SHORT COMMUNICATION

Sulfonylurea resistance in *Amaranthus hybridus* from southern Brazil

Rafael Romero Mendes[1], Vanessa Francieli Vital Silva[1], Luiz Augusto Inojosa Ferreira[1], Rubem Silvério de Oliveira Jr[1]*

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ABSTRACT

Amaranthus hybridus is a C4 broadleaf species widely spread across Brazilian agricultural territory. Recently, several herbicide resistance reports have been documented in southern Brazil, including the reports for enolpyruvilshikymate-3-phosphate (EPSPS)- and acetolactate-synthase (ALS)- inhibitors. The objective of this study was to confirm the existence of an ALS resistant (R) *A. hybridus* population from Paraná state. Dose-response experiments were conducted with R and a known susceptible (S) population with herbicides from three different chemical groups of ALS inhibitors. Biomass relative to untreated control was quantified and GR_{50} (dose for 50% of biomass reduction), GR_{90} (dose for 90% of biomass reduction) and resistant index (RI) were calculated based on non-linear regression analysis. The R population was 6.9-fold resistant to chlorimuron-ethyl and 6.5-fold resistant to metsulfuron-ethyl (sulfonylureas - SUL). Additionally, the recommended rates from each herbicide was not sufficient to reach 90% control to R based on GR_{90} parameter estimation. There was no resistance to imazethapyr (imidazolinone - IMI) and cloransulan-methyl (triazolopyrimidine - TRI) due to the low doses of GR_{90} and non-significant RIs. The R *A. hybridus* population investigated was resistant to ALS inhibitors chlorimuron-ethyl and metsulfuron-ethyl (SUL), but susceptible to IMI and TRI herbicides.

Keywords: chlorimuron-ethyl, metsulfuron-ethyl, acetolactate synthase, smooth pigweed.

INTRODUCTION

Acetolactate-synthase inhibiting (ALS)-herbicides have been globally commercialized since the 1960's (Garcia *et al.*, 2017). More than 50 different molecules are classified in five chemical groups: imidazolinones (IMI), sulfonylureas (SUL), triazolopyrimidines (TRI), *pyrimidinethiobenzoates* (PIR) and sulfonylamides (ST). These products are used in low doses, have low toxicity to mammals, are broad spectrum herbicides and selective to many crops due to the high number of molecules available in the market (Tranel & Wright, 2002).

Specially involving target site mutations at ALS gene, the frequency of ALS-resistant individuals in a weed community is usually high compared to other mechanisms of action (Preston & Powles, 2002). Despite that, ALS-inhibitors are still important tools in burndown programs and in weed management systems including glyphosate-resistant species for major crops such as soybeans (Santos *et al*., 2016; Zobiole *et al*., 2018).

Although metabolism or reduced translocation may participate in non-target site mechanisms for ALS resistance in some weeds, target site mutations are certainly the most frequent mechanisms of resistance found in the nature (Murphy & Tranel, 2019). By now, eight amino acid positions in *ALS* gene may harbor nucleotide changes that reduce the affinity of ALS herbicides to the enzyme: Ala122, Pro197, Ala205, Arg376, Asn377, Trp574, Ser653, and Gly654. Among them, many amino acid substitutions can confer distinct levels and patterns of ALS resistance (see ALS mutation database, Tranel *et al*., 2020).

One of the most relevant weeds from *Amaranthus* genus is *Amaranthus hybridus*. It is a C4 broadleaf species that can grow as much as 120 cm and produces more than 250.000 seeds per plant growing without competition during the season as evaluated by Sellers *et al*. (2003) in Missouri. Hence, *A. hybridus* has demonstrated to be a strong competitor for resources with several crops, such

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[1] Universidade Federal de Maringá, Maringá, Paraná, Brazil. rafaromero.mendes@gmail.com; vfvitalsilva@gmail.com; luizinojosaf@gmail.com; rsoliveirajr@gmail.com
*Corresponding author: rafaromero.mendes@gmail.com

as soybean, cotton, and sunflower (Carvalho *et al*., 2008; Soares *et al*., 2019). The concern about this species has increased in the last few years due to the reports of selection of resistant populations, specially to 5-*enolpyruvylshikimate*-3-phosphate (EPSPS)-, ALS-inhibitors, and synthetic auxins in Argentina (Dellaferrera *et al*., 2018; García *et al*., 2019; Perotti *et al*., 2019) or to EPSPS- and ALS- inhibitors in Brazil (Heap, 2021).

