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CROP PROTECTION

Multiple resistance of *Conyza sumatrensis* to three mechanisms of action of herbicides

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ABSTRACT. Fleabane (*Conyza* spp.) is an important weed in grain production systems and is currently one of the most problematic weeds in Brazil. An important factor related to weeds such as fleabane is the characteristic of herbicide-resistant biotypes developed under selection pressure, with multiple resistance previously detected for *Conyza* spp. Thus, the aim of this study was to demonstrate the multiple resistance of *Conyza sumatrensis* to the herbicides paraquat, glyphosate, and chlorimuron. From the F2 seeds of biotypes with suspected resistance to paraquat, glyphosate, and chlorimuron, dose-response greenhouse experiments were conducted for the three herbicides. Herbicides were applied when the plants had 6-8 leaves that were at a height of 8 cm. At the end of the evaluations, 28 days after application, multiple resistance to paraquat, glyphosate, and chlorimuron was observed, with resistance factors (RF_{50}) for the control of 7.43, 3.58, and 14.35 and for the reduction of dry mass of 2.65, 2.79, and 11.31, respectively. All the established criteria for demonstrating new cases of weed resistance were met; thus, the first case worldwide of a *Conyza* species with resistance to herbicides with three different mechanisms of action was confirmed.

Keywords: *Conyza* spp.; dose-response; paraquat; glyphosate; chlorimuron.

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Introduction

Plants of the genus *Conyza*, known as fleabane, originated in the Americas, according to most reports. *Conyza canadensis* originates in the United States and Canada and is most commonly found in temperate regions of the Northern Hemisphere but is also in subtropical climates in the Southern Hemisphere. *Conyza bonariensis* originates in the temperate region of South America and is in Argentina, Uruguay, Paraguay, Colombia, Venezuela and Brazil (Michael, 1977). The origin of *C. sumatrensis* is hypothesized to be in the subtropical region of South America, and therefore, the species is distributed to the warm regions of continents (Thebaud & Abbott, 1995); however, other authors identify Indonesia as the probable place of origin (Kostermans et al., 1987).

In recent years, fleabane infestation (*Conyza* spp.) has gradually increased in cultivated agricultural areas in Brazil, primarily in soybean crops, becoming one of the primary weeds that interfere with this crop (Albrecht et al., 2018). After the harvest of off-season corn, interval occurs before sowing soybeans, and in this period, between the planting of the crops, fleabane develops significantly (Krenchinski et al., 2019; Cesco et al., 2019). This fact is due to the high adaptability of this plant to the production systems and the evolution of biotypes resistant to herbicides, which cause increasing losses to crops (Lamego et al., 2013; Albrecht et al., 2017; Cesco et al., 2019).

The first cases of herbicide resistance in the genus *Conyza* spp. were recorded in Japan in 1980 for paraquat, a photosystem I inhibitor herbicide (Fuerst, Nakatani, Dodge, Penner, & Arntzen, 1985), in *C. canadensis* and in the same year in Taiwan, in *C. sumatrensis*. Since these first cases, *C. canadensis* has 65 cases of resistance to five mechanisms of action in 18 countries, and *C. bonariensis* has spread to 12 countries and has 20 cases of resistance to four mechanisms of action. By contrast, 20 cases of resistance to five mechanisms of action in 11 countries are reported for *C. sumatrensis* (Heap, 2020). Nevertheless, until 2017, no case was reported worldwide of multiple resistance simultaneously to herbicides with three different mechanisms of action.

In Brazil, the first case of resistant fleabane was recorded in 2005, in relation to EPSPs (5-enolpyruvylshikimate-3-phosphate synthase) inhibitors, for the species *C. bonariensis* and *C. canadensis*. In 2010, the same type of resistance was found in *C. sumatrensis*, and afterward in 2011, resistance to ALS (acetolactate synthase) inhibitors and multiple resistance to ALS and EPSPs inhibitors were reported (Santos et al., 2014; Heap, 2020). Additionally, *C. sumatrensis* resistance to paraquat (photosystem I inhibitor) was confirmed in 2016 and to Protox inhibitors in 2017. More recently, multiple resistance was reported for ALS inhibitors, EPSPs inhibitors and photosystem I inhibitors (Heap, 2020) as the first case reported worldwide of a species of the genus *Conyza* presenting resistance to three mechanisms of action, which is the focus of the present study.

