Agrária - Revista Brasileira de Ciências Agrárias
ISSN (on line) 1981-0997
v.12, n.1, p.51-59, 2017
Recife, PE, UFRPE. www.agraria.ufrpe.br
DOI:10.5039/agraria.v12i1a5423
Protocolo 5423 - 01/09/2016 • Aprovado em 01/03/2017

Agronomic factors involved in low-level wild poinsettia resistance to glyphosate

André da Rosa Ulguim[1], Dirceu Agostinetto[2], Leandro Vargas[3],
Jessica Dias Gomes da Silva[2], Bruno Moncks da Silva[2], Nixon da Rosa Westendorff[2]

[1] Universidade Federal de Santa Maria, Centro de Ciências Rurais, Departamento de Defesa Fitossanitária, Av. Roraima, 1000, CEP 97105-900, Santa Maria, Brasil. E-mail: andre_ulguim@yahoo.com.br
[2] Universidade Federal de Pelotas, Faculdade de Agronomia Eliseu Maciel, Departamento de Fitossanidade, Campus Universitário, s/n, CEP 960109-00, Pelotas-RS, Brasil. Caixa Postal 354. E-mail: dirceu.agostinetto@pq.cnpq.br; jessidadiasgomes@hotmail.com; brunomoncks@gmail.com; nwestendorff@gmail.com
[3] Embrapa Trigo, BR 285, km 174, Industrial, CEP 99001-970, Passo Fundo-RS, Brasil. Caixa Postal 451. E-mail: leandro.vargas@embrapa.br

ABSTRACT

The awareness of agronomic practices that affect the resistance selection of weeds to a particular herbicide is of utmost importance in order to advocate management practices that aim to avoid or prolong the resistance. The objectives of this study were to identify and map the occurrence of the wild poinsettia's low-level resistance to glyphosate in Rio Grande do Sul State (RS), Brazil, and to define the main agronomic factors associated with the selection of these biotypes. Seed and / or soil samples in areas with suspected resistance were collected in Roundup Ready® (RR) soybean crops in different counties in the RS region during the 2010/11 and 2011/12 seasons. A questionnaire on the operations history in the area was applied at each site in order to establish relationships between the distribution of wild poinsettia with cases of suspected resistance to glyphosate and the likely agronomic factors involved in this occurrence. The study included the response of wild poinsettia biotypes with suspected resistance to glyphosate and an analysis of the questionnaires carried out with producers. The main agronomic practices used by producers that favor the emergence of resistant biotypes were the continued cultivation of the RR® soybean; an overdependence on the use of glyphosate; a low rate of crop rotation and efficient chemical alternatives for wild poinsettia control and management.

Key words: crop rotation; 5-enolpiruvil chiquimato 3-fosfato sintase; *Euphorbia heterophylla*; selection pressure

Fatores agronômicos envolvidos na resistência de nível baixo de leiteira a glyphosate

RESUMO

O conhecimento das práticas agronômicas que interferem na seleção de plantas resistentes a determinado herbicida é de extrema importância para preconizar práticas de manejo que visem evitar ou retardar a resistência. Os objetivos do trabalho foram identificar e mapear a ocorrência da resistência de nível baixo de leiteira ao glyphosate no Rio Grande do Sul (RS), e definir os pricipais fatores agronômicos associados na seleção desses biótipos. Amostras de sementes e/ou solo em áreas com a suspeita de resistência foram coletadas em lavouras de soja Roundup Ready® em diferentes Municípios do RS nas safras 2010/11 e 2011/12. Em cada local, foi aplicado questionário sobre o histórico de manejo da área, visando estabelecer relações entre a distribuição de casos de leiteira com suspeita de resistência ao glyphosate e os prováveis fatores agronômicos envolvidos nessa característica. O estudo constou da resposta dos biótipos de leiteira com suspeita de resistência ao glyphosate e análise dos questionários realizados com os produtores. As principais práticas agronômicas utilizadas pelos produtores que favorece o surgimento de biótipos resistentes foi o cultivo continuado de soja RR®, excessiva dependência do uso de glyphosate, baixo índice de rotação de culturas e de alternativas químicas eficientes para o controle de leiteira.

Palavras-chave: rotação de culturas; 5-enolpiruvil chiquimato 3-fosfato sintase; *Euphorbia heterophylla*; pressão de seleção

Introduction

Weed management is an important practice in agricultural production systems, as well as the application of alternative herbicides for weed control to prevent the competition of weeds with crops and consequent reduced productivity. However, the exclusive use of the chemical control method has resulted in increasing numbers of resistant weeds.

There are 250 species of weeds with resistance to at least one herbicide site of action and distributed worldwide (Heap, 2016). In Brazil, there are 37 cases of resistance to 27 different species, and eight of these cases relate to resistance to the glyphosate herbicide (Heap, 2016). The intensive use of glyphosate for weed management, intensified with the advent of transgenic crops, resulted in increased selection of weeds resistant to this herbicide.

Resistance is defined as the inherent and heritable capacity of a biotype to survive and reproduce after exposure to the herbicide rate label that is usually lethal to susceptible populations of the same species. Scientific definition of resistance or low resistance is characterized by the difference in control between populations when doses below that record are used, and yet has satisfactory control when using the recommended dose from the manufacturer written on the package (Gazziero et al., 2014).

In the case of wild poinsettia (*Euphorbia heterophylla* L.), in which certain biotypes showed less susceptibility to glyphosate, even if controlled by the maximum dose record (Vargas et al., 2011), it can be attributed to the occurrence of low-level resistance. However, most producers (91%) reported wild poinsettia control difficulty when applying glyphosate in Rio Grande do Sul State (RS) (Nohatto, 2010), indicating the need for new studies to characterize the current situation of this issue.

Besides confirmation of resistance, knowledge of the characteristics that lead to resistant biotype selection of a particular herbicide is of great importance. Some parameters such as the distribution and mapping of resistance, tracking the control of species, the type of crop rotation, the frequency of a certain herbicide and its dose being applied, the application season and stage of plants as well as the history of the area, have been analyzed as potential indicators of resistance, and may promote better understanding of the practices that favor the emergence of resistance (Givens et al., 2009).

