

# Red Rice Control and Soybean Tolerance to S-Metolachlor in Association with Glyphosate

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## Abstract

Red rice is one of the major troublesome and difficult weeds to control in rice production regions. The introduction of the Clearfield® technology allowed producers to control red rice using rice genotypes tolerant to the imidazolinone herbicides. However, because the consecutive use of this technology red rice biotypes have evolved resistance to imidazolinone herbicides, the rice-soybean rotation has been an alternative used by producers to control this weed. This system allows the use of herbicides with different modes of action to control red rice, such as S-metolachlor. Thus, greenhouse and field experiments were carried out during the 2011 to 2012 and 2012 to 2013 growing seasons to evaluate: 1) sensitivity of imidazolinone-resistant red rice to S-metolachlor; 2) red rice control and soybean tolerance in response to associations of S-metolachlor and glyphosate. In greenhouse, S-metolachlor effectively controlled both susceptible and imidazolinone-resistant red rice in preemergence. In field, preemergence applications of S-metolachlor provided greater red rice control in comparison to S-metolachlor alone in early postemergence. The association of S-metolachlor with glyphosate did not improve red rice control in preemergence application. However, association of S-metolachlor with glyphosate significantly improved red rice control in early post-emergence applications. S-metolachlor injury to soybean increased with early postemergence applications. These results indicate that S-metolachlor effectively control imidazolinone-resistant red rice in rice-soybean rotation.

## Keywords

Crop Rotation, Weed Control, Crop Injury, Application Timing

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## 1. Introduction

Red rice (*Oryza sativa* L.) is one of the major troublesome weeds of irrigated rice in the Southern Brazil and in various rice producing regions worldwide [1]–[3]. This weed belongs to the same species of cultivated rice sharing many morphological and physiological characteristics [4]. However, red rice typically exhibits seed dormancy, grows taller, and produces more tillers and biomass than cultivated rice [5]. It also has greater nitrogen efficiency under competitive conditions, absorbing up to 60% of applied N fertilizer [2].

Morphophysiological similarities between red rice and cultivated rice make its management difficult using conventional herbicides [6]. Since 2002, the introduction of the Clearfield® technology allowed producers to selectively control red rice in irrigated rice by using rice genotypes tolerant to the imidazolinone herbicides [7]–[9]. For example, the adoption of this technology resulted in more than 50% of rice acreage planted with Clearfield® rice in Southern Brazil specifically in the state of Rio Grande do Sul by 2011 [10]. On the other hand, because the continued use of the Clearfield® rice and minimal alternative cultural practices being adopted concomitantly, several red rice biotypes have evolved resistance to the imidazolinone herbicides [10]–[12].

Facing the widespread distribution of imidazolinone-resistant red rice, rice-soybean rotation has been the most effective practice adopted by producers to control red rice, reduce seed bank and prevent rice grain yield and quality losses caused by its interference [13] [14]. In this system, glyphosate is widely used to control a broad spectrum of weeds on glyphosate-resistant soybean (GR) and it is also applied as a burndown treatment to control red rice prior to rice emergence [15] [16]. Thus, continued exposure of red rice populations to glyphosate might increase risks of resistance development to this herbicide. Related studies showed that selection pressure has already resulted in resistance to glyphosate in many other weed species [17]–[19].

Additionally, as glyphosate is a foliar-applied herbicide without soil residual activity, it must be combined with an effective soil residual herbicide or followed by a postemergence (POST) herbicide to ensure season-long weed control [20]. A common approach in soybean production is to combine glyphosate with *S*-metolachlor, applying the association before or at-planting time to control existing vegetation and provide residual weed control. *S*-metolachlor has demonstrated acceptable activity on annual grasses, such as barnyardgrass (*Echinochloa crus-galli* L.) and broadleaf sinalgrass (*Urochloa platyphylla* (Griseb.) Nash), yellow nutsedge (*Cyperus esculentus* L.) and many small-seeded broadleaf weeds [21]. However, limited information is available concerning its efficacy on imidazolinone-resistant red rice. Thus, this study aimed to evaluate: 1) sensitivity of imidazolinone-resistant red rice biotypes to *S*-metolachlor; and 2) red rice control and soybean tolerance in response to association of *S*-metolachlor with glyphosate.

