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Article

Paraquat Resistance of Sumatran Fleabane (*Conyza sumatrensis*)

Buva (**Conyza sumatrensis**) *Resistente a Paraquat*

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ABSTRACT - In Brazil, some populations of *Conyza bonariensis* and *C. canadensis* are glyphosate resistant and there are populations of *C. sumatrensis* (Sumatran fleabane) presenting multiple resistance to both glyphosate and chlorimuron. During the 2014/2015 and 2015/2016 seasons, growers reported failures to control Sumatran fleabane with paraquat. This study investigated the potential of paraquat resistant Sumatran fleabane populations in Paraná state, Brazil. Populations with suspected paraquat resistance were tested in the field in 2016 season. In 2017, seeds from these populations were collected, sown and grown in a greenhouse. Paraquat dose-response curve experiments were performed in Mogi Mirim, SP; Londrina, PR and Palotina, PR using doses of 0, 50, 100, 200, 400, 800, 1,600 and 3,200 g ha^{-1}, following all standard criteria for confirmation of weed resistance cases. Percentage control was assessed at 3, 7, 14, 21 and 28 days after application and data were fitted to a nonlinear, log-logistic model, and dose response curves were generated. The results of this study confirmed significant levels of resistance of Sumatran fleabane biotypes to paraquat with resistance factors between 3,57 to 34,29. Therefore, the first case of *C. sumatrensis* resistance to paraquat was confirmed in biotypes from the western area of Paraná state, Brazil.

Keywords: control, dose-response, PSI inhibitor.

RESUMO - No Brasil, ***Conyza bonariensis*** *e* ***C. canadensis*** *apresentam biótipos resistentes a glyphosate e* ***C. sumatrensis*** *apresenta resistência múltipla a glyphosate e chlorimuron. Durante as safras de 2014/2015 e 2015/2016 foram relatadas falhas no controle de buva submetida à aplicação de paraquat. Este estudo teve por objetivo investigar a possibilidade da resistência ao paraquat em populações de* ***C. sumatrensis****. Populações com suspeita de resistência foram testadas em campo na entressafra de 2016, sendo depois coletadas sementes e plantas durante a safra de 2017, que foram posteriormente, estudadas em casa de vegetação. Experimentos com curva de dose-resposta foram instalados em Mogi-Mirim, SP, Londrina-PR e Palotina-PR, utilizando doses de 0, 50, 100, 200, 400, 800, 1.600 e 3.200 g ha^{-1}. Foi avaliada a porcentagem de controle aos 3, 7, 14, 21 e 28 dias após aplicação e utilizado um modelo não linear log-logístico para o ajuste da curva de dose-resposta. Com os resultados obtidos neste estudo, foi confirmada a hipótese inicial do primeiro caso de resistência da buva ao paraquat na região oeste do Estado do Paraná, com fator de resistência variando entre 3,57 e 34,29, seguindo todos os critérios para a confirmação da resistência.*

Palavras-chave: controle, dose-resposta, inibidor do fotossistema I.

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INTRODUCTION

Conyza sp. is an annual dicot weed that often emerges in the autumn and winter (Tozzi and van Acker, 2014). In Brazil, the winter fallow period (May to September) corresponds to the emergence peak of Sumatran fleabane; this period allows the plant to grow undisturbed in fallow and reach sizes where control with herbicides becomes very difficult (Oliveira Neto et al., 2010). Such biological characteristics, agronomic practices, and the selection for herbicide-resistant biotypes contribute to the wide expanding areas of *Conyza* populations (Dauer et al., 2009).

In Brazil, populations of *C. bonariensis*, *C. canadensis* have been selected for glyphosate resistant and populations of *C. sumatrensis* can exhibit multiple resistance to both glyphosate and chlorimuron (Santos et al., 2014; Heap, 2017). In this genus, there are also several cases of resistance to other herbicide modes of action such as photosystem I inhibitors worldwide. There are many countries where *Conyza* sp. has been reported to be resistant to paraquat since 1980, e.g., Hungary (Pölös et al., 1987), Taiwan, Japan, Egypt, Malaysia, Canada, Unites States, Belgium, Sri Lanka, South Africa; the last report in 2016 was in Australia (Chiang and Chiang, 2006; Heap, 2017; Preston, 2017).

