

## *Amaranthus hybridus* L. RESISTANT TO GLYPHOSATE AND CHLORIMURON IN PARAGUAY

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### ABSTRACT

*Amaranthus* species are extremely problematic weeds due to their aggressiveness and resistance, being *Amaranthus palmeri* S. Watson, *Amaranthus retroflexus* L., *Amaranthus tuberculatus* (Moq.) J. D. Sauer, and *Amaranthus hybridus* L. prominent worldwide. The present research aimed to study *A. hybridus* populations in Paraguay where the use of glyphosate and chlorimuron herbicides presented management challenges. This research was conducted in the municipalities of Corpus Christi and Hernandarias, Paraguay, in 2016, 2017, 2018, 2019, and 2020. Weed seeds with resistance indicators were collected, cultivated, and following a screening process, cultivated for seed production (heritability up to F<sub>2</sub>), and the seeds were collected to obtain the herbicide dose-response curve. The herbicides glyphosate and chlorimuron were tested in portions of 1/8, 1/4, 1/2, 1, 2, 4, and 8 times the recommended dose on the package inserts (720 g acid equivalent [ae] ha<sup>-1</sup> and 20 g active ingredient [ai] ha<sup>-1</sup>, respectively). Among the *A. hybridus* populations evaluated in Paraguay, biotype from Hernandarias with multiple resistance to ALS-inhibiting (HRAC 2) and EPSPs-inhibiting (HRAC 9) herbicides, and biotype from Corpus Christi with resistance to ALS-inhibiting herbicides (HRAC 2) were found. Therefore, rotation and combination of herbicides, as well as integration with non-chemical measures, are essential to control and prevent selection of resistant biotypes. Studies aimed to develop integrated weed management plans are essential for effective control. In countries like Paraguay, this becomes imperative because of the lack of research on this topic.

**Keywords:** smooth pigweed, herbicides, acetolactate synthase (ALS) inhibitors, 5-enol-pyruvyl-shikimate-3-phosphate (EPSPS) inhibitors, weed.

## INTRODUCTION

The high prolificacy, competitive ability, and resistance to treatments of *Amaranthus* species are a concern, especially palmer amaranth (*Amaranthus palmeri* S. Watson), redroot pigweed (*Amaranthus retroflexus* L.), and tall waterhemp (*Amaranthus tuberculatus* [Moq.] J. D. Sauer), and smooth pigweed (*Amaranthus hybridus* L.) (Meyer et al., 2015; Roberts and Florentine, 2022) being prominent weeds worldwide. In recent years, increased occurrence, and challenges in controlling smooth pigweed have been highlighted in South America.

Smooth pigweed is native to America (Sauer, 1967) and is sub-cosmopolitan (Costea et al., 2004). It propagates through seeds, and a single plant can produce approximately 60,000 seeds (Weaver, 1984; Costea et al., 2004). *Amaranthus* species are resistant to several herbicides worldwide, mainly protoporphyrinogen oxidase (PPO), 5-enolpyruvyl-shikimate-3-phosphate (EPSP), and acetolactate synthase (ALS) inhibitors (Heap, 2023).

Resistance to ALS-inhibiting herbicides in *A. hybridus* has been observed in neighboring Paraguay, like Brazil, where, there are cases of *A. hybridus* resistance to glyphosate (Resende et al., 2022), multiple resistance to glyphosate and chlorimuron (Heap, 2023), and resistance to chlorimuron and metsulfuron (Mendes et al., 2022). In Argentina, multiple resistance to glyphosate and ALS inhibitors has been highlighted (García et al., 2020). Therefore, monitoring resistance in plants and treatment using rotation and mixtures of herbicides, as well as integration with non-chemical measures, is essential to control and prevent selection of resistant biotypes (Meyer et al., 2015).

