PLANTA DANINHA
SBCPD
SOCIEDADE BRASILEIRA DA CIÊNCIA DAS PLANTAS DANINHAS
<http://www.sbcpd.org>
ISSN 0100-8358 (print)
1806-9681 (online)

Article

Occurrence of Horseweed Biotypes with Low Susceptibility to Glyphosate in the States of Rio Grande do Sul, Paraná and Mato Grosso do Sul, Brazil

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Ocorrência de Biótipos de Buva com Reduzida Suscetibilidade ao Glyphosate nos Estados do Rio Grande do Sul, Paraná e Mato Grosso do Sul

ABSTRACT - Horseweed is an annual cycle weed naturally controlled by the herbicide glyphosate. However, the continued use of this product has selected tolerant and resistant populations. The objective of this study is to analyze the occurrence of horseweed biotypes with a low glyphosate susceptibility in the states of Rio Grande do Sul, Paraná and Mato Grosso do Sul. Seeds of horseweed plants (*Conyza* spp.) that survived glyphosate applications were collected in RR soybean crops, totaling 137 samples from 37 municipalities. The seeds of the biotypes were sown, and the plants originated from them, when they reached the stage of four to six leaves, were subjected to a treatment with 1,440 g a.e. ha^{-1} of glyphosate. According to the results, 108 biotypes were not controlled and 29 were controlled by the application of glyphosate. Thus, 78% of horseweed biotypes evaluated are not controlled by the dose 1,440 g a.e. ha^{-1} of glyphosate, and 22% are susceptible to the same dose. Evaluating the distribution per state, Paraná, Rio Grande do Sul and Mato Grosso do Sul presented 83%, 77% and 77%, respectively, of biotypes not controlled by glyphosate at the dose used. Thus, most of the collected horseweed biotypes present a low sensitivity to glyphosate, thus confirming the high horseweed distribution with a low susceptibility to glyphosate in Mato Grosso do Sul, Paraná and Rio Grande do Sul.

Keywords: *Conyza bonariensis, Conyza sumatrensis, Conyza canadensis*, resistance, chemical control.

*RESUMO - Buva é uma planta daninha de ciclo anual, naturalmente controlada pelo herbicida glyphosate, no entanto, o uso continuado desse produto selecionou populações tolerantes e resistentes. Objetivou-se neste estudo avaliar a ocorrência de biótipos de buva com reduzida suscetibilidade ao herbicida glyphosate nos Estados do Rio Grande do Sul, Paraná e Mato Grosso do Sul. Sementes de plantas de buva (**Conyza** spp.) que sobreviveram a aplicações de glyphosate foram coletadas em lavouras de soja RR, totalizando 137 amostras de 37 municípios. As sementes dos biótipos foram semeadas, e as plantas originadas delas, quando atingiram o estádio de quatro a seis folhas, submetidas ao tratamento de 1.440 g e.a. ha^{-1} de glyphosate. De acordo com os resultados, 108 biótipos não foram controlados e 29 foram controlados pela aplicação do herbicida glyphosate. Dessa forma, 78% dos biótipos de buva avaliados não são controlados pela dose de 1.440 g e.a. ha^{-1} de glyphosate, e 22% apresentam suscetibilidade à mesma dose. Avaliando a distribuição por Estado, Paraná, Rio Grande do Sul e Mato*

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Received: May 31, 2018
Approved: October 8, 2018

Planta Daninha 2019; v37:e019201666

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Doi: 10.1590/S0100-83582019370100093

Grosso do Sul apresentaram 83%, 77% e 77%, respectivamente, dos biótipos não controlados pelo glyphosate, na dose utilizada. Assim, conclui-se que a maioria dos biótipos de buva coletados apresenta baixa sensibilidade ao glyphosate, confirmando assim a elevada distribuição de buva com reduzida suscetibilidade ao glyphosate no Mato Grosso do Sul, Paraná e Rio Grande do Sul.

Palavras-chave: *Conyza bonariensis, Conyza sumatrensis, Conyza canadensis*, resistência, controle químico.

INTRODUCTION

A world production of grains, such as wheat, rice, soybeans, maize, among others, is essential for the world food supply. Any threat to the production compromises food safety. Weeds that infest these crops threaten productivity. Herbicides are effective alternatives to control weeds in such agricultural crops. However, the biological repercussion of an excessive dependence on herbicides is the evolution of weed populations with resistance to herbicides. Worldwide, 252 weed species are resistant to herbicides, totaling 479 cases (Heap, 2018).

