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Article

Multiple-and Cross-Resistance of *Amaranthus retroflexus* to Acetolactate Synthase (ALS) and Photosystem II (PSII) Inhibiting Herbicides in Preemergence

*Resistência Múltipla e Cruzada de **Amaranthus retroflexus** a Herbicidas Inibidores da Enzima Acetolacto Sintase (ALS) e do Fotossistema II (PS II) em Pré-Emergência*

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ABSTRACT - Herbicide resistance in *Amaranthus* genus occurs frequently around the word and has become a big problem in cotton producing areas. The objective of this work was to evaluate cross-and multiple-resistance of redroot pigweed (*A. retroflexus*) to herbicides used in preemergence in cotton fields in Brazil. Seven dose-response experiments were conducted with herbicides atrazine, prometryn, diuron, S-metolachlor, trifluralin, trifloxysulfuron-sodium and pyrithiobac-sodium, and the treatments consisted of application rates of 0, ¼, ½, 1, 2 and 4 times the recommended label rate. Eight *A. retroflexus* byotipes with suspect of resistance were sampled for experiments in three brazilian states of cotton producing. Resistance to prometryn was confirmed for one biotype in Goiás (GO), and one biotype from Mato Grosso (MT) showed cross-resistance to atrazine and prometryn. One byotipe from GO was identified with cross-resistance to trifloxysulfuron-sodium and pyrithiobac-sodium. One of the GO samples was identified with multiple resistance to prometryn and ALS inhibitors, another one to atrazine and ALS inhibitors, while MT byotipe was confirmed with multiple resistance to triazines and pyrithiobac. The herbicides S-metolachlor, diuron, and trifluralin were efficient for control of this species, therefore, they can be used as managment alternative in those regions.

Keywords: redroot pigweed, *Gossypium hirsutum* L., triazines, sulfonylureas.

*RESUMO - A resistência a herbicidas em espécies do gênero **Amaranthus** ocorre frequentemente no mundo e está se tornando um grande problema em regiões algodoeiras no Brasil. O objetivo deste trabalho foi verificar a resistência cruzada e múltipla de biótipos de caruru-gigante (A. retroflexus) aos herbicidas utilizados em pré-emergência na cultura do algodão no Brasil. Sete experimentos de dose-resposta foram realizados em casa de vegetação com os herbicidas atrazine, prometryn, diuron, S-metolachlor, trifuralin, trifloxysulfuron-sodium e pyrithiobac-sodium, sendo os tratamentos equivalentes a 0, ¼, ½, 1, 2 e 4 vezes a dose recomendada. Foram avaliados oito biótipos de A. retroflexus coletados em três Estados produtores de algodão do Brasil com suspeita de resistência a esses herbicidas. Confirmou-se a resistência em um biótipo de Goiás (GO) para prometryn, assim como um de Mato Grosso (MT), que apresentou resistência cruzada a atrazine e prometryn. Um biótipo do Estado do Mato Grosso do Sul (MS) e quatro de GO apresentaram resistência cruzada a trifloxysulfuron-sodium e pyrithiobac-sodium. Um biótipo de GO foi identificado com resistência múltipla a prometryn e inibidores*

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da ALS, outro para atrazine e inibidores da ALS, enquanto para o bipótipo do MT foi confirmada resistência múltipla para triazinas e pirimiditio-benzoatos (pyrithiobac). Os herbicidas S-metolachlor, diuron e trifuralin foram eficientes no controle de todas as populações e, portanto, enquadram-se como alternativas de manejo nas regiões onde foram constatados os biótipos resistentes.

Palavras-chave: caruru-gigante, *Gossypium hirsutum* L., triazinas, sulfonilureias.

INTRODUCTION

Currently, in Brazil, the average number of confirmed cases of herbicide resistance is two per year. Forty-three cases have been recorded to date, which places Brazil in the fifth position in the ranking of countries with the greatest number of resistance reports, behind the United States, Australia, Canada and France (Heap, 2019).

The mechanism of action that present the greatest number of cases are acetolactate synthase inhibitors (ALS) and photosystem II inhibitors (PS II). Weed populations may present a great number of individuals with genotypes that confer resistance to both mechanisms, which makes selection of resistant plants faster than other herbicide groups (Powles and Yu, 2010; Yu and Powles, 2014).

