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Identification of *Echinochloa* spp. populations resistant and susceptible to acetolactate synthase-inhibiting and auxin-mimic herbicides

Identificação de populações de *Echinochloa* spp. resistentes e suscetíveis a herbicidas inibidores da enzima acetolactato sintase e mimetizadores de auxina

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Abstract

This study evaluated the control of *Echinochloa* spp. collected in four irrigated rice producing regions of Rio Grande do Sul State, Brazil. We identified populations susceptible and resistant to the herbicides quinclorac (auxin mimic), bispyribac-sodium, penoxsulam, and imazethapyr (ALS inhibitors). The experiment was carried out in a greenhouse in a randomized block design with four replicates. Treatments were arranged in a 40x4x6 factorial scheme (40 populations, 4 herbicides, and 6 rates, corresponding to zero, one-half label rate, full label rate, and two, four, and eight times the label rate of each herbicide tested). *Echinochloa* spp. plants were counted and collected 25 days after emergence. Percent damage was analyzed as a function of plant dry mass. The dose-response curves showed different resistances between the populations collected in the different regions. The Central Depression region accounted for a higher number of populations resistant to ALS-inhibiting herbicides. Some *Echinochloa* spp. populations showed multiple resistance to ALS inhibitors and auxin mimics. Thus, the identification of resistant and susceptible populations and their regions of occurrence allows for more environmentally appropriate weed management.

Additional keywords: chemical control; herbicide resistance; weed.

Resumo

O objetivo foi avaliar o controle de *Echinochloa* spp. coletadas em quatro regiões produtoras de arroz irrigado do Rio Grande do Sul, Brasil, para identificar populações suscetíveis e resistentes aos herbicidas quincloraque (mimetizador de auxina) e bispiribaque-sódico, penoxsulam e imazetapir (inibidores de ALS). O experimento foi conduzido em casa de vegetação, em delineamento experimental de blocos ao acaso, com quatro repetições. Os tratamentos foram arranjados em esquema fatorial 40x4x6 (40 populações, 4 herbicidas e 6 doses, correspondentes a zero, meia dose de registro, dose de registro e duas, quatro e oito vezes a dose de registro de cada herbicida testado). As plantas de *Echinochloa* spp. foram contadas e coletadas 25 dias após a emergência. A análise do percentual de dano foi realizada por meio da massa seca das plantas. Analisando-se as curvas dose-resposta, verificou-se que houve diferença entre as populações coletadas nas diferentes regiões, quando analisado o fator de resistência. Na Depressão Central, encontrou-se maior número de populações resistentes aos herbicidas inibidores da enzima ALS. Resistência múltipla aos inibidores de ALS e ao mimetizador de auxina foi observada em algumas populações de *Echinochloa* spp. Assim, a identificação das populações resistentes e suscetíveis e das respectivas regiões de ocorrência permite o manejo de plantas daninhas mais adequado ambientalmente.

Palavras-chave adicionais: controle químico; planta daninha; resistência a herbicida;

Introduction

Rice grass (*Echinochloa* spp.) is considered one of the main weeds in irrigated rice (*Oryza sativa* L.) in southern Brazil (Agostinetto et al., 2010). Belonging to the family Poaceae, the genus *Echinochloa* P. Beauv comprises about 30 to 50 species, being associated mainly with moist soil conditions. In Brazil, species *Echinochloa colona* (L.) Link. is highlighted along with some varieties of *Echinochloa crus-galli* (L.)

Beauv. [*E. crus-galli* var. *crus-galli* (L.) Beauv. and *E. crus-galli* var. *mitis* (Pursh) Peterm], *Echinochloa helodes* (Hackel) Parodi., *Echinochloa crus-pavoris* (Kunth) Hitchc, and *Echinochloa polystachya* (Kunth) Hitchc (Moreira & Bragança, 2010).

The negative effects of rice grass on irrigated rice can be attributed to its high competitive capacity for water, light, nutrients, and CO_2, as well as its high adaptability to different production environments. The presence of weeds in irrigated rice fields has a direct influence on yield and may reduce the total Brazilian production by 70% (Conab, 2016).

Chemical control represents the main weed control method, using pre- and/or postemergence herbicides (Castro, 2011). Postemergence application is the most widely used method, in which 50% of herbicides have acetolactate synthase (ALS) inhibitors as a mechanism of action (Sosbai, 2009). ALS inhibitors were introduced to the world market in 1982 and represent the group of herbicides with the most cases of resistant weeds worldwide (~64%) (Heap, 2017).

The biological activity of an herbicide in susceptible plants occurs as a function of the absorption, translocation, metabolism, and sensitivity of the plant to this herbicide and/or to its produced primary and secondary metabolites. ALS inhibitors act on the first enzyme of the branched-chain amino acid synthesis pathway (valine, leucine, and isoleucine), affecting cell division and DNA synthesis (Duggleby et al., 2008). However, several genotypes of weed species have already acquired resistance to ALS-inhibiting herbicides (Heap, 2015).

Another mechanism of action used to control rice grass are auxin mimics, such as the herbicide quinclorac. This herbicide interferes with RNA polymerase enzyme activity and with the synthesis of nucleic acids and proteins, inducing tissue cell proliferation, causing shoot epinasty, and interrupting phloem activity, preventing the movement of photoassimilates from the leaves to the root system (Ferreira & Cataneo, 2010). After application of these herbicides in sensitive plants, there is a rapid increase in the cellulase enzyme, especially carboxymethylcellulase (CMC), mainly in the roots.

