MULTIPLE RESISTANT OF SUMATRAN FLEABANE IN PARAGUAY

CONTRIBUTING WEED SCIENTISTS

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INTRODUCTION

After several years of study, in 2017, part of this team, confirmed scientifically a new case of multiple resistance of *Conyza sumatrensis* (Sumatran Fleabane) to herbicides glyphosate, chlorimuron and paraquat (http://www.weedscience.org/Details/Case. aspx? ResistID = 17102), in a study that was carried out in the western region of the State of Paraná, Brazil.

After that, resistance monitoring work carried out by this team, found plants with these characteristics in other regions of Brazil and Paraguay, a country that borders the State of Paraná.

As in Paraguay there was no scientific proof of any *Conyza* species, resistant to any herbicide, this team that already had research in Paraguay decided to increase its partnerships and expand its research structure in that country.

This study was done with the objective of monitoring and investigating the herbicide resistance of *Conyza Sumatrensis* and other weed species. The work was carried out jointly with researchers, technicians and farmers in Paraguay, a country bordering Brazil, presenting similar problems, but it presents a great lack of in-depth research about weed science.

MATERIAL AND METHODS

Seeds of *Conyza Sumatrensis* were collected during the crop season 2017/2018, then in the first half of 2018 screening was performed to select the biotypes that would then be used for dose response curves, as susceptible and as resistant. Plants of these biotypes were cultivated, and their seeds collected for the accomplishment of dose response curves, which were carried out in the second half of 2018, in a greenhouse, located in the Municipality of Katuete, Department of Canindeyu - Paraguay (24°09'27"S 54°52'10"W).

Simultaneously, between June and December 2018, field experiments were performed in the area that presented the biotype used as resistant in dose response curves, located in the Municipality of Corpus Christ, Department of Canindeyu - Paraguay (24°03'34.8"S 55°00'20.1"W). These experiments were for practical field verification of the problem of the resistance of *Conyza Sumatrensis* to the three herbicides studied and also to find efficient control alternatives in areas with this problem. Plants in reproductive stage were properly identified as *Conyza sumatrensis*.

Dose Response Experiment

The experimental units were pots containing 1.0 dm^{-3} filled with vermiculite, at greenhouse conditions. The F1 generation seeds were sown and after emergence were thinned, keeping one seedling per pot.

The treatments were applied when the plants reached 8 cm in height and 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 applications were made using a CO_2 pressurized backpack sprayer equipped with four flat-fan nozzles AIXR-110015 (TeeJet Technologies, Wheaton, IL) at a pressure of 240 kPa and a speed of 1 ms^{-1}, delivering an application volume equivalent to 200 L ha^{-1}.

The experiment was a completely randomized design, with four replications. The treatments were: paraquat at doses of 0, 50, 100, 200, 400, 800, 1600 and 3200 g a.i. ha^{-1}, associated with nonionic adhesive spreader at 0.1% (v/v); glyphosate at doses of 0, 90, 180, 360, 720, 1440, 2880 and 5760 (g a.e. ha^{-1}) and chlorimuron at 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 doses used represent the normal field doses at 1/8, ¼, ½, 1, 2, 4 and 8X doses.

The visual control was evaluated at 7, 14, 21 and 28 days after application (DAA) of the herbicides, through visual evaluations (0 for no injuries, up to 100% for plant death) in this case, symptoms significantly visible in plants, according to their development (SBCPD, 1995).

The dry mass evaluation was performed at 28 days after application of the herbicides. 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 the weighted.

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%). In this way, it was decided to use the mathematical calculation through the inverse equation of Streibig (1988), allowing to calculate the C_{50}, according to what was proposed by Souza et al. (2000). The models used to obtain C_{50} were the same as those used in other important recent works found in relevant literature in the area (Takano et al., 2016; Takano et al., 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

According to the proven, by a part of this team, in 2016, in Brazil, resistance to paraquat in Paraguay was also confirmed (Table 1), reaching a RF of 6.79 for control at 28 DAA (Figure 1) and for dry mass reduction a RF value of 3.92 was obtained for the same biotype (Figure 2).

Table 1. Doses of paraquat required to control 50% of the population (28 DAA), reduce dry mass of shoots by 50% and resistance factor (RF) for populations of *Conyza sumatrensis*. Municipality of Katuete, Departament of Canindeyu - Paraguay, 2018.

