RESISTANCE OF AMARANTHUS HYBRIDS POPULATION TO IMAZETHAPYR

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ABSTRACT

Purpose: It was to evaluate the possibility of the occurrence of resistance to imazethapyr in a population of *Amaranthus hybridus* from São Paulo.

Theoretical framework: Plants of the genus *Amaranthus* spp. are native to America and present a diversity of species. Currently, there are 515 cases of weeds resistant to herbicides in the world. Among these species, plants of the genus *Amaranthus* have shown an increase in cases of resistance in recent years, which generates concern about its chemical management, with six cases of resistance recorded for this species in Brazil.

Method/design/approach: The experimental design was entirely randomized in a 2x9 factorial scheme, with six replications. The first factor consisted of two populations of *A. hybridus*, one with putative resistance to imazethapyr herbicide from Cândido Mota and a susceptible population from Florínea. The herbicides dose were applied when the plants presented 4 to 6 leaves. The evaluations made were the percentage of control and dry matter.

Results and conclusion: Suspected resistance of *Amaranthus hybridus* populations to imazethapyr has been confirmed in the city of Cândido Mota.

Research implications: The main contributions of the study are in the sense of demonstrating the existence of a new resistance problem in the Brazilian agricultural areas, which will demand new control alternatives.

Originality/value: This study aimed to investigate the possible occurrence of resistance to the herbicide imazethapyr in *Amaranthus hybridus*, which will be the first report of its kind in Brazil.

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RESISTÊNCIA DE POPULAÇÕES DE AMARANTHUS HYBRIDUS AO HERBICIDA IMAZETHAPYR

RESUMO

Objetivo: Avaliar a possibilidade de ocorrência de resistência ao imazethapyr em população de Amaranthus hybridus proveniente da localidade de São Paulo.

Referencial teórico: As plantas do gênero Amaranthus spp. são originárias da América e apresentam uma diversidade de espécies. Atualmente 515 casos de plantas daninhas resistentes à herbicidas no mundo foram relatados. Dentre estas espécies, as plantas do gênero Amaranthus vêm mostrando um aumento de casos de resistência nos últimos anos, o que gera preocupação quanto ao seu manejo químico, existindo seis casos de resistência registrados para essa espécie no Brasil.

Método/design/abordagem: O delineamento experimental foi inteiramente casualizado em esquema fatorial 2x9, com seis repetições. O primeiro fator foi constituído por duas populações de A. hybridus, sendo, uma com suspeita de resistência ao herbicida imazethapyr, oriunda de Cândido Mota e uma população suscetível de Florínea. As aplicações foram realizadas quando as plantas apresentavam de 4 a 6 folhas. As avaliações realizadas foram a percentagem de controle e a matéria seca.

Resultados e conclusão: Foi confirmada a resistência da população de Amaranthus hybridus ao imazethapyr na cidade de Cândido Mota.

Implicações da pesquisa: O estudo demonstra a existência de um novo problema de resistência nas áreas agrícolas do Brasil e a necessidade de novas alternativas de manejo químico.

Originalidade/valor: Este estudo teve como objetivo investigar a possível ocorrência de resistência ao herbicida imazethapyr em Amaranthus hybridus, o que constituirá no primeiro relato desse tipo no Brasil.


1 INTRODUCTION

Currently Brazil is a reference in the world agricultural scenario, standing out as one of the largest producers of soybeans (ZANATA, RIZZI, SCHORR, 2022) and corn (DE ALMEIDA VILLANUEVA at al., 2022). However, the productivity of grain crops can be directly affected by numerous factors, among which is the competition with weeds.

Several factors limit the productivity potential of commercial crops (FRANCISCHINI et al., 2013). Among them, the main one is the interference of weeds, which in certain cases can cause losses of up to 90% of productivity (FREITAS et al., 2006).

Caruru is a species that has become very important in the cultivation of soybeans, corn and cotton, because this weed competes for light, water and nutrients (GUO, AL-KHATIB, 2003). Losses greater than 78% can be observed in the soybean crop containing a Amaranthus spp. plant every 0.125 meters in the soybean line (BENSCH et al., 2003).

Recurrent use of the same herbicide or herbicide of the same mechanism of action during a given period may lead to population selection of resistant weeds (ADEGAS et al., 2017).

