International Journal of Agriculture Innovations and Research
Volume 7, Issue 1, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy):
Received: 27/07/2018 | Accepted on: 04/08/2018 | Published: 11/08/2018

Growth and Development of Glyphosate-Resistant *Amaranthus palmeri* Identified in the State of Mato Grosso, Brazil

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Abstract **– Considering the importance of the weed *Amaranthus palmeri* (Palmer amaranth) to world agriculture, as well as its recent identification in Brazil, the objective of this work was to evaluate the growth and development of the Brazilian biotype of *A. palmeri* in non-competitive condition. Therefore, three similar experiments were carried out in a greenhouse to evaluate the phenology and accumulation of dry matter (shoot, roots and total) of this plant. Eight growth evaluations were carried out in each experiment, spaced 12 days apart, starting at 28 days after sowing (DAS), totaling a mean cycle of 105 days. Data of total dry matter was used to calculate the absolute and relative growth. Brazilian biotype of *A. palmeri* has a rapid phenological development, with onset of inflorescences at approximately 50 DAS. The phenological development of the species fitted to a linear model (y = 0.8866 x). Plants evaluated in greenhouse conditions showed maximum accumulated dry weight of 45 g plant^{-1}, on average, with absolute growth peak at 60 DAS. Thus, the growth of this species were considered moderate compared with those from other Brazilian *Amaranthus* species and international data on *A. palmeri*.**

Keywords **– Weed Biology, Integrated Management, Palmer Amaranth, Phenology.**

I. Introduction

Infestations of *Amaranthus palmeri* (Palmer amaranth) were recently identified on cotton farms in the central-northern region of the State of Mato Grosso, Brazil [1]. *A. palmeri* is a very important global weed that had never been identified in Brazil before. As a result of these infestations and control failures in the fields, experiments were carried out and proved the resistance of this biotype to the herbicide glyphosate and to ALS inhibitors, i.e., multiple resistance EPSPS-ALS [2], [3]. Therefore, this species has great agricultural importance. There are fifty-nine cases of resistant *A. palmeri* biotypes to herbicides of several modes of action reported in the world, including cases of multiple resistance [4].

A. palmeri is indigenous to arid regions of the southern center United States and northern Mexico, however, it is found in several countries around the world. This species has become the main cotton weed in the USA, due to its biological characteristics and resistance to herbicides of different modes of action [5], [6]. This species has morphological similarities with the *Amaranthus* species commonly found in Brazil, however, *A. palmeri* differs from these species because it is dioecious, i.e., plant populations having male and female reproductive organs in separate individuals [3], [7].

Amaranthus plants are strongly competitive with crops for light, water and nutrients, due to their aggressive growth habit and great seed production [8], [9]. The negative effect of *A. palmeri* on the growth, development and yield of cultivated plants varies according to the present species, infestation density and time of emergence in relation to the crop [10], [11].

Studies on weed growth and development provide information of the different phenological stages and patterns of plant growth. These results allow analyzing plants response to ecological factors, as well as their effect on the environment, mainly regarding to their effect on the development of other plants, which may contribute to the development of integrated weed management systems [12], [13]. Growth characteristics of a given species are important indicators of its competitive ability [14], [15]; species that grow rapidly and produce a large leaf area are likely to be more competitive than those of low growth [16]. Thus, the description of the phenological phases and evaluation of the dry biomass of the different plant structures have major importance [17], [18].

The use of growth analyzes is an accessible and precise method to infer the contribution of different physiological processes to plant growth, which reveals the kinetics, distribution and efficiency of plant biomass production along their ontogenesis [18], [19]. Thus, considering the importance of the weed *A. palmeri* (Palmer amaranth) to world agriculture, as well as its recent identification in Brazil, the objective of this work was to evaluate the growth and development of the Brazilian biotype of *A. palmeri* in non-competitive condition.

II. Material and Methods

Three similar experiments were performed to evaluate the growth and development of *Amaranthus palmeri*. Two of these experiments were carried out in the greenhouse of the Federal Institute of Education, Science and Technology of the South of Minas Gerais, Machado Campus, in the municipality of Machado - MG, Brazil. (21° 40' S, 45° 55' W, 850 m). Seeds of *A. palmeri* were sowed on November 13, 2014 for the first experiment, with plants developing until February 2015; and on January 14, 2015 for the second experiment, with plants developing until April 2015. The third experiment was carried out in the greenhouse of the Crop Science Department, University of Sao Paulo, in Piracicaba - SP, Brazil, with sowing on August 29, 2015 and development until December 2015.