Currently, herbicide resistant *A. hybridus* populations are spread across cereal fields in southern Brazil, mainly in soybean areas in Rio Grande do Sul and in *Campos Gerais* region in Paraná. This study aimed to characterize an ALS-resistant *A. hybridus* population from *Campos Gerais* (Ventania, PR).

MATERIAL AND METHODS

Seeds from a putative resistant population were collected in a field located in Ventania (24°23'12" S, 50°09'27" W). The field has been cultivated with soybean in the summer and cereals (wheat or oat rotation) in the winter for more than ten years. Plants were collected and identified as *A. hybridus* running an identification key of *Amaranthus* species proposed by Senna (2015) and Milani *et al*. (2020). Seeds from different plants that survived to ALS-inhibitors applications were collected and bulked in paper bags and stored at room temperature. Resistant (R) and a known ALS-susceptible population (collected in Maringá, PR - 23°20'56" S, 52°04'26" W) were sown in 200-cell flats filled with potting soil (Horta3®, MecPlant). Seedlings were transplanted into 1 L-pots containing the same soil and kept in a greenhouse under 10 mm daily irrigation and average temperature of 30 °C (day) / 20 °C (night); and 60% humidity.

Preliminary single-dose herbicide screenings were performed with post emergence applications of glyphosate at 960 g ae ha^{-1} and chlorimuron-ethyl at 20 g ha^{-1}. Twenty-one days after treatment (DAT), 100% survival was observed for R population after chlorimuron application while all plants died after glyphosate application.

Dose-response experiments were conducted in two *A. hybridus* populations (resistant - R and susceptible – S) and the experiments were composed by seven herbicide doses and an untreated control (check). Four experiments of dose-response were performed, one for each ALS-inhibitor. For imazethapyr, cloransulam-methyl, and metsulfuron-ethyl, the doses were equivalent to 1/16, 1/8, 1/4, 1/2, 1, 2, and 4 times the labeled dose for S and 1/8, 1/4, 1/2, 1, 2, 4, and 8 times the labeled dose for R. For chlorimuron, R and S plants were treated with 1/8, 1/4, 1/2, 1, 2, 4, and 8 times the labeled dose. Labeled doses were chosen based on the recommendation of each herbicide (Rodrigues & Almeida, 2018), as follows: imazethapyr 106 g ha^{-1}, cloransulam 25.2 g ha^{-1}, chlorimuron 20 g ha^{-1}, and metsulfuron 2.4 g ha^{-1}.

Treatments were sprayed when plants had four fully expanded leaves (approximately 5 cm-tall). Each experimental unit (or replicate) was composed by 1L pot with three plants. The experiments were conducted in a completely randomized design with four replications. One experiment at the same conditions was conducted preliminarily to define a logical range of doses for each herbicide/population (data not shown).

Treatments were applied with a backpack sprayer and a hand boom equipped with three ST 110.015® nozzle (TeeJet Technologies), delivering 150 L ha^{-1} of solution and CO_2-pressurized at 30 psi. Treatments were applied when the air temperature was lower than 30 °C, humidity higher than 60 %, and wind speed under 3 km h^{-1}.

At 28 DAT, shoots were collected and kept in an oven at 65 °C for three days before biomass of each experimental unit had been quantified. Aboveground biomass data of each replication was expressed in percentage of untreated check for each population (Li *et al*., 2017; Schwartz-Lazaro *et al*., 2017). Data were submitted to non-linear regression analysis using the *dcr* package in R software (Ritz *et al*., 2015). A three-parameter log-logistic equation was fit:

$$Y = \frac{a}{\left[1 + \left(\frac{x}{GR50}\right)^{b}\right]}$$

Where, Y is the relative biomass (%), a is the asymptote, x is herbicide dose, GR_{50} is the dose to promote 50% of biomass reduction, and b is the sloop around GR_{50}. These parameters are illustrated in the Table 1. Resistant index (RI) was calculated by GR_{50} R/GR_{50} S ratio. GR_{50} of both populations were compared by t-test ($p > 0.05$) to identify significant differences between R and S GR_{50} parameters. The dose to provide efficient control (90%) was estimated through each model adjustment (Rana & Jhala, 2016).

RESULTS AND DISCUSSION

According to GR_{50} values, R population demonstrated two times less sensitive to imazethapyr than S (Table 1 and Figure 1a). However, t-test comparing GR_{50}-R and -S was not significant (p-value=0.0833), not confirming resistance to imazethapyr. Besides, a dose of 73.9 g ha^{-1} was sufficient to provide 90% control of R population, while the recommended dose is 106 g ha^{-1} (Rodrigues & Almeida, 2018). R and S plants demonstrated similar response to cloransulam (Figure 1b). RI value lower than one for R population and the relatively low dose required to provide GR_{90} (0.03 g ha^{-1}) evidentially illustrated sensibility to this herbicide (Table 1). Furthermore, the GR_{50}'s values were not different between R and S (p-value = 0.3871 in the t-test).