Population	C50	RF50	GR50	RF50
Susceptible	49.65	--	52.46	--
Resistant	337.19	6.79	205.94	3.92

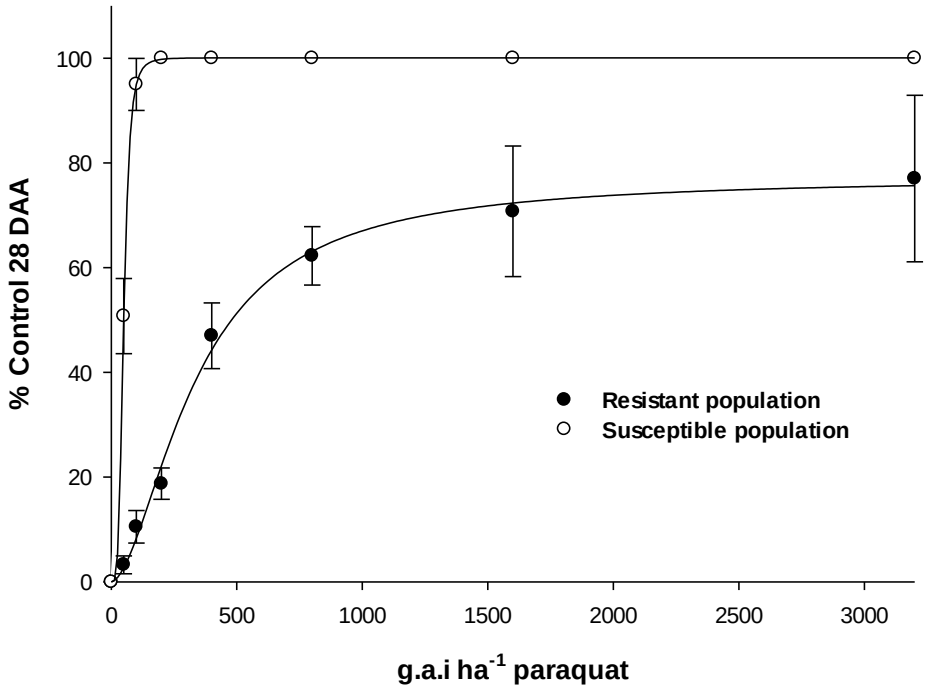


Figure 1. Percent control at 28 days after paraquat application. Municipality of Katuete, Departament of Canindeyu - Paraguay, 2018.

