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Short title: Multiple-resistant fleabane

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

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Nomenclature: 2,4-D; chlorimuron; glyphosate; glufosinate; paraquat; saflufenacil; fleabane, *Erigeron* spp.; Sumatran fleabane, *Erigeron sumatrensis* Retz.

Abstract

Monitoring herbicide-resistant weeds makes it possible to study the evolution and spread of resistance, providing important information for 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 pre-plant 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-resistant Sumatran fleabane were identified. Herbicides were used in single or sequential (seq.) 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 4 replicates), segregated (<80% control for 1 to 3 replicates), or susceptible (>80% control for all 4 replicates). There was no evidence of resistance to glufosinate or saflufenacil in any of the 461 accessions, while 65 showed possible resistance or segregation only for glyphosate, 235 for glyphosate + chlorimuron, 79 to glyphosate + chlorimuron + paraquat, 59 to glyphosate + chlorimuron + 2,4-D, and 23 with 4-way resistance (glyphosate, chlorimuron, paraquat and 2,4-D). Of these 23 accessions, 7 were analyzed using dose-response curves (F2 generation), all from PR, confirming 4-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. Sequential application is needed for plants >5 cm in height, it was recommended glyphosate + synthetic auxin followed by glufosinate or glyphosate + saflufenacil.

Keywords: Herbicide, sequential application, weed, rapid necrosis, monitoring

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Introduction

The genus *Erigeron* (sin.: *Conyza*) belongs to the family Asteraceae and contains 150 species worldwide (Flann 2016). Hairy fleabane (*Erigeron bonariensis* L.), Sumatran fleabane, and horseweed (*Erigeron 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 having the potential to produce around 110,000 seeds for hairy fleabane or up to 200,000 seeds for horseweed (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 that facilitates wind dispersal (Liu et al. 2022), allowing them to travel great distances (Shields et al. 2006). However, Dauer et al. (2007) found 99% of seeds were found within a 100m radius, with smaller amounts reaching up to 500 m.

Worldwide, there are 108 cases of herbicide resistance for the three *Conyza* species combined. 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, whereas hairy fleabane is also observed, especially in the southern region (Marochio et al. 2017; Ruiz et al. 2022). There are cases of Sumatran fleabane with resistance to paraquat (Zobiole et al. 2019), 2,4-D (Queiroz et al. 2020), glyphosate, chlorimuron, and saflufenacil (Heap 2023). Also, multiple resistance to chlorimuron and glyphosate (Santos et al. 2014a), chlorimuron, glyphosate, and paraquat (Albrecht et al. 2020a), 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 non-chemical 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 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.

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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 the agricultural system (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 Parana (PR) and Mato Grosso do Sul (MS), Brazil and their response to soybean pre-plant 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 pre-sowing, 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 PR, 53 from MS) 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, 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 12 h photoperiod. Seeds collected from each accession were sown in 0.8 L plastic pots filled with potting mix (Humusfértil®, Toledo, PR), and once plants had 1 to 2 true leaves, they were transplanted into pots (0.8 L) at two plants per pot, showing no signs of transplant shock.

At the 6-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

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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, the highest dose 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 AIXR110015 nozzles (TeeJet Technologies, Wheaton, 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 days after application (DAA) on a visual score scale from 0 to 100%, where 0% indicates no control and 100% plant death (Velini et al. 1995). Control scores at 28 DAA were used to classify the accessions as putative resistant (<80% control for all 4 replicates), segregated (<80% control for 1 to 3 replicates), or susceptible (>80% control for all 4 replicates), based on an adaptation of the classifications proposed by Lopez-Ovejero et al. (2017) and Mendes et al. (2021).

Dose-response curve

Plants were identified as Sumatran fleabane, being 23 accessions 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, 7 accessions were selected for the dose-response curve (F2 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: SILV4-R (24º18’58”S 53º30’25”W), TN1-R (24º17’29”S 53º30’10”W), TN3-R (24º19’31”S 53º31’15”W) and 514-R (24º17’09”S 53º30’42”W), and 3 from Palotina - PR: 480-R (24º21’53”S 53º52’49”W), 521-R (24º12’13”S 53º47’35”W) and 522-R (24º21’19”S 53º53’08”W) were tested. The susceptible accession was collected in Palotina (24°16'28.9"S 53°40'12.6"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, 1/8x, 1/4x, 1/2x, 1x, 2x, 4x, and 8x the dose used in the initial screening.

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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 non-linear logistic regression model (Streibig 1988):

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

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

The non-linear logistic model provides an estimate of the GR_{50} parameter (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):

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

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

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

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 the AIC-values. The SigmaPlot® 15 (Systat Software Inc.) 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).

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Chemical control at three stages height of Sumatran fleabane with 4-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 pre-plant chemical burndown management strategies. They were carried out were conducted between August and October 2020 in three locations at Palotina - PR, Brazil, which contained accessions identified as Sumatran fleabane with 4-way resistance to glyphosate, chlorimuron, paraquat, and 2,4-D, according to initially screening and the dose-response curve (Table 1). Climate in the region is classified as mesothermal subtropical humid - Cfa (*C*: mild temperate; *f*: fully humid; *a*: hot summer). 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 pre-emergence. In post-emergence, the application of glyphosate alone or in mixtures with ALS-inhibiting herbicides may be used. In maize at succession, it is common to use atrazine in mixtures with glyphosate.

