

## BARNYARDGRASS WITH MULTIPLE RESISTANCE TO SYNTHETIC AUXIN, ALS AND ACCASE INHIBITORS<sup>1</sup>

*Capim-Arroz com Resistência Múltipla a Auxina Sintética, Inibidores de ALS e ACCase*

EBERHARDT, D.S.<sup>2</sup>, OLIVEIRA NETO, A.M.<sup>3</sup>, NOLDIN, J.A.<sup>2</sup>, and VANTI, R.M.<sup>4</sup>

**ABSTRACT** - The objective of this research was to confirm the resistance of a barnyardgrass biotype (*Echinochloa crus-galli*) to herbicides quinclorac (synthetic auxin), penoxsulam (ALS inhibitor) and cyhalofop-butyl (ACCase inhibitor). Two experiments were carried out in a greenhouse located in Itajaí, State of Santa Catarina, Brazil. One experiment evaluating the biotype ECH 18 (known as susceptible) and one with the ECH 141 biotype (suspected to be multiple resistant). The experimental design used was completely randomized, in a 4 x 7 factorial arrangement with four replications. The first factor was the herbicide: cyhalofop-butyl (Clincher®), penoxsulam (Ricer®), quinclorac (Facet®) and propanil (Grassaid®) and the second factor consisting of seven rates of each herbicide (0.00; 0.25; 0.50; 1; 2; 4 and 8 times the label rate). The evaluations of percentage of control, plant density per pot and dry mass of shoots were performed 35 days after treatments. The rates to control 50% and 80% and the dose to provide 50% and 80% of the weed biomass accumulation and the resistance factor were estimated based on data analysis. The initial hypothesis was confirmed; the biotype ECH 141 presented multiple resistance (synthetic auxin, ALS and ACCase inhibitor). However, the herbicide propanil controlled biotype ECH 141 adequate.

**Keywords:** Paddy rice, *Echinochloa crus-galli*, chemical control, herbicide resistance.

**RESUMO** - Este estudo teve como objetivo confirmar a resistência múltipla de um biótipo de capim-arroz (*Echinochloa crus-galli*) aos herbicidas auxínicos (quinclorac), inibidores da ALS (penoxsulam) e ACCase (cyhalofop-butyl). Foram conduzidos dois experimentos em casa de vegetação localizada no município de Itajaí/SC: um com o biótipo ECH 18 (sabidamente suscetível) e outro com o biótipo ECH 141 (com suspeita de resistência múltipla). O delineamento experimental utilizado foi o inteiramente casualizado (DIC), organizado em arranjo fatorial 4 x 7, com quatro repetições. O primeiro fator avaliado foram os herbicidas: cyhalofop-butyl, penoxsulam, quinclorac e propanil (Clincher®, Ricer®, Facet® e Grassaid®, respectivamente). O segundo fator consistiu de sete doses de cada herbicida (0,00, 0,25, 0,50, 1,00, 2,00, 4,00 e 8,00 vezes a dose de registro). Aos 35 dias após a aplicação dos tratamentos, foram realizadas as avaliações de porcentagem de controle, densidade de plantas por vaso e massa seca da parte aérea. Após a análise dos dados, estimou-se a dose para proporcionar controle de 50% e 80%, a dose para proporcionar redução de 50% e 80% no acúmulo de biomassa e o fator de resistência. A hipótese inicial foi confirmada, pois o biótipo ECH 141 apresentou resistência múltipla aos herbicidas quinclorac (auxina sintética), penoxsulam (inibidor da ALS) e cyhalofop-butyl (inibidor de ACCase). Contudo, o herbicida propanil o controlou com eficiência.

**Palavras-chave:** arroz irrigado, controle químico, *Echinochloa crus-galli*, resistência a herbicidas.

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<sup>2</sup> Epagri, Estação Experimental de Itajaí-SC, Brazil, <noldin@epagri.sc.gov.br> e <dsavioe@gmail.com>; <sup>3</sup> Universidade Federal de Pelotas, Pelotas-RS; <sup>4</sup> Agroas Agronomia Ltda. Tubarão-SC, Brazil.