Based on the confirmation that some *C. sumatrensis* biotypes show resistance to paraquat, the study investigated the possible multiple resistance to other herbicides, because the resistance of this species to glyphosate (EPSPs inhibitor) and to chlorimuron (ALS inhibitor) has been previously identified in Brazil. In this context, the goal of the present study was to demonstrate the existence of multiple resistance of *C. sumatrensis* to herbicides with three different mechanisms of action: photosystem I inhibitors (paraquat), EPSPs inhibitors (glyphosate), and ALS inhibitors (chlorimuron).

Material and methods

Seeds of *C. sumatrensis* from plants demonstrated as resistant to paraquat were grown to produce new seeds (F2 generation), with the objective of testing the resistance of these biotypes to other herbicides as follows.

In September 2016, an experiment was conducted in the field, with doses of paraquat (0, 800, and 1,600 g active ingredient – a.i. ha^{-1}) applied on plants with 4 to 8 leaves. In early January 2017, seeds characterized as the F1 generation were collected from plants that had been properly marked after receiving the highest dose of paraquat and were not significantly damaged. These plants were located in Assis Chateaubriand, Paraná State, Brazil (24°17'00.4" S, 53°30'55.7" W).

F1 seeds were then sown in 5 dm^3 pots, and when fleabane plants had 6-8 leaves (8 cm), they received applications of glyphosate at the dose of 1,200 g acid equivalent - a.e. ha^{-1} and chlorimuron at the dose of 30 g a.i. ha^{-1}. Herbicides were applied on March 3, 2017, using a CO_2 pressurized backpack sprayer equipped with four AIXR-110015 flat-fan nozzles (TeeJet Technologies, Wheaton, IL) under a pressure of 240 kPa and a speed of 1 m s^{-1}, delivering an application volume equivalent to 200 L ha^{-1}.

Approximately 120 days after sowing, plants that survived the application of glyphosate and chlorimuron without major injuries produced seeds, which were collected, characterizing the F2 generation. Then, using the seeds from each plant (generation F2), dose-response curve experiments were conducted between May and July 2017 at the Federal University of Paraná (UFPR), Palotina, Paraná State, Brazil.

Seeds from a biotype considered susceptible to glyphosate, chlorimuron and paraquat were collected also in the area located in Assis Chateaubriand, Paraná State, Brazil. Professor Jimi Naoki Nakajima at the Institute of Biology of the Federal University of Uberlândia, Minas Gerais State, Brazil, properly identified plants at the reproductive stage as *Conyza sumatrensis*.

Dose-response curve experiments

F2 generation seeds were sown in perforated plastic trays containing potting substrate covered with newspapers and soon after emergence were transplanted, always keeping one single well-developed plant per pot. The experimental units were pots of 1 dm^3 containing substrate placed in a greenhouse (Van der Hoeven, model Researches 161), irrigated by sprinklers twice a day, and day/night temperatures were set at 25 ± 5°C/16 ± 5°C under natural lighting.

The treatments were applied when the plants reached 8 cm in height with approximately 6-8 leaves. The herbicides tested were paraquat (200 g a.i. L^{-1}), glyphosate (480 g a.e. L^{-1}) and chlorimuron (250 g a.i. kg^{-1}). All herbicides were applied with a CO_2 pressurized backpack sprayer equipped with four AIXR-110015 flat-fan nozzles (TeeJet Technologies, Wheaton, IL) at a pressure of 240 kPa and a speed of 1 m s^{-1}, delivering an application volume equivalent to 200 L ha^{-1}.