The genus *Conyza* includes approximately 50 species distributed almost all over the world (Kissmann and Groth, 1999). The most prominent species, due to their negative character, are *C. bonariensis, C. canadensis* and *C. sumatrensis*. They are popularly known as horseweeds. They infest abandoned areas, pastures, perennial crops and areas with annual crops (Thebaud and Abbott, 1995).

In no-tillage systems or in orchards, the control of horseweed is usually carried out with the application of non-selective herbicides. Glyphosate is the most used. However, because of resistance to this herbicide, there is a clear need to associate it with other herbicides with different mechanisms of action aiming to overcome the problem of resistance and, at the same time, protect other active ingredients. The use of glyphosate for this purpose is due to its high efficiency at different vegetative stages of the species, combined with its low cost compared to other herbicides (Christoffoleti and López-Ovejero, 2003). However, the intensive use of an herbicide imposes a high selection pressure on plant populations, resulting in the selection of preexisting resistant biotypes (Christoffoleti and López-Ovejero, 2003; Vargas et al., 2009; Powles and Yu, 2010). In 2005, the repeated use of glyphosate, in both desiccation and post-emergence of soybean cultivation, selected horseweed biotypes (*Conyza bonariensis*) resistant to this herbicide (Heap, 2018).

Weed resistance to herbicides is an evolutionary process, and the dynamics and the impacts depend on several factors. The factors capable of determining how the resistance is driven are the genetic diversity of the population, the existence of genes resistant to a specific herbicide, and operational factors (Powles and Yu, 2010).

Resistance of weeds to herbicides is a worldwide problem. In Brazil in recent years, it has gained importance due to the appearance of numerous cases of species resistant to different mechanisms of herbicide action. In Brazil, there are species resistant to glyphosate, among them *Conyza bonariensis, C. sumatrensis, C. canadensis, Lolium multiflorum, Digitaria insularis, Chloris elata, Amaranthus palmeri* and *Eleusine indica* (Heap, 2018). In addition, *C. sumatrensis* has simple resistance to the herbicides paraquat, saflufenacil and chlorimurom, and multiple resistance to glyphosate and chlorimurom, as well as glyphosate, chlorimuron and paraquat (Heap, 2018). Globally, three horseweed species are resistant to four mechanisms of herbicidal action (Heap, 2018).

Faced with a scenario of increased occurrence of weed resistance to herbicides, measurements that recommend resistance management are fundamental. The constant monitoring of the area to identify the outbreaks of resistance and elimination of suspect plants is important (Hugh, 2006; Lazaroto et al., 2008). After the resistance is identified in the field, the adoption of practices such as crop rotation, mixture of herbicides, restricted applications and association of control methods reduce the impacts of the problem (Hugh, 2006). The economic impacts of weed resistance may triple in many cases with respect to areas without the occurrence of resistance. The proactive management of resistance with the use of pre-emergence herbicides can be costly for the producer (a 26% higher cost). However, this cost increase is justified in face of the cost of reactive management, when plants are resistant to glyphosate. The herbicide mixture is then required, increasing the cost by 358% to enable a cultivation free from the interference of resistant weeds (Mueller et al., 2005). Thus, it is assumed that the monitoring of resistance as a proactive tool is an important tool to be implemented by the chain involved in this sector.

The knowledge of the extent of the infested area, by either resistant weeds or weeds with a low susceptibility, provides evidence for the beginning and the future proportion of the problem, and allows determining resistance management and slowing the selection process of resistant biotypes, thus helping in the decision-making regarding the control of these populations (Owen et al., 2014). In addition, the mapping of areas allows establishing the costs of resistance and defining the specific public policies and technical assistance for each producer.

In Brazil, there is a survey of the frequency and dispersion of resistance of sourgrass (*Digitaria insularis*) to glyphosate (López-Ovejero et al., 2017). However, there is no such survey on an infested area with resistant horseweed (*Conyza* spp.) or with a reduced glyphosate susceptibility. The survey of horseweed populations in response to glyphosate is an important tool to identify the sites of occurrence and determine management strategies specific to each region according to its characteristics. With this information in hand, decision-making and the adoption of resistance prevention and control strategies become feasible, recovering the viability of cultivation in these areas.

