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# MULTIPLE RESISTANCE OF *Amaranthus palmeri* TO ALS AND EPSPS INHIBITING HERBICIDES IN THE STATE OF MATO GROSSO, BRAZIL[1]

*Resistência Múltipla de **Amaranthus palmeri** aos Herbicidas Inibidores da ALS e EPSPS no Estado do Mato Grosso, Brasil*

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ABSTRACT - This work was carried out in order to evaluate the susceptibility to ALS-inhibiting herbicides of the Brazilian biotype of glyphosate-resistant *A. palmeri*, considering different chemical groups. For that, four experiments were performed, each with one of the following herbicides: glyphosate, chlorimuron-ethyl, cloransulan-methyl and imazethapyr. In each trial, treatments were organized according to a 2x8 factorial scheme, in which two were the species of *Amaranthus* (*A. palmeri* and *A. spinosus*) and eight were the herbicide rates (16D, 8D, 4D, 2D, D, 1/2D, 1/4D and herbicide absence; being D the commercial rate of each product). For glyphosate, D = 720 g a.e. $ha^{-1}$; for chlorimuron-ethyl, D = 20 g $ha^{-1}$; for cloransulan-methyl, D = 30 g $ha^{-1}$; for imazethapyr, D = 100 g $ha^{-1}$. Glyphosate was not applied on *A. spinosus*. In all the trials, the Brazilian biotype of *A. palmeri* had low herbicide susceptibility, so it was possible to conclude this biotype has ALS-EPSPs multiple resistance. Therefore, considering only ALS-inhibiting herbicides, this population has sulfonilurea-triazolopirimidine-imidazolinone cross-resistance.

**Keywords:** Palmer amaranth, dose-response, management, control.

*RESUMO - Este trabalho foi desenvolvido com o objetivo de avaliar a suscetibilidade do biótipo brasileiro de **A. palmeri**, resistente ao herbicida glyphosate, a herbicidas inibidores da ALS de diferentes grupos químicos. Para isso, quatro experimentos foram realizados, um com cada um dos seguintes herbicidas: glyphosate, chlorimuron-ethyl, cloransulan-methyl e imazethapyr. Em cada experimento, os tratamentos foram organizados em esquema fatorial 2 x 8, em que duas foram as espécies de **Amaranthus** (**A. palmeri** e **A. spinosus**) e oito foram as doses de herbicida (16D, 8D, 2D, 4D, D, 1/2D, 1/4D e ausência do herbicida, sendo D a dose comercial recomendada de cada produto). Para glyphosate, D = 720 g e.a. $ha^{-1}$; para chlorimuron-ethyl, D = 20 g $ha^{-1}$; para cloransulan-methyl, D = 30 g $ha^{-1}$; e para imazethapyr, D = 100 g $ha^{-1}$. Não foi aplicado glyphosate sobre **A. spinosus**. Em todos os experimentos, os biótipos de **A. palmeri** tiveram baixa suscetibilidade aos herbicidas, de modo que se pôde concluir tratar-se de um caso de resistência múltipla aos inibidores da EPSPs e ALS. Ainda, considerando somente os inibidores da ALS, conclui-se que o biótipo possui resistência cruzada às sulfonilureias - triazolopirimidinas - imidazolinonas.*

**Palavras-chave:** caruru-palmeri, dose-resposta, manejo, controle.

## INTRODUCTION

Among weeds more commonly found in agriculture the ones classified as *Amaranthus* (pigweeds) genus are those that stand out, represented by around 60 species, and approximately 20 from those are important as weeds (Kissmann and Groth, 1999). In Brazil, there are around 10 species of this genus with agricultural importance, the

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following standing out: *A. deflexus*, *A. hybridus*, *A. lividus*, *A. retroflexus*, *A. spinosus* and *A. viridis* (Carvalho et al., 2008).

The Brazilian *Amaranthus* species have annual life cycle, reproduce exclusively by seeds and, generally, are difficult to visually differentiate among the species, especially as seedlings. In the agricultural areas, they can be considered as difficult management plants due to the extended emergency period, to the fast growth and long viability of their seed bank (Horak and Loughin, 2000). Yet, a large sized plant can produce more than 200,000 seeds (Kissmann and Groth, 1999; Lorenzi, 2000). They compete with the agricultural crops for water, light and nutrients; reduce the amount and quality of the harvested product; and, especially the large-sized species, interfere in the harvest procedures (Klingaman and Oliver, 1994; Knezevic et al., 1997; Rowland et al., 1999; Carvalho and Christoffoleti, 2008).