The experiment was a completely randomized design, with four replications. The treatments were the following: paraquat at doses of 0, 50, 100, 200, 400, 800, 1,600, and 3,200 g a.i. ha^{-1}, associated with 0.1% (v/v) Agral® adjuvant (Agral®, Syngenta Crop Protection, Greensboro, NC); glyphosate at doses of 0, 90, 180, 360, 720, 1,440, 2,880, and 5,760 (g a.e. ha^{-1}); and chlorimuron at doses of 0, 2.5, 5, 10, 20, 40, 80, and 160 (g a.i. ha^{-1}) associated with 0.5% (v/v) emulsifiable mineral oil. The rates used represented 1/8, ¼, ½, 1, 2, 4, and 8X the normal field rate.

The visual control was evaluated 7, 14, 21, and 28 days after application (DAA) of the herbicides. Plants were visually evaluated (0 for no injuries, up to 100% for plant death) for significantly visible symptoms, according to their development (SBCPD, 1995).

Dry mass was evaluated 28 days after herbicide application. Plants were cut at the soil surface, placed in paper bags, oven-dried at 70°C for 4 days (to reach constant mass) and then weighed. The dose-response curves were performed with the biotype that was controlled the least, in relation to the susceptible biotypes, and that provided the best fit. The susceptible biotype was identified in a previous report (Heap, 2020; Zobiole et al., 2019).

Data were tested by analysis of variance and regression and when significant, were fitted to the logistic model of non-linear regression proposed by Streibig (1988):

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

where: *y* is the response variable (percentage control or dry mass of shoot); *x* is the dose of the herbicide (g ha^{-1}); and *a*, *b* and *c* are the estimated parameters of the equation, such that: *a* is the amplitude between the maximum point and the minimum point of the variable; *b* is the dose that provides 50% response; and *c* is the slope of the curve around *b*.

The nonlinear logistic model provides an estimate of the parameter C_{50} (*Control by* 50%) or GR_{50} (*Growth Reduction by* 50%). Therefore, the mathematical calculation through the inverse equation of Streibig (1988) was used to calculate the C_{50}, as proposed by Souza, Ferreira, Silva, Cardoso, and Ruiz (2000). The models used to obtain the C_{50} were the same as those used in other important recent works found in relevant literature (Takano et al., 2016; Takano, Oliveira Jr, Constantin, Braz, & Gheno, 2017).

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

Based on the values of C_{50} and GR_{50}, we calculated the resistance factor (RF = C_{50} or GR_{50} of the resistant biotype/C_{50} or GR_{50} of the susceptible biotype). The resistance factor expresses the number of times in which the dose required to control 50% of the resistant biotype is greater than the dose that controls 50% of the susceptible biotype (Burgos et al., 2013).

Results and discussion

Based on the results of the dose-response curves for the three herbicides studied for the suspected biotype, the resistance of *C. sumatrensis* to paraquat, reported in 2016 (Heap, 2020) for biotypes collected in the same microregion, was confirmed (Table 1), with a resistance factor (RF) of 7.43 for the control at 28 DAA (Figure 1) and of 2.65 for the reduction in shoot dry mass (Figure 2).

In Brazil, the resistance of Sumatran fleabane to paraquat is recent (Zobiole et al., 2019), but cases report the resistance of *C. sumatrensis* to paraquat since 1980 in Taiwan, and current reports are from several other countries, including Japan, Egypt, Malaysia, Canada, the United States, Belgium, Sri Lanka, South Africa, and Australia (Fuerst et al., 1985; Chiang & Chiang, 2016; Preston, 2018).

Table 1. Rate of paraquat required to control 50% of the biotype (28 DAA) or to reduce shoot dry mass by 50% and resistance factors (RF) for the biotype of *Conyza sumatrensis*. Palotina, Paraná State, Brazil, 2017.

Biotype	% Control		Shoot biomass reduction	
	C50	RF50	GR50	RF50
Susceptible	56.93	--	57.95	--
Resistant	422.82	7.43	153.72	2.65

Resistance to glyphosate (EPSPs inhibitor) (Table 2) was also observed, confirming the biotype resistance to paraquat and glyphosate. For the control at 28 DAA (Figure 3), the resistance factor for glyphosate was 3.58, and for the dry mass, the RF was 2.79 (Figure 4).