The experiment was conducted as a randomized complete block design and double factorial arrangement (30 x 3), with 4 x 6 m plots and 4 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 at three heights, 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 m^{-2}, 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 the identification of heights in subsequent control evaluations. A CO_2-pressurized backpack sprayer equipped with 4 AIXR110.015 nozzles (TeeJet Technologies, Wheaton, IL), spaced 0.5 m apart, at a constant pressure of 196 kPa and flow rate of 1 m s^{-1} was used, 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% 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 analysis of variance (ANOVA) using the F-test (p <

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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 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 PR and MS

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 were segregating for resistance to glyphosate +/- one or more of the herbicides tested (Figure 2).

Of the accessions analyzed, 65 showed putative resistance or segregation only for glyphosate, 235 for glyphosate + chlorimuron, 79 for glyphosate + chlorimuron + paraquat, 59 for glyphosate + chlorimuron + 2,4-D, and 23 exhibiting 4-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 only segregation, 4 segregated for glyphosate, 25 for chlorimuron, 17 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 PR and MS, 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 MS, those identified as putative resistant or segregated were from Caarapó, Amambai, Dourados, and Maracaju (center and south of MS), regions with large-scale grain cultivation operations. In PR, most accessions at risk of resistance or segregation for other herbicides were concentrated in the western region of the state (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 - PR, 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,

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Maripá, and Assis Chateaubriand - PR. 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 pappi (Liu et al. 2022), also be dispersed over short distances by agricultural machinery. In severe infestations, fleabane seeds can travel up to 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 - PR or Assis Chateaubriand - PR could reach Dourados - MS 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 done 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 4-way resistance to glyphosate, chlorimuron, paraquat and 2,4-D

After initial screening, the 7 accessions from PR (F2 generation) of Sumatran fleabane were analyzed using dose-response curves. The 4-way resistance to glyphosate (EPSPs inhibitors - group G), chlorimuron (ALS inhibitors - group B), paraquat (PSI electron diversion - group D) , and 2,4-D (synthetic auxins - group O) 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 523 to 786 g ai ha^{-1} for paraquat, with an RF between 10 and 14, while for 2,4-D, GR_{50} values were 718 to 1,932 g ai ha^{-1} and RF value 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 (RS) 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 these studies, the possible resistance mechanisms involved were not elucidated.

Chlorimuron-resistant accessions obtained GR_{50} values from 42 to 122 g ai ha^{-1}, with an RF of up to 122. In Sumatran fleabane, 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 twelve 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 Sumatran fleabane accessions in the states of PR and São Paulo (SP), 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. 2019). 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 RF 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 4-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 4-way resistance to glyphosate, chlorimuron, paraquat, and 2,4-D. In location 1 (480-R accession), for plants <5 cm in height, a single application of the triple mixtures or sequential (seq.) application of double mixtures regardless of the interval, achieved greater control. Additionally, the application of glufosinate only provided greater control of these plants. For <5 and 5 to 10 cm plant height, the best control was achieved with seq. applications, glufosinate alone, and triple mixtures containing glufosinate. Regarding taller plants, treatments with a 14- to 21-day interval between applications showed the greatest control ($\geq$98%) (Table 6).

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In location 2 (521-R accession), treatments were equally effective on plants <5 cm high, whereas a single application of glyphosate + synthetic auxin mixtures was less effective in 5-10 cm plants. In seq. application, only glyphosate + 2,4-D followed by glyphosate + saflufenacil or glufosinate at an interval of 21 days were not among the most effective treatments. This loss of effectiveness occurred due to greater plant recovery but was not observed for the 7 and 14-day intervals, demonstrating that the interval should be shortened in some cases. In plants >10 cm in height, seq. application with a 7-day interval consistently achieved the greatest control (Table 7).

In location 3 (522-R accession), for plants <5 cm, a single application of glyphosate + synthetic auxin was statically or numerically the less effective option. In intermediate-sized plants, seq. applications typically performed better than single applications, especially 2-way mixtures, while in plants >10 cm in height, a seq. application interval of 7 days was consistently the most effective treatment. In taller fleabane plants, treatments with an interval of 7 days achieved the most effective control (Table 8).

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

Across locations, Sumatran fleabane plants <5 cm in height, triple mixtures can be applied in a single as opposed to seq. applications, just as glufosinate alone in a single application was effective. For intermediate-sized plants, all treatments with a seq. interval of 7 days achieved greater control, while for plants >10 cm in height, with a 7-day seq. application interval 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 seq. application of glufosinate 14 days later was also among the most effective options across the 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

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(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. 2019).

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 pre-plant 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; Piasecki et al. 2020), with the results indicating similar effectiveness. 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 strategies (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 PR and MS. Seven Sumatran fleabane accessions with 4-way resistance to glyphosate, chlorimuron, paraquat, and 2,4-D were identified in western PR. 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 4-way resistance, 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. Sequential application is needed for plants >5 cm in height, it was recommended

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glyphosate + synthetic auxin followed by glufosinate or glyphosate + saflufenacil. 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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Conflicts of Interest

No conflicts of interest have been declared.

