Article

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RESISTANCE OF *Echinochloa crusgalli* var. *mitis* TO IMAZAPYR+IMAZAPIC HERBICIDE AND ALTERNATIVE CONTROL IN IRRIGATED RICE

*Resistência de **Echinochloa crusgalli** var. **mitis** ao Herbicida Imazapyr+Imazapic e Controle Alternativo em Arroz Irrigado*

ABSTRACT - The introduction of Clearfield® system for irrigated rice production provided an effective management strategy in the selective control of weeds. However, the selection pressure caused by the continuous use of herbicides such as imazapyr+imazapic belonging to the imidazolinone chemical group, without the proper integrated management of weeds, has favored the selection of resistant accessions. The goals of this work were to confirm the resistance to ALS inhibitors in accessions of *E. crusgalli* var. *mitis* collected in rice fields of the state of Rio Grande do Sul and to evaluate the control with alternative herbicides registered for weed control. Three experiments were conducted in a greenhouse using plant seeds that survived the application of the herbicide imazapyr+imazapic, collected in irrigated rice producing regions with suspected resistance escapes. For dose-response curve study, three resistant accessions (ECH1 - Pelotas/Rio Grande do Sul, ECH27 - Arroio Grande/Rio Grande do Sul and ECH38 - Rio Grande/Rio Grande do Sul) and two susceptible accessions (ECH14 - Pelotas/Rio Grande do Sul and ECH44 - Rio Grande / Rio Grande do Sul) (factor A) and 11 doses of the herbicide imazapyr+imazapic (factor B) were selected. The results indicate that barnyardgrass resistant accessions present a high resistance level to imazapyr+imazapic. ALS inhibiting herbicides such as imazethapyr+imazapic, bispyribac-sodium and penoxsulam did not control the resistant accessions either. Integrated weed management should be adopted and alternative mechanisms of action as ACCAse (cyhalofop-butyl, profoxydim and clethodim) and EPSPS (glyphosate) inhibitors are still eficients controlling barnyardgrass ALS resistant accessions.

Keywords: barnyardgrass, herbicide, alternative chemical control, ALS-inhibiting resistance.

*RESUMO - A introdução do sistema Clearfield® de produção de arroz irrigado proporcionou estratégia eficaz no controle seletivo de plantas daninhas. No entanto, a pressão de seleção causada pelo uso contínuo de herbicidas como imazapyr+imazapic, pertencente ao grupo químico das imidazolinonas, sem o adequado manejo integrado de plantas daninhas, tem favorecido a seleção de acessos de plantas daninhas resistentes. Os objetivos deste trabalho foram confirmar a resistência aos inibidores da ALS em acessos de **E. crusgalli** var. **mitis** coletados em lavouras do RS e avaliar o controle desses acessos com herbicidas alternativos registrados para o controle da espécie daninha na cultura do arroz irrigado. Foram conduzidos três experimentos em casa de vegetação, utilizando-se sementes provenientes de plantas que sobreviveram à aplicação do herbicida*

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imazapyr+imazapic, coletadas em lavouras com escapes suspeitos de resistência, em regiões produtoras de arroz irrigado. Para o estudo de curva de dose-resposta, foram selecionados três biótipos resistentes (ECH1 - Pelotas/RS, ECH27 - Arroio Grande/RS e ECH38 - Rio Grande/RS) e dois suscetíveis (ECH14 - Pelotas/RS e ECH44 - Rio Grande/RS) (fator A) e 11 doses do herbicida imazapyr+imazapic (fator B). Os resultados indicam que os acessos resistentes de capim-arroz apresentam elevado fator de resistência ao imazapyr+imazapic. Os herbicidas inibidores da ALS como imazethapyr+imazapic, bispyribac-sodium e penoxsulam, não controlaram os acessos resistentes também. O manejo integrado de plantas daninhas deveria ser adotado e mecanismos de ação alternativos como inibidores da ACCAse (cyhalofop-butyl, profoxydim e clethodim) e da EPSPS (glyphosate) ainda são eficientes em controlar acessos de capim-arroz resistentes a ALS.

Palavras-chave: capim-arroz, herbicida, controle químico alternativo, resistência aos inibidores da ALS.

INTRODUCTION

Barnyardgrass (*Echinochloa* spp.) is an important weed in rice cultivation. Its initial management is important and has generally been performed with herbicides inhibiting the acetolactate synthase (ALS) enzyme, which is the main tool to control weeds in different cropping systems (Tranel and Wright, 2002). ALS inhibiting herbicides act on the first enzyme in the synthesis route of the side chain amino acids (valine, leucine and isoleucine), where the blocking of the route affects cell division and the synthesis of DNA, cause the accumulation of ketobutyrate and the blockage of the Acetyl-CoA synthesis (Duggleby et al., 2008).

ALS inhibiting herbicides were introduced on the world market in 1982 and are currently the leading class with cases of resistance in the world, with 63% of cases (Heap, 2017). Resistant biotypes have the natural and inherited ability to survive and reproduce after the exposure to herbicide rates that would be lethal to susceptible individuals from the same species (Powles and Yu, 2010). Weed genetic factors can strongly contribute to the emergence of resistance, such as a high initial frequency of the gene responsible for resistance, which is more evident in cases involving ALS inhibiting herbicides (Tranel and Wright, 2002).

