SENSITIVITY OF IMIDAZOLINONE-RESISTANT RED RICE (*Oryza sativa L.*) TO GLYPHOSATE AND GLUFOSINATE

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INTRODUCTION

Imidazolinone-resistant (IMI) rice, Clearfield® technology, was developed using either induced mutation by gamma radiation or chemical transformation by ethyl methane sulfonate and it is commercialized since 2004 in Brazil (SANTOS et al., 2007). The introduction of Clearfield® technology allowed rice growers to selectively control red rice in rice areas with little effect on crop safety (VILLA et al., 2006). The adoption of this technology was rapid, resulting in more than 50% of rice acreage planted with IMI-rice in brazilian state of Rio Grande do Sul by 2011 (IRGA, 2012). On the other hand, due to the continued use of this technology and with minimal alternative cultural practices being adopted concomitantly, several red rice biotypes have evolved resistance to imidazolinone herbicides (MENEZES et al., 2009).

In response to the widespread occurrence of red rice resistance in the late season rice growers to included multiple management practices to successfully control this weed. The most effective traditional practice used is to rotate rice with soybean, allowing the use of non-selective herbicides and alternative pre-emergent treatment options (BURGOS et al., 2011). However, studies have been reported differential sensitivity of some red rice ecotypes and accessions to glufosinate and glyphosate (NOLDIN et al., 1999; BURGOS et al., 2011). Based on these findings, additional information on herbicide sensitivity are needed to help farmers in weed management decisions and to avoid resistance evolution to alternative herbicides. Therefore, this study aimed to evaluate the control of red rice biotypes resistant to imidazolinone herbicides with glyphosate and glufosinate.

MATERIAL AND METHODS

An whole-plant bioassay was carried out during October of 2011 at the Centro de Estudos em Herbologia, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Capão do Leão, Rio Grande do Sul - RS, Brazil. Two red rice biotypes, identified as AV 109 and AVsus, were obtained from Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. The biotypes cited above were collected in rice fields located in Southern Brazil (RS state). The AV 109 biotype was determined as imidazolinone-resistant due to ALS gene mutation $Gly_{654}Glu$ (ROSO et al., 2010). AVsus was confirmed as susceptible to imidazolinone in whole-plant bioassay and molecular characterization previous studies.

The experiment was conducted in randomized complete block design in a factorial arrangement with four replications. The factor A included the herbicides glyphosate (Roundup Transorb®), glufosinate (Finale®) and imazapyr plus imazapic (Kifix®). The factor B was the red rice biotypes previously described. The factor C included nine herbicide rates (0.001; 0.01; 0.1; 0.25; 0.5; 1.0; 2.0 and 5.0 times the labeled rate) plus an untreated check.

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The labeled rates of glyphosate was 1440 g a.e. ha^{-1}, for glufosinate it was 400 g a.i. ha^{-1} and for imazapyr + imazapic it was 73,5 + 24,5 g a.i. ha^{-1}.

Ten seeds of each red rice biotype were placed in 700 mL plastic cups previously filled with 500 g of rice soil. Treatments were applied at 3 to 4-leaf stage of red rice plants including adjuvant accordingly to the label. Applications were performed using a CO_2 pressurized backpack sprayer couple to a boom equipped with three flat-fan nozzles (Teejet XR110015) spaced at 50 cm and calibrated to deliver 150 L ha^{-1} of spray solution at 172 kPa.

Red rice control was evaluated at 7, 14, 21 e 28 days after herbicide applications. Control results were estimated visually using a scale of 0 to 100% where 0 = no red rice control and 100 = total red rice control (death of red rice plants). After 28 days red rice plants were harvested and dried in an oven at 60 ºC to determinate shoot dry weight. Results were expressed as percentage of untreated check to standardize comparisons between biotypes and herbicides.

Data were tested to the assumptions of experimental design (independence, homogeneity and normality). Then, the data were subjected to analysis of variance (ANOVA). A non-linear log-logistic model was used to indicate overall patterns of treatments in dose-response curves accordingly to SEEFELDT et al. 1995 (equation 1):

$$Y = C + \frac{D - C}{1 + \left(\frac{X}{X50}\right)^{b}} \quad \text{eq. (1)}$$

Where Y= response variable; X= herbicide rate; D= upper limit; C= lower limit; b= slope of the curve around X_{50}; X_{50}= herbicide rate that causes 50% red rice control (CT_{50}) or herbicide rate required to 50% dry weight reduction (GR_{50}). Resistance ratio was based on CT_{50} and GR_{50} values of resistant and susceptible biotypes. Ninety-five percent confidence intervals were used to compare CT_{50} and GR_{50} values between biotypes and calculated based on standard error of this estimated parameter.

RESULTS AND DISCUSSION

Red rice biotypes showed differential tolerance level to imazapyr plus imazapic at 28 days after treatment (DAT) (Figure 1A). The herbicide rate required to control 50% of population was lower for IMI-susceptible than IMI-resistant biotype (Table 1). Similar response was observed for shoot dry weight variable (Figure 1A). IMI-resistant biotype was > 3-fold tolerant than IMI-susceptible according to tolerance ratio values. Higher tolerance level of the IMI-resistant biotype is due to amino acid substitution $Gly_{654}Glu$ in the functional protein that decreases its sensitivity to the inhibitory effect of the herbicide (ROSO et al., 2010). This substitution in the ALS sequence have been reported to confer resistance of the most red rice populations to imidazolinone herbicides in Rio Grande do Sul (MENEZES et al., 2009; ROSO et al., 2010).