References

1. Agostinetto D, Silva DRO, Vargas L (2017) Soybean yield loss and economic thresholds due to glyphosate resistant hairy fleabane interference. Arq Inst Biol 84:e0022017
2. Albrecht AJP, Albrecht LP, Silva AFM (2022b). Agronomic implications of paraquat ban in Brazil. Adv Weed Sci 40:e020220040
3. Albrecht AJP, Pereira VGC, Souza CNZ, Zobiole LHS, Albrecht LP, Adegas FS (2020a) Multiple resistance of *Conyza sumatrensis* to three mechanisms of action of herbicides. Acta Scientiarum. Agronomy 42:e42485
4. Albrecht AJP, Thomazini G, Albrecht LP, Pires A, Lorenzetti JB, Danilussi MTY, Silva AFM, Adegas FS (2020b) *Conyza sumatrensis* resistant to paraquat, glyphosate and chlorimuron: confirmation and monitoring the first case of multiple resistance in Paraguay. Agriculture 10:582
5. Albrecht LP, Albrecht AJP, Silva AFM, Ramos RA, Costa KYR, Araújo GV, Mundt TT, Colombari C (2022c). Sequential application of herbicide options for controlling *Conyza sumatrensis* in soybean pre-sowing. Rev Fac Cienc Agrar 54:83-93
6. Albrecht LP, Albrecht AJP, Silva AFM, Silva LM, Neuberger DC, Zanfrilli G, Antunes VMS (2022d) Sumatran fleabane (*Conyza sumatrensis* [Retz.] E. Walker) control in soybean with combinations of burndown and preemergence herbicides applied in the off-season. Arq Inst Biol 89:e00052022

7. Albrecht LP, Heimerdinger N, Albrecht AJP, Silva AFM, Piccin ES, Silva LM, Larini WF (2022a). Chemical control of fleabane resistant to 2, 4-D. Outlook Pest Manag 33:239-243
8. Angonese PS, Queiroz ARS, Angonese LS, Machado FM, Napier R, Markus C, ... Merotto A (2023) Rapid necrosis: Implications of environmental conditions and plant growth stage on 2, 4-D resistance and effect of other auxinic herbicides in Sumatran fleabane (*Conyza sumatrensis*). Weed Technol 37:174-184
9. Baccin LC, Albrecht AJP, Albrecht LP, Silva AFM, Victoria Filho R (2022) Mechanisms of multiple resistance to herbicides in *Conyza* sp. complex. J Plant Protect Res 62:113-121
10. Bajwa AA, Sadia S, Ali HH, Jabran K, Peerzada AM, Chauhan BS (2016) Biology and management of two important *Conyza* weeds: a global review. Environ Sci Pollut Res 23:24694-24710
11. Banzatto DA, Kronka SN (2013) Experimentação agrícola. Jaboticabal, SP, Brazil, Funep. 237 p
12. Beckie HJ, Harker KN (2017) Our top 10 herbicide-resistant weed management practices. Pest Manag Sci 73:1045-1052
13. Bhowmik PC, Bekech MM (1993) Horseweed (*Conyza canadensis*) seed production, emergence, and distribution in no-tillage and conventional-tillage corn (*Zea mays*). Agronomy (Trend Agric Sci) 1:67-71
14. Burgos NR (2015) Whole-plant and seed bioassays for resistance confirmation. Weed Sci 63:152-165
15. Burgos NR, Tranel PJ, Streibig JC, Davis VM, Shaner D, Norsworthy JK, Ritz C (2013) Confirmation of resistance to herbicides and evaluation of resistance levels. Weed Sci 61:4-20
16. Cantu RM, Albrecht LP, Albrecht AJ, Silva AFM, Danilussi MT, Lorenzetti JB (2021) Herbicide alternative for *Conyza sumatrensis* control in pre-planting in no-till soybeans. Adv Weed Sci 39:e2021000025
17. Crose JA, Manuchehri MR, Baughman TA (2020) Horseweed (*Conyza canadensis*) management in Oklahoma winter wheat. Weed Technol 34:229-234
18. Dalazen G, Kruse ND, Machado SL, Balbinot A (2015) Synergism of the glyphosate and saflufenacil combination for controlling hairy fleabane. Pesqui Agropecu Trop 45:249-256

19. Dauer JT, Mortensen DA, Vangessel MJ (2007) Temporal and spatial dynamics of long-distance *Conyza canadensis* seed dispersal. J Appl Ecol 44:105-114
20. Dilliott M, Soltani N, Hooker DC, Robinson DE, Sikkema PH (2022) Strategies to improve the control of glyphosate-resistant horseweed (*Erigeron canadensis*) with glufosinate applied preplant to soybean. Weed Technol 36:289-294
21. Ferreira DF (2011) Sisvar: a computer statistical analysis system. Cienc Agrotecnol 35:1039-1042
22. Flann C (2016) GCC: global compositae checklist (version 5 (Beta)), Jun 2014. *in* Roskov Y et al., eds. Species 2000 and ITIS catalogue of life. Leiden: Naturalis
23. Gazola T, Dias MF, Carbonari CA, Velini ED (2019) Monitoring of resistance of sourgrass to glyphosate herbicide in urban areas of the State of São Paulo, Brazil. Planta Daninha 37:e019207210
24. Gazziero DLP, Christoffoleti PJ, Vargas L, Kruse ND, Galli AJB, Trezzi MM (2009) Critérios para relatos oficiais estatísticos de biótipos de plantas daninhas resistentes a herbicidas. Pages 91-101 *In* Agostinetto D, Vargas L, eds. Resistência de plantas daninhas a herbicidas no Brasil. Passo Fundo, RS, Brazil: Berthier
25. Grint KR, Arneson NJ, Arriaga F, DeWerff R, Oliveira M, Smith DH, Stoltenberg DE, Werle R (2022) Cover crops and preemergence herbicides: An integrated approach for weed management in corn-soybean systems in the US Midwest. Front Agron 4:80
26. Grossmann K (2010) Auxin herbicides: current status of mechanism and mode of action. Pest Manag Sci 66:113-120
27. Hall LM, Stromme KM, Horsman GP, Devine MD (1998) Resistance to acetolactate synthase inhibitors and quinclorac in a biotype of false cleavers (*Galium spurium*). Weed Sci 46:390-396
28. Hanson BD, Shrestha A, Shaner DL (2009) Distribution of glyphosate-resistant horseweed (*Conyza canadensis*) and relationship to cropping systems in the Central Valley of California. Weed Sci 57:48-53
29. Heap I (2023) The international survey of herbicide resistant weeds. www.weedscience.com. Accessed: March 4, 2023
30. Lamego FP, Kaspary TE, Ruchel Q, Gallon M, Basso CJ, Santi AL (2013) Management of glyphosate resistant *Conyza bonariensis*: winter cover crops and herbicides in soybean pre-seeding. Planta Daninha 31:433-442

