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Biological characteristics, resistance to herbicides and management of *Amaranthus palmeri* in agroecosystems[1]

Características biológicas, resistência a herbicidas e manejo de ***Amaranthus palmeri*** *em agroecossistemas*

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Abstract - Herbicide-resistant populations of the species *A. palmeri* were detected in Brazilian crops in 2014. Plants from this species were never identified in Brazil before. In other places of the world, *A. palmeri* causes many crop losses due to its biological characteristics and its competitivity in agricultural systems. Furthermore, herbicide-resistant populations become an important management issue, especially in the case of multiple herbicide resistance. This literature review describes the biological characteristics of this species in order to understand its adaptation and potential for crop losses and, also, to help to identify weakness which might aid the plant eradication. The cases described are those of resistance of *A. palmeri* around the world, as well as the mechanisms responsible for this resistance. It is presented possible actions that allow the management and control of plants from this species in cotton, soybean and corn crops.
Keywords: biologic characteristics; eradication; chemical control; cultivated species

Resumo - Populações da espécie *A. palmeri* com resistência a herbicidas foram detectadas em lavouras brasileiras em 2014. Plantas dessa espécie nunca foram identificadas anteriormente no Brasil. Em outros locais do mundo, *A. palmeri* causa muitos prejuízos, em função das características biológicas que a torna muito competitiva em sistemas agrícolas. Além disso, populações resistentes aos herbicidas são problemas importantes no manejo das plantas, especialmente quando ocorre resistência múltipla. Na presente revisão são descritas as características biológicas dessa espécie que auxiliam no entendimento da sua adaptação, os prejuízos potenciais para as culturas e, também, na expectativa de encontrar vulnerabilidades que possam ser utilizadas no manejo das plantas. São descritos os casos de resistência de *A. palmeri* registrados no mundo, bem como os mecanismos causais da mesma. Também são discutidas ações que permitam o manejo e o controle dessa espécie nas culturas do algodão, soja e milho.
Palavras-chaves: características biológicas; erradicação; controle químico; espécies cultivadas

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Introduction

The species of the genus *Amaranthus* spp., commonly known as pigweed, are important as weeds that infest several crop species (Kissmann and Groth, 1999). In Brazil, around 10 pigweed species occur in crops. Plants of *A. palmeri* were first identified in Brazil in crops located in the state of Mato Grosso. One disturbing characteristic identified on the first biotypes detected is their resistance to herbicides such as glyphosate and ALS inhibitors (Andrade Júnior et al., 2015; Carvalho et al., 2015).

The infestations of *A. palmeri* in the state of Mato Grosso raise important issues to the national agricultural industry. The potential for crop losses is very high because the plants are resistant to herbicides from two mechanisms of action, which were widely used to control the plants in several annual and/or perennial crops, from north to south of Brazil. The plants from this species also present biological characteristics that allow them to be very competitive in agricultural systems.

A. palmeri is a type of pigweed originated from the arid regions of the South-Central parts of the United States and north of Mexico. The plants were reported as weeds in several countries. In the United States, in recent years, it has been one of the main weeds to invade agricultural areas, especially in cotton crops (Carvalho et al., 2015; Neve et al., 2011.; Webster and Grey, 2015).

The first step to take with an invading species is to adopt eradication strategies. Thus, on the populations detected in Mato Grosso state is important to establish a weed eradication program. The production of seeds and its dissemination to other areas must be stopped. The adoption of control measures, such as preventive, cultural, biological, physical, mechanical and chemical methods, as well as the integrated management of weeds, is necessary for a successful plant eradication and control (Andrade Júnior et al., 2015).

This literature review describes the biological characteristics of this species in order to understand its adaptation and potential for crop losses and to help to identify weakness that might aid to manage and eradicate *A. palmeri* plants in the main agricultural crops.

