Resistance of *Amaranthus retroflexus* to Acetolactate Synthase Inhibitor Herbicides in Brazil[1]

*Resistência de **Amaranthus retroflexus** a Herbicidas Inibidores da Enzima Acetolactato Sintase no Brasil*

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ABSTRACT - When in competition with cotton, *Amaranthus retroflexus* can cause high yield losses. Due to the limited availability of selective herbicides registered for post emergence control of this weed, the same herbicides have been used repeated times over the last few years, which may have selected resistant biotypes. Biotypes of *A. retroflexus* collected from the main areas of cotton cultivation in Brazil were submitted to dose-response trials, by applying the herbicides trifloxysulfuron-sodium and pyrithiobac-sodium in doses equivalent to 0, ¼, ½, 1, 2 and 4 times the recommended rates. Resistance to ALS inhibitors was confirmed in biotypes of *A. retroflexus*. Biotype MS 2 from Mato Grosso do Sul, was cross-resistant to both trifloxysulfuron-sodium and pyrithiobac-sodium, while biotype MS 1 was resistant to trifloxysulfuron-sodium only. Likewise, singular and cross resistance was also confirmed in biotypes from Goiás (GO 3, GO 4 and GO 6), in relation to trifloxysulfuron-sodium and pyrithiobac-sodium. One biotype from Mato Grosso (MT 13) was not resistant to any of the ALS inhibitors evaluated in this work.

Keywords: trifloxysulfuron-sodium, pyrithiobac-sodium, cross-resistance, ALS inhibitors.

*RESUMO - Quando em competição com a cultura do algodoeiro, Amaranthus retroflexus é capaz de promover grande perda de produtividade. Devido à limitada disponibilidade de herbicidas seletivos para controle em pós-emergência dessa espécie daninha, algumas moléculas têm sido usadas por safras seguidas, o que pode ter levado à seleção de biótipos resistentes. Biótipos de **A. retroflexus** coletados das principais regiões produtoras de algodão do Brasil foram submetidos a ensaios de dose-resposta, por meio da aplicação de doses dos herbicidas trifloxysulfuron-sodium e pyrithiobac-sodium equivalentes a 0, ¼, ½, 1, 2 e 4 vezes a dose recomendada. Foi confirmada a ocorrência de biótipos de A. retroflexus resistentes aos herbicidas inibidores da enzima ALS. O biótipo MS 2, oriundo do Mato Grosso do Sul, apresentou resistência cruzada ao trifloxysulfuron-sodium e ao pyrithiobac-sodium, ao passo que o biótipo MS 1 mostrou resistência apenas ao trifloxysulfuron-sodium. Da mesma maneira, foram confirmados casos de resistência nos biótipos coletados no Estado de Goiás (GO 3, GO 4 e GO 6) aos herbicidas trifloxysulfuron-sodium e ao pyrithiobac-sodium, demonstrando resistência singular e cruzada. Um biótipo oriundo do Mato Grosso (MT 13) não apresentou resistência aos herbicidas inibidores da ALS testados.*

Palavras-chave: trifloxysulfuron-sodium, pyrithiobac-sodium, resistência cruzada, inibidores da ALS.

INTRODUCTION

Amaranthus retroflexus is a weed with fast development and intense production of seeds, and is able to produce up to 1.5 million seeds per plant (Gliessman, 1989; Gidea et al, 2010; Damian, 2011).

Due to its C4 physiology, *A. retroflexus* plants can reach up to 1.5 m tall, which makes them especially competitive in capturing light and other resources such as water and nutrients (Jones Jr. et al., 1997). Therefore, they are considered strong initial competitors, causing significant losses when they grow

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simultaneously with those crops with low density of plants or slow initial growth, such as cotton.

The ability of *A. retroflexus* to promote yield reduction has been documented in crops such as cotton, soybeans and peanuts (Dowler, 1995; Knezevic et al., 1999; Aguyohe & Masiunas, 2003; Bensch et al, 2003; Culpepper et al., 2006). Aguyoh & Masiunas (2003) evaluated the interference of *A. retroflexus* on beans and found yield losses up to 58%. There are further reports of yield losses of 5-34% in maize (*Zea mays*), while in sorghum (*Sorghum bicolor*) these losses can reach 46% (Knezevic et al., 1994, 1997). Cotton production fields infested with *A. retroflexus* may suffer losses from 5 to 70%, depending on the density of infestation and emergence period of the weed in the crop (Buchanan & Burns, 1971).