Another factor that contributes to the expressive number of species that are resistant to ALS inhibitors is the presence of nuclear dominance and semidominance alleles, which allow rapid dispersion of the resistance, due to the ability of cross-fertilization via pollen (Tranel and Wright, 2002). The predominance of weeds that are resistant to these herbicides in different crops makes this herbicide class the most problematic, mainly in irrigated rice cultivation, due to the continuous use of imazapyr + imazapic, a product of the Clearfield® technology.

Herbicide resistance can be diagnose using dose-response curves (Perez-Jones, 2007), where non-linear regression determine the C_{50} index, that is, the herbicide dose necessary to control 50% of the population, allowing the comparison of resistant and susceptible biotypes (Christoffoleti, 2002). Once resistance is confirmed, control measures should be taken to minimize the negative effects of weed accessions. Among the chemical management ways for herbicide-resistant biotypes, there is the use of different mechanisms of action or the association of herbicides with distinct mechanisms of action (Powles and Yu, 2010); they are effective alternatives in controlling resistant weeds, as well as avoiding the selection of new resistant accessions (Oliveira Neto et al., 2010).

This study had the goal to confirm the resistance to ALS inhibitors in accessions of *E. crusgalli* var. *mitis* in the state of Rio Grande do Sul, and to evaluate the control of these accessions with alternative herbicides registered for the control of weed species in irrigated rice crops.

MATERIAL AND METHODS

In the 2013/2014, seeds of barnyardgrass plants that survived the application of imazapyr + imazapic herbicide, were harvested in crops of the southern region of Rio Grande do Sul state. Seeds of 49 accessions were collected in the cities of Rio Grande, Arroio Grande, Pelotas, Santa

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Vitória do Palmar and Capão do Leão; they came from individual plants. After harvested, seeds from each accession were cleaned and stored individually for six months in brown paper bags, at room temperature, until the tests were implanted.

In August 2014, the seeds of suspected resistant accessions were sown in trays in a greenhouse at Embrapa CPACT/ETB, Capão Leão-RS. After their emergence, plants were transplanted to plastic pots, with a volumetric capacity of 0.5 L, containing dystrophic haplic Planosol type soil. Soil analysis presented pH in water 5.0; CTC_{pH7} = 7.1 $cmol_c$ dm^{-3}; organic matter = 1.8%; clay = 14%; texture = 4; Ca = 4.1 $cmol_c$ dm^{-3}; Mg = 1.1 $cmol_c$ dm^{-3}; Al = 1.6 $cmol_c$ dm^{-3}; P = 6.5 mg dm^{-3}; and K = 0.15 $cmol_c$ dm^{-3}. Soil fertilizing correction was performed according to the recommendations for irrigated rice (SOSBAI, 2012).

A screening of the harvested accessions was carried out in a completely randomized design (CRD) study with four replications, with the application of the formulated mixture of imazapyr+imazapic (Kifix®), at the maximum registered dose to control barnyardgrass, corresponding to 73.5+24.5 g e.a. ha^{-1}, when they reached the 3-4 leaf stage.

Herbicide application was performed with a CO_2 pressurized backpack sprayer, calibrated to provide a spray volume of 140 L ha^{-1}, equipped with 110.015 fan-type spraying nozzles, spaced 50 cm apart.

The control evaluation was performed 28 days after the treatment application, adopting a percentage scale where zero (0) and one hundred (100) corresponded to absence of damage and death of plants, respectively (Frans and Crowley, 1986). In this preliminary study, five accessions were selected, three resistant and two susceptible ones. The accessions were then isolated in a greenhouse to avoid cross-pollination, in order to obtain the following generation of seeds as material for subsequent experiments.

Samples from the accessions were sent to the Herbário PEL from Universidade Federal de Pelotas – UFPel and identified as *Echinochloa crusgalli* var. *mitis.*

Dose-response curve study

The determination of the imazapyr+imazapic dose necessary to control 50% of the population (C_{50}) and to reduce 50% of the dry mass production by barnyardgrass (GR_{50}) in susceptible and resistant accessions was performed by a greenhouse study, installed on December 2nd and 3rd 2014, using a CRD with four replications. After their emergence, plants were transplanted to plastic pots with a volumetric capacity of 0.7 L, containing dystrophic haplic Planosol type soil. Seeds from the accessions selected for the studies were sown and, subsequently, seedlings were thinned in order to etablish one plant per pot.

The treatments were arranged in a factorial scheme, where factor A was constituted by the barnyardgrass accessions (ECH1, ECH14, ECH27, ECH38 and ECH44), and factor B was the herbicide doses (imazapyr + imazapic): 0; 0.125; 0.25; 0.5; 1; 2; 4; 8; 16; 32; and 64 x the maximum recommended dose of 73.5+24.5 g a.e. ha^{-1}.