Rio Grande do Sul State has confirmed cases of rice grass biotypes resistant to auxin-mimic and ALS-inhibiting herbicides. Mariot et al. (2010) and Eberhardt et al. (2016) reported the occurrence of rice grass biotypes resistant to the herbicide quinclorac. Menezes & Mariot (2009) found *E. crus-galli* biotypes resistant to herbicides imazethapyr and imazapic, showing problems with the Clearfield® system, which combines a rice variety resistant to imidazolinone herbicides (ALS inhibitors) with the use of these herbicides to control red rice and rice grass.

Resistance is confirmed by dose-response experiments. Biotypes are resistant when the resistance factor is greater than 2.0 (Saari et al., 2018), with resistance factors lower than 2.0 representing natural variability in biotype susceptibility, not characterizing herbicide resistance (Nicolai et al., 2010). Therefore, the identification of herbicide resistance in rice grass can help to quantify the problem and facilitate an efficient weed control program in each producing region of Rio Grande do Sul State.

This study analyzed rice grass populations in Rio Grande do Sul State, as well as the presence and degree of resistance or susceptibility to the herbicides used in the chemical management of weeds in irrigated rice.

Materials and methods

The experiment was conducted during the agricultural years of 2010 and 2011, under controlled conditions, in a greenhouse in Santa Maria city. According to the Köppen classification, the climate of the region is type Cfa, humid subtropical with hot summers and no defined dry season (Heldwein et al., 2009).

Rice grass was sown in pots on October 17, 2010; January 14, 2011; and October 5, 2011, at a depth of 1 cm, measured with a graduated ruler. Fourteen (14) populations were evaluated at each sowing season. The seeds were sown in 0.75-liter polyethylene pots filled with soil and lined with plastic bags to prevent water and herbicide losses at irrigation. After emergence, plants were thinned for uniformity to five plants per pot.

A randomized complete block design with four replicates was used, with treatments arranged in a factorial 40x4x6. Factor A consisted of 40 rice grass populations collected in Rio Grande do Sul State, which were divided into their respective regions of occurrence according to the Instituto Rio-Grandense do Arroz (IRGA) zoning (Table 1 and Figure 1). Factor B corresponded to four herbicides, quinclorac (375 g a.i. ha^{-1}), bispyribac-sodium (50 g a.i. ha^{-1}), penoxsulam (60 g a.i. ha^{-1}), and imazethapyr (106 g a.i. ha^{-1}). Factor C was represented by increasing herbicides rates (zero, one-half label rate, full label rate, and two, four, and eight times the label rate).

The soil used as substrate was collected in Santa Maria-RS, being classified as arenic Eutrophic Hydromorphic Planosol (SiBCS, 2013). The soil belongs to the Vacacaí mapping unit, and had the following characteristics: pH water (1:1) = 5.4; P = 8.2 mg dm^3; K = 107 mg dm^{-3}; Clay = 18%; O.M. = 1.4%; Ca = 2.0 $cmol_c.dm^{-3}$; Mg = 1.75 $cmol_c$ dm^{-3}; Al = 1.1 $cmol_c$ dm^{-3}; and SMP index of 6.6. Soil fertilization was performed according to the soil chemical analysis and following the technical recommendations for irrigated rice cultivation (Sosbai, 2010). After correction, the soil was homogenized with a centrifugal mixer.

Herbicides were applied on October 27, 2010; January 26, 2011; and October 17, 2011, in the postemergence of rice grass plants, when they had two to three leaves. Applications were performed with a CO_2-pressurized backpack sprayer, equipped with a 1.5-meter bar containing four 110.02 nozzles, with a syrup volume of 150 L ha^{-1}.

Table 1 - Identification and localization of rice grass (*Echinochloa* spp.) biotypes collected in irrigated rice producing regions of Rio Grande do Sul state.

Localization Map	Biotype	City	Specie
		Campanha region	
11	BAGÉ 1	Bagé	*E. colona*
11	BAGÉ 2	Bagé	*E. crusgalli* var. crusgalli
9	*S*GABRIEL 1	São Gabriel	*E. crusgalli* var. crusgalli
9	*S*GABRIEL 2	São Gabriel	*E. colona*
10	DPEDRITO 1	Dom Pedrito	*E. crusgalli* var. mitis
10	DPEDRITO 2	Dom Pedrito	*E. crusgalli* var. crusgalli
10	DPEDRITO 3	Dom Pedrito	*E. crusgalli* var. crusgalli
6	LIVRAMENTO 1	Santana do Livramento	*E. crusgalli* var. cruspavonis
6	LIVRAMENTO 2	Santana do Livramento	*E. crusgalli* var. crusgalli
8	CACEQUI 1	Cacequi	*E. colona*
		Depressão Central region	
Localization Map	Biotype	City	Specie
13	STAMARIA 1	Santa Maria	*E. colona*
13	STAMARIA 2	Santa Maria	*E. crusgalli* var. crusgalli
13	STAMARIA 3	Santa Maria	*E. crusgalli* var. mitis
7	ROSÁRIO 1	Rosário do Sul	*E. crusgalli* var. crusgalli
7	ROSÁRIO 2	Rosário do Sul	*E. crusgalli* var. crusgalli
12	SPEDRO 1	São Pedro do Sul	*E. crusgalli* var. crusgalli
14	SSEPÈ 1	São Sepé	*E. colona*
15	CACHO 1	Cachoeira do Sul	*E. crusgalli* var. mitis
15	CACHO 2	Cachoeira do Sul	*E. colona*
15	CACHO 3	Cachoeira do Sul	*E. crusgalli* var. crusgalli
		Fronteira Oeste region	
Localization Map	Biotype	City	Specie
2	ITAQUI 1	Itaqui	*E. crusgalli* var. crusgalli
2	ITAQUI 2	Itaqui	*E. crusgalli* var. cruspavonis
2	ITAQUI 3	Itaqui	*E. colona*
1	URUG 1	Uruguaiana	*E. crusgalli* var. crusgalli
1	URUG 2	Uruguaiana	*E. crusgalli* var. cruspavonis
1	URUG 3	Uruguaiana	*E. crusgalli* var. crusgalli
4	ALEG 1	Alegrete	*E. crusgalli* var. crusgalli
4	ALEG 2	Alegrete	*E. crusgalli* var. mitis
3	SBORJ 1	São Borja	*E. crusgalli* var. crusgalli
5	MVIANA 1	Manoel Viana	*E. crusgalli* var. mitis
		Zona Sul region	
Localization Map	Biotype	City	Specie
19	STAVITP 1	Santa Vitória do Palmar	*E. crusgalli* var. crusgalli
19	STAVITP 2	Santa Vitória do Palmar	*E. crusgalli* var. crusgalli
18	JAGUAR 1	Jaguarão	*E. crusgalli* var. crusgalli
18	JAGUAR 2	Jaguarão	*E. crusgalli* var. cruspavonis
18	JAGUAR 3	Jaguarão	*E. crusgalli* var. crusgalli
16	PEL 1	Pelotas	*E. crusgalli* var. mitis
16	PEL 2	Pelotas	*E. colona*
16	PEL 3	Pelotas	*E. crusgalli* var. crusgalli
17	ARRGRA 1	Arroio Grande	*E. crusgalli* var. crusgalli
17	ARRGRA 2	Arroio Grande	*E. colona*