*Amaranthus* plants are extremely competitive and have adapted to grain crops (Korres et al., 2019). Studies indicate that 4.6 plants m<sup>-2</sup> of *A. hybridus* can reduce soybean yield by 25–30% (Toler et al., 1996). Considering the magnitude of the problem, it is essential to focus on integrated weed management involving a multiple set of effective practices (Harker et al., 2013; Soltani et al., 2023). This includes strategies that range from monitoring through phytosociological surveys and identification of resistance, which results in efficient agricultural control measures, such as cultural, mechanical, and chemical practices (Marochi et al., 2018; Albrecht et al., 2020a, Chauhan, 2020). For example, the combination of pre- and post-emergence herbicide application with a population of 247,000 soybean plants ha<sup>-1</sup> was advantageous in the management of *Amaranthus* spp. (Butts et al., 2016). The

association of cover crops with herbicides in the integrated management of *A. hybridus* was also effective (Bunchek et al., 2020).

Nevertheless, there are many challenges in weed management that Paraguay share, which emphasizes the need for further investigation for better control. Furthermore, the problems encountered in this region can be applicable throughout Latin America and serve as a study model to configure actions in all parts of the world. Paraguay has an area of 406,752 km<sup>2</sup>, with approximately 31 million ha arable land, of which 3.7 million ha are cultivated with soybeans in the 2022–2023 growing season (Ministerio de Agricultura y Ganadería [MAG], 2022). In Paraguay, the first case of herbicide-resistant weed was reported in 1995 in Itapúa by Adolfo Benegas. It was reported *Euphorbia heterophylla* L. resistant to imazethapyr, an herbicide with an ALS-inhibiting mode of action (Heap, 2023).

The objective of this study was to monitor and investigate the resistance of *A. hybridus* to herbicides, specifically to analyze cases with an indication of multiple resistance. The work was conducted in conjunction with researchers, technicians, and farmers from Paraguay, a country bordering Brazil, which faces similar weed management challenges but lacks extensive weed science research.

## MATERIAL AND METHODS

### *Amaranthus hybridus* monitoring and screening

*Amaranthus hybridus* seeds were collected during the 2018–2019 and 2019–2020 growing seasons. Seed collection followed the methodology proposed by Burgos et al. (2013). Seeds were collected after herbicide application from one or more plants with similar characteristics, at specific control failure points. Collections were carried out on different farms in the regions studied, based on information received from farmers and agronomists, with only one collection per plot.

In 2020, screening was performed to select susceptible and resistant biotypes to be used for determining herbicide dose-response curves. Plants were cultivated in greenhouses in the Districts of Hernandarias and Corpus Christi, Department of Alto Paraná and Canindeyú, Paraguay, respectively.

Seeds of the first generation (F<sub>1</sub>) were sown and thinned after emergence, leaving one seedling per pot, with six replicates. The herbicides tested in F<sub>1</sub> were glyphosate (Roundup Full® II, Monsanto Paraguay S.A., Asunción, Paraguay) at dose of 720 g acid equivalent [ae] ha<sup>-1</sup> and chlorimuron (Poker® 75 WG, Glymax Paraguay S.A., Hernandarias, Paraguay) at dose of 20 g

active ingredient [ai] ha<sup>-1</sup>. The experimental units were pots containing 1 dm<sup>3</sup> of vermiculite under greenhouse conditions.

Treatments were applied to plants with four leaves, one plant per pot. All herbicides were applied using a CO<sub>2</sub> pressurized backpack sprayer equipped with four flat-fan nozzles AIXR 110.015 (TeeJet Technologies, Wheaton, IL) at a pressure of 240 kPa and a speed of 1 ms<sup>-1</sup>, delivering an application volume equivalent to 200 L ha<sup>-1</sup>. The pots were removed from the greenhouse for application and brought back 1 h after application. The weed control was evaluated at 28 days after application (DAA) of herbicides, through visual inspection (0 for no injuries, up to 100% for plant death) (Velini et al., 1995).

### Dose-response curve

After screening, two biotypes (susceptible and suspected resistance) were selected for the dose-response curve (F<sub>2</sub> generation). The experiments were conducted using a completely randomized design with six replicates. The sowing process, growing conditions and growth stage for herbicide application were the same as those used in screening.