In most cases, farmers are unaware of the implications of selection pressure on the weed community and the evolution of resistance, although they observe the changes that occur in the population of weeds (Prince et al., 2012). Thus, one of the main measures to mitigate the evolution of resistance is the constant monitoring of the crop, in order to identify possible pockets of resistance, and the suspect plants should be systematically eliminated (Lazaroto et al., 2008).

Studies that aimed to survey and monitor the agricultural practices of farmers are excellent tools to monitor the impacts on weed populations and changes in the management of them, and to help identify research needs (Norsworthy et al., 2013). Thus, it is important to continually observe those plants with suspected resistance, so that they can identify outbreaks early, before the resistant biotype predominates in the area, thereby facilitating the adoption of management measures.

Studies that evaluate the occurrence of wild poinsettia populations resistant to glyphosate will thus identify the locations where resistance occurs, as well as the agronomic factors involved in this process. A good command of this information, together with the knowledge of the biological characteristics of the species, will be important for the future definition of prevention strategies, management and control of weed resistance to herbicides. The objectives in this study were to identify and map the occurrence of resistance of this species to the herbicide in the state of RS, Brazil, and define the main agronomic factors associated with the selection of wild poinsettia biotypes resistant to glyphosate.

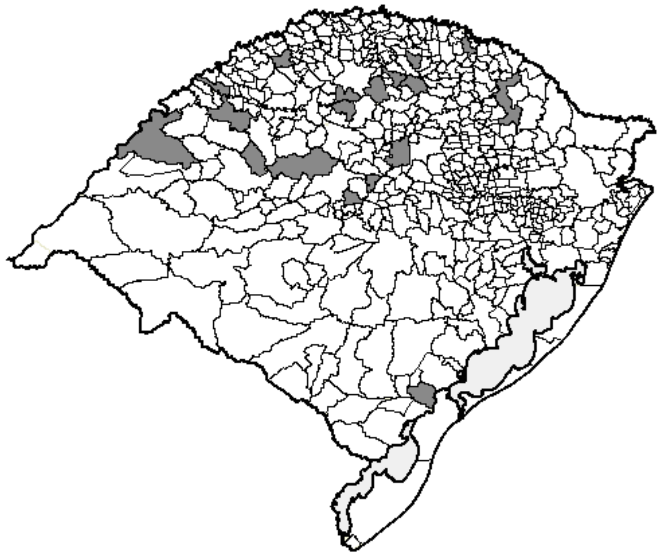
Material and Methods

In order to carry out the study, samples of seeds and / or soil in areas where the suspected occurrence of wild poinsettia resistance to glyphosate were collected in Roundup Ready® (RR®) soybean crops in different counties of the Rio Grande do Sul State (RS). The samples were collected between the harvests of 2010/11 and 2011/12, in properties located in the traditional soy producing counties and with the highest production recorded in 2010 (IBGE, 2010). Samples were collected by technicians who provide agricultural assistance in the selected counties.

In the 2010/11 harvest, eleven soil samples were collected in the counties of Campinas do Sul, Chapada, Condor, Espumoso, Lagoa Vermelha, Panambi, Roque Gonzales, Três de Maio and Tupanciretã. Then, in the 2011/12 harvest, seeds were collected from a single plant that survived the treatment with glyphosate, individually wrapped and labeled, totaling 41 samples. The collection sites were as follows: Capão do Cipó, Condor, Estrela Velha, Lagoa Vermelha, Nova Palma, Panambi, Pontão, Roque Gonzales, São Borja, São Luiz Gonzaga, Sarandi and Viadutos. Each collection point was identified by geographical coordinates using the Global Positioning System (GPS) (Table 1). A collection of wild poinsettia biotype seeds was also carried out, in an area without a history of glyphosate application, in the Capão do Leão County, characterized as notoriously susceptible to the herbicide. The area of counties covered during the collections can be seen in Figure 1.

On the occasion of the collections, a questionnaire on the management history of the area was given to the producers, seeking to establish relationships between the distribution of wild poinsettia cases with suspected resistance to glyphosate and with likely agronomic factors involved in that feature. Thus, the study was divided into two stages, as follows: the response of wild poinsettia biotypes with suspected resistance to glyphosate and analysis of the questionnaires carried out with producers.

For the first stage, there were three completely randomized design experiments: the first between November and December 2011 using soil samples, and the second and third from October 2012 to February 2013 using the seed samples, all in a greenhouse. In the first experiment, eleven soil samples were placed in trays with a capacity of 8L, each tray corresponded



Source: GeoLivre (2011).

Figure 1. Geographical location of the counties of the Rio Grande do Sul state where wild poinsettia seed collections (*Euphorbia heterophylla* L.) with suspected resistance to glyphosate were carried out.

to a single collection site. When the plants were at the four-leaf stage, glyphosate was applied at a dosage of 1080 g a.e. ha^{-1}, with the aid of a knapsack sprayer, pressurized with CO_2, with fan-shaped nozzles 110,015, and a liquid volume equivalent to 150 L ha^{-1}. The dose was established to be that which is most common in the RR® soybean crops in RS (Ulguim et al., 2013). The variable control was evaluated 28 days after the application of the herbicide treatment (DAA), in which the plants that survived the herbicide application were selected (data not shown).

Plants from each location that were alive were transplanted to pots with a capacity 8L for seed production. The plants from Condor (21.1) and Panambi (11.5) were the only samples that produced seeds and were therefore selected for the realization of the second and third experiments in the next stage of the study.

For the second experiment, conducted in boxes containing Argissolo Vermelho-Amarelo soil, with a sandy loam texture, and a commercial surface substrate GerminaPlant® with a volume of 8L, 43 seed samples of wild poinsettia biotypes were planted, 2 samples from the first experiment and the rest from the 2011/12 crop seed collections, establishing 42 biotypes.Then, in the third experiment, seeds from the same samples were used, in which 40 wild poinsettia biotypes were established, the same procedure being carried out in plastic trays with a capacity of 8L, filled with a mixture of soil and commercial substrate at a ratio of 1:1. We opted to repeat the experiment to obtain repetition in results and also due to the failure to establish some samples in the second experiment.