31. Lamego FP, Vidal RA (2008) Resistance to glyphosate in *Conyza bonariensis* and *Conyza canadensis* biotypes in Rio Grande do Sul, Brazil. Planta Daninha 26:467-471
32. Liu J, Zhao Q, Huang H, Ye R, Stewart CN, Wang J (2022) Dynamic seed emission, dispersion, and deposition from horseweed (*Conyza canadensis* (L.) Cronquist). Plants 11:1102
33. Lopez-Ovejero RF, Takano HK, Nicolai M, Ferreira A, Melo MS, Cavenaghi AL, ... Oliveira Junior RS (2017) Frequency and dispersal of glyphosate-resistant sourgrass (*Digitaria insularis*) populations across Brazilian agricultural production areas. Weed Sci 65:285-294
34. Marochio CA, Bevilaqua MRR, Takano HK, Mangolim CA, Oliveira Junior RS, Machado MFPS (2017) Genetic admixture in species of *Conyza* (Asteraceae) as revealed by microsatellite markers. Acta Sci Agron 39:437-445
35. McCauley CL, Young BG (2019) Differential response of horseweed (*Conyza canadensis*) to halauxifen-methyl, 2, 4-D, and dicamba. Weed Technol 33:673-679
36. Mellendorf TG, Young JM, Matthews JL, Young BG (2013) Influence of plant height and glyphosate on saflufenacil efficacy on glyphosate-resistant horseweed (*Conyza canadensis*). Weed Technol 27:463-467
37. Mendes RR, Takano HK, Gonçalves Netto A, Picoli Junior GJ, Cavenaghi AL, Silva VF, Nicolai M, Christoffoleti PJ, Oliveira Junior RS, Melo MSC, Lopez-Ovejero RF (2021) Monitoring glyphosate-and chlorimuron-resistant *Conyza* spp. populations in Brazil. An Acad Bras Cienc 93:e20190425
38. Moretti ML, Van Horn CR, Robertson R, Segobye K, Weller SC, Young BG, ... Schulz B (2018) Glyphosate resistance in *Ambrosia trifida*: Part 2. Rapid response physiology and non-target-site resistance. Pest Manag Sci 74:1079-1088
39. Nandula VK, Eubank TW, Poston DH, Koger CH, Reddy KN (2006) Factors affecting germination of horseweed (*Conyza canadensis*). Weed Sci 54:898-902
40. Pereira VGC (2019) Caracterização da resistência de *Conyza sumatrensis* ao herbicida paraquat. MSc. Dissertation. Botucatu, SP, Brazil: Unesp, Faculdade de Ciências Agronômicas. 75 p
41. Piasecki C, Carvalho IR, Avila LA, Agostinetto D, Vargas L (2020) Glyphosate and saflufenacil: Elucidating their combined action on the control of glyphosate-resistant *Conyza bonariensis*. Agriculture 10:236
42. Pinho CF, Leal JFL, Souza AS, Oliveira GFPB, Oliveira C, Langaro AC, Machado AFL, Christoffoleti PJ, Zobiole LHS (2019) First evidence of multiple resistance of

Sumatran fleabane (*Conyza sumatrensis* (Retz.) E. Walker) to five-mode-of-action herbicides. Aust J Crop Sci 13:1688-1697

