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RESEARCH ARTICLE

***Amaranthus* spp.: a genus of troublesome weeds**

Geographical distribution and resistance level to chlorimuron of *Amaranthus* spp. populations in the main soybeans producing regions of Brazil

Acácio Gonçalves Netto[a], Laís S. Resende[a], Marcelo R. Malardo[a], Jéssica C. Presoto[a], Jeisiane de F. Andrade[a], Marcelo Nicolai[b], Ramiro F. L. Ovejero[c], Saul J. P. de Carvalho*[d]

[a] Luiz de Queiroz College of Agriculture, Piracicaba, SP, Brazil. [b] Agro do Mato Soluções Agronômicas, Santa Bárbara d'Oeste, SP, Brazil. [c] Bayer Crop Science Brasil, São Paulo, SP, Brazil. [d] Federal Institute of Education, Science and Technology of the South of Minas Gerais, Machado, MG, Brazil.

Abstract: Background: Species of *Amaranthus* genus are common in agricultural areas of Brazil. Such weeds are problematic, and they bring complexity to the management mainly due to herbicide resistance. Thus, the monitoring and mapping of chlorimuron-resistant *Amaranthus* spp. is necessary to detect resistance in different locations of Brazil.

Objective: Elaborate discriminating dose of distinction between *Amaranthus* spp. populations that are susceptible and resistant to chlorimuron and monitor the resistance dispersal throughout five crops.

Methods: 33 pigweed populations from the main grain producing properties in Brazil by means of dose-response curves were evaluated. For the D dose, chlorimuron dose of 20 g ha^{-1} ai was considered. Once the discriminating dose was identified, a monitoring screening of the dispersal of resistance of *Amaranthus* spp. to chlorimuron was conducted with 226 samples between the 2016 and 2020 crops.

Results: The discriminating dose ("base line") considered ideal to control susceptible plants was 20 g ha^{-1} of chlorimuron. Among 226 pigweed samples evaluated in the five years of monitoring, 74% of populations were considered susceptible (S), while those classified as resistant (R) and segregating (r) did not exceed 26.0%.

Conclusions: By comparing susceptible biotypes of *Amaranthus* spp. with international scientific literature standards and leaflet averages, it could be safely concluded that the discriminating dose of chlorimuron is 20 g ha^{-1}. Resistance of *Amaranthus* spp. to chlorimuron in Brazil is present in the main soybean producing regions evaluated, with a frequency of 26% of the total samples evaluated.

Keywords: Acetolactate synthase inhibitor; Resistance monitoring; Dose-response assay

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* Corresponding author:
<sjpcarvalho@yahoo.com.br>

1. Introduction

Amaranthus genus has agronomically important weeds, including *Amaranthus palmeri* S. Wats, *Amaranthus hybridus* L., *Amaranthus retroflexus* and *Amaranthus viridis* (Kissman, 2000). Such species are problematic in agricultural areas mainly due to the rapid growth and large amount of seeds that they produce (Horak, Loughin, 2000).

Furthermore, these four species have resistance reported in Brazil to herbicides with different mechanisms of action (Heap, 2021). The first report of *Amaranthus* resistance in Brazil refers to *Amaranthus viridis* resistant to ALS and FSII inhibitor herbicides (Heap, 2021). *Amaranthus retroflexus* with multiple resistance to ALS and FSII inhibitor herbicides, and another case of resistance to PROTOX was also reported in Brazil (Heap, 2021). *Amaranthus palmeri* has also been reported with multiple resistance to glyphosate and ALS inhibitor herbicides in cotton agricultural areas (Gonçalves Netto et al., 2016). The most recent report of resistance in Brazil refers to *Amaranthus hybridus* with multiple resistance to glyphosate and ALS inhibitors (Penckowski, Maschietto, 2019).

ALS inhibitor herbicides have been commercially produced since the early 1980s (Wise et al., 2009). Those herbicides are widely used to manage glyphosate-resistant populations, which has probably led to the evolution of multiple resistance (Peterson et al., 2018). Among them, chlorimuron is a common choice among farmers for pre- or post-emergence chemical control of *Amaranthus* spp. mainly in grain crops. The cases of Amaranthus resistance to chlorimuron are complex and of concern for the production system. Control failures are recurrent complaints among farmers. However, it is not clear whether they are being caused due to resistance or not.