For the implementation of these experiments (second and third), the wild poinsettia seeds were sown in a line, each sowing line consisting of a wild poinsettia biotype with suspected resistance. After the emergence of weeds, thinning was performed and five plants of each biotype were maintained by line sowing. In all, 43 wild poinsettia biotypes were tested with suspected resistance to glyphosate.

When the plants were in the stage with five to six leaves, the potassium glyphosate herbicide at a dose of 720 g a.e. ha^{-1} was applied, which is the registered dose for wild poinsettia control post-emergence of the RR® soy crop (Agrofit, 2012). To apply the herbicide, a knapsack sprayer pressurized with CO_2 was used, equipped with a fan-shaped nozzle and 110.01 points, and a spray volume equivalent to 120 L ha^{-1}. The variable control was visually assessed by two evaluators, at 30 DAA, using a binary scale where zero (0) represented death (susceptible) and one (1) represented survival (resistant) of the plants.

The second stage of the study consisted of the analysis of the producers' responses in relation to the management adopted in the locations where biotypes were collected. The information obtained in the questionnaire was the time of consecutive cultivation of the RR® soybean in the area; the number of glyphosate applications annually and during the soybean cycle; the doses of this herbicide for the management of pre-sowing desiccation and application in post-emergence; the occurrence of herbicide mixtures with glyphosate in both applications; the type of rotation and succession of crops, when applicable; if the producer thought there existed resistant weeds on his property, itemizing by species; and if management was carried out differently in order to control these species.

Data were analyzed by percentage, using descriptive statistics and then graphically expressing the results. Based on the results, we tried to establish relationships between the distribution of wild poinsettia in cases of suspected resistance to glyphosate and agronomic factors involved in the observation of this feature.

Results and Discussion

The first stage of the study consisted of evaluating the response of wild poinsettia biotypes with suspected resistance to glyphosate. The result observed for wild poinsettia biotypes collected in RS showed that the biotype 21.1 was the only one in which all plants survived the glyphosate, although observed injury followed regrowth (Table 1).

In a study that used a dose of 2160 g a.e. ha^{-1}, greater than 95% control of wild poinsettia was observed, derived from RR® soybean crops in RS (Vargas et al., 2011). Thus, due to the observed result and the conditions in which the experiment was conducted, the authors concluded that most wild poinsettia biotypes were not resistant to glyphosate.

It was found that among the 43 tested biotypes, approximately 74% had at least one surviving plant in the 30 DAA assessment (Table 1). The fact that there are reports of wild poinsettia control failures by applying glyphosate and the results of this study that confirm the occurrence of high levels of weeds out of the herbicide's control (survivors), shows the need for studies to be done to characterize the problem. In addition, due to high selection pressure observed in the RR® soybean crops in RS, it is likely that the weeds are developing resistance to glyphosate.

The weed resistance to herbicides is an evolutionary process that goes through three main stages: elimination of highly sensitive biotypes; elimination of all biotypes except

Table 1. Glyphosate control, 30 days after application (DAA), at a dose of 720 g a.e. ha^{-1}, of the wild poinsettia biotypes (*Euphorbia heterophylla* L.) with suspected resistance, collected in Roundup Ready® soybean crops in Rio Grande do Sul.

County	Biotype	Geographic Coordinates		Survivors[1]	
		Latitude	Longitude	Experiment 2	Experiment 3
São Borja	2.1	28°46'8,27”	53°52'41,85”	1[2]	0
Roque Gonzales	3.1	28°06'8,47"	55°01'3,88"	1	1
São Luiz Gonzaga	4.1	28°15'48,96"	54°49'25,63"	1	1
	4.2	28°34'24,8"	55°04'8,37"	1	1
	4.3	28°40'40"	54°49'24"	0	1
	4.4	28°08'38,5"	55°14'31,3"	0	0
	4.5	28°25'28,60"	54°52'17,02"	0	1
	4.6	28°04114'	55°13,040'	1	1
Capão do Cipó	5.1	29°00'28,0438"	54°33'45,65"	1	1
	5.3	29°00'28,0438"	54°33'45,65"	0	1
	5.4	29°00'28,0438"	54°33'45,65"	0	1
Sarandi	6.1	27°54'05"	52°57'43,01"	0	1
	6.2	28°00'57,4"	52°46'50,2"	1	1
	6.3	27°56'54,21"	52°52'35,74"	1	1
	6.4	27°56'35,58"	52°52'42,31"	0	1
	6.5	27°58'58,97"	52°46'39,31"	1	1
	6.6	28°0'42,645"	58°54'28,85"	0	-
Viadutos	7.2	27°35'44,94”	50°05'07,98”	0	1
	7.3	27°35'44,94”	50°05'07,98”	0	-
	7.4	27°35'44,94”	50°05'07,98”	1	1
	7.6	27°35'44,94”	50°05'07,98”	0	1
Lagoa Vermelha	9.1	28°06'130"	51°20'230"	0	1
	9.2	28°04'129"	51°20'130"	1	1
	9.3	28°00'6,65'	51°24'29,4"	1	1
	9.4	28°06'12,5"	51°22'290"	1	1
	9.6	28°09,128'	51°25,060'	1	1
	9.7	28°04,133'	51°20,270'	1	1
Panambi	11.1	28°17'46"	53°25'81"	0	0
	11.3	28°14'24"	53°43'22"	-[3]	1
	11.4	28°26'02"	53°29'60"	0	1
	11.5	28°18'836"	53°53'433"	1	1
Estrela Velha	14.1	29°11,620'	53°07,490'	1	1
	14.2	29°09,853'	53°07,386'	0	1
	14.3	29°11'64"	53°30'51"	1	1
	14.4	29°10,983'	53°11,597'	0	1
Nova Palma	15.1	29°26'38,15"	53°25'12"	0	-
	15.2	29°27'24,1"	53°25'12"	1	1
Pontão	20.1	28°1'42,78"	52°47'6,94"	0	1
	20.2	28°1'42,78"	52°47'6,94"	1	1
	20.3	28°1'42,78"	52°47'6,94"	0	0
Condor	21.1	28°14'005"	53°36'582"	1[4]	1
	21.2	28°08'45"	53°23'00"	1	1
Capão do Leão	22.1	31°48'14,67”	52°42'53,75”	0	0