In Brazil, *Amaranthus retroflexus* has been reported in fields of soybeans, cotton, peanuts, among others. Specifically in the case of the cotton-growing areas, it is considered a difficult plant to control by herbicides registered for use in this crop, both due to the high infestations that occur, and to its intermittent flow of emergence during the early stages of plant development.

Due to the limited number of herbicides available for use in cotton aimed at the control of broadleaved weeds and the continuous use of herbicides with the same mechanism of action, the escape of *Amaranthus* plants has been observed after the use of the main herbicide alternatives for this crop (Francischini, 2012).

There is evidence that acetolactate synthase (ALS) inhibitor herbicides, such as pyrithiobac-sodium, which traditionally controlled several *Amaranthus* species efficiently, can show a lack of control in some areas, indicating a selection of resistant biotypes (Norsworthy et al., 2008). The selection of herbicide resistant weeds in cotton may impair or disable the use of the few herbicides registered for control of dicotyledons in this culture.

In response to the growing number of complaints of control failures after the use of ALS-inhibiting herbicides in cotton, this study aimed to evaluate the possible selection of resistant biotypes of *Amaranthus retroflexus* to trifloxysulfuron-sodium and pyrithiobac-sodium in samples from major cotton producing regions of Brazil.

MATERIAL AND METHODS

The seeds of *Amaranthus retroflexus* biotypes with suspected resistance used in this experiment were collected in cotton producing regions in the states of Mato Grosso do Sul (MS 1 and MS 2), Mato Grosso (MT 13) and Goiás (GO 3, GO 4 and GO 6) during March 2010 (Table 1). The sample of seeds of *A. retroflexus* susceptible biotype (SB) came from areas without any previous application of herbicides for more than five years in the state of São Paulo. The areas with suspected resistant biotypes had in common a history of applications for more than five years with ALS inhibitors, both for post-emergence in cotton as for pre and post-emergence in soybean and corn.

In each location, plants with mature seeds were collected in paper bags and dried in the

Table 1 **-** Geographical coordinates of the collection sites of species of *Amaranthus retroflexus*

Location	Sample Identification	Coordinates of the seed collection site	Number of Plants
Chapadão do Sul, MS	MS 1	18°40'41.94"S/ 52°53'58.90"O	30
Chapadão do Sul, MS	MS 2	18°41'13.47"S/ 52°52'33.33"O	48
Chapadão do Céu, GO	GO 3	18°35'6.58"S/ 52°43'41.43"O	12
Chapadão do Céu, GO	GO 4	18°34'22.94"S/ 52°47'52.21"O	24
Chapadão do Céu, GO	GO 6	18°40'13.03"S/ 52°47'19.99"O	12
Sorriso, MT	MT 13	12°35'13.55"S/ 55°44'27.18"O	10
Engenheiro Coelho, SP	(SB*)	22°28'43"S /47°12'56.10"O	-

* Susceptible biotype.

shade. Thereafter, 50 seeds from each sample were sown in pots with capacity for 3 dm^3 soil (experimental unit) at a depth of 0.5 cm, and soon after emergence were thinned to 10 plants per pot.

The soil used for this experiment was submitted to the chemical and physical analysis and presented pH in water of 5.5; 4,13 g dm^{-3} of C; 15% of coarse sand; 38% of fine sand; 5% of silte; and 42% of clay.

When *A. retroflexus* was at the stage of 4 to 6 leaves, plants were sprayed with increasing doses of herbicides trifloxysulfurom-sodium and pyrithiobac-sodium equivalent to 0, ¼, ½, 1, 2 and 4 times the dose of 0.00750 kg ha^{-1} (trifloxysulfuron-sodium) or 0.14 kg ha^{-1} (pyrithiobac-sodium), respectively: 0; 0.00187; 0.00375; 0.0075; 0.01500; and 0.03 kg ha^{1}(trifloxysulfuron-sodium) and 0; 0.035; 0.07; 0.14; 0.28; and 0.56 kg ha^{-1} (pyrithiobac-sodium).