43. Queiroz AR, Delatorre CA, Lucio FR, Rossi CVS, Zobiole LHS, Merotto Júnior A (2020) Rapid necrosis: a novel plant resistance mechanism to 2,4-D. Weed Sci 68:6-18
44. Quinn J, Soltani N, Ashigh J, Hooker DC, Robinson DE, Sikkema PH (2020) Halauxifen-methyl controls glyphosate-resistant horseweed (*Conyza canadensis*) but not giant ragweed (*Ambrosia trifida*) in winter wheat. Weed Technol 34:607-612
45. Rodrigues BN, Almeida FS (2018) Guia de Herbicidas. 7th edn. Londrina, PR, Brazil: Ed. Authors. 764 p
46. Ruiz MR, Mangolin CA, Oliveira Junior RS, Mendes RR, Takano HK, Eisele TG, Fátima PSM (2022) Mechanisms that may lead to high genetic divergence and to the invasive success of tall fleabane (*Conyza sumatrensis*; Asteraceae). Weed Sci 70:64-78
47. Santos G, Oliveira Junior RS, Constantin J, Francischini AC, Machado MF, Mangolin C. A, Nakajima JN (2014b) *Conyza sumatrensis*: A new weed species resistant to glyphosate in the Americas. Weed Biol Manag 14:106-114
48. Santos G, Oliveira Junior RS, Constantin J, Francischini AC, Osipe JB (2014a) Multiple resistance of *Conyza sumatrensis* to chlorimuron-ethyl and to glyphosate. Planta Daninha 32:409-16
49. Schultz JL, Chatham LA, Riggins CW, Tranel PJ, Bradley KW (2015) Distribution of herbicide resistances and molecular mechanisms conferring resistance in Missouri waterhemp (*Amaranthus rudis* Sauer) populations. Weed Sci 63:336-345
50. Seefeldt SS, Jensen JE, Fuerst EP (1995) Log-logistic analysis of herbicide dose-response relationships. Weed Technol 9:218-227
51. Sharma G, Shrestha S, Kunwar S, Tseng TM (2021) Crop diversification for improved weed management: A review. Agriculture 11:461
52. Shields EJ, Dauer JT, VanGessel MJ, Neumann G (2006) Horseweed (*Conyza canadensis*) seed collected in the planetary boundary layer. Weed Sci 54:1063-1067.
53. Streibig JC (1988) Herbicide bioassay. Weed Res 28:479-484
54. Takano HK, Beffa R, Preston C, Westra P, Dayan FE (2019) Reactive oxygen species trigger the fast action of glufosinate. Planta 249:1837-1849
55. Takano HK, Oliveira Junior RS, Constantin J, Braz GBP, Gheno EA (2017) Goosegrass resistant to glyphosate in Brazil. Planta Daninha 35:e017163071

https://doi.org/10.1017/wet.2024.10 Published online by Cambridge University Press

56. Trezzi MM, Vidal RA, Patel F, Miotto Júnior E, Debastiani F, Balbinot Júnior AA, Mosquen R (2015) Impact of *Conyza bonariensis* density and establishment period on soyabean grain yield, yield components and economic threshold. Weed Res 55:34-41
57. Trezzi MM, Vidal RA, Xavier E, Rosin D, Balbinot JR, Prates MA (2011) Resistance to glyphosate in *Conyza* spp. biotypes in Western and Southwestern Parana, Brazil. Planta Daninha 29:1113-1120
58. Vargas L, Bianchi MA, Rizzardi MA, Agostinetto D, Dal Magro T (2007) *Conyza bonariensis* biotypes resistant to the glyphosate in southern Brazil. Planta Daninha 25:573-578
59. Velini ED, Osipe R, Gazziero DLP (1995) Procedimentos para instalação, avaliação e análise de experimentos com herbicidas. Londrina, PR, Brazil: SBCPD. 42 p
60. Wu C, Song M, Zhang T, Zhou C, Liu W, Jin T, Zhao N (2022) Target-site mutation and cytochrome P450s confer resistance to multiple herbicides in Italian ryegrass (*Lolium multiflorum* Lam.) from China. Crop Prot 161:106068
61. Wu H, Walker S, Rollin MJ, Tan DKY, Robinson G, Werth J (2007) Germination, persistence, and emergence of flaxleaf fleabane (*Conyza bonariensis* [L.] Cronquist). Weed Biol Manag 7:192-199
62. Yang Q, Yang X, Zhu J, Wei T, Lv M, Li Y (2022) Metabolic resistance to acetyl-CoA carboxylase-inhibiting herbicide cyhalofop-butyl in a Chinese *Echinochloa crus-galli* population. Agronomy 12:2724
63. Zheng D, Kruger GR, Singh S, Davis VM, Tranel PJ, Weller SC, Johnson WG (2011) Cross-resistance of horseweed (*Conyza canadensis*) populations with three different ALS mutations. Pest Manag Sci 67:1486-1492.
64. Zobiole LHS, Pereira VGC, Albrecht AJP, Rubin RS, Adegas FS, Albrecht LP (2019) Paraquat resistance of Sumatran fleabane (*Conyza sumatrensis*). Planta Daninha 37:e019183264

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

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	24º21’53”S	53º52’49”W	480-R	1,452	14	44	44	655	12	718	8
2	24º12’13”S	53º47’35”W	521-R	909	9	52	52	523	10	1,423	15
3	24º21’19”S	53º53’08”W	522-R	2,387	23	42	42	674	11	1,197	16

GR_{50} (dose required to reduce dry mass by 50%), RF (resistance factor).

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Table 2. Herbicide treatments to control Sumatran fleabane.