However, recently, Andrade Junior et al. (2015) identified Palmer amaranth (*A. palmeri*) for the first time in Brazil, in the cotton north-central region of the state of Mato Grosso, in areas where cotton, soybeans and corn are grown in rotation. Palmer amaranth comes from the arid regions of the South-Central of the United States of America (USA) and the north of Mexico, appearing in several countries. In recent years, this species has become the main weed in cotton areas in the USA, due to its biologic characteristics and resistance to herbicides of different action mechanisms (Ward et al., 2013).

Palmer amaranth is dioecious, which means that, in a population, part of the plants will only have female flowers ("female" plants) and the other part will only have male flowers ("male" plants), where seeds will only be produced by female flowers (Ward et al., 2013). This characteristic enables the identification of *A. palmeri* in the field, once the other species of amaranth that are important for Brazilian agriculture have male and female flowers in the same plant, being classified as monoecious.

In a paper done to verify susceptibility of *A. palmeri* to glyphosate in Brazil, the same population that was also used in the present paper, Carvalho et al. (2015) saw that this is a glyphosate resistant population and it demands dosages above 4,500 g a.e. $ha^{-1}$ in order to reach $DL_{80}$, which are economically and environmentally unfeasible.

In the USA, in addition to glyphosate, there are reports of Palmer amaranth populations resistant to other action mechanisms, such as ALS inhibitors, HPPD inhibitors, tubulin synthesis inhibitors (mitosis) and photosystem II inhibitors (FSII). The management of these populations becomes even more complex, especially for the populations with multiple resistance, which has been reported for two or three of these action mechanisms: ALS/glyphosate, ALS/glyphosate/FSII and ALS/FSII/HPPD (Beckie and Tardif, 2012; Ward et al., 2013; Heap, 2016). Besides the USA borders, there have been reports of *A. palmeri* resistant to ALS inhibitors in Israel (Heap, 2016) and EPSPs inhibitors in Argentina (Morichetti, 2013).

The ALS inhibitor herbicides can be used in pre-emergence or post-emergence in the control of glyphosate-resistant weeds in Brazil (Nicolai et al., 2008). Likewise, herbicides with this action mechanism have been used successfully in the control of *A. palmeri* resistant to glyphosate in the USA (Wise et al., 2009; Mohseni-Moghadam et al., 2013), which could possibly be an alternative to the control of resistant *A. palmeri* in Brazil.

Therefore, this work was developed with the objective of assessing the susceptibility of the Brazilian biotype of *A. palmeri*, considered resistant to glyphosate, to ALS inhibiting herbicides from different chemical groups.

## MATERIAL AND METHODS

Four experiments were developed in a greenhouse of the Department of Crop Production at ESALQ/USP, in the city of Piracicaba, São Paulo (22°42'S; 47°37'W; 545 m in altitude), between August and November 2015. Simultaneously, two populations of amaranth were evaluated, one of *A. palmeri* and one of *A. spinosus* (susceptible). The seeds of *A. palmeri* were the same that were used in the research developed by Carvalho et al. (2015); therefore, this population is proven to be resistant to glyphosate, while the seeds of *A. spinosus* were collected in the state of Mato Grosso.

For the installation of the experiments, seeds were distributed in excess in rectangular plastic boxes, with capacity for 2.0 L of commercial substrate (peel of *Pinus*, peat and vermiculite) and vermiculite (3:1; v:v). In the vegetative development stage of completely expanded cotyledons, that is, stage 10 (Hess et al., 1997), seedlings were transplanted into plastic pots with capacity for 1.0 L. filled with the same substrate mixture, where they remained until the end of the experiment, in an average density of three plants per pot.

The susceptibility of the populations was quantified by dose response curves. All the experiments were installed in an experimental design of randomized blocks with five replicates. For each herbicide studied (Table 1), treatments were organized in a 2 x 8 factorial design, in which two were the species of *Amaranthus* (*A. palmeri* and *A. spinosus*) and eight were the herbicide doses, namely: 16D, 8D, 4D, 2D, D, 1/2D, 1/4D and absence of herbicide, D being the maximum commercial dose indicated on the label of each product (Table 1). Glyphosate was not applied on *A. spinosus*.