Paraquat remains important as a preplant burndown herbicide as it is one of only three herbicide active ingredients (the other two being glyphosate and glufosinate) that are truly non-selective, broad spectrum, and without residual soil activity (Hawkes, 2013). Non-selective herbicides such as paraquat are widely used in no-till systems to control weeds prior to planting. Emerged *Conyza* requires a burndown application utilizing paraquat, sometimes in a sequential program that utilizes sequential preplant application combinations of glyphosate + 2,4-D followed by paraquat (Oliveira Neto et al., 2010; Oliveira Jr. et al., 2013).

In the western region of Paraná State, in the 2014/2015 season, farmers reported failure to control *Conyza* sp. following typical paraquat applications. In this case, the applications normally occurred in the fall, by spraying a split program ("double shot") using glyphosate + 2,4-D or glyphosate + chlorimuron followed by a sequential application of paraquat. A similar situation occurred in the 2015/2016 season, with reduced efficacy of the sequential application of paraquat. Further in the 2016/17 season, there were several reports by farmers to external consultants and researchers, regarding control failures and increased tolerance of *Conyza* to paraquat in some agricultural fields located near Palotina and Assis Chateaubriand. Therefore, the aim of this research was to test and evaluate the presence of resistance to paraquat in these Sumatran fleabane populations.

MATERIAL AND METHODS

First experiment – preliminary investigation

In 2016, growers reported failures to control paraquat using the commercial label rate at 400 g ha^{-1}, applied in burndown prior soybean sowing. The location was a soybean field located in Assis Chateaubriand-PR (24^{o}16'53.8"S 53^{o}30'47.5"W). In the same area, a strip test using a CO_2 pressurized backpack sprayer (2 m boom) was performed, spraying paraquat at the same rate used by farmers. No symptoms were noted. Thus, in November 2016, live plants of *C. sumatrensis* (~6 cm, 8 leaves) were collected randomly in the areas of fields where the postemergence paraquat treatment did not provide control. These plants were taken to the greenhouse of Federal University of Paraná (UFPR), located in Palotina-PR, Brazil.

The plants were transplanted to pots (5 dm^{-3}) and irrigated twice a day under greenhouse conditions; day/night temperatures were set at 25 ± 5 ^oC/16 ±5 ^oC with no supplemental lighting. Approximately five weeks after being transplanted, the plants reached 12 cm height and had 10-12 leaves. The first experiment was conducted to evaluate the level of control after paraquat application.

The experimental design was a randomized complete block with three replications. The treatments were a commercial formulation of registered paraquat (200 g L^{-1}, Paraquate ALTA 200 SL, trademark of ALTA – América Latina Tecnologia Agrícola Ltda) at 800 and 1,600 g ha^{-1}

mixed with the adjuvant Agral at 0.1% (v/v) (Agral®, trademark of Syngenta Crop Protection, Greensboro, NC) and an untreated group without herbicide. The recommended label rate to control *Conyza* sp. ranges between 300 and 400 g ha^{-1}, depending on the manufacturer (SEAB, 2017). Herbicide applications were made with a CO_2 pressurized backpack sprayer fitted with four XR-110015 flat-fan nozzles (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}.

Control percentage (visual scale from 0 to 100%) was assessed at 14 and 28 days after application according to SBCPD (1995). The results were submitted to analysis of variance (F) and treatments were considered significantly different when probability was less than 5% ($p \leq 0.05$). When the F-test was significant, Tukey's test at 5% ($P \leq 0.05$) was used for comparisons of means.

Second experiment

In March 2017, the F1 generation seeds from the previous experiment were collected, which were sent to three different locations for greenhouse studies. Three experiments were conducted simultaneously in Londrina-PR (Embrapa Soja) – Biotype 1 (B1), in Palotina-PR (Universidade Federal do Paraná-UFPR) Biotype 2-3 (B2-B3) and in Mogi Mirim-SP (Mogi Mirim Field Station, Dow AgroSciences) – Biotype 4 (B4). Seeds for the susceptible biotypes were collected in Palotina-PR, Londrina-PR and Mogi Mirim-SP in areas without known issues to control using the herbicide paraquat, in order to understand the level of susceptibility of such species.

The experimental units were 1.0 dm^{-3} pots containing common planting mix substrate. The seeds were sown and after emergence were thinned to one seedling per pot. Plants at the reproductive stage were properly identified anatomically by Professor Jimi Naoki Nakajima in the Biology Institute (INBIO) of the Universidade Federal de Uberlandia-MG (UFU), although more studies have been conducted to properly confirm the species.