The origin of the *A. palmeri* seeds was the same as those used in previous experiments conducted by [2] and [3], therefore, it is a confirmed resistant population to the herbicide glyphosate and to ALS inhibitors in the State of Mato Grosso, Brazil. These seeds were stored in paper bags, in a dry place at room temperature, until the beginning of the experiments.

Seeds of *A. palmeri* were placed in 2-L plastic boxes filled with a commercial substrate (pinus bark + peat + vermiculite) for germination. Than, plants were transplanted to pots (one plant per pot), at the phenological stage 10 (fully expanded cotyledonary leaves) [20], where they remained until the end of the experiment.

The experimental plots consisted of 4.0-L plastic pots filled with a commercial substrate, disaggregated sieved clay soil and vermiculite (6:3:1 v/v). The substrate of all plots were fertilized with the following nutrients (mg plot^{-1}): N (450), P_2O_5 (650), K_2O (600), Ca (200), S (195), Mg (15), Zn (3), B (0.6), Fe (6), Cu (1.5), Mn (3) and Mo (0.6). Approximately 30 to 60 days after sowing (DAS), a supplemental fertilization was carried out with the following nutrients (mg plot^{-1}): N (100), Ca (40), S (70) and Mg (45). Pots were irrigated by an automated irrigation system, thus, no water or nutritional deficiency occurred.

The phenological development of the plants was monitored from the transplants, thus, it was carried out during all growth samplings. The phenology of the entire remaining population was initially identified at each evaluation, using the scale proposed by [20]. The phenological stage was defined by the identification of the developmental characteristics in 50% + 1 of the total remaining plants. After flowering, the reproductive structures were bagged to avoid seed dispersal. In the experiment carried out in Piracicaba - SP, plant phenology was not evaluated.

A randomized complete block experimental design, with eight treatments and four replications was used for the growth evaluations. Eight growth evaluations (treatments) were performed during the experiment, on average, at each 12 days, except the first evaluation, which was performed at 28 days after sown (DAS), totaling a 105-day average cycle. In each evaluation, four plants (replicates) were then randomly sampled by the destructive method and washed in running water to remove the remaining substrate of the roots, to analyze their variables. The sampled material was dried in an oven at 70 ºC for 72 h and the root, shoot and total dry weight (g plant^{-1}) was evaluated.

In addition, absolute (G, g day^{-1}) and relative (R, g g^{-1} day^{-1}) growth rate were calculated based on the average total dry weight [11], [18], [21]. The absolute growth rate was calculated using the formula:

$$G_M = \frac{Mt_2 - Mt_1}{t_2 - t_1} \qquad (1)$$

Wherein M_{t2} and M_{t1} are the total dry weight of two successive samples and $_{t2}$ and $_{t1}$ are the days between these samplings.

The relative growth rate was calculated using the formula:

$$R_M = \frac{\ln Mt_2 - \ln Mt_1}{t_2 - t_1} \qquad (2)$$

The technique of analysis by group of experiments was used to compare the variability of the experiments by the test of Hartley [22]. Analyzes of the phenological development of the plants were fitted to the linear model, as performed by [19]. The quantitative variables related to plant growth were analyzed using nonlinear regressions. The root, shoot and total dry weight averages were fitted to the nonlinear logistic regression model, and the absolute and relative growth data were fitted to exponential nonlinear models.

III. Results and Discussion

The joint analysis of phenological development of *A. palmeri* in the two experiments carried out in Machado - MG showed a rapid phenological development for the species (Figure 1), with onset of inflorescence at approximately 50 days after sowing (DAS). Rapid development was also found in five other species of *Amaranthus*, which showed visible reproductive structures (onset of inflorescence) at 40 DAS (stage 50), especially *A. deflexus*, whose flowering was more advanced [13].