In cotton growing fields, the use of herbicides with these two mechanisms of action is frequent (ALS inhibitor and PS II inhibitor), e.g., trifloxysulfuron-sodium, pyrithiobac-sodium, atrazine, prometryn and diuron. These herbicides are recommended for several mono- and dicotyledonous weed control, besides having crop selectivity in applications over the entire area or by spraying it over inter-rows (Rodrigues and Almeida, 2011).

Species of *Amaranthus* genus, commonly known as redroot pigweed, are largely found in the world. In Brazil, five to six species of this genus are present and adapted, both under conventional and no-tillage systems, including cotton fields. They are annual species that reproduce from seeds, have C4 photosynthesis cycle, and can produce more than 200 thousand seeds per plant (Carvalho, 2015). Weed interference, e.g., *A. palmeri*, may cause losses of up to 60% in cotton yields (McRae et al., 2013). In corn crops, *A. retroflexus* species may cause yield losses of up to 3,000 kg ha^{-1} (Vazin et al., 2012). Thus, the genus *Amaranthus* is composed of highly competitive weed species, requiring control actions.

Globaly, there are 226 reports of herbicides resistance by the genus *Amaranthus*. In Brazil, the most recent cases include *A. retroflexus* resistant to protoporphyrinogen oxidase (PPO) inhibitors (Heap, 2019) and *A. palmeri,* simultaneously resistant to glyphosate and ALS inhibitors (Gonçalves Netto et al., 2016). In cotton fields, *A. retroflexus* populations resistant to trifloxysulfuron-sodium and pyrithiobac-sodium were found (Francischini et al., 2014b) as well as *A. viridis* resistant to trifloxysulfuron-sodium (Francischini et al., 2014a), applied in postemergence.

In previous crop seasons in the states of Goiás, Mato Grosso do Sul and Mato Grosso, poor control of redroot pigweed (*A. retroflexus*) was observed for herbicides used in cotton preemergence, such as ALS and PS II inhibitors. Therefore, the objective of this research was to evaluate the occurrence of cross resistance to ALS-and PS II-inhibiting herbicides in redroot pigweed (*A. retroflexus*) biotypes, studied by Francischini et al. (2014b), but this time with preemergence applications of products used in cotton fields.

MATERIAL AND METHODS

Redroot pigweed samples were harvested in March 2010 from seven locations, four in the state of Goiás, two in Mato Grosso do Sul and one in Mato Grosso (Table 1). Five to ten plants per site were sampled. After being collected, the plants were dried at 45 °C in forced-air oven for 24 hours and the seeds were removed. The seeds were grouped according to each sampling site, stored in paper bags and kept at room temperature.

One sample was collected in the state of São Paulo, where had no herbicide application at least five years. After being previously tested, it was considered a susceptible biotype in the

Table 1 - Identification of the sampling sites of redroot pigweed (*A. retroflexus*)

State	Town	Identification	Coordinate
Mato Grosso do Sul	Chapadão do Sul	MS 1	18º40'41.94"S; 52º53'58.90"W
Mato Grosso do Sul	Chapadão do Sul	MS 2	18º41'13.47"S; 52º52'33.33"W
Goiás	Chapadão do Céu	GO 3	18º35'6.58"S; 52º43'41.43"W
Goiás	Chapadão do Céu	GO 4	18º34'22.94"S; 52º47'52.21"W
Goiás	Chapadão do Céu	GO 5	18º30'22.94"S; 52º45'52.21"W
Goiás	Chapadão do Céu	GO 6	18º40'13.03"S; 52º47'19.99"W
Mato Grosso	Sorriso	MT 13	12º35'13.55"S; 55º44'27.18"W
São Paulo	Engenheiro Coelho	Susceptible biotype	22º28'51.28"S; 47º12'56.10"W

experiments. The sampling sites are soybean or corn crops grown in rotation with cotton. In these areas, control of dicotyledonous weed plants was often made with atrazine or prometryn + clomazone in preemergence by spraying these herbicides directly into between the crop rows and with trifloxysulfuron + pyrithiobac-sodium in postemergence.

In corn crops, atrazine herbicide was often used in pre-and postemergence applications, and in soybean crops the weeds control was basically done with glyphosate herbicide, both in pre-burndow and during the soybean growing cycle.