One of the cases of resistance to herbicides that are of great concern are those related to ALS inhibitors, mainly because of the demand for residual activity and because they have low
toxicity to animals and high selectivity for crops. There are five chemical groups of herbicides that act on the ALS enzyme: imidazolinones, sulfonylureas, sulfonanilides, pyrimidilbenzoates, and sulfonlamino-carbonyltriazolinones.

In 2018, in the state of Rio Grande do Sul, the first report of the species *Amaranthus hybridus* occurred, with multiple resistance to the herbicide glyphosate (inhibitor of EPSPs) and also to chlorimuron-ethyl (inhibitor of ALS). Currently, ALS resistance is reported in *Amaranthus hybridus* in five countries: Argentina (imazethapyr, chlorimuron-ethyl, glyphosate) Bolivia (imazethapyr, chlorimuron-ethyl, oxasulfuron, imazamox), Brazil (chlorimuron-ethyl, glyphosate), Paraguay (chlorimuron-ethyl, glyphosate) and the United States (imazethapyr, imazaquin, thifensulfuron-methyl, chlorimuron-ethyl, nicosulfuron n, primisulfuron-methyl, flumetsulam, primisulfuron-methyl, atrazine, imazamox, thifensulfuron-methyl, flumetsulam) However, to date there is no case in Brazil of resistance of the herbicide imazethapyr in the populations of *Amaranthus hybridus*, but they have been found in other places in the world such as Argentina, Bolivia and the United States (HEAP, 2023).

Thus, the objective of the study is to evaluate the possibility of occurrence of resistance to imazethapyr in a population of *Amaranthus hybridus* originating from the state of São Paulo.

2 THEORETICAL FRAME

Plants of the *Amaranthaceae* family are represented by species known throughout the country, occurring in all Brazilian biomes. Certain plants are used in landscaping or even consumed as food in certain regions, with both human and animal destination, especially small ones. Most of these species have annual cycles, alternating leaves, herbaceous size, globose or cylindrical inflorescences, branched or not. The genus *Amaranthus*, belonging to this family, is considered invasive (MOREIRA, BRAGANÇA, 2010).

2.1 Origin and Morphology

The plants of the genus *Amaranthus* spp. originate from tropical and subtropical America, presenting a diversity of species. They are well adapted plants, have a vigorous root system and a short cycle, which makes it possible to tolerate water stresses (MADEIRA et al., 2010).

Several weeds demonstrate more than one mechanism of dormancy. The *Amaranthus* spp. plants are examples of weeds that have water and gas impermeability of the integument, besides showing physiological dormancy of the embryo and mechanical resistance of the integument. They may also host whitefly and nematodes, such as *Pratylenchus brachiurus* and *Meloidogyne incognita* (OAK et al., 2019).

Weeds have the potential to interfere with agricultural crops, causing injury. Among them, the species of *Amaranthus stand out*, whose family *Amaranthaceae* is divided into more than 70 genera and 850 species (AMAYA-FARFAN et al., 2005).

There are approximately 60 species of plants classified within the genus *Amaranthus* (carurus), and about 10 of these are important weeds of Brazilian crops (KISSLMANN, GROTH, 1999). Among the species of the genus Amaranthus most present in Brazil, one can cite: A. hybridus var. patulus (purple caruru), A. hybridus var. paniculatus (white caruru), A. retroflexus (giant caruru), A. deflexus (creeper), A. spinosus (thorny caruru), A. viridis (spot caruru) (EMBRAPA, 2017). Also according to Embrapa (2017), the presence of *A. palmeri* in the country was recently registered, causing great concern for being an extremely aggressive exotic plant, with high potential to affect the productivity of corn, cotton, soybeans or any other crop in which it is present.
When carrying out a morphoanatomical study of these species of *Amaranthus* spp., it was found that they are plants of the annual life cycle, since they germinate, develop, flower, produce seeds and die within a year, dicotyledonous, erect and with reproduction exclusively by seeds. Large plants can produce more than 200 thousand seeds.

