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LOW LEVEL RESISTANCE OF WILD POINSETTIA BIOTYPES TO GLYPHOSATE AND ALTERNATIVE CHEMICAL CONTROL

RESISTÊNCIA DE NÍVEL BAIXO DE BIÓTIPOS DE LEITEIRA AO GLYPHOSATE E ALTERNATIVAS QUIMICAS DE CONTROLE

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ABSTRACT: In the State of Rio Grande do Sul (RS), Southern Brazil, glyphosate has not been capable of controlling wild poinsettia (*Euphorbia heterophylla* L.) in soybean fields, thus, suggesting resistance to this herbicide. Therefore, this study aimed at evaluating sensitivity of wild poinsettia biotypes to glyphosate, identifying the occurrence of resistance of wild poinsettia to the herbicide in RS state and determining the resistance factor of wild poinsettia biotypes under suspicion, besides assessing other herbicides as alternative controls. Two greenhouse experiments, which lasted two years, were conducted by a completely randomized design with four replications. Six biotypes (Factor A) and eight doses of glyphosate (Factor B) were used for getting the dose-response curve. Regarding the alternative control, post-emergence herbicides for soybean and corn crops were tested. Control and dry mass of the shoot were analyzed as variables. Resistance factors of resistant biotypes 20.2 and 21.1 were 4.83 and 5.29, respectively, by comparison with the susceptible biotype (11.4). In RS state, there has currently been high selection pressure due to the intensive use of glyphosate against wild poinsettia plants, as the result of the occurrence of biotypes 20.2 and 21.1 which have low levels of resistance to glyphosate and very little control by ALS-inhibiting herbicides. Therefore, an alternative to mitigate the problem is the use of herbicides with different mechanisms of action.

KEYWORDS: *Euphorbia heterophylla*. Dose response. Resistance factor. Weed.

INTRODUCTION

Wild poinsettia (*Euphorbia heterophylla* L.) is a weed Euphorbiaceae which has been known as one of the most important infestant of soybean crops (TREZZI et al., 2006; RIZZARDI; SILVA, 2014). The species causes economic loss, since it features high ability to compete for resources in the environment and may decrease productivity, affect the quality of the product and hinder harvesting operations (CARVALHO et al., 2010, VARGAS et al., 2011).

In order to mitigate productivity loss, weed control has been almost exclusively carried out by herbicides because of their efficiency and high benefit-cost ratio. However, the exclusive use of chemical control with herbicides that have the same mechanism of action has resulted in increasing numbers of resistant weeds.

Resistance has been defined as the inherent and inheritable capacity a biotype has, in a certain population, to survive and reproduce after exposure to the registered herbicide dose, which is usually lethal to the susceptible population of the same species (GAZZIERO et al., 2014). However, there are differences in the control of biotypes of certain weeds in cases in which doses are below the ones recommended by the information booklet. In these cases, scientific resistance, also known as low level of resistance, can be considered. It does not necessarily imply that the herbicide does not control the species when the maximum registered dose is applied (GAZZIERO et al., 2014). In Brazil, wild poinsettia has been reported as being resistant to herbicides which inhibit acetolactate synthase (ALS) (VIDAL; MEROTTO JÚNIOR, 1999) and protoporphyrinogen oxidase (PROTOX) (TREZZI et al., 2005).

The introduction of transgenic soybean resistant to glyphosate favored the control of wild poinsettia which is resistant to ALS and PROTOX inhibitors with efficiency and low cost (TREZZI et al., 2006). However, due to reports of failure to control this species, selection of either resistance or more tolerant biotypes to this herbicide may be occurring (VARGAS et al., 2011; VARGAS et al.,

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2013a). Cases in which certain wild poinsettia biotypes were less susceptible to glyphosate, even controlled by the maximum registered dose (VARGAS et al., 2011), may be attributed to the occurrence of low level of resistance. However, most farmers (91%) in Rio Grande do Sul (RS) state mention their difficulty in controlling wild poinsettia with the application of glyphosate (NOHATTO, 2010). It shows the need to carry out new studies to characterize the present situation of this problem.

In order to use prevention and handling measures of wild poinsettia in rationally, based on consistent information, studies must be carried out to evaluate the sensitivity of populations of this species to herbicides – with other mechanisms of action – which are used when crop rotation is done and, thus, decrease selection pressure due to rotation of mechanisms of action (TALBERT; BURGOS, 2007). Therefore, the study of alternatives to chemical control is fundamental to handle resistant biotypes adequately (MOREIRA et al., 2010), since changes in handling practices are needed.