Seven experiments were carried out in Maringá (PR). The experimental units consisted of pots with a capacity of 3 dm^{-3} (20 cm in diameter and 15 cm in height) containing soil with 42% of clay and pH_{H2O} of 5.5, classified as Distroferric Red Latosol. In each pot, 50 seeds were scattered on the surface and covered with a 0.5 cm soil layer. Application of the treatments occurred in preemergence, soon after sowing.

For herbicides application, a CO_2-pressurized knapsack sprayer with constant pressure (2.46 kgf cm^{-2}) was used, with an application rate of 200 L ha^{-1}. The application bar was equipped with three spraying nozzles, model XR 110.02, spaced 0.5 m apart and positioned at 0.5 m from the ground. At the time of application, air temperature was 26 °C, relative humidity was 68%, the sky was overcast and wind speed less than 1.2 km h^{-1}. The seven experiments were applied on July 10, 2010, and the greenhouse was irrigated with 5 mm water rate sprinkling per day, until completion of the experiments.

Each experiment consisted of a dose-response assay with herbicide, with four replications, in a randomized block design and 8x6 factorial arrangement. Factor A consisted of eight redroot pigweed biotypes, and factor B of six different doses of herbicide (0, ¼, ½, 1, 2 and 4 times the full label recommended dose). The herbicides used were atrazine (Proof®), prometryn (Gesagard® 500 SC), diuron (Diuron® 500 SC), trifluralin (Premerlin® 600 EC), S-metolachlor (Dual Gold®), trifloxysulfuron-sodium (Envoke®) and pyrithiobac-sodium (Staple® 280 SC), at the respective recommended rates: 1.5, 1.0, 2.0, 1.08, 1.44, 0.0075 and 0.14 kg a.i. ha^{-1}. The recommended rate was defined based on information of Rodrigues and Almeida (2011), except for the herbicides trifloxysulfuron-sodium and pyrithiobac-sodium, which do not have a record for this species, and so the rates used by Carvalho et al. (2006) were considered, in postemergence application.

Visual control evaluations were conducted at 28 days after application (DAA), using 0 to 100% percent scores, where 0% means absence of symptoms and 100% the plant death. For these evaluations, it was considered the reduced dry matter and in the treated plants emergence compared to the (untreated) control. Data were subjected to F-test of significance (p=0.05) using the SISVAR software; to generate dose-response curves, the SigmaPlot software was used, according to the model proposed by Streibig et al. (1988):

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

where: *y* = percent control; x = herbicide rate (kg a.i. ha^{-1}); *a*, *b* and *c* = estimated parameters of the equation, so that; *a* = asymptote between the maximum and minimum point of the variable; *b* = dose that provides 50% of asymptote; *c* = slope of the curve around *b*.

Based on the adjusted models, the herbicide rate was calculated, in kg ha^{-1}, which would provide 50% and 90% of control (I_{50} and I_{90}). For this calculation, we decided to reverse the log-logistic model, leaving it as a function of *y*:

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

The model was adjusted to the experiments (herbicides) in which there was interaction of dose *versus* biotypes in the F-test. To confirm resistance, it was considered that the I_{90} rate should be higher in suspect biotypes compared to what was considered susceptible, and also higher than the doses recommended for field control. In addition, the resistance factor (RF), or the ratio of I_{50} of the suspect biotype to the I_{50} of the susceptible biotype should be greater than 3.0.

RESULTS AND DISCUSSION

PS II inhibitors (atrazine, prometryn, diuron)

Biotype GO3 exhibited a 10.9 RF for atrazine, and I_{90} was 1.6 kg ha^{-1}, above the recommended rate for field control (1.5 kg ha^{-1}), which confirms the hypothesis of resistance (Table 2). Similarly, for biotype MT13, a high RF value was observed (59.7). This fact, combined with the finding that the I_{90} for this biotype (3.79 kg ha^{-1}) was 2.52 times higher than the recommended dose (1.5 kg ha^{-1}) and 27 times higher than the I_{90} of biotype MS2, indicated a high level of resistance to atrazine. Although biotype GO4 has exhibited a RF higher than 1.0, the I_{90} was 3.6 times lower compared to the intermediate dose used in the experiment (1.5 kg ha^{-1}), which rejects the hypothesis of resistance. Regarding the other biotypes, no criterion for confirmation of resistance was met (Figure 1).