Biological Characteristics

Amaranthus palmeri is one of the 75 species of its genus (Steckel, 2007). It is a summer annual plant which grows between 1 m and 3 m in height (Sauer, 1955; Sellers et al., 2003). It is a native species of the Southwest of North America, and it has been introduced into Central America, South America, Europe, Asia and Oceania (Rzedowski and Rzedowski, 2005; Steckel, 2007; Legleiter and Johnson, 2013).

The plants are glabrous or almost completely glabrous, with erect and branched stems, leaves with long petioles and panicle-like inflorescences. *A. palmeri* has morphological characteristics similar to *A. retroflexus*, *A. hybridus* and *A. rudis*. Six characteristics help differentiate *A. palmeri* from those species. They are: absence of hair in all the tissues of the plant (except individuals that have only one hair at the tip of the leaf), oval shape of the leaf, petiole bigger in length than the leaf, apical meristems in the shape of rosette when seen from above, female plant with long panicles with thorny bracts and variegated leaf with a V shaped stain (Steckel, 2007; Legleiter and Johnson, 2013).

The plants have a C4 photosynthetic system (Sage et al., 2007) which give them photosynthetic ability and adaptation to high temperatures, when compared to other species (Ehleringer, 1983). The plants also have great adaptability to different lighting conditions (Jha et al., 2008; Guo and Al-Khatib, 2003).

A. palmeri is one of the few species of dioecious plants, that is, which presents female and male reproductive organs in separate individuals. The reproduction occurs through the production of seeds, with flowering between spring and autumn. During the reproductive period of the year, only one plant of *A. palmeri* can produce from 250.000 (Sellers et al., 2003) to 600.000 seeds (Keeley et al., 1987), with the highest production occurring under a competition-free condition. The production of seeds can occur by the crossing of individuals of the same species, among species (*A. rudis*) or even through apomixis. Apomixis can be a determining factor in the fixation of genes in populations resistant to glyphosate (Ribeiro et al., 2014).

Temperature is a determining factor in the germination of *A. palmeri* seeds. There is greatest seed germination under alternate temperatures. Indeed, the greatest germination percentage (about 40%) was documented with alternate day/night temperatures of 35/30 °C (Guo and Al-Khatib, 2003). The extreme temperatures, below and above which there was no germination, were 15/10 and 50/45 °C, respectively (Guo and Al-Khatib, 2003), which explains the extended germination periods throughout the year (Jha et al., 2008).

A. palmeri seeds can remain viable in the seeds bank for a period of more than 3 years (Sosnoskie et al., 2013). However, that depends on the depth in which the seeds are available in the soil and on the activity of seed predators such as arthropods, and rodents. Seeds positioned at 40 cm depth during 36 months presented 22% germination, whereas the seeds placed at 1 cm depth presented only 9% germination. There is an exponential reduction of the germination percentage in relation to the time in which the seeds are in the soil (Sosnoskie et al., 2013).

Amaranthus palmeri presents a positive photoblastic response of seed germination. Thus, good management strategies to prevent seed germination includes reduction of the passage of solar radiation to the soil using cover crops or straw in no-tillage cropping systems (Jha and Norsworthy, 2009).

The roots of *A. palmeri* plants present great length and small diameter (Forseth et al., 1984). This characteristic probably explains the plant adaptability to low rainfall conditions and its ability to explore the soil volume, ensuring an advantage in the competition for water and nutrients (Berger et al., 2015).

A. palmeri plants accumulate great amount of dry matter over time. This fact is interpreted as an evidence of resource competition with crops. Also, it may explain the difficulty for the chemical control of the plants (Sellers et al., 2003; Ward et al., 2013).

Soybean plants in coexistence during the whole season with eight *A. palmeri* plants per linear meter reduced the crop yield by 78% (Bensch et al., 2003). At the same weed density, the corn grain yield was reduced by 91% (Massinga et al., 2001). In cotton, however, only one *A. palmeri* plant per meter of row reduced the crop yield by 54% (Morgan et al., 2001).

