Ciência Rural, Santa Maria, v.45, n.9, p.1557-1563, set, 2015 http://dx.doi.org/10.1590/0103-8478cr20140896
ISSN 0103-8478
CROP PRODUCTION

Sensitivity of imidazolinone-resistant red rice (*Oryza sativa* L.) to glyphosate and glufosinate

Sensibilidade de arroz-vermelho (*Oryza sativa* L.) resistente às imidazolinonas, aos herbicidas glyphosate e glufosinate

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ABSTRACT

Dose-response experiments were carried out to evaluate the sensitivity of imidazolinone-resistant red rice to nonselective herbicides currently used in rice-soybean rotation in Rio Grande do Sul. Two red rice biotypes previously identified as resistant and susceptible to the imidazolinone herbicides were treated with imazapic plus imazapic, glyphosate and glufosinate under nine herbicide rates. A non-linear log-logistic analysis was used to estimate the herbicide rate that provided 50% red rice control and dry weight reduction (GR_{50}). Imidazolinone-resistant red rice exhibited greater GR_{50} values than imidazolinone-susceptible biotype for imazapyr plus imazapic. In contrast, both imidazolinone-resistant and susceptible red rice showed similar GR_{50} values for glyphosate and glufosinate. These results indicate that glyphosate and glufosinate effectively control imidazolinone-resistant red rice at similar herbicide rates used to control imidazolinone-susceptible; however, integrated weed management practices must be adopted in rice-soybean rotation to delay resistance evolution of red rice populations to glyphosate and glufosinate.

Key words: *Clearfield® rice, nonselective herbicides, red rice management, rice-soybean rotation, weed resistance.*

RESUMO

Curvas de dose-resposta foram conduzidas para avaliar a sensibilidade de arroz-vermelho resistente às imidazolinonas para herbicidas não-seletivos, comumente utilizados em áreas de rotação soja-arroz irrigado no Rio Grande do Sul. Dois biótipos de arroz-vermelho, previamente identificados como suscetível e resistente às imidazolinonas, foram aspergidos com imazapyr+imazapic, glyphosate e glufosinate sob nove concentrações herbicidas. Utilizou-se análise de regressão não linear do tipo log-logística para estimar a concentração herbicida que proporcionou 50% de controle e redução na massa de matéria seca da parte aérea (GR_{50}) de arroz-vermelho. O biótipo de arroz-vermelho resistente às imidazolinonas demonstrou maior GR_{50}, quando comparado ao biótipo suscetível para imazapyr+imazapic. Valores similares de GR_{50} foram observados para ambos os biótipos tratados com glyphosate e glufosinate. Esses resultados indicam que os herbicidas glyphosate e glufosinate controlam efetivamente biótipos de arroz-vermelho resistente às imidazolinonas em doses recomendadas para controlar biótipos suscetíveis às imidazolinonas. No entanto, práticas integradas de manejo devem ser utilizadas para retardar a evolução da resistência de arroz-vermelho aos herbicidas glyphosate e glufosinate na rotação soja-arroz irrigado.

Palavras-chave: *arroz Clearfield®, herbicidas não-seletivos, manejo do arroz-vermelho, resistência de plantas daninhas, rotação soja-arroz irrigado.*

INTRODUCTION

Clearfield® rice was developed using either induced mutation by gamma radiation or chemical transformation by ethyl methane sulfonate and it is commercialized since 2004 in the southern Brazil (SANTOS et al., 2007). Clearfield® rice genotypes exhibit tolerance to the imidazolinone herbicides (IMI), which inhibit acetolactate synthase (ALS), a key enzyme in

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Received 06.12.14 Approved 11.20.14 Returned by the author 04.22.15
CR-2014-0896.R1

the biosynthesis of branched-chain amino acids leucine, isoleucine and valine (AVILA et al., 2005).

The introduction of Clearfield® technology allowed producers to selectively control red rice in irrigated rice areas with little effect on crop safety (AVILA et al., 2005; SUDIANTO et al., 2013). The adoption of this technology was rapid, resulting in more than 50% of rice acreage planted with Clearfield® rice in Rio Grande do Sul by 2012 (MENEZES et al., 2013). On the other hand, because the continued use of this technology and minimal alternative cultural practices being adopted concomitantly, several red rice biotypes have evolved resistance to imidazolinone herbicides (MENEZES et al., 2009; ROSO et al., 2010; SUDIANTO et al., 2013).