Therefore, this study aimed to identify the occurrence of resistance of wild poinsettia to the herbicide in RS state and determining the resistance factor of wild poinsettia biotypes, besides assessing alternative herbicides to control the weed.

MATERIAL AND METHODS

Two experiments were carried out in greenhouses: dose-response curve and alternative control. Both were repeated in 2014 and 2015. In order to determine the dose-response curve of the herbicide glyphosate, the following five biotypes of wild poinsettia were used: 11.4, from Panambi, RS, with suspect susceptibility (28°26'02"S 53°29'60"W); 20.2 and 20.3, from Pontão, RS, with suspect resistance and susceptibility, respectively (28°1'42,78"S 52°47'6,94"W); 21.1, from Condor, RS, possibly resistant (28°14'005"S 53°36'582"W); and 22.1, collected in an area in Capão do Leão, RS, with no records of glyphosate application (28°14'005"S 53°36'582"W).

The experimental design was thoroughly randomized, with four replications. Experimental units were 0.75 L pots which were filled with substrate and a plant. Treatments were arranged in a factorial scheme whose Factor A comprised wild poinsettia biotypes and Factor B tested the following doses of the herbicide glyphosate potassium salt (Roundup Transorb R®, 480 g e.a. L^{-1}): 0, 90, 180, 360, 720, 1440, 2880 and 5760 g a.e ha^{-1}.

When plants were in the 5th-7th leaf growth stage, doses of the herbicide were applied by a coastal sprayer, pressurized with CO_2, with 110.015 fan-type nozzle, 0.5 m apart, and product volume equivalent to 120 L ha^{-1}. The following environmental conditions were used throughout application: mean temperature was 26.9°C; mean relative air humidity (RH) was 78% and wind velocity was 3.4 km h^{-1}.

Variables under analysis were control 20 and 30 days after the application (DAA) of treatments and dry matter of the shoot (DM) 30 DAA. The evaluation of control was carried out by a percentage scale, in which zero represented absence of symptoms and a hundred meant plant death (SBCPD, 1995). In order to determine DM, material was collected and then dried in an oven with forced air circulation at 60°C for 72 hours. Afterwards, conversion to percentages was carried out and DM found by treatments with herbicides were compared with the control treatment, which was considered 100%.

Data were analyzed regardless their normality (Shapiro-Wilk test) and homoscedasticity (Hartley test) and then submitted to the analysis of variance ($p \leq 0.05$). When statistical significance was observed, the analysis of the logistic-type sigmoid non-linear regression was carried out, as follows:

$$y = a / [1 + (x / x_0)^{b}]$$

where: “y” = percentage of DM control or reduction; “x” = dose of herbicide; and “a”, “x_0” and “b” = equation parameters, i. e., *a* corresponds to the difference between both maximum and minimum points estimated for the curve, “x_0” is the dose that provides 50% of the variable response and *b* is the declivity of the curve.

Values of the dose needed either to promote 50% control(DL_{50}) and to decrease 50% dry mass yield (GR_{50}) were found by the arithmetic calculus of the value needed to provide 50% of the response, in agreement with the parameters generated by the equations of the curves. Both values led to the resistance factor (RF) of every biotype with suspect resistance, by comparison with biotypes with suspect susceptibility. In order to use the RF, the confidence interval ($p \geq 0.95$) of the susceptible biotype in relation to the others was verified. Overlap of the confidence interval of the biotype which is susceptible in relation to the resistant ones under evaluation shows that there was no significant difference between the DL_{50} and GR_{50} of the biotypes.

After the experiment of the dose-response curve, three biotypes (11.4, 20.2 and 21.1) were selected for the experiment of alternative control.

Seeds of the biotypes were sown on plastic trays with the commercial substrate Germina Plant®. When plants were in the 1st-2nd leaf growth stage, they were transplanted to individual vases.

The design was thoroughly randomized, with four replications. Experimental units were 0.75 L plastic vases which were filled with substrate. Treatments were arranged in a factorial scheme whose Factor A comprised three wild poinsettia biotypes and Factor B consisted of nine herbicides registered for soybean and corn crops, besides the control treatment with no application.