For chlorimuron, GR_{50} was 43.5 g ha^{-1} for R and 6.3 g ha^{-1} for S populations, respectively, resulting in an RI of 6.9 (Table 1). Doses higher than the highest dose sprayed in this experiment (640 g ha^{-1}) are needed to provide GR_{90}, suggesting that the recommended dose is no longer effective, which confirms the resistance to chlorimuron. (Figure 1c). Similar results were found for metsulfuron, another SUL herbicide (Table 1 and Figure 1d). GR_{50} values resulted in an RI of 6.5, and the GR_{90} was 7.1 g ha^{-1}, which is at least three times the labeled dose to control *Amaranthus* species (Rodrigues & Almeida, 2018).

Recently, ALS resistance in *A. hybridus* populations was broadly studied in the United States (Maertens *et al*., 2004; Whaley *et al*., 2006), Argentina (Larran *et al*., 2018; Dellaferra *et al*., 2018; García *et al*., 2019), and Italy (Milani *et al*., 2020). For the most populations already investigated, ALS resistance is often associated with cross-resistance to all chemical groups. Whaley *et al*. (2006) observed four *A. hybridus* populations resistant to IMI, SUL and PYR, and three of them were also resistant to TRI. Romagnoli *et al*. (2013) found several biotypes resistant to IMI, SUL, and TRI across central and northern Argentina. Differently, ALS-resistant *A. hybridus* from this research were considered resistant to SUL but susceptible to IMI and TRI (Table 1).

Herbicides from SUL group play important roles in cereal crop production due to their efficiency in weed control in modalities such as pre- and post-emergence in burndown applications (*e.g.* chlorimuron), post-emergence in wheat or oats (*e.g.* metsulfuron), and post-emergence in corn (*e.g.* nicosulfuron) (Oliveira Neto *et al*., 2019; Cholette *et al*., 2019). Therefore, the loss of SUL to control *A. hybridus* limits the options of herbicide treatments in these crops and crops and make difficult to plan weed control practices. The populations of *Amaranthus palmeri* resistant to glyphsoate and ALS inhibitors found in Brazil (Küpper *et al*., 2017) were introduced from Argentina (Alcántara-de-la-Cruz *et al*., 2020). Because the multiple resistance of *A. hybridus* to glyphosate and ALS inhibitors from Argentina is governed by multiple mutations in the target enzymes (triple mutation for high glyphosate resistance) (Larran *et al*., 2018, García *et al*., 2019), the risks that the populations of *A. hybridus* with these resistance profiles found in southern Brazil have also been introduced from Argentina is very great for high (Alcántara-de-la-Cruz *et al*., 2020).

Different cross-resistance profiles to ALS inhibitors are extremely dependent on the mechanism of resistance involved (Powles & Yu, 2010). Target site mutation from *A. hibrydus* biotypes was identified in four positions *ALS*