*Amaranthus hybridus* is popularly known as bredo, giant bredo, caruru, broad-leaved caruru, giant caruru, wild caruru, purple caruru, rooster crest, purple rooster crest and white caruru (MOREIRA, BRAGANÇA, 2010; LORENZI, 2014). The species has been studied and used as food, as salads, being considered an unconventional food plant, becoming the target of studies due to its potential as a source of protein and occasionally used in popular therapy (BRIGHENTI, 2010; FIGUEIRA, MILOCH JUNIOR, 2013). Species of *A. hybridus* have been found in phytosociological surveys on caupi bean crops (LIMA et al., 2016), passion fruit (SILVA et al., 2019), banana (SARMENTO et al., 2015), coffee conilon (FORNACIARI et al., 2019), and sugar cane (CORDEIRO JUNIOR et al., 2015).

Caruru (*Amaranthus hybridus*) is an annual herbaceous, branched, erect, pigmented plant, about 40-100 cm tall, native to Tropical America. Its propagation occurs through seeds. It is a weed plant of great importance and relatively frequent in the south of the country, infesting mainly cultivated soils from annual crops in general, orchards, coffee plantations and wasteland. It has ample reproductive capacity, and a single plant can produce 117 thousand seeds (LORENZI, 2008).

Another important characteristic of this species is the presence of two main varieties, being the *paniculatus* and the *patulus* (SPEHAR, 2003). The *Amaranthus hybridus* var. *paniculatus* presents an approximate mean height ranging from 0.5 - 1.0 m and the inflorescence is panicle type with purple coloration. The stalk and petioles also have purple pigmentation. *Amaranthus hybridus* var. *patulus* has an average height ranging from 0.4 to 1.0 m and a panicle-like inflorescence of greenish coloration. The stalk and petioles may or may not have purplish pigmentation (LORENZI, 2014).

### 2.2 Strength

Resistance is defined as the ability of a population to survive and reproduce, even after applying a herbicide in the dose of the bull that would normally be lethal to this population. Thus, resistance can be an acquired characteristic that is passed on to their descendants, therefore inheritable, presented by a population of a given species (VARGAS et al., 1999).

Resistance occurs as a function of the evolutionary process that has been imposed by modern agriculture through selection pressure (CHRISTOFFOLETI, VICTORIA FILHO, SILVA, 1994). The selection process takes place in the production areas, as a result of repeated or continuous applications of the same herbicide or herbicides of the same mechanism of action during a given period (ADEGAS et al., 2018).

There are currently 515 unique cases of herbicide-resistant weeds worldwide, in a total of 267 different species (HEAP, 2023). Among these species, plants of the genus *Amaranthus* have been showing an increase of cases of resistance in recent years, which generates concern regarding their chemical management (Tables 1 and 2).

**Table 1** - *Amaranthus* species with herbicide resistance to date in Brazil.

<table>
<thead>
<tr>
<th>Year</th>
<th>Species</th>
<th>Mechanism of Action</th>
<th>Active</th>
<th>Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011</td>
<td><em>Amaranthus</em> retrofit</td>
<td>Inhibition of Acetolactate Synthase, PSII inhibitors</td>
<td>atrazine, prometryne, trifloxysulfuron-Na</td>
<td>Cotton</td>
</tr>
<tr>
<td>2012</td>
<td><em>Amaranthus</em> retrofit</td>
<td>Acetolactate Synthase Inhibition</td>
<td>pyrithiobac sodium, trifloxysulfuron-Na</td>
<td>Cotton</td>
</tr>
<tr>
<td>2014</td>
<td><em>Amaranthus</em> retrofit</td>
<td>Protoporphyrinogen Oxidase Inhibition</td>
<td>fomesafen</td>
<td>Cotton, Soya</td>
</tr>
</tbody>
</table>
Resistance of *Amaranthus Hybrids* Population to Imazethapyr

<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Mechanism of action</th>
<th>Active</th>
<th>Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td><em>Amaranthus palmeri</em></td>
<td>Inhibition of Enolpiruvil Chiquimate Phosphate Synthase</td>
<td>Imazethapyr</td>
<td>Cotton</td>
</tr>
<tr>
<td>2016</td>
<td><em>Amaranthus palmeri</em></td>
<td>Acetolactate Synthase Inhibition, Enolpiruvil Chiquimate Phosphate Synthase Inhibition</td>
<td>Imazethapyr, chlorimuron-ethyl, chloransulam-methyl, glyphosate</td>
<td>Corn, Cotton, Soybeans</td>
</tr>
<tr>
<td>2018</td>
<td><em>Amaranthus hybridus</em></td>
<td>Acetolactate Synthase Inhibition, Enolpiruvil Chiquimate Phosphate Synthase Inhibition</td>
<td>Chlorimuron ethyl glyphosate</td>
<td>Soybeans</td>
</tr>
</tbody>
</table>