Table 2 - Estimates of parameters (*a*, *b* and *c*), 50% (I_{50}) and 90% (I_{90}) control rates and resistance factors (RF) of *A. retroflexus* obtained in the control evaluation at 28 days after preemergence applications of photosystem II-inhibiting herbicides

Atrazine								
Biotype	*A*	*b*	*C*	R^2	I_{50}	I_{90}	RF[(1)]	Identification of resistance
GO3	109.47	0.20	-0.73	0.99	0.1608	1.6739	10.90	X
GO4	98.27	0.19	-2.95	0.99	0.1888	0.4195	12.80	
GO5	99.72	0.01	-0.61	0.99	0.0086	0.3209	0.58	
GO6	100.72	0.00	-0.49	0.99	0.0012	0.0928	0.08	
MS1	102.60	0.00	-0.98	0.99	0.0026	0.0200	0.17	
MS2	99.48	0.01	-0.99	0.99	0.0148	0.1433	1.00	
MT13	119.80	1.24	-0.98	0.99	0.8809	3.7990	59.70	X
Susceptible	102.10	0.00	-0.38	0.99	0.0003	0.0565	0.02	
Prometryn								
Biotype	*A*	*b*	*C*	R^2	I_{50}	I_{90}	RF[(2)]	Identification of resistance
GO3	99.98	0.20	-1.84	0.99	0.20183	0.66709	7.12	
GO4	99.23	0.15	-2.44	0.99	0.14592	0.36837	5.15	
GO5	151.70	0.74	-0.35	0.99	0.09805	2.15393	3.46	X
GO6	103.42	0.03	-0.74	0.99	0.02834	0.40900	1.00	
MS1	99.51	0.20	-5.41	0.99	0.20527	0.31030	7.24	
MS2	115.40	0.00	-0.16	0.99	0.00002	0.26731	0.00	
MT13	94.77	0.08	-0.95	0.99	0.09090	1.78797	3.21	X
Susceptible	98.12	0.15	-2.34	0.99	0.15299	0.41993	5.40	

[(1)] I_{50} biotype evaluated / I_{50} MS2; [(2)] I_{50} biotype evaluated / I_{50} MS1.