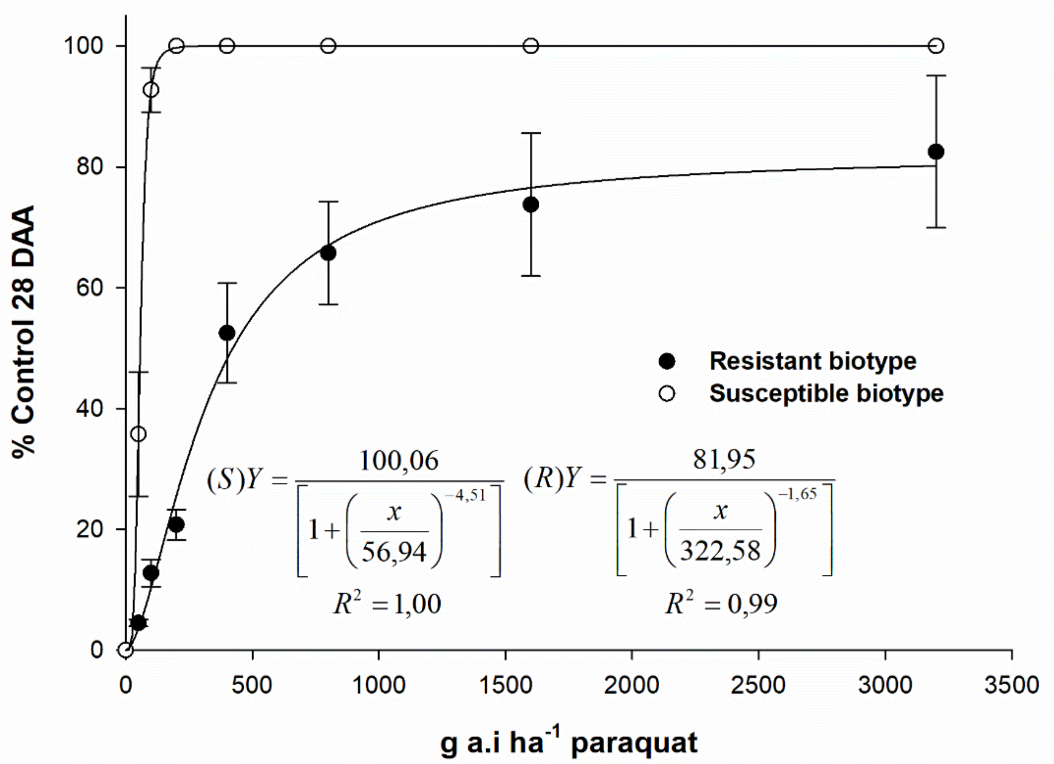

Figure 1. Percent control at 28 days after paraquat application. Palotina, Paraná State, Brazil, 2017.