The treatments consisted of glyphosate (0, 90, 180, 360, 720; 1,440; 2,880 and 5,760 g ae ha<sup>-1</sup>; Roundup Full® II) or chlorimuron (0, 2.5, 5, 10, 20, 40, 80, and 160 g ai ha<sup>-1</sup>; Poker® 75 WG) combined with 0.5% (v/v) emulsifiable mineral oil. The doses used represent the normal field doses at 0, 1/8, 1/4, 1/2, 1, 2, 4 and 8 times.

The shoots were collected at 28 DAA of herbicides to determine dry mass. Plants were cut at the soil surface, collected in paper bags, oven-dried at 70 °C for 4 d (to constant mass), and then weighed. Data were subjected to analysis of variance and regression, the SigmaPlot® 13 (Systat Software Inc.) was used. When significant, were fitted to the non-linear logistic regression model proposed by Streibig (1988):

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

where  $y$  is the response variable (percentage dry mass reduction of shoot);  $x$  is the dose of the herbicide (g ha<sup>-1</sup>);  $a$ ,  $b$  and  $c$  are the parameters estimated, in which  $a$  is the amplitude between the maximum and the minimum value of the variable;  $b$  is the dose that elicited 50% response; and  $c$  is the slope of the curve around  $b$ .

The non-linear logistic model provides an estimate of the growth reduction by 50% (GR<sub>50</sub>). In this way, we decided to use mathematical calculation through the inverse equation of Streibig (1988), allowing the calculation of GR<sub>50</sub>

as proposed by Souza et al. (2000). The models used to obtain GR<sub>50</sub> were the same as those used in other studies (Takano et al., 2016; Takano et al., 2017; Albrecht et al., 2020b).

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

Based on the values of GR<sub>50</sub>, we calculated the resistance factor (RF = GR<sub>50</sub> of the resistant biotype/GR<sub>50</sub> of the susceptible biotype). The RF expresses the number of times the dose required to control 50% resistant biotypes is greater than the dose to control 50% susceptible biotypes (Burgos et al., 2013). Experimental procedures, the development of the dose-response curve and the statistical analysis were adopted in line with the current literature and recent publications (Zobiolo et al., 2019; Albrecht et al., 2020b; Albrecht et al., 2020c).

## RESULTS AND DISCUSSION

After F<sub>2</sub>, *A. hybridus* resistance to glyphosate and chlorimuron was identified in the Paraguayan locations. The biotypes were identified in Hernandarias, Alto Paraná department, and Corpus Christi, Canindeyú department, in areas cultivated with soybean and maize (Table 1). For the resistant biotype located in Hernandarias, the GR<sub>50</sub> was 257.34 g ae ha<sup>-1</sup> with RF of 2.56 for glyphosate, while for chlorimuron it was 16.79 g ai ha<sup>-1</sup> with RF of 20.78 (Table 2).

For the resistant biotype located in Corpus Christi, the GR<sub>50</sub> was 26.33 g ai ha<sup>-1</sup> with RF of 12.4 for chlorimuron, while it was 142.86 g ae ha<sup>-1</sup> with RF of 1.06 for glyphosate (Table 2). For a biotype to be considered resistant, the FR must be >1 and the growth reduction by 80 (GR<sub>80</sub>) > than the recommended dose of the herbicide (Takano et al., 2017). Even with FR >1, GR<sub>80</sub> did not exceed the recommended dose (720 g ae ha<sup>-1</sup>) of glyphosate. This does not point to agronomic resistance but shows a low level of resistance.

This proves the resistance of the biotypes in the F<sub>2</sub> generation after evaluation. Biotype from Hernandarias with multiple resistance to ALS - (HRAC 2) and EPSPs - (HRAC 9) inhibiting herbicides, and biotype from Corpus Christi with resistance to ALS - (HRAC 2) inhibiting herbicides were found. After statistical analysis and application of non-linear regression, models and graphs (Fig. 1) were generated as commonly described in the literature (Burgos et al., 2013; Takano et al., 2016; Takano et al., 2017; Zobiolo et al., 2019; Albrecht et al., 2020b; Albrecht et al., 2020c).