Monitoring weed resistance to herbicides allows the detection of it at low frequencies in order to provide an alert system and develop assertive management strategies in the field (Davis et al., 2008). In order to compare different populations, it is common to determine the dose that causes 50% mass reduction (GR50) and/or establish the dose required to kill 50% of the plants (LD50) (Burgos et al., 2013). To determine the discriminating dose and differentiate susceptible and resistant populations, ideally one should compare the responses of multiple susceptible populations to obtain sensitivity data for a given herbicide (Burgos et al., 2013). With such data, it is possible to make comparisons among distinct populations and detect changes in sensitivity and resistance evolution in different locations (Escorial et al., 2019).

Thus, this work has been developed aiming to elaborate the "base line" of *Amaranthus* spp., thus generating the discriminating dose of distinction between susceptible and resistant populations of *Amaranthus* spp. to chlorimuron, by means of dose-response curves, in post-emergence stage of the weed, as well as monitoring the resistance dispersal throughout five crops.

2. Material and Methods

The study has been developed in the greenhouse of *Agro do Mato Soluções Agronômicas*, in Santa Bárbara d'Oeste, São Paulo, Brazil (22º 48'S; 47º 280' W; 605 m altitude). The work has been divided into two stages: the first stage consisted of the identification and separation of susceptible biotypes from resistant biotypes. In the second stage, a chlorimuron base line for susceptible biotypes was effectively elaborated.

2.1 Plant Material

The first stage consisted of evaluating 33 populations of *Amaranthus* spp. from the main soybean producing properties located in the states of Bahia, Goiás, Maranhão, Minas Gerais, Mato Grosso do Sul, Mato Grosso, Pará, Piauí, Paraná, Rio Grande do Sul, Santa Catarina, São Paulo e Tocantins. In each area, seeds were collected from at least 20 plants per population, at the stage of full physiological maturity. At the time of collection, the geographic coordinates of each sample point were written down (Table 1).

2.2 Identification of susceptible and resistant biotypes by means of dose-response curve

In order to install the experiment, seeds were distributed in 2.0-L plastic boxes, filled with a proportion of commercial substrate (Pinus bark, peat and vermiculite) and vermiculite (3:1; v:v). At the two true leaf stage, the seedlings were transplanted to 1L-pots filled with the same substrate mixture, where they remained until the end of the experiment, at an average density of three plants per pot. During the experiment, the plots were equally fertilized and irrigated for plant growth and development.

The susceptibility of the populations was quantified using dose-response curves. The treatments were arranged in randomized blocks, with 6 treatments and 4 replicates. The herbicide doses used were 8D, 4D, D, 1/4D, 1/8D, and absence of herbicide. For the D dose, a dose of 20 g e.a. ha^{-1} was considered. The dose-response curves were performed only one time with all populations. The spraying

Table 1 - Sample populations of *Amaranthus* spp. state of collection and geographic coordinates. Santa Bárbara D'Oeste – SP, 2021.

State	Geographic coordinates	
	Latitude	Longitude
BA	-5.1867	-45.6906
BA	-12.0969	-45.8417
BA	-11.6381	-45.4636
BA	-11.6728	-45.4317
MA	-9.4900	-45.5622
MA	-7.5331	-46.0350
MG	-16.3517	-46.9119
MS	-22.2208	-54.8058
MT	-13.6906	-57.8919
MT	-13.6906	-57.8917
MT	-13.6914	-57.8914
MT	-15.5625	-54.2947
MT	-13.0331	-55.9439
MT	-15.4447	-54.6669
MT	-13.9269	-55.6669
MT	-15.3225	-54.8519
MT	-15.3208	-54.8564
MT	-16.8431	-53.9650
MT	-17.3464	-54.4589
MT	-12.0450	-55.9725
MT	-12.0458	-55.9725
PR	-24.7214	-53.7872
PR	-24.5967	-53.7872
PR	-24.5208	-50.3500
PR	-24.5331	-50.8111
PR	-25.9978	-52.8111
PR	-24.1006	-52.3597
PR	-22.1589	-43.5811
RS	-29.3761	-52.0442
RS	-27.9914	-51.4597
RS	-28.1508	-52.4164
RS	-29.0289	-51.1817
RS	-28.2611	-52.4083

BA: Bahia, MA: Maranhão, MG: Minas Gerais, MS: Mato Grosso do Sul, MT: Mato Grosso, PR: Paraná, RS: Rio Grande do Sul was performed at the 3 to 4 leaf pairs stage. For this, a CO_2 pressurized precision knapsack sprayer was used, coupled to a boom with two TeeJet 110.02 type tips, positioned at 0.50 m from the targets, with relative syrup consumption of 200 L ha^{-1}.