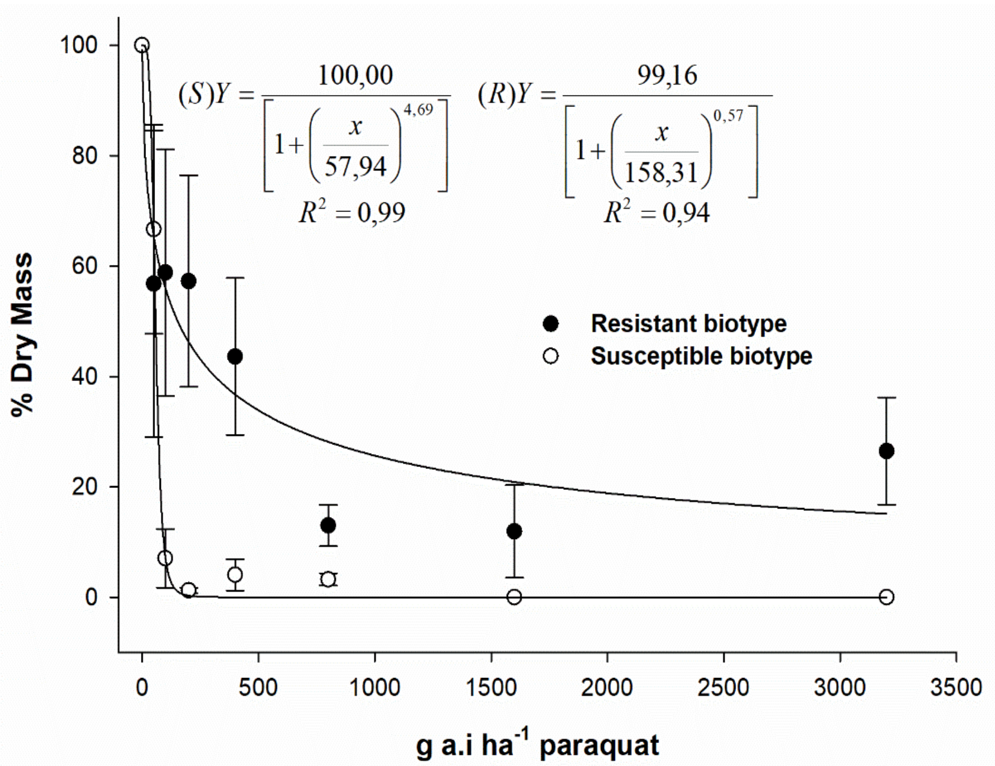

Figure 2. Dry mass at 28 days after paraquat application. Palotina, Paraná State, Brazil, 2017.

The resistance of different species of *Conyza* to glyphosate has been widely known for several years. Cases of resistance of *C. bonariensis* and *C. canadensis* to glyphosate were previously presented by Vargas, Bianchi, Rizzardi, Agostinetto, and Dal Magro (2007) and Lamego and Vidal (2008) in the state of Rio Grande do Sul, and Moreira, Nicolai, Carvalho, and Crhistoffoleti (2007) in the state of São Paulo. More recently, Santos, Oliveira Constantin, Francischini, and Osipe (2014) first identified *C. sumatrensis* and then reported resistance to glyphosate.

Table 2. Rate of glyphosate required to control 50% of the biotype (28 DAA) or to reduce shoot dry mass by 50% and resistance factors (RF) for the biotype of *Conyza sumatrensis*. Palotina, Paraná State, Brazil, 2017.

Biotype	% Control		Shoot biomass reduction	
	C50	RF50	GR50	RF50
Susceptible	72.06	--	90.65	--
Resistant	257.72	3.58	252.69	2.79