Allelopathy is another strategy of *A. palmeri* interference with other plant species. This negative form of plant interference is proposed to happen mainly due to the presence of volatile compounds present in the shoots, mainly in the leaves and flowers. Substances recovered from these tissues reduced the germination speed of carrot, onion and tomato seeds (Bradow and Connick, 1987).

Cases of Resistance in the World and in Brazil

In the world, biotypes of *A. palmeri* with resistance to herbicides were documented in the United States, Israel (Heap, 2015), Argentina (Morichetti, 2013) and Brazil (Andrade Júnior et al., 2015).

The resistance of *A. palmeri* occurs to herbicides from one mechanism of action (MeA) (simple) or to several MeA (multiple). The first case of simple herbicide resistance was reported in 1989, with biotypes from South Carolina, USA, resistant to the active ingredient trifluralin (tubulin polymerization inhibitor), in cotton and soybeans crops (Table 1). Afterwards, the next records of simple resistance to herbicides from other MeA were documented in 1993 (ALS and II photosystem inhibitors), in 2005 to EPSPS inhibitor, and in 2011 to HPPD and PROTOX inhibitors. The first case of multiple resistance in *A. Palmeri* was evidenced in 2008, with biotypes insensitive to ALS and EPSPs inhibitors. In 2009, it was registered resistance to ALS, FSII and HPPD inhibitors and, in 2010, to ALS, FSII and EPSPS inhibitors (Table 1).

Simple resistance to glyphosate is the most disseminated, being found in 20 American states. However, although not widely spread, the multiple resistance cases are of great concern because they make the management of this species more complex and difficult.

In Israel, there are records of resistance of *A. palmeri* to ALS inhibitor herbicides. In Argentina, the first reports happened in Cordoba, in 2013. Later on, there was an expansion of resistance to glyphosate, and in 2013 the infestations were more focused on the Southwest quadrant of Cordoba province and in the Southeast quadrant of San Luis province (Morichetti, 2013).

Table 1. Reported cases of biotypes of *A. palmeri* resistant to herbicides.

Resistance type	Modes of action	First year of report Location	Other places and years	Crops affected
Simple	Tubulin polym. inhibitors	1989 (South Carolina)	1998 (Tennessee)	Cotton, soybeans,
Simple	FS II inhibitors	1993 (Texas)	1995 (Kansas); 2008 (Georgia); 2011 (Nebraska)	Corn, sorghum
Simple	ALS inhibitors	1993 (Kansas)	1994 (Arkansas and Tennessee); 1995 (North Carolina), 1997 (South Carolina); 2000 (Georgia); 2008 (Florida; Israel); 2013 (Illinois)	Alfalfa, corn, sorghum and soybeans, cotton, peanut
Simple	EPSPs inhibitor	2005 (Georgia and North Carolina)	2006 (Arkansas, Tennessee, North Carolina); 2007 (New Mexico); 2008 (Alabama and Missouri); 2010 (Louisiana; Illinois; Ohio; Kentucky); 2011 (Michigan; Virginia; Kansas; Texas); 2012 (Delaware; Indiana); 2013 (Pennsylvania; Florida; Wisconsin); 2014 (New Jersey)	Cotton, soybeans, corn, orchards and walnut pecan, among others
Multiple	ALS and EPSPs inhibitors	2008 (Mississippi and Georgia)	2009 (Tennessee); 2010 (South Carolina); 2012 (Arizona); 2013 (Illinois; Florida); 2014 (Delaware; Maryland)	Cotton, corn, soybeans, among others
Multiple	ALS, FSII and HPPD inhibitors	2009 (Kansas)		Corn and sorghum
Multiple	ALS, FSII and EPSPs inhibitors	2010 (Georgia)		Corn
Simple	HPPD inhibitors	2011 (Nebraska)		Corn
Simple	PROTOX inhibitors	2011 (Arkansas)		Soybeans

Source: Adapted from Heap (2016).