The treatments were applied when the plants reached the 3-4 leaf stage, with a CO_2 pressurized backpack sprayer, with 110.015 fan type spraying nozzles, spaced 50 cm apart, at a constant pressure of 210 KPa, adjusted to a spray volume equivalent to 140 L ha^{-1}.

The evaluated variables were: control on day 14 and 28 after application (DAA) and shoot dry matter (SDM) on day 28 DAA. Control evaluations were performed using the percentage scale adapted from Frans and Crowley (1986). SDM was determined by drying the plant material in an oven at the temperature of 60°C, until reaching constant mass.

The obtained data were analyzed for their homoscedasticity and were later submitted to analysis of variance ($p \leq 0.05$). When statistically significant, data were adjusted to the log-logistic nonlinear regression model using the SigmaPlot 12.0 software (Sigmaplot, 2012), and C_{50} and GR_{50} values were calculated from the parameters of the equation (Seefeldt et al., 1995), which relates the response of the plant (control) to the herbicide dose. Values were adjusted to the logistic-type sigmoid regression equation: $y = a / [1 + (x / x_0)^b]$, where: y = control percentage;

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x = herbicide dose; and *a*, x_0 and *b* = equation parameters, where *a* is the difference between the maximum and minimum points of the curve, x_0, is the dose providing 50% of the variable response, and *b* is the curve gradient.

The resistance factor (RF) was calculated by the ration between C50 or GR50 of the resistant accession and its corresponding susceptible accession.

Alternative chemical control

An experiment was conducted in a greenhouse, during November and December 2014, using the CRD with six replications. The experimental units were composed of 0.7 L plastic pots, filled with soil containing one barnyardgrass plant per pot, as described in the previous study. The establishment of the accessions, the application of the treatments, the application stage, the maintenance of the water blade and the methodology used to evaluate each variable were the same described in the dose-response study.

The treatments were defined based on herbicides recommended to weed management, taking into account the selectivity within the crop. Herbicides, and their respective doses, were: imazethapyr + imazapic (56.2 + 18.7 g a.e. ha^{-1}), imazapyr + imazapic (73.5 + 24.5 g a.e. ha^{-1}), bispyribac sodium (50 g a.i. ha^{-1}), penoxsulan (57.6 g a.i. ha^{-1}), cyhalofop-butyl (315 g a.i. ha^{-1}), profoxydim (170 g a.i. ha^{-1}), clethodim (240 g a.i. ha^{-1}), Quinclorac (375 g a.i. ha^{-1}), glyphosate (1,440 g a.i. ha^{-1}) and propanil (4,000 g a.i. ha^{-1}).

The following adjuvants were used according to the recommendations for each product: Dash® (0.5% v/v) for imazethapyr+imazapic and imazapyr+imazapic; Assist® (0.5% v/v) for clethodim and quinclorac; Assist® (0.3% v/v) for profoxydim; Veget Oil® (3.0 L ha^{-1}) for cyhalofop-butyl; Veget Oil® (1.0 L ha^{-1}) for penoxsulam; and the adhesive spreader (0.25% v/v) for bispyribac-sodium.

The evaluation on plant control was made visually 28 days after the application of the herbicides (DAA), together with the plant collection in order to determine SDM.

The obtained data were analyzed as for their homoscedasticity and were later submitted to analysis of variance (p≤0.05). When statistically significant, the accessions were compared by the t test (p≤0.05) and the herbicide treatments by the Duncan test (p≤0.05).

RESULTS AND DISCUSSION

The performed screening showed that 38 (78%) of the 49 accessions collected and analyzed were not controlled by the maximum dose of the used herbicide, based on the percentage scale adapted from Frans and Crowley (1986). The maximum dose was used since, in order to classify a plant as resistant; it should survive and reproduce after the exposure to the labelled rate of the product to control the species, according to the application criteria (vegetative stage indicated, climate conditions, among others) (Gazziero et al., 2009). Thus, plants from the accessions that survived the screening were determined as originated from resistant accessions: accessions ECH1 from Pelotas (latitude 31°30'33" S and longitude 52º10'45" W), ECH27 from Arroio Grande (latitude 32º18'27" S and longitude 52º52'26" W) and ECH38 from Rio Grande (latitude 32º12'57" S and longitude 52º30'09" W), as resistant. Plants that not survived as accessions ECH14 (latitude 31º29'26" S and longitude 52º10'55" W) and ECH44 (latitude 32º16'54" S and longitude 52º28'20" W) were considered as susceptible, coming from Pelotas and Rio Grande, respectively.