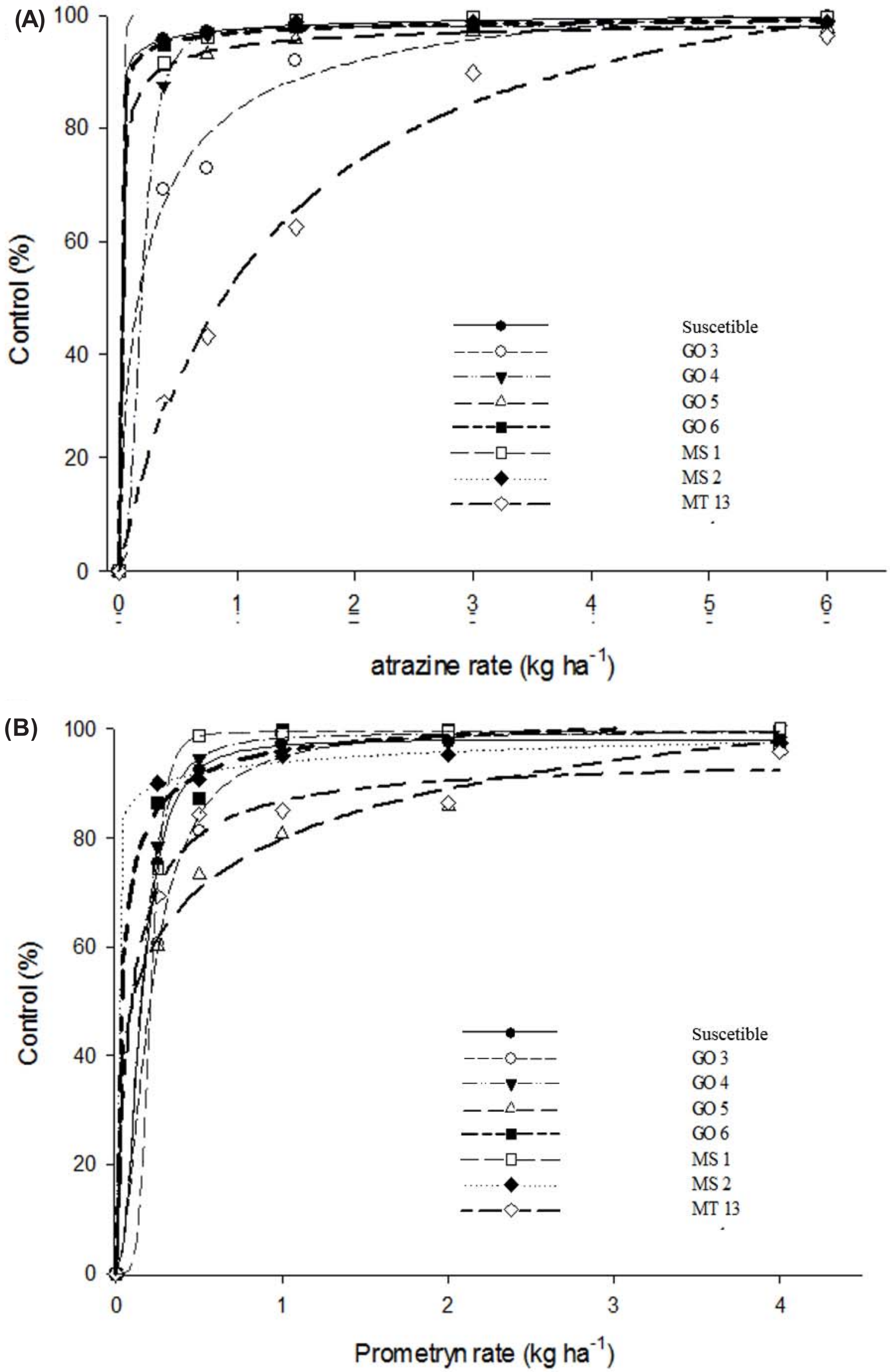

Figure 1 **-** Dose-response curves of biotypes of redroot pigweed (*A. retroflexus*) to atrazine (A) and prometryn (B) in preemergence. Maringá (PR), 2010.

For prometryn, three biotypes were found with RF > 3.0 (GO3, GO5, MS1 and MT13). In spite of this, treatments with biotypes GO3 and MS1 exhibited a control rate of 90% with doses of 0.66 and 0.31 kg ha^{-1}, lower than doses used in the field (1.0 kg ha^{-1}). Resistance for GO5 and MT13 was confirmed, which exhibited I_{90} over 1.7 kg ha^{-1} (Table 2). The other biotypes were considered susceptible, as well as SP 12, considered as susceptible pattern.

Analysis of data by the F-test for the diuron herbicide was not significant for the sources of variation doses, biotypes and in the interaction (biotypes x doses), eliminating the need to adjust the model to dose-response curves and the possibility of resistance.

Inhibitors of initial growth (S-metolachlor and trifluralin)

For S-metolachlor, there was no significant interaction between the biotypes and doses in the F-test. By analyzing the RFs observed from the control levels in different biotypes, all of them showed values below 1.0 (data not shown), indicating no resistance levels of these biotypes to S-metolachlor, when applied in preemergence.

The variation sources relating to biotypes and biotypes x doses interaction were not significant in the F-test in the trifuralin experiment. Therefore, similar control levels were evaluated for all biotypes, irrespective of the dose used, which eliminates the possibility of resistance of these biotypes to this herbicide, when applied in preemergence.

The results found for S-metolachlor and trifluralin show that these herbicides are still effective for the biotypes sampled, thus being important tools for the management of resistance to other mechanisms of action.

ALS inhibitors (trifloxysulfuron-sodium and pyrithiobac-sodium)

For trifloxysulfuron-sodium herbicide, only biotype MS1 did not indicate resistance, because I_{90} was lower than the recommended dose (I_{90} = 0.0039 kg ha^{-1}). All other biotypes exhibited high resistance levels (RF between 39 and 339.03) (Table 3) and less than 70% of control even on the highest dose used (Figure 2). Indefinite I_{90} values suggest that rates higher than 0.03 kg ha^{-1} are needed, more than four times the recommended dose for the control of these biotypes.