The sprays were done on plants in the stage from 3 to 4 pairs of leaves. For that, a $CO_2$ pressurized precision backpack sprayer was used, coupled in a TeeJet 110.02 two-tip bar, positioned at 0.50 m from the target, with a spray relative consumption of 200 L $ha^{-1}$.

At 28 days after application (DAA), the percentage control was evaluated, as well as the residual dry matter of the plots. The control was analyzed by using a variable percentage scale between zero and 100, in which zero was attributed to the absence of symptoms caused by the herbicide and 100% to the death of the plants. Plant dry mass was obtained from the collection of the plant material remaining in the plots, with later drying in an oven at 70 °C for 72 hours. Dry matter was corrected for the percentage values through the comparison of mass obtained in herbicide treatments with the mass of the check plots, considered 100%.

Data analysis was performed through the application of the F test in the variance analysis. The control variable was adjusted to the logistic model proposed by Streibig (1988):

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

where: $y$ = control percentage; $x$ = herbicide dosage; and $a$, $b$ and $c$ = curve parameters, so that $a$ is the difference between the maximum and minimum point of the curve, $b$ is the dosage that provides 50% of variable response and $c$ is the slope of the curve.

For the residual dry matter, the model adopted was the one proposed by Seefeldt et al. (1995):

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

where: $y$ = mass quantification; $x$ = herbicide dosage; and $a$, $b$ and $c$ = curve parameters, so that $a$ is the inferior limit of the curve, $b$ is the difference between the maximum and minimum point of the curve, $c$ is the dosage that provides 50% of variable response and $d$ is the slope of the curve.

The logistic model has advantages, once one of the terms of the equation ($b$ or $c$) is an estimate of the $DL_{50}$ or $GR_{50}$ value (ChristoffoletI, 2002). $DL_{50}$ (lethal dosage for

***Table 1*** - Herbicides and dosages used in the experiments. Piracicaba /SP, 2015

| Herbicide | Commercial name | 1/ Trade dosage (g or L $ha^{-1}$) | g a.i. $ha^{-1}$ |
|---|---|---|---|
| Glyphosate | Roundup® | 2.0 | 720 |
| Chlorimuron-ethyl | Classic® | 80.0 | 20 |
| Cloransulan-methyl | Pacto® | 35.7 | 30 |
| Imazethapyr | Pivot® | 1.0 | 100 |

1/ Rodrigues and Almeida (2011).

50% of the population) and $GR_{50}$ (growth reduction by 50%) are the dosages of herbicides that provide 50% of control or reduction of the weed mass, respectively (Christoffoleti, 2002; Christoffoleti and López-Ovejero, 2004). Although one of the parameters of the logistic model is an estimate of $DL_{50}$, its mathematical calculation was also made through an inverse equation, according to the discussion proposed by Carvalho et al. (2005).

## RESULTS AND DISCUSSION

Low sensitivity was detected in the biotype of *Amaranthus palmeri* to all the herbicides assessed. In some cases, it was not possible to discriminate the necessary doses to obtain 50% of control or reduction of the dry matter. This difficulty was even more frequent for the obtaining of doses of 80% control or reduction of dry matter (Table 2).

This Palmer amaranth population is known to be resistant to glyphosate (Carvalho et al., 2015), which was proven again in this paper. Considering the percentage control or dry matter, an average dosage of 8,500 g $ha^{-1}$ of glyphosate was necessary to obtain $DL_{50}$ or $GR_{50}$. It was not possible to estimate the necessary dosage to obtain $DL_{80}$ or $GR_{80}$, seeing that the demand was superior to the higher dosage used in the experiment (Table 2; Figure 1), that is, above 11,520 g a.e. $ha^{-1}$.

These values corroborate the report of resistance in the population to glyphosate and justify the need to evaluate alternative herbicides for the control of this species, such as ALS inhibitors. Worldwide, there are currently 50 reported cases of *A. palmeri* resistant to herbicides, of several action mechanisms, including cases of multiple resistance involving EPSPs-ALS inhibiting herbicides (Heap, 2016).

