99 aA
Gly + dicamba	Glufosinate	21	100 aA	100 aA	99 aA
Gly + triclopyr	Glufosinate	21	100 aA	100 aA	100 aA

[a]Data are from Location 1 (accession 480-R).
[b]Abbreviation: Gly, glyphosate.
[c]Doses: glyphosate (1,242 g ae ha^{-1}), 2,4-D (804 g ae ha^{-1}), dicamba (288 g ae ha^{-1}), triclopyr (576 g ae ha^{-1}), saflufenacil (35 g ai ha^{-1}), glufosinate (500 g ai ha^{-1}).
[d]Means followed by different lowercase letters (herbicide treatments) differ according to the Scott-Knott test at 5%. Means followed by different uppercase letters (plant height) differ according to Tukey's test at 5%.

ED_{50} was 6.75 to 47 g ai ha^{-1} for resistant accessions and 1 g ai ha^{-1} for their susceptible counterparts (Santos et al. 2014a). Monitoring carried out in nine Brazilian states established control doses of glyphosate and chlorimuron for 12 accessions, with average GR_{50} values of 887 and 47 g ai ha^{-1}, respectively (Mendes et al. 2021).

Paraquat resistance in Sumatran fleabane is recent in Brazil, the first report was in 2019 for accessions obtained in the states of Paraná and São Paulo, with GR_{50} values of 244, 699, 1,166, and 2,007 g ai ha^{-1} for resistant accessions, and 20, 60, and 67 g ai ha^{-1} for their susceptible counterparts (Zobiole et al. 2019). In the present study, the GR_{50} for resistant accessions ranged from 523 to 786 g ai ha^{-1}.

Sumatran fleabane resistance to 2,4-D was reported in 2019, with cell death or rapid necrosis in plants at an ED_{50} of 1,133 g ae ha^{-1} (Queiroz et al. 2020). This symptomatology has also been reported in giant ragweed (*Ambrosia trifida* L.) after glyphosate exposure, hydrogen peroxide accumulation resulting in cell death (Moretti et al. 2018). The accessions studied exhibited GR_{50} values of 718 to 1,932 g ae ha^{-1} for 2,4-D and an RF value of up to 21.

The results from the dose-response study based on criteria for establishing resistance, heritability, confirmation via protocols, and proven practical impact (Gazziero et al. 2009) confirm multiple resistance to glyphosate, chlorimuron, paraquat, and 2,4-D in Sumatran fleabane.

Chemical Control at Three Stages Height of Sumatran Fleabane with Four-Way Resistance to Glyphosate, Chlorimuron, Paraquat, and 2,4-D

The experiments were conducted in three locations, which contained accessions identified as Sumatran fleabane with four-way resistance to glyphosate, chlorimuron, paraquat, and 2,4-D. At Location 1 (accession 480-R), for plants <5 cm in height, a single application of the triple mixtures or sequential application of double mixtures regardless of the interval, achieved greater control. Additionally, the application of glufosinate provided greater control of these plants. For plants that were <5 and 5 to 10 cm high, the best control was achieved with sequential applications, glufosinate alone, and triple mixtures containing glufosinate. Treatment of taller plants with a 14- to 21-d interval between applications showed the greatest control (≥98%; Table 6).

At Location 2 (accession 521-R), treatments were equally effective on plants <5 cm high, whereas a single application of glyphosate + synthetic auxin mixture was less effective on plants that were 5 to 10 cm tall. In sequential application, glyphosate + 2,4-D followed by glyphosate + saflufenacil or glufosinate at an interval of 21 d were not among the most effective treatments. This loss of effectiveness occurred due to greater plant recovery, but it was not observed for the 7- and 14-d intervals, demonstrating that the interval should be shortened in some cases. For plants >10 cm

Table 7. Control of Sumatran fleabane resistant to glyphosate, chlorimuron, paraquat and 2,4-D, at 28 d after application in plants <5 cm, 5–10 cm, and >10 cm [a–d]