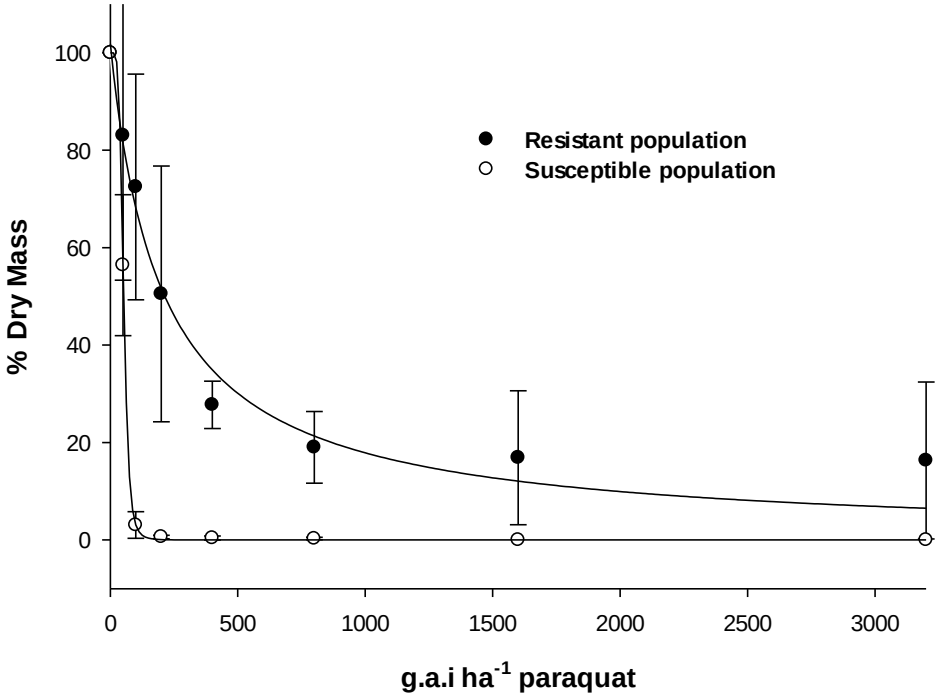
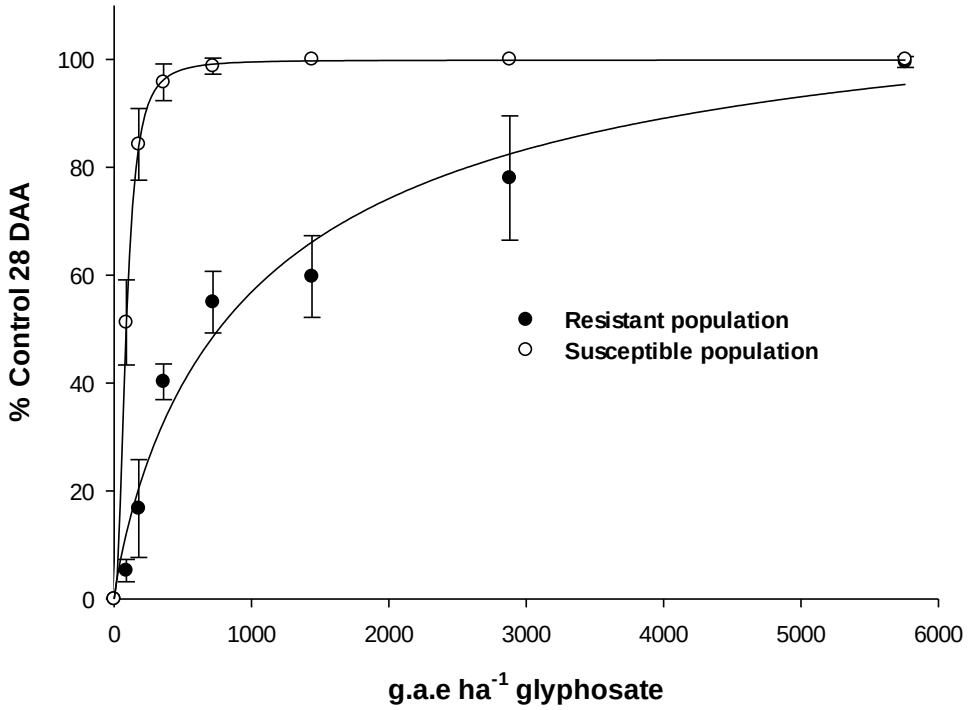


Figure 2. Dry mass at 28 days after paraquat application. Municipality of Katuete, Departament of Canindeyu - Paraguay, 2018.

Resistance to glyphosate (EPSPs inhibitor) was observed (Table 2), thus confirming resistance to paraquat and glyphosate in the same biotype. For the control at 28 DAA (Figure 3), glyphosate resistance factor was 12.32 and 4.15 to dry mass (Figure 4).

Table 2. Doses of glyphosate required to control 50% of the population (28 DAA), reduce dry mass in aerial part by 50% and resistance factor (RF) for populations of *Conyza sumatrensis*. Municipality of Katuete, Departament of Canindeyu - Paraguay, 2018.

Population	**C50**	**RF50**	**GR50**	**RF50**
Susceptible	87.85	--	126.10	--
Resistant	1082.36	12.32	523.35	4.15



