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Resistence of Radish Biotypes to Iodosulfuron and Alternative Control[1]

Resistência de Biótipos de Nabo ao Herbicida Iodosulfurom e Controle Alternativo

CECHIN, J.[2], VARGAS, L.[3], AGOSTINETTO, D.[2], ZIMMER, V.[2], PERTILE, M.[2], and GARCIA, J.R.[2]

ABSTRACT - The repetitive use of iodosulfuron for the control of weeds in winter cereals in the south of Brazil has favored the emergence of resistant *Raphanus sativus* biotypes. The objective of this study was to evaluate: the response of *Raphanus sativus* biotypes susceptible and resistant to different dosages of iodosulfuron; the control of biotypes with alternative registered herbicides for the control of the species in crops of wheat, corn and soybean; and the existence of cross-resistance of the biotypes. Thus, four experiments were done in a greenhouse, with a completely randomized design and four replicates. The experimental units were composed of vases with a volumetric capacity of 0.75 L filled with substrate, containing a plant each. For the dose-response curve, three biotypes (factor A) and nine doses of the iodosulfuron herbicide (factor B) were used. For the alternative control, the recommendation was herbicides in pre or postemergence of the crops, and the crossed-resistance was evaluated by using herbicides that inhibit the ALS enzyme of different chemical groups. The analyzed variables were control and shoot dry matter. GR_{50} of the susceptible biotype (B_1) was 0.11 g a.i. ha^{-1}, whereas GR_{50} of resistant biotypes (B_4 and B_{13}) was 102.9 and 86.8 g a.i. ha^{-1} of the iodosulfuron herbicide, respectively. The resistant biotypes presented crossed resistance to herbicides that inhibit the ALS enzyme, where the control can be efficient with the use of herbicides with different action mechanisms.

Keywords: *Raphanus sativus*, dose-response, acetolactate synthase, weed.

RESUMO - O uso repetido do herbicida iodosulfurom para controle de plantas daninhas em cereais de inverno no Sul do Brasil favoreceu o surgimento de biótipos de ***Raphanus sativus*** *resistentes. O objetivo deste estudo foi avaliar: a resposta de biótipos de* ***Raphanus sativus*** *suscetível e resistentes a diferentes doses do herbicida iodosulfurom; o controle dos biótipos com herbicidas alternativos registrados para controle da espécie nas culturas de trigo, milho e soja; e a existência de resistência cruzada dos biótipos. Assim, foram conduzidos quatro experimentos em casa de vegetação, em delineamento inteiramente casualizado com quatro repetições. As unidades experimentais constituíram-se de vasos com capacidade volumétrica de 0,75 L preenchidos com substrato, contendo uma planta cada. Para a curva de dose-resposta foram utilizados três biótipos (fator A) e nove doses do herbicida iodosulfurom (fator B). Para o controle alternativo, foram preconizados herbicidas na pré e pós-emergência das culturas, e a resistência cruzada foi avaliada utilizando herbicidas inibidores da enzima ALS de diferentes grupos químicos. As variáveis analisadas foram controle e massa da matéria seca da parte aérea. A* GR_{50} *do biótipo suscetível* (B_1) *foi de 0,11 g i.a.* ha^{-1}*, enquanto a* GR_{50} *dos biótipos resistentes* (B_4 *e* B_{13}) *foi de 102,9 e 86,8 g i.a.* ha^{-1} *do herbicida iodosulfurom, respectivamente. Os biótipos resistentes apresentaram resistência cruzada a herbicidas inibidores da enzima ALS, onde o controle pode ser eficiente com a utilização de herbicidas com mecanismos de ação distintos.*

Palavras-chave: *Raphanus sativus*, dose-resposta, acetolactato sintase, planta daninha.

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[2] Universidade Federal de Pelotas (UFPel), Pelotas-RS, Brazil, <joaneicechin@yahoo.com.br>; [3] Embrapa Trigo, Passo Fundo-RS, Brazil.

INTRODUCTION

Raphanus sativus (radish) is a type of soil coverage that happens in autumn/winter which is highly used in agricultural systems of the south of Brazil due to its fast growth and because it suppresses the growth of weeds (Balbinot Jr et al., 2007). Although it is very important, radish is one of the main dicotyledon weeds when present in the winter and summer crops, resulting in significant losses in the final yield of the crops (Vargas & Roman, 2005; Bianchi et al., 2011).

The control of radish with herbicides that inhibit the ALS enzyme is the main management tool due to its high efficiency and selectivity to winter crops (Vargas & Roman, 2005). These herbicides act in the first enzyme of the synthesis rout of the valine, leucine and isoleucine side chain amino acids, where there is a blockage of cell division and DNA synthesis (Duggleby et al., 2008). Although it is efficient, the dependence on a single action mechanism enabled the emergence of resistance cases (Walsh et al., 2007). In Brazil, the first records of resistance of *R. sativus* biotypes to herbicides inhibitors of the ALS enzyme, such as the metsulfuron-methyl, chlorimuron-ethyl, imazethapyr, cloransulam-methyl and nicosulfuron, date back to 2001. Years later, biotypes of *R. sativus* were found with crossed resistance to herbicides inhibitors of the ALS enzyme in Argentina and Chile (Heap, 2015).