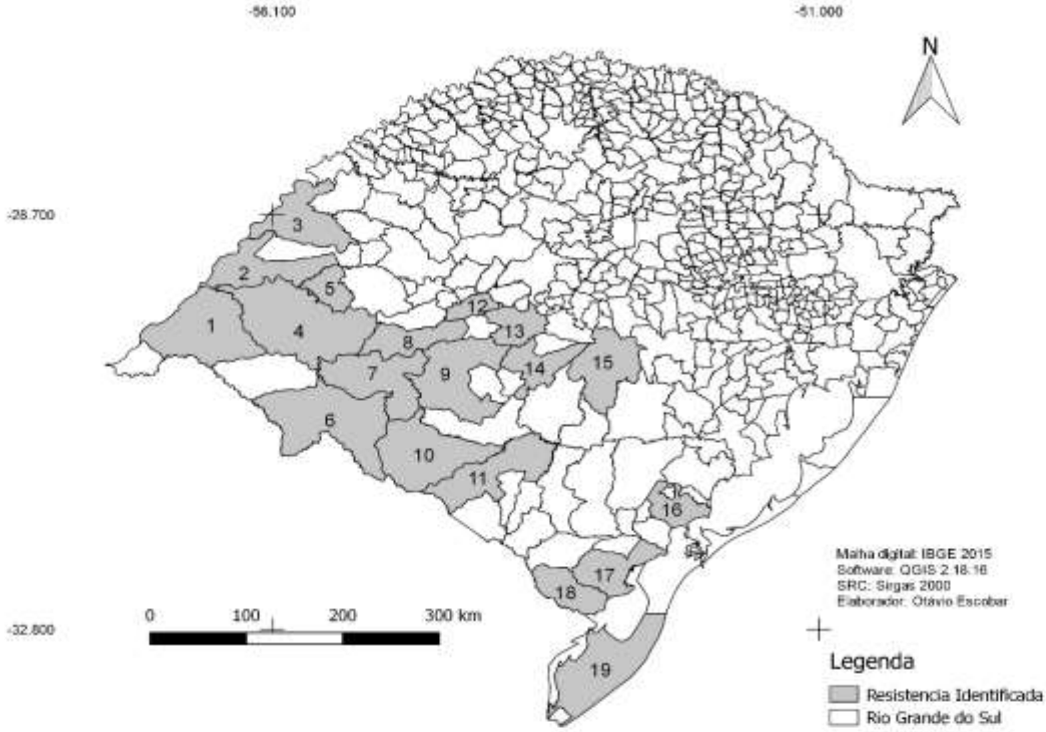

Figure 1 - Infestation map in the state of Rio Grande do Sul.

Applications occurred outside the greenhouse, where the pots remained separated in an open area for 24 hours to avoid contamination between treatments through possible herbicide volatilization. After this period, the pots returned to the greenhouse, receiving irrigation every two days for 30 days. The average temperature and relative humidity during the experiment were 25 °C and 58%, respectively.

Twenty-five days after the application of the treatments, the number of rice grass plants per pot was counted, and plants were collected for dry mass (DM) determination. The samples were oven dried at 60 ºC until constant mass. The dose-response curves considered the dry mass results (DM_{50}) at 20 days after application, when the injuries caused by the treatments were already visible, mainly on the rice grass biotypes susceptible to ALS-inhibiting and auxin-mimic herbicides.

The data obtained were tested for compliance with the model assumptions. All data were submitted to the F test ($P \geq 0.05$) in the analysis of variance to verify interactions between the factors. For significant interactions, the data were fitted to the log-logistic nonlinear regression model, using the model proposed by Seefeldt et al. (1995).

$$y = \frac{a}{\left[1 + \left(\frac{x}{b}\right)^{c}\right]}$$

wherein: y = dry mass at 20 DAA; x = herbicide rate; and a, b, and c = curve parameters, so that a is the difference between the maximum and minimum point of the curve, b is the rate that provides 50% of variable response, and c is the slope of the curve.