Regarding the selection of herbicides, the ones recommended for wild poinsettia control post-emergence of soybean and corn crops were advocated (Table 1). Adjuvants was added when the manufacturer recommended it (AGROFIT, 2014). Herbicides were applied when wild poinsettia plants were in the 4th-6th leaf growth stage. The equipment and its calibration were the same described by the previous study. The atmospheric conditions throughout application were the following: mean temperature was 27.3ºC; mean relative air humidity (RH) was 75% and wind velocity was 3.1 km h^{-1}.

Table 1. Mechanism of action, chemical group, active ingredient and dose of the herbicides used in the experiment of chemical control alternatives of wild poinsettia biotypes (*Euphorbia heterophylla* L.) with suspected resistance to glyphosate

Machanism of action	Chemical group	Active ingredient	Dose
			g a.i./a.e. ha^{-1}
Inhibition of ALS[1]	Sulfonylureas*	Nicosulfuron	60
	Sulfonylureas**	Chlorimuron-ethyl[6]	20
	Triazolopyrimidines**	Cloransulam-methyl[7]	40
	Imidazolinones**	Imazethapyr	100
Inhibition of PS II[2]	Triazines*	Atrazine	2500
Inhibition of GS[3]	Phosphinic acids*	Glufosinate-ammonium	400
Synthetic Auxins	Phenoxy-carboxylic acids**	2,4-D	1390
Inhibition of 4-HPPD[4]	Tricetone*	Tembotrione[7]	100.8
	Tricetone*	Mesotrione[6]	192
Inhibition of EPSPs[5]	Glycines**	Glyphosate	1920

Font: Adapted from AGROFIT, 2014. [1]Acetolactate synthase; [2]Photosystem II; [3]Glutamine synthetase; [4]4-hydroxyphenyl-pyruvate dioxygenase; [5]-enolpyruvylhikimate-3-phosphate synthase; [6]Addition of oily adjuvant. [7]Addition of surfactant. Registration dose for corn* and soybean**

Variables under evaluation were visual control of wild poinsettia plants after 14 and 28 DAA, by a scale which is similar to the one used by the previous study, and DM 28 DAA, which was determined as described by the study of the dose-response curve. Data were analyzed regarding their normality and homoscedasticity and then submitted to the analysis of variance ($p \leq 0.05$). When statistical significance was found, means were compared by the Duncan test ($p \leq 0.05$).

RESULTS AND DISCUSSION

The analysis of results found by the experiments of the dose-response curve and the alternative control showed that data transformation was not necessary. The analysis of variance gave evidence of that fact that there was interaction among factors tested for all variables under investigation. Data adjustment to the logistic-type sigmoid regression equation was also observed; values of the coefficient of determination (R^2) ranged between 0.79 and 0.98 (Table 2).

In general, there was less control of wild poinsettia biotypes 21.1, 20.2 and 20.3 in the interval of doses up to 360 g a.e. ha^{-1} by comparison with biotypes 11.4 and 22.1. This effect decreased at higher doses (Figure 1). This result is similar to the one found by previous studies of wild poinsettia biotypes collected in RS state (NOHATTO, 2010; VARGAS et al., 2011; VIDAL et al., 2007). Differences in weed susceptibility to glyphosate were reported in *Chloris polydactyla* (L.) Sw., i. e., groups of biotypes with high tolerance, intermediate tolerance and susceptibility to the herbicide were classified (BARROSO et al., 2014). In the case of wild poinsettia 30 DAA, there was lower control of biotype 21.1 than the one of the others up to 360 g a.e. ha^{-1}. It may imply that this biotype is more tolerant to glyphosate than the others (Figure 1). Therefore, variations in the resistance levels of weed biotypes may show that more than a single mechanism of resistance act on this characteristic (CARVALHO et al., 2011).

Table 2. Logistic-type equation for control 20 and 30 days after application (DAA) and dry mass, coefficient of determination (R^2), values of the dose needed either to promote 50% control (DL_{50}) or to decrease dry mass yield (GR_{50}) in 50% with confidence intervals (CI) and the resistance factor (RF) of wild poinsettia biotypes (*Euphorbia heterophylla* L.) as the response to the application of different doses of the herbicide glyphosate