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

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Gly + triclopyr[b]	1,242 + 576	Glufosinate[a]	500	7
Gly + 2,4-D	1,242 + 804	Gly + saflufenacil[a]	1,242 + 35	14
Gly + dicamba[a]	1,242 + 288	Gly + saflufenacil[a]	1,242 + 35	14
Gly + triclopyr[b]	1,242 + 576	Gly + saflufenacil[a]	1,242 + 35	14
Gly + 2,4-D	1,242 + 804	Glufosinate[a]	500	14
Gly + dicamba[a]	1,242 + 288	Glufosinate[a]	500	14
Gly + triclopyr[b]	1,242 + 576	Glufosinate[a]	500	14
Gly + 2,4-D	1,242 + 804	Gly + saflufenacil[a]	1,242 + 35	21
Gly + dicamba[a]	1,242 + 288	Gly + saflufenacil[a]	1,242 + 35	21
Gly + triclopyr[b]	1,242 + 576	Gly + saflufenacil[a]	1,242 + 35	21
Gly + 2,4-D	1,242 + 804	Glufosinate[a]	500	21
Gly + dicamba[a]	1,242 + 288	Glufosinate[a]	500	21
Gly + triclopyr[b]	1,242 + 576	Glufosinate[a]	500	21

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

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

Individual	Location 1			Location 2			Location 3		
Source	MS	F	P	MS	F	P	MS	F	P
Herbicide (H)	4,499.2	330.9	0.00	4,651.8	132.4	0.00	4,641.3	59.5	0.00
Plant height (PH)	5,463.5	401.8	0.00	10,467.8	297.9	0.00	18,001.3	230.8	0.00
H x 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 (L)	66.4	0.00
Herbicide (H)	265.5	0.00
Plant height (PH)	642.7	0.00
L x H	7.6	0.00
L x PH	18.6	0.00
H x PH	12.3	0.00
L x H x PH	3.5	0.00
Block	3.5	0.00
Mean	86.6	
CV (%)	8.1	

MS (mean square).

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Table 4. Fleabane accessions with possible herbicide resistance or segregation in PR and MS.

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%)

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}).

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Table 5. GR_{50} and RF values of Sumatran fleabane accessions (F2 generation) with multiple resistance to glyphosate, chlorimuron, paraquat, and 2,4-D.

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}	
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	-

GR_{50} (dose required to reduce dry mass by 50%), RF (resistance factor).

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Table 6. Control of Sumatran fleabane resistant to glyphosate, chlorimuron, paraquat, and 2,4-D, at 28 days after application in plants <5 cm, 5 to 10 cm, and >10 cm. Location 1 (480-R accession).

1st application[a]	Sequential application[a]	Days between applications	Sumatran fleabane control		
			<5 cm	5 to 10 cm	>10 cm
			---------------- % ----------------		
Control (without application)	-	-	0 dA	0 dA	0 eA
Glyphosate (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

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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] 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}). * Significant ($p < 0.5$), 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%.

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Table 7. Control of Sumatran fleabane resistant to glyphosate, chlorimuron, paraquat and 2,4-D, at 28 days after application in plants <5 cm, 5 to 10 cm, and >10 cm. Location 2 (521-R accession).

1st application[a]	Sequential application[a]	Days between applications	Sumatran fleabane control		
			<5 cm	5 to 10 cm	>10 cm
			----------------- % -----------------		
Control (without application)	-	-	0 bA	0 fA	0 gA
Glyphosate (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

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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] 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}). * Significant (p < 0.5), 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.

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Table 8. Control of Sumatran fleabane resistant to glyphosate, chlorimuron, paraquat, and 2,4-D, at 28 days after application in plants <5 cm, 5 to 10 cm, and >10 cm. Location 3 (522-R accession).

1st application[a]	Sequential application[a]	Days between applications	Sumatran fleabane control					
			<5 cm		5 to 10 cm		>10 cm	
			---------------- % ----------------					
Control (without application)	-	-	0	cA	0	fA	0	gA
Glyphosate (gly) + 2,4-D	-	-	69	bA	52	eB	44	fB
Gly + dicamba	-	-	89	aA	68	dB	54	eC
Gly + triclopyr	-	-	73	bA	55	eB	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

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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] 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}). * Significant ($p < 0.5$), 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%.

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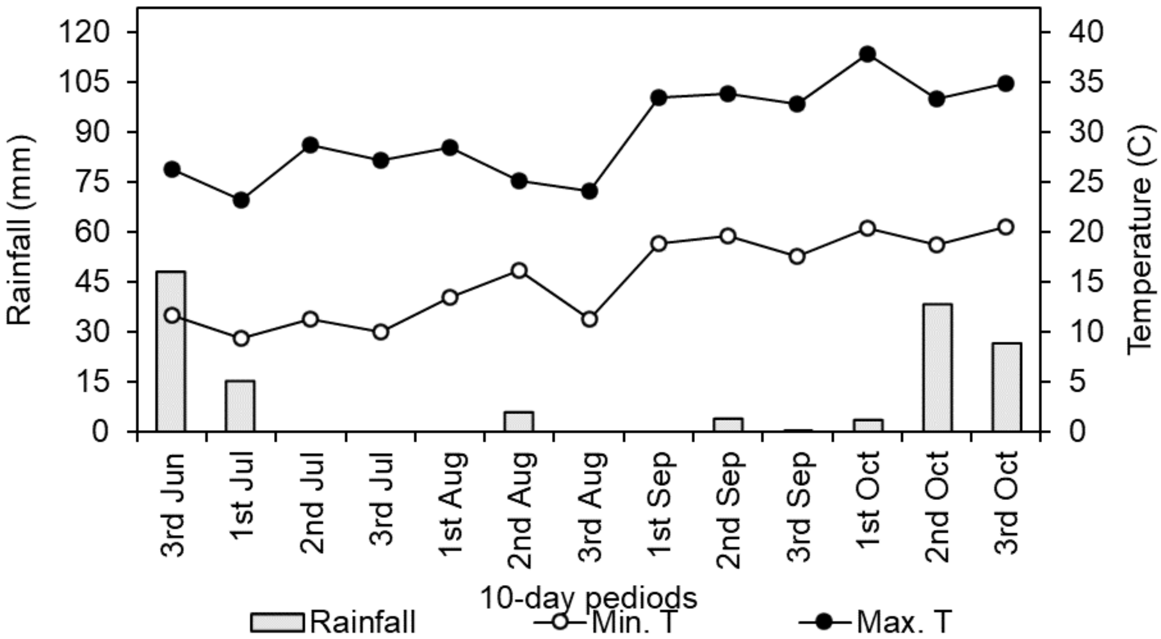