Treatments were sprayed on Sumatran fleabane when the plants reached 10 cm of height and had 8-10 leaves. The registered herbicide in use was paraquat (200 g a.i. L^{-1}, Paraquate ALTA 200 SL, a trademark of ALTA – América Latina Tecnologia Agrícola Ltda) associated with the adjuvant Agral at 0.1% (v/v) (Agral®, a trademark of Syngenta Crop Protection, Greensboro, NC) at 0, 50, 100, 200, 400, 800, 1,600 and 3,200 g ha^{-1}. All herbicide applications were made with a CO_2-pressurized backpack sprayer using the same settings as in the first experiment. The experimental design was a randomized complete block with five replications.

Unlike the first experiment, efficacy was assessed visually as control percentage (0 = no effect, 100 = complete control) at 3, 7, 14, 21 and 28 days after application of paraquat. Plants were considered to be alive when green tissue was present. Dry weight evaluation was performed at 28 days after herbicide applications. The plants were clipped at the soil surface, placed in paper bags, and oven dried at 70 °C for 4 days, and weight was then recorded. The dose response curves were performed with the biotype that presented the least control relative to the susceptible biotype.

Data were submitted to analysis of variance and regression analysis, and when significant, they were adjusted to logistic nonlinear regression model proposed by Streibig (1988):

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

where: y is the response variable, a is the amplitude between the maximum and minimum point of the variable, x is the dose of the herbicide (g ha^{-1}), b is the dose that promotes 50% response of the variable, c is the declivity of the curve around b.

The logistic nonlinear model provides an estimate of the parameter C_{50} or GR_{50}. In this way, a decision was made to use the mathematical calculation through the inverse equation of Streibig

(1988), hence C_{50} could be calculated according to the proposition of Souza et al. (2000). The models used to obtain C_{50} were the same ones used in other recent reports cited in the literature (Takano et al., 2016, 2017).

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

With the values for C_{50} and $GR_{50,}$ we calculated the resistance factor (RF) resulting from the difference between C_{50} or GR_{50} of the possible resistant population and C_{50} or GR_{50} of the susceptible population.

RESULTS AND DISCUSSION

First experiment

The results found with the first experiment, using paraquat at 800 and 1,600 g ha^{-1}, showed that the level of control of resistant C. *sumatrensis* was poor, providing less than 10% control at 14 and 28 DAA, and there was no statistical difference between the two doses applied (Table 1). In Brazil, the registered commercial label rate used of paraquat to control *Conyza bonariensis* is 400 g ha^{-1} (SEAB, 2017), although there is no substantial difference to control other species, such as *Conyza summatrensis* or *Conyza canadensis*, using such rate. In the literature, susceptible

Table 1 - Control of resistant *Conyza summatrensis* at 14 and 28 days after application. Palotina-PR

Treatment	Active ingredient	Rate (g ha^{-1})	% Control	
			14 DAA	28 DAA
1	Paraquat	800	6.3 a	4.0 a
2	Paraquat	1600	7.7 a	4.7 a
3	Untreated	-	0.0 b	0.0 b
HSD (p≤0.05)*			3.14	2.27
Standard deviation			1.08	0.78
Coefficient of variation			23.15	27.06

Means within a column followed by the same letter are not significantly different according to Tukey's HSD (honest significant difference) at P≤0.05. DAA = days after application.

biotypes of *C. bonariensis* can be 100% controlled when sprayed with paraquat at 400 g ha^{-1} (Vargas et al., 2007). Such result was found for plants at the 4-leaf growth stage. However, Yamauti (2010) found 84% control when they sprayed paraquat at 600 g ha^{-1} to *C. canadensis* and *C. bonariensis*.

The results of the preliminary study in the first experiment indicated a high level of resistance to paraquat and motivated the collection of F1 seeds to create a more robust dose-response analysis in further experiments.

Second experiment

The analysis of the dose response experiments, from the adjusted non-linear model utilizing the equation proposed by Streibig (1988), enabled the adjusted curves for the variables analyzed, with R^2 being close to 1.00. Thus, the parameters a, b and c of the equation could be determined, allowing the calculation of the paraquat dose required to control 50% of the population evaluated (C_{50}) for control at 28 DAA, as well as the dose required to reduce weight by 50% (GR_{50}) of all biotypes with suspected resistance. Table 2 shows the values of C_{50}, GR_{50} and resistance factor (RF) for both variables of all tested biotypes.