**Table 1.** Geographical location of *A. hybridus* seed collection areas with proven resistance.

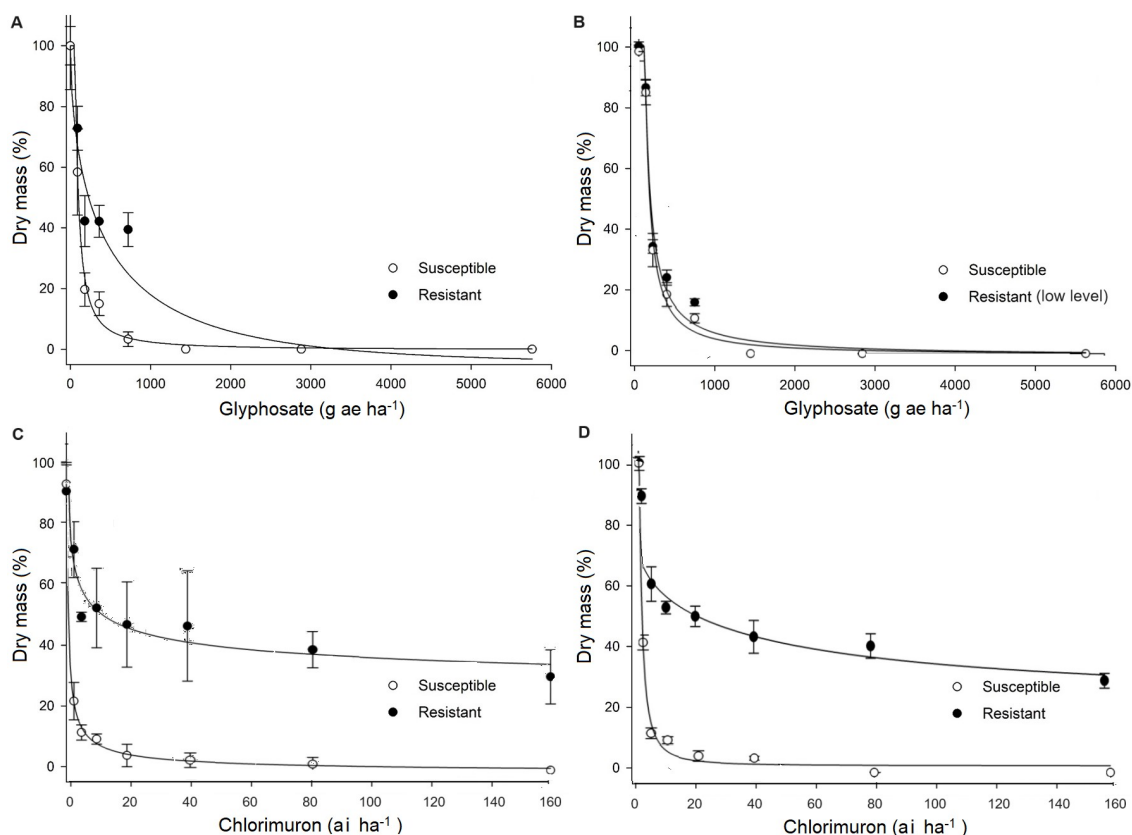
Location	Susceptibility	Coordinates	Agricultural crops
1. Hernandarias, Alto Paraná	Resistant	25°02'58"S 54°54'12"W	Soybean and maize
	Susceptible	25°00'01"S 54°52'59"W	
2. Corpus Christi, Canindeyú	Resistant	24°26'16"S 55°34'42"W	
	Susceptible	24°27'48"S 55°39'50"W	

**Table 2.** Herbicide dose for 50% growth reduction ( $GR_{50}$ ) and the resistance factor (RF) for *A. hybridus*.

Location	Glyphosate		Chlorimuron	
	$GR_{50}$	RF	$GR_{50}$	RF
1. Hernandarias, Alto Paraná	100.66	-	0.81	-
	257.34	2.56	16.79	20.78
2. Corpus Christi, Canindeyú	142.86	-	2.12	-
	151.80	1.06	26.33	12.40

Doses in g active ingredient  $ha^{-1}$  for chlorimuron and g acid equivalent  $ha^{-1}$  for glyphosate.

RF =  $GR_{50}$  of the resistant biotype/ $GR_{50}$  of the susceptible biotype.