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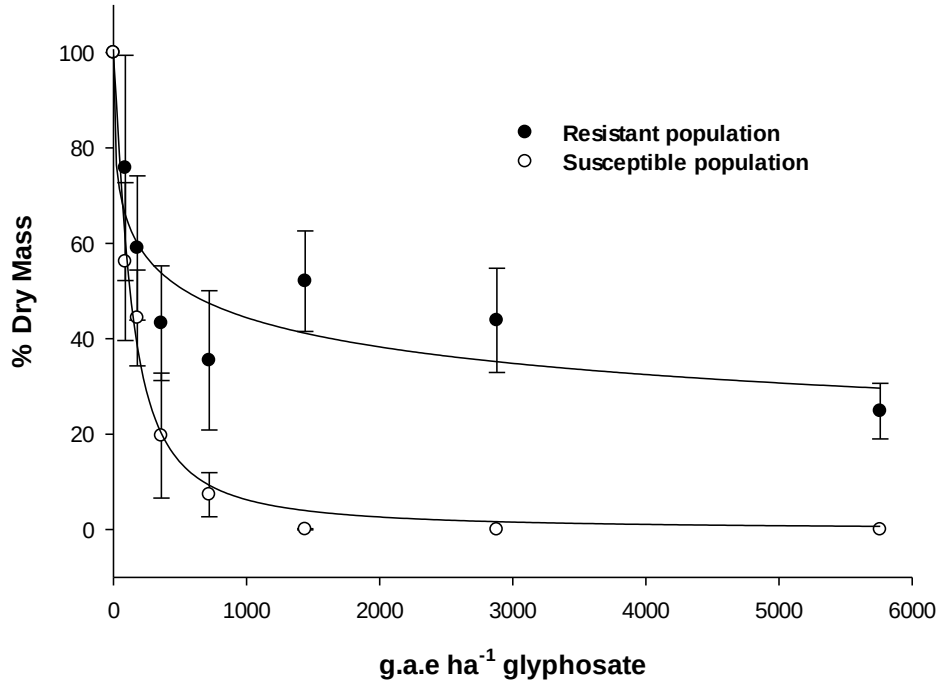
124 Figure 3. Percent control at 28 days after glyphosate application. Municipality of Katuete,
125 Departament of Canindeyu - Paraguay, 2018.



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127 Figure 4. Dry mass at 28 days after glyphosate application. Municipality of Katuete,
128 Departament of Canindeyu - Paraguay, 2018.

Triple resistance was confirmed when resistance to chlorimuron-ethyl herbicide was observed in the same biotype (Table 3). A resistance factor of 11.32 was found for control at 28 DAA (Figure 5) and 10.96 for dry matter mass (Figure 6).

Table 3 Doses of chlorimuron-ethyl required to control 50% of the population (28 DAA), reduce dry mass in aerial part by 50% and resistance factor (RF) for populations of *Conyza sumatrensis*. Municipality of Katuete, Departament of Canindeyu - Paraguay, 2018.

Population	**C50**	**RF50**	**GR50**	**RF50**
Susceptible	1.25	--	2.26	--
Resistant	14.16	11.32	24.78	10.96

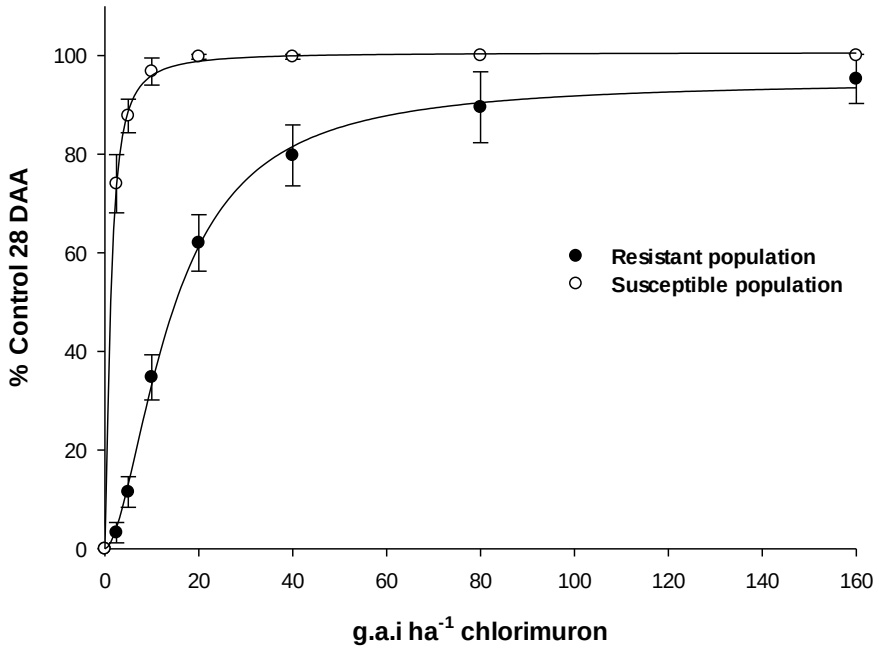

Figure 5. Percent control at 28 days after chlorimuron-ethyl application. Municipality of Katuete, Departament of Canindeyu - Paraguay, 2018.