The resistance provides the biotypes with survival and capacity of reproducing after being submitted to a dosage of herbicide that would be lethal on the other individuals of the same population (Vargas et al., 2009). The survival of biotypes to herbicides can happen due to factors related to the target-site (associated with gene mutations or enzyme overexpression) or due to the non-target-site of the herbicide (increase in the metabolization, compartmentalization or reduction of absorption and/or translocation of the herbicide), affecting the efficacy of the herbicide (Délye, 2013).

Among the alterations caused by the resistance is the increase of the necessary dosage to control these biotypes, which can be obtained through curves of dose-response, which allow to determine the C_{50} (control of 50%) and GR_{50} (reduction of 50% of the dry matter), in addition to the resistance factor (RF) of the biotypes (Christoffoleti, 2002). The increase of the herbicide dosage in radish biotypes resistant to herbicides that inhibit the ALS enzyme may make its use impracticable, and the use of alternative herbicides for the control may be recommended in order to minimize the negative effects they have on the crops (Monjardino et al., 2003; Pandolfo et al., 2013), besides avoiding the selection of new resistant biotypes (Oliveira Neto et al., 2010).

The intensification of control failure on radish populations with iodosulfuron leads us to believe that there are biotypes resistant to this and other herbicides that inhibit the ALS enzyme, calling for the use of higher control doses when compared to susceptible biotypes. Therefore, the objective of this study was to evaluate the response of *Raphanus sativus* biotypes susceptible and resistant to different dosages of iodosulfuron; evaluate the control of these biotypes with alternative herbicides recommended for the control of the species in the crops of wheat, corn and soybean; and to evaluate the existence of a cross resistance of the tested biotypes.

MATERIAL AND METHODS

Thus, to carry out this study, four experiments were done in a greenhouse, with a completely randomized design and four replicates, containing a plant per plastic vase. First, werecollected seeds of radish from crops of wheat in the north and northwest regions of Rio Grande do Sul that survived the application of iodosulfuron. In total, 16 biotypes were collected; the seeds contained in each sample were from one plant, and they were identified and georeferenced through their geodetic coordinates.

Screening

The seeds of the biotypes were sown in trays and, after emergence, the plants were transplanted to plastic vases with a volumetric capacity of 0.75 L, containing soil of the type

Red Yellow Argisol and GerminaPlant® substrate in the proportion 2:1. The analysis of the soil showed pH in water = 5.6; CTC_{pH7} = 7.2 $cmol_c$ dm^{-3}; organic matter = 1.5%; clay = 16%; texture = 4; Ca = 4,1 $cmol_c$ dm^{-3}; Mg = 1.1 $cmol_c$ dm^{-3}; Al = 1.8 $cmol_c$ dm^{-3}; P = 6.5 mg dm^{-3}; and K = 0.15 $cmol_c$ dm^{-3}. The correction of fertility was done before the mixture with the substrate, according to the recommendations for the radish crop.

The biotypes were submitted to the application of the iodosulfuron herbicide in the maximum registered dose for the wheat crop, corresponding to 5.0 g a.i. ha^{-1}, to which was added the spreader-sticker (Hoefix) in the dose of 0.3% of spray (Agrofit, 2015) when the plants reached a vegetative stage of 3-4 leaves.

The herbicide was applied using the backpack sprayer, pressured at CO_2, calibrated to provide spray volume of 120 L ha^{-1}, equipped with spray nozzles in the form of a fan 110.02, with a space of 50 cm among each other.

The evaluation of the visual control was done 28 days after the application of the treatment (DAA), adopting the percentage scale in which zero (0) and one hundred (100) corresponded to the absence of damage and complete death of the plants, respectively (Frans & Crowley, 1986). The choice of the susceptible biotypes was done by the greatest control, and the resistant ones by the smallest control.

Dose-response curve

To determine the dose-response curve of the iodosulfuron herbicide, a study was carried out in a greenhouse, using an entirely randomized experimental design with four replicates; the experimental units, the establishment, the conduction of the plants and the application of the herbicide happened under the same conditions described in the *screening.*

The treatments were arranged in a factorial design in which factor A was composed of radish biotypes (B_1, B_4 e B_{13}) and factor B of doses of iodosulfuron. For the susceptible biotype (B_1) the used dosages were 0, 6.25, 12.5, 25, 50, 100, 200, 400 and 800%; for the resistant biotypes (B_4 e B_{13}) the dosages used were 0, 50, 100, 200, 400, 800, 1.600, 3.200 and 6.400% of the recommended dose of iodosulfuron for the control of radish (3.5 g a.i. ha^{-1}), to which the spreader-sticker (Hoefix) was added in the doses of 0.3% of spray (Agrofit, 2015).