## INTRODUCTION

The genus *Echinochloa* (Poaceae) contains several species infesting rice crops in southern of Brazil (Andres & Machado, 2004), among which *Echinochloa crus-galli* (barnyardgrass), native to Europe and Asia, highlights as the main specie (Talbert & Burgos, 2007). The occurrence of in-field natural hybrids results in a high level of polymorphism through the genus, leading botanists to use the term “*Echinochloa complex*” to encompass these species (Kissmann & Groth, 1997). Concerning to the field management, barnyardgrass species present different sensitivity levels to herbicides in general, and the most sensitive species are the *E. crus-galli*, *E. phyllopogon* and *E. oryzoides* (Damalas et al., 2008).

Resistant plants have inherited ability to survive and reproduce after exposure to a dose of herbicide normally lethal to the natural population (Gazziero et al., 2008; Inoue & Oliveira Jr, 2011). Weed biotypes with multiple resistance, i.e., resistant to herbicides with different mechanisms of action, have greatly hindered weed control (Moura et al., 2015), constituting a major problem faced by farmers in paddy rice fields.

Selection of resistant biotypes is common in weeds population due to the wide genetic variability, which allows the adaptation and survival in different environmental conditions (Christoffoleti, 2008). Two main factors further aggravate the selection pressure in Santa Catarina rice fields: a) lack of crop rotation; and, b) repeated use of herbicides with the same mechanism of action. This situation became worse in recent years, especially because the selection pressure is on populations that are already resistant to some herbicides. Five resistant species have been already identified, two with multiple resistance (Heap, 2015).

The Arkansas State (USA) situation exemplifies this process, since the repeated use of propanil led to resistant barnyardgrass selection in 1989. The measure adopted to control these biotypes was the replacement of propanil by quinclorac, however, barnyardgrass with resistance to propanil and quinclorac was

confirmed in 1999 (Burgos & Talbert, 2007). This situation was similar in Brazil, where firstly was identified barnyardgrass biotype with resistance to synthetic auxin (quinclorac), and then occurred a selection of biotypes with resistance to synthetic auxin and ALS inhibitors (Heap, 2015). There were also reported cases of barnyardgrass with multiple resistances to herbicides in rice in Italy, Philippines, South Korea, Thailand and Turkey (Heap, 2015). These global cases of resistant barnyardgrass to herbicides with different mechanisms of action confirmed the ability to develop resistance mechanisms (Talbert & Burgos, 2007).

Areas with barnyardgrass biotypes with resistance to synthetic auxin and ALS inhibitors are increasing in Santa Catarina. An alternative control used by some rice farmers is the application of cyhalofop-butyl. The first suspicion of multiple resistance involving three mechanisms of action rose with the intensification of ACCase inhibitor applications. A farmer reported the lack of control of barnyardgrass after applying the herbicide cyhalofop-butyl in the 2014/15 crop season in a field with population resistant to quinclorac, bispyribac-sodium, penoxsulam and imazapic + imazethapyr.

Therefore, dose-response experiments were conducted to confirm or disprove the multiple resistances of a barnyardgrass biotype (*E. crus-galli*) involving synthetic auxinic (quinclorac), ALS (penoxsulam) and ACCase (cyhalofop-butyl) inhibitor herbicides.

## MATERIAL AND METHODS

Two experiments were conducted in a greenhouse located in Itajaí, Santa Catarina, Brazil (26°57'06,34" S, 48°45'41,33" W), during December 2014 and January 2015.

The experiments were conducted at the same time, with the same methodology and treatments, differing only by the barnyardgrass biotypes. One experiment was conducted with a susceptible biotype (ECH 18), using seeds collected in Itajaí, SC, prior to the herbicide resistance confirmation, which were maintained in cold storage. The other experiment was conducted with seeds of the

barnyardgrass biotype (ECH 141) from a commercial area of irrigated rice, located in Tubarão, SC with suspicious to be resistant to synthetic auxin, ALS and ACCase inhibitors.

Preliminary tests were conducted to verify a possible multiple resistance, which consisted of applications of (imazapyr+imazapic) (147 + 49 g a.i. ha<sup>-1</sup>) (Kifix®), penoxsulam (48 g a.i. ha<sup>-1</sup>), (Ricer®), quinclorac (500 g a.i. ha<sup>-1</sup>) (Facet®), cyhalofop-butyl (360 g a.i. ha<sup>-1</sup>) (Clincher®) and a control, without herbicide application. The evaluations of control showed that the biotype ECH 141 had plants that survived in all treatments. This result led to the conduction of a dose response experiment.