Biotypes	Equation	R^2	DL_{50}/GR_{50}[1]		RF[3]
			g a. e. ha^{-1}	CI (95%)[2]	11.4
		Control at 20 DAA			
11.4	$y = 100.0/1+(x/112.71)^{-2.22}$	0.97	112.71	103-122	-
20.2	$y =100.0/1+(x/208,71)^{-1.60}$	0.94	208.72	174-244	1.85*
20.3	$y = 100.0/1+(x/110,43)^{-2.00}$	0.95	210.44	184-237	1.87*
21.1	$y = 99.10/1+(x/275.55)^{-3.23}$	0.94	277.10	247-307	2.46*
22.1	$y = 100.0/1+(x/96.33)^{-1.53}$	0.91	96.34	74-119	0.85^{ns}
		Control at 30 DAA			
11.4	$y = 100.0/1+(x/96.51)^{-2.53}$	0.97	96.51	88-105	-
20.2	$y = 100.0/1+(x/180.90)^{-1.63}$	0.91	180.90	143-219	1.87*
20.3	$y = 100.0/1+(x/149.79)^{-1,71}$	0.94	149.79	128-172	1.55^{ns}
21.1	$y = 100.0/1+(x/263.46)^{-3.55}$	0.95	263.46	236-291	2.73*
22.1	$y = 100.0/1+(x/96.07)^{-1.46}$	0.86	96.08	67-125	1.00^{ns}
		Dry mass			
11.4	$y =100.0/1+(x/58.64)^{0.54}$	0.92	58.65	22-96	-
20.2	$y = 100.0/1+(x/283.28)^{0.80}$	0.88	283.28	206-360	4.83*
20.3	$y =100.0/1+(x/4.40)^{0.35}$	0.98	4.40	1-8	0.08*
21.1	$y =100.0/1+(x/310.35)^{1.38}$	0.84	310.36	233-388	5.29*
22.1	$y = 100.0/1+(x/179.08)^{0.72}$	0.88	197.08	144-250	3.36*

[1] DL_{50} related to the variable control 20 and 30 days after application (DAA) and GR_{50} related to the variable dry mass 30 DAA. [2] Confidence intervals at 95% significance ($p \geq 0.95$). [3] * and ^{ns} show either significant difference or non-significant difference, respectively, shown by the overlap of the confidence interval (CI), or lack of it, related to the susceptible biotype 11.4, respectively.

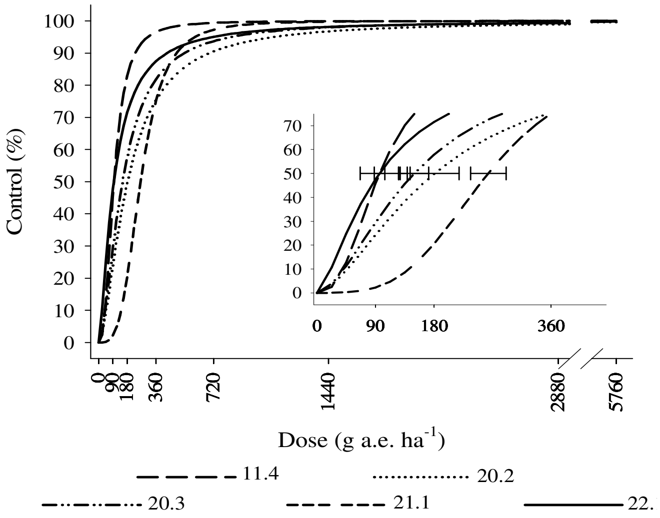

Figure 1. Control (%) of wild poinsettia (*Euphorbia heterophylla* L.) biotypes with suspect resistance to glyphosate 30 days after the application of different doses of the herbicide. Capão do Leão, 2014/15. Horizontal bars represent the confidence interval at 95% significance ($p \geq 0.95$) in the case of doses that represent 50% of biotype control. Coefficient of variation (%) = 18.64.

In this study, control above or close to 90% was found in all wild poinsettia biotypes 30 DAA, at the dose considered the registered herbicide one (720 g a.e. ha^{-1}) (Figure 1). This result corroborates the one found by a study of different wild poinsettia biotypes in which the same dose was enough to cause plant death (NOHATTO, 2010; VARGAS et al., 2011; ZANATTA et al., 2007).

Even having been collected in an area with no records of glyphosate application, biotype 22.1 seems to have lower susceptibility than biotype 11.4 (Figure 1). Low susceptibility of biotypes which originate from areas that have no record of glyphosate application was also observed in *Chloris polydactyla*, whose biotype that had not got any glyphosate application for four years showed intermediate susceptibility (BARROSO et al., 2014).