## 2. Material and Methods

### 2.1. Greenhouse Study

The experiment was carried out under greenhouse conditions at the Weed Science Research Group, Federal University of Pelotas, Capão do Leão, RS, Brazil, from October to December in 2011. The experimental design was a randomized complete block design in factorial arrangement ( $4 \times 2 \times 10$ ) with four replications. Factor A included four red rice biotypes, two resistant (Av75 and Av109) and two susceptible (Av01 and SC608) to the imidazolinone herbicides. Factor B was composed by two application timings (preemergence and postemergence) and factor C included nine rates of *S*-metolachlor (0.001, 0.01, 0.1, 0.5, 1.0, 1.5, 2.0, 5.0 and 10-fold the recommended rate of 1680 g a.i ha<sup>-1</sup>) plus an untreated check.

Red rice biotypes were obtained from Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil. All biotypes mentioned above were collected during the 2006 to 2007 and 2007 to 2008 growing seasons in rice fields of Rio Grande do Sul. The Av109 and Av75 biotype were previously identified as imidazolinone-resistant due to ALS gene mutation Gly<sub>654</sub>Glu [12]. Av01 and SC608 were confirmed as susceptible to imidazolinone after a screening carried out in 228 red rice populations [11].

Ten seeds of each red rice biotype were placed in 700 mL plastic cups previously filled with 500 g of lowland soil. Cups were surface irrigated every other day to allow red rice germination. Preemergence (PRE) treatments were applied after three days red rice sowing and postemergence (POST) applications at 3- to 4-leaf red rice stage. Applications were performed using a CO<sub>2</sub>-pressurized backpack sprayer coupled to a boom equipped with three flat-fan nozzles (Teejet XR110015, Spraying system Co., IL, 1609) spaced at 50 cm and calibrated to deliver 150 L·ha<sup>-1</sup> of spray solution at 172 kPa.

Red rice control was visually estimated at 28 days after herbicide applications using a grade from 0% to 100% where 0 = no red rice control and 100 = total red rice control (death of red rice plants) [22]. Plant height was determined by measuring the length (cm) from the soil surface to the oldest leaf tip. After the final evaluation, red rice plants were harvested and dried in an oven at 60°C to determinate shoot dry matter. Data were expressed as percentage of untreated check to standardize comparisons between biotypes and application timings.

Red rice control, plant height and shoot dry matter were tested to assumptions of experimental design (independence, homogeneity and normality) and then subjected to analysis of variance ( $P \leq 0.05$ ). A non-linear log-logistic model describing by Equation (1) was used to indicate overall patterns of treatments in dose-response curves:

$$Y = a / (1 + (x/x_0)^b) \quad (1)$$

where  $Y$  = response variable;  $x$  = herbicide rate;  $a$  = limiting value of red rice control on dry weight reduction;  $b$  = slope of the dose-response curve;  $x_0$  = herbicide rate that provides 50% red rice control ( $CT_{50}$ ); 50% plant height reduction ( $EST_{50}$ ) or 50% dry weight reduction ( $GR_{50}$ ). Ninety-five percentage confidence intervals were calculated based on standard error of estimated parameters and used to compare  $CT_{50}$ ,  $EST_{50}$  and  $GR_{50}$  values between biotypes.

## 2.2. Field Study

Experiments were conducted at University Farm (Centro Agropecuário da Palma), located in Capão do Leão, Rio Grande do Sul, Brazil, during the 2011 to 2012 and 2012 to 2013 growing seasons. The experimental design was a randomized complete block with four replications. Herbicide treatments included *S*-metolachlor alone or in combination with glyphosate applied both in preemergence (PRE) and early postemergence (EPOST) of soybean (Table 1). Prior to soybean planting, the SC608 red rice biotype was drilled at 2 cm deep at 20 kg·ha<sup>-1</sup> to

**Table 1.** Soybean injury (%) at 14 and 28 days after treatment applications (DAA) in the 2011 to 2012 and 2012 to 2013.