The percentage control and residual dry mass were evaluated at the 28th day after application (DAA). For the control evaluation, 0% was assigned in the case of absence of symptoms caused by the herbicide and 100% for plant death. The plant mass was obtained from the harvest of the remaining material in the plots, with subsequent drying in an oven at 70°C for 72 hours. The dry mass was corrected to percentage values by comparing the mass obtained in the herbicide treatments with the mass of the control considered 100%.

Data analysis was performed by applying the F test in the variance analysis. The dose-response curves were fitted to a logistic non-linear regression model. The control variable was adjusted to the model proposed by Streibig (1988).

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

In which: y = percentage of control; x = dose of herbicide; and a, b and c = parameters of the curve, so that a is the difference between the maximum and minimum points of the curve; b is the dose that provides 50% response of the variable and c is the slope of the curve.

For the variables residual fresh and dry mass, the model proposed by Seefeldt et al. (1995) was adopted;

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

In which: y = control percentage; x = dose of herbicide; and a, b, c and d = parameters of the curve, so that a is the lower limit of the curve, b is the difference between the maximum and minimum points of the curve, c is the dose that provides 50% response of the variable and d is the slope of the curve.

After the analyses of the dose-response curves, the response pattern of the cumulative C_{50} and GR_{50} of the populations was evaluated, aiming to separate resistant and susceptible individuals by means of these parameters for the elaboration of the "base line" of susceptibility.

2.3 Elaboration of discriminating dose ("base line") of susceptibility of *Amaranthus* sp. to chlorimuron herbicide

At this step, we used only the individuals considered susceptible to chlorimuron in the previous one. The susceptibility of the populations was quantified by means of dose-response curves according to the methodology described in the first step of the experiments. In this step, the susceptibiliy was verify only one time.

After the analyses of the dose-response curves, the response pattern of the accumulated C_{80} and GR_{80} of the populations was evaluated, as well as their confidence interval, given by the formula:

$$\hat{m} \pm t_o \frac{S}{\sqrt{r}}$$

In which $\hat{m}$ = estimated average of the repetitions; t_o = value present in the t-test table; s = standard deviation and r = number of replications.

2.4 Monitoring the spread of *Amaranthus* spp. to the herbicide chlorimuron

An amount of 226 *Amaranthus* spp. samples were collected during the five years of monitoring. These *Amaranthus* spp. seeds from different soybean producing regions of Brazil were collected throughout the 2016 (19 samples), 2017 (63 samples), 2018 (51 samples) and 2019 (44 samples) and 2020 (49 samples) crops. Populations were originary from the states of Bahia, Goiás, Maranhão, Minas Gerais, Mato Grosso, Mato Grosso do Sul, Pará, Piauí, Paraná, Rio Grande do Sul, Santa Catarina, São Paulo and Tocantins. Seed collection occurred between the months of January and March of each crop, in areas where control failures were observed after the application of chlorimuron.

The collections were made in bulk, being sampled approximately 50 plants per collection site, forming a composite sample of at least 1,000 seeds (Burgos et al., 2013). Seeds were stored in paper bags and identified as to the geographic coordinates, municipalities and state pertinent to each one.

For the installation of the experiment, seeds were distributed in excess in plastic trays, with capacity for 1 liter of substrate. When the plants were in the vegetative development stage of fully expanded cotyledonary leaves (Hess et al., 1997), they were transplanted into 200 mL pots filled with commercial substrate, where they were kept until the end of the experiment at the density of 3 plants per pot.

The experimental design was entirely randomized, with four repetitions. The dose of 20 g ha^{-1} of chlorimuron for post-emergence control used in the experiment was determined according to the results obtained in the previous stage of the research. A single dose can be used for the classification of populations for resistance if it results in the survival of resistant plants and the death of susceptible ones (Burgos et al., 2013).

Applications were made when plants had 3 to 4 pairs of leaves. The sprays were made prioritizing favorable environmental conditions: relative humidity above 60%, temperature below 30 °C and moist soil. A CO_2-based constant pressure backpack sprayer was used, composed of two commercial brand XR 110.02 fan spray tips, calibrated at an application volume of 200 L ha^{-1}.

The control of the plants was evaluated using a scale of 0 to 100%, where 0% means no damage caused and 100% means plant death. The evaluations occurred at 28 days after application (DAA) of the treatments and were used to classify the populations as resistant (R), segregating (r) or susceptible (S), based on the methodology used by López-Ovejero et al. (2017) (Table 2).

Based on the geographic coordinates of each collection site and the results of the sample evaluation, maps with the spatial distribution of the collected samples were prepared using QGIS 2.14.12 software (QGIS Development Team, 2017). The points of each pigweed population were colored on the maps according to their respective classification (Figure 1) after the control evaluation at 28 DAA. The frequency of populations in the different states with resistance to chlorimuron as well as the percentage of susceptibility were calculated.