The variables evaluated were visual control and shoot dry matter (SDM) 28 days after application (DAA). The SDM was determined by drying the vegetable material in an oven with forced air circulation at 60 °C until it reached a constant mass, being expressed in g per plant.

The data obtained was analyzed as to its homoscedasticity and then submitted to the variance analysis by the F test ($p \leq 0.05$). The data was adjusted to the non-linear regression model of the logistic type, using the SigmaPlot 12.0 (Sigmaplot, 2012) software; the values of C_{50} and GR_{50} were calculated from the parameters of the equation (Seefeldt et al., 1995), to which is related the response of the plant with the dose of the herbicide. The values were adjusted to the logistic type sigmoidal regression equation: $y = a/[1 + (x / x_{50})^b]$, in which: y = percentage of visual control or SDM; x = dose of the herbicide; and a, x_{50} and b = equation parameters, being a the difference between the maximum and minimum points of the curve; x_{50}, the dose that provides 50% of the variable response; and b, the curve declivity.

The resistance factor (RF) was calculated by the ration between C_{50} or GR_{50} of the resistant biotype and its corresponding susceptible biotype.

Alternative control

Three studies in greenhouse were done for an alternative control. The experimental units, the establishment and conduction of plants, the application of the herbicides, the application stage and the variables analyzed were the same as described in the *screening* study, with the addition of a visual control evaluation at 14 DAA.

The treatments used recommend chemical management strategies considering the dissecting operation and the management of the weed in the postemergence state of the wheat, corn and soybean crops, considering the selectivity of the herbicide inside each crop. The herbicides used in the pre-sowing of the cultures were: glyphosate, in the dosage of 720 g a.e. ha^{-1}; glufosinate ammonium, in the dosage of 400 g a.i. ha^{-1}; paraquat, in the dosage of 400 g a.i. ha^{-1}; diuron+paraquat, in the dosage of 200+400 g a.i. ha^{-1}; and saflufenacil, in the dosage of 49 g a.i. ha^{-1}.

The herbicides used in the postemergence management in the wheat crops were: metsulfuron-methyl, in the dosage of 4.0 g a.i. ha^{-1}; bentazon, in the dosage of 720 g a.i. ha 1; metribuzin, in the dosage of 144 g a.i. ha^{-1}; and 2,4-D amine, in the dosage of 1.005 g a.e. ha^{-1}. The herbicides used in the postemergence management in the corn crops were: mesotrione, in the dosage of 192 g a.i. ha^{-1}; atrazine, in the dosage of 2.500 g a.i. ha^{-1}; tembotrione, in the dosage of 76 g a.i. ha^{-1}; and nicosulfuron, in the dosage of 60 g a.i. ha^{-1}. The herbicides used in the postemergence management in the soybean crops were: clorimurom-ethyl, in the dosage of 20 g a.i. ha^{-1}; fomesafen, in the dosage of 250 g a.i. ha^{-1}; imazethapyr, in the dosage of 106 g a.i. ha^{-1}; and cloransulam-methyl, in the dosage of 30 g a.i. ha^{-1}. An adjuvant or spreader-sticker was added, according to the manufacturer's recommendation (Agrofit, 2015).

The data obtained was analyzed as to its homoscedasticity and submitted to the variance analysis by the F test ($p \leq 0.05$). For the biotypes factor, the data was compared by the t test ($p \leq 0.05$) and, for the herbicide treatments, they were compared by the Duncan test ($p \leq 0.05$).

Crossed resistance

Regarding crossed resistance, a study was carried out in a greenhouse. The experimental units, the establishment and conduction of plants, the application of the herbicides, the application stage and the methodology used to evaluate each variable were the same as described in *screening.*

The herbicide treatments recommend the use of at least one herbicide for each chemical group of inhibitors of the ALS enzyme in the different radish biotypes (B_1, B_4 e B_{13}). The herbicides used were: iodosulfuron, in the dose of 3.5 g a.i. ha^{-1} (sulfonylurea); metsulfuron-methyl, in the dosage of 4.0 g a.i. ha^{-1} (sulfonylurea); imazethapyr, in the dosage of 106 g a.i. ha^{-1} (imidazolinone); cloransulam-methyl, in the dosage of 40 g a.i. ha^{-1} (triazolopyrimidine); bispyribac sodium, in the dosage of 50 g a.i. ha^{-1} (pyrimidinyl thiobenzoate); and flucarbazone-sodium, in the dosage of 21 g a.i. ha^{-1} (sulfonyl aminocarbonyl thiazolinone), which were compared to the control without application. An adjuvant or spreader-sticker was added, according to the manufacturer's recommendation.