First application	Sequential application	Days between applications	Sumatran fleabane control		
			<5 cm	5–10 cm	>10 cm
				%	
Control (without application)			0 bA	0 fA	0 gA
Gly + 2,4-D			85 aA	49 eB	33 fC
Gly + dicamba			91 aA	63 dB	55 dB
Gly + triclopyr			94 aA	73 cB	47 eC
Gly + 2,4-D + saflufenacil			100 aA	98 aA	78 bB
Gly + dicamba + saflufenacil			100 aA	100 aA	87 bB
Gly + triclopyr + saflufenacil			100 aA	99 aA	89 bB
Gly + 2,4-D + glufosinate			99 aA	95 aA	59 dB
Gly + dicamba + glufosinate			97 aA	88 bB	71 cC
Gly + triclopyr + glufosinate			97 aA	90 bA	69 cB
Gly + saflufenacil			100 aA	97 aA	70 cB
Glufosinate			99 aA	94 aA	58 dB
Gly + 2,4-D	Gly + saflufenacil	7	100 aA	100 aA	98 aA
Gly + dicamba	Gly + saflufenacil	7	100 aA	100 aA	99 aA
Gly + triclopyr	Gly + saflufenacil	7	100 aA	100 aA	99 aA
Gly + 2,4-D	Glufosinate	7	100 aA	99 aA	97 aA
Gly + dicamba	Glufosinate	7	100 aA	100 aA	99 aA
Gly + triclopyr	Glufosinate	7	100 aA	100 aA	98 aA
Gly + 2,4-D	Gly + saflufenacil	14	100 aA	96 aA	70 cB
Gly + dicamba	Gly + saflufenacil	14	100 aA	100 aA	88 bB
Gly + triclopyr	Gly + saflufenacil	14	100 aA	100 aA	100 aA
Gly + 2,4-D	Glufosinate	14	100 aA	99 aA	84 bB
Gly + dicamba	Glufosinate	14	100 aA	99 aA	92 bA
Gly + triclopyr	Glufosinate	14	100 aA	100 aA	99 aA
Gly + 2,4-D	Gly + saflufenacil	21	100 aA	88 bB	58 dC
Gly + dicamba	Gly + saflufenacil	21	100 aA	98 aA	88 bB
Gly + triclopyr	Gly + saflufenacil	21	100 aA	100 aA	95 aA
Gly + 2,4-D	Glufosinate	21	95 aA	82 bB	60 dC
Gly + dicamba	Glufosinate	21	100 aA	97 aA	83 bB
Gly + triclopyr	Glufosinate	21	100 aA	100 aA	92 bB

[a]Data are from Location 2 (accession 521-R).
[b]Abbreviation: Gly glyphosate.
[c]Doses: glyphosate (1,242 g ae ha^{-1}), 2,4-D (804 g ae ha^{-1}), dicamba (288 g ae ha^{-1}), triclopyr (576 g ae ha^{-1}), saflufenacil (35 g ai ha^{-1}), glufosinate (500 g ai ha^{-1}).
[d]Means followed by different lowercase letters (herbicide treatments) differ according to the Scott-Knott test at 5%. Means followed by different uppercase letters (plant height) differ according to Tukey's test at the 5% level.

in height, sequential applications with a 7-d interval consistently achieved the greatest control (Table 7).

At Location 3 (accession 522-R) a single application of glyphosate + synthetic auxin to plants <5 cm tall was statically or numerically the less effective option. Sequential applications to intermediate-sized plants typically performed better than single applications, especially two-way mixtures, a sequential application interval of 7 d was consistently the most effective treatment for plants that were >10 cm tall. Treatment of taller fleabane plants at an interval of 7 d achieved the most effective control (Table 8).

Interaction between herbicides and plant height demonstrated that sequential applications were superior to those involving a single application, especially for plants that were 5 to 10 cm and >10 cm in height. Thus, control declines as the height of fleabane plants increases, as has been reported in other studies (Crose et al. 2020; Mellendorf et al. 2013). With respect to the sequential application interval, although behavior differed between areas, intervals of 7 and 14 d achieved better control.

At all locations, a triple mixture was applied to Sumatran fleabane plants that were <5 cm in height in a single application as opposed to sequential applications, just as glufosinate alone in a single application was effective. For intermediate-sized plants, all treatments with a sequential interval of 7 d achieved greater control, while a 7-d sequential application interval for plants >10 cm in height demonstrated greater control effectiveness, including glyphosate + dicamba or triclopyr, followed by glyphosate + saflufenacil and glyphosate + 2,4-D, or triclopyr followed by glufosinate. In addition, glyphosate + triclopyr with a sequential application of glufosinate 14 d later was also among the most effective options at all locations.

Despite resistance to 2,4-D, double mixtures of this herbicide were among the most effective treatments in plants <5 cm in height. Probably, this is because of the resistance mechanism of rapid necrosis. This symptom was observed at experiment sites and accession collection points with 2,4-D resistance. Rapid necrosis results in cell death due to increased hydrogen peroxide production with subsequent recovery of resistant plants (Queiroz et al. 2020), but may not occur the recovery in smaller plants, even for resistant accessions (Angonese et al. 2023). However, this hypothesis needs to be further corroborated by other studies. Dicamba and triclopyr can be substituted for 2,4-D to control Sumatran fleabane, with symptoms of rapid necrosis identified for these two herbicides. Synthetic auxins are important because their systemic effect and mode of action weaken the entire plant (Grossmann 2010). Other studies also reported satisfactory control results with these herbicides (McCauley and Young 2019; Queiroz et al. 2020).