[1] At least two surviving plants. [2] 1 and 0 represent survival and death, respectively. [3] Not available because of insufficient sample. [4] All surviving plants.

the resistant ones, and selection of these within the population with a high tolerance; and interbreeding among survivor biotypes, generating new individuals with a higher degree of resistance (Mortimer, 2011). Even though a low level of resistance to glyphosate was observed in wild poinsettia, it is likely that other factors are involved in the observation of control failures in the soybean crops in RS, and that these factors also contribute to the reduction of herbicide efficiency.

Based on the analysis of the questionnaires given to the soybean producers where the collection of wild poinsettia with suspected resistance to glyphosate was carried out, it was observed that a majority of the producers cultivated RR® soybean for more than seven consecutive years (Figure 2A). This result confirmed that the RS soybean producers prefer to use this technology, being that the adoption of this technology can be attributed to the fact of the simplicity and flexibility of the use of the herbicide (Riar et al., 2013b). Thus, for the most part, RS farmers use glyphosate consecutively as the only herbicide for RR® crops, in some cases for up to nine years (Ulguim et al., 2013).

Continued use of RR® cultivation in the same area and for several consecutive years promotes the elevation of selection pressure of weeds resistant to repeated use of the same herbicide. Accordingly, the likelihood of resistance of *Amaranthus tuberculatus* (Moq.) Sauer glyphosate was estimated to be 87% where the management was carried out exclusively with glyphosate for several consecutive harvests, in comparison to a probability of 38% when a rotation of herbicides with different mechanisms of action in the same area was held (Rosenbaum & Bradley, 2013), indicating the risks of frequent use of RR® technology.

Besides the exclusive use of glyphosate, it was found that most producers carried out applications of the herbicide more than three times annually in the area (Figure 2B), where 43% of the producers reported proceding with three applications of glyphosate per soybean cycle (Figure 2C). This result confirms previous results, which found that the number of glyphosate applications were three (Vargas et al., 2013) and two (Ulguim et al., 2013) times annually and in the cultivation of soybean in RS, respectively. In contrast, in the United States it was

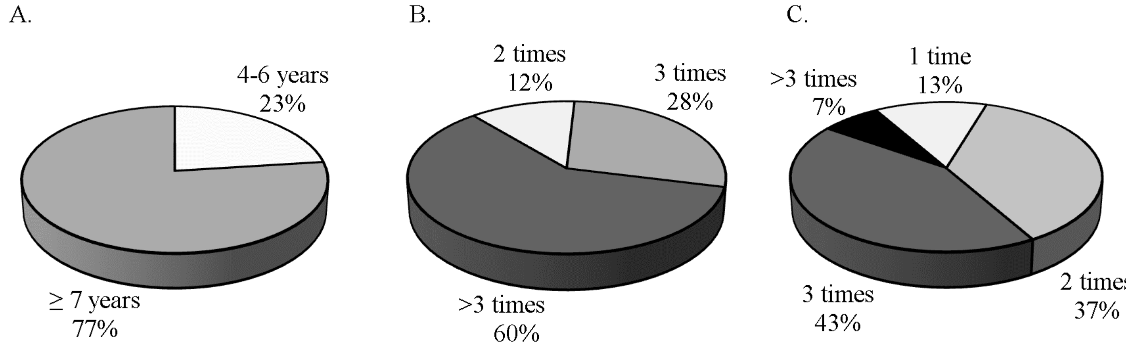


Figure 2. Roundup Ready® soybean cultivation time (a), number of annual applications (b) and the soybean crop cycle (c) of glyphosate in areas with suspicion of the presence of the wild poinsettia (*Euphorbia heterophylla* L.) resistant herbicide.

found that most producers used up to two post-emergence applications per RR® cultivation cycle (Givens et al. 2009).

For different RR® crops, an average number of glyphosate applications at 1.8, 1.3 and 2.3 times for transgenic soybean, corn and cotton were observed, respectively (Prince et al., 2012). Thus, it is possible to see that there is a high selection pressure for weed resistance to glyphosate in RR® soybean cultivation in RS. However, other factors contribute to the high selection pressure observed, such as the dose of glyphosate.

The dose of the herbicide interferes with the strength of selection, regardless when high or low doses of glyphosate are used. Doses below the recommended levels, between 150 and 350 g a.e. ha^{-1} of glyphosate, select populations resistant to *Lolium rigidum* after three cycles of selection (Busi & Powles, 2009). It was suggested that this mechanism occurs because subdoses of glyphosate cease the flavonoid biosynthesis in plants treated with the herbicide and, together with the knowledge of the key role of these compounds in blocking the ultraviolet radiation, a large number of mutations can be generated, some of which afford resistance (Gressel, 2011). Furthermore, in those cases in which resistance is governed by a series of genes and when it is not due to a change in location of action, low doses of herbicides can promote accumulation of genes responsible for resistance in the treated plants (Busi et al., 2013).

It is worth noting that overdoses of herbicides are also harmful, and may cause increased selection pressure, which can accelerate the emergence, in the field of resistant weed biotypes, as they select plants with a higher degree of resistance. Thus, mistakenly, the use of the maximum dose recorded was elected by RR® soybean and cotton farmers as one of the most important factors for weed management, in order to reduce cases of resistance (Riar et al., 2013a).

In the present study, it was found that 80% of the producers have used a range of doses varying from 1080 to 1440 g a.e. ha^{-1} of glyphosate, for pre-sowing desiccation applications (Figure 3A) and for applications in post-emergence of the culture, with a majority of the producers having used doses of 720-1080 g a.e. ha^{-1} (Figure 3B). In this case, it is clear that most of the farmers interviewed used a dose range that covers that which is recommended for wild poinsettia control in post-emergence cultivation of genetically modified soybean (Agrofit, 2015). These results were similar to those observed in other studies conducted in the State of RS, for the control of *Eleusine indica* (Ulguim et al., 2013) and wild poinsettia (Vargas et al., 2013).