Source: Heap (2023)

Table 2 - Data from *Amaranthus hybridus* with ALS resistance in the world

A. *palmeri*, *A. retroflexus*, and *A. hybridus* are the species that have reported resistance in Brazil in the last decade, with cases of multiple resistance in all species, including at least one herbicide from the ALS inhibitor group. *Amaranthus hybridus* is the most recent case of resistance of this group in the country, presenting resistance to chlorimuron-ethyl (ALS inhibitors) and glyphosate (EPSP inhibitors) (Heap, 2023)

For herbicide resistance, some mechanisms of action present greater problems than others, for example, the highest number of cases of resistant populations is found in the

Source: Heap (2023)
mechanism of action of ALS inhibitors (Group B). Evidently this number is a reflection of its intense use in agriculture, but in a way some mechanisms of action have the tendency to select resistant weeds more easily than others, which in the case of ALS inhibitors may be related to the frequency of resistant individuals in the population present in the area. (CHRISTOFFOLETI, NICOLAI, 2016).

2.3 ALS inhibitors

Acetolactate synthase (ALS) is the first enzyme of the metabolic pathway of branched amino acid biosynthesis, leucine, valine and isoleucine figure 1 (YU, POWLES, 2014). The action of these herbicides reduces or blocks the catalytic activity of the ALS enzyme resulting in the deficiency of these amino acids and, as a consequence, interrupts the synthesis of proteins, interfering in DNA synthesis and cell growth (TAN, 2006). With the herbicide bound to the enzyme, the production of amino acids is jeopardized, causing the death of the plants (SHIMIZU et al., 2002).

This mechanism of action is presented in five chemical groups, being imidazolinones (IMI), sulfonylureas (SOUTH), triazolopyrimidines (TRI), pyrimidiniothio- (oxy)benzoates (PIR) and sulfonylamino-carbonyltriazolinones (SCT) (RIZZARDI et al., 2002). The diversity of molecules guarantees the wide spectrum of control of this mechanism of action. They are herbicides that control monocots and dicots, have systemic action and have low toxicity to humans and animals (ROSO et al., 2006).

The discovery of ALS-inhibiting herbicides was very significant in the history of Weed Science. These herbicides became famous for their use in low dosages, while at the same time it was common to use other herbicides in higher dosages (TRANEL, WRIGHT, 2002). ALS inhibitors were largely responsible for the drop in the total value of the active herbicide ingredient applied to crops during the 1980s (BELLINDER et al., 1994). In addition, these herbicides presented broad spectrum, ensuring selectivity for cultivated crops, residual period, wide application windows and low toxicity to mammals (MAZUR, FALCO, 1989).

Most cases of resistance to ALS enzyme inhibitors reported involve mutation in the target gene, which reduces the affinity of the enzyme to the herbicide (BURGOS et al., 2015). Alterations in the action site usually result from mutations in the genes that encode the enzyme.
(DEVINE, EBERKEIN, 1997), resulting in a reduction in its affinity with the inhibitors, but with absence or reduced loss of enzyme function (TRANEL, WRIGHT, 2002).

The participation of this group of herbicides in the soybean crop was greater before the advent of the Roundup Ready (RR) technology, in particular the herbicides imazethapyr and imazaquin. However, currently, chlorimuron-ethyl, imazethapyr, diclosulam and chloransulam herbicides are still important components within the chemical management systems of soybean crops.

3 METHOD

Caruru seeds were collected at the stage of physiological maturation, and samples were made up of five to ten plants per location. The collection sites were properties whose main activity is the cultivation of grains in which *Amaranthus hybridus* control failures were observed after the application of herbicides, in the 2019/2020 crop. The seeds were packed in paper bags and identified as to the geographical coordinates, the name of the producer and/or the name of the farm, the municipality and the date of collection. A total of four *Amaranthus hybridus* populations were evaluated in the vegetation house at the Technical Irrigation Center of the State University of Maringá, in the period of October 2020, one of them coming from the areas of suspected resistance and one of them considered as a susceptible standard (Table 3).