The required parameters in the equation were obtained by plotting dry mass (DM) data from treated plants against the dry mass of control plants. The graphs were obtained from a basic data matrix analyzed in the SigmaPlot® program (version 11).

From DM_{50} values, the resistance factor (RF) was obtained for each combination between populations with suspected resistance and susceptible populations. The resistance factor is the result of the ratio between the DM_{50} of the resistant biotype (DM_{50} R) and the DM_{50} of the susceptible biotype (DM_{50} S) (RF = DM_{50} R / DM_{50} S), and expresses the number of times in which the dose required to control the resistant population (R) is greater than dose that controls 50% of the susceptible population (S).

Results

For the control variable of rice grass, the effect of ALS-inhibiting herbicides occurred between 10 and 15 days after the application of the treatments. Visual symptoms in the plants were leaf chlorosis followed by leaf necrosis and plant death, most noticeable mainly at higher herbicide rates. Herbicides and biotypes differed ($P < 0.05$) for plant dry mass (DM_{50}) (Table 2).

Table 2 - Doses of quincloraque, bispiribaque-sodium, penoxsulam and imazetapyr, in g a.i. ha^{-1}, that cause 50% reduction of plant dry mass (DM_{50}) as a function of the studied populations of rice grass (*Echinochloa* spp.) and respective confidence intervals. at 95% probability.

Biotype	Quincloraque	Bispiribaque-sodium	Penoxsulam	Imazetapyr
Campanha region				
	DM_{50} (g a.i. ha^{-1})			
BAGÉ 1	143.12±18.43	21.75±9.46	19.06±1.76	39.13±6.76
BAGÉ 2	156.20±11.72	24.11±13.14	27.21±2.41	48.47±4.94
SGABRIEL 1	120.90±17.31	22.51±8.22	30.37±4.55	61.47±6.62
SGABRIEL 2	175.90±12.91	132.60±15.38	199.11±22.91	602.81±51.87
DPEDRITO 1	513.00±56.76	231.47±25.70	230.22±27.78	589.00±39.26
DPEDRITO 2	166.90±13.65	19.69±2.00	24.31±3.56	50.43±2.67
DPEDRITO 3	154.20±12.29	83.59±7.44	285.54±19.55	372.25±35.39
LIVRAMENTO 1	85.42±23.51	21.61±3.97	22.85±3.75	47.93±4.37
LIVRAMENTO 2	178.60±12.04	16.29±1.92	24.63±2.44	45.31±4.47
CACEQUI 1	153.70±8.60	20.85±1.68	24.66±1.80	43.43±2.43
Depressão Central region				
Biotype	Quincloraque	Bispiribaque- sodium	Penoxsulam	Imazetapyr
	DM_{50} (g a.i ha^{-1})			
STAMARIA 1	166.31±11.06	120.86±14.58	257.92±24.99	582.83±52.20
STAMARIA 2	190.88±11.98	242.34±22.98	224.62±20.13	537.18±41.47
STAMARIA 3	180.67±14.99	24.32±1.97	25.07±3.31	63.36±5.53
ROSÁRIO 1	215.11±16.91	30.15±3.13	24.24±3.72	593.73±51.28
ROSÁRIO 2	259.26±29.55	241.15±22.96	179.69±19.62	582.47±44.02
SPEDRO 1	185.20±12.26	28.21±3.65	30.00±4.87	59.37±3.07
SSEPÈ 1	245.86±20.62	27.35±3.40	24.54±2.68	325.66±34.84
CACHO 1	1823.65±160.37	229.61±17.01	249.41±12.27	534.09±70.90
CACHO 2	251.95±25.55	19.90±1.86	30.38±3.16	49.84±5.57
CACHO 3	205.46±22.23	31.76±2.73	30.94±2.96	56.85±4.39
Fronteira-Oeste region				
Biotype	Quincloraque	Bispiribaque- sodium	Penoxsulam	Imazetapyr
	DM_{50} (g a.i ha^{-1})			
ITAQUI 1	189.29±16.90	40.24±2.01	42.12±4.07	60.28±8.27
ITAQUI 2	246.92±15.31	42.55±3.58	25.65±1.27	56.88±5.58
ITAQUI 3	269.55±14.34	25.71±1.72	29.74±2.14	54.16±4.62
URUG 1	223.76±13.14	24.72±2.46	25.18±1.44	64.61±4.46
URUG 2	305.77±27.50	40.30±3.76	25.97±1.42	57.54±3.26
URUG 3	235.13±18.64	32.67±2.66	22.82±3.21	45.72±4.04
ALEG 1	241.76±14.12	29.64±2.25	35.58±3.61	70.54±4.16
ALEG 2	696.22±77.38	128.74±14.00	157.75±14.49	256.03±37.81
SBORJ 1	260.87±17.85	35.54±3.52	32.87±2.63	65.27±6.03
MVIANA 1	825.31±71.59	26.15±2.31	29.15±3.57	75.90±7.51
Zona Sul region				
Biotype	Quincloraque	Bispiribaque- sodium	Penoxsulam	Imazetapyr
	DM_{50} (g a.i ha^{-1})			
STAVITP 1	173.66±14.18	27.16±2.50	46.99±6.06	50.77±4.70
STAVITP 2	310.50±26.99	25.71±1.27	34.56±3.56	47.82±5.36
JAGUAR 1	1221.92±179.72	28.66±2.11	145.27±16.77	434.01±52.16
JAGUAR 2	249.97±10.74	40.04±3.73	28.97±2.42	56.60±5.48
JAGUAR 3	247.64±28.49	23.75±1.93	35.88±1.65	55.20±2.14
PEL 1	817.69±120.39	158.66±15.38	133.57±17.26	435.15±85.21
PEL 2	165.20±11.05	27.26±2.11	32.35±3.08	56.51±3.37
PEL 3	225.80±16.83	150.47±22.33	114.05±13.91	252.36±44.69
ARRGRA 1	176.33±9.35	24.48±2.16	25.80±2.10	262.84±33.71
ARRGRA 2	206.35±17.06	46.06±3.88	27.90±2.92	56.48±3.93