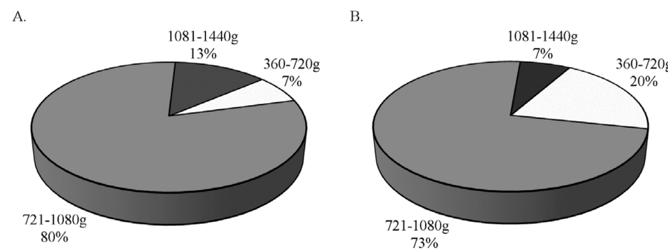


Figure 3. Dose (g a.e. ha^{-1}) for desiccation (a) in post-emergence application (b) of glyphosate used for Roundup Ready® soybean farmers in areas with the occurrence of wild poinsettia (*Euphorbia heterophylla* L.) resistant to glyphosate. Capão do Leão, 2012.

It is noted that the range of doses recorded for the management of wild poinsettia by applying glyphosate is from 480 to 2220 g a.e. ha^{-1}, depending on the brand, the herbicide application time and the culture (Agrofit, 2015). Thus, using this dose range as a comparison, it can be considered that those used by the interviewed producers, mainly related to pre-sowing desiccation, fall short of what is considered high and may favor the survival of the weed. On the other hand, based on the results observed in this study and the dose range registered for post-emergence applications of the RR® soybean, it is not possible to say that the dose of glyphosate used by the producers is responsible for the control failures observed.

For the management of weeds resistant to herbicides, it is essential to carry out practices such as reducing the use of the same herbicide and the application of herbicides with other mechanisms of action. For this, crop rotation is essential to obtain satisfactory results in weed management, as it also allows the rotation of herbicides. However, only 25% of producers interviewed admitted to rotating crops (Figure 4A), and of these, the vast majority uses corn as an option (Figure 4B). Thus, the high selection pressure exerted by the herbicide glyphosate in areas of RR® soybean cultivation is evident, particularly by continued cultivation and the absence of crop rotation. It is worth noting that, although not quantified, the producers who perform rotation with corn crops are using RR® corn and glyphosate application, which does not aid in resistance management.

Crop rotation was reported among the six most important factors for resistance management and is used by approximately 50% of RR® soybean producers in the South-Central region of the United States (Riar et al., 2013a). However, it was observed that the risk of evolution of resistance in weeds is less in culture

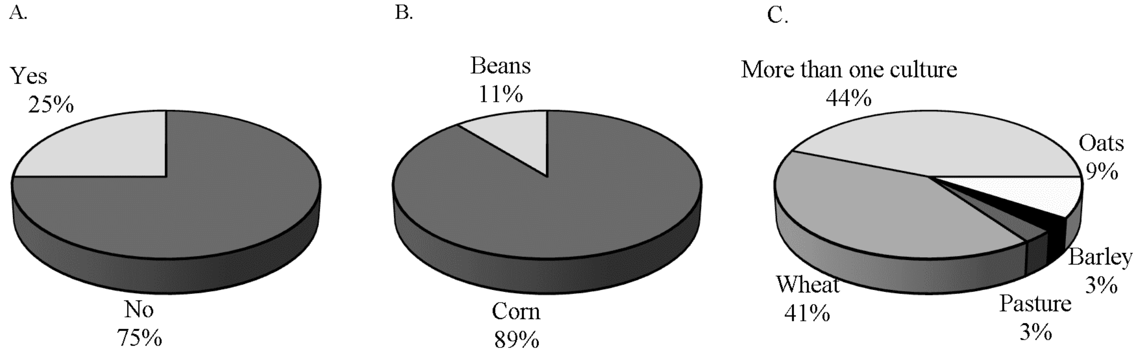


Figure 4. Percentage of producers that perform rotation (a), most commonly adopted alternatives for rotating (b) and sequence (c) of cultures used by producers of Roundup Ready® soybean, in areas with the occurrence of wild poinsettia (*Euphorbia heterophylla* L.) with suspected resistance to glyphosate. Capão do Leão, 2012.

systems with regular crop rotation, including the rotation of herbicides, compared to the cultivation system with little or no crop rotation (Neve et al. 2011). In 89% of the areas where RR® soybean was cultivated for two consecutive years, the presence of glyphosate-resistant *Amaranthus tuberculatus* was observed, while those areas where some form of crop rotation was adopted, the percentage of the weeds with resistance was 45% (Rosenbaum & Bradley, 2013).

Even with a growing number of resistance cases and the difficulty of managing these weeds, a majority of the RS RR® soybean producers have not been using crop rotation, although aware of the benefits of the system. Thus, outreach education to the producers about the importance of crop rotation, herbicides, mechanisms of action and other resistance management practices is essential, in order to reduce the intensity of selection of new biotypes of weeds.

The low rate of crop rotation observed, coupled with the fact that the RR® soybean is being grown consecutively and with more than three applications of glyhosate in areas with suspicion of wild poinsettia resistance, shows the high selection pressure that the species undergoes. Thus, alternatives should be adopted to reduce the selection pressure, and to give crop succession better chances.

The succession of cultivation or crops in the winter permits the reduction of weed infestation in the area by the continuous occupation of the land. To this end, it was observed that 80% of producers cultivate the area during the time between harvests (data not shown), and largely cultivate wheat in the area (41%) or use more than one alternative culture in succession on the property due to the division of the area (44%) (Figure 4C). Few producers use the area for grazing during the winter season, which favors the use of crops that allow straw cultivation for the direct sowing system.