The dose of trifloxysulfuron-sodium equivalent to 1x (0.0075 kg ha^{-1}) was determined by the commercial registratated dose for post-emergence control of *Amaranthus retroflexus*. In addition, preliminary tests confirmed the efficacy of such dose to control susceptible biotypes of four *Amaranthus* species, including *A. retroflexus* (Francischini, 2012).

Although the herbicide pyrithiobac-sodium has no registration for post-emergence control of *A. retroflexus*, there is evidence that shows control of *Amaranthus* species (Sibony et al., 2001). In addition to the evidence from literature, preliminary investigations have also demonstrated the susceptibility of this species to pyrithiobac-sodium (Francischini 2012). In this case, the adopted 1x (recommended dose) was 0.14 kg ha^{-1}, according to susceptibility studies conducted by Carvalho et al. (2006) and Francischini (2012).

Thus, as the objective of this study was not to compare the efficacy of the two herbicides, isolated experiments were conducted for each herbicide. In experiment 1, the application of trifloxysulfuron-sodium doses was carried out on biotypes of *A. retroflexus* from the locations MS 1, MS 2, GO 3, GO 4, GO 6 and MT 13, in addition to the application to the susceptible biotype (SB). In experiment 2, application of increasing doses of pyrithiobac-sodium in the same circumstances and biotypes of experiment 1 was performed.

For all herbicide applications, a pressurized backpack sprayer was used at a constant pressure of 35 lb in^{-2} equipped with three XR 110.02 tips, spaced 0.5 m apart and positioned 0.5 m above the surface of the target, which delivered an application rate of 200 L ha^{-1}. At the time of application the sky was partly cloudy, with a temperature of 27 °C, 78% relative humidity and wind speed of 1.7 km h^{-1}.

Each experiment was designed as a randomized blocks with treatments arranged in a factorial scheme 7 x 6; the first factor was composed by biotypes (MS 1, MS 2, GO 3, GO 4, GO 6, MT 13 and SB), and the second by the doses of the herbicide in each experiment.

In both experiments, control evaluations (visual scale, 0-100%, where 0% means no symptoms and 100% means death of weeds) were performed at 28 days after application (DAA). Satisfactory contol was considered when grades were ≥80%. The aerial parts of the remaining plants of *A. retroflexus* biotypes were harvested by cutting close to the ground and encased in paper bags, placed in a forced ventilation oven at 65 °C to constant weight; later they were weighed to obtain the dry mass from the experimental units (g per plant). The dry mass was corrected for percentage values by comparing the mass obtained in treatments with the mass of the dose zero (considered 100%).

The data were initially subjected to analysis of variance and the application of the F test. Significant effects for the factor doses of herbicide were subjected to regression analysis. The data to construct the dose-response curves were fitted to the nonlinear regression of the log-logistic-type model. The control variable was adjusted by the model proposed by Streibig et al. (1988):

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

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

Based on the log-logistic equations, dose-response curves were elaborated. Based on the adjusted models, we performed the calculation of the herbicide dose in kg ha^{-1}, which would provide either 50 or 80% of control (I_{50} and I_{80}), both in relation to visual evaluations and in relation to the shoot dry matter. To perform the calculation, we opted for the reversal of the log-logistic model, making it a function of y, according to Carvalho et al. (2005):

$$x = b * \sqrt[c]{\frac{a}{y} - 1}$$

Since not always the value of 100% control is achieved in dose-response curves, the value of the parameter b was disregarded and the I_{50} value was calculated by substituting the y of the inverse equation with 50 (control of 50%). Likewise, when replacing y by 80, the dose providing I_{80} is obtained.

The resistance factor (RF) was calculated by dividing the value of I_{50} of the biotype suspected of resistance by the I_{50} of the susceptible biotype. The resistance factor expresses the number of times the necessary dose to control 50% of the resistant biotypes is higher than the dose that controls 50% of the susceptible biotypes. Values above 1.0 are considered as one of the criteria for considering a biotype as resistant (Christoffoleti, 2002).

In this work, biotypes were considered as resistant when RF>1.0 and simultaneously satisfied two other additional conditions: I_{80} values > I_{80} of by the susceptible biotype and I_{80} value > recommended dose (1x) to control this species.