3. Results and Discussion

3.1 Identification of susceptible and resistant biotypes by means of dose-response curves

After the susceptibility analysis of the 33 individuals from the states of Goiás, Mato Grosso and Paraná, 27 susceptible populations with mean C_{50} of 6.08 and GR_{50} of 5.37 g ha^{-1} ai have been identified; while the other 6 populations were considered resistant, obtaining mean C_{50} of 69.70 and GR_{50} of 61.53 g ha^{-1} ai, reaching a mean resistance factor of 11.47 for control and 11.46 for mass (Tables 3 and 4).

Table 2 - Criterion, classification and color for resistance in Brazil.

Criterion	Classification	Color
All repetitions with control > 80%	S- Susceptible	White
One or two repetitions with control < 80%	r- Segregant	Yellow
Three or more repetitions with control < 80%	R- Resistant	Red

Source: Adapted from López-Ovejero et al. (2017).

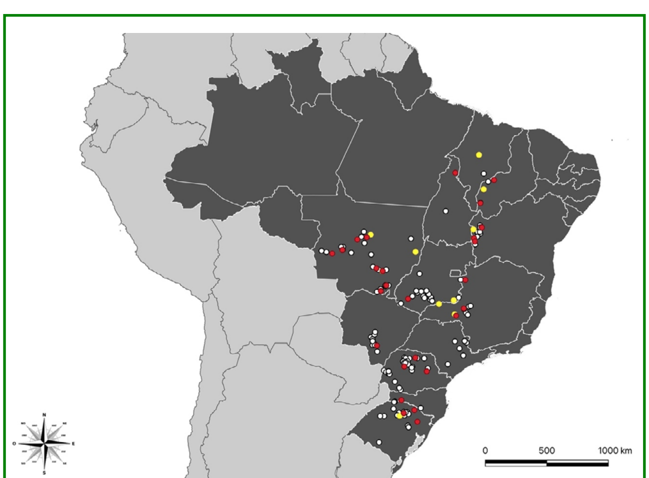

Figure 1 - Dispersion of chlorimuron-resistant *Amaranthus* spp. populations in Brazil between the 2016 to 2019 seasons.

In relation to the literature review performed, regarding susceptible populations (Table 5), variable values have been found between 0.08 and 10.82 g ha^{-1} ai for C_{50} and 1.72 and 3.82 g ha^{-1}ai for GR_{50}, with an overall average calculated at 3.07 and 2.76 g ha^{-1} ai for C_{50} and GR_{50}, respectively.

It has also been observed that even when comparing the susceptible individual with the highest C_{50} or GR_{50} to the resistant individual with the lowest C_{50} or GR_{50}, the resistance factor remained high, - higher than 2.0 - characterizing resistance and allowing the separation between susceptible and resistant populations, with the separation limit being in the range of 20 g ha^{-1} (Figure 1). According to Saari et al. (1994), resistance is confirmed when the R/S factor > 1.0.

Weed resistance to herbicides is the result of an evolutionary process. It occurs due to the repetitive application of a particular herbicide or different herbicides, but that have the same mechanism of action, changing the genetic composition of weed populations, increasing the frequency of resistance alleles and consequently the number of resistant individuals in the population. Evolution occurs whenever the frequency of a gene within a population is altered as a result of selection, mutation, migration or random distribution (Christoffers, 1999).

The natural genetic variability that exists in any weed population is responsible for the initial source of resistance in a susceptible population. Generally, gene mutations that occur in a susceptible population that has not yet been subjected to herbicide selection pressure are the result of spontaneous genetic variability and are therefore not induced by the selection agent, i.e., the herbicide.

3.2 Elaboration of discriminating dose ("base line") of susceptibility of *Amaranthus* spp. to the chlorimuron herbicide

The data obtained after the application of chlorimuron doses on pigweed susceptible populations (*Amaranthus* spp.), selected in the first experiment, indicated C_{80} of 18.66 g ha^{-1} ai (± 2.66) and GR_{80} of 10.98 g ha^{-1}ai (± 1.12) (Figure 2).

In the leaflet survey of commercial formulations of chlorimuron registered in Brazil, 16 products were found with recommendation of use for *Amaranthus* spp. control, with doses ranging from 15 to 20 g ha^{-1} ai (Rodrigues, Almeida, 2018). Considering only post-emergence applications to provide efficient control of broadleaf with 2 to 6 leaves, the average recommended dose is 17.5 g ha^{-1} ai (Table 6).