Thus, the objective of this study is to analyze the occurrence of horseweed biotypes with a low glyphosate susceptibility in the states of Rio Grande do Sul, Paraná and Mato Grosso do Sul.

MATERIAL AND METHODS

Seeds of plants of *Conyza* spp. were collected in areas with a history of glyphosate application for weed desiccation and Roundup Ready (RR) soybean cultivation. In these areas, producers observed a lack of control of the weed after using glyphosate. Each plant was placed individually in a paper bag in order not to mix seeds from different collection sites.

The seeds were collected in municipalities of the states of Rio Grande do Sul, Paraná and Mato Grosso do Sul. The collection of seeds occurred in properties in the states and municipalities listed below. The number of biotypes per municipality was recorded. Rio Grande do Sul: Bagé (5), Carazinho (4), Chapada (2), Coqueiros do Sul (2), Cruz Alta (5), Jari (11), Julio de Castilhos (6), Panambi (2), Pontão (3), Quevedos (5), Saldanha Marinho (2), Santa Bárbara do Sul (3), Tio Hugo (1) and Tupanciretã (10); Paraná: Cambé (2), Capitão Leonidas Marques (3), Cascavel (4), Céu Azul (1), Diamante do Oeste (1), Matelândia (1), Medianeira (1), Missal (9), Ouro Verde (1), Romilândia (1), Santa Izabel do Oeste (3), Santa Helena (1), Santa Terezinha de Itaipú (1), Serranópolis do Iguaçú (1) and Três Barras (1); Mato Grosso do Sul: Bonito (10), Costa Rica (1), Douradina (1), Dourados (2), Indápolis (1), Itaporã (2), Maracaju (12) Nova Alvorada do Sul (5) and Ponta Porã (11) (Figure 1), totaling 137 seed samples collected in 37 municipalities in three states.

After collection, the seeds were cleaned, identified and stored until they were used in the experiments. The experiment to evaluate the sensitivity of biotypes to glyphosate was carried out between April and July 2016 in a greenhouse of the University of Passo Fundo (UPF) in the city of Passo Fundo, RS, in a completely randomized design. The seeds of each biotype were placed in plastic cups, submerged in water, and stored in a refrigerator at 5 ^{o}C for 24 hours, after which they were seeded. The treatments consisted of horseweed biotypes with a suspected resistance, and the application of glyphosate herbicide at 1,440 g a.e. ha^{-1}. The commercial product, Glyphosate Nortox®, was used. The experimental units consisted of plastic pots with a volume of 500 mL containing commercial substrate composed of peat, vermiculite, agroindustrial organic residue, and limestone. Each pot contained three plants.