RESULTS AND DISCUSSION

Identification of *Amaranthus retroflexus* resistance to trifloxysulfuron-sodium

Pergentage control data of *A. retroflexus* biotypes adjusted accordingly to the logistic model proposed by Streibig (1988). With parameters a, b and c set, it was possible to calculate the values of I_{50} and I_{80}, characterizing susceptibility levels of these biotypes to trifloxysulfuron-sodium (Table 2). Coefficients of determination values were close to 1, indicating and excellent fit of the model to the data collected.

Amaranthus retroflexus biotypes collected in the State of Mato Grosso do Sul (MS 1 and MS 2) showed 50% control with doses 22.63 and 42.13 times higher than those used to obtain the same control level of the susceptible biotype. Satisfactory control (80%) of the MS 1 biotype would only be obtained with a dose of 0.4547 kg ha^{-1} of trifloxysulfuron-sodium, which is 61 times the recommended dose (0.0075 kg ha^{-1}) to control the species (Table 2). A similar phenomenon can be observed with the MS 2 biotype, which would only be satisfactorily controlled (80%) with a dose equivalent to 90 times the recommended dose or 445 times higher than that which would control 80% of the susceptible biotype. For the susceptible biotype, there was a drastic reduction on dry matter accumulation of plants with increasing doses of trifloxysulfuron, much more significant than that observed for both biotypes from Mato Grosso do Sul (MS 1 and MS 2) (Figure 1).

The samples of *A. retroflexus* from Goiás showed relatively high resistance factors (between 15 and 58), indicating that they require doses higher than that provides 50% control of the SB. Depending on the adjusted models, we can be observe the need for doses equivalent to 0.0104, 0.0282 and 0.0407 kg ha^{-1} to achieve 50% control of biotypes GO 3, GO 4 and GO 6, respectively (Table 2). It was not possible to perform the calculation of doses required for 80% control of biotypes GO 3, GO 4 and GO 6, since they required doses well above those used in this study (> 4 x recommended dose). The results of dry matter by spraying increasing doses of tryfloxysulfuron confirmed the control rate observed in evaluations of biotypes GO 3, GO 4 e GO 6 (Figure 1).

The results for biotypes from both Goiás and Mato Grosso do Sul suggest that reports of control failure observed in locations MS 1,

Table 2 - Estimates of the parameters a, b and c and the coefficient of determination (R^2) of the log-logistic model, adjusted for trifloxysulfuron-sodium and doses for 50% (I_{50}) and 80% (I_{80}) control of *A. retroflexus* in relation to the percentage of control at 28 days after application (DAA). Maringá, PR – 2011

Location	a	b	c	R2	I80 (kg ha^{-1})	I50 (kg ha^{-1})	RF
MS 1	82.7222	0.0199	-1.0804	0.99	0.4547	0.0294	42.13
MS 2	91.9554	0.0108	-4.59E-01	0.99	0.6797	0.0158	22.63
GO 3	50.6280	0.0060	-2.2849	0.96	-	0.0407	58.27
GO 4	76.6019	0.0124	-0.7657	0.99	-	0.0282	40.43
GO 6	62.6440	0.0056	-2.1882	0.99	-	0.0104	15.01
MT 13	100.0311	0.0007	-2.4992	0.99	0.0013	0.0007	1.07
SB*	100.1069	0.00070	-1.9542	0.99	0.0014	0.0006	1.00

* SB – susceptible biotype of *Amaranthus retroflexus*.

MS 2, GO 3, GO 4 and GO 6 may be related to the selection of biotypes resistant to trifloxysulfuron-sodium, selected by continuous and repeated use of ALS herbicides.

In the case of the biotype collected in the location MT 13, both the values of I_{50} and I_{80} were very similar to those observed for the SB, which indicates that this biotype can be adequately controlled with doses similar to those required to control SB. Once the dose required for 80% control of this biotype is lower than the recommended dose (0.0075 kg ha^{-1}) (Table 2 and Figure 1), it was considered as a susceptible biotype. Any control failure in this area could be linked to other factors unrelated to the resistance, such as inadequate sampling of plants in this area.

The results suggest the occurrence of typical cases of resistance, where there is a selection of resistant biotypes due to prolonged use of herbicides with the same mechanism of action. The recurrent use of ALS inhibitor herbicides for several consecutive years in cotton-producing areas in Goiás and Mato Grosso do Sul selected biotypes of *A. retroflexus* resistant to trifloxysulfuron-sodium.