The experimental units consisted of plastic pots with capacity of 8 dm<sup>3</sup>. The soil used as a substrate had a sandy texture, which was collected from the subsurface of a non-agricultural field, to ensure that the substrate was free from weed seeds. After collection, the soil was shadow dried and sieved to remove crop residues, clods and coarse materials.

Soil fertilization was performed as described for paddy rice, grown in pre-germinated system, for low fertility areas, according to the recommendations Knoblach et al. (2011).

The experimental design was completely randomized, in a 4 x 7 factorial arrangement, with four replications. The first factor was the herbicides: cyhalofop-butyl (Clincher®), penoxsulam (Ricer®), quinclorac (Facet®) and propanil (Grassaid®), and the second factor consisting of seven herbicide rates (0.0, 0.25, 0.50, 1, 2, 4 and 8X (X= label rate)). The registration rates of the herbicides are: cyhalofop-butyl - 315 g a.i. ha<sup>-1</sup>, penoxsulam - 36 g a.i. ha<sup>-1</sup>, quinclorac - 375 g a.i. ha<sup>-1</sup> and propanil - 2,880 g a.i. ha<sup>-1</sup>.

The adjuvant Veget'Oil® was used for the cyhalofop-butyl (1.5 L ha<sup>-1</sup>) and penoxsulam (1.0 L ha<sup>-1</sup>) and Assist® (1.0 L ha<sup>-1</sup>) was used for quinclorac. Adjuvant was not used for propanil application.

Barnyardgrass seeds (100 seeds per pot) were planted on December 4, 2014. The amount of seeds used was defined according to the seed availability of the biotype

ECH 141. A high density was used due to the heterogeneity of the biotype ECH 141 and the low initial frequency of resistant individuals observed in the preliminary experiment.

The application of the herbicides was performed in December 15, 2014, with seedlings at the stage of 1 to 2 leaves and an average density of 74 (ECH 18) and 47 (ECH 141) plants per pot. The density difference between the two biotypes was due to their different germination index. One day after the application, the pots were flooded with 4 - 5 cm of water depth and maintained through the end of the experiment.

The herbicide spray was performed with a CO<sub>2</sub>-pressurized backpack sprayer, equipped with a spray boom with four flat-fan nozzles (Mod. 110.015), spaced 0.50 m, a constant pressure of 220 kPa psi and a speed of 1.0 m s<sup>-1</sup>, which resulted in an application rate of 150 L ha<sup>-1</sup>. The weather conditions during application were air temperature of 27.1 °C, air humidity of 64% and average wind speed of 3.0 km h<sup>-1</sup>.

Evaluations of percentage of control, plant density per pot and dry mass of shoots were performed 35 days after treatment application. The percentage of control was performed by visual assessments using a percentage scale, in which zero means no control and 100% death of the plant (SBCPD, 1995).

After counting the number of plants per pot, the results were converted to percentage of plants compared to the control.

The dry mass of the plants was verified by collecting shoots and roots of the plants that survived the treatments and drying at temperature of 60 °C to a constant mass and measuring the dry mass in a precise digital scale.

The data were submitted to analysis of variance (p<0.05) and, when significant, a non-linear regression model was used, relating the plant response to the herbicide dose used. The three-parameter logistic model was used, as follows:

$$y = \frac{a}{1 + \left(\frac{x}{x_0}\right)^b}$$



where  $y$  is the dependent variable,  $x$  is the independent variable and  $a$ ,  $b$  and  $x_0$  are the parameters of the logistic model.

The regression model enabled to estimate the rate for controlling 50% and 80% of the barnyardgrass ( $C_{50}$  and  $C_{80}$ ) and the rate for reducing 50% and 80% of its biomass ( $GR_{50}$  and  $GR_{80}$ ).

The resistance factor (RF) was calculated by dividing the  $C_{50}$  of the resistant biotype by the  $C_{50}$  of the susceptible biotype (Vidal et al., 2006). The resistance was confirmed when  $RF > 1.0$  (Saari et al., 1994) and the  $C_{80}$  was higher than the herbicide maximum recommended rate (Christoffoleti et al., 2009).