Except for MS1, MS 2 and MT13, all other biotypes were considered resistant to pyrithiobac-sodium (Figure 2), meeting all criteria defined, with RFs > 3 and control rates above that used in the field (> 0.14 kg ha^{-1}). As for trifloxysulfuron-sodium, some I_{90} values for pyrithiobac-sodium were not possible to obtain. To calculate it, additional tests would be necessary with higher rates, higher than than four times the recommended dose of the herbicide, which indicates the need for extremely high doses to achieve 90% of control of resistant biotypes in the field.

In the present work, it was identified one biotype of redroot pigweed (*A. retroflexus*) with cross-resistance to atrazine and prometryn (MT 13) herbicides as well as one biotype with resistance to only atrazine (GO3) and another one only to prometryn (GO5) in preemergence (Table 4).

Cross-resistance to the triazines group was also found in populations of *Chenopodium album* (Mechant et al., 2008), *Echinochloa colona* (Elahifard et al., 2013) and *Poa annua* (Perry et al., 2012) in postemergence. In these cases, the mechanism that confers resistance to the herbicides of this group is Ser_{264}Gly mutation in *psb*A gene, which changes the configuration of D1 protein of PS II, preventing the herbicides binding to the target binding site. In the case of simultaneous resistance to triazines and urea herbicides, the mechanisms most commonly found is Val_{219}Ile mutation in the *psb*A gene (Mengistu et al., 2000, 2005; Dumont et al., 2016).

In preemergence, resistance to atrazine was also found in *A. retroflexus* populations in Illinois (USA) (Ma et al., 2016). It is possible that the intensive use of triazine herbicides in the areas where cross-resistance was found (GO3, GO5 and MT13) has led to the selection of *A. retroflexus* biotypes with mechanisms of resistance only to this group, specifically. It should also be

Table 3 **-** Estimates of parameters (*a*, *b* and *c*), 50% (I_{50}) and 90% (I_{90}) control rates and resistance factors (RF) of *A. retroflexus* obtained in the control evaluation at 28 days after preemergence applications of ALS-inhibiting herbicides

Trifloxysulfuron-sodium								
Biotype	*A*	*B*	*C*	R^2	I_{50}	I_{90}	RF[(1)]	Confirmation of resistance
GO3	290.27	1.14	-0.44	0.99	0.0324	DI	339.03	x
GO4	92.54	0.01	-0.58	0.99	0.0192	DI	200.38	x
GO5	134.00	0.09	-0.50	0.99	0.0319	DI	333.92	x
GO6	110.96	0.02	-0.46	0.99	0.0132	DI	138.33	x
MS1	101.27	0.00	-0.57	0.99	0.0001	0.0039	1.00	
MS2	497.32	1.68	-0.51	0.99	0.0238	DI	248.67	x
MT13	96.74	0.00	-1.82	0.96	0.0037	0.0150	39.05	x
Susceptible	111.94	0.00	-0.23	0.99	0.0000	0.0082	0.07	
Pyrithiobac-sodium								
Biotype	*A*	*B*	*C*	R^2	I_{50}	I_{90}	RF[(2)]	Confirmation of resistance
GO3	51.83	0.13	-1.68	0.97	0.92968	UD	70.947	x
GO4	63.39	0.02	-1.24	0.99	0.04682	UD	3.573	x
GO5	74.08	0.03	-2.32	0.99	0.04342	UD	3.314	x
GO6	62.86	0.03	-1.12	0.99	0.09323	UD	7.114	x
MS1	99.96	0.01	-2.48	0.99	0.01310	0.032	1.000	
MS2	81.95	0.01	-0.59	0.99	0.01408	UD	1.074	
MT13	103.74	0.00	-0.59	0.99	0.00195	0.053	0.149	
Susceptible	124.90	0.00	-0.18	0.99	0.00003	0.051	0.003	

[(1)] I_{50} biotype evaluated / I_{50} MS2; [(2)] I_{50} biotype evaluated / I_{50} MS1. UD: undetermined dose by nonlinear equation.

emphasized that in addition to the application of these herbicides in cotton field, atrazine was often used in corn crops at the site of sample MT13.