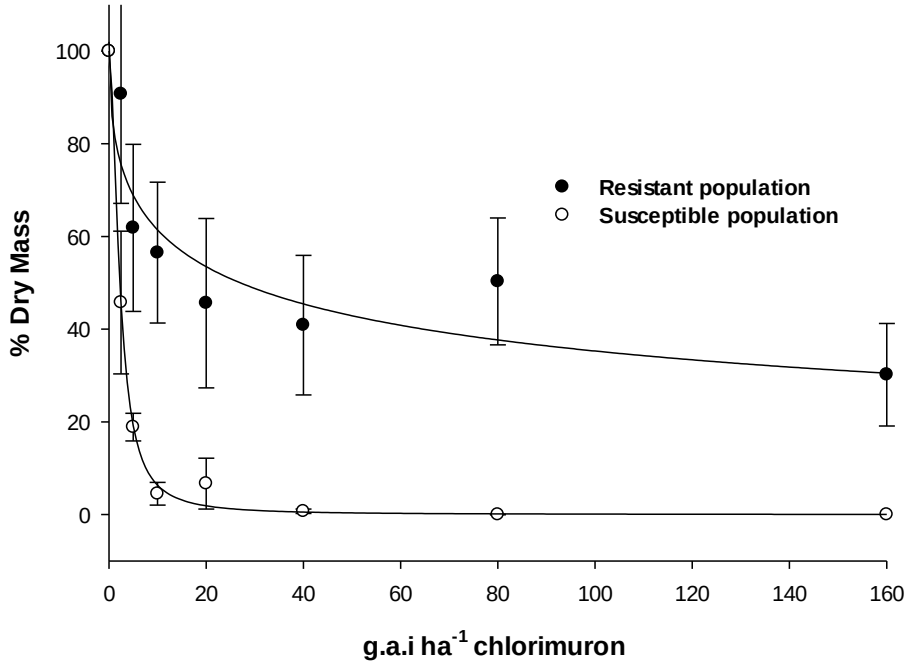

Figure 6. Dry mass at 28 days after chlorimuron-ethyl application. Municipality of Katuete, Departament of Canindeyu - Paraguay, 2018.

This population of *Conyza sumatrensis* meets all the criteria set to confirm a new case of multiple resistance to paraquat, glyphosate and chlorimuron-ethyl (Heap 2005). Criterion 1: the plants from these populations have survived and reproduced after their exposure to a herbicide dose that was lethal to the susceptible population; Criterion 2: the resistance factors were high and the recommended dose to the species did not provide satisfactory control; Criterion 3: the plants from the F1 generation of these populations were considered resistant; Criterion 4: control flaws complaints are being observed on the field; Criterion 5: random plants of these population were properly classified as *Conyza sumatrensis*.

The Brazilian Weed Science Society has been notified about this multiple resistance case and following the HRAC-Brazil. For even though it is a case found in Paraguay, this problem affects both countries and there is a large agricultural frontier region that is potentially affected by this problem.

At this time, actions are being taken to monitor the areas where resistant biotypes were collected, as well as other areas with suspected resistance. This work is being carried out in partnership with Semillas Pires and with the collaboration of farmers and

technicians from different institutions working in Paraguay. Focusing on alerting farmers about this problem and reducing its spread in Paraguay and Brazil, to avoid the loss of these important tools.

It is noteworthy that since the first week of 2019, these results were divulged and discussed with professionals and farmers from Paraguay and Brazil. This was done personally and also by different social media. In this sense, technical papers were prepared and disseminated by the authors of this study to inform and raise awareness of the people affected by this problem in Brazil and Paraguay.

This team is expanding its research structure in Paraguay and increasing its work with weed resistance in this country that presents a great lack of information in this area. Thus, soon this team will be finalizing further studies, with other weed species, and will be communicating new reports of resistance to herbicides.

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