Biotype 20.3, which was selected as a possible susceptible one, was found to have low percentage of control, thus, showing that it cannot be used in this role. When a species has been investigated concerning resistance to certain herbicide, a population with probability to be susceptible must be collected in the closest place (BURGOS et al., 2013). This fact is important because genetic diversity among weed species may be highly influenced by climatic and geographical differences. Besides, plants in places nearby must have similarities regarding genetic characteristics that can impact their responses to herbicides (BURGOS et al., 2013).

Thus, biotype 11.4 was selected to represent the susceptible biotype which was collected close to the place where biotype 21.1 was collected. The latter can be compared to the former, since there was overlap of the confidence interval in relation to biotype 22.1, which is known as a susceptible one (Figure 2; Table 2). In general, considering all evaluation periods, there was no overlap of its confidence interval with the ones of biotypes 21.1, 20.2 and 20.3 and resistance factors were above one (Table 2).

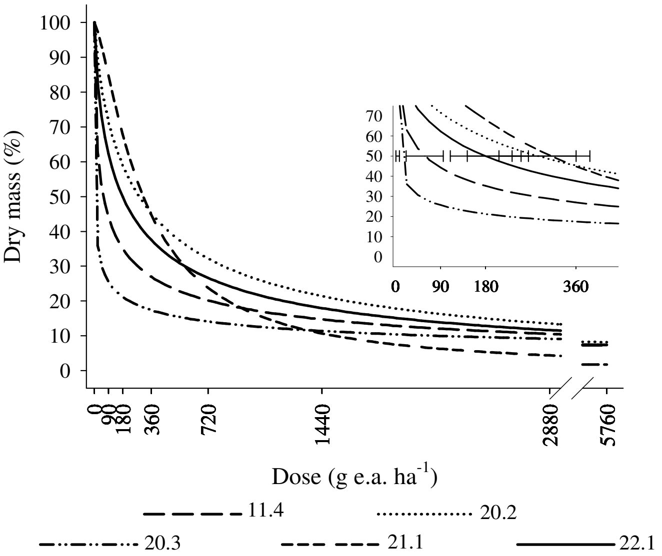

Figure 2. Dry mass of the aerial part (%) of wild poinsettia (*Euphorbia heterophylla* L.) biotypes with suspect resistance to glyphosate 30 DAA of different herbicide doses. Capão do Leão, RS, 2014/15. Horizontal bars represent the confidence interval at 95% significance ($p \geq 0.95$) in the case of doses that represent 50% of the dry mass of the aerial part of the biotypes. Coefficient of variation (%) = 45.30.

On all dates of control evaluation, the RF of biotype 21.1 was found to be significant, a fact that is shown by the absence of overlap of confidence intervals in relation to the susceptible biotype (11.4) (Table 2). In addition, in the case of biotypes 20.2 and 20.3, there was also absence of overlap of the confidence interval with the susceptible biotype (Figure 2; Table 2). However, the DL_{50} of biotype 21.1 (263.46) was higher than the others, thus, suggesting the need for higher doses to reach the same control level, a fact that contributed to a high RF (2.74) for the control 30 DAA (Table 2).

Due to the natural variation of the same population in relation to sensitivity to a certain herbicide, a plant may have high RF, by comparison with more susceptible plants, but, even so, may be susceptible to the registered herbicide dose (BURGOS et al., 2013). DL_{50} of wild poinsettia biotypes were 141 and 433 g a.e. ha^{-1} glyphosate, with RF equal to 3.06 (Table 2). The herbicide dose of 769 g a.e. ha^{-1} was enough to control 90% of the biotype which was considered resistant (VIDAL et al., 2007). In the case of *Raphanus raphanistrum* L. biotypes, with moderate resistance to glyphosate, RF values ranged from 2.3 to 3.2 (ASHWORTH et al., 2014).

Based on the results, wild poinsettia biotypes 21.1, 20.2 and 20.3 were found to have low resistance not only because the RF was low 30 DAA but also because plants were controlled in the registered herbicide dose (GAZZIERO et al., 2014; STECKEL et al., 2008). The *Chloris polydactyla* biotype had high tolerance to glyphosate; at 720 g a.e. ha^{-1}, it reached 100% control. Besides, based on the RF equal to 2.5, it was also attributed to low resistance (BARROSO et al., 2014).

Data on DM corroborate the results of control found for biotype 21.1, which showed lower DM decrease than the others up to 360 g a.e. ha^{-1} (Figure 2). In general, above 720 g a.e. ha^{-1}, there was decrease in DM above 70%, a fact that shows efficient control of the biotypes (Figure 2). However, there were changes in the behavior of the variable in wild poinsettia biotypes, by comparison with the control.