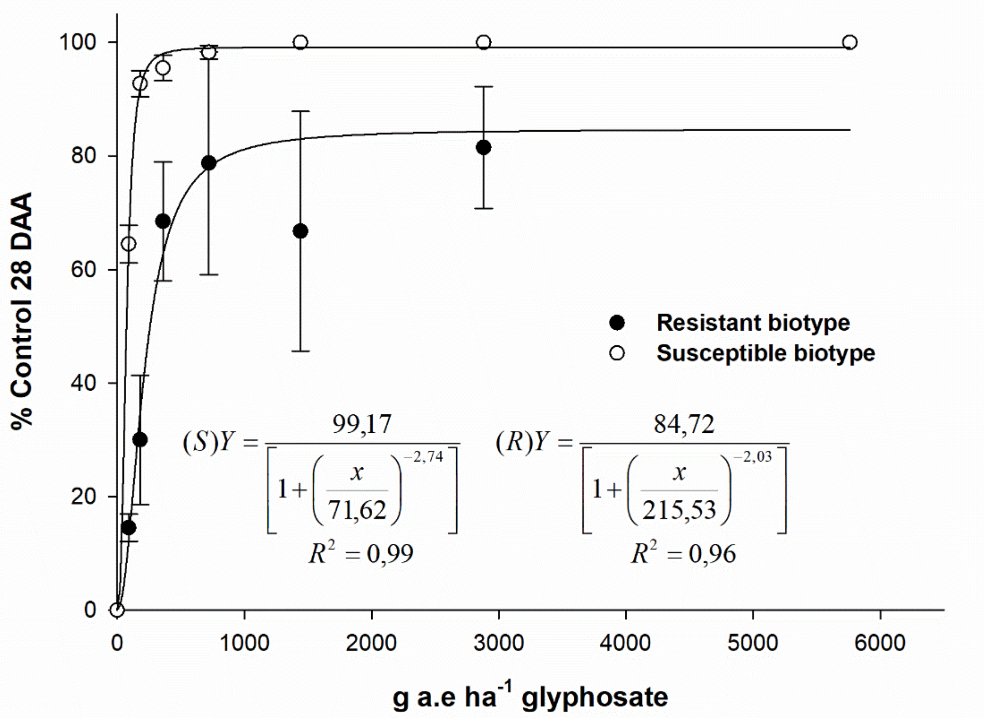

Figure 3. Percent control at 28 days after glyphosate application. Palotina, Paraná State, Brazil, 2017.

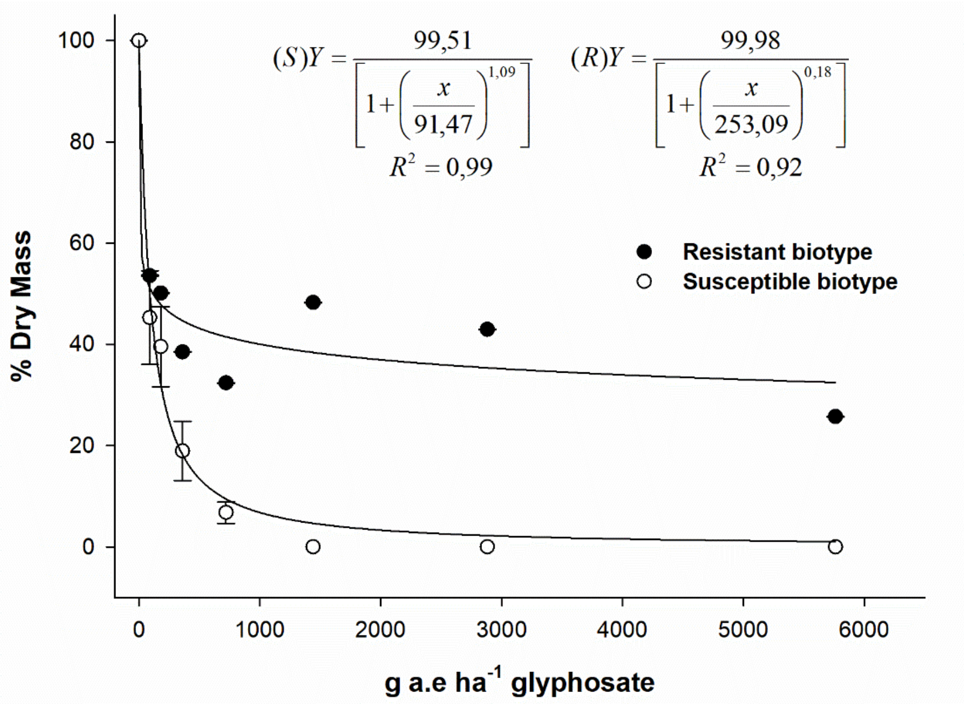

Figure 4. Dry mass at 28 days after glyphosate application. Palotina, Paraná State, Brazil, 2017.

Triple multiple resistance was confirmed when resistance to chlorimuron was found in the same biotype (Table 3). The resistance factor was 14.35 for % control at 28 DAA (Figure 5) and 11.31 for dry mass (Figure 6).

Such data for the resistance to glyphosate and chlorimuron herbicides are consistent with the results of Santos et al. (2014), and these authors also note that resistance factors found for chlorimuron are usually higher than those found for glyphosate due to the resistance conferred by the ALS inhibitor herbicides.

Table 3. Rate of chlorimuron required to control 50% of the biotype (28 DAA) or to reduce shoot dry mass by 50% and resistance factors (RF) for the biotype of *Conyza sumatrensis*. Palotina, Paraná State, Brazil, 2017.