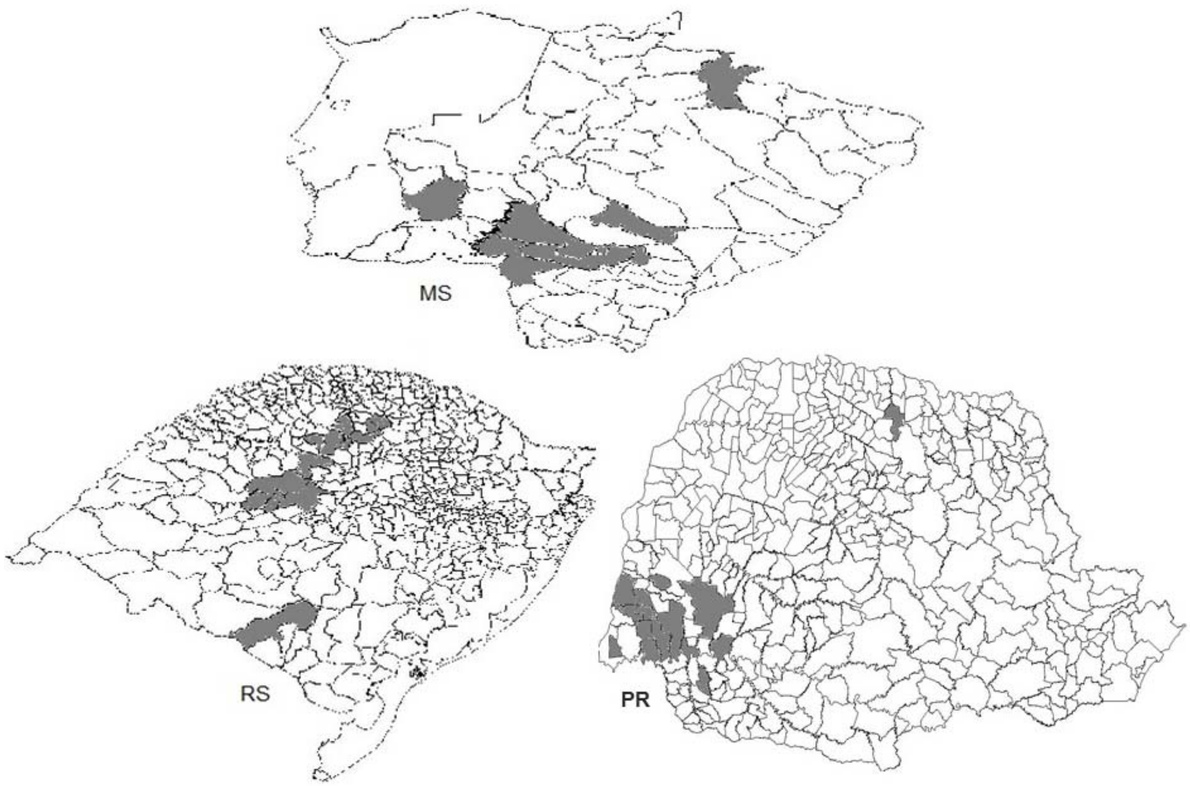

Figure 1 - Geographic location of the municipalities where the collection of seeds of horseweed plants with a suspected resistance to the herbicide glyphosate was carried out in the states of Rio Grande do Sul (RS), Paraná (PR) and Mato Grosso do Sul (MS).

When the plants reached the four- to six-leaf stage, the herbicide glyphosate was applied using a backpack sprayer, pressurized with CO_2, calibrated to provide an application volume of 150 L ha^{-1} of herbicide syrup, and equipped with spray tips of the fan type 110.015.

The evaluated variable was visual control at thirty days after the application of the treatment (DAT). The biotypes were classified according to the response to the herbicide as susceptible or with a reduced susceptibility, adopting a binary scale, where zero (0) represented plant susceptibility and one (1) represented reduced susceptibility. The data obtained were analyzed by descriptive statistics to establish relationships among the horseweed cases with a suspected resistance to the herbicide glyphosate.

RESULTS AND DISCUSSION

The data analysis revealed that, of the 137 collected biotypes, 108 were not controlled by the application of 1,440 g a.e. ha^{-1} of glyphosate (Table 1). However, 29 biotypes did not survive the application of the same dose of the herbicide (Table 1). Thus, 78% of the horseweed population evaluated in this work presented a reduced susceptibility to glyphosate, and only 22% was still susceptible to the dose of 1,440 g a.e. ha^{-1} of glyphosate.

When the analysis is performed by state, the results show that in Paraná there is the highest percentage of biotypes with a low susceptibility (83%); Mato Grosso do Sul and Rio Grande do Sul presented 77% of biotypes with a reduced susceptibility (Figure 2). It is not possible to relate the occurrence of the differential response to glyphosate, but the results become more important and attractive since in Rio Grande do Sul the first report of horseweed resistance to glyphosate occurred. The results indicate that the proportion of low susceptibility is similar in all three states. Although the number of populations studied does not match the totality of the municipalities from the three states mentioned above, the volume of the samples provides a reliable idea of the geographic distribution of resistance of horseweed populations in these states.

Table 1 - Location and response (0 = susceptible, 1 = low susceptibility) of *Conyza* spp. with a suspicion of resistance in function of the application of 1,440 g a.e. ha^{-1} of glyphosate visually evaluated at 30 days after treatment (DAT). Passo Fundo, RS. 2016