Until 2014, the presence of *A. palmeri* had not been reported in Brazil. This year, the first report of *A. palmeri* in Brazil happened in crops with crop rotation (cotton, soybeans and corn) in the cotton center region located on the north-center area of Mato Grosso state (Andrade Júnior et al., 2015). Biotypes of A. palmeri were not controlled by herbicides to which plants of the same species were susceptible. It has been shown that populations harvested in the crops presented multiple resistance to ALS and EPSPs inhibitors (Andrade Júnior et al., 2015).

Resistance Mechanisms

The resistance of weeds to herbicides occurs due to several mechanisms including (but not limited to): the alteration (or no alteration) of the site of action of the herbicide; the increase of the target enzyme expression (Gaines et al., 2010), increasing the number of places for herbicide coupling. The alteration of the site of action happens due to the replacement of one or more amino acid in a target protein/enzyme, preventing the correct coupling of the herbicide (Powles and Yu, 2010). The mechanisms not related to the site of action include: the reduction of absorption or translocation of the herbicides; the increased herbicide detoxification or its sequestration on the cell vacuoles (Perez-Jones et al., 2007; Powles and Yu, 2010).

The mechanism of resistance of *A. palmeri* to glyphosate is related to those not involving the herbicide site of action (Ward et al., 2013). Investigations on the resistance mechanisms of this species to glyphosate, carried out by several authors, found conflicting results, which may be explained by the fact that they are populations with different origins. No differences in glyphosate absorption and translocation were observed between resistant and susceptible biotypes of *A. palmeri* collected in the state of Georgia (USA) (Culpepper et al., 2006). Furthermore, the accumulation of shikimate was not detected in tissues of the resistant biotype previously treated with this herbicide.

In studies of glyphosate S and R *A. palmeri* biotypes harvested in Mississippi (USA) did not find differences in detoxification of this herbicide (Nandula et al., 2012). But the authors describe that glyphosate R biotype absorbed less herbicide 48 hours after the application, compared to S biotype (Nandula et al., 2012). The glyphosate S biotype accumulated more herbicide molecules in branches and leaves located above the treated leaf in which the apical meristem is located. Contrarily, the glyphosate R biotype accumulated more herbicide in branches and leaves located below the treated leaf. The possible cause of glyphosate resistance was attributed to reduced herbicide absorption and translocation (Nandula et al., 2012).

In another paper involving *A. palmeri* biotypes R to glyphosate, collected in Georgia (USA), it was observed that the activity of the EPSPs enzymes of both R and S plants was also inhibited by the herbicide (Gaines et al., 2010). Nevertheless, the genome of R plants had five times more additional copies of the EPSPs gene than the S plant genome. Further studies carried out with glyphosate S and R *A. palmeri* biotypes collected from New Mexico, USA, also concluded that the resistance to this herbicide is due to the increased EPSPs gene expression (Mohseni-Moghadam et al., 2013).

Eradication and Management of *A. palmeri*

Eradication

In areas where the *A. palmeri* plants were detected for the first time, as it happened in the properties located in the state of Mato Grosso, in Brazil, eradication campaigns of resistant populations are highly recommended because they have great potential for elimination of the plants located on the initial places of detection.

Theoretically, the higher the dissemination of infestations, the harder it is to successfully eradicate the plants. The analysis of the biological characteristics of *A. palmeri* plants detected a fragility of this species under the United States conditions, which is low viability of seeds in the soil (Jha et al., 2014). These studies indicated that almost 100% of the *A. palmeri* in the soil seed bank is reduced within four years, when new propagules are prevented to be reintroduced. Currently, crop lands in which *A. palmeri* infestations were detected in the state of Mato Grosso are under intense supervision by public agencies. The agronomists instruct the farmers to remove the plants before they set flowers to prevent the production and the dissemination of seeds.