Figure 1. Rainfall and minimum and maximum temperatures during the experimental period. Source: weather station in Palotina - PR, Brazil (24°10'44.5"S 53°50'16.4"W).

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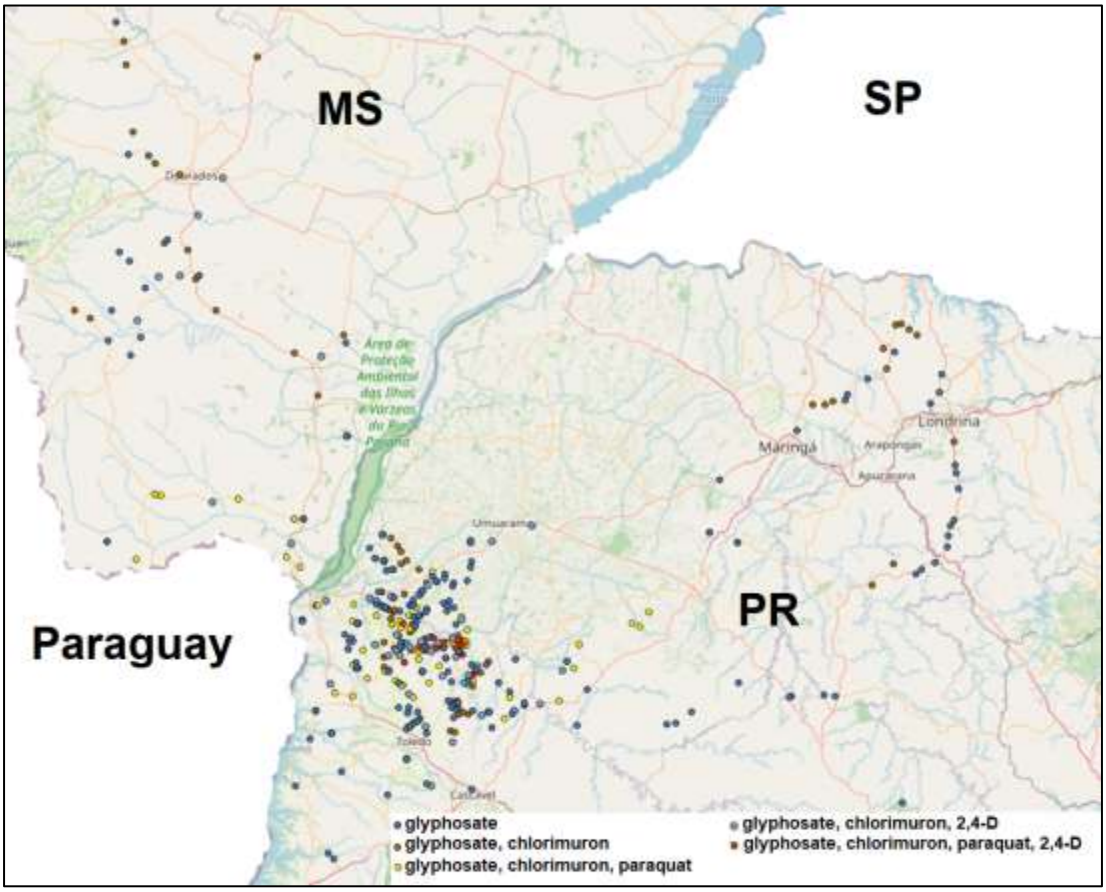

Figure 2. Location of the fleabane accessions with possible resistance or segregation for glyphosate, chlorimuron, paraquat, and 2,4-D in PR and MS.