The GR_{50} value of a certain biotype may be affected by environmental conditions during growth, whereas the DL_{50} may be less affected (BURGOS et al, 2013). Therefore, differences related to decrease in the dry mass of the aerial part may result from intrinsic characteristics each biotype has, since they lead its dry mass production. In this study, the GR_{50} of biotype 21.1 was found to be 310.36, about 81% above the one of biotype 11.4 (Table 2). Previous studies of wild poinsettia found that GR_{50} ranged from 48 to 127 g a.e. ha^{-1} among susceptible biotypes, with high tolerance to glyphosate (VARGAS et al., 2011). It may be inferred that both values of biotypes 21.1 and 11.4 found by this study are comparatively high.

Based on GR_{50} values for the calculation of RF, differences were found between biotypes 21.1 and 11.4 due to the absence of overlap of confidence intervals, whose RF value was 5.29 (Figure 2; Table 2). Likewise, biotype 20.2 may be considered to have low resistance, by comparison with biotype 11.4, whose RF is 4.83. As a result, it may be confirmed that biotypes 21.1 and 20.2 had low resistance to glyphosate.

Even though wild poinsettia showed low resistance to glyphosate, other factors may be involved in the observation of failure of control in soybean crops in RS. They also contribute to decrease the efficiency of the herbicide. It is believed that the developmental stage of wild poinsettia plants when glyphosate is applied may favor the occurrence of plant escape from the control with the herbicide. The control of wild poinsettia did not reach 100% when 960 g a.e. ha^{-1} glyphosate was applied to the 4th-6th-leaf growth stage (RAMIRES et al., 2010). Low efficiency in the control of this species was also reported when doses of 480, 960 and 1.440 g a.e. ha^{-1} were applied to plants in the 4th-8th-leaf stage (PROCOPIO et al., 2007). This observation is mainly due to the morphological characteristics of the species.

The more developed the plant, the lower the deposit of the sprayer product on wild poinsettia plants (GAZZIERO et al., 2006). Besides, the species has leaf barriers against herbicide penetration, such as the high content of epicuticular wax, high density of laticifers and large thickness of the cuticle, which may be more intense when plants are in an advanced developmental stage (FERREIRA et al., 2003). Thus, the application of the herbicide glyphosate to wild poinsettia plants in an early developmental stage may favor control, even the one of resistant biotypes, such as *Eleusine indica* (VARGAS et al., 2013b). Application of herbicides at the right moment, when plants are in early developmental stages, was considered the most efficient practice to manage resistant weeds (PRINCE et al., 2012), and should be recommended to manage wild poinsettia.

Regular observation of crops is important and should be emphasized to know any possible alteration in the infectious community before resistance develops. Besides, it should be highlighted that it is important to use integrated practices of weed management which aim at mitigating the damage caused by the negative interference of these individuals in agricultural practices.

Both crop rotation and the use of herbicides which have different mechanisms of action are essential tools to decrease selection pressure of biotypes that are resistant to certain herbicides (MOREIRA et al., 2010). Based on the results of the alternative control, different levels of control of wild poinsettia biotypes were found by the herbicides under investigation 14 and 28 DAA (Table 3). However, taking into account that satisfactory control meant that herbicides led to control above 80%, atrazine, glufosinate-ammonium, 2,4-D and glyphosate were found to be efficient to control all biotypes (11.4, 20.2 and 21.1) 14 DDA. In the same period, excellent control of biotypes 11.4 and 21.1 by the herbicide nicosulfuron was also observed. Regarding the herbicide glyphosate, the high level of control of the biotypes results from the use of its maximum registered dose against such species.

Table 3. Control (%) of susceptible and low resistant wild poinsettia biotypes (*Euphorbia heterophylla* L.) to glyphosate and alternative herbicides in the management of soybean and corn crops 14 and 28 days after application (DAA)