Phenological development of *A. palmeri* was fitted to a linear model, according to the equation y = 0.8866 x (Figure 1). The parameter *a* of this equation allows us estimating the development speed of plants during a season or according to their sowing date [19], [23]. According to this estimate model, *A. palmeri* plants have a development of 10 units on the BBCH scale every 12 DAS [20]. This information has important practical application, since the best effectiveness of the herbicides is achieved by applications on young plants, which must be carried out before 30 DAS.

[19] Evaluated the development of *Cyperus rotundus* and also found its phenology fitted to the linear model (*a* = 0.8877). On the other hand, considering *Digitaria insularis*, it was found the parameter *a* = 0.4423, i.e., a slower phenological development [15]. The ability to predict weed phenological stages, such as flowering, development and dispersal of seeds, can assist in the development of integrated management practices [12], [23].

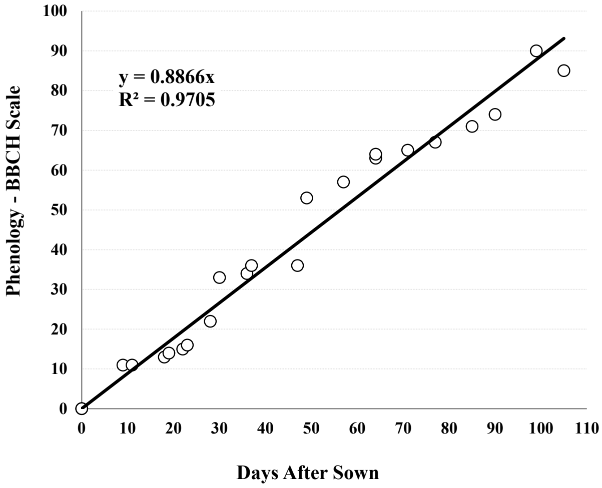

Fig. 1. Phenological development of *Amaranthus palmeri* plants throughout their development cycle, according to the BBCH scale [20]. Machado - MG, Brazil, 2014/2015.

The three experiments showed homogeneity of variance for all quantitative variables (shoot, root and total dry weight), thus justifying the joint analysis of the data. The averages of dry matter accumulation of *A. palmeri* plants fitted to the sigmoid model, with coefficients of determination always higher than 90% (Figures 2, 3 and 4). The variables related to the growth of plants showed a point of inflection in the curves at approximately 60 DAS, time of in which the plants reached maximum growth and then decelerated, possibly due to the differentiation of the flowers and fruits (Figures 2, 3 and 4).

The maximum total dry weight of *A. palmeri* was 45 g plant^{-1}, at 100 DAS (Figure 4). These data are below the maximum production found in the literature, which shows a *A. palmeri* biomass usually higher than the other *Amaranthus* species found in Brazil, such as *A. retroflexus*, *A. hybridus* and *A. spinosus* [16], [24], [25].

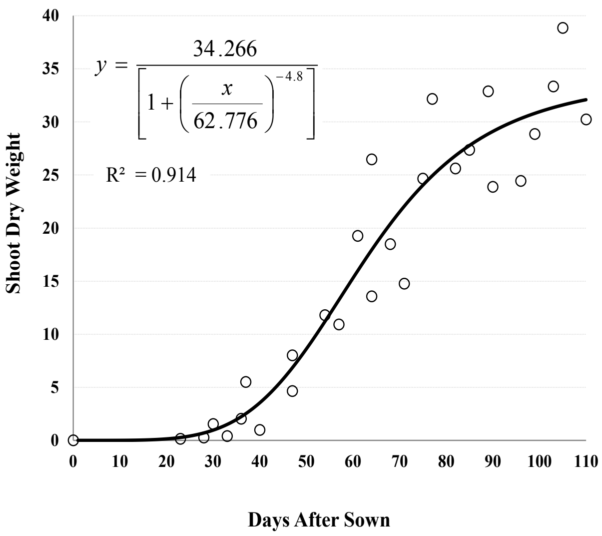

Fig. 2. Shoot dry weight accumulation (g plant^{-1}) by *Amaranthus palmeri* plants throughout their development cycle. Machado - MG, Piracicaba - SP, Brazil, 2014/2015.