A study evaluating wild poinsettia control with different soil covering in the winter season observed that in areas where oat or ryegrass covering was used, herbicide application in the pre-sowing of the crop was not required, since two applications of glyphosate in the post-culture emergence were carried out (Rizzardi & Silva, 2014). This fact can be attributed to the physical effect of the straw, which hinders germination and emergence of wild poinsettia plants in these areas. However, this benefit was not observed when the soybean was cultivated in the area of straw stubble, where the best control levels of weeds were achieved when using herbicides in soybean pre-sowing (Rizzardi & Silva, 2014). Thus, the producer must be aware of whether or not to use herbicides complementary to glyphosate in the pre-sowing of soybean, under the penalty of having difficulty in managing wild poinsettia during crop cultivation.

The use of herbicides with different mechanisms of action on the selecting agent is among the main management methods to reduce the frequency of resistant biotypes (Neve et al. 2011). To this end, we observed in this study that the vast majority (90%) of the producers use herbicides associated with glyphosate in pre-sowing soybean desiccation (Figure 5). In 61% of the RR® soybean crop area in the South Central Region of the United States, an additional herbicide was used in the pre-sowing application of the crop, followed by glyphosate applications in post-emergence (Riar et al., 2013b). In comparison, between 2005 and 2010, most producers have introduced in their management systems the combination of additional herbicides and glyphosate in continuously cultivated areas with RR® soybean (Prince et al., 2012), due to the increasing problems related to weed resistance to glyphosate.

It is possible to observe in this study that 68% of producers used inhibiting herbicides acetolactate synthase (ALS) to complement weed control in desiccation (Figure 5). The application of glyphosate associated with diclosulam in the soybean pre-seeding yielded effective control of wild poinsettia, eliminating the need for application of herbicides post-emergence (Rizzardi & Silva, 2014). However, the use of the ALS inhibitor herbicide may not be an adequate strategy for wild poinsettia control, since the species is resistant to this herbicide mechanism of action (Heap, 2016; Vidal & Merotto Jr., 1999; Vargas et al, 2013).

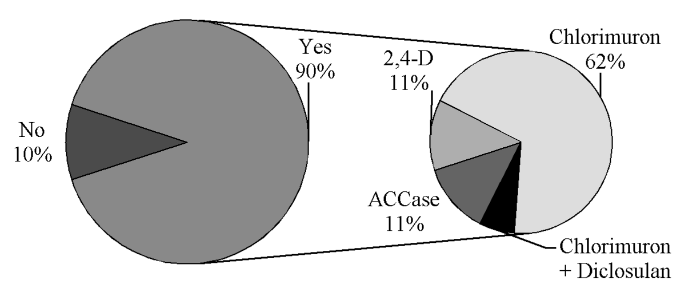


Figure 5. Percentage of Roundup Ready® soybean producers that use a combination of herbicides in the pre-sowing application, and the main herbicides used. Capão do Leão, 2012.

The herbicide 2,4-D was reported by 11% of the producers as an alternative in the combination of herbicides in desiccation for sowing RR® soybean in areas with suspected wild poinsettia resistance to glyphosate (Figure 5). The herbicide 2,4-D was effective in the management of wild poinsettia resistant to different mechanisms of action, including the ALS inhibitors (Vidal & Merotto Jr., 1999). Similarly, inhibitor herbicides of the enzyme acetyl coenzyme-A carboxylase (ACCase) were also reported by 11% of producers, with its main purpose being the control of *Lolium multiflorum* L. resistant to glyphosate. Furthermore, it was observed that all producers pointed out that they did not make an herbicide mixture with glyphosate in the post-emergence applications (data not shown).

There are alternatives in the market to replace the ALS inhibitors in pre-sowing desiccation, such as saflufenacil herbicides, carfentrazone and flumioxazin (Agrofit, 2015). In this case, it is important to note that these are herbicides with different mechanism of action than the previously listed, and can further reduce the selection pressure for glyphosate and ALS inhibitors. On the other hand, all the herbicides belong to the inhibitors of protoporfirinogêmio oxidades (PROTOX) and its continued use was responsible for the selection of wild poinsettia biotypes with multiple resistances (ALS and PROTOX) in the State of Paraná (Trezzi et al., 2005).

In the study, few alternatives for herbicide rotation of the RR® soybean culture were observed, covering basically three different mechanisms of action, raising overdependence on the use of glyphosate in those areas. Herbicides with residual activity in the soil were considered crucial for the management of *Amaranthus palmeri* S.Wats. (Neve et al. 2011) and it is also the recommended practice to delay the development and reduce the selection pressure of resistant weeds. Therefore, it is imperative that the producer knows and can identify the main problems of weeds in their area of cultivation in order to plan the best way to manage the weeds.

The realization of specific practices for weed management with resistance was reported by most producers (Figure 6). In addition, producers described believing that the principal practices to mitigate the emergence of resistance to herbicides are the use of a mixture of herbicides, crop rotation and rotation of herbicides (Figure 6). This result confirms previous findings, where 90% of farmers use herbicide combinations in the management of pre-sowing soybean (Figure 5). However, it was observed that all of the producers interviewed do not mix herbicides with glyphosate in post-emergence applications as discussed above.

The use of more than one herbicide mechanism of action for weed control has been considered an effective practice

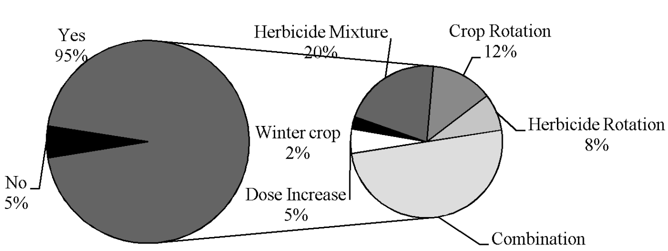


Figure 6. Percentage of Roundup Ready® soybean producers using some specific management practice for weed resistance in the area of cultivation, and leading practices that they believe are more efficient to avoid the emergence of new cases. Capão do Leão, 2012.

for resistance management (Riar et al., 2013a). In addition, practices that aim to prevent seed production by resistant weeds are adopted by approximately half of the soybean producers in the South Central Region of the United States (Riar et al., 2013a), with no reports of this type of tool in areas where wild poinsettia seeds with suspected resistance to glyphosate were collected.