## RESULTS AND DISCUSSION

The barnyardgrass biotype ECH 18 was sensitive to all herbicides evaluated, since it was efficiently controlled (>90%) even at the lower rates (Figure 1). However, the ECH 141 biotype was efficiently controlled up to the registration rate only by propanil (PSII inhibitor).

The herbicide cyhalofop-butyl reached the minimum acceptable level of control ( $C_{80}$ ) with a rate 60% higher than the label recommended rate (Table 1), while the herbicides penoxsulam and quinclorac did not achieve 80% control at the evaluated rates (0- to 8x). These results confirmed the initial hypothesis that the biotype 141 is multiple resistant to three different mechanism of action (ACCase, ALS inhibitors and synthetic auxins). Two important criteria for resistance confirmation have been met in this study: 1) the resistance factor was higher than 1.0; and 2) the percentage of control of ECH 141 was not adequate using the label rate.

The biotype ECH 18 reduced dry matter accumulation with increasing rate of the herbicides cyhalofop-butyl, penoxsulam, quinclorac and propanil (Figure 2), which reduced the dry mass close to zero with rates lower than 0.5 the label rate. The biotype ECH 141 presented varied response depending on the herbicide used. There was less interference in the dry matter accumulation with penoxsulam and quinclorac. High reduction in shoot biomass was observed with

the application of 2x rates of cyhalofop-butyl label rate (630 g a.i. ha<sup>-1</sup>). The application of propanil had similar performance for the two biotypes. In general, the results of dry mass and control were similar for both biotypes.

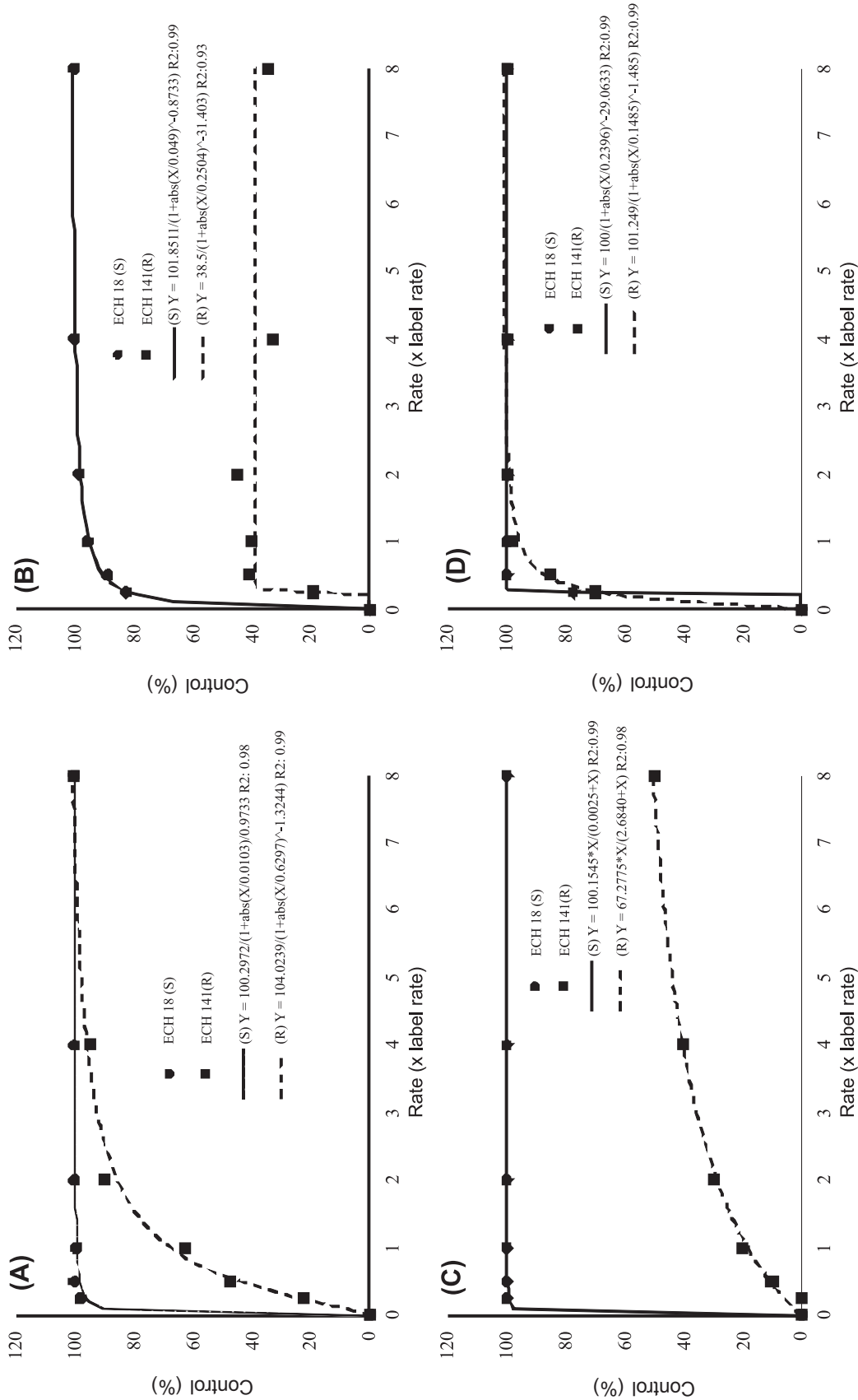
The susceptible biotype (ECH 18) presented  $GR_{80}$  values lower than the label rate for all herbicides (Table 1). Similar results occurred with application of cyhalofop-butyl and propanil in biotype ECH 141.