Biotype	State	Municipality	Response to Glyphosate
BAG 01	Rio Grande do Sul	Bagé	1
BAG 02		Bagé	0
BAG 03		Bagé	1
BAG 04		Bagé	1
BAG 05		Bagé	1
CAR 01		Carazinho	1
CAR 02		Carazinho	1
CAR 03		Carazinho	1
CAR 04		Carazinho	1
CHAP 01		Chapada	1
CHAP 02		Chapada	1
COQ 01		Coqueiros do Sul	1
COQ 02		Coqueiros do Sul	1
CRA 01		Cruz Alta	1
CRA 02		Cruz Alta	1
CRA 03		Cruz Alta	0
CRA 04		Cruz Alta	1
CRA 05		Cruz Alta	1
JAR 01		Jari	1
JAR 02		Jari	0
JAR 03		Jari	0
JAR 04		Jari	1
JAR 05		Jari	1
JAR 06		Jari	1
JAR 07		Jari	0
JAR 08		Jari	1
JAR 09		Jari	1
JAR 10		Jari	1
JAR 11		Jari	0
JUC 01		Julio de Castilhos	0
JUC 02		Julio de Castilhos	0
JUC 03		Julio de Castilhos	1
JUC 04		Julio de Castilhos	1
JUC 05		Julio de Castilhos	1
JUC 06		Julio de Castilhos	1
PAN 01		Panambi	1
PAN 02		Panambi	1
PON 01		Pontão	1
PON 02		Pontão	0
PON 03		Pontão (SUS)	0
QUEV 01		Quevedos	1
QUEV 02		Quevedos	1
QUEV 03		Quevedos	0
QUEV 04		Quevedos	1
QUEV 05		Quevedos	0
SAM 01		Saldanha Marinho	1
SAM 02		Saldanha Marinho	1
SAB 01		Santa Bárbara do Sul	1
SAB 02		Santa Bárbara do Sul	1
SAB 03		Santa Bárbara do Sul	1
TIH 01		Tio Hugo	1
TUP 01		Tupanciretã	1

To be continued ...

Table 1, cont.

Biotype	State	Municipality	Response to Glyphosate
TUP 02	Rio Grande do Sul	Tupanciretã	1
TUP 03		Tupanciretã	1
TUP 04		Tupanciretã	1
TUP 05		Tupanciretã	0
TUP 06		Tupanciretã	1
TUP 07		Tupanciretã	0
TUP 08		Tupanciretã	1
TUP 09		Tupanciretã	1
TUP 10		Tupanciretã	1
CAM 01	Paraná	Cambé	0
CAM 02		Cambé	1
CLM 01		Capitão Leonidas Marques	1
CLM 02		Capitão Leonidas Marques	0
CLM 03		Capitão Leonidas Marques	1
CAS 01		Cascavel	1
CAS 02		Cascavel	1
CAS 03		Cascavel	1
CAS 04		Cascavel	1
CEA 01		Céu Azul	1
DIO 01		Diamante do Oeste	1
MAT 01		Matelândia	1
MED 01		Medianeira	1
MIS 01		Missal	1
MIS 02		Missal	1
MIS 03		Missal	1
MIS 04		Missal	1
MIS 05		Missal	1
MIS 06		Missal	1
MIS 07		Missal	1
MIS 08		Missal	1
MIS 09		Missal	0
OUV 01		Ouro Verde	1
ROM 01		Romilândia	1
SIO 01		Santa Izabel do Oeste	1
SIO 02		Santa Izabel do Oeste	0
SIO 03		Santa Izabel do Oeste	1
SAH 01		Santa Helena	1
STI 01		Santa Terezinha de Itaipu	1
SEI 01		Serranópolis do Iguaçu	0
TRB 01		Três Barras	1
BON 01	Mato Grosso do Sul	Bonito	0
BON 02		Bonito	1
BON 03		Bonito	1
BON 04		Bonito	1
BON 05		Bonito	1
BON 06		Bonito	1
BON 07		Bonito	1
BON 08		Bonito	1
BON 09		Bonito	0
BON 10		Bonito	1
COR 01		Costa Rica	0
DOU 01		Douradina	1
DOU 01		Dourados	1
DOU 02		Dourados	0
IND 01		Indápolis	1

To be continued ...

Table 1, cont.

Biotype	State	Municipality	Response to Glyphosate
ITA 01	Mato Grosso do Sul	Itaporã	1
ITA 02		Itaporã	1
MAR 01		Maracaju	1
MAR 02		Maracaju	1
MAR 03		Maracaju	1
MAR 04		Maracaju	1
MAR 05		Maracaju	1
MAR 06		Maracaju	1
MAR 07		Maracaju	1
MAR 08		Maracaju	1
MAR 09		Maracaju	1
MAR 10		Maracaju	1
MAR 11		Maracaju	1
MAR 12		Maracaju	1
NAS 01		Nova Alvorada do Sul	1
NAS 02		Nova Alvorada do Sul	1
NAS 03		Nova Alvorada do Sul	1
NAS 04		Nova Alvorada do Sul	0
NAS 05		Nova Alvorada do Sul	1
POP 01		Ponta Porã	0
POP 02		Ponta Porã	0
POP 03		Ponta Porã	0
POP 04		Ponta Porã	1
POP 05		Ponta Porã	0
POP 06		Ponta Porã	1
POP 07		Ponta Porã	1
POP 08		Ponta Porã	0
POP 09		Ponta Porã	1
POP 10		Ponta Porã	1
POP 11		Ponta Porã	1