The use of logistic-type mathematical models provided perfect fit of the data set, with determination coefficients always greater than 99% (Table 7). Therefore,

Table 3 - Variables evaluated. parameters of the logistic model[1]. coefficient of determination (R^2) and control (C) for the susceptibility of *Amaranthus* spp. biotypes to chlorimurom. Santa Bárbara d'Oeste – SP, 2021.

Biotype	Parameters for control			R^2	C_{50}
	a	b	c		
1	105.54	2.38	-0.31	1.00	1.71
2	101.80	2.36	-2.61	0.99	2.33
3	101.58	2.38	-2.38	0.99	2.35
4	98.63	2.36	-2.15	0.99	2.39
5	101.56	2.42	-2.64	0.99	2.39
6	100.77	2.51	-1.92	1.00	2.49
7	96.73	2.74	-2.33	0.99	2.83
8	98.63	2.81	-1.73	0.98	2.86
9	101.45	3.07	-1.93	1.00	3.03
10	97.99	3.09	-2.44	1.00	3.14
11	96.24	3.04	-1.76	0.99	3.18
12	100.69	3.43	-2.07	1.00	3.41
13	101.07	3.54	-2.05	1.00	3.50
14	107.85	5.63	-1.24	0.96	5.01
15	99.01	5.11	-1.47	1.00	5.18
16	97.97	5.46	-1.00	0.98	5.69
17	96.80	5.98	-2.64	0.98	6.13
18	97.83	6.28	-1.27	0.99	6.51
19	106.18	7.74	-0.94	0.96	6.84
20	103.20	7.55	-1.16	0.99	7.16
21	109.06	9.59	-1.44	0.97	8.55
22	101.27	9.65	-0.79	0.99	9.35
23	103.28	10.96	-0.83	0.98	10.15
24	103.07	11.79	-0.81	0.98	10.95
25	108.35	14.01	-0.67	0.99	11.12
26	108.87	22.15	-0.74	0.98	17.78
27	101.23	18.39	-1.64	0.98	18.12
28	101.19	37.09	-1.22	0.99	36.38
29	134.11	134.84	-0.62	0.99	58.58
30	107.29	80.04	-0.92	1.00	68.99
31	106.32	83.21	-0.98	1.00	73.72
32	100.44	78.19	-2.72	0.99	77.94
33	105.99	113.35	-1.13	1.00	102.57

[1]Mathematical model: $y = a/(1+(x/b)^c)$

the discriminatory dose (base line) considered ideal for the control of susceptible plants was 20 g ha^{-1} ai of chlorimuron. A dose that, when compared to the average recommendations of 17.5 g ha^{-1} ai (Table 4), is characterized as an effective dose for the control of susceptible biotypes and ineffective for the control of resistant biotypes. This being also the sufficient dose for the control of susceptible plants and control levels below 80% of resistant plants (Carvalho et al., 2006; Gonçalves Netto et al., 2016; Larran et al., 2017), which supports the use of this discriminating dose.

The approach of using single discriminating dose used to characterize the level of resistance is widely described in the literature (Owen et al., 2015; Schultz et al., 2015). The discriminating dose is defined as the minimum rate that provides the maximum difference between dose-response curves for resistant (R) and susceptible (S) biotypes, resulting in a minimum 80% control of the S biotype (Beckie et al., 1990).

3.3 Monitoring the spread of *Amaranthus* spp. to the herbicide chlorimuron

A total of 226 *Amaranthus* spp. samples were evaluated over the five years of monitoring. Susceptible populations (S) totaled 74.0%, while those classified as resistant (R) and segregant (r) did not exceed 26.0% (Table 8). With the exception of the states of Pará and São Paulo, where no chlorimuron-resistant populations were found, in all other states evaluated, resistant or segregating individuals (R or r) were found in at least one evaluation year (Table 8).

The states most represented by the sampling were Paraná and Mato Grosso, where 19.46% (44 samples) of the total evaluated between the years 2016 and 2020 were sampled (Table 9). The states with fewer R or r populations are Piauí and Pará. While the rest of them showed higher frequencies of R or r for chlorimuron (Table 9).

ALS inhibitor herbicides are widely used in agriculture due to their high agronomic efficacy in controlling several weed species, low recommended doses, low mammalian toxicity and selectivity to several crops (Tan et al., 2005). However, the inappropriate use of these herbicides has led to the selection of resistant weeds, totaling 167 cases worldwide which represents 33% of all resistance cases worldwide (Heap, 2021).