There was significant reduction in dry matter accumulation with cyhalofop application in the ECH 141 biotype despite the unsatisfactory control, which may be justified by the fact that not all plants survived the herbicide application (Figure 3). However, the surviving plants development was similar to the control. Regarding the penoxsulam and quinclorac herbicides,  $GR_{80}$  were much higher than the label rates.

According to Huan et al. (2013), the *E. crus-galli* resistance to ACCase inhibitors (quizalofop-ethyl) is due to a change in sensitivity of the chloroplast enzyme ACCase, and the gene amplification had an important role in this species.

There was no significant reduction in plant density of biotype ECH 141 treated with quinclorac (Figure 3C). The other herbicides reduced plant density with increasing herbicide rates regardless of the biotype evaluated (Figure 3). These results indicate that the frequency of resistant plants of the biotype ECH 141 was low, and that the minority of the population survived penoxsulam and cyhalofop-butyl applications.

According to Silva et al. (2007), the initial frequency of resistant plants in a population can be very low, however, a gradual increase in the frequency of these plants occurs when the selection pressure persists, with a population of about 30% in seven years. Matzenbacher et al. (2015) evaluated 624 populations of *E. crus-galli* in southern Brazil and found frequency of plants with resistance to imidazolinones of 0.81, and to quinclorac of 0.19. These authors also reported that barnyardgrass resistance is due to its multiple resistance mechanisms and multiple mutations in the ALS enzyme gene, however,



**Figure 1** - Percentage of control of two barnyardgrass (*E. crus-galli*) biotypes, 35 days after application (DAA) of the herbicides cyhalofop-butyl (A), penoxsulam (B), quinclorac (C) and propanil (D). Itajai, SC, 2014/2015.



**Table 1** - Estimated rate (g ha<sup>-1</sup>) to provide control of 50% and 80% (C<sub>50</sub> and C<sub>80</sub>), estimated rate to reduce dry matter accumulation by 50 and 80% (GR<sub>50</sub> and GR<sub>80</sub>) and resistance factor (RF) of two biotypes of barnyardgrass. Itajaí, SC, 2014/2015

Variable	Cyhalofop		Penoxsulam		Quinclorac		Propanil	
	S	R	S	R	S	R	S	R
C <sub>50</sub>	3.2	198.0	1.8	- <sup>1/</sup>	0.9	2925	691.2	427.7
C <sub>80</sub>	12.6	504.0	7.9	-	3.7	-	724.3	1045.4
GR <sub>50</sub>	1.6	145.8	0.14	<0.01	2.6	555.6	73.2	114
GR <sub>80</sub>	6.0	220.0	0.6	>288	9.2	1541.3	288	316.8
RF	1.0	61.9	1.0	-	1.0	3250	1.0	0.6

Maximum label rate: cyhalofop-butyl (315 g a.i. ha<sup>-1</sup>), penoxsulam (50 g a.i. ha<sup>-1</sup>), quinclorac (375 g a.i. ha<sup>-1</sup>) and propanil (3,600 g a.i. ha<sup>-1</sup>). S = ECH 18; R = ECH 141. <sup>1/</sup> = not reached 50 or 80% of control within the evaluated rates.

they had not found barnyardgrass biotypes resistant to cyhalofop-butyl.