Dose-response curve study

According to the visual control, there was an interaction (p≤0.05) for accessions and doses (Figure 1), 14 DAA. For the ECH1, ECH14, ECH27 and ECH44 accessions, the sigmoid logistic regression model was the one that fitted data (Table 1), whereas for the ECH38 accession there was no curve adjustment, since 100% of plant control was not obtained. On day 14 DAA, it was already possible to confirm that the ECH14 and ECH44 accessions are susceptible to the herbicide (Figure 1), and that the determination coefficients (R^2) presented values   of 0.99 and 0.98, respectively. C_{50} was calculated only for two resistant accessions; ECH1 was equivalent to 7.01
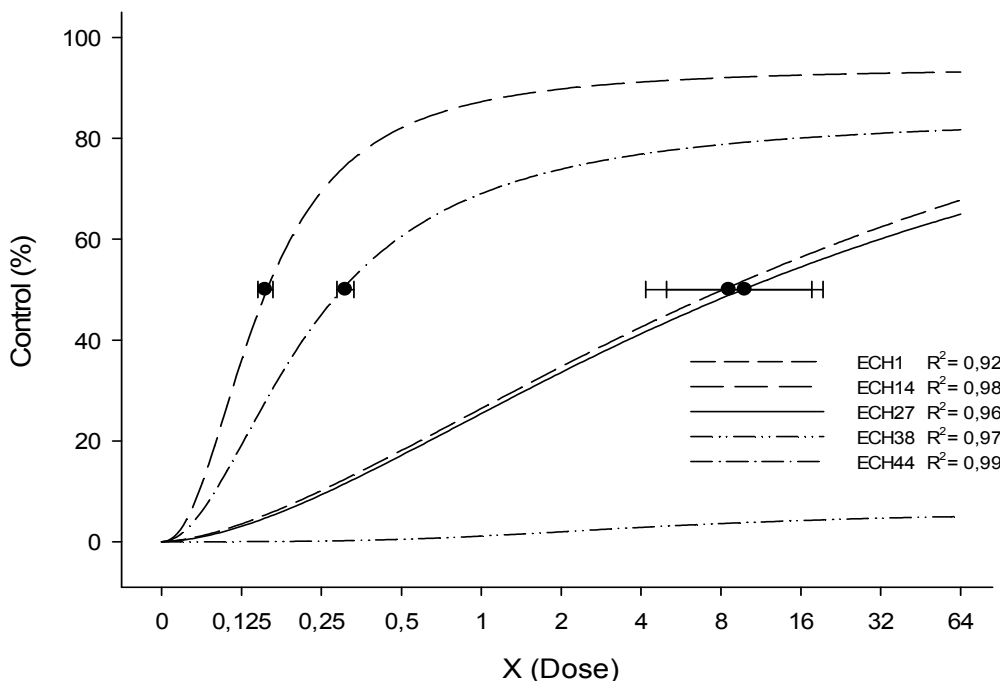
The horizontal bars represent the confidence intervals for the dose causing 50% control over the plant, with 95% significance.

Figure 1 - Control (%) of *Echinochloa crusgalli* var. *mitis* (barnyardgrass) accessions resistant (ECH1-Pelotas/Rio Grande do Sul, ECH27-Arroio Grande and ECH38-Rio Grande/Rio Grande do Sul) and susceptible (ECH14-Pelotas/Rio Grande do Sul and ECH44-Rio Grande/Rio Grande do Sul) to imazapyr+imazapic herbicide, evaluated 14 days after the application of the treatment (DAA), according to different doses.

Table 1 - Logistic type sigmoid regression equation to control *Echinochloa crusgalli* var. *mitis* (barnyardgrass) accessions on day 14 and 28 after the application of the treatment (DAA) and SDM on day 28 DAA, with the respective C_{50} values (x dose), confidence interval (CI) and resistance factor (RF), in response to the application of imazapyr+imazapic doses

Accession	Equation	R^2	$(C_{50})^{(1)}$ or $(GR_{50})^{(2)}$		$(RF)^{(4)}$	
			X0	$CI^{(3)}$	ECH14	ECH44
14 DAA						
ECH1	$y=105.71/[1+(x/9.25)^{-1.59}]$	0.92	9.25	2.13	74.61	25.01
ECH14	$y=98.13/[1+(x/0.12)^{-2.18}]$	0.98	0.12	0.19	-	-
ECH27	$y=101.63/[1+(x/8.23)^{-1.69}]$	0.96	8.23	1.97	66.37	22.18
ECH38	$y=5.97/[1+(x/x0)^{-3.36}]$	0.97	> 64 x	-	-	-
ECH44	$y=98.41/[1+(x/0.37)^{-1.95}]$	0.99	0.37	0.21	-	-
28 DAA						
ECH1	$y=104.68/[1+(x/1.54)^{-1.02}]$	0.98	1.54	2.09	15.2	10.2
ECH14	$y=100.14/[1+(x/0.10)^{-4.28}]$	0.99	0.10	0.05	-	-
ECH27	$y=103.81/[1+(x/2.52)^{-1.06}]$	0.96	2.82	2.74	27.9	18.6
ECH38	$y=51.31/[1+(x/x0)^{-2.02}]$	0.96	> 64 x	-	-	-
ECH44	$y=103.01/[1+(x/0.15)^{-2.49}]$	0.99	0.15	0.07	-	-
SDM						
ECH1	$y=98.97/[1+(x/1.75)^{2.22}]$	0.98	1.75	1.81	15.91	13.46
ECH14	$y=100.01/[1+(x/0.11)^{2.86}]$	0.99	0.11	0.09	-	-
ECH27	$y=98.02/[1+(x/7.96)^{1.12}]$	0.91	7.96	2.23	72.36	61.23
ECH38	$y=100.99/[1+(x/x0)^{1.28}]$	0.94	> 64 x	-	-	-
ECH44	$y=99.84/[1+(x/0.13)^{1.33}]$	0.99	0.13	0.14	-	-