Herbicide Treatment	Rate (g·ha <sup>-1</sup> )	Timing <sup>a</sup>	Soybean injury (%) <sup>b</sup>			
			2011 to 2012		2012 to 2013	
			14 DAA	28 AA	14 DAA	28 DAA
<i>S</i> -metolachlor	768	PRE	5	5	10	5
<i>S</i> -metolachlor	1152	PRE	8	5	12	3
<i>S</i> -metolachlor	1680	PRE	10	5	12	5
<i>S</i> -metolachlor + glyphosate	768 + 1860	PRE	5	5	8	3
<i>S</i> -metolachlor + glyphosate	1152 + 1860	PRE	6	5	11	4
<i>S</i> -metolachlor + glyphosate	1680 + 1860	PRE	6	5	13	5
<i>S</i> -metolachlor	768	EPOST	15	5	7	9
<i>S</i> -metolachlor	1152	EPOST	20	7	20	10
<i>S</i> -metolachlor	1680	EPOST	27	9	17	8
<i>S</i> -metolachlor + glyphosate	768 + 1860	EPOST	14	5	12	6
<i>S</i> -metolachlor + glyphosate	1152 + 1860	EPOST	18	6	15	7
<i>S</i> -metolachlor + glyphosate	1680 + 1860	EPOST	18	6	16	5
Untreated check	-	-	N/A	N/A	N/A	N/A
LSD (0.05)	-	-	5	2	8	4

<sup>a</sup>Abbreviation: PRE, preemergence; EPOST, early postemergence. <sup>b</sup>Data were presented as a percent of soybean injury compared with the untreated check (no herbicide).

ensure properly red rice infestation in the experimental area. An early-maturity variety (FUNDACEP 62 GR) was drill seeded perpendicularly to red rice rows at 400,000 seeds per hectare in December for both growing seasons. Soybean was planted in 45cm row spacings. Seeds were previously inoculated with *Bradyrhizobium japonicum* SEMIA 5079 (CPAC 15) and SEMIA 5080 (CPAC 7) strains to provide biological nitrogen fixation. Soybean plots measured 4 m long and 2 m wide. All the other agronomic practices followed local recommendations for soybean production [23].

Treatment applications were performed with a CO<sub>2</sub>-pressurized backpack sprayer coupled to a boom equipped with three flat-fan nozzles (Teejet XR11002, Spraying system Co., IL, 1609) spaced at 50 cm and calibrated to deliver 150 L·ha<sup>-1</sup> of spray solution at 172 kPa. Red rice control and herbicide injury to soybean were estimated visually at 14 and 28 days after herbicide applications (DAA). Visual ratings were based on a grade from 0 to 100% where 0 = no herbicide injury symptoms or red rice control and 100 = total herbicide injury (death of soybean plants) or red rice control [22].

Data were tested to assumptions of experimental design (independence, homogeneity and normality) and followed by analysis of variance ( $P \leq 0.05$ ). Means of significant main effects were separated by Fisher's protected LSD test ( $P \leq 0.05$ ).

### 3. Results and Discussion

#### 3.1. Greenhouse Study

According to analysis of variance, there was no significant effect of biotype ( $P \geq 0.05$ ) for all of the variables evaluated in the experiment. This indicates that both imidazolinone-resistant and imidazolinone-susceptible biotypes showed similar sensitivity to *S*-metolachlor and therefore data were pooled across biotypes to generate the dose-response curves.