In Brazil, the first report of resistance of *Amaranthus* spp. to ALS-inhibiting herbicides occurred in 2011 with the species *A. viridis* and *A. retroflexus* (Heap, 2021). This resistance quickly spread in Brazil and other resistant *Amaranthus* spp. species subsequently emerged. Several factors contribute to the high number of ALS resistance cases, including residual activity (Tranel, Whright, 2017), high level of resistance, lack of adaptive cost, and high initial frequency of resistant individuals (Preston and Powles, 2002).

Table 4 - Variables evaluated. parameters of the logistic model[1]. coefficient of determination (R^2) and growth reduction (GR) for the susceptibility of *Amaranthus* spp. biotypes to chlorimuron herbicide. Santa Bárbara d'Oeste – SP, 2021.

Biotype	Parameters for residual dry mass				R^2	GR_{50}
	Pmín	a	b	c		
1	-1.59	99.98	2.25	2.23	0.99	2.19
2	-1.45	101.35	2.23	1.87	1.00	2.20
3	-2.50	102.46	2.32	1.30	1.00	2.23
4	-8.96	109.13	3.14	0.76	0.98	2.54
5	-1.36	101.42	2.62	1.54	1.00	2.58
6	-0.88	95.74	2.75	3.65	0.99	2.65
7	1.66	98.60	2.71	1.56	1.00	2.78
8	-0.81	100.74	2.82	2.25	1.00	2.80
9	0.47	99.58	2.89	2.08	1.00	2.91
10	-1.13	101.21	2.97	1.37	1.00	2.92
11	-0.31	100.30	2.99	2.55	1.00	2.98
12	-2.04	101.75	3.19	1.77	1.00	3.11
13	-6.78	98.71	4.31	1.47	0.98	3.50
14	-0.59	100.45	3.64	3.62	1.00	3.62
15	4.08	128.25	3.70	2.78	0.99	4.56
16	-1.76	98.92	4.77	3.13	0.99	4.63
17	-2.27	101.70	5.13	1.23	0.96	4.90
18	-2.67	102.53	5.05	1.79	1.00	4.90
19	-8.81	108.23	6.16	1.22	0.95	5.34
20	3.40	96.92	5.80	2.12	0.99	6.02
21	-10.79	110.33	7.43	1.12	0.98	6.19
22	-9.32	109.42	8.61	1.19	0.99	7.47
23	2.73	97.80	7.87	2.63	1.00	8.07
24	-1.26	101.46	11.05	1.53	0.99	10.90
25	-6.90	107.05	15.11	0.67	0.99	12.52
26	-8.72	107.44	15.71	1.39	0.96	13.74
27	-14.99	114.15	23.00	0.86	0.99	16.62
28	-25.16	125.22	123.36	0.34	0.99	37.03
29	-13.12	109.09	58.77	0.85	0.98	40.50
30	-3.67	102.57	47.32	1.24	1.00	43.90
31	-3.03	104.36	55.05	0.71	0.99	52.58
32	3.51	102.79	112.46	-1.09	1.00	94.37
33	-2.89	103.70	105.69	0.85	0.99	100.81

[1]Mathematical model: $y = Pmín + a/(1+(x/b)^c)$

Weeds of *Amaranthus* genus are competitive, have an annual life cycle, C4 photosynthetic cycle, high fecundity, and rapid growth. A large plant can produce more than 200,000 seeds (Kissmann, Groth 2000). In addition to these characteristics, pigweed plants have extensive germination period of the seed bank, long viability of their seeds in the soil, and are difficult species to identify in the field (Horak, Loughin, 2000). These characteristics enable the rapid establishment and dispersal of this weed in agricultural areas.

The dispersal of pigweed seeds occurs mainly through irrigation water, birds, and mammals. Another form of dispersal is related to the movement of agricultural machinery such as harvesters and grain sowers. The flow

Table 5 - Susceptibility level to chlorimuron herbicide of *Amaranthus* spp. populations available in scientific literature. estimated by dose-response curves. Santa Bárbara d'Oeste – SP, 2021.

Authors	Species	Application Stage	Results[1]	
			C_{50}	GR_{50}
Burgos et al., 2001	*A. palmeri*	3–4 leaves	0.41	-
	A. hybridus		0.30	-
Carvalho et al., 2006	*A. deflexus*	5–6 leaves	5.86	-
	A.hybridus		1.69	-
	A. retroflexus		1.48	-
	A. spinosus		4.08	-
	A. hybridus		3.49	-
Gonçalves Netto et al., 2016	*A. spinosus*	2–4 leaves	4.97	1.72
Larran et al., 2018	*A. palmeri*	5–6 leaves	10.82	3.82
Average			3.07	2.76

[1] Result expressed in active ingredient of chlorimuron needed to obtain 50% population control (C50) or to obtain 50% reduction in dry matter mass (GR50); [2] DAA: days after application; [3] Average value among susceptible populations.