Identification of *Amaranthus retroflexus* resistance to pyrithiobac-sodium

Amaranthus retroflexus biotypes collected in the cotton producing regions of Brazil have shown great variability in relation to the control provided by pyrithiobac-sodium. Data from I_{80}, I_{50} and the resistance factor values found for these biotypes are shown in Table 3. This variation in susceptibility may be associated with the high genetic variability observed in plants of some species of *Amaranthus*, such as *A. tuberculatus*, where its high reproductive potential (production of hundreds of thousands of seeds) offers a wealth of genetic variants, which ultimately promotes greater development of resistance (Tranel & Trucco, 2009).

Biotypes derived from locations MS 1 and MT 13 exhibited resistance factors to pyrithiobac-sodium lower than 1.0 (Table 3). Moreover, the doses needed for 80% of control of these biotypes are lower than the dose required for the same level of control of the SB. Similarly, the data related to the *A. retroflexus* dry mass of the aerial part of biotypes MS 1 e MT 13 indicate similar sensitivity to SB (Figure 2). This evidence leads to the conclusion that both can be considered as susceptible biotypes, and possible control failures observed in the areas where these samples came from are not associated with the selection of resistant individuals.

The MS 2 and GO 4 biotypes showed levels of RF between 7 and 8. Although these values of RF can be considered relatively low, by observing the values of I_{80}, it appears that it is necessary to apply doses 38-42 times higher than those required for the same control level of the SB.

The highest values of RF in relation to pyrithiobac (> 30) were observed for the GO 3 and GO 6 biotypes. Doses of pyrithiobac-sodium required to reach 80% control of these biotypes