Low herbicide sensitivity of *A. palmeri* was evident for ALS inhibitors as well, especially after comparing with the species *A. spinosus*, which had very high susceptibility and validated the sprays properly (Table 2). In the case of chlorimuron-ethyl, for instance,

***Table 2*** - Variables assessed (percentage control at 28 DAA [1/] or dry matter), parameters of the logistic model [2/], determination coefficient ($R^2$) and lethal dosage (DL) or growth reduction (GR) for the susceptibility of *Amaranthus* populations to different herbicides. Piracicaba /SP, 2015

| Species | Variable | Parameters | | | | $R^2$ | DL or GR | |
|---|---|---|---|---|---|---|---|---|
| | | Pmin | a | b | c | | 50 | 80 |
| Glyphosate | | | | | | | | |
| *A. palmeri* | 28 DAA | --- | 107.769 | 9301.508 | -1.428 | 0.977 | 8406.76 | > 11,520 |
| | Dry Matter | 12.353 | 76.048 | 8538.133 | 4.042 | 0.941 | 8580.12 | > 11,520 |
| Chlorimuron-ethyl | | | | | | | | |
| *A. palmeri* | 28 DAA | --- | 108.694 | 214.342 | -1.022 | 0.991 | 183.22 | > 320 |
| | Dry Matter | -6.347 | 105.855 | 189.195 | 0.542 | 0.912 | 232.08 | > 320 |
| *A. spinosus* | 28 DAA | --- | 98.422 | 1.674 | -1.101 | 0.977 | 1.72 | 6.35 |
| | Dry Matter | 5.491 | 94.583 | 4.313 | 0.831 | 9.950 | 4.97 | 33.69 |
| Cloransulan-methyl | | | | | | | | |
| *A. palmeri* | 28 DAA | --- | 179.262 | 5445.886 | -0.486 | 0.958 | > 480 | > 480 |
| | Dry Matter | 10.672 | 66.253 | 906.970 | 3.066 | 0.891 | > 480 | > 480 |
| *A. spinosus* | 28 DAA | --- | 94.701 | 1.942 | -1.381 | 0.986 | 2.11 | 6.62 |
| | Dry Matter | -0.671 | 101.419 | 4.310 | 0.302 | 0.882 | 4.33 | < 7.5 |
| Imazethapyr | | | | | | | | |
| *A. palmeri* | 28 DAA | --- | 123.272 | 1716.888 | -0.669 | 0.983 | 969.75 | > 1,600 |
| | Dry Matter | 1.159 | 87.019 | 1787.977 | 1.048 | 0.927 | 1,342.13 | > 1,600 |
| *A. spinosus* | 28 DAA | --- | 100.157 | 11.069 | -1.957 | 0.985 | 11.05 | 22.39 |
| | Dry Matter | 14.848 | 85.637 | 10.920 | 1.386 | 0.975 | 14.18 | 79.34 |

[1/] DAA - days after application; [2/] $y = a/(1+(x/b)^c)$ or $y = a+b/(1+(x/c)^d)$.

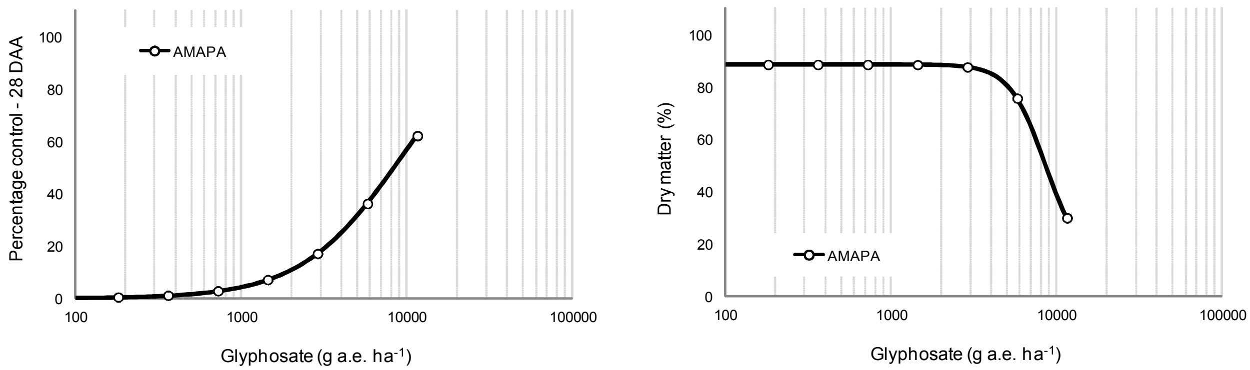


***Figure 1*** - Percentage control and dry matter of *Amaranthus palmeri* (AMAPA) subjected to different dosages of glyphosate, assessed at 28 days after application (DAA). Piracicaba /SP, 2015.

less than 10 g $ha^{-1}$ was enough to control *A. spinosus* above 80%. However, in the case of *A. palmeri*, it was not possible to estimate $DL_{80}$ or $GR_{80}$, seeing that the demanded rate was above 320 g $ha^{-1}$ of chlorimuron, that is, *A. spinosus* has high sensitivity to the molecule, while *A. palmeri* is insensitive to the same herbicide, considering commercial doses and the same spraying conditions (Table 2; Figure 2).