[1] C_{50} = necessary dose to control 50% of the population. [2] G_{R50} = necessary dose to control 50% of the dry matter. [3] CI = confidence interval at 95% parameter error probability. [4] RF = calculation performed through the ratio between C_{50} or GR_{50} of the resistant accession and its correspondent of the susceptible accession.

and ECH27 was equivalent to 7.27 times the maximum dose for the post-emergence control of barnyardgrass (73.5+24.5 g a.e. ha^{-1}) (Table 1).

Starting from the established C_{50} values, it was possible to calculate the RF for each accession; for ECH1 from Pelotas and ECH27 from Arroio Grande, RF varied from 25.01 and 22.18 (related to ECH44) to 74.61 and 66.37 (related to ECH14), respectively. Depending on the overlapping of the intervals, the resistant accesses ECH1 and ECH27, although coming from different places, show very similar C_{50} values, whereas ECH38 from Rio Grande presented only 5% of control when submitted to the application at the maximum recommended dose. Thus it was not possible to calculate C_{50} for ECH38 access, since it presented only 15% of control when submitted to the application of up to 64 times the recommended dose. The low control level observed for ECH38, when the ALS inhibiting herbicide was applied demonstrates the high level of resistance presented by the accession.

For control results on day 28 DAA, the sigmoid logistic regression model also fitted the data (Table 1); the use of sub-doses of 0.10 for ECH14 and 0.15 times the register dose for ECH44, respectively, was enough to control 50% of the population of susceptible accessions (Figure 2). However, in order to obtain the same control percentage in the resistant accessions, it is necessary to use higher doses than the registered one, that is, 1.54 and 2.82 times for the resistant accesses ECH1 and ECH27, respectively.

Using the C_{50} result obtained for the herbicide imazapyr + imazapic, the RF value was calculated, where it was possible to observe that the ECH1 and ECH27 accessions required 15.2 and 27.9 times, respectively, the dose that controlled 50% of their populations, in relation to susceptible access ECH14 (Table 1). When compared to the susceptible access ECH44, the RF of the same resistant accessions presented lower values, equivalent to 10.2 and 18.6, respectively. As for the ECH38 accession, it was not possible to calculate the RF, since the control levels for all doses did not reach 50% control, even when overdoses of the herbicide imazapyr + imazapic were used. Similar results were found for *Raphanus sativus* plants resistant and susceptible to the ALS inhibiting herbicide (iodosulfuron-methyl sodium), where it was necessary to use 33 and 274 times the dose indicated to reach a satisfactory level of control (80%), making impossible to use the herbicide to control resistant turnips (Cechin et al., 2016). In order to this control level to be reached for the ECH1 and ECH27 accessions, it is necessary to use 14.2 and 32 times the registered dose to control this species in irrigated rice crops.

In studies conducted with *Sagittaria montevidensis* under controlled conditions, it was observed that an increase in the imazethapyr + imazapic dose, equivalent to 64 times the recommended dose, did not increase the mortality of the ALS inhibitor-resistant *Sagittaria montevidensis*, where control did not reach 10%, highlighting the high resistance degree of this population to the ALS inhibiting herbicides. In the same study, the susceptible *Sagittaria* biotype, when receiving the application of a recommended half dose of imazethapyr + imazapic, demonstrated total control of the plants, highlighting their high susceptibility to these herbicides (Moura et al., 2015). This behavior is similar to the one of the results obtained in this study, where the recommended dose of the herbicide imazapyr + imazapic totally controlled the susceptible accessions, whereas for resistant accessions, there were few or no phytotoxicity symptoms.
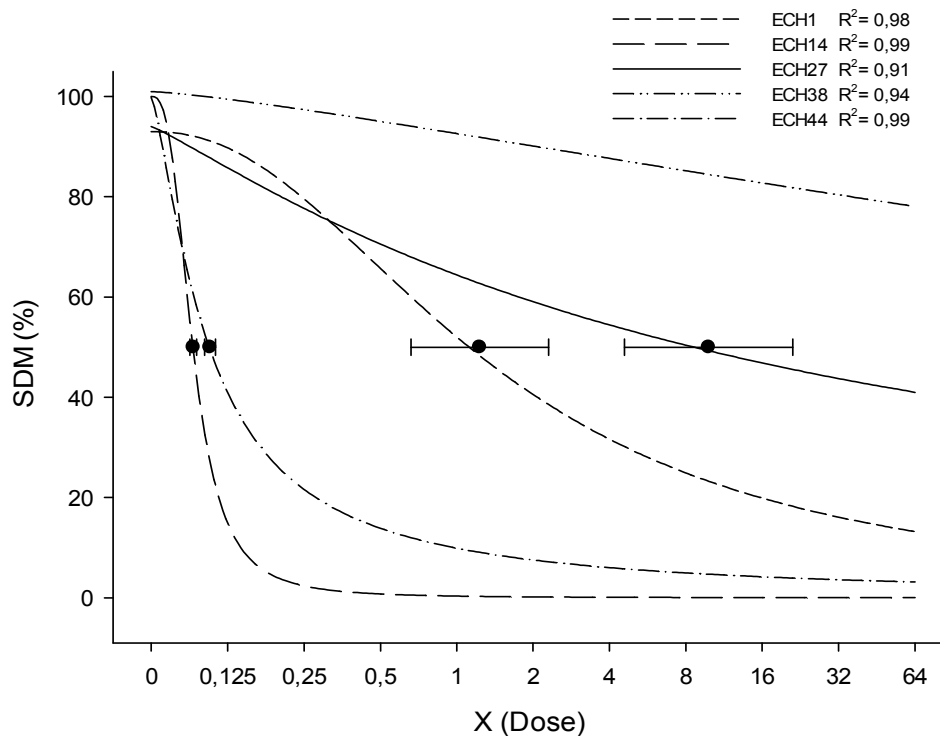
Weeds resistant to ALS enzyme inhibiting herbicides tend to present high resistance levels. According to Han et al. (2012), this situation is common, since the mutation in the ALS enzyme may result in high RF and, therefore, in the need of using higher doses than those used to control susceptible plants.