Preventive and Cultural Management

The main goal of the preventive method is to avoid the introduction of plants in not-yet-infested areas and to prevent the entry of new propagules in already-infested areas. The potential magnitude of the impact of glyphosate R *A. palmeri* justifies the prevention effort. Among the recommended measures, it can be included cleaning of vehicles, machinery and equipment before entering an area, focusing on the harvesters. Likewyse, roads and pathways, crop edges, fallow lands, terraces and contour lines must be monitored to eliminate weed plants before they set flowers and seeds (Tuesca et al., 2015). The infested crop areas must always be the last ones to be harvested in order to reduce the risk of introduction in non-contaminated fields.

The fundament of the cultural method of weed management is to adopt measures that give the crop plants a competitive advantage against the weeds. Among the cultural measures, it is highlight: crop rotation, which enables the alternation and use of herbicides from different MeA; the use of cultivars with high competitive ability against weeds, including high initial vigor and with potential of quickly shading the crop inter-rows (Tuesca et al., 2015). In cotton areas infested with *A. palmeri*, it is recommend to grow the corn crop for at least two years, in order to improve weed control efficacy (Legleiter and Johnson, 2013).

The use of cover crops such as rye, wheat or crimson clover (*T. incarnatum*) can effectively reduce early emergence of *A. palmeri*, both independent of the soil tillage systems (Aulakh et al., 2011; De Vore et al., 2013). Indeed, the association of soil tillage with rye cover crop was able to reduce the infestation of *A. palmeri* between 68 and 85% (De Vore et al., 2013). In Brazilian conditions, it would be important to investigate the cover crops with highest potential to reduce the germination of seeds from *A. palmeri*. These crops species would be the key component of an integrated weed management approach for *A. palmeri*.

Chemical Control in Cotton, Corn and Soybeans

Since this species was unknown in Brazil until 2014, there are no registered products for its control in the country. However, there are management alternatives for other pigweed species, such as *A. deflexus*, *A. hybridus* var. patulatus, *A. hybridus* var. paniculatus, *A. retroflexus*, *A. spinosus* and *A. viridis*. All these species have different sensitivity to herbicides (Inoue et al., 2015). The chemical control of *A. palmeri* in cotton crop must consider herbicides available for applications in pre-sowing, pre-emergence, post-emergence and directed spray. A weed control program starts with the application of herbicides such as 2,4-D or paraquat to eliminate emerged weeds or voluntary plants of crop species. Sequential applications, in general, result in higher efficacy, when compared to one burndow spray alone.

Among the residual herbicides recommended for applications in pre-sowing, pre-emergence or pre-plant-incorporated within the cotton crops, the ones that stand out includes flumioxazin, trifluralin and S-metolachlor, usually associated to diuron (Cavenaghi and Guimarães, 2015). Other herbicides like pendimethalin, alachlor, prometryn and fomesafen, can be used in pre-emergence of the cotton crops. An appropriate strategy to reduce *A. palmeri* infestation would be the application of herbicides in pre-emergence at the moment of the second sequential application of the burndown operation. For instance, in the United States, the herbicide fomesafen is used in glyphosate-resistant *A. palmeri* management programs, sprayed from 10 to 15 days before the crop sowing (Bangarwa et al., 2010).

It is possible to apply residual herbicides such as metribuzin, flumioxazin or sulfentrazone sequentially during the crop cycle, overlaping their effects in order to avoid new fluxes of weed emergence and to reduce the development of *A. palmeri* plants (Scott and Smith, 2011; Ward et al., 2013; Tuesca et al., 2015). The interval between the herbicide sprays may range from 15 to 30 days, depending on the characteristics of the herbicide.

The application of herbicides in the post-emergence of the cotton crop may include conventional cultivars or the ones resistant to glyphosate and ammonium-glufosinate. In areas infested by glyphosate and ALS inhibitors R *A. palmeri*, the main herbicides are the PROTOX inhibitors, such as fomesafen and lactofen (Tuesca et al., 2015) and also in ammonium-glufosinate. Special care should be taken with herbicide-resistant *A. palmeri* infestations in the soybean crop. In Southern USA, crops with inappropriate herbicide-resistant *A. palmeri* control usually require the fields to be ploughed and to be replanted (Steckel et al., 2012), increasing the production costs.