Wise et al. (2009), in a study done with populations of *A. palmeri* from 30 different locations of the state of Georgia (USA), obtained satisfactory control when they used 13 g $ha^{-1}$ of this herbicide (chlorimuron-ethyl), reaching mass reduction of 64% in relation to the check plots without treatment.

As for the Brazilian biotypes of *Amaranthus*, the same response pattern observed for chlorimuron was kept for cloransulan-methyl and imazethapyr, considering high susceptibility of *A. spinosus* and very low sensitivity of *A. palmeri* (Figures 3 and 4). In a previous report, Andrade Júnior et al. (2015) made comments on the low sensitivity of the *A. palmeri* population to glyphosate and also to ALS inhibitors. These authors already considered the possibility of multiple resistance to glyphosate - ALS, but that still needed scientific proof.

The species *A. palmeri* and *A. spinosus* share a common ancestral, more than other species of amaranth, seeing that they have the same number of chromosomes (2n=34), pollen grain morphological similarities (Franssen et al., 2001) and similarities in the genome size (Rayburn et al., 2005). Moreover, *A. palmeri* and *A. spinosus* were classified as sisters by the analysis of the phylogenetic rate, once all the other studied rates for other species of *Amaranthus* were excluded, which indicates that other species of amaranth are more distant from *A. palmeri* and *A. spinosus* (Wassom and Tranel, 2005; Riggins et al., 2010).

In the evaluation of the different ALS inhibitor herbicides belonging to three different chemical groups — sulphonylurea (chlorimuron-ethyl), triazolopyrimidine (cloransulan-methyl) and imidazolinone (imazethapyr) —, none of the herbicides evaluated was able to appropriately control the population of *A. palmeri*. It was not possible, at least, to estimate $DL_{80}$ or $GR_{80}$ even using doses 16 times higher than the ones commercially recommended of the products (Rodrigues and Almeida, 2011), showing the insensitivity to the molecules (Table 2; Figures 2, 3 and 4).

In Brazil, until now, a biotype of *A. palmeri* susceptible to glyphosate or ALS inhibitors has not been identified in order to enable scientific comparison of the $DL_{50}$ and $GR_{50}$ values. However, from the values identified on Table 2, it is possible to affirm that it is a population with multiple resistance to EPSPs and ALS inhibitors. Also, considering only the ALS inhibiting herbicides, this population has cross-resistance among sulfonylureas - triazolopyrimidines - imidazolinones.

In that sense, recommendations of herbicides based on EPSPs and ALS inhibitors should be avoided for the control of *A. palmeri*

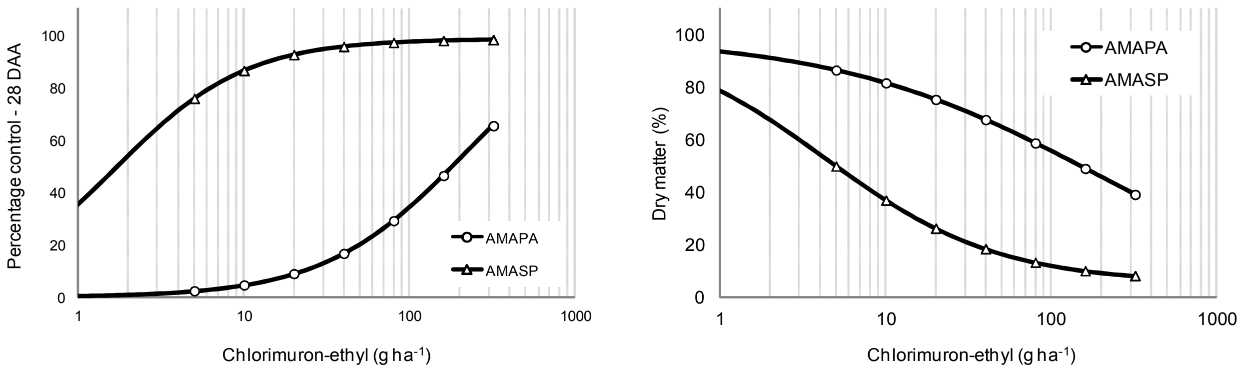


***Figure 2*** **-** Percentage control and dry matter of *Amaranthus palmeri* (AMAPA) and *A. spinosus* (AMASP) subjected to different dosages of chlorimuron-ethyl, assessed at 28 days after application (DAA). Piracicaba /SP, 2015,