The data obtained was analyzed as to its homoscedasticity and submitted to the variance analysis by the F test ($p \leq 0.05$). The biotypes factor was compared by the t test ($p \leq 0.05$) and, the herbicide treatments were compared by the Duncan test ($p \leq 0.05$).

RESULTS AND DISCUSSION

The results and discussion will be presented obeying the sequence of activities presented in material and methods.

Screening

The results have shown the existence of two susceptible biotypes and 14 biotypes resistant to the iodosulfuron herbicide at 28 DAA (non-sampled data). The selected biotypes were: B_1 (susceptible to the herbicide, original from the city of Três de Maio-RS), which presented 99% of control; and B_4 and B_{13}, considered resistant, original rom the city of Três de Maio and Boa Vista do Cadeado-RS, respectively, with 1% control (non-sampled data). Later on, each biotype was duly classified as to its taxonomy and deposited at the herbarium PEL of the Department of Botany, belonging to the Federal University of Pelotas (UFPel), under the numbers 26.495 (B_1), 26.496 (B_4) and 26.497 (B_{13}).

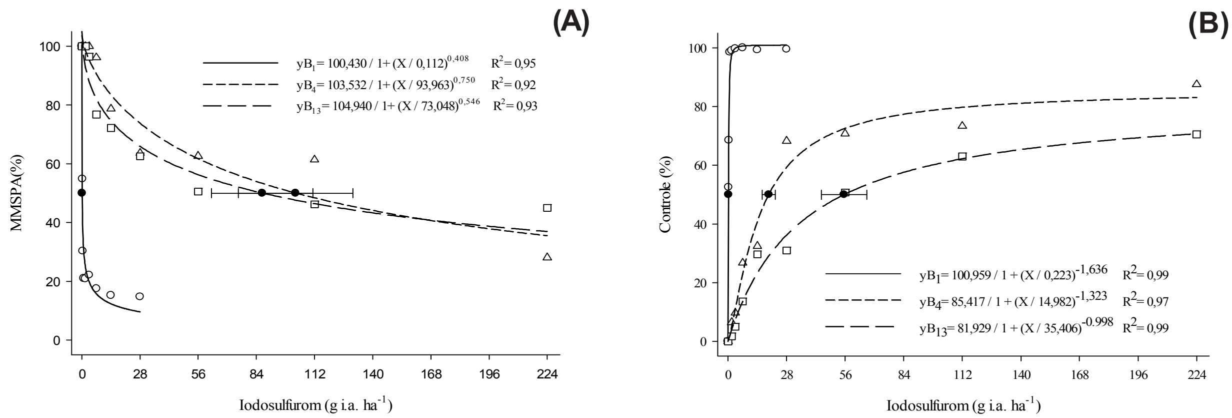
Dose-response curve

The variance analysis indicated interaction among the biotypes factors and doses of iodosulfuron; the control data and the

SDM were adjusted to the logistic type sigmoid curves (Figure 1). The control results, obtained at 28 DAA, showed that C_{50} of the susceptible biotype (B_1) and the resistant biotypes (B_4 and B_{13}) was 0.22, 19.5 and 55.5 g a.i. ha^{-1}, respectively (Table 1). These doses are 33 and 274 times superior to the one recommended for the iodosulfuron herbicide, which makes its use impracticable for the control of resistant radish. Based on the values of C_{50}, a resistance factor (RF) was calculated for the biotypes B_4 and B_{13}, which was 89 and 252, respectively, at 28 DAA (Table 1). Similar results were found by Yu et al. (2012) in biotypes of *Raphanus raphanistrum* submitted to the application of chlorsulfuron, in which the RF was 131.

Regarding the SDM results, it was seen that the accumulation was inversely proportional to the dosage of the herbicide (Figure 1B). For the susceptible biotype (B_1), GR_{50} was 0.11 g a.i. ha^{-1}, whereas GR_{50} of resistant biotypes (B_4 and B_{13}) was 102.9 and 86.8 g a.i. ha^{-1}, respectively, showing the need for elevated doses of the herbicide to provide a 50% reduction of the SDM, causing an elevated RF (Table 1). The results corroborate the ones found in biotypes of *R. sativus* resistant to metsulfuron-methyl, in which GR_{50} was 7.2 g a.i. ha^{-1}, resulting in RF of 144 (Pandolfo et al., 2013).

The resistance levels of tested biotypes were elevated for the iodosulfuron herbicide



The open symbols represent the average values of four repetitions, and the closed symbols and the horizontal bars represent the confidence intervals for the doses that cause 50% of the control or 50% reduction of the SDM.

Figure 1 **-** Visual control (%) (A) and shoot dry matter (SDM in %) (B) of biotypes of susceptible (B_1) and resistant (B_4 e B_{13}) *Raphanus sativus* submitted to doses of iodosulfuron at 28 days after application of the treatments (DAA). Capão do Leão-RS, 2014.