The substitution of glyphosate by other herbicides in the soybean crops of may be troublesome (Legleiter and Johnson, 2013; Tuesca et al., 2015), because there is a limited number of post-emergence herbicides recommended. Due to these limitations, to reach elevated levels of *A. palmeri* control, the farmers usually spray residual herbicides during burndown or pre-emergence. Afterwards, they supplement the weed management with post-emergence herbicides.

High levels of weed control with burndown herbicides reduce the probability of interference between *A. palmeri* and soybean plants, thus, avoiding crop yield losses. In the case of glyphosate and ALS inhibitors-resistant *A. palmeri*, the herbicide options for burndown include ammonium glufosinate, 2,4-D, saflufenacil, paraquat or metribuzin. In general, better weed control are achieved with herbicide association.

Pre-emergence herbicide spray in the soybean crop is important for glyphosate R *A. palmeri* plant management. Adequate weed control reduces the weed-soybean competition and decreases the crop yield losses (Scott et al., 2011). S-metolachlor, flumioxazin, fomesafen, saflufenacil, sulfentrazone, chlorimuron, imazethapyr, metribuzin, alachlor and pendimenthalin were used to control *A. palmeri* (Dillon et al., 2011).

Post-emergence herbicide spray on soybean crop may consider conventional or glyphosate R cultivars. Glyphosate and ALS inhibitors R *A. palmeri* control with post-emergence herbicides selective for soybean was achieved with s-metolachlor, fomesafen, lactofen and glufosinate ammonium. Applications of herbicides in post-emergence (with compounds with different MeA) during early growth stages of *A. palmeri* plants may increase the efficacy of weed control, reducing the number of remaining plants.

Crop rotation including corn is considered strategic to control *A. palmeri* because it allows the use of different herbicides in either in burndown, or in pre-emergence, post-emergence and directed spray. During burndown prior to corn sowing, the herbicides which impact glyphosate R *A. palmeri* plants include 2,4-D and paraquat. In pre-emergence, the herbicide options consist of atrazine, isoxaflutole, S-metolachlor, acetochlor and dimethenamid (Legleiter and Johnson, 2013). The HPPD inhibitors herbicides isoxaflutole and tembotrione may have better performance when associated to atrazine (Ward et al., 2013).

Post-emergence herbicides selective for the corn crop include atrazine, mesotrione and tembotrione. Considering that in the state of Mato Grosso there are *A. palmeri* biotypes R to EPSPs and ALS, the use of glyphosate R corn crop should include weed management systems that include herbicides other than glyphosate, such as the ones mentioned above. With the use of Liberty Link (LL) cultivars, it is possible to use ammonium glufosinate. However, best performance of this herbicide in the corn crop was achieved when associated with atrazine (Jones et al., 1998).

Final Remarks

The aspects mentioned in this review help estimate the impact of plants from *A. palmeri* species on crop fields not infested previously. Environmental characteristics, biological characteristics and the existence of resistant populations are determining factors of the rates of invasion of this species in new environments.

The eradication of *A. palmeri* in environments where this species was recently introduced and presents a small infestation area is a priority action that must be supported by public and private institutions. In the United States, where the species is a major issue to farmers, the strategy named "zero tolerance" has gained attention, due to the low longevity of the seeds on the soil seed-bank. In summary, the idea is to avoid seed set and dispersion. However, because it is a newly introduced species in Brazil and due to the differences between environments and management systems, it is not possible to predict if this strategy would have positive outcomes in our agroecosystems.

Biological characteristics of the species must support preventive and cultural plant management. These methods, associated to chemical options, are likely to be needed for best *A. palmeri* weed management. The chemical method should consider the alternation of herbicides with different mechanisms of action and the use of pre-emergence herbicides, and of crop rotation systems.

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