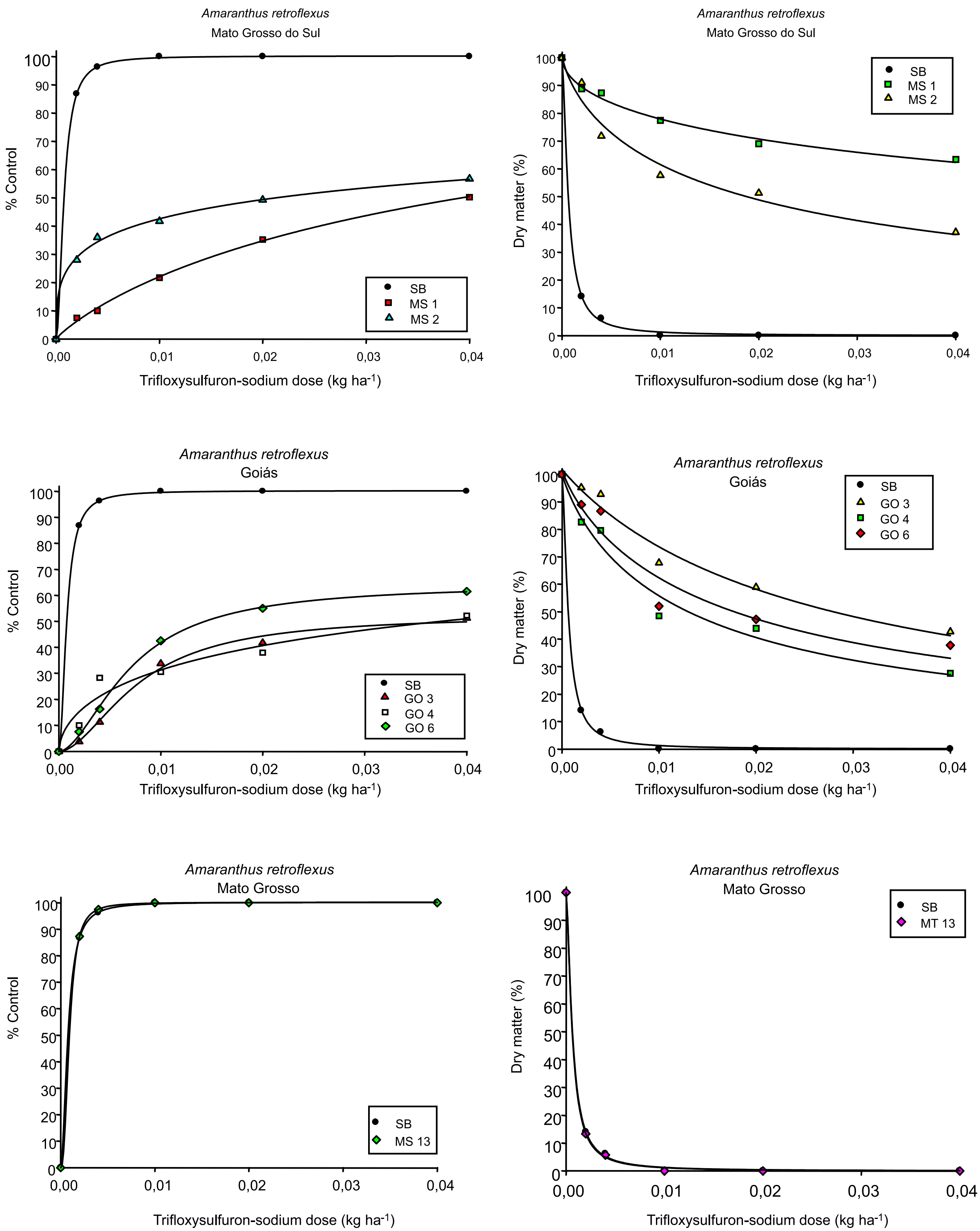

Figure 1 - Dose-response curve of the percentage of control (left) and relative production of dry mass of shoots (% relative to zero dose) (right) provided by the application of trifloxysulfuron-sodium on *Amaranthus retroflexus* biotypes from locations in the states of Mato Grosso do Sul, Goiás and Mato Grosso.

Table 3 - Estimates of the parameters a, b and c and the coefficient of determination (R^2) of the log-logistic model, adjusted for pyrithiobac-sodium and doses for 50% (I_{50}) and 80% (I_{80}) control of *Amaranthus retroflexus* in relation to the percentage of control at 28 days after application (DAA). Maringá, PR – 2011

Location	A	B	C	R2	I80 (kg ha^{-1})	I50 (kg ha^{-1})	RF
MS 1	100.3566	0.0059	-1.4779	0.99	0.0148	0.0058	0.42
MS 2	93.1602	0.0823	-7.09E-01	0.99	1.0482	0.1012	7.31
GO 3	62.9846	0.1262	-1.1038	0.99	-	0.4280	30.90
GO 4	120.4485	0.2491	-0.4469	0.99	1.1458	0.1156	8.34
GO 6	138.3409	1.0750	-0.7886	0.98	1.6042	0.5223	37.70
MT 13	100.7693	0.0085	-1.4074	0.99	0.0221	0.0084	0.60
SB*	100.3437	0.0139	-1.9997	0.99	0.0275	0.0138	1.00

* SB – susceptible biotype of *Amaranthus retroflexus*.

are at least 58 times the dose for this control relative to the SB.

In contrast, the dose required to control 80% of the susceptible biotype is only 0.0275 kg ha^{-1}, which represents only 19% of the recommended dose of pyrithiobac-sodium (Figure 2). The results obtained for the dry matter of shoots (Figure 2) are consistent with those of the control percentage and confirm the evidence that the pattern of dry mass accumulation of the aerial part of biotypes MS 2, GO 3, GO 4 and GO 6 differs significantly from that observed for the SB.

Therefore, it can be concluded that the biotypes MS 2, GO 3, GO 4 and GO 6 present resistance to pyrithiobac-sodium.

Considering the results for both herbicides, it is concluded also that although the natural occurrence of unique cases of resistance (such as the case of biotype MS 1, resistant only to trifloxysulfuron-sodium), in most cases the biotypes selected were cross-resistant to both trifloxysulfuron and pyrithiobac (MS 2, GO3, GO 4 and GO 6).

Different mechanisms are known in the process of inactivation of the herbicide in the plant, which can therefore be triggers of weed resistance, such as a reduction in the absorption and translocation of the herbicide in the plant, reducing the sensitivity of the site of action of the herbicide, increasing detoxification rate and/or decreasing activation rate of the herbicide, as well as its sequestration into the vacuole or apoplast (Devine & Eberlein, 1997).