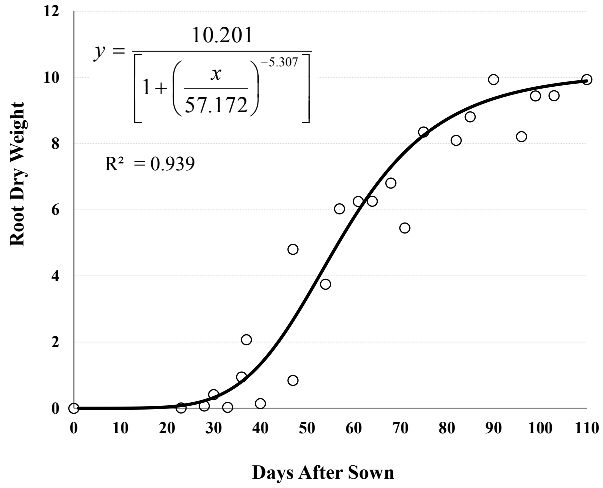

Fig. 3. Root dry weight accumulation (g plant^{-1}) by *Amaranthus palmeri* plants throughout their development cycle. Machado - MG, Piracicaba - SP, Brazil, 2014/2015.

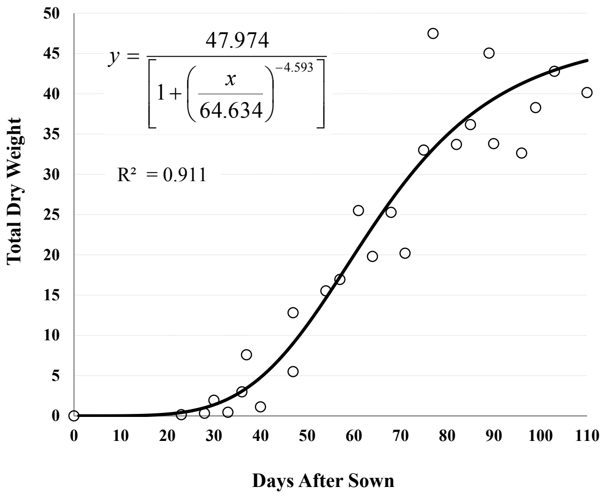

Fig. 4. Total dry weight accumulation (g plant^{-1}) by *Amaranthus palmeri* plants throughout their development cycle. Machado - MG, Piracicaba - SP, Brazil, 2014/2015.

[25] Compared six species of *Amaranthus* and found greater growth potential for *A. palmeri*, with total accumulation weight of up to 800 g plant^{-1}, which was higher than the weights found for the other species. On the other hand, [24] found biomass production of 18 to 70 g plant^{-1} on plants grown under temperatures of 15/10 and 35/30°C, respectively. [16] Also found variable biomass production for *A. palmeri* (40 to 120 g plant^{-1}) in different years and sowing times. These authors considered that the photoperiod probably affect the biology of this species, due to the physiological changes from the induction for flowering.

Weeds from the genus *Amaranthus* have a C4 photosynthetic cycle, thus, temperature is one of the ecological factors that most affect the plant growth and productivity [24]. [26] Evaluated 24 populations of *A. palmeri* from different locations and found ecotypes for the species, which showed different development regarding their leaf area ratio, specific leaf area and net assimilation rate. Thus, species of this genus may have different development under other temperatures, and possibly be from different biotypes.

The averages of absolute growth of the species fitted to a parabolic curve, with peak at 60 DAS, and a maximum accumulation of 0.9 g day^{-1} (Figure 5). The maximum relative growth was 0.2 g g^{-1} day^{-1}, which decreased over time (Figure 6). These results are low compared with those from the *Amaranthus* species commonly found in Brazil, which can reach absolute growth rates higher than 1.5 g day^{-1} and decreasing relative growth from 0.3 g g^{-1} day^{-1} [13]. The growth of other Brazilian traditional species can be similar (*A. spinosus* and *A. viridis*) or higher (*A. hyrbridus* and *A. retroflexus*) to the growth of the *A. palmeri* biotype, except *A. deflexus* [13]. However, this result is not confirmed in the literature, which commonly reports *A. palmeri* as the highest-growth species [16], [24], [25].