Table 1 - Values of C_{50} and GR_{50} with confidence interval (CI) and resistance factor (RF) of biotypes of *Raphanus sativus* susceptible (B_1) and resistant (B_4 and B_{13}) to iodosulfuron, at 28 days after application of the treatments (DAA). Capão do Leão-RS, 2014

Biotype	C_{50} or GR_{50} [1/]		Resistance factor[2/] (RF)
	g i.a. ha^{-1}	95 CI	
	C_{50}		
Susceptible (B_1)	0.22	0.24 — 0.20	-
Resistant (B_4)	19.50	24.64 — 16.37	89.00
Resistant (B_{13})	55.50	66.62 — 44.38	252.00
	GR_{50}		
Susceptible (B_1)	0.11	0.19 — 0.03	-
Resistant (B_4)	102.90	130.54 — 75.26	935
Resistant (B_{13})	86.80	111.21 — 62.39	789

[1/] C_{50} or GR_{50} = necessary dosage to obtain control of 50% or reduction of 50% of the SDM. [2/] RF obtained by the division of the C_{50} or GR_{50} of the resistant biotype by C_{50} or GR_{50} of the susceptible biotype.

when compared to the susceptible biotype (B_1), which shows the need for alternative strategies for the management of the prevention and to avoid the spread of resistance.

Alternative control

The results of the variance analysis have shown that there was a significant difference for all the analyzed variables, with interaction between the factors biotypes and herbicides (Tables 2, 3 and 4).

The control at 14 DAA was above 90% in all tested biotypes (B_1, B_4 and B_{13}), except for the metsulfuron-methyl herbicide applied in resistant biotypes (B_4 and B_{13}), where the control was of 5% and 16%, respectively (Table 2). All recommended herbicides for the desiccation were efficient and are considered options for the control of the resistant radish. Agostinetto et al. (2009) reported control above 97% in radish when glyphosate was used in the dose of 1.440 g a.e. ha^{-1}, corroborating the data from this study (Table 2).

The control at 28 DAA was excellent in all herbicide treatments with control levels above 99%, except for the metsulfuron-methyl herbicide applied in resistant biotypes, in which the control levels were of 4% and 5% for B_4 and B_{13}, respectively (Table 2). Similar results were diagnosed in resistant biotypes of *R. raphanistrum*, where the application of mestulfuron-methyl did not enable the appropriate control (Costa & Rizzardi, 2013). In the wheat crop, the application of metribuzin, bentazon and 2,4-D amine in postemergence is an option for the management of resistant radish, because they are herbicides selective to the crop and allow an appropriate control (Table 2). Results show that 2,4-D amine in the dosage of 670 g a.e. ha^{-1} is highly efficient in the control of radish biotypes (Farinelli et al., 2005).

For the SDM accumulation variable, evaluated at 28 DAA, the reduction was superior to 80% in the biotypes of *R. sativus,* resistant and susceptible in all herbicide treatments, except in the application of metsulfuron-methyl in the B_4 e B_{13} biotypes in which the reduction was null and inferior to 21%, respectively (Table 2), possibly related to biotypes sensitivity differences. Costa & Rizzardi (2013) reported that the application of metsulfuron-methyl causes a small

Table 2 - Control (%) (A) and shoot dry matter (SDM in g per plant) of biotypes of *Raphanus sativus* susceptible (B_1) and resistant (B_4 and B_{13}) to iodosulfuron at 14 and 28 days after application of the treatments (DAA) with alternative herbicides for the wheat crop management. Capão do Leão-RS, 2014

Treatment	14 DAA		
	B_1	B_4	B_{13}
Ammonium-glufosinate	98 ^{ns}A	98 A	97 A
Diuron + paraquat	100 ^{ns}A	100 A	100 A
Paraquat	100 ^{ns}A	100 A	100 A
Glyphosate	98 ^{ns}A	97 A	99 A
Saflufenacil	100 ^{ns}A	100 A	100 A
Metribuzin	99 ^{ns}A	99 A	99 A
Bentazon	100 ^{ns}A	100 A	99 A
2,4-D amine	98 ^{ns}A	97 A	97 A
Metsulfuron-methyl	90 aB	5.0 cB	16 bB
Control	0.0 ^{ns}C	0.0 C	0.0 C
VC (%)	1.99		
Treatment	28 DAA		
Ammonium-glufosinate	100 ^{ns}A	100 A	100 A
Diuron + paraquat	100 ^{ns}A	100 A	100 A
Paraquat	100 ^{ns}A	100 A	100 A
Glyphosate	99 ^{ns}A	100 A	100 A
Saflufenacil	100 ^{ns}A	100 A	100 A
Metribuzin	100 ^{ns}A	100 A	100 A
Bentazon	100 ^{ns}A	100 A	100 A
2,4-D amine	99 ^{ns}A	100 A	100 A
Metsulfuron-methyl	100 aA	4.0 bB	5.0 bB
Control	0.0 ^{ns}B	0.0 C	0.0 C
VC (%)	1.25		
Treatment	SDM		
Ammonium-glufosinate	0.19 ^{ns}BC	0.25 C	0.26 C
Diuron + paraquat	0.15 ^{ns}C	0.17 C	0.25 C
Paraquat	0.18 ^{ns}BC	0.20 C	0.24 C
Glyphosate	0.19 bBC	0.25 bC	0.33 aC
Saflufenacil	0.18 bBC	0.24 bC	0.30 aC
Metribuzin	0.19 bBC	0.29 aC	0.32 aC
Bentazon	0.17 bBC	0.23 bC	0.52 aB
2,4-D amine	0.20 bBC	0.32 aC	0.37 aC
Metsulfuron-methyl	0.31 cBC	2.34 aA	1.58 bA
Control	1.01 ^{ns}A	1.69 B	1.76 A
VC (%)	26.70		