Herbicide	14 DAA			28 DAA		
	11.4	20.2	21.1	11.4	20.2	21.1
Nicosulfuron	91 aA[1]	30 dB	95 aA	100 aA	28 deB	100 aA
Chlorimuron-ethyl	44 d^{ns}	33 d	23 f	56 dA	25 eB	55 dA
Cloransulam-methyl	70 cA	30 bB	48 dB	62 cA	28 deB	67 cA
Imazethapyr	45 dA	14 eB	43 eA	95 aA	41 cC	70 cB
Atrazine	98 a^{ns}	97 a	99 a	100 a^{ns}	100 a	100 a
Glufosinate-ammonium	99 a^{ns}	99 a	99 a	100 a^{ns}	100 a	100 a
2,4-D	97 aA	91 aB	89 bB	100 a^{ns}	100 a	100 a
Tembotrione	71 cB	56 cC	77 cA	25 eC	36 dcB	87 bA
Mesotrione	81 bA	70 bB	77 cAB	88 bA	58 bB	84 bA
Glyphosate	98 aA	98 aA	89 bB	100 aA	100 aA	99 aB
Control	0 e^{ns}	0 f	0 g	0 f^{ns}	0 f	0 e
V.C. (%)		8.06			7.64	

[1]means followed by the same small letter (in the column) and the same capital letter (on the line) do not differ significantly by the Duncan's test ($p \leq 0.05$). ns = non-significant

Concerning biotype 20.2, which had previously shown low resistance to the herbicide glyphosate, unsatisfactory levels of control by HPPD-inhibiting herbicides (tembotrione and mesotrione) were observed 14 DAT. It was even lower 28 DAT, due to plant re-sprouting (Table 3). Satisfactory control of biotypes 11.4 and 21.1 by the herbicide mesotrione was found 28 DAA whereas the same only happened to biotype 21.1 in the case of tembotrione. Such results show that mesotrione has a wider spectrum of control than tembotrione. On the other hand, studies that aim at identifying the causes of failure in the control of biotypes 11.4 and 20.2 by the herbicide tembotrione and of biotype 20.2 by the herbicide mesotrione must be carried out so as to check their resistance to these herbicides.

Regarding ALS-inhibiting herbicides chlorimuron-ethyl and cloransulam-methyl, control below 80% was found for all biotypes under analysis, in both periods. Likewise, unsatisfactory control of biotypes 20.2 and 21.1 by the herbicide imazethapyr and of biotype 20.2 by nicosulfuron was observed 28 DAA. In addition, it should be highlighted that no ALS-inhibiting herbicide controlled biotype 20.2 satisfactorily. Such results suggest that wild poinsettia biotypes have crossed resistance to ALS-inhibiting herbicides.

Crossed resistance of wild poinsettia has already been shown by other studies (VIDAL; MEROTTO, 1999; XAVIER et al., 2013; PRIGOL et al., 2014). Resistance to ALS-inhibiting herbicides was found for chemical groups of imidazolinones, sulfonylureas and triazolopyrimidines (PRIGOL et al., 2014), as observed by this study. Unsatisfactory control of biotypes 20.2 and 21.1 by ALS-inhibiting herbicides, associated with low resistance to the herbicide glyphosate, may even hinder its management in soybean crops since their mutual and abusive use may submit wild poinsettia plants to high selection pressure (TREZZI et al., 2011; COSTA; RIZZARDI, 2014).

Regarding the variable DM, there was decrease in its amount in all treatments by comparison with the one of the control treatment. It corroborates results that had previously been found for the control. Such results show lower DM amount in all biotypes submitted to the application of herbicides atrazine, glufosinate-ammonium, 2,4-D, glyphosate and mesotrione, by comparison with amounts found by other treatments, a fact that implies efficient control of these biotypes (Table 4).

Concerning biotype 11.4, low DM amounts were also related to herbicides nicosulfuron and imazethapyr, whereas, in the case of biotype 21.1, such effect was observed in the nicosulfuron treatment. Since DM values of biotypes 11.4, 20.2 and 21.1 in both chlorimuron-ethyl and cloransulam-methyl treatments did not differ, suspicion of crossed resistance to ALS-inhibiting herbicides was reinforced. It should also be mentioned that, in the case of biotype 20.2, herbicides nicosulfuron, cloransulam-methyl and imazethapyr were the treatments that had the highest DM amount, by comparison with the other treatments (Table 4).