Won et al. (2012) evaluated 155 barnyardgrass accessions from 15 sites in five locations in South Korea and found 25% of population resistant to ACCase inhibitors, 22% resistant to ALS inhibitors and 8.2% with multiple resistance (ACCcase + ALS).

The resistance factor (RF) of the biotype ECH 141 for cyhalofop-butyl and quinclorac were high, with values of 61.9 and 3250, respectively (Table 1). The RF of the biotype ECH 141 for penoxsulam was not possible to estimate because the control percentage did not reach 50%, even at very high herbicide rates. According to Lopez-Ovejero et al. (2005), high values of RF may be related to the high susceptibility of the known sensitive biotype, which may be one of the factors contributing to the high RF values in this study.

The ECH 141 biotype was sensitive to propanil, presenting an RF of 0.6, i.e., less than 1.0. This result shows that propanil is an effective alternative to control this biotype, and can be used in integrated management systems to contain the resistance evolution in this population. However, propanil must be used in rotation with different mechanism of action, since its repeated use can also select resistant individuals. In the United States there are reports of barnyardgrass biotypes resistant to propanil since 1990 (Malik et al., 2010; Heap, 2015).

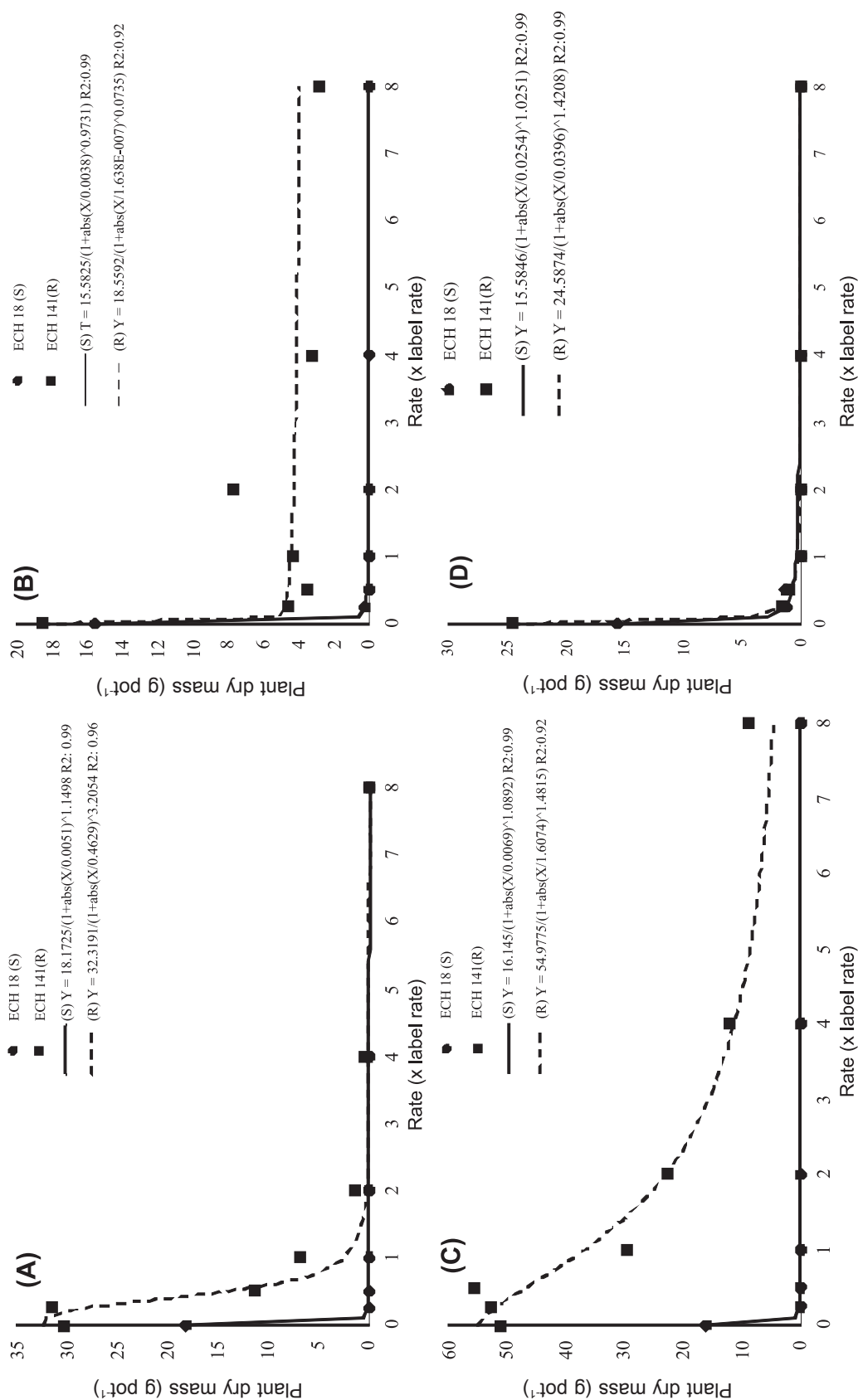
These are worrying results, since they present the first case of an *Echinochloa* population with resistance to three mechanisms of action in Brazil. The