In a similar study, producers considered the use of the recommended herbicide dose; the right time of application; constant surveillance of areas; crop rotation; use of herbicides with different mechanisms of action in pre and post-emergence; and soil preparation, as more efficient practices for the control of weeds resistant to glyphosate (Riar et al., 2013a). In the present study, most producers reported that weed management practices should be connected to favoring the control of the weeds and to prevent the development of resistant weeds (Figure 6).

A majority of the producers that indicated the combination of practices as the most efficient method to manage the emergence of weed resistance in the area of cultivation, considered crop rotation and winter cultivation as the main methods. However, this information contrasts with the percentage of producers that rotate crops in their areas (Figure 4A), since 75% of the respondents admitted to not rotating crops in their areas. Therefore, there is evidence that even though the producers are aware of the benefits of the use of crop rotation, they do not use this practice in their areas for unknown reasons.

One of the main causes of not adopting certain management practices to prevent resistance arises from the producers' lack of information about the risks of continued use of glyphosate. Thus some authors have expressed the need for specific training related to the basic practices of weed management for the producers (Riar et al., 2013b).

It is possible to consider that the strategies for managing resistance and mitigation are well understood by producers, even if few strategies have been implemented, primarily considering the economic argument (Edwards et al., 2014). In most cases, producers have adopted the most convenient and economical management practices until a critical event promotes the paradigm shift (Burgos et al., 2013). Thus, detecting the occurance of resistance as soon as possible makes it easier to establish strategies to reduce productivity losses, and this goal can be achieved with the constant monitoring of crops.

The selection pressure of weeds resistant to glyphosate in RR® soybean crops in RS is high and few alternatives and efficient management practices are being adopted by the producers to reduce the development of new cases of resistance. For example, reports of weed control problems in these areas is recurrent, involving several species (Nohatto, 2010; Ulguim et al, 2013; Vargas et al, 2011). Thus, it is important to conduct studies in order to confirm or refute the occurrence of wild poinsettia resistance to glyphosate in the State of Rio Grande do Sul and on this basis, to draw up alternatives to the management and control of this weed.

Based on the results obtained in this study, you can infer that there is a high selection pressure of weeds resistant to glyphosate in RR® soybean crops, where wild poinsettia seeds were collected. This inference is based on the fact that generally inefficient practices have been observed to prevent the emergence or management of resistant weeds, such as the continued cultivation of RR® soybean; the excessive number of glyphosate applications annually and per cycle in these areas; the low crop rotation rate and (when observed) the exclusivity of corn rotation; and the low level of chemical control alternatives, mostly ALS inhibitor herbicides.

The producers' awareness of weed management practices is essential in order to reduce the high selection pressure observed. However, even with high selection pressure, it cannot be said that the wild poinsettia biotypes studied are resistant to glyphosate, due to the fact that they are controlled by the herbicide within the recorded dose range, thus making it possible to infer the occurrence of low level resistance and to warn of the risk of resistance development in these populations, especially with the high selection pressure applied.

Periodic monitoring of crops is important and should be emphasized, in order to observe possible changes in the weed community before the dispersion of resistance. In addition, it is worth noting the importance of the adoption of integrated weed management practices, in order to reduce the damage caused by the negative interference of these individuals in agricultural crops.

Conclusions

The main agronomic factors observed that are responsible for increased selection pressure were continued cultivation of the RR® soybean; overdependence on the use of glyphosate; low rate of crop rotation; and efficient chemical alternatives for wild poinsettia control. It cannot be affirmed that the studied biotypes present resistance because they were controlled with the recommended dose.

Acknowledgments

To FAPERGS and CNPq for financial support. Edital 22/2010. To Maggie Palmer for help in writing the work.

Literature Cited

Agrofit. Sistema de agrotóxicos fitossanitários. http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons. 20 Mar. 2015.

Agrofit. Sistema de agrotóxicos fitossanitários. http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons. 09 Jun. 2012.

Burgos, N.R.; Tranel, P.J.; Streibig, J.C.; Davis, V.M.; Shaner, D.; Norsworthy, J.K.; Ritz, C. Review: confirmation of resistance to herbicides and evaluation of resistance levels. Weed Science, v.61, n.1, p.4-20, 2013. http://dx.doi.org/10.1614/WS-D-12-00032.1.

Busi, R.; Neve, P.; Powles, S. Evolved polygenic herbicide resistance in *Lolium rigidum* by low-dose herbicide selection within standing genetic variation. Evolutionary Applications, v.6, n.2, p.231–242, 2013. http://dx.doi.org/10.1111/j.1752-4571.2012.00282.x.

Busi, R.; Powles, S.B. Evolution of glyphosate resistance in a *Lolium rigidum* population by glyphosate selection at sublethal doses. Heredity, v.103, n.4, p.318-325, 2009. http://dx.doi.org/ 10.1038/hdy.2009.64.

Edwards, C.B.; Jordan, D.L.; Owen, M.D.K.; Dixon, P.M.; Young, B.G.; Wilson, R.G.; Weller, S.C.; Shaw, D.R. Benchmark study on glyphosate-resistant crop systems in the United States. Economics of herbicide resistance management practices in a 5 year field-scale study. Pest Management Science, v.70, n.12, p.1924-1929, 2014. http://dx.doi.org/10.1002/ps.3759.

Gazziero, D.L.P.; Christoffoleti, P.J.; Vargas, L.; Kruse, N.D.; Galli, A.J.B.; Trezzi, M.M. Critérios para relatos oficiais estatísticos de biótipos de plantas daninhas resistentes a herbicidas. In: Agostinetto, D.; Vargas, L. (Eds.) Resistência de plantas daninhas a herbicidas no Brasil. Pelotas: Editora UFPel, 2014. p.91-101.

GeoLivre. Mapas temáticos RS. http://www.geolivre.rs.gov.br/. 03 Abr. 2011.

Givens, W.A.; Shaw, D.R.; Johnson, W.G.; Weller, S.C.; Young, B.G.; Wilson, R.G.; Owen, M.D.K.; Jordan, D. A grower survey of herbicide use patterns in glyphosate-resistant cropping systems. Weed Technology, v.23, n.1, p156-161, 2009. http://dx.doi.org/10.1614/WT-08-039.1.