Averages followed by the same lower case letter (on the row) and the same upper case letter (on the column) do not differ significantly among themselves by the Duncan test ($p \leq 0.05$). ^{ns} = non-significant ($p \leq 0.05$).

reduction of SDM in resistant biotypes, and it is not considered a management option. Thus, the application of alternative herbicides recommended in the desiccation and in postemergence selective to wheat, such as metribuzin, bentazon and 2,4-D amine is considered an option for the resistant radish management, because they cause a reduction of more than 70% of the SDM (Table 2).

Regarding the management in desiccation in the crops of corn, glufosinate-ammonium, paraquat, diuron + paraquat, glyphosate and saflufenacil are options for the control of resistant radish, with levels superior to 98% at 14 DAA (Table 3). In a paper conducted in biotypes of *R. raphanistrum* that the application of glyphosate in desiccation, in the dosage of 720 g a.e. ha^{-1}, provided 98% of control, corroborating the results obtained in this study (Vitorino et al., 2014). Furthermore, ammonium-glufosinate and glyphosate may also be used in the control of radish in corn postemergence, in case the producer uses a Liberty Link® or Roundup Ready® cultivar (Bohm et al., 2011).

At 28 DAA, the control of resistant radish was excellent for all the herbicides indicated for corn crops, except nicosulfuron, in which the control was below 2% in biotypes B_4 and B_{13}, not being different from the control without the application of herbicide (Table 3). The use of mesotrione, tembotrione and atrazine provided control above 99% in resistant biotypes, showing themselves as excellent alternatives for the management in crop postemergence (Table 3). However, it is observed that the application of nicosulfuron did not control the biotypes resistant to iodosulfuron, possibly because they belong to the same group in the chemical class. Therefore, the use of herbicides with alternative action mechanisms on corn crops composes a highly efficient strategy in the control of resistant biotypes.

The results of SDM accumulation at 28 DAA showed that all tested herbicides caused a reduction of the SDM in resistant and susceptible biotypes, except nicosulfuron applied on resistant biotypes (B_4 and B_{13}) where, there was no reduction of the SDM, and did not differ from the control without application (Table 3). Similar results were found in biotypes of radish resistant to inhibitors of ALS, where the application of 22.5 g a.i. ha^{-1} of nicosulfuron provided a

Table 3 - Control (%) and shoot dry matter (SDM in g per plant) of biotypes of *Raphanus sativus* susceptible (B_1) and resistant (B_4 and B_{13}) to iodosulfuron at 14 and 28 days after application of the treatments (DAA) with alternative herbicides for the corn crop management. Capão do Leão-RS, 2014

Treatment	14 DAA		
	B_1	B_4	B_{13}
Ammonium-glufosinate	99 ^{ns}A	99 A	100 A
Diuron + paraquat	100 ^{ns}A	100 A	100 A
Paraquat	100 ^{ns}A	100 A	100 A
Glyphosate	98 ^{ns}AB	100 A	99 A
Saflufenacil	100 ^{ns}A	100 A	100 A
Mesotrione	96 ^{ns}B	94 B	95 B
Tembotrione	94 aB	89 bC	90 bC
Atrazine	95 bB	99 aA	99 aA
Nicosulfuron	91 aC	1.0 bD	6.0 bD
Control	0.0 ^{ns}D	0.0 D	0.0 E
VC (%)	2.71		
Treatment	28 DAA		
Ammonium-glufosinate	100 ^{ns}A	100 A	100 A
Diuron + paraquat	100 ^{ns}A	100 A	100 A
Paraquat	100 ^{ns}A	100 A	100 A
Glyphosate	100 ^{ns}A	100 A	100 A
Saflufenacil	100 ^{ns}A	100 A	100 A
Mesotrione	100 ^{ns}A	100 A	100 A
Tembotrione	100 ^{ns}A	100 A	100 A
Atrazine	99 ^{ns}A	100 A	100 A
Nicosulfuron	100 aA	0.0 bB	2.0 bB
Control	0.0 ^{ns}B	0.0 B	0.0 B
VC (%)	0.82		
Treatment	MDMAP		
Ammonium-glufosinate	0.20 ^{ns}B	0.25 B	0.26 B
Diuron + paraquat	0.18 ^{ns}B	0.18 B	0.24 B
Paraquat	0.18 ^{ns}B	0.18 B	0.25 B
Glyphosate	0.19 bB	0.25 aB	0.33 aB
Saflufenacil	0.16 bB	0.24 aB	0.30 aB
Mesotrione	0.25 ^{ns}B	0.30 B	0.29 B
Tembotrione	0.18 bB	0.29 aB	0.32 aB
Atrazine	0.25 bB	0.34 aB	0.29 aB
Nicosulfuron	0.24 bB	1.64 aA	1.55 aA
Control	1.12 ^{ns}A	1.45 A	1.76 A
VC (%)	30.60		