All redroot pigweed biotypes in Goiás were considered cross-resistant to ALS-inhibiting herbicides, i.e., trifloxysulfuron-sodium and pyrithiobac-sodium (GO3, GO4, GO5 and GO6) applied in preemergence. In contrast, biotypes MS2 and MT13 were identified as having resistance only to trifloxysulfuron-sodium, and MS1 was susceptible to both ALS-inhibiting herbicides.

The different cross-resistance patterns to ALS inhibitors in weed populations also depend on the mechanisms of resistance of each biotype (Deng et al., 2014). The $Asp_{376}Glu$ mutation found in gene ALS in other resistant *Amaranthus* species (*A. hybridus* and *A. powellii*) confers resistance to triazolopyrimidines and pyrimidinylthiobenzoate (Whaley et al., 2007; Ashigh et al., 2009). In contrast, other mutations in ALS, responsible for conferring resistance to these groups, were also found in *Amaranthus* species, such as $Trp_{574}Leu$ and $Ser_{653}Asn$ (Beckie and Tardif, 2012).

For the MS2 and MT13 biotypes, it is possible that other substitution of amino acids is involved, making that resistance occurs only to the group of sulphonylureas. In *A. retroflexus*, the mechanisms of resistance already found are related with substitution of $Ala_{205}Val$, which confers resistance to the group of imidazolinones, but not to sulphonylureas. In contrast, substitution of $Trp_{574}Leu$ provides resistance to both groups (McNaughton et al., 2005).

Mechanisms of resistance such as metabolization, absorption and translocation reduction and vacule compartimentalization may cause different patterns of cross-resistance. However, as the experiments in this work were carried out with preemergence applications, it is possible that these mechanisms are not related with the resistant biotypes identified. So, even in situations where herbicides of with a single mechanism of action were available for the control of certain weed species, diversification of different chemical groups within the same mechanism of action may be important to reduce the selection pressure for resistance.

A summary of resistance reports confirmed in this work is found in Table 4. Biotype MT13 showed resistance in preemergence, but not in postemergence, considering the results found