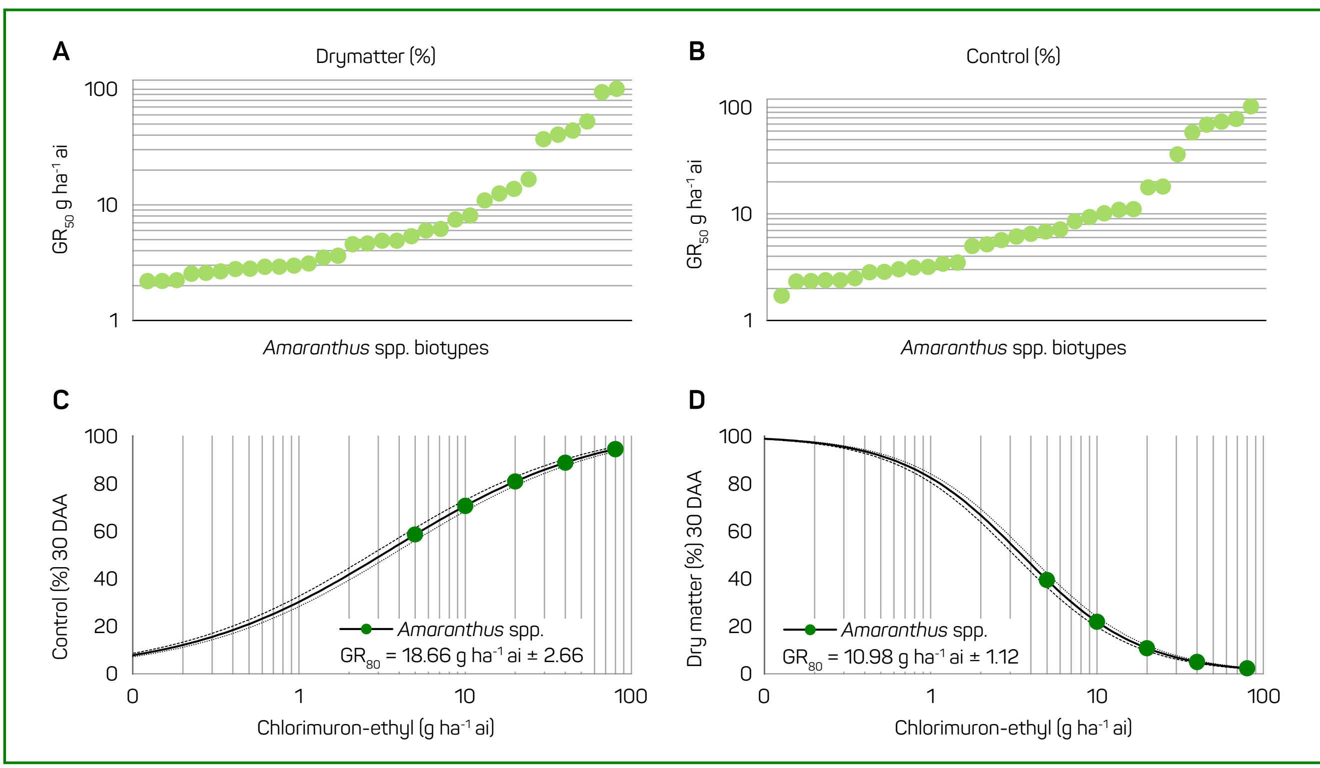

Figure 2 - Dispersion for C_{50} (a) and GR_{50} (b), percentage control (c) and residual dry mass (d) of susceptible biotypes of *Amaranthus* spp., submitted to different doses of the herbicide chlorimuron, evaluated at 30 days after application (DAA). Santa Bárbara d'Oeste - SP, 2021.

of rented equipment for grain harvesting, and the local selection pressure exerted by using the same mechanism of action repeatedly are factors that contribute to the dispersal of resistant populations (Takano et al., 2018).

The impact of ineffective control of *Amaranthus* spp. plants due to lack of management can bring losses to the agricultural production system. These productivity losses, in the case of *Amaranthus palmeri*, can reach 91% in corn crop, 65% in cotton, 68% in sorghum, 79% in soybean, 68% in peanut and 94% in sweet potato (Ward et al., 2013). In addition to the characteristics of aggressiveness and competition, resistance in agricultural production

Table 6 - Instructions for commercial formulations of chlorimuron registered in Brazil with recommendations for use for *Amaranthus* spp. from 2 to 4 leaves (lower dose) and 6 to 8 leaves (higher dose). Santa Bárbara D'Oeste - SP, 2021.