Herbicides quinclorac and bispyribac-sodium affected rice grass biotypes collected in the Campaign region of Rio Grande do Sul State. DPEDRITO 1 was the only population to show resistance to the herbicide quinclorac, in which the rate of 513 g a.i. ha^{-1} reduced 50% of the total dry mass of the evaluated plants. Population DPEDRITO 1 showed multiple and cross-resistance, since it was also resistant to ALS-inhibiting herbicides: bispyribac-sodium (DM_{50} rate = 231.47 g a.i. ha^{-1}), penoxsulam (DM_{50} rate = 230.22 g a.i. ha^{-1}), and imazethapyr (DM_{50} rate = 590.0 g a.i. ha^{-1}).

Similar results confirming resistance to the herbicide quinclorac were obtained with the following populations: CACH 1 (Central Depression), DM_{50} rate= = 1823.65 g a.i. ha^{-1}; MVIANA 1 (West Frontier), DM_{50} rate = 825.31 g a.i. ha^{-1}; JAGUA 1 and PEL 1 (South region), DM_{50} rate = 1221.92 g a.i. ha^{-1} and 817.69 g a.i. ha^{-1}, respectively. Populations CACH 1, PEL 1, and MVIANA 1 were identified as *E. crusgalli* var. *mitis*, and JAGUA 1 as *E. crus-galli* var. *crusgalli*. Thus, resistance to the herbicide quinclorac persists among rice grass populations in Rio Grande do Sul State, occurring in all irrigated rice producing regions of the state and in different weed species.

Populations SGABRIEL 2 and DPEDRITO 3, in the Campaign region (Table 1), showed resistance to two mechanisms of action: auxin mimics (quinclorac) and ALS inhibitors (bispyribac-sodium, penoxsulam, and imazethapyr). Therefore, they also present multiple and cross-resistance simultaneously.

Biotypes from the Campaign region (BAGÉ 1, BAGÉ 2, SGABRIEL 1, SGABRIEL 2, DPEDRITO 2, DPEDRITO 3, LIVRAMENTO 1, LIVRAMENTO 2, and CACEQUI 1); Central Depression (STAMARIA 1, STAMARIA 3, ROSÁRIO 1, SPEDRO 1 , SSEPÉ 1, CACHO 2, and CACHO 3); South region (STAVITP 1, STAVITP 2, JAGUAR 2, JAGUAR 3, PEL 2, ARRGRA, 1 and ARRGRA 2); and West Frontier (ITAQUI 1, ITAQUI 2, ITAQUI 3, URUG 1, URUG 2, URUG 3, ALEG 1, and SBORJ 1) were susceptible to the herbicide quinclorac, and the active ingredient rate to reduce 50% of plant dry mass was below 50% of the labeled herbicide rate for rice grass control. These populations were also found to be susceptible to ALS-inhibiting herbicides (bispyribac-sodium and penoxsulam at usual levels recorded for these active ingredients).

Moreover, populations STAMARIA 2, ROSÁRIO 2, and CACHO 1 showed resistance to the herbicide bispyribac-sodium, with DM_{50} values of 242.34 g a.i. ha^{-1}, 241.15 g a.i. ha^{-1}, and 229.61 g a.i. ha^{-1}, respectively, tolerating up to four times the label rate of this herbicide for irrigated rice. These populations were also found to be resistant to herbicides penoxsulam and imazethapyr, characterizing multiple resistance to ALS-inhibiting herbicides.

There were more populations resistant to the herbicide imazethapyr than to the other ALS-inhibiting herbicides (bispyribac-sodium and penoxsulam). These results were observed for populations STAMARIA 1, STAMARIA 2, ROSÁRIO 1, ROSÁRIO 2, SSEPÉ 1, and CACHO 1, requiring DM_{50} rates of 582.83 g a.i. ha^{-1}, 537.18 g a.i. ha^{-1}, 593.73 g a.i. ha^{-1}, 582.47 g a.i. ha^{-1}, 325.66 g a.i. ha^{-1}, and 534.09 g a.i. ha^{-1}, respectively, values that can be up to 5 times the label rate of imazethapyr for irrigated rice (106 g a.i. ha^{-1}).

Table 2 also shows that population ALEG 2 showed multiple resistance to auxin-mimic and ALS-inhibiting herbicides, with DM_{50} values of 696.22 g a.i. ha^{-1}, 128.74 g a.i. ha^{-1}, 157.75 g a.i. ha^{-1}, and 256.03 g a.i. ha^{-1}, respectively, for herbicides quinclorac, bispyribac-sodium, penoxsulam, and imazethapyr.