Monitoring red rice populations from Rio Grande do Sul, MENEZES et al. (2009) found that 56% of accessions were resistant to imazethapyr plus imazapic. Additional studies using the same populations showed that the main herbicide resistance mechanism was ALS insensitivity indicating the occurrence of gene flow of the herbicide resistance allele from the Clearfield® rice variety to red rice (GOULART et al., 2012; ROSO et al., 2010). In addition, long term and continued exposure of red rice populations to ALS-inhibitor herbicides used over decades in rice production may have contributed to select natural populations of IMI-tolerant red rice due to ALS polymorphism (KUK et al., 2008; RAJGURU et al., 2005; SHIVRAIN et al., 2009).

Amino acid substitutions in the ALS sequence have been reported to alter fitness, competitive traits and sensitivity of red rice hybrids to herbicides (KUK et al., 2008; RAJGURU et al., 2005; SHIVRAIN et al., 2009). F_1 plants from hybrids between Clearfield® rice and red rice flowered 1-5 days later and produced 20-50% more seeds than the rice parent (SHIVRAIN et al., 2009). Also, a related study found that germination rate was higher in a rice genotype carrying Ala122Thr substitution than others imidazolinone-resistant and susceptible genotypes at low temperatures (GOULART et al., 2012).

The widespread occurrence of imidazolinone-resistant red rice led producers to integrate multiple management practices to successfully control this weed. The most effective practice adopted is to rotate rice with soybean allowing the application of nonselective herbicides and other pre-emergent treatments (BURGOS et al., 2011). In this rotation, glyphosate and glufosinate are commonly used as desiccation treatment prior rice planting and, in particular, glyphosate is also applied several times during the soybean season to control imidazolinone-resistant red rice. However, studies carried out in the United States have been reported differential sensitivity of some red rice ecotypes and accessions to glufosinate and glyphosate suggesting that selection pressure imposed by the continued use of these herbicides could increase resistance development in red rice populations (BURGOS et al., 2011; NOLDIN et al., 1999).

Based on these findings, red rice biotypes carrying mutations in the ALS gene might have differential sensitivity to nonselective herbicides currently used in rice-soybean rotation in Rio Grande do Sul. Moreover, additional research on herbicide sensitivity is needed to improve red rice management and delay resistance evolution to nonselective herbicides. Thus, this study was carried out to evaluate the sensitivity of imidazolinone-resistant red rice to glyphosate and glufosinate.

MATERIAL AND METHODS

A greenhouse experiment was carried out in 2011 and repeated in 2012 at the Centro de Estudos em Herbologia, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Capão do Leão, Rio Grande do Sul. The experiment was conducted in a randomized 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 composed by two red rice biotypes collected during the 2006/07 and 2007/08 growing seasons in rice fields of Rio Grande do Sul. The AV109 biotype was identified as imidazolinone-resistant due to ALS gene mutation Gly654Glu (ROSO et al., 2010) and AVsus was confirmed to be susceptible after a screening for imidazolinone resistance carried out in 228 populations (MENEZES et al., 2009). 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 recommended rate) plus an untreated check. The recommended rate for glyphosate was 1440g e.a. ha^{-1}, 400g i.a. ha^{-1} for glufosinate and 73.5+24.5g i.a. ha^{-1} for imazapyr plus imazapic, respectively.

Ten seeds of each red rice biotype were placed in 700mL plastic pots previously filled with 500g of paddy soil. Pots were daily surface irrigated to keep the soil moisture at the field capacity. After red rice emergence, seedlings were thinned to three per pot. Treatments were applied at 3- to 4-leaf stage of red rice plants including adjuvant according to specific recommendation for each herbicide. Applications were performed using a CO_2-pressurized backpack sprayer coupled to a boom equipped with three flat-fan nozzles (Teejet XR110015) spaced at 50cm and calibrated to deliver 150L ha^{-1} of spray solution at 172kPa.

Red rice control was evaluated at 28 days after herbicide treatment applications by visual ratings using a scale from 0 to 100% where 0 represents no red rice control and 100 total red rice control achieved (death of the red rice plants). After 28 days, red rice plants were harvested and dried at 60ºC to determine shoot dry weight. Results were expressed as percentages of untreated check to standardize comparisons between herbicides and biotypes.