**Fig. 1.** Dose-response curve for dry mass reduction of *A. hybridus* biotypes under glyphosate (A: Hernandarias, B: Corpus Christi) and chlorimuron (C: Hernandarias, D: Corpus Christi) application.

Resistance to ALS-inhibiting herbicides in *A. hybridus* has been observed in neighboring Paraguay, like Brazil, where there are cases of *A. hybridus* resistance to glyphosate (Resende et al., 2022), multiple resistance to glyphosate and chlorimuron (Heap, 2023), and resistance to chlorimuron and metsulfuron (Mendes et al., 2022). In Argentina, multiple resistance to glyphosate and ALS inhibitors has been highlighted (García et al., 2020). To our knowledge, the present multiple resistance case is the first report for this species in Paraguay.

Regarding the mechanisms of resistance to the EPSPS (glyphosate) and ALS inhibitors, the triple substitution of amino acids TAP□IVS was identified in the resistant biotype of *A. hybridus* in Argentina (Perotti et al., 2019). Furthermore, the plants also showed increased EPSP expression compared to that in susceptible plants. A Ser653Asn substitution was found in the ALS sequence, explaining the cross-resistance pattern to the ALS-inhibiting families of herbicides (pyrimidylthiobenzoates, sulfonylureas, and triazolopyrimidines) (García et al., 2020).

*Amaranthus* species are highly problematic weeds because of their aggressive dispersal and resistance. In recent years, there has been an increase in the occurrence and difficulty in controlling *A. hybridus*. As previously indicated, *Amaranthus* species show resistance to several herbicides, mainly PPO, glyphosate, and ALS-inhibiting herbicides. Resistance gene flow is mediated by seeds, playing an important role in the dissemination of *Amaranthus* spp. herbicide resistant (Yannicari et al., 2023). Therefore, rotation and combination of herbicides, as well as integration with non-chemical measures, are essential for controlling and preventing the selection of resistant biotypes (Braz and Takano, 2022; Soltani et al., 2023). The combination of pre- and post-emergence herbicide application with a population of 247,000 soybean plants ha<sup>-1</sup> was advantageous in the management of *Amaranthus* spp. (Butts et al., 2016). The association of cover crops with herbicides in the integrated management of *A. hybridus* was also effective (Bunchek et al., 2020).

Coffman et al. (2021) observed that the application of dicamba could be an alternative for the control of the PPO-resistant *A. palmeri*; however, lower levels of control were observed in the PPO-resistant population than in the susceptible population. Other studies have reported the use of glufosinate to control *Amaranthus* spp. (Hay et al., 2019; Browne et al., 2020), especially in sequential applications and management programs including synthetic auxins (Cuvaca et al., 2020), with better efficacy in

young plants and cover crops.

A significant finding is the use of herbicides with residual effects that can be applied in the off-season, especially in the pre-sowing period of soybeans. Flumioxazin, metribuzin (De Sanctis et al., 2021; Houston et al., 2021), sulfentrazone + cloransulam (Houston et al., 2021), pyroxasulfone + flumioxazin, pyroxasulfone, and acetochlor (Perkins et al., 2021) have shown good results in controlling *Amaranthus* spp.

Therefore, population monitoring is important for effective control. Monitoring weed resistance is a useful and essential practice to understand, identify, and quantify the frequency of resistant plants in advance (Schultz et al., 2015). Resistance monitoring studies have led to increased research and thus the development of new techniques for controlling resistant or tolerant plants (Rubione and Ward, 2016; Comont and Neve, 2021).

## CONCLUSIONS

Among the *A. hybridus* populations evaluated in Paraguay, biotype from Hernandarias with multiple resistance to ALS-inhibiting (HRAC 2) and EPSPs-inhibiting (HRAC 9) herbicides, and biotype from Corpus Christi with resistance to ALS-inhibiting herbicides (HRAC 2) were found. Therefore, rotation and combination of herbicides, as well as integration with non-chemical measures, are essential to control and prevent selection of resistant biotypes.

Studies aimed to develop integrated weed management plans are essential for effective control. In countries like Paraguay, this becomes imperative because of the lack of research on this topic.

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