There was difference between red rice biotypes to glufosinate sensitivity based on CT_{50} values (Figure 1B). IMI-resistant biotype required greater herbicide rate compared to IMI-susceptible to achieve 50% of control. However, GR_{50} values from shoot dry matter data indicated no differences within biotypes. Furthermore, tolerance ratio was similar for both variable evaluated suggesting minimal differences observed in this experiment. Differential sensitivity of red rice populations to glufosinate have also been reported in literature. Blackhulled red rice TX 4 was less sensitive to paraquat and glufosinate than other ecotypes and rice cultivars. Glufosinate at 1.12 kg a.i ha^{-1} was required to provide 94% control of TX 4 (NOLDIN et al., 1999).

Table 1. Regression equation, CT_{50}, GR_{50} and resistant to susceptible ratio (R/S) values for three herbicides and two biotypes in whole-plant bioassay estimated by log-logistic analysis. Capão do Leão, RS, 2012.

Herbicide	Biotype	Regression equation	R^2	CT_{50} [1/]	R/S [2/]
imazapyr+imazapic	resistant	$Y=70/1+(X/0.94)^{-3.14}$	0.93	0.94	3.48*
	susceptible	$Y=104/1+(X/0.27)^{-0.94}$	0.96	0.27	
glufosinate	resistant	$Y=99/1+(X/0.87)^{-7.54}$	0.99	0.87	1.16*
	susceptible	$Y=100/1+(X/0.74)^{-5.52}$	0.97	0.74	
glyphosate	resistant	$Y=104/1+(X/0.81)^{-2.64}$	0.92	0.81	1.03^{ns}
	susceptible	$Y=101/1+(X/0.78)^{-4.39}$	0.99	0.78	
Herbicide	**Biotype**	**Regression equation**	R^2	GR_{50}	**R/S**
imazapyr+imazapic	resistant	$Y=112/1+(X/0.90)^{1.13}$	0.86	0.90	4.50*
	susceptible	$Y=116/1+(X/0.20)^{1.01}$	0.83	0.20	
glufosinate	resistant	$Y=133/1+(X/0.64)^{1.72}$	0.85	0.64	1.06^{ns}
	susceptible	$Y=114/1+(X/0.60)^{1.59}$	0.84	0.60	
glyphosate	resistant	$Y=132/1+(X/0.31)^{1.24}$	0.86	0.31	1.00^{ns}
	susceptible	$Y=113/1+(X/0.31)^{1.78}$	0.96	0.31	

[1/] CT_{50} and GR_{50} are the herbicide rate that causes 50% red rice control and shoot dry weight reduction, respectively.
[2/] R/S ratio were calculated based on CT_{50} and GR_{50} values of resistant and susceptible biotypes.
* ratio is significant as the 95% confidence interval of the two CT_{50} or GR_{50} did not overlap.
[ns] ratio is not significant different as the 95% confidence interval of the two CT_{50} or GR_{50} did overlap.

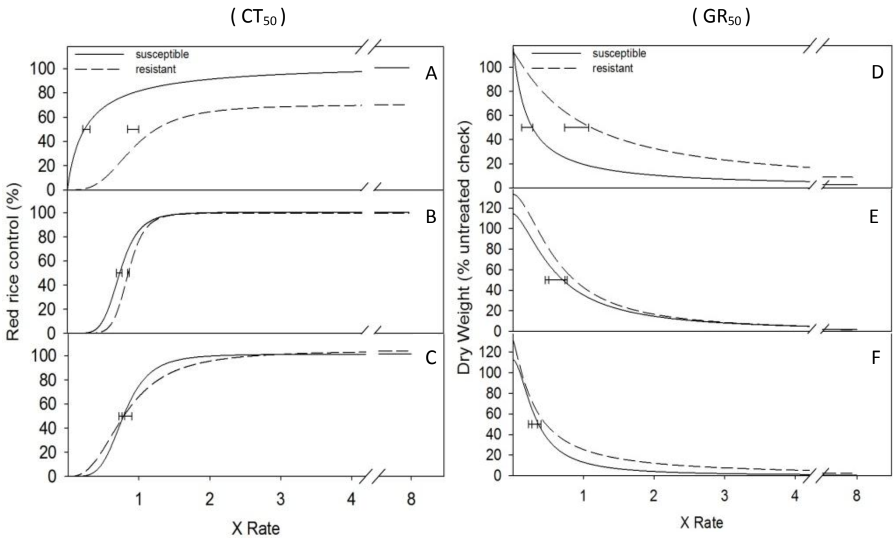

Figure 1. Dose response curves for red rice control (A-C) and shoot dry weight (D-F) with imazapyr plus imazapic (A, D), glufosinate (B, E) and glyphosate (C, F) at 28 DAT. Capão do Leão, RS, 2012. Biotypes are compared at CT_{50} and GR_{50} values using overlapping of 95% confidence intervals.

Glyphosate controlled both IMI-resistant and susceptible biotypes with no differences on CT_{50} and GR_{50} values (Figures 1C and 1F). Fifty percent of control and shoot dry matter reduction was obtained with less than 1X the labeled rate. Efficacy of this herbicide on red rice control resulted of the alternative mode of action, inhibiting 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a key enzyme in the shikimate biosynthetic pathway which is necessary for the production of the aromatic amino acids, auxin, phytoalexins, folic acid, lignin, plastoquinones and many other secondary products. Similar results were reported by KUK et al. (2008), who found that imazethapyr-tolerant accessions were susceptible to glyphosate.

CONCLUSIONS

Based on the red rice accessions evaluated in this study glufosinate and glyphosate can be used successfully in rice before planting as a burndown treatment or at-planting by dissecation of emerged IMI-resistant red rice. Glyphosate can also be employed in rice-soybean rotation programs to control red rice flushes during soybean growing season. Nevertheless, other cultural practices must be associated in the red rice management to extend use of the Clearfield® technology and avoid resistance evolution of red rice populations to glyphosate and glufosinate.

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