Red rice control and shoot dry weight were tested to the assumptions of experimental design (independence, homogeneity and normality) and subjected to analysis of variance (ANOVA) to test interaction between the main effects. The ANOVAs showed no significant treatment by replication in time interaction and therefore data were combined over two experiments. A non-linear log-logistic model was used to indicate overall patterns of treatments in dose-response curves according to equation 1.

$$Y=a/1+(X/GR_{50})^b$$

Where Y = predicted red rice control or shoot dry weight reduction (%), a = maximum red rice control or shoot dry weight reduction observed, X = predicted herbicide rate; b = slope of the dose response curve and GR_{50} = herbicide rate that provides 50% red rice control or shoot dry weight reduction. Resistance ratio was calculated based on GR_{50} values of resistant and susceptible biotypes (BURGOS et al., 2013). Also, 95% confidence intervals were calculated based on standard error of the estimated parameters and used to compare GR_{50} values between treatments evaluated.

RESULTS AND DISCUSSION

Red rice biotypes showed differential sensitivity to imazapyr plus imazapic at 28 days after treatment (DAT) (Figure 1A and 2A). The herbicide rate required to provide 50% red rice control was lower for IMI-susceptible than IMI-resistant biotype. Similar response was observed for shoot dry weight variable. IMI-resistant biotype was at least 3-fold more resistant than IMI-susceptible according to resistance ratio values for red rice control and shoot dry weight (Table 1 and 2). Greater resistance level of the IMI-resistant biotype has been attributed to an amino acid substitution Gly654Glu in the functional protein that decreases its sensitivity to the inhibitory effect of the herbicide (ROSO et al., 2010). This substitution in the ALS gene have also been reported to confer resistance of the most red rice populations to imidazolinone herbicides in Rio Grande do Sul (ROSO et al., 2010).

According to GR_{50} values for red rice control, red biotypes exhibited differential sensitivity to glufosinate (Figure 1B). IMI-resistant biotype required 16% greater herbicide rate to achieve 50% red rice control compared to IMI-susceptible. In contrast, GR_{50} values from shoot dry weight indicated no difference between red rice biotypes (Figure 2B). Also, resistance ratio was similar for both variable evaluated suggesting minimal differences on herbicide sensitivity observed (Table 2). Nevertheless, differential sensitivity of red rice populations to glufosinate has been reported in literature. A research showed that blackhulled red rice TX 4 was less sensitive to paraquat and glufosinate than other ecotypes and rice cultivars when these herbicides were applied at 0.35 and 0.56kg a.i. ha^{-1}, respectively (NOLDIN et al., 1999). As a result, glufosinate at 1.12kg a.i ha^{-1} was required to provide 94% control of TX 4 (NOLDIN et al., 1999).

Glyphosate controlled both IMI-resistant and IMI-susceptible biotypes with similar GR_{50} values for red rice control and shoot dry weight variables (Figures 1C and 2C). Fifty percent red rice control and shoot dry matter reduction were obtained with less than 1X the