<p>| Table 3 - Origin and identification of seed samples from Amaranthus hybridus populations collected for the experiments. Maringá, PR, 2021. |
|---------------------------------|------------|---------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Municipality</th>
<th>Status</th>
<th>Population</th>
<th>Geographical coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cândido Mota</td>
<td>SP</td>
<td>Suspected resistance</td>
<td>Coord. (S) Coord. (W)</td>
</tr>
<tr>
<td>Florine</td>
<td>SP</td>
<td>Susceptible</td>
<td>22° 44' 02&quot; 50° 25' 27&quot;</td>
</tr>
</tbody>
</table>

Source: Prepared by the authors (2022)

The seeds from each location were sown in trays containing commercial Carolina® substrate, at 0.5 cm depth and the seedlings were later transplanted into 0.2 L vessels. The physicochemical characteristics of the soil used in the vessels are detailed in Table 4.

| Table 4 - Results of the physical-chemical analysis of the soil used in the greenhouse. |
|---------------------------------|---------------|----------------|-------------|-----------------|
| pH | H+ | Al | Ca | Mg | K | SB | CTC | P | C | MO | V | Sand | Clay | Silt |
| CatCl2 | — cmolv dm$^3$ — | mg dm$^{-3}$ | g dm$^{-3}$ | — % — |
| 5.2 | 2.5 | 4.5 | 1.1 | 0.53 | 6.13 | 8.63 | 15 | 215 | 2.6 | 71.03 | 33.3 | 50.3 | 16.4 |

Source: Prepared by the authors (2022)

The experimental design was entirely casualized, with treatments arranged in a 2 x 9 factorial with six repetitions. Factor A was composed of a population with suspected resistance (Cândido Mota) and a susceptible (Florínea). Factor B levels were composed of doses of the herbicide imazethapyr, applied in post-emergence, at the 4-6 leaf stage. The nine doses of herbicide applied to the population with suspected resistance were 0; 25; 50; 100; 200; 400; 800; 1600 and 3200 g i.a ha$^{-1}$. For the susceptible population, the doses of the herbicide were composed of 0; 6.25; 12.5; 25; 50; 100; 200; 400 and 800 g i.a ha$^{-1}$. The commercial formulation used was Imazethapyr Plus Nortox, which has a concentration of 106 g of active ingredient per liter, equivalent to 100 g of acid equivalent per liter.

The applications were carried out with a costal sprayer with a constant pressure based on CO$_2$. The application bar was equipped with three spray tips XR 110.015 and the volume of syrup applied was 150 L ha$^{-1}$. The applications occurred in the morning period (8:00-10:00),

prioritizing the most favorable conditions possible, i.e. UR above 70%, air temperature below 28°C and no wind gusts.

At all stages, control evaluations were performed (visual scale, 0-100%, where 0% means no symptoms and 100% death of weeds) at 28 days after application (DAA), being considered acceptable controls values ≥ 80% and satisfactory values ≥ 90%, due to the severity of the weed. In the 28-day evaluation, the aerial part of the remaining plants was collected by cutting close to the ground and closed in paper bags, placed in an oven at 65°C until a constant weight and weighed so that an accumulation of dry mass was obtained from each experimental unit (g vessel⁻¹). The dry mass was corrected for percentage values by comparing the mass obtained in the herbicide treatments with the mass of the control (zero dose), considered 100%, according to the following equation:

% dry matter related to the control= ((Repeat value*100)/(Witness averages))

Then, the data from the dose-response experiments were submitted to the analysis of variance (ANOVA) and the Tukey test. The control % and dry mass % data for the controls was used to obtain the dose-response curves were adjusted to the logistic type nonlinear regression model proposed by Streibig (1988): (Equation 1).

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

Where, y is the dependent variable (control and dry mass relative to the control); x is the independent variable (dose g i.a ha⁻¹), a, b and c are the estimated parameters of the equation, such that: a is asymptotic, b is the angular coefficient in c; c is the dose to provide 50% of a (DC₅₀ for control and DM₅₀ for dry matter).

From the logistic equations, the dose-response curves were drawn up. Based on the adjusted models, the dose of the herbicide was calculated, in g i.a. ha⁻¹, which would provide 50% and 90% control (DC₅₀ and DC 90).