Gressel, J. Low pesticide rates may hasten the evolution of resistance by increasing mutation frequencies. Pest Management Science, v.67, n.3, p.253-257, 2011. http://dx.doi.or/10.1002/ps.2071.

Heap, I. Internacional survey of herbicide resistant weeds**.** http://www.weedscience.org. 29 Mai. 2016.

Instituto Brasileiro de Geografia e Estatística - IBGE. Produção Agrícola Municipal - culturas Temporárias e Permanentes. Rio de Janeiro: IBGE, 2010. v.37, p.1-91. http://www.ige.gov.r/home/estatistica/economia/pam/2010/PAM2010_Publicacao_completa.pdf. 10 Jun. 2016.

Lazaroto, C.A.; Fleck, N.G.; Vidal, R.A. Biologia e ecofisiologia de buva (*Conyza bonariensis* e *Conyza canadensis*). Ciência Rural, v.38, n.3, p.852-860, 2008. http://dx.doi.org/10.1590/S0103-84782008000300045.

Mortimer, A. M. Review of graminicide resistance. http://www.hracglobal.com/Publications/AReviewofGraminicideResistance/tabid/223/Default.aspx.13 de Mar. de 2011.

Neve, P.; Norsworthy, J.K.; Smith, K.L.; Zelaya, I.A. Modeling glyphosate resistance management strategies for Palmer amaranth (*Amaranthus palmeri*) in cotton. Weed Technolohy, v.25, n.3, p.335-343, 2011. http://dx.doi.org/10.1614/WT-D-10-00171.1.

Nohatto, Marcos André. Resposta de *Euphorbia heterophylla* proveniente de lavouras de soja Roundup Ready® do Rio Grande do Sul ao herbicida glyphosate. Pelotas: Universidade Federal de Pelotas, 2010. 76p. Dissertação Mestrado.

Norsworthy, J.K.; Bond, J.; Scott, R.C. Weed management practices and needs in Arkansas and Mississippi rice. Weed Technology, v.27, n.3, p.623-630, 2013. http://dx.doi.org/10.1614/WT-D-12-00172.1.

Prince, J.M.; Shaw, D.R.; Givens, W.A.; Newman, M.E.; Owen, M.D.K.; Weller, S.C.; Young, B.G.; Wilson, R.G.; Jordan, D.L. Benchmark study: III. survey on changing herbicide use patterns in glyphosate-resistent cropping systems. Weed Technology, v.26, n.3, p.536-542, 2012. http://dx.doi.org/10.1614/WT-D-11-00093.1.

Riar, D.S.; Norsworthy, J.K.; Steckel, L.E.; Stephenson IV, D.O.; Eubank, T.W.; Bond, J.; Scott, R.C. Adoption of best management practices for herbicide-resistant weeds in Midsouthern United States cotton, rice, and soybean. Weed Technology, v.27, n.4, p.788-797, 2013a. http://dx.doi.org/10.1614/WT-D-13-00087.1.

Riar, D.S.; Norsworthy, J.K.; Steckel, L.E.; Stephenson IV, D.O.; Eubank, T.W.; Scott, R.C. Assessment of weed management practices and problem weeds in the Midsouth United States - soybean: a consultant's perspective. Weed Technology, v.27, n.3, p.612-622, 2013b. http://dx.doi.org/10.1614/WT-D-12-00167.1.

Rizzardi, M.A.; Silva, L. Manejo de plantas daninhas eudicotiledôneas na cultura da soja Roundup Ready®. Planta Daninha, v.32, n.4, p.683-697, 2014. http://dx.doi.org/10.1590/S0100-83582014000400003.

Rosenbaum, K.K.; Bradley, K.W. A survey of glyphosate-resistant waterhemp (*Amaranthus rudis*) in Missouri soybean fields and prediction of glyphosate resistance in future waterhemp populations based on in-field observations and management practices. Weed Technology, v.27, n.4, p.656-663, 2013. https://doi.org/10.1614/WT-D-13-00042.1.

Trezzi, M.M.; Felippi; C.L.; Mattei, D.; Silva, H.L.; Nunes, A.L.; Debastiani, C.; Vidal, R.A.; Marques, A. Multiple resistance of acetolactate synthase and protoporphyrinogen oxidase inhibitors in *Euphorbia heterophylla* biotypes. Journal of Environmental Science and Health Part B, v. 40, n. 1, p. 101-109, 2005. http://dx.doi.org/ 10.1081/PFC-200034254.

Ulguim, A. da R.; Vargas, L.; Agostinetto, D.; Dal Magro, T.; Westendorff, N. da R.; Holz, M.T. Manejo de capim pé-de-galinha em lavouras de soja transgênica resistente ao glifosato. Pesquisa Agropecuária Brasileira, v.48, n.1, p.17-24, 2013. http://dx.doi.org/ 10.1590/S0100-204X2013000100003.

Vargas, L.; Nohatto, M.A.; Agostinetto, D.; Bianchi, M.A.; Gonçalves, E.M.; Toledo, R.E. Resposta de biótipos de *Euphorbia heterophylla* a doses de glyphosate. Planta Daninha, v.29, n.esp., p.1121-1128, 2011. http://dx.doi.org/10.1590/S0100-83582011000500020.

Vargas, L.; Nohatto, M.A.; Agostinetto, D.; Bianchi, M.A.; Paula, J.M.; Polidoro, E.; Toledo, R.E. Práticas de manejo e a resistência de *Euphorbia heterophylla* aos inibidores da ALS e tolerância ao glyphosate no Rio Grande do Sul. Planta Daninha, v.31, n.2, p.427-432, 2013. http://dx.doi.org/10.1590/S0100-83582013000200021.

Vidal, R.A.; Merotto Jr., A. Resistência de amendoim-bravo aos herbicidas inibidores da enzima acetolactato sintase. Planta Daninha, v.17, n.3, p.267-373, 1999. https://doi.org/10.1590/S0100-83581999000300005.