*Echinochloa* genus had already been confirmed with resistance to quinclorac (Eberhardt et al., 2000; Andres et al., 2007) and to quinclorac and ALS inhibitors (Heap, 2015). In the USA (Mississippi State), there was a reported case of *E. crus-galli* resistant to fenoxaprop-ethyl, imazamox, imazethapyr, propanil and quinclorac (Heap, 2015), which have distinct mechanism of action. In California, *E. phyllopogon* biotype was found with resistance to four herbicides (molinate, thiobencarb, fenoxaprop-ethyl and bispyribac-sodium), however, this was a sensitive biotype to propanil (Fischer et al., 2000).

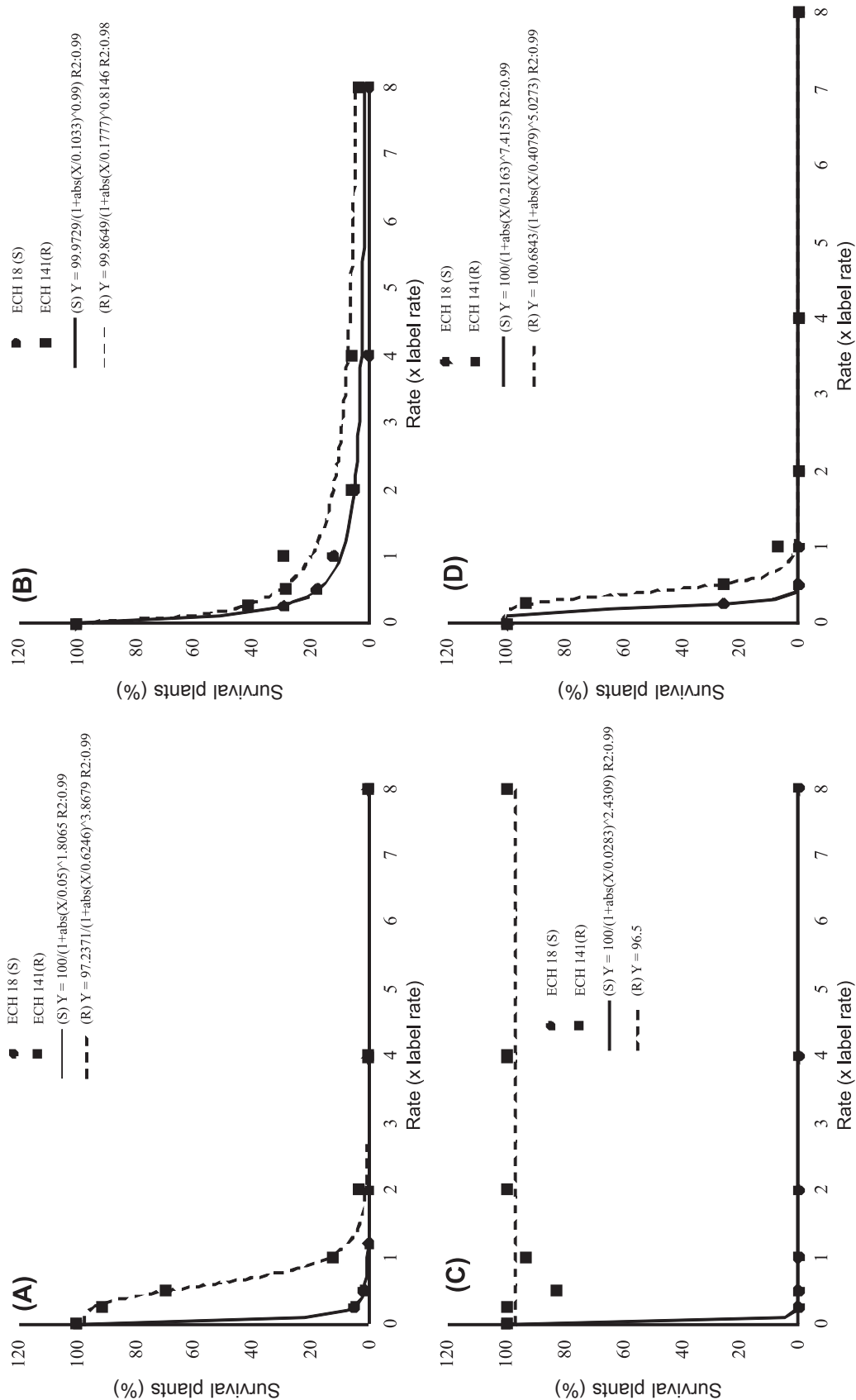
The occurrence of multiple resistance in rice fields brings serious consequences for weed management, since limits the number of herbicides and active ingredients available for controlling resistant biotypes, reduce the control efficiency, productivity and rice grains quality, besides the increase in production costs, resulting in reduction of rice production profitability.