From the DC₅₀ values (50% control), the resistance factor (RF) was obtained for each combination of population with suspected resistance and susceptible population. The resistance factor (RF = DC₅₀ of the suspected resistance/CD₅₀ population considered susceptible) expresses the number of times the dose required to control 50% of the resistant population is higher than the dose controlling 50% of the susceptible population.

In order to state that the population is resistant, two concepts were used, the resistance factor (RF) and DC₈₀ (dose necessary to control 80% of the population), so we joined the concept of scientific resistance and agronomic resistance, respectively. That is, for the population to be considered resistant, it must necessarily present FR > 1.0 and DC₈₀ > 100 g i.a. ha⁻¹ (recommended dose).

4 RESULTS AND DISCUSSIONS

Analysis of data variance showed that there was significance for the sources of variation as well as for the interaction between populations and doses. The parameters of the logistic models adjusted for the controls obtained with the application of increasing doses of Imazethapyr on the populations of A. hybridus are presented in Table 5 and 6.
Table 5. Model parameters for % control, doses (g ha<sup>-1</sup>) for 50% and 90% control of Imazethapyr herbicide applied in two populations of *Amaranthus hybridus*.

<table>
<thead>
<tr>
<th>Place</th>
<th>Population</th>
<th>to B</th>
<th>DC&lt;sub&gt;50&lt;/sub&gt; (c)</th>
<th>DC&lt;sub&gt;90&lt;/sub&gt;*</th>
<th>FR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florine</td>
<td>Susceptible</td>
<td>100.80 ± 4.32</td>
<td>-1.62 ±0.34</td>
<td>17.78 ± 2.41</td>
<td>103.63</td>
</tr>
<tr>
<td>Cândido Mota</td>
<td>Resistant</td>
<td>115.62 ±30.05</td>
<td>-0.56±0.20</td>
<td>233.97 ± 258</td>
<td>16</td>
</tr>
</tbody>
</table>

Note: *DM<sub>50</sub>: dose for 50% control. DM<sub>90</sub>: dose to 90% control. DM: Calculated by DM<sub>50</sub> of resistant population / DM<sub>50</sub> of susceptible population.

*Source: Prepared by the authors (2022)*

Table 6. Model parameters for Relative dry mass (% relative to zero dose of population S), doses (g ha<sup>-1</sup>) for 50% and 90% control of the herbicide Imazethapyr applied in two populations of *Amaranthus hybridus*.

<table>
<thead>
<tr>
<th>Place</th>
<th>Population</th>
<th>to B</th>
<th>DM&lt;sub&gt;50&lt;/sub&gt; <em>(c)</em></th>
<th>DM&lt;sub&gt;90&lt;/sub&gt;*</th>
<th>FR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florine</td>
<td>Susceptible</td>
<td>99.66 ± 8.00</td>
<td>1.65 ± 0.40</td>
<td>10.56 ± 1.89</td>
<td>39.99</td>
</tr>
<tr>
<td>Cândido Mota</td>
<td>Resistant</td>
<td>100.76 ± 7.86</td>
<td>0.61 ± 0.11</td>
<td>99.18 ± 36.26</td>
<td>6332.54</td>
</tr>
</tbody>
</table>

Note: *DM<sub>50</sub>: dose for 50% control. DM<sub>90</sub>: dose to 90% control. DM: Calculated by DM<sub>50</sub> of resistant population / DM<sub>50</sub> of susceptible population.

*Source: Prepared by the authors (2022)*

From the adjusted equations, the DC<sub>50</sub> and DC<sub>90</sub> values were calculated, with the DC<sub>50</sub> values characterizing the susceptibility levels of the population of *A. hybridus* to herbicide and the DC<sub>90</sub> characterizes the doses for achieving satisfactory control (90%) of the weeds. Resistance factor (RF) values are also shown (Tables 5).

The response of the susceptible population (S) to imazethapyr resulted in CD<sub>50</sub> of 17.78 g i.a ha<sup>-1</sup> indicating that the population is actually sensitive to the evaluated herbicide. For the evaluations performed with imazethapyr at 28 AAD, none of the populations suspected of resistance showed control with the recommended dose of the bull.

The resistance factors characterized by the control relationship between DC<sub>50</sub> of the R/DC<sub>50</sub> population of the S population was 13.17 for the Cândido Mota population, respectively. For the resistance factors characterized by dry mass in relation to the witness had an RF of 9.4 for the population of Cândido Mota. Proving that the population has an FR>1.