Company name	Formulation	Registration Holder	[1]Dose g p.c. ha^{-1}	[2]Dose g ha^{-1} ai
Classic®	250	FMC	60 - 80	15 - 20
Climur®	250	Rainbow	60 - 80	15 - 20
Clipper®	250	Sinon	60 - 80	15 - 20
Clomom®	250	UPL	60 - 80	15 - 20
Clorim®	250	UPL	60 - 80	15 - 20
Clorimurom Nortox®	250	Nortox	40 - 80	10 - 20
Clorimurom Master®	250	Nortox	60 - 80	15 - 20
Clorimurom CCAB®	250	CCAB	60 - 80	15 - 20
250 WG Rainbow®	250	Rainbow	60 - 80	15 - 20
Conquest®	250	ADAMA	60 - 80	15 - 20
Fullmuron®	250	AgroImport	60 - 80	15 - 20
Higon®	250	HELM	60 - 80	15 - 20
Kromo 250 WG®	250	Sumitomo	40 - 80	10 - 20
Rajer 250 WG®	250	Loveland	60 - 80	15 - 20
Panzer 250 WDG®	250	CropChem	60 - 80	15 - 20

[1]Rodrigues e Almeida 2018, [2]Result expressed in grams of chlorimurom (active ingredient).

Table 7 - Variables evaluated, parameters of the logistic model[1], coefficient of determination (R^2), control (C) or growth reduction (GR) for susceptibility of *Amaranthus* spp. to chlorimurom biotypes. Santa Bárbara D'Oeste - SP, 2021.

Variable	Parameters				R^2	C_{80} ou GR_{80}	Standard Error
	Pmín	a	b	c			
Control	-	105.21	3.62	-0.70	0.99	18.66	± 2.66
Residual Dry Mass	0.13	100.05	3.51	1.22	0.98	10.98	± 1.12

[1]$y = a/(1+(x/b)^c)$ or $y = P_{min} + a/(1+(x/b)^c)$

Table 8 - Frequency (%) of susceptible (S), segregant (r) and resistant (R) *Amaranthus* spp. populations to chlorimuron in Brazil, sampled between the years 2016 to 2020. Santa Bárbara D'Oeste - SP, 2021.

Herbicide	Classification	2016 to 2020
Chlorimuron 20 g ha^{-1} ai	S	170
	r	19
	R	37
	Total	226

systems brings complexity to management. These include restriction of the use of important herbicides, loss of planting areas, and loss of quality and yield of agricultural products (Christoffoleti, López-Ovejero, 2008). Therefore, the adoption of sustainable management strategies is necessary to prevent the selection of resistant biotypes in the field.

4. Conclusions

By comparing susceptible biotypes of *Amaranthus* spp. with international scientific literature standards

Table 9 - Number (n°) and frequency (%) of populations with 1(R+r) resistance to chlorimuron in the states sampled between the years 2016 to 2020. Santa Bárbara D'Oeste - SP, 2021.

State	Chlorimuron (20)		
	N°	Σ(R+r)	%
BA	16	7	63%
GO	26	5	19%
MA	5	1	20%
MG	14	4	29%
MS	19	1	5%
MT	44	14	32%
PA	1	0	0%
PI	1	1	100%
PR	44	5	11%
RS	35	14	40%
SC	7	2	29%
SP	11	0	0%
TO	3	2	67%
Total	226	56	26%

[1](R + r = Resistant + Segregant). BA: Bahia, GO: Goiás, MA: Maranhão, MG: Minas Gerais, MS: Mato Grosso do Sul, MT: Mato Grosso, PA: Pará, PI: Piauí, PR: Paraná, RS: Rio Grande do Sul, SC: Santa Catarina, SP: São Paulo, TO: Tocantins.

and leaflet averages, it could be safely concluded that the discriminating dose obtained through the susceptibility "base line" among pigweed populations is 20 g ha^{-1} ai of chlorimuron.

Resistance of *Amaranthus* spp. to chlorimuron in Brazil is present in the main soybean producing regions evaluated, with a frequency of 26% of the total samples evaluated.

Author's contributions

All authors read and agreed to the published version of the manuscript. AGN: conceptualization of the manuscript and development of the methodology. RFLO and SJPC: supervision and project administration. LSR: data analysis and data interpretation. LSR, JCP, JAF, MRM: data collection and curation.

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