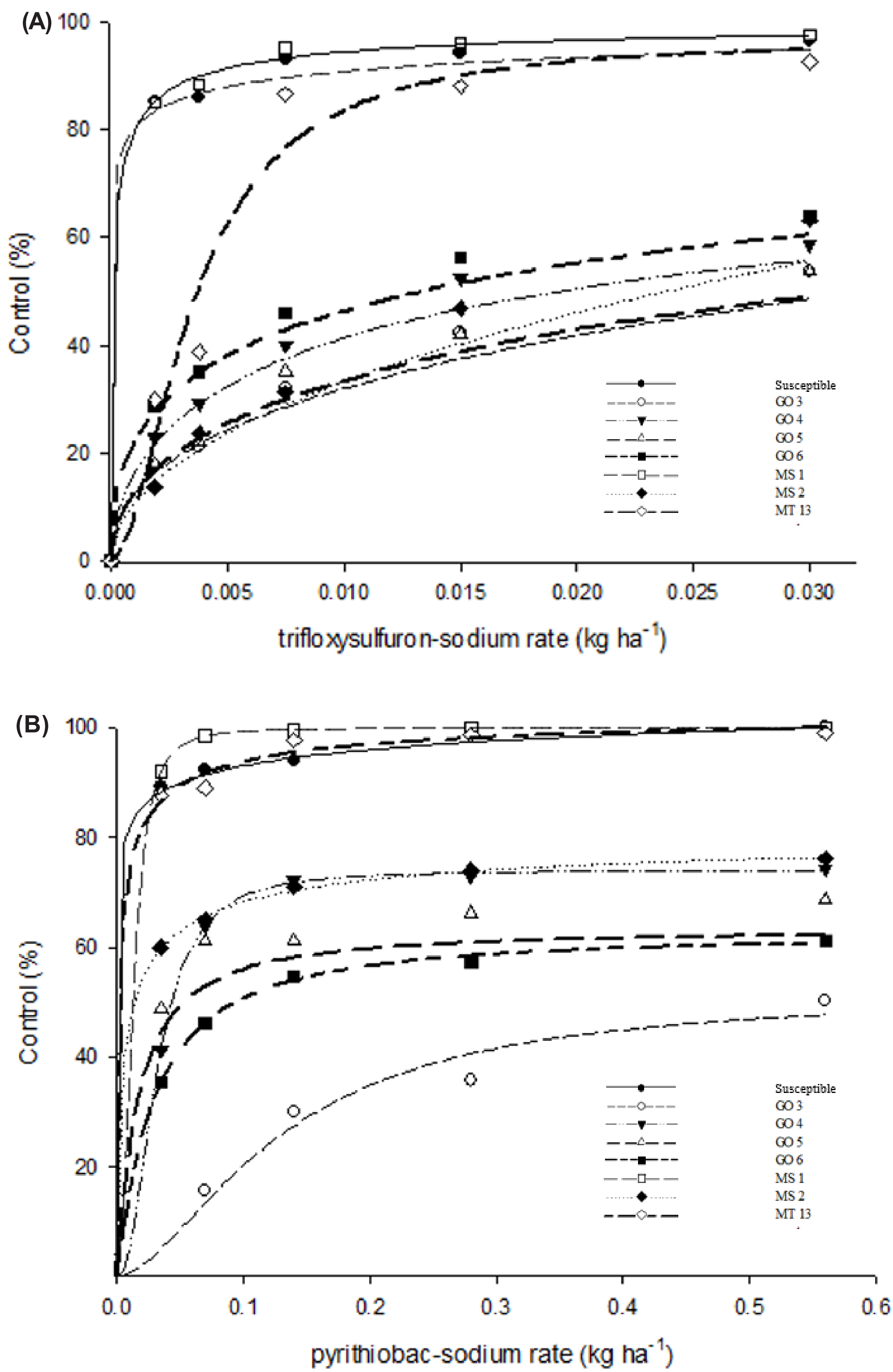

Figure 2 - Dose-response curves of biotypesf of redroot pigweed (*A. retroflexus*) to trifloxysulfuron-sodium (A) and pyrithiobac-sodium (B) in preemergence. Maringá (PR), 2010.

Table 4 - Summary of *A. retroflexus* biotypes identified with cross-resistance and multiple resistance to PS II- and ALS-inhibiting herbicides applied in preemergence

Mechanism of action	Chemical group	Herbicide	Sites						
			MS 1	MS 2	GO 3	GO 4	GO 5	GO 6	MT 13
PS II	Triazines	Atrazine			X				X
PS II	Triazines	Prometryn					X		X
ALS	Sulfonylureas	Trifloxysulfuron-sodium		X	X	X	X	X	X
ALS	Oxy-benzoic Pyrimidines	Pyrithiobac-sodium			X	X	X	X	

in previous studies (Francischini et al., 2014b) (Table 3). In the present work, three biotypes were identified with multiple resistance to ALS-and PS II inhibitors (GO3, GO5 and MT13), which further increases limits of control options for this species in cotton fields.

In overall, 98% of the resistance cases of *A. retroflexus* are related to PS II- or ALS inhibitors (Heap, 2019). *A. retroflexus* is a fast-growing plant, with great competitive ability, high seeds production and great genetic variability (Horak and Loughin, 2000; Steckel, 2007; Sammour, 2015), characteristics that may contribute to the species selection process.

Even after finding *A. retroflexus* biotypes with cross-resistance and multiple resistance to ALS-and PS II-inhibiting herbicides used in the sampled areas, either in preemergence or postemergence, it was observed that an effective control can be achieved with S-metolachlor, diuron and trifluralin in preemergence. Other choices for an effective control of other *Amaranthus* species such as *A. lividus* and *A. hybridus* are glufosinate-ammonium and glyphosate (Braz et al., 2012). However, the use of these herbicides in postemergence requires the use of tolerant cultivars to these herbicides. Also, it is necessary to investigate the mechanisms of these biotypes to ALS-and PS II-inhibiting herbicides.

Multiple resistance of *A. retroflexus* to triazines (atrazine and prometryn) and trifloxysulfuron-sodium (biotype MT13), prometryn and ALS inhibitors (biotype GO5) and atrazine and ALS inhibitors (GO3) was confirmed. Cross-resistance to ALS inhibitors (trifloxysulfuron-sodium and pyrithiobac-sodium) was confirmed for biotypes GO3, GO4, GO5 and GO6. Biotype MS2 was resistant only to trifloxysulfuron-sodium.

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