Given this scenario, it was possible to observe that the populations have a low resistance factor compared to the work of Whaley, Wilson and James (2006), where they found populations of *Amaranthus hybridus* resistant to the herbicide imazethapyr with RF that varied from 261 to 537 times. The elevated RF occurred due to a change/mutation in the site of action of the target enzyme. Manley et al. (1999) observed resistant populations with an RF of 71. For the *Amaranthus quitensis* population, a resistance factor of 24.55 was found showing that this population is more resistant than that studied in this work (MONQUEIRO, CHRISTOFFOLETI, DIAS, 2000).

Work related to the red rice population (*Oryza sativa*) that presented escape in six regions of the state of Rio Grande do Sul for herbicide Only (imazethapyr + imazapic - 75 + 25 g L<sup>-1</sup>) in the Clearfield cultivar, confirmed that 56% of the populations were resistant. The RF found were 5.2 and 11.6.

The S population at the recommended herbicide dose resulted in a 96.77% control, whereas the R population (Candido Mota) resulted in a 47.77% control (figure 2). For the reduction of mass in the dose of the bull for the population S and R resulted in 2.39% and 40.61%, respectively, of the dry matter compared to the witness.
Resistance of *Amaranthus Hybrids* Population to Imazethapyr

Figure 2. Dose response curves for the Cândido Mota population. For the control percentage (left) and relative dry mass (right) variables for populations suspected of resistance and susceptible to *Amaranthus hybridus* to the herbicide imazethapyr.

Source: Prepared by the authors (2022)

The higher doses of the herbicide Imazethapyr (3200 g i.a ha\(^{-1}\)), which is equivalent to 32 times the dose of the bull, caused high levels of injury and reduced dry mass. However, they were not sufficient to result in 100% control (Figure 2). Furthermore, even at the end of the evaluations (28 DAA), it was possible to observe that the plants were recovering from the injuries caused by the herbicide.

The main mechanism of resistance for ALS inhibitors involves the mutation of genes encoding the target herbicide protein (POWLES E YU, 2010). Accordingly, with this mutation that alters the structure of the herbicide action site impeding the molecule from binding itself and promoting phytointoxication in the plant.

Nowadays, it is normal to find weeds that are highly resistant to ALS inhibitors. In *Amaranthus palmeri* populations cross-resistance due to mutation in the enzyme with A122S substitution has been reported. This can be explained by a lower number of hydrophobic interactions between the herbicide and the mutant enzyme (PALMIERI et al., 2021). In another study with grass-rice (*Echinochloa crusgalli*), it was possible to identify the mechanism of resistance to imazethapyr in resistant populations as increased metabolism of the herbicide by P450 enzymes (MATZENBACHER et al., 2015; DALAZEN et al., 2018a; DALAZEN et al., 2018b).

In 2021, also in a study conducted with grass rice, the first case of negative cross-resistance (NCR) was found (CUTTI et al., 2021). Negative cross-resistance is resistance to a particular pesticide that can result in increased sensitivity of other pesticides. Poston et al. (2001) have shown that NCR occurs in populations with target site resistance to ALS herbicide.

A population of *Amaranthus retroflexus* located in China found in soybean area has survived a recommended dose of tifensulfuron-methyl. Dose-response assays show that it was highly resistant to herbicide 61 times. In addition, they observed that it was cross-resistant to Imazethapyr. It is a phytochrome P450-mediated resistance (CAO et al., 2021)

The results obtained in this work showed that the population of Cândido Mota met all the criteria mentioned for the confirmation of a case of resistance by Heap (2005) and therefore are resistant to the herbicide imazethapyr.

In this sense, it is necessary to understand the whole scenario to think about complementary management solutions. The selection pressure imposed by ALS-inhibiting
herbicides can be reduced, for example, by combining and using herbicides with different mechanisms of action.

5 FINAL CONSIDERATIONS

Under the conditions under which the experiment was conducted, it is concluded that: The present study confirmed the suspicion of resistance of the *Amaranthus hybridus* populations to the herbicide imazethapyr in the city of Cândido Mota of the state of São Paulo, being the first report in Brazil. These results contribute to more assertive decision-making by the rural producer in the chemical management of *Amaranthus hybridus*. In addition, it helps the positioning of the industry for the development of new technologies for the handling of resistant weeds.

REFERENCES