The moderate growth of the Brazilian *A. palmeri* biotype, compared with those found in the national and international literature, is due to its early floral differentiation, which is possibly affected by the photoperiod, reducing the plant growth and directing photo assimilates to flowers and fruits. Similar results were found for *Digitaria insularis* [15] and *Cenchrus echinatus* [23], which produce less dry biomass due to early flowering, thus, when flowering is delayed, there is more time for growth, resulting in greater biomass accumulation.

Lower growth of Brazilian *A. palmeri* biotype may also be a consequence of the greenhouse experiment, or due to expression of resistance to the herbicide glyphosate. According to [4], the first case of resistance of *A. palmeri* to glyphosate in the USA was in 2005, indicating that the works cited above were developed with susceptible biotypes. In addition to the comparison of the dates, there is no mention of the use of glyphosate resistant biotypes in the works found [16], [24], [25].

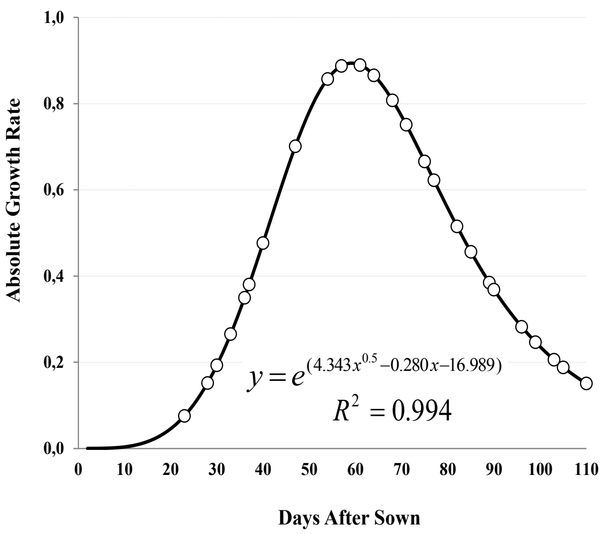

Fig. 5. Absolute growth (g day^{-1}) of *Amaranthus palmeri* plants throughout their development cycle. Machado - MG, Piracicaba - SP, Brazil, 2014/2015

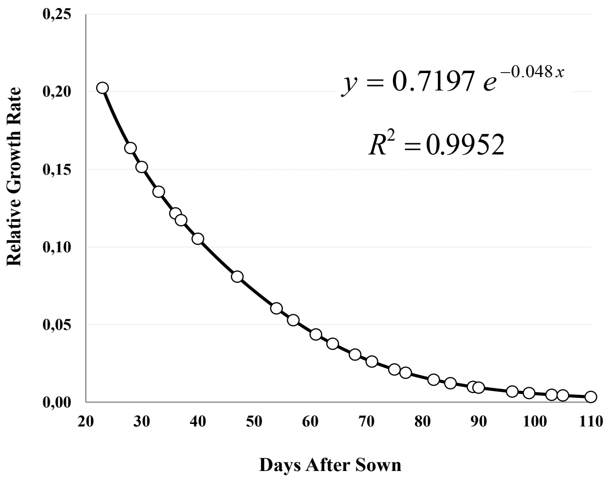

Fig. 6. Relative growth (g g^{-1} day^{-1}) of *Amaranthus palmeri* plants throughout their development cycle. Machado - MG, Piracicaba - SP, Brazil, 2014/2015.

Resistant *A. palmeri* biotypes to glyphosate are currently related to the enzymatic overproduction, also resulting from the genomic amplification of the EPSPs gene [27], [28]. Plants that employ more energy in the overexpression of a single enzyme may have less availability of amino acids for the synthesis of other vital proteins and enzymes, resulting in greater general nitrogen demand, i.e., they would be less efficient plants on nitrogen use. Thus, a field environment with low nutritional availability, especially nitrogen, may result in plants with low development, size and dry biomass accumulation.

IV. Conclusions

Brazilian biotype of *A. palmeri* has a rapid phenological development, with onset of inflorescences at approximately 50 DAS; phenological development of the species fitted to a linear model ($y = 0.8866x$); plants evaluated in greenhouse conditions, showed maximum accumulated dry weight of 45 g plant^{-1}, with absolute growth peak at 60 DAS. The growth of this species was considered moderate compared with those from other Brazilian *Amaranthus* species and international data on *A. palmeri*.

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