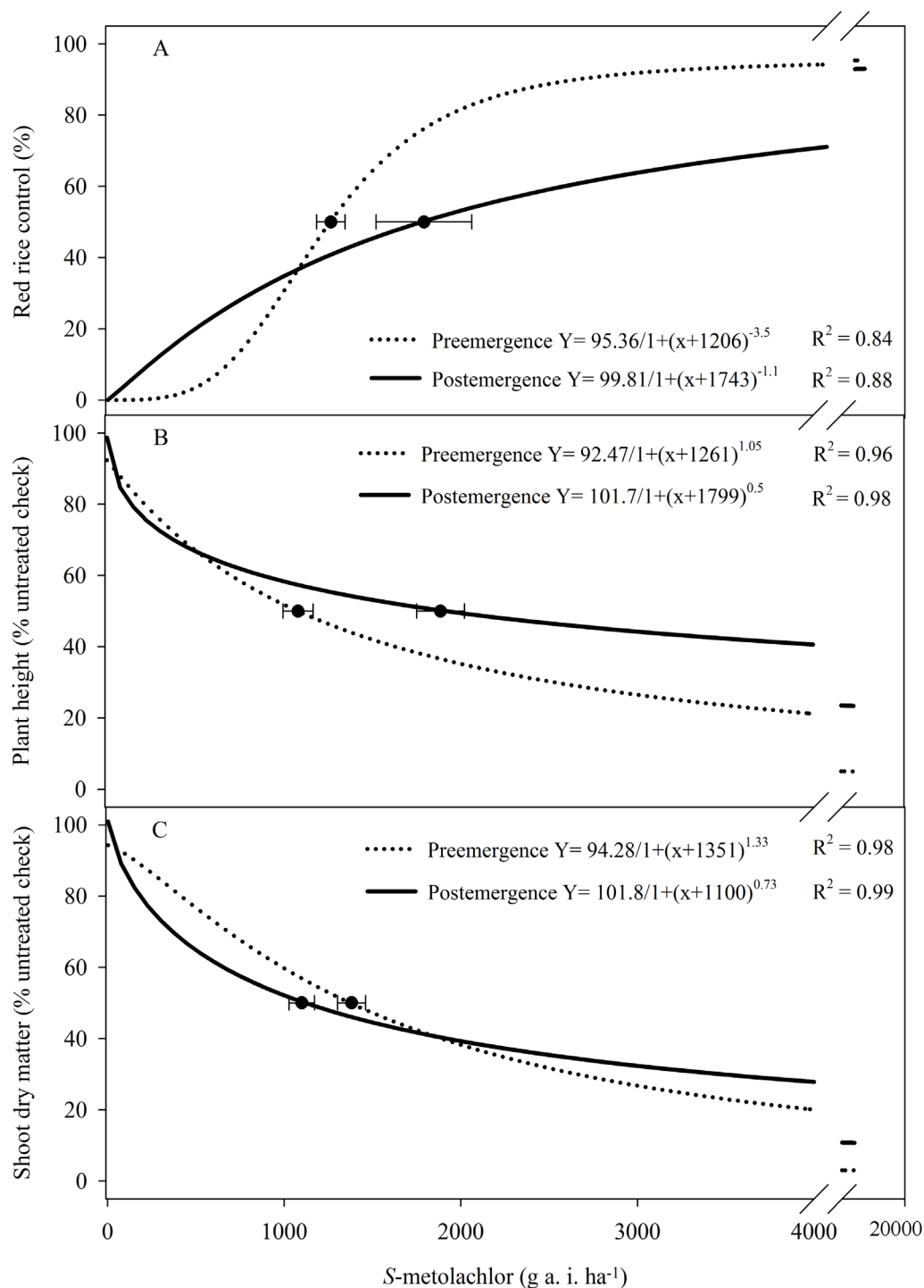
Differences on CT<sub>50</sub>, EST<sub>50</sub> and GR<sub>50</sub> were observed between application timings. Preemergence (PRE) application of *S*-metolachlor provided greater red rice control (**Figure 1(A)**). Lower *S*-metolachlor rate was required to achieve 50% red rice control in PRE compared to POST application. Similar tendency was verified to plant height variable (**Figure 1(B)**). Only the GR<sub>50</sub> value was lower for POST in comparison to PRE application (**Figure 1(C)**). Greater *S*-metolachlor efficacy on red rice in PRE application may be also related to its mode of action, which inhibits the biosynthesis of several plant components such as fatty acids, lipids, proteins, isoprenoids and flavonoids [24]. *S*-metolachlor is primarily absorbed by emerging shoots, especially grass coleoptiles whereas weeds beyond the seedling stage can absorb the herbicide by the root system and translocate to the shoots, but its translocation is limited [24]. Thus, these characteristics enhance *S*-metolachlor efficacy in younger weeds. Related studies have been reported greater annual grasses control when *S*-metolachlor was PRE applied in corn, soybean and dry bean [21]–[25].