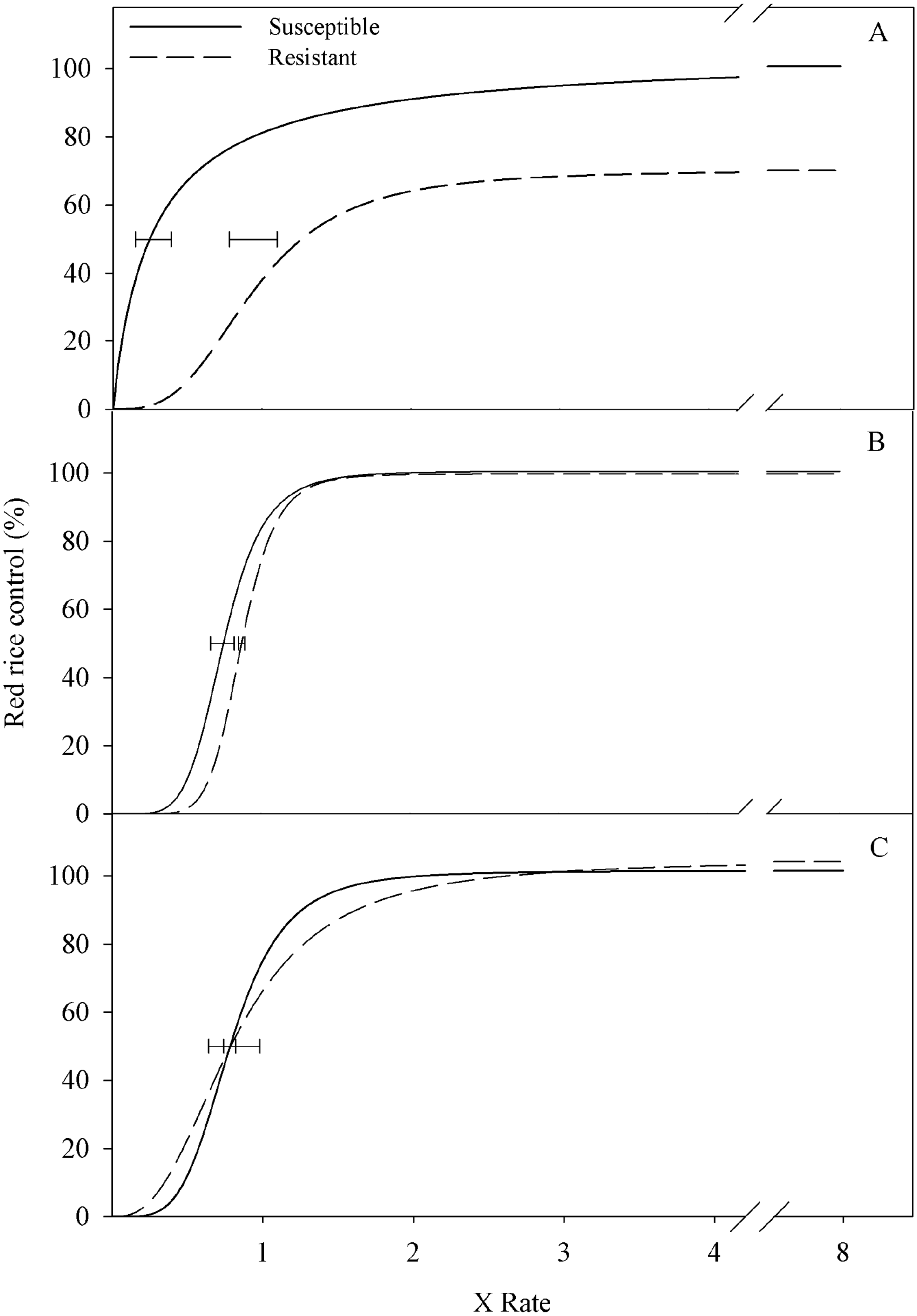

Figure 1 - Dose-response curves for red rice control with imazapyr plus imazapic (A), glufosinate (B) and glyphosate (C) at 28 DAT. Capão do Leão, RS, 2012. Biotypes were compared at GR_{50} values using overlapping of the 95% confidence intervals.