Paraquat resistance in Sumatran fleabane, identified here and in other studies (Zobiole et al. 2019), and the banning of this herbicide in Brazil, mean that alternative herbicides are needed for soybean preplant chemical burndown (Albrecht et al. 2022b). In the present study, glufosinate and glyphosate + saflufenacil were used, which have a synergistic effect on fleabane control (Dalazen et al. 2015;

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Table 8. Control of Sumatran fleabane resistant to glyphosate, chlorimuron, paraquat, and 2,4-D, at 28 d after application in plants <5 cm, 5–10 cm, and >10 cm[a–d]

			Sumatran fleabane control		
1st application	Sequential application	Days between applications	<5 cm	5–10 cm	>10 cm
			%		
Control (without application)			0 cA	0 fA	0 gA
Gly + 2,4-D			69 bA	52 Be	44 fB
Gly + dicamba			89 aA	68 dB	54 eC
Gly + triclopyr			73 bA	55 Be	52 eB
Gly + 2,4-D + saflufenacil			99 aA	82 cB	42 fC
Gly + dicamba + saflufenacil			99 aA	86 bB	56 eC
Gly + triclopyr + saflufenacil			99 aA	98 aA	94 aA
Gly + 2,4-D + glufosinate			96 aA	91 bA	58 eB
Gly + dicamba + glufosinate			99 aA	91 bA	83 bB
Gly + triclopyr + glufosinate			99 aA	96 aA	85 bB
Gly + saflufenacil			89 aA	71 dB	45 fC
Glufosinate			100 aA	94 bA	53 eB
Gly + 2,4-D	Gly + saflufenacil	7	100 aA	96 aAB	91 aB
Gly + dicamba	Gly + saflufenacil	7	100 aA	100 aA	99 aA
Gly + triclopyr	Gly + saflufenacil	7	100 aA	100 aA	97 aA
Gly + 2,4-D	Glufosinate	7	100 aA	100 aA	88 aB
Gly + dicamba	Glufosinate	7	100 aA	100 aA	96 aA
Gly + triclopyr	Glufosinate	7	100 aA	100 aA	93 aA
Gly + 2,4-D	Gly + saflufenacil	14	100 aA	98 aA	64 dB
Gly + dicamba	Gly + saflufenacil	14	99 aA	95 aA	86 bB
Gly + triclopyr	Gly + saflufenacil	14	100 aA	99 aA	86 bB
Gly + 2,4-D	Glufosinate	14	100 aA	94 bA	58 eB
Gly + dicamba	Glufosinate	14	95 aA	90 bA	67 dB
Gly + triclopyr	Glufosinate	14	100 aA	100 aA	98 aA
Gly + 2,4-D	Gly + saflufenacil	21	98 aA	89 bA	43 fB
Gly + dicamba	Gly + saflufenacil	21	100 aA	98 aA	85 bB
Gly + triclopyr	Gly + saflufenacil	21	99 aA	88 bB	71 cC
Gly + 2,4-D	Glufosinate	21	97 aA	93 bA	49 fB
Gly + dicamba	Glufosinate	21	99 aA	95 aA	79 cB
Gly + triclopyr	Glufosinate	21	100 aA	94 bA	77 cB

[a]Data are from Location 3 (accession 522-R).
[b]Abbreviation: Gly, glyphosate.
[c]Doses: glyphosate (1,242 g ae ha^{-1}), 2,4-D (804 g ae ha^{-1}), dicamba (288 g ae ha^{-1}), triclopyr (576 g ae ha^{-1}), saflufenacil (35 g ai ha^{-1}), glufosinate (500 g ai ha^{-1}).
[d]Means followed by different lowercase letters (herbicide treatments) differ according to the Scott-Knott test at 5%. Means followed by different uppercase letters (plant height) differ according to Tukey's test at 5%.

Piasecki et al. 2020), with the results indicating an effectiveness similar to that of paraquat. The control effectiveness obtained here corroborates the findings of other studies that tested these herbicides, reinforcing their use in fleabane control (Albrecht et al. 2022d; Cantu et al. 2021; Dilliott et al. 2022; Piasecki et al. 2020).

In cases of herbicide resistance, one of the ways to manage these plants is through herbicide rotation, using other mechanisms of action or the same mechanism when there is no cross-resistance. Other forms of management include crop rotation and mechanical management (Grint et al. 2022; Sharma et al. 2021). Fleabane plants produce positive photoblastic seeds; that is, they do not germinate in the absence of light (Nandula et al. 2006; Wu et al. 2007). As such, rotation with cover crops that leave sufficient and/or uniform soil cover helps reduce fleabane emergence. Maize straw, *Urochloa*, ryegrass, vetch, turnip, wheat, and black oat have also been found to mitigate fleabane emergence (Lamego et al. 2013). Following this same principle, soil turning can also be used in more severe cases (Beckie and Harker 2017).

Practical Implications

Fleabane plants with either single or multiple resistance to glyphosate, chlorimuron, paraquat, or 2,4-D were found in the states of Paraná and Mato Grosso do Sul. Seven Sumatran fleabane accessions with four-way resistance to glyphosate, chlorimuron, paraquat, and 2,4-D were identified in western Paraná. It is believed that this region is the focal point for the dissemination and selection of accessions resistant to these herbicides. In order to control Sumatran fleabane with four-way resistance, smaller plants should be the priority. Despite resistance to 2,4-D, double mixtures containing this herbicide were among the most effective treatments of plants <5 cm in height. Sequential application is needed for plants taller than 5 cm, and glyphosate + synthetic auxin followed by glufosinate or glyphosate + saflufenacil is recommended. This type of research is essential to developing integrated fleabane management strategies. Both mapping and ongoing research are important in confirming herbicide resistance and advancing strategies to control target weeds.

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