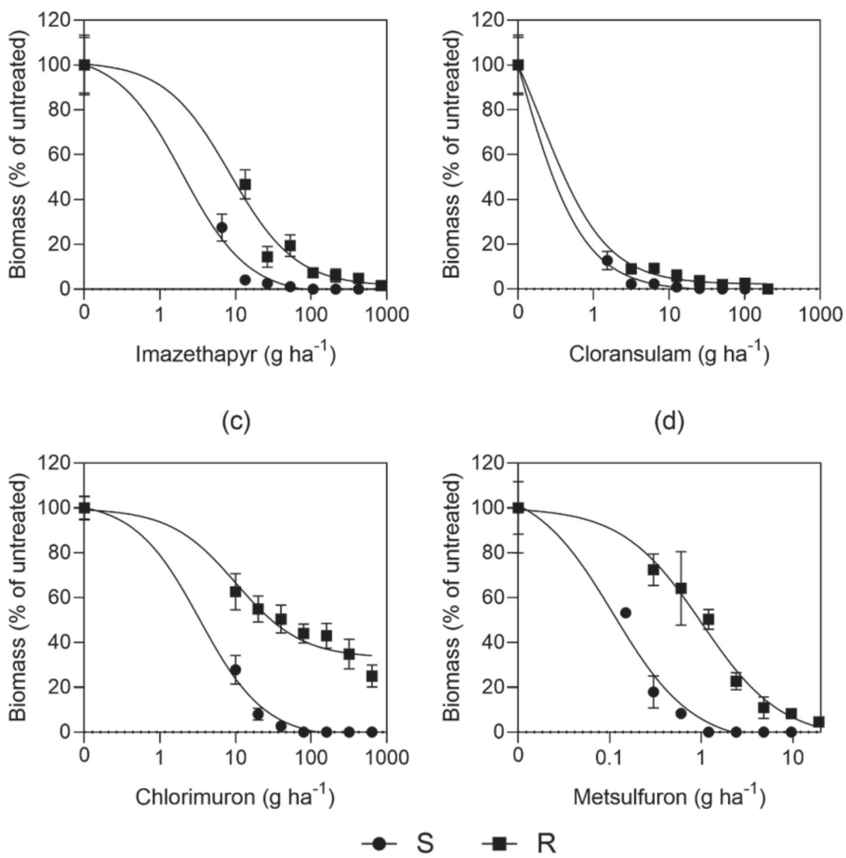

Figure 1: Dose-response curves for acetolactate-synthase-resistant (R) and susceptible (S) *Amaranthus hybridus* populations. (a) imazethapyr, (b) cloransulam-methyl, (c) chlorimuron-ethyl, and (d) metsulfuron-ethyl.

Table 1: Dose (g ha^{-1}) for 50% (GR_{50}) and 90% (GR_{90}) of biomass reduction, and resistant index (RI) of acetolactate-synthase-resistant (R) and susceptible (S) *Amaranthus hybridus* populations

Herbicide	Population	p-value	GR_{50}	RI(R/S) ratio	GR_{90}
Imazethapyr	R	<0.0001	9.7 (±2.6)	2 ns	73.9 (±27.8)
	S	<0.0001	4.7 (±1.1)		10 (±2.5)
Cloransulan-methyl	R	<0.0001	0.03 (±0.07)	0.05 ns	3.3 (±2.1)
	S	<0.0001	0.6 (±0.6)		1.7 (±0.3)
Chlorimuron-ethyl	R	<0.0001	43.5 (±15.2)	6.9 *	> 640
	S	<0.0001	6.3 (±1.4)		18.1 (±3.8)
Metsulfuron-ethyl	R	<0.0001	0.98 (±0.22)	6.5 ***	7.1 (±2.4)
	S	<0.0001	0.15 (±0.02)		0.4 (±0.1)

Significant by t-test at *0.05, **0.01, and ***0.001. ns: non-significant by t-test (p > 0.05).

gene so far. Ala122Thr conferred resistance only to IMI herbicides and susceptibility to other groups (Whaley *et al*., 2007). The remaining mutations documented (Asp376Glu, Trp574Leu, and Ser653Asn) endowed all chemical groups with simultaneous resistance (Whaley *et al*., 2006; Whaley *et al*., 2007). Typically, cross-resistance only to SUL herbicides is conferred by mutations at Pro197 (Tranel *et al*., 2020), which is probably the mechanism of resistance in this population. Further investigation on the mechanism of resistance must be conducted for the R population in future researches.

Once many glyphosate resistant *A. hybridus* biotypes have been reported in southern Brazil, including the *Campos Gerais* region (Penkcowski & Maschietto, 2019), understanding ALS resistance is one of the most important keys to implement efficient management strategies for *A. hybridus* control. This study identified a specific resistance pattern to ALS inhibitors in an *A. hybridus* population that is susceptible to glyphosate. Additional investigations are necessary in multiple resistant populations to identify resistance or susceptibility to SUL herbicides and other chemical groups of ALS inhibitors. Likewise, integrated weed management practices must be adopted to mitigate multiple resistance evolution, not only to *A. hybridus*, but also to other *Amaranthus* species present in Brazilian agricultural areas, such as *A. retroflexus* and *A. palmeri* (Francischini *et al*., 2014; Küpper *et al*., 2017). These practices should include mode of action rotation, pre-emergent applications, cultural control, mechanical control, and weed border control (Beckie & Harker, 2017).

CONCLUSION

The R *A. hybridus* population was resistant to ALS inhibitors herbicides chlorimuron-ethyl and metsulfuron-ethyl due to the RI calculated (6.5-fold and 6.9-fold, respectively) and also to the recommended rate for each herbicide no longer be enough to control R plants (GR_{90} > 640 g ha^{-1} for chlorimuron-ethyl and 7.2 g ha^{-1} for metsulfuron-ethyl). On the other hand, this population is not resistance to other ALS inhibitors imazethapyr (imidazolinone) and cloransulam-methyl (triazolopyrimidine).

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