Averages followed by the same lower case letter (on the row) and the same upper case letter (on the column) do not differ significantly by the Duncan test ($p \leq 0.05$). ^{ns} = non-significant ($p \leq 0.05$).

reduction of 35% of the SDM, when compared to the susceptible biotype (Costa & Rizzardi, 2013). However, because the other herbicides used presented different action mechanisms, they are considered options for the management of radish in the corn crops (Table 3).

For the alternative management in crops of soybean, at 14 DAA, it was seen that ammonium-glufosinate, paraquat, diuron + paraquat, glyphosate and saflufenacil showed control superior to 98%, being options for the management of radish in desiccation. In biotypes of *R. raphanistrum* with multiple resistance to herbicides inhibitors of the ALS and EPSPs enzyme, the application of diquat and diuron provided total control in resistant and susceptible biotypes (Ashworth et al., 2014).

At 28 DAA, the level of radish control was above 99% in all herbicide treatments, except for resistant biotypes treated with herbicides that inhibit the ALS enzyme (Table 4). Among the tested selective herbicides, fomesafen, in the dosage of 250 g a.i. ha^{-1}, was the only one that controlled the resistant biotypes. Among the herbicides that inhibit the ALS enzyme selective to the crop, it was seen a higher level of control when cloransulam-methyl was used in the dosages of 30 g a.i. ha^{-1}, with 58% and 74% of the control in the biotypes B_4 and B_{13}, respectively (Table 4). Similar results were obtained in resistant biotypes of *R. raphanistrum* where the application of chlorsulfuron, imazamox and metosulam did not provide an efficient control (Yu et al., 2012).

For the SDM variable at 28 DAA, all herbicides with different mechanism enabled a lower accumulation of dry matter in resistant biotypes (B_4 and B_{13}), with reduction above 72% and 80%, respectively (Table 4). These results corroborate the ones obtained by Costa & Rizzardi (2013) where no herbicide inhibitor of the ALS enzyme provided efficient control in biotypes of resistant radish, suggesting that the biotypes presented crossed-resistance.

Crossed resistance

Through the variance analysis, an interaction was seen among the biotype factors and the tested herbicides inhibitors of the ALS enzyme. At 28 DAA, the control was above 98% in the susceptible biotype (B_1) and below 11% in the biotypes B_4 and B_{13} when there was use of iodosulfuron, metsulfuron-methyl,

Table 4 - Control (%) and shoot dry matter (SDM in g per plant) of biotypes of *Raphanus sativus* susceptible (B_1) and resistant (B_4 and B_{13}) to iodosulfuron at 14 and 28 days after application of the treatments (DAA) with alternative herbicides for the soybean crop management. Capão do Leão-RS, 2014

Treatment	14 DAA		
	B_1	B_4	B_{13}
Ammonium-glufosinate	99 ^{ns}A	99 A	100 A
Diuron + paraquat	100 ^{ns}A	100 A	100 A
Paraquat	100 ^{ns}A	100 A	100 A
Glyphosate	98 ^{ns}A	100 A	99 A
Saflufenacil	100 ^{ns}A	100 A	100 A
Clorimuron	64 aC	15 bC	20 bC
Fomesafen	100 ^{ns}A	100 A	100 A
Imazethapyr	66 aC	12 bC	8.0 bC
Cloransulan	91 aB	60 bB	56 bB
Control	0.0 ^{ns}D	0.0 D	0.0 D
VC (%)	2.93		
Treatment	28 DAA		
Ammonium-glufosinate	100 ^{ns}A	100 A	100 A
Diuron + paraquat	100 ^{ns}A	100 A	100 A
Paraquat	100 ^{ns}A	100 A	100 A
Glyphosate	100 ^{ns}A	100 A	100 A
Saflufenacil	100 ^{ns}A	100 A	100 A
Clorimuron	99 aA	20 bC	25 bC
Fomesafen	100 ^{ns}A	100 A	100 A
Imazethapyr	100 aA	2.0 bD	0.0 bD
Cloransulan	100 aA	58 cB	74 bB
Control	0.0 ^{ns}B	0.0 D	0.0 D
VC (%)	2.37		
Treatment	MDMAP		
Ammonium-glufosinate	0.20 ^{ns}B	0.25 C	0.26 C
Diuron + paraquat	0.18 ^{ns}B	0.18 C	0.24 C
Paraquat	0.18 ^{ns}B	0.18 C	0.25 C
Glyphosate	0.19 bB	0.25 aC	0.33 aC
Saflufenacil	0.16 bB	0.24 aC	0.30 aC
Clorimuron	0.29 bB	1.47 aA	1.43 aA
Fomesafen	0.17 bB	0.40 aC	0.28 aC
Imazethapyr	0.24 bB	1.51 aA	1.42 aA
Cloransulan	0.29 bB	0.80 aB	0.66 aB
Control	1.12 ^{ns}A	1.45 A	1.76 A
VC (%)	30.60		