SDM results demonstrate difference between the accessions for the dose that controls 50% of the population (Figure 3). The highest SDM accumulation was observed for ECH38, which showed a 20% drop only in the application of very high doses of the herbicide imazapyr + imazapic. However, for ECH1, the dose required to reduce 50% of the SDM (GR$_{50}$) was 1.75, and for ECH27 it was 7.96 times the recommended dose. For the susceptible accessions ECH14 and ECH44, the GR$_{50}$ was needed 0.11 and 0.13 of the recommended rate 73.5 + 24.5 g a.e. ha^{-1} (Table 1). With this, it is possible to state that barnyardgrass accessions ECH1, ECH27 and ECH38 from Pelotas, Arroio Grande and Rio Grande, respectively, are resistant to the herbicide imazapyr + imazapic, and the resistant biotype ECH38 shows the higher resistance level.
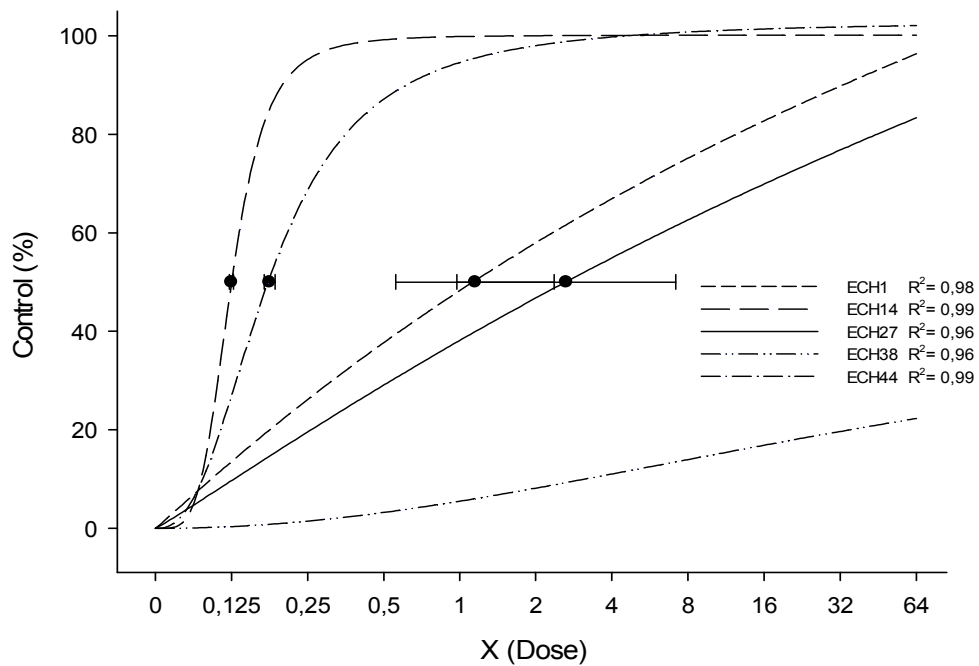
The horizontal bars represent the confidence intervals for the dose causing 50% control over the plant, with 95% significance.

Figure 2 - Control (%) of *Echinochloa crusgalli* var. *mitis* (barnyardgrass) accessions that are resistant (ECH1-Pelotas/Rio Grande do Sul, ECH27-Arroio Grande and ECH38-Rio Grande/Rio Grande do Sul) and susceptible (ECH14-Pelotas/Rio Grande do Sul and ECH44-Rio Grande/Rio Grande do Sul) to the herbicide imazapyr+imazapic, evaluated on day 28 after the application of the treatment (DAA), according to different doses.