This context may lead to modifications on planning the selection of herbicides for weed control. Regarding to barnyardgrass control, high efficiency herbicides such as penoxsulam and cyhalofop-butyl, would be alternatives to propanil, which has a lower efficiency (Ntanos et al., 2000 and Damalas et al., 2008). An alternative would be pre-emergent herbicides, however, their use has restrictions in pre-germinated systems (predominant in Santa Catarina) and also may raise the production cost.

According to Fischer et al. (2000), the selection of barnyardgrass biotypes with



**Figure 2** - Plant dry mass of two barnyardgrass (*E. crus-galli*) biotypes in grams per pot, 35 days after application (DAA) of the herbicides cyhalofop-butyl (A), penoxsulam (B), quinclorac (C) and propanil (D). Itajai, SC, 2014/2015.



**Figure 3** - Survival plants of two barnyardgrass (*E. crus-galli*) biotypes, in percentage compared to control, 35 days after application (DAA) of the herbicides cyhalofop-butyl (A), penoxsulam (B), quinclorac (C) and propanil (D). Itajai, SC, 2014/2015.





multiple resistances in California (USA) occurred due to repeated application of few different herbicides associated with monoculture. This situation was also registered in areas of irrigated rice in Santa Catarina, Brazil. The use of herbicides integrated with other control measures is essential for controlling multiple herbicide resistant weeds (Fischer et al., 2000; Talbert and Burgos, 2007).

The increasing cases of resistance has narrowed herbicide alternatives for rice production in Santa Catarina and hindering the plant breeding area, especially studies dedicated to develop herbicide-resistant crops, since the occurrence of barnyardgrass biotypes that are resistant to three mechanisms of action may decrease the control efficiency in rice cultivars resistant to imidazolinones (Clearfield Production System).

The initial hypothesis was confirmed, i.e., the ECH 141 biotype presented resistance to herbicides quinclorac (synthetic auxin), penoxsulam (ALS inhibitor) and cyhalofop-butyl (ACCase inhibitor). These findings showed that the control strategies used by rice farmers in Santa Catarina have not been effective to control resistance cases in rice. The integration of control methods is increasingly important in this present situation, with the use of technical recommendations, associating chemical and non-chemical control measures, emphasizing measures that favors the rice crops and not the weeds (cultural control). The ECH 141 biotype was sensitive to the herbicide propanil, which figures as an alternative for integrated weed management systems.

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