Biotype	% Control		Shoot biomass reduction	
	C50	RF50	GR50	RF50
Susceptible	0.96	--	1.88	--
Resistant	13.80	14.35	21.23	11.31

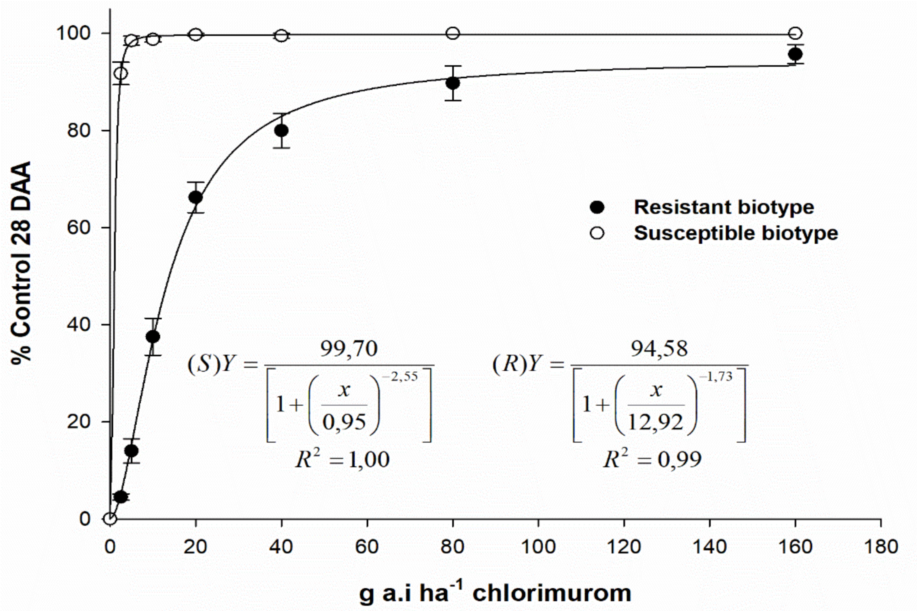

Figure 5. Percent control at 28 days after chlorimuron application. Palotina, Paraná State, Brazil, 2017.

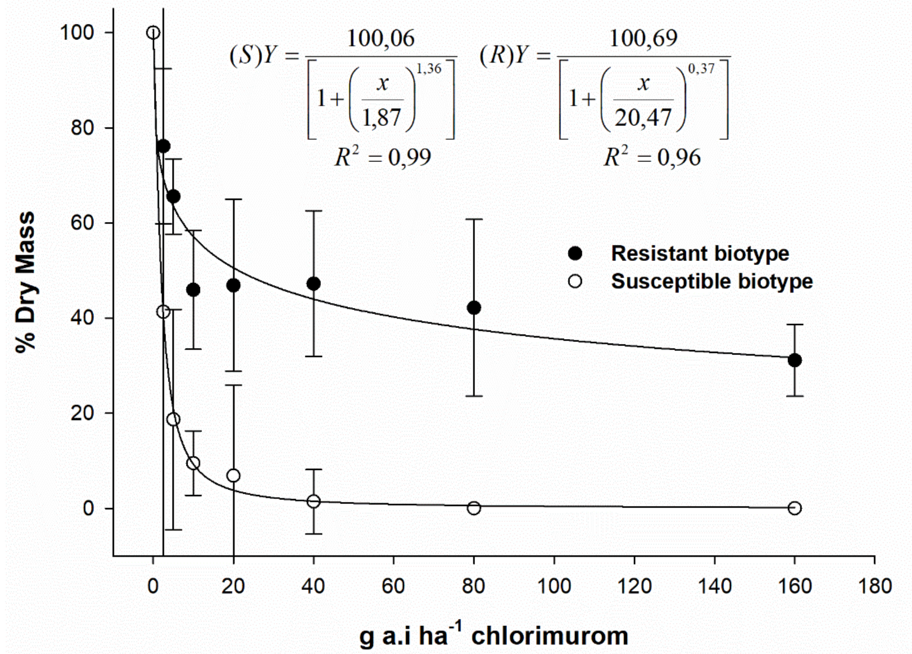

Figure 6. Dry mass at 28 days after chlorimuron application. Palotina, Paraná State, Brazil, 2017.