The horizontal bars represent the confidence intervals for the dose causing 50% control over the plant, with 95% significance.

Figure 3 - Shoot dry matter (%) of *Echinochloa crusgalli* var. *mitis* (barnyardgrass) accessions that are resistant (ECH1-Pelotas/Rio Grande do Sul, ECH27-Arroio Grande and ECH38-Rio Grande/Rio Grande do Sul) and susceptible (ECH14-Pelotas/Rio Grande do Sul and ECH44-Rio Grande/Rio Grande do Sul) to the herbicide imazapyr+imazapic, evaluated on day 28 after the application of the treatment.

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The fact that it is impossible to calculate C_{50} for ECH38 may be explained by the possible genetic variability in the different populations of barnyardgrass, which is affected by several evolutionary factors, such as the interaction between crop and weed, the gene flow through pollen and seed dispersion, the geographic distribution and the natural selection (Huangfu et al., 2009). Thus, the differences observed between C_{50} and GR_{50} may be due to genetic characteristics among barnyardgrass accessions, possibly influencing their responses to the herbicide. The genetic constitution of a species may determine varying degrees of herbicide resistance or susceptibility (Hartwing et al., 2008).

It was possible to confirm the resistance of barnyardgrass accessions from areas where the herbicide was the main selection agent. As for the losses caused by the resistance of weeds in a certain area, the population of resistant accession plants is enough to limit the production of agricultural cultures, with the need of changes in the used management practices (Moreira et al., 2010). As for the prevention and management of resistance, the most important aspect is the recommendation of practices and production systems in which the selection pressure of plants resistant to a certain herbicide is reduced (Boerboom, 1999).

Alternative chemical control

For this study, a significant interaction between the accession and herbicide factors was observed (Tables 2 and 3). The controlling effect of herbicides varied according to the evaluated accession. In the evaluation performed on day 28 DAA (Table 2), all herbicides satisfactorily controlled susceptible accessions. For the resistant accession ECH1, the alternative herbicides cyhalofop-butyl, profoxydim, clethodim, quinclorac, propanil and glyphosate were effective in controlling, whereas the herbicide quinclorac presented only 4% and 15% of control for the accessions ECH27 and ECH38, respectively. However, ALS inhibiting herbicides, such as imazethapyr + imazapic, imazapyr + imazapic, bispyribac-sodium and penoxsulam, did not control the other resistant accessions. On the other hand, propanil presented a control level below 70% for ECH27 and did not differ significantly from the other treatments.

According to studies conducted by Tranel and Wright (2002), ALS inhibiting herbicides have the characteristic of high effectiveness, when used at commercial doses on sensitive biotypes. Even if these herbicides controlled effectively the susceptible accessions ECH14 and EH44, it is importance not depend on a few or exclusively one or more herbicides belonging to a sole mechanism of action to control and manage barnyardgrass in irrigated rice areas, since resistance can occur quickly for herbicides with other target site (Walsh et al., 2006).

In the SDM evaluation performed on day 28 DAA (Table 3), the susceptible accessions ECH14 and ECH44 showed reduction, since all herbicides controlled these accessions. However, for the resistant accessions ECH27 and ECH38, the production of SDM was practically zero for plants submitted to the application of the registered dose of cyhalofop-butyl, profoxydim, clethodim and glyphosate. However, when ALS inhibitors (imazethapyr + imazapic, imazapyr + imazapic, bispyribac-sodium, penoxsulam) and auxin mimics (quinclorac) were used at the maximum registered doses, there was small SDM reduction, suggesting that for these accessions there is a possible cross-resistance with ALS inhibitors and a multiple resistance with auxin mimics. For the resistant accession ECH1, there was a SDM reduction by 24% for the herbicide imazapyr + imazapic, when compared to the control sample. However, this significant difference did not prevent the development of the plant and the seed production of this accession (data not presented).

Treatments with profoxydim, clethodim, propanil and glyphosate were the most effective, not allowing plants growth thus demonstrating the effectiveness of these herbicides as an alternative control when there is resistance to ALS inhibitors in *Echinochloa crusgalli* accessions. In this study, the accessions ECH1 and ECH14, coming from Pelotas, were controlled by cyhalofop-butyl (955 and 90%, respectively), but in the destructive evaluation on day 28 DAA, there was still dry matter production by the plants.

The results indicate that the application of ALS inhibiting herbicides from three different chemical groups did not satisfactorily control the resistant access ECH1, suggesting that it presents cross resistance, since weeds selected with an ALS inhibitor also presented cross

Table 2 - Control (%) of *Echinochloa crusgalli* var. *mitis* (barnyardgrass) accessions that are resistant (ECH1-Pelotas/Rio Grande do Sul, ECH27-Arroio Grande and ECH38-Rio Grande/Rio Grande do Sul) and susceptible (ECH14-Pelotas/Rio Grande do Sul and ECH44-Rio Grande/Rio Grande do Sul) to the herbicide imazapyr+imazapic with alternative herbicides, evaluated on day 28 after the application of the treatment (DAA)

Treatment	28 DAA				
	ECH1 (R)	ECH14 (S)	ECH27 (R)	ECH38 (R)	ECH44 (S)
Control sample	0.0 cA	0.0 cA	0.0 eA	0.0 eA	0.0 Ae
Imazethapyr+imazapic	0.0 cC	97 aA	10 dB	2.0 eC	95 bcA
Imazapyr+imazapic	5.0 Bc	100 aA	10 dB	5.0 eC	99 abA
Bispyribac-sodium	5.0 bC	100 aA	21 cC	10 cdC	100 aA
Penoxsulam	0.0 cC	92 bA	2.0 eC	12 bcB	95 dA
Cyhalofop-butyl	98 aA	96 aA	100 aA	98 aA	95 cdA
Profoxydim	100 aA	100 aA	100 aA	100 aA	100 aA
Clethodim	100 aA	100 aA	100 aA	100 aA	100 aA
Quinclorac	98 aA	98 aA	4.0 eC	15 bB	98 bcA
Propanil	100 aA	100 aA	68 bB	100 aA	100 aA
Glyphosate	100 aA	100 aA	100 aA	100 aA	100 aA
VC (%)	4.76				

* Averages followed by the same lowercase letter in the column and the same capital letter on the line do not differ significantly by Duncan's test ($p \leq 0.05$).

Table 3 - Shoot dry matter (SDM) (g per plant) on day 28 after the application of treatments on *Echinochloa crusgalli* var. *mitis* (barnyardgrass) accessions that are resistant (ECH1-Pelotas/Rio Grande do Sul, ECH27-Arroio Grande and ECH38-Rio Grande/Rio Grande do Sul) and susceptible (ECH14-Pelotas/Rio Grande do Sul and ECH44-Rio Grande/Rio Grande do Sul) to the herbicide imazapyr+imazapic with alternative herbicides

Treatment	SDM				
	ECH1 (R)	ECH14 (S)	ECH27 (R)	ECH38 (R)	ECH44 (S)
Control sample	2.28 aB	2.21 aBC	2.38 aA	2.23 aC	2.15 aC
Imazethapyr+imazapic	2.17 bA	0.11 bcB	2.22 cA	2.21 aA	0.01 cC
Imazapyr+imazapic	2.12 bA	0.08 cB	2.16 cA	2.18 aA	0.0 cC
Bispyribac-sodium	2.31 aA	0.12 bcC	2.24 cB	2.17 aB	0.01 cD
Penoxsulam	2.02 cC	0.15 bD	2.36 bA	2.15 aB	0.02 bE
Cyhalofop-butyl	0.11 bA	0.13 bA	0.0 cC	0.0 cC	0.05 bB
Profoxydim	0.03 eA	0.04 dA	0.0 eB	0.0 cB	0.0 cB
Clethodim	0.01 eB	0.04 dA	0.0 eB	0.0 cB	0.0 cB
Quinclorac	0.03 eC	0.11 bcC	2.45 aA	1.77 bB	0.04 bC
Propanil	0.01 eC	0.09 cB	0.79 dA	0.0 cC	0.0 cC
Glyphosate	0.01 eB	0.11 bcA	0.01 eB	0.0 cB	0.0 cB
VC (%)	12.1				

* Averages followed by the same lowercase letter in the column and the same capital letter on the line do not differ significantly by Duncan's test ($p \leq 0.05$).

resistance to other compounds with activity from the same enzyme (Primiani et al., 1990; Ribas and Merotto, 1999). Most studies report that herbicide-resistant biotypes from the imidazolinone group, with a mutation at the site of action of the herbicide in the ALS enzyme, demonstrate varying levels of cross-resistance for the chemical groups sulfonylureas and triazolopyrimidines (Walsh et al., 2004). Variations in the cross-resistance level within ALS inhibitors may be possibly due to the differentiated and particular binding of each herbicide to the site of action and the different mutations occurring in the gene encoding the ALS enzyme (Powles and Preston, 2013).

In the case of the ECH27 and ECH38 accessions, the quinclorac treatment was also not effective, since both produced large amounts of SDM. Barnyardgrass resistant to quinclorac were also found in rice fields in Southern Brazil (Andres et al., 2007); since then, cases involving the resistance to this herbicide has increased. For weeds showing resistance to more than one action mechanism, the management in irrigated rice fields becomes more complex, since the herbicide options for chemical control decrease.

Herbicides with alternative mechanism of action then ALS inhibitors, such as ACCase inhibitors (cyhalofop-butyl, profoxydim and clethodim), glyphosate and propanil, are effective and can be used to control imazapyr + imazapic resistant barnyardgrass accessions. However, other ALS inhibiting herbicides, as well as the auxin mimic (quinclorac), are not effective in controlling.

Accessions of *Echinochloa crusgalli* var *mitis* on rice fields in Rio Grande do Sul show resistance to ALS inhibiting herbicides, thus making the correct use of technology necessary, not consecutively cultivating rice in the same areas, as well as adopting a good planning of the herbicides to be used, rotating action mechanisms and avoiding to accelerate the evolution of resistance.

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