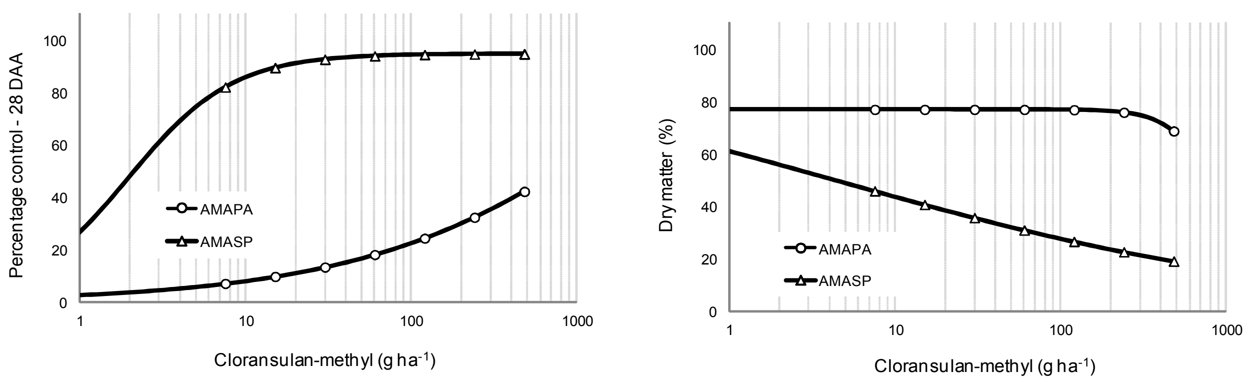


***Figure 3*** **-** Percentage control and dry matter of *Amaranthus palmeri* (AMAPA) and *A. spinosus* (AMASP) subjected to different dosages of cloransulan-methyl, assessed at 28 days after application (DAA). Piracicaba /SP, 2015,

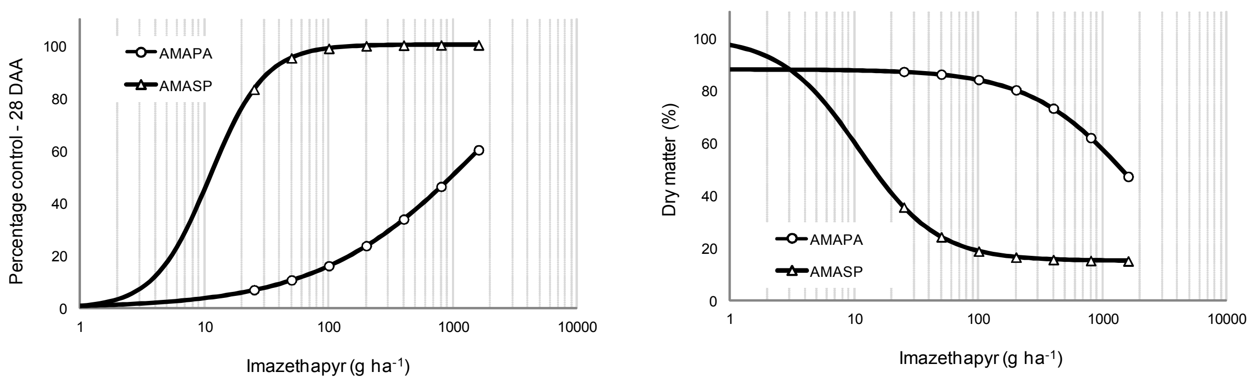


***Figure 4*** **-** Percentage control and dry matter of *Amaranthus palmeri* (AMAPA) and *A. spinosus* (AMASP) subjected to different dosages of imazethapyr, assessed at 28 days after application (DAA). Piracicaba /SP, 2015.

identified in areas of Mato Grosso. Therefore, new management alternatives must be evaluated, including photosystems I and II inhibitors, auxin-mimetic herbicides, PROTOX inhibitors and carotenoid synthesis inhibitors.

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