Averages followed by the same lower case letter (on the row) and the same upper case letter (on the column) do not differ significantly among themselves by the Duncan test ($p \leq 0.05$). ^{ns} = non-significant ($p \leq 0.05$).

flucarbazone-sodium, imazethapyr and cloransulam-methyl (Table 5). Among the herbicides that inhibit the ALS enzyme, bispyribac-sodium was the one that provided a higher level of control, with 78% and 68% in the biotypes B_4 and B_{13}, respectively (Table 5). The control differences diagnosed in the resistant biotypes may be related to the differential affinity of the herbicide molecule with the enzyme target-site, due to the possible mutation of the ALS gene (Délye, 2013). Similar results were obtained in biotypes of *Xanthium strumarium* resistant to herbicides inhibitor of the ALS enzyme, where the resistant biotypes were less sensitive to herbicides of the chemical group of the imidazolinones, when compared to herbicides of the group of the sulfonylureas and triazolopyrimidines (Schmitzer et al., 1993).

Regarding the SDM accumulated by biotypes, it was observed that the application of herbicides in the recommended doses reduced around 70% of the SDM of B_1, when compared to the control without application (Table 5). For the resistant biotypes, the only herbicide that caused reduction of the SDM was bispyribac-sodium, in which there was a reduction of 35% and 60% of the SDM in the biotypes B_4 and B_{13}, respectively, when compared to their respective controls (Table 5). A similar result was found in resistant biotypes of *R. raphanistrum*, where the accumulation of the SDM was different among herbicides inhibitors of the ALS enzyme and greater when cloransulam-methyl was used in the dosage of 30 g a.i. ha^{-1}, with a reduction of 61% when compared to the control without application (Costa & Rizzardi, 2013).

The results showed that the radish biotype B_1 is susceptible, while biotypes B_4 and B_{13} are resistant to iodosulfuron. The results proved that there was no efficient control with any of the herbicides that inhibit the ALS enzyme due to the existence of crossed resistance. To the resistant biotypes control it's recommended the use of ammonium-glufosinate, glyphosate, diuron+paraquat, paraquat and saflufenacil in dessecation. The selective herbicides metribuzin, bentazon and 2,4-D amine in wheat; mesotrione, tembotrione and atrazine in corn; and fomesafen in soybean are also options for resistant biotypes management.

Table 5 - Control (%) and SDM (per plant) of biotypes of *Raphanus sativus* susceptible (B_1) and resistant (B_4 and B_{13}) to iodosulfuron submitted to different chemical groups inhibitors of the ALS enzyme at 28 days after application of the treatments (DAA). Capão do Leão-RS, 2014

Treatment	28 DAA		
	B_1	B_4	B_{13}
Metsulfuron-methyl	99 aA	2.0 bCD	2.0 bC
Iodosulfuron	99 aA	1.0 bCD	4.0 cB
Flucarbazone	98 aA	0.0 bD	4.0 bB
Imazethapyr	98 aA	3.0 bC	2.0 bC
Bispyribac-sodium	99 aA	78 bA	68 cA
Cloransulam-methyl	99 aA	10 bB	11 bB
Control	0.0 ^{ns}B	0.0 D	0.0 D
VC (%)	7.00		
Treatment	MDMAP		
Metsulfuron-methyl	0.50 bB	1.31 aAB	1.41 aBC
Iodosulfuron	0.43 bB	1.41 aAB	1.51 aAB
Flucarbazone	0.44 bB	1.37 aAB	1.82 aA
Imazethapyr	0.38 bB	1.59 aA	1.40 aBC
Bispyribac-sodium	0.42 bB	0.75 aC	0.58 abD
Cloransulan-methyl	0.43 bB	1.22 aB	1.06 bC
Control	1.42 ^{ns}A	1.15 B	1.42 BC
VC (%)	14.63		

Averages followed by the same lower case letter (on the row) and the same upper case letter (on the column) do not differ significantly among themselves by the Duncan test (p≤0.05). ^{ns} = non-significant (p≤0.05).

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