The suspected biotype presented RFs greater than 1 based on the dose-response curves (Figures 1 to 6). The results confirmed the initial suspicion of multiple resistant biotypes to the three mechanisms of action tested in the western region of the state of Paraná, following the criteria for official reports of herbicide-resistant weed biotypes according to a document prepared by Gazziero et al. (2009). This document includes suggestions from the Committee for Action on Herbicide Resistant Weeds, the Brazilian Weed Science Society (SBCPD) and the Brazilian Association of Action to Herbicide Resistance in Weeds (HRAC-BR). These suggestions were based on the article "Criteria for confirmation of herbicide resistant weeds" (Heap, 2005).

Following a reasoning similar to that of other recent works, in addition to conducting a procedure previously described in the methodology (Burgos et al., 2013), we also used the concept of resistance based on the dose required to provide at least 80% control of a suspected biotype, which combines the concepts of scientific and agronomic resistance. To summarize, for a biotype to be considered as resistant, the RF must be > 1.0 and the C_{80} > the recommended dose of the herbicide (Santos et al., 2014; Takano et al., 2016; Takano et al., 2017). Thus, for this study, the C_{80} values for paraquat, glyphosate and chlorimuron were 3,037.48 g a.i. ha^{-1}, 862.79 g a.e. ha^{-1}, and 34.43 g a.i. ha^{-1}, respectively.

After 2011, with the confirmation in Paraná State of biotypes of *C. sumatrensis* presenting multiple resistance to glyphosate and chlorimuron, other non-selective herbicides, such as paraquat, became more widely used in cropping systems, on burndown applications. Additionally, in the western region of Paraná State, the use of paraquat for pre-harvest soybean desiccation is extremely common, which further increases the selection pressure (Albrecht et al., 2017; Albrecht et al., 2018; Cesco et al., 2019).

Control of this weed species before soybean sowing usually requires sequential applications in burndown, combining glyphosate + 2,4-D followed by paraquat. However, the control provided by paraquat to fleabane has become less effective over time, most likely due to the selection of resistant biotypes caused by the intensive use of paraquat, which has been applied two to four times per growing season (Albrecht et al., 2017; Albrecht et al., 2018; Cesco et al., 2019).

Therefore, research must be conducted that evaluates the efficient control of this weed with alternative herbicides and mechanisms of action (Peterson, Covallo, Ovejero, Shivrain, & Walsh, 2018) available on the market, such as ammonium glufosinate, which is promising. Research conducted before the discovery of fleabane resistance to paraquat demonstrated satisfactory results for the use of ammonium glufosinate (Silva et al., 2014; Oliveira Neto et al., 2011; Moreira, Melo, Carvalho, Nicolai, & Christoffoleti, 2010). Also, to saflufenacil, which is a Protox inhibitor, and other new herbicides, positioned isolated and associated, in unique and sequential applications (Albrecht et al., 2018; Krenchinski et al., 2019; Cesco et al., 2019).

In this context, Beckie and Harker (2017) discuss the primary herbicide-resistant weed management practices. Only crop and herbicide diversity is not sufficient; rather, cultural practices are required to promote crop competition and biological control, proper use of pre- and post-emergent herbicides, and use of herbicides in combinations and to avoid heavy use of ALS inhibitors, combat weeds out of the crop where major damage is caused, avoid dissemination of these problematic plants, organize strategic soil preparation and maintain coverage, and maintain a database and history of an area. This approach would prevent serious problems caused by weed resistance, such as those discussed here (Peterson et al., 2018).

Although these results provided evidence of fleabane resistance to different herbicides with three mechanisms of action, information is lacking on the status and extent of this resistance and other cases that may exist. Thus, based on these results, activities to monitor this case of resistance are being conducted, in addition to characterization of the actual situation regarding the use of the primary herbicides widely used for fleabane control in production systems.

Conclusion

Our results confirmed the multiple resistance of fleabane to a photosystem I inhibitor herbicide (paraquat), EPSPs inhibitor (glyphosate), and ALS inhibitor (chlorimuron) because all established criteria for demonstrating new cases of weed resistance were met.

Studies are underway to map and monitor the resistance of fleabane to these herbicides, in addition to the search for new cases of resistance to other mechanisms of action and for efficient and sustainable alternatives to control this weed.

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