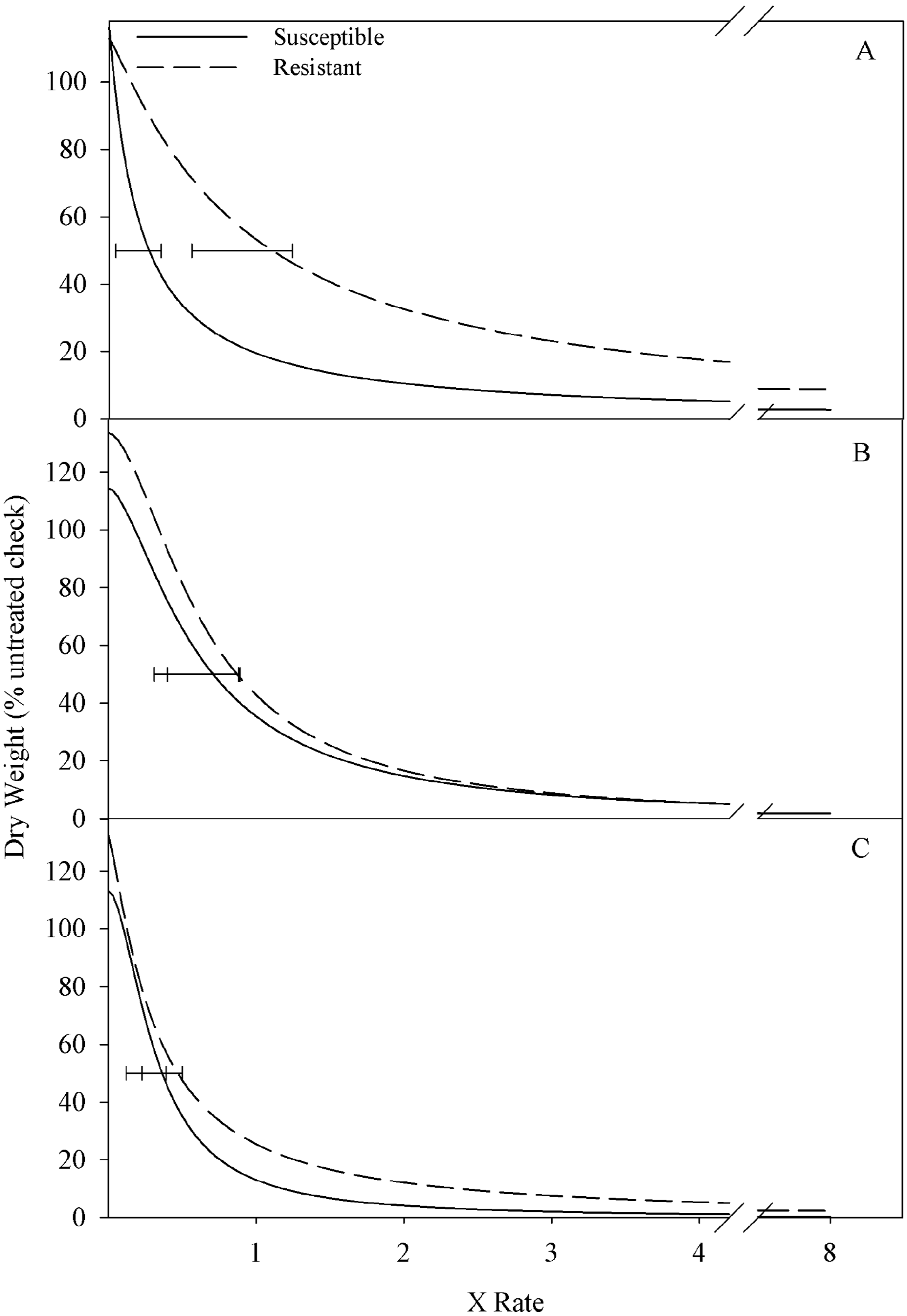

Figure 2 - Dose-response curves for shoot dry weight of red rice treated with imazapyr plus imazapic (A), glufosinate (B) and glyphosate (C) at 28 DAT. Capão do Leão, RS, 2012. Biotypes were compared at GR_{50} values using overlapping of the 95% confidence intervals.

Table 1 - Regression equation, GR_{50} and resistant to susceptible ratio (R/S) values of red rice control for three herbicides and two red rice biotypes in dose-response curves estimated by log-logistic analysis. Capão do Leão, RS, 2013.

Herbicide	Biotype	Regression equation	R^2	GR_{50}[a]	R/S[b]
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	

[a] GR_{50} is the herbicide rate that provides 50% red rice control.
[b] R/S ratio was calculated based on GR_{50} values of resistant and susceptible biotypes.
[*] Ratio is significant as the 95% confidence interval of the two GR_{50} did not overlap.
[NS] Ratio is not significant different as the 95% confidence interval of the two GR_{50} did overlap.

recommended rate for glyphosate. The efficacy of this herbicide on red rice control resulted from its alternative mode of action, inhibiting 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a key enzyme in the shikimate biosynthetic pathway that 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 naturally imazethapyr-tolerant accessions showed susceptibility to glyphosate at the recommended rate.

In summary, the sensitivity of IMI-resistant red rice for glyphosate and glufosinate applied at 3- to 4-leaf stage was similar to IMI-susceptible biotype. These results indicate that glyphosate and glufosinate can be used successfully in rice before planting as a burndown treatment or at-planting by dissecation of emerged IMI-resistant red rice. In particular, glyphosate can also be applied during the soybean growing season to control new flushes of red rice emergence and reduce red rice seed bank. However, it is extremely important to note that there is no ‘silver bullet’ to control red rice and therefore integrated weed management practices must be adopted in rice-soybean rotation to extend the use of the Clearfield® technology and delay resistance evolution of red rice populations to glyphosate and glufosinate.

CONCLUSION

Imidazolinone-resistant red rice carrying the Gly654Glu mutation in the ALS gene exhibits similar sensitivity to imidazolinone-susceptible red rice when treated at 3- to 4-leaf stage with glyphosate and glufosinate.

Table 2 - Regression equation, GR_{50} and resistant to susceptible ratio (R/S) values of shoot dry weight for three herbicides and two biotypes in dose-response curves estimated by log-logistic analysis. Capão do Leão, RS, 2013.

Herbicide	Biotype	Regression equation	R^2	GR_{50}[a]	R/S[b]
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	

[a] GR_{50} is the herbicide rate that provides 50% shoot dry weight reduction.
[b] R/S ratio was calculated based on GR_{50} values of resistant and susceptible biotypes.
[*] Ratio is significant as the 95% confidence interval of the two GR_{50} did not overlap.
[NS] Ratio is not significant different as the 95% confidence interval of the two GR_{50} did overlap.

AKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support of Conselho Nacional de Desenvolvolvimento Científico e Tecnológico (CNPq). They also would like to acknowledge the Environmental Fate of Herbicides team for the assistance with this research.

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