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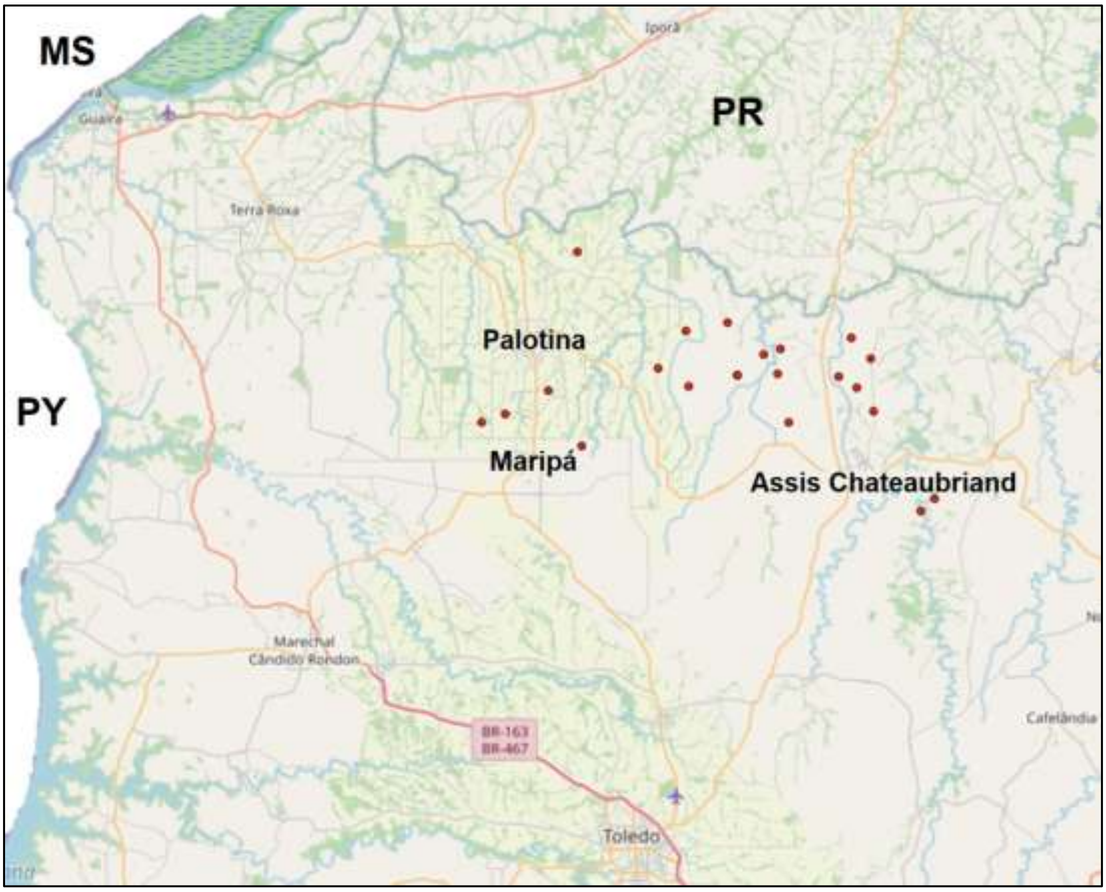

Figure 3. Location of Sumatran fleabane with possible multiple resistance or segregation for glyphosate, chlorimuron, paraquat, and 2,4-D in the region of Palotina and Assis Chateaubriand - PR.

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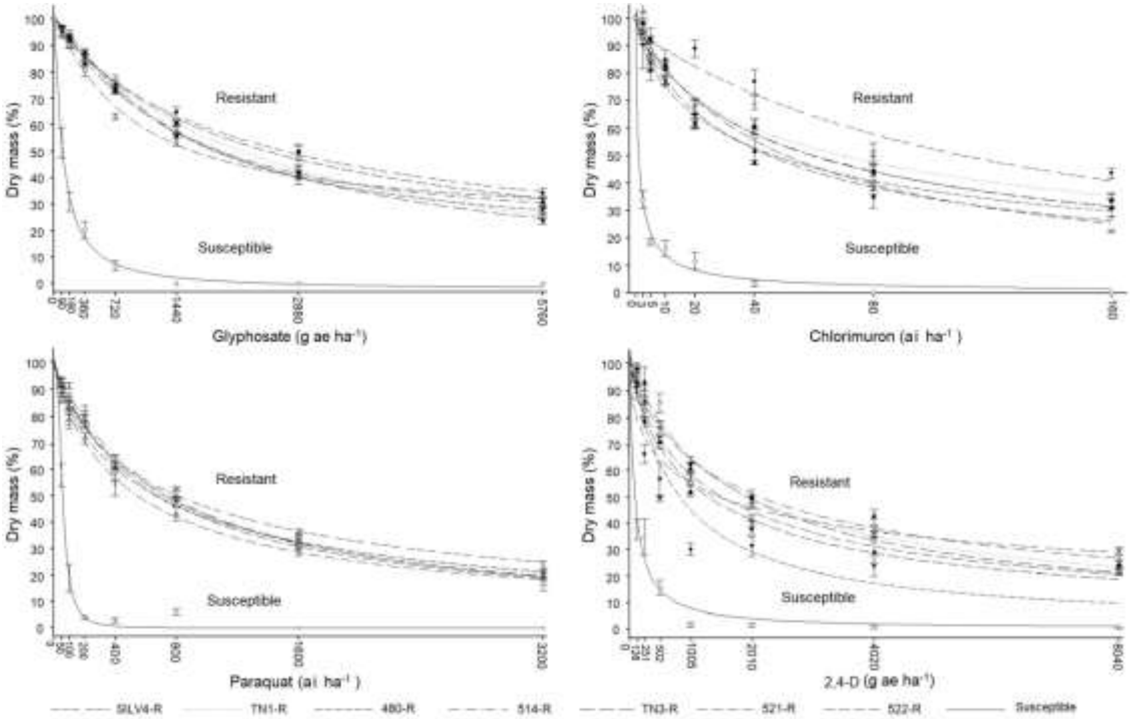

Figure 4. Dose-response curve for dry mass of Sumatran fleabane susceptible and resistant (SILV4-R, TN1-R, 480-R, 514-R, TN3-R, 521-R, 522-R) accessions under glyphosate, chlorimuron, paraquat, and 2,4-D application. Palotina and Assis Chateaubriand - PR.

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