Table 2 - Rate of paraquat required to control 50% of the population (28 DAA), to reduce shoot dry weight by 50% and resistance factor (RF) for populations of *Conyza sumatrensis*. 2016/2017

Population	C_{50}	RF	GR_{50}	RF
	Control		Dry weight	
	(g ha^{-1})			
Susceptible - Londrina (PR)	60	-	60	-
Londrina (PR) – B1	1844	31	2007	33
Susceptible – Palotina (PR)	63	-	67	
Palotina (PR) - B2	913	14	244	3.6
Palotina (PR) - B3	2468	39	1166	17
Susceptible – Mogi Mirim (SP)	54	-	20	-
Mogi Mirim (SP) – B4	1491	28	699	34

C_{50} - dose of paraquat required to control 50% of the population; GR_{50} - dose required to reduce weight by 50%. RF – resistant factor.

In general, for all susceptible biotypes, C_{50} ranged between 54 to 63 g ha^{-1} and GR_{50} from 20 to 67 g ha^{-1} (Table 2). These results provided high RF, as in Londrina, PR, C_{50} for the B1 at 28 DAA was 1844 (g ha^{-1}) and GR_{50} was 2007 (g ha^{-1}), demonstrating a RF for control of 31 and RF for dry weight of 33. In Palotina, PR (B2 and B3) showed C_{50} of 913 and 2468 (g ha^{-1}) and RF of 14 and 39, respectively. In addition, GR_{50} was 244 and 1166 (g ha^{-1}) for B2 and B3, providing a RF of 3.6 and 17. The same results were found for the B4 population in Mogi Mirim, SP which had C_{50} and GR_{50} of 1491 and 699 (g ha^{-1}) respectively, with a RF to control of 28 and 34.

Moretti et al. (2013) found similar GR_{50} values to those found in this study in paraquat-resistant *C. bonariensis*. However, GR_{50} of paraquat varied across seasons (940, 240 and 70 g a.i. ha^{-1} during summer, fall, and winter, with a RF of 33, 22, and 6 times higher than the resistant population, respectively). Turcsányi et al. (1998) found different levels of resistance factors according to stage of weed development, and the resistant factor to paraquat in *C. canadensis* ranged from 450 at the vegetative rosette stage to 1000 at the flowering stage.

In paraquat, cationic amino acid transporters (CAT) were hypothesized to be involved in paraquat sequestration into the vacuole based on the transcription increase of a certain CAT gene in a *C. canadensis* resistant biotype (Jóri et al., 2007). However, it is not currently known what the mechanism of resistance is in this case, five possible mechanisms of resistance have been proposed: (a) altered compartmentation of paraquat; (b) restricted cuticular penetration; (c) detoxification of reactive forms of O_2 produced in the presence of paraquat; (d) inactivation of paraquat through plant metabolism and (e) sequestration of paraquat, preventing translocation to the site of action (Fuerst and Vaughn, 1990). There is a case of resistant to paraquat in *Solanum nigrum*, in which the possible mechanism of resistance is due to reduced electron flow in PSI (Chase et al., 1998).

In all biotypes, RF was greater than 1 (RF>3.0). The dose response curves are shown in Figure 1. Although the four biotypes tested in this study had been collected in the same location, they showed differential response to paraquat, and RF ranged from 3.6 to 34. The results confirmed the initial suspicion of the presence of paraquat-resistant biotypes in the western region of Paraná, after the experiments had followed the criteria for confirming a new case of herbicide resistance of weeds according to HRAC, (2017); Criterion 1: definition of weed resistance: 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: confirmation by defined protocols in the scientific base: the resistance factors were higher and the recommended dose to the species (400 g ha^{-1}) did not provide satisfactory control; Criterion 3: characterization of heritability: the F1 plants from these populations were also considered to be resistant; Criterion 4: demonstration of the practical impact in the field by the herbicide resistant weed: control flaws complaints were clearly observed on the field; Criterion 5: botanical identification: random plants of these populations were properly classified as *C. sumatrensis*.