However, in the particular case of the ALS inhibitor herbicides, reduced sensitibility of the site of action caused by mutation points in the ALS gene chain is the most common mechanism reported as a cause of causing weed resistance (Mallory-Smith and Namuth, 2012).

This type of resistance is usually caused by single amino acid substitution that can occur at various positions of the ALS enzyme gene (Shanner, 1999). The mutation of the acetolactate synthase enzyme reduces its sensitivity to these herbicides (Saari et al., 1994). The first case of *Amaranthus retroflexus* resistance to ALS inhibitors in Israel, for example, was caused by mutations in the ALS enzyme (Sibony et al., 2001). Seventeen different amino acid substitutions in the DNA sequence of the ALS enzyme are associated with the finding of resistance to ALS inhibitors in living organisms (Duggleby & Pang, 2000). However, only seven of these substitutions in the ALS enzyme have been described as causes of resistance in plants: Ala122, Pro197, Ala205, Asp376, Trp574, Ser653 and Gly654 (Powles & Yu, 2010).

There may yet be cases of weeds resistant to two or more herbicides from the same mechanism of action, which is characterized as cross-resistance. Cases of cross-resistance involving different herbicides with the same site and/or mechanism of action are usually also a result of the change in the site of enzymatic action (Christoffoleti & López-Ovejero, 2004). The cross resistance between ALS inhibitors depends on the position of the