Table 4. Dry matter of the shoot (DM) (g plant^{-1}) 28 DAA of treatments of susceptible and resistant wild poinsettia (*Euphorbia heterophylla* L.) biotypes to the herbicide and alternative herbicides in the management of soybean and corn crops

Herbicide	Dry matter					
	11.4		20.2		21.1	
Nicosulfuron	0.154	eB[1]	1.454	bA	0.189	dB
Chlorimuron-ethyl	1.317	b^{ns}	0.786	c	1.233	b
Cloransulam-methyl	0.870	c^{ns}	1.232	b	0.850	c
Imazethapyr	0.429	deB	1.309	bA	0.923	cAB
Atrazine	0.146	eB	0.213	deA	0.095	dB
Glufosinate-ammonium	0.091	e^{ns}	0.189	e	0.182	d
2,4-D	0.198	e^{ns}	0.222	de	0.247	d
Tembotrione	0.604	cdA	0.615	cdA	0.119	dB
Mesotrione	0.158	eB	0.428	cdeA	0.188	dB
Glyphosate	0.245	e^{ns}	0.136	e	0.222	d
Control	2.863	a^{ns}	2.463	a	3.213	a
V.C. (%)			31.65			

[1]means followed by the same small letter (in the column) and the same capital letter (on the line) do not differ significantly by the Duncan's test ($p \leq 0.05$). ns = non-significant

Herbicides that had satisfactory control and low DM amount are used either for post-emergence management of corn crops or for desiccation management to implement soybean and corn crops. It should be pointed out that, in the post-emergence management of soybean crops, continuous and excessive use of the herbicide glyphosate may result in increase in the resistance levels of wild poinsettia biotypes, a fact that hinders control. Thus, management of wild poinsettia in soybean crops is restricted to some ALS- and/or PROTOX-inhibiting herbicides, even though there are some reports of resistance of this species to herbicides with such mechanisms of action.

It should be highlighted that failure in the control of wild poinsettia – found by this study – by HPPD-inhibiting herbicides need to be deeply studied in order to clarify the resistance this species may have to such herbicides. This is an important fact because, if the resistance of these biotypes is confirmed, new technologies, such as soybean resistant to HPPD-inhibiting herbicides, will not be a viable tool to control this species.

Wild poinsettia control in crops can also be carried out by the herbicide glyphosate due to low resistance to desiccation and in post-emergence of cultivars with Roundup Ready® technology. However, the association and rotation of glyphosate and herbicides with different mechanisms of action is recommended because this strategy enables increase in the spectrum of activity, cost decrease, fewer residues in the environment – since smaller doses are used – and management and prevention of the appearance of weeds that are resistant to herbicides. Application of the herbicide 2,4-D associated with glyphosate and sequential applications of the combination of glyphosate and paraquat+diuron led to efficient control of *Conyza bonariensis* (LAMEGO et al., 2013), considering that all these herbicides controlled resistant wild poinsettia in soybean pre-sowing.

CONCLUSIONS

There are wild poinsettia biotypes, from Condor (21.1) and Pontão (20.2), with low-level resistance to the glyphosate.

The control of this biotypes by ALS-inhibiting herbicides is smaller, but they are controlled by herbicides atrazine, glufosinate-ammonium and 2,4-D, which have different mechanisms of action.

RESUMO: As falhas de controle de leiteira (*Euphorbia heterophylla* L.) após aplicação de glyphosate em lavouras de soja do Rio Grande do Sul (RS) são frequentes, sugerindo a resistência ao herbicida. Diante disso, os objetivos foram avaliar a sensibilidade de biótipos de leiteira ao herbicida glyphosate, identificar a ocorrência da resistência, determinar o fator de resistência de biótipos de leiteira com suspeita de resistência e avaliar herbicidas alternativos para o seu controle. Foram conduzidos dois experimentos em casa de vegetação, em delineamento inteiramente casualizado com quatro repetições ambos realizados em dois anos. No experimento de curva dose-resposta foram utilizados cinco biótipos (fator A) e oito doses do herbicida glyphosate (fator B). Para o controle alternativo, foram testados herbicidas em pós emergência das culturas de soja e milho. As variáveis analisadas foram controle e massa seca da parte aérea. O fator de resistência dos biótipos resistentes (20.2 e 21.1) foram 4,83 e 5,29 comparativamente ao biótipo suscetível (11.4) respectivamente. Existe elevada pressão de seleção pelo glyphosate em plantas de leiteira no RS, observando-se a ocorrência de biótipos 20.2 e 21.1 com resistência de nível baixo ao herbicida e com controle reduzido pelos herbicidas inibidores de ALS. Portanto, uma alternativa para atenuar o problema é o uso de herbicidas com diferentes mecanismos de ação.

PALAVRAS-CHAVE: *Euphorbia heterophylla.* Dose resposta. Fator de resistência. Planta daninha.

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