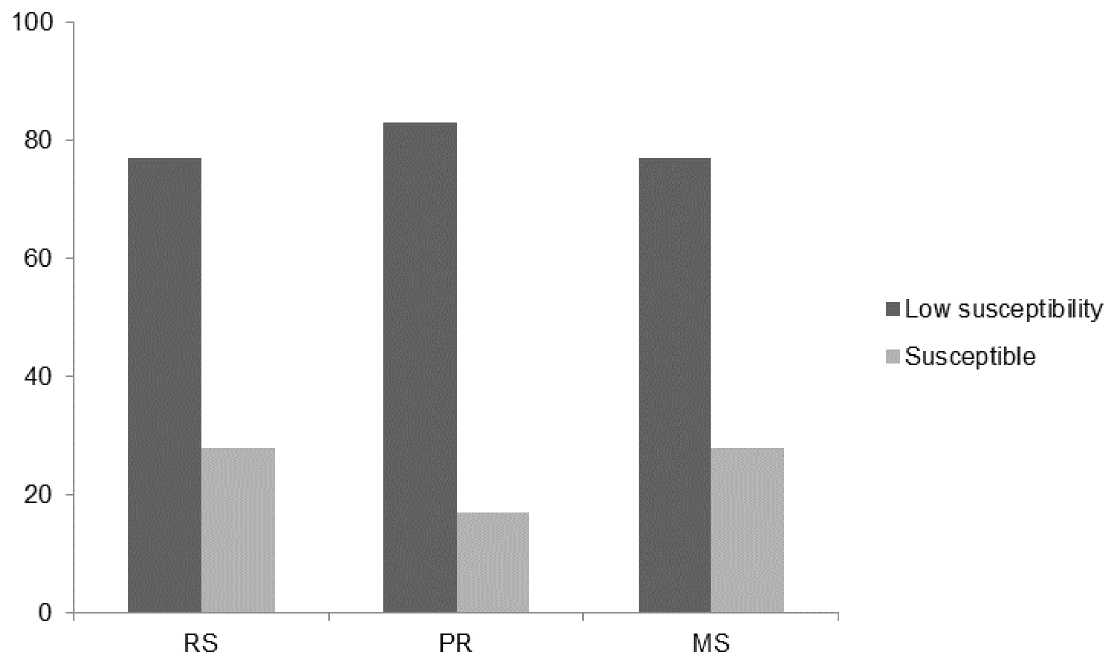

Figure 2 - Percentage of biotypes with a reduced susceptibility and susceptible to the herbicide glyphosate in each state analyzed: Rio Grande do Sul (RS), Paraná (PR) and Mato Grosso do Sul (MS). Passo Fundo - 2017.

The justification for the use of the dose 1,440 g a.e. ha^{-1} is because the manuals of commercial glyphosate-based products indicate this dose for the control of horseweed (AGROFIT, 2018). In addition, in the field, this dose is used in most situations. Although this work is not a direct identification of resistance, the criteria were the same as adopted for the identification of resistance, mainly obeying the application criteria (indicated vegetative stage, climate conditions, among others) (Owen and Powles, 2010; Gazziero et al., 2014).

The 29 biotypes that did not survive the application are from the same regions where there were biotypes with less susceptibility, and there was no geographic relation between them. Thus, with the confirmation of horseweed populations susceptible to glyphosate, the importance and viability of using glyphosate in rotation with other herbicides with different mechanisms of action are emphasized. The continued use of glyphosate in combination with other herbicides should be maintained due to the important characteristics of this herbicide, such as the action on other weed species, combined with the synergistic and/or additive effects of such associations. Even in areas with resistant populations, this reasoning must be followed since in a same area there may be resistant and susceptible individuals and other species on which glyphosate exerts a satisfactory control.