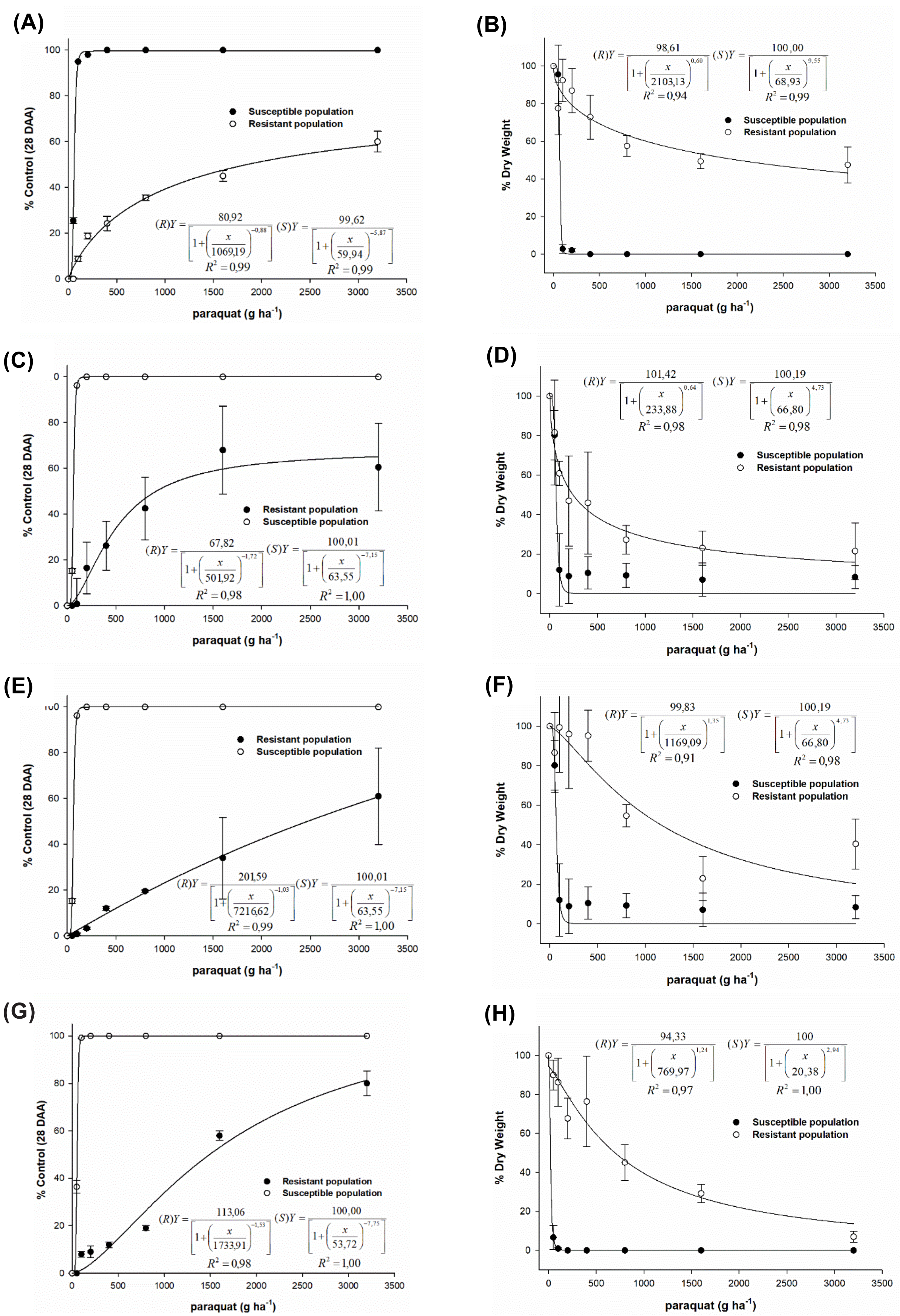

Figure 1 - Percent control (A, C, E G) and dry weight (B, D, F, H) at 28 DAA in Londrina-PR (A,B) Palotina-PR (C, D, E, F) and Mogi Mirim-SP (G, H), respectively.

Based on the results found in this study, the initial hypotheses were confirmed that this report is the first case of Sumatran fleabane resistance to paraquat in Brazil. Further studies should be conducted to understand the mechanisms that lead to resistance to paraquat.

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