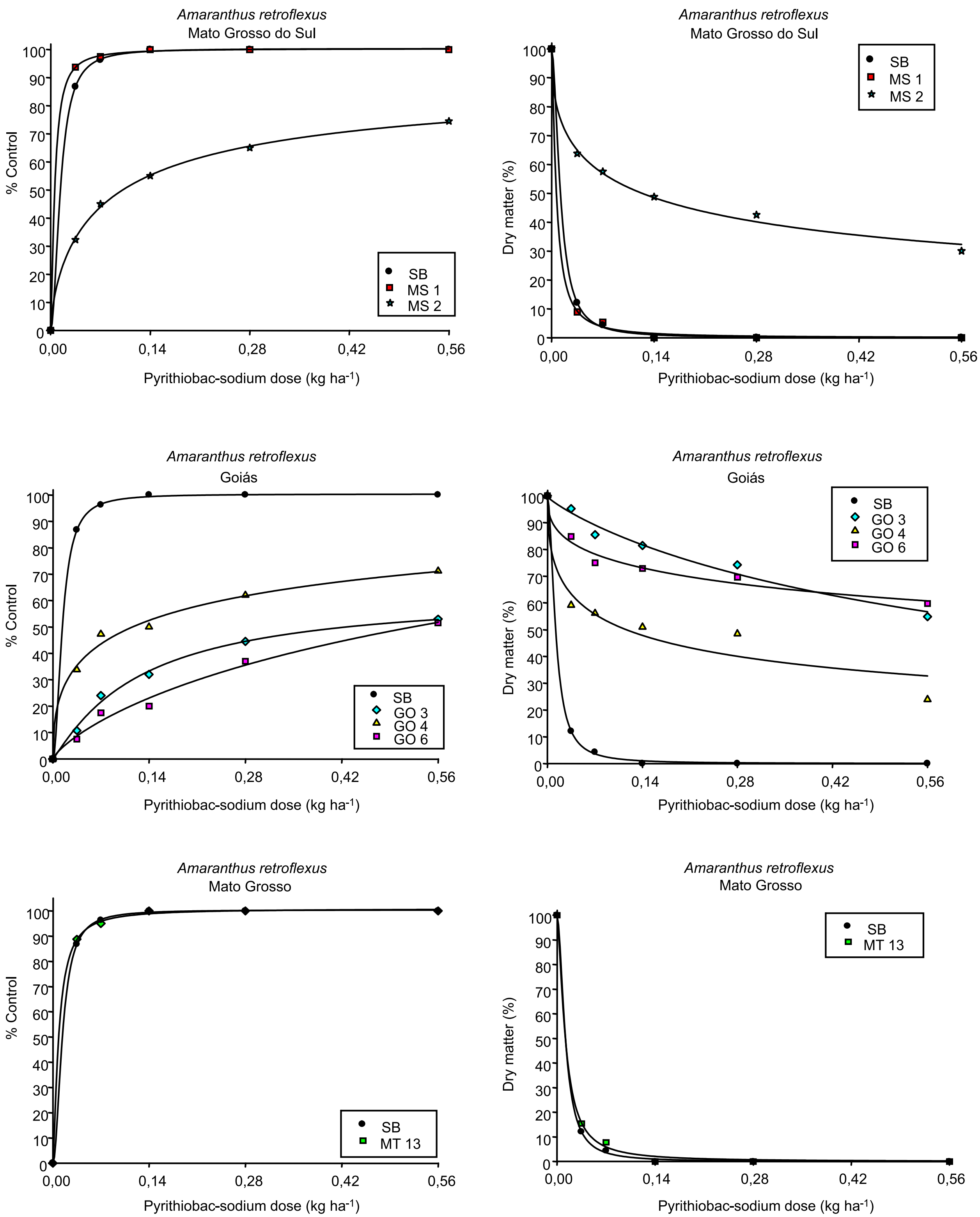

Figure 2 - Dose-response curve of the percentage of control (left) and relative production of dry mass of shoots (% relative to zero dose) (right) provided by the application of pyrithiobac-sodium on *Amaranthus retroflexus* biotypes from locations in the states of Mato Grosso do Sul, Goiás and Mato Grosso.

Table 4 - Biotypes of *Amaranthus retroflexus* that demonstrated resistance to trifloxysulfuron-sodium and pyrithiobac-sodium

Action mechanism	Chemical group	Herbicide	Location					
			MS 1	MS 2	GO 3	GO 4	GO 6	MT 13
ALS	Sulfonilureia	Trifloxysulfuron-sodium	X	X	X	X	X	
ALS	Pirimidil(tio)benzoate	Pyrithiobac-sodium		X	X	X	X	

substitution of amino acids and the specific substitution (Shaner, 1999). Substitutions at position Ala_{122} or Ser_{653}, for example, provide little or no resistance to herbicides of the sulphonylurea group and a high rate of resistance to herbicides from the imidazolinone group (Bernasconi et al., 1995; Devine & Eberlein, 1997; Patzoldt & Tranel, 2001). Likewise, substitutions at Pro_{197} confer resistance to sulfonylureas (Guttieri et al., 1992), but little or no resistance to the imidazolinone group. Substitutions at Trp_{574} or Ala_{205} provide resistance to the four chemical groups that inhibit ALS (sulphonyureas, imidazolinones, triazolopyrimidines and pyrimidyl(thio)benzoates) (Oliveira Jr., 2011). However, when the mutation occurs at position Ala_{205}, resistance levels are lower than those provided when the mutation occurs at position Trp_{574} in the amino acid sequence of the DNA of ALS (Whaley et al., 2007).

Taking into account the levels of RF found and the fact that resistance has manifested itself in relation to more than one chemical group within the ALS inhibitors, it is possible that the resistance mechanism involved is related to the inability of herbicides to bind to the ALS enzyme. This suggests that possible mutations in the ALS enzyme could be involved, although this confirmation depends on further studies.

Thus, cases of resistance to trifloxysulfuron-sodium in *A. retroflexus* biotypes from cotton growing areas in the locations of Mato Grosso do Sul (MS 1 and MS 2) and Goiás (GO 3, GO 4 and GO 6) were confirmed. Resistance to pyrithiobac-sodium in biotypes from Mato Grosso do Sul (MS 2) and Goiás (GO 3, GO 4 and GO 6) were also found. In addition, cases of cross-resistance to both herbicides were identified in MS 2, GO 3, GO 4 and GO 6 biotypes.

The summary of the data presented in this study (Table 4) indicates that, of the six suspected biotypes of *A. retroflexus*, five were resistant to trifloxysulfuron-sodium and four to pyrithiobac-sodium. Furthermore, four biotypes of *A. retroflexus* were resistant simultaneously to both ALS inhibitors tested, characterizing them as cases of cross-resistance. Thus, it was possible to confirm the suspected resistance in five biotypes of *Amaranthus retroflexus* derived from cotton producing areas of Mato Grosso do Sul and Goiás to ALS inhibitor herbicides (trifloxysulfuron-sodium and pyrithiobac-sodium).

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