Populations JAGUAR 1 and PEL 1 showed resistance to the herbicide quinclorac, with DM_{50} values of 1221.92 g a.i. ha^{-1} and 817.69 g a.i. ha^{-1}, respectively. Populations STAVITP 1, STAVITP 2, JAGUAR 2, JAGUAR 3, PEL 2, ARRGRA 1, and ARRGRA 2 were susceptible to the herbicide quinclorac, with DM_{50} values lower than the label rate for irrigated rice (375.0 g a.i. ha^{-1}). Populations PEL 1 and PEL 3 showed resistance to the herbicide bispyribac-sodium, with DM_{50} values of 158.66 g a.i. ha^{-1} and 150.47 g a.i. ha^{-1}, respectively. The results allow inferring that populations STAVITP 1, STAVITP 2, JAGUAR 2, JAGUAR 3, PEL 2, ARRGRA 1, and ARRGRA 2 were susceptible to the herbicide penoxsulam, with DM_{50} values below 60 grams of active ingredient per hectare.

It is noteworthy that in the South region of Rio Grande do Sul State, population JAGUAR 1 showed resistance to herbicides penoxsulam and imazethapyr, a fact not observed for the herbicide bispyribac-sodium. Populations JAGUAR 1, PEL 1, PEL 3, and ARRGRA 1 showed resistance to the herbicide imazethapyr, with DM_{50} values of 434.01 g a.i. ha^{-1}, 435.15 g a.i. ha^{-1}, 252.36 g a.i. ha^{-1}, and 262.84 g a.i. ha^{-1}, respectively. Populations PEL 1 and PEL 3 showed cross-resistance to ALS-inhibiting herbicides (bispyribac-sodium, penoxsulam, and imazethapyr). Population PEL 1 showed multiple resistance to ALS-inhibiting and auxin-mimic herbicides.

To calculate the resistance factor (Tables 3 and 4), we considered the susceptible populations LIVRAMENTO 1, LIVRAMENTO 2, BAGÉ 1, and BAGÉ 2, which showed lower DM_{50} for herbicides quinclorac, bispyribac-sodium, penoxsulam, and imazethapyr, respectively. For quinclorac, only BAGÉ 1, BAGÉ 2, SGABRIEL 1, DPEDRITO 2, DPEDRITO 3, LIVRAMENTO 1, CACEQUI 1, STAMARIA 1, and PEL 2 were considered susceptible, since they presented RF values lower than 2.0. Populations SGABRIEL 2, DPEDRITO 1, DPEDRITO 3, STAMARIA1, STAMARIA 2, ROSÁRIO 2, CACHO 2, ITAQUI 1, ITAQUI 2, URUG 2, URUG 3, ALEG 2, SBORJ 1, JAGUAR 2, PEL 1, PEL 3, and ARRGRA 2 showed resistance (RF greater than 2.0) to bispyribac-sodium. Populations SGABRIEL 2, DPEDRITO 1, DPEDRITO 3, STAMARIA 1, STAMARIA 2, ROSÁRIO 2, CACHO 1, ITAQUI 1, ALEG 2, STAVITO 1, JAGUAR 1, PEL 1, and PEL 3 showed resistance (FR above 2.0) to penoxsulam. Populations SGABRIEL 2, DPEDRITO 1, DPEDRITO 3, STAMARIA 1, STAMARIA 2, ROSÁRIO 1, ROSÁRIO 2, SSEPÉ1, CACHO 1, ALEG 2, JAGUAR 1, PEL 3, and ARRGRA 1 showed resistance (FR above 2.0) to imazethapyr.

Discussion

The dose-response curves for the rice grass populations evaluated showed different responses to herbicide application among populations from different regions of Rio Grande do Sul State. The Central Depression region showed a higher number of biotypes resistant to ALS-inhibiting herbicides. These results are associated with the type of management adopted by irrigated rice producers in this region and the high red rice infestation in these areas (Dornelles et al., 2010). In this way, rice crops are succeeded without adopting red rice control programs including rotation of areas and herbicides with different mechanisms of action. Therefore, weed selection pressure on Clearfield® crops is inevitable through continuous and intensive use of the herbicide imazethapyr. This leads to the selection of resistant populations of both red rice, as observed by Kuk et al. (2008), and grass rice, as verified in the present study.

The use of herbicides bispyribac-sodium and penoxsulam in areas with cultivation of conventional varieties may also have provided the selection of rice grass populations resistant to these herbicides. The Central Depression region of Rio Grande do Sul State is characterized by intensive use of the Clearfield® system, thus enabling the emergence of large numbers of rice grass populations resistant to ALS-inhibiting herbicides. In addition, these populations may also show cross-resistance between these herbicides. Vidal et al. (1997) verified cross-resistance of rice grass (*E. colona*) biotypes collected at the West Frontier of Rio Grande do Sul State when submitted to the application of different ALS-inhibiting herbicides, corroborating the results obtained in different regions of the state.

Quinclorac-resistant rice grass biotypes were found in all evaluated regions of Rio Grande do Sul State, mainly of species *E. crusgalli* var. *mitis*, with one case being identified as *E. crusgalli* var. *crusgalli*. These results show that there are still populations of species *E. crusgalli* var. *mitis* resistant to the herbicide quinclorac in the state, despite the reduced use of this herbicide over the last years. This species was identified as resistant to this herbicide in the 1990s (Andres, 2007), having been identified in five populations from different regions of the state. Mariot et al. (2010) reported the occurrence of quinclorac-resistant rice grass biotypes in rice-growing regions of the States of Rio Grande do Sul and Santa Catarina.

There were fewer rice grass populations resistant to quinclorac compared to ALS-inhibiting herbicides. These results may have been influenced by the reduced use of the herbicide quinclorac from the 2000s onwards with the emergence of resistance to this active ingredient and the evolution of the use of ALS-inhibiting herbicides from this decade. This allowed a control of the seed bank of these quinclorac-resistant rice grass biotypes, mainly of species *E. crusgalli* var. *mitis*, which is still found, as shown in this study. The results of multiple resistance found in rice grass populations resistant to auxin-mimic and ALS-inhibiting herbicides may be related to gene flow among rice grass species (Mariot et al., 2010; Eberhardt et al., 2016). Dornelles et al. (2010) highlight the gene flow between red rice biotypes and Clearfield® cultivars in Rio Grande do Sul State, leading to the emergence of red rice plants resistant to herbicides belonging to the imidazolinone chemical group.