The horseweed control failures observed in areas with glyphosate application to soybean and corn crops and the result of this study evidence the occurrence of differential responses by horseweed populations to the herbicide glyphosate. Horseweeds have a great dispersion capacity over long distances, which facilitates dispersion to different geographic regions. Horseweed seeds have structures called *papus*, which can be twice the size of the seed, making it hover in the air for a long period (Regehr and Bazzaz, 1979; Andersen, 1993).

Although the RR soybean cultivation was approved in Brazil in 2005, it is known informally that between 1998 and 2005, RR soybean was cultivated irregularly mainly in the state of Rio Grande do Sul. The control of weeds in the soybean crop was carried out almost exclusively with glyphosate. Thus, the selection of resistant biotypes was high, confirming the first report of a glyphosate-resistant horseweed in 2005. This occurred in Rio Grande do Sul (Heap, 2018).

Considering the foregoing, although this work does not contemplate horseweed populations from the entire soybean producing region of Brazil, the number and the geographic location of the populations studied shows that the distribution of horseweed with a low susceptibility to glyphosate is extensive and comprises a large part of the producing region. The states of Rio Grande do Sul (RS), Paraná (PR) and Mato Grosso do Sul (MT) are significant to the Brazilian soybean production. In 2017, the soybean area in RS, PR and MS was 5,570, 5,250 and 2,520 million hectares, respectively, with a production of 18,714, 19,534 and 9,071 million tons (Conab, 2017). Thus, the study of the area with the occurrence of horseweed populations with resistance, susceptibility or even reduced susceptibility to glyphosate in these states is justified since they alone represent 40% of the volume of soybeans produced in Brazil.

The resistance of weeds to herbicides is an evolutionary process from preexisting genes in nature influenced by the biology of the species, genetic factors and the herbicide (Maxwell and Mortimer, 1994). Among them, the pressure exerted by the volume of application of herbicides is an important factor of selection of resistant biotypes (Powles and Yu, 2010). The occurrence of reduced susceptibility in the studied populations may indicate the beginning of the process of selection of resistant biotypes. Thus, these results serve as a warning for the development of strategies to delay resistance.

In addition to the increased herbicide dependence on management in either desiccation or post-emergence, the evolution of horseweed resistance to glyphosate can be aided by several other factors, such as gene flow, seed movement through agricultural machines, and seed lots contaminated with horseweed seeds (Barroso et al., 2006; Busi et al., 2008; Michael et al., 2010).

Alternatively to glyphosate, several other herbicides with different mechanisms of action (2,4-D, saflufenacil, paraquat, atrazine, glufosinate, diclosulan, among others) remain effective for horseweed control, although resistance to these herbicides is constant. With the recent identification of horseweed resistance to paraquat and saflufenacil, the alternatives used in the management of resistance to glyphosate should be maintained and emphasized since the impacts of these last substances will aggravate the scenario of the management of resistant weeds. Agronomic practices, such as crop rotation, integration of weed control methods, associated with the rotation of the herbicide action mechanism, although already used, are increasingly in need of development. Thus, this wide range of integrated horseweed management options may achieve herbicide sustainability and consequently system productivity.

Of the 137 evaluated biotypes, 78% presented a reduced susceptibility to glyphosate and 22% were susceptible. Moreover, 83% of the biotypes from Paraná and 77% from Mato Grosso do Sul and Rio Grande do Sul showed a reduced susceptibility to glyphosate. These results confirm the high geographical distribution of horseweed biotypes with a low susceptibility to glyphosate in the states of Rio Grande do Sul, Paraná and Mato Grosso do Sul.

Based on the results obtained, the hypothesis of a high distribution of horseweed populations with a reduced susceptibility to glyphosate in a large part of the soybean-producing region in Brazil is confirmed. However, it must be ensured that efficient management measurements are adopted in an extremely efficient way so that this situation is not reversed for other important herbicides used as a management tool.

The need to evaluate the occurrence of these results from an economic and political point of view is evident. It may serve as a subsidy for the preparation of public policies directed to the evolution of the resistance of weeds to herbicides, since, certainly, a great part of the yield of grains is reduced due to the occurrence of resistance, and even the financial cost with the control of these populations is high because of resistance.

The importance of maintaining the herbicide glyphosate in the system, together with other herbicides with different mechanisms of action, and the integration of several strategies in an integrated weed management is highlighted. In addition, a strategic and safe integration of management practices of this weed is necessary.

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