#### 3.2. Field Study

Herbicide injury to soybean was 27% or less in both years of experiment. Early postemergence (EPOST) applications of *S*-metolachlor plus glyphosate or *S*-metolachlor alone increased herbicide injury to soybean at 14 DAA in 2011 to 2012 and 2012 to 2013 growing seasons (**Table 1**). In this study the symptoms of soybean injury were similar to the symptoms observed by Clewis *et al.* (2006) [26], mainly characterized by growth reduction and transient necrotic speckling on exposed leaves. Increased injury with EPOST applications may be related to direct exposure of soybeans leaves to *S*-metolachlor reducing plant ability to overcome the oxidative stress caused by herbicide application.

Red rice control was significantly affected by application timing and association of herbicides. *S*-metolachlor rate had little effect on red rice control regardless of the association with glyphosate (**Table 2**). Poor red rice control was obtained when *S*-metolachlor was applied alone in EPOST applications. In contrast, PRE applications of *S*-metolachlor alone significantly improved red rice control in both years of experiment. Greater *S*-metolachlor efficacy in PRE applications might be related to the fact that red rice plants can easily absorb and translocate the herbicide during the germination and early growth stages.

Association with glyphosate in PRE applications did not improve red rice control in comparison to *S*-metolachlor alone. However, EPOST applications of *S*-metolachlor plus glyphosate resulted in greater red rice control at 28 DAA in 2011 to 2012 and 2012 to 2013 growing seasons (**Table 2**). The efficacy of this associa



**Figure 1.** Red rice control (A), plant height (B) and shoot dry matter (C) in response to rates and application timings of *S*-metolachlor at 28 days after treatment applications. Errors bars represent 95% confidence intervals of four replications.

tion in EPOST applications may be explained by a combination of factors. Glyphosate is a postemergence non-selective herbicide that controls many annual and perennial weeds. It is particularly very active on annual grasses such as barnyard grass, broadleaf signal grass and red rice [15]–[27]. As a result, POST applications of glyphosate ensured almost total control of the existing red rice in this study. In addition, association with *S*-me-

**Table 2.** Red rice control (%) at 14 and 28 days after treatment applications (DAA) in the 2011 to 2012 and 2012 to 2013.

Herbicide Treatment	Rate (g·ha <sup>-1</sup> )	Timing <sup>a</sup>	Red rice control (%) <sup>b</sup>			
			2011 to 2012		2012 to 2013	
			14 DAA	28 AA	14 DAA	28 DAA
<i>S</i> -metolachlor	768	PRE	65	74	82	53
<i>S</i> -metolachlor	1152	PRE	69	79	72	55
<i>S</i> -metolachlor	1680	PRE	65	84	90	64
<i>S</i> -metolachlor + glyphosate	768 + 1860	PRE	56	84	90	79
<i>S</i> -metolachlor + glyphosate	1152 + 1860	PRE	70	91	89	74
<i>S</i> -metolachlor + glyphosate	1680 + 1860	PRE	68	87	98	95
<i>S</i> -metolachlor	768	EPOST	0	0	23	23
<i>S</i> -metolachlor	1152	EPOST	0	0	28	20
<i>S</i> -metolachlor	1680	EPOST	0	0	33	27
<i>S