The frequent use of the herbicide imazethapyr to control red rice has led to selection pressure on its populations, which may have influenced rice grass populations, as evidenced when analyzing the results of this study in different regions of Rio Grande do Sul State. This statement corroborates the results obtained by Matzenbacher (2012) with rice grass populations in southern Brazil, in which resistance to imazethapyr was confirmed in five populations. Moreover, it is found that there are more populations of rice grass resistant to this herbicide than to bispyribac-sodium and penoxsulam. Christoffoleti (2002) describes that there may be levels of tolerance to herbicides with the same mechanism of action due to differences in metabolization and translocation between the different chemical groups of ALS-inhibiting herbicides, as observed when analyzing the results for the herbicide imazethapyr in comparison with other ALS-inhibiting herbicides (bispyribac-sodium and penoxsulam).

Conclusions

There is evidence that the chemical management used for weed control in irrigated rice has directly influenced the selection of herbicide-resistant rice grass populations. The degree of resistance of rice grass biotypes may be related to the intensive use of a given herbicide in a region of Rio Grande do Sul State. This is evidenced with the highest number of cases of resistance to ALS-inhibiting herbicides being found in regions where weed management programs including the herbicide imazethapyr were the most adopted, as in the Central Depression. In this region, the practice of rice monoculture may have influenced this process.

The present study found multiple resistance of rice grass populations to auxin-mimic and ALS-inhibiting herbicides in Rio Grande do Sul State. This may be a result of the crossing between quinclorac-resistant rice grass species, such as observed in populations of *E. crusgalli* var. *mitis* that are still found in the state, and populations (including several species) resistant to ALS-inhibiting herbicides, of recent occurrence.

Further studies are needed to investigate the influence of crossings between rice grass species, the problem of increased resistance to ALS-inhibiting herbicides, and the maintenance of populations with auxin-mimic resistant biotypes.

Table 3 - Resistance factor of studied populations of rice grass (*Echinochloa* spp.) to the herbicides quincloraque, bispiribaque-sodium, penoxsulam and imazetapyr, in different regions of Rio Grande do Sul state.

Campanha region					Depressão Central region				
Biotypes	Quincloraque	Bispiribaque-sodium	Penoxsulam	Imazetapyr	Biotypes	Quincloraque	Bispiribaque-sodium	Penoxsulam	Imazetapyr
	Resistence factor					Resistence factor			
BAGÉ 1	1.68	1.34	1.00	1.00	STAMARIA 1	1.95	7.42	13.53	14.89
BAGÉ 2	1.83	1.48	1.43	1.24	STAMARIA 2	2.23	14.88	11.78	13.73
*S*GABRIEL 1	1.42	1.38	1.59	1.57	STAMARIA 3	2.12	1.49	1.32	1.62
*S*GABRIEL 2	2.06	8.14	10.45	15.41	ROSÁRIO 1	2.52	1.85	1.27	15.17
DPEDRITO 1	6.01	14.21	12.08	15.05	ROSÁRIO 2	3.04	14.80	9.43	14.89
DPEDRITO 2	1.95	1.21	1.28	1.29	SPEDRO 1	2.17	1.73	1.57	1.52
DPEDRITO 3	1.81	5.13	14.98	9.51	SSEPÈ 1	2.88	1.68	1.29	8.32
LIVRAMENTO 1	1.00	1.33	1.20	1.22	CACHO 1	21.35	14.10	13.09	13.65
LIVRAMENTO 2	2.09	1.00	1.29	1.16	CACHO 2	2.95	1.22	1.59	1.27
CACEQUI 1	1.80	1.28	1.29	1.11	CACHO 3	2.41	1.95	1.62	1.45

Table 4 - Resistance factor of studied populations of rice grass (*Echinochloa* spp.) to the herbicides quincloraque, bispiribaque-sodium, penoxsulam and imazetapyr, in different regions of Rio Grande do Sul state.

Fronteira-Oeste region					Zona Sul region				
Biotypes	Quincloraque	Bispiribaque-sodium	Penoxsulam	Imazetapyr	Biotypes	Quincloraque	Bispiribaque-sodium	Penoxsulam	Imazetapyr
	Resistence factor					Resistence factor			
ITAQUI 1	2.22	2.47	2.21	1.54	STAVITP 1	2.03	1.67	2.47	1.30
ITAQUI 2	2.89	2.61	1.35	1.45	STAVITP 2	3.63	1.58	1.81	1.22
ITAQUI 3	3.16	1.58	1.56	1.38	JAGUAR 1	14.30	1.76	7.62	11.09
URUG 1	2.62	1.52	1.32	1.65	JAGUAR 2	2.93	2.46	1.52	1.45
URUG 2	3.58	2.47	1.36	1.47	JAGUAR 3	2.90	1.46	1.88	1.41
URUG 3	2.75	2.01	1.20	1.17	PEL 1	9.57	9.74	7.01	11.12
ALEG 1	2.83	1.82	1.87	1.80	PEL 2	1.93	1.67	1.70	1.44
ALEG 2	8.15	7.90	8.28	6.54	PEL 3	2.64	9.24	5.98	6.45
SBORJ 1	3.05	2.18	1.72	1.67	ARRGRA 1	2.06	1.50	1.35	6.72
MVIANA 1	9.66	1.61	1.53	1.94	ARRGRA 2	2.42	2.83	1.46	1.44

References

Andres A, Concenço G, Melo, PTBS, Schmidt M, Resende RG (2007) Detecção da resistência de capim-arroz (*Echinochloa* sp.) ao herbicida quinclorac em regiões orizícolas do sul do Brasil. Planta Daninha 25(1): 221-226. Doi: 10.1590/S0100-83582007000100025

Agostinetto D, Galon L, Silva, JMBV, Tironi SP, Andres A (2010) Interferência e nível de dano econômico de capim-arroz sobre o arroz em função do arranjo de plantas da cultura. Planta Daninha 28:993-1003. Doi: 10.1590/S0100-83582010000500007.

Castro GSA (2011) Sistemas de produção de grãos e incidência de plantas daninhas. Planta Daninha, 29:1001-1010. Doi: 10.5935/1806-6690.20160060.

Christoffoleti P.J. (2002) Curvas de dose-resposta de biótipos resistente e suscetível de *Bidens pilosa* L. aos herbicidas inibidores da ALS. Scientia Agricola 59 (3):513-519. Doi: 10.1590/S0103-90162002000300016

Companhia nacional de abastecimento - Conab (2016) A cultura do arroz.

Duggleby RG, McCourt JA, Guddat LW (2008) Structure and mechanism of inhibition of plant acetohydroxyacid synthase. Plant Physiology and Biochemistry 46(3):309-324. Doi: 10.1016/j.plaphy.2007.12.004

Dornelles SHB, Garcia DC, Loreto EL, Canto-dorow TS, Manfron PA, Sanchotene DM, Oliveira LFV (2010) Suscetibilidade de biótipos de arroz-vermelho e de cultivares de Arroz Irrigado ao herbicida Imazetaphyr. Planta Daninha 28:1097-1106. Doi: S0100-83582010000500017.

Eberhardt DS, Oliveira Neto AM, Noldin JA, Vanti RM (2016) Barnyardgrass with multiple resistance to synthetic auxin, ALS and ACCase inhibitors. Planta Daninha 34(4):823-832. Doi: S0100-83582016000400823.

Ferreira LC, Cataneo AC (2010) Aspectos bioquímicos da ação “de safeners”. Boletim informativo da SBCPD 8(2):5-6. Doi: 10.13140/RG.2.1.1911.7846.

Heap I (2015) International survey of herbicide resistant weeds. Disponível em: <http://www.weed science.org>. Acesso em: 30 ago. 2018. Doi: S0100-83582008000100011.

Heap I (2017) The International Survey of Herbicide Resistant Weeds. Disponível em: <www.weedscience.org>. Acesso em 09 de julho de 2018.

Heldwein AB, Buriol GA, Streck NA (2009) O clima de Santa Maria. Ciência e Ambiente 38:43-58.

Kuk YI, Burgos NR, Shivrain VK (2008) Natural tolerance to imazethapyr in red rice (*Oryza sativa*). Weed Science 56(1): 1-11. Doi: 10.1614/WS-07-073.1

Mariot CHP, Menezes VG, Souza PA (2010) Resistência múltipla e cruzada de capim-arroz a herbicidas na cultura do arroz irrigado no Rio Grande do Sul. In: Resumos do 27º Congresso Brasileiro da Ciência das Plantas Daninhas. Londrina, PR: Sociedade Brasileira da Ciência das Plantas Daninhas 1-26.

Matzenbacher F (2012) Caracterização e controle de capim-arroz (*Echinochloa crusgalli*) resistente aos herbicidas do grupo das imidazolinonas e quinclorac em arroz irrigado. 212 f. Dissertação (Mestrado em Agronomia) - Faculdade de Agronomia, Universidade Federal do Rio Grande Do Sul.

Menezes VG, Mariot CHP, Kalsing A, Goulart ICGR (2009) Red rice (*Oryza sativa*) resistant to the herbicides imidazolinones. Planta Daninha, 27: 1047-1052. Doi: 10.1590/S0100-83582009000500018

Moreira HDC, Bragança HBN (2010) Manual de identificação de plantas infestantes: cultivos de verão. Campinas: FMC, 266.

Nicolai M, Melo MSC, López-Ovejero RF, Christoffoleti PJ (2010) Monitoramento de infestações de populações de capim-amargoso (*Digitaria insularis*) suspeitas de resistência ao glifosato. In: Congresso Brasileiro da Ciência das Plantas Daninhas 27:943-946.

Saari LL, Cotterman JC, Thill DC (2018) Resistance to acetolactate synthase inhibiting herbicides. In: Herbicide resistance in plants 83-140.

Seefeldt SS, Jensen JE, Fuerst EP (1995) Log-logistic analysis of herbicide dose-response relationships. Weed technology 9(2): 218-227.

SiBCS - Sistema Brasileiro de Classificação de Solos (2013). Brasília: Embrapa, 2013. 353 p.

SOSBAI - Sociedade Sul-Brasileira De Arroz Irrigado (2009) Reunião Técnica da Cultura do Arroz Irrigado. Arroz irrigado: recomendações técnicas da pesquisa para o Sul do Brasil. Bento Gonçalves; Pelotas; 2009

SOSBAI - Sociedade Sul-Brasileira De Arroz Irrigado (2010). Reunião Técnica da Cultura do Arroz Irrigado. Arroz irrigado: recomendações técnicas da pesquisa para o Sul do Brasil. Bento Gonçalves; Porto Alegre 2010.

Vidal RA (1997) Herbicidas: mecanismos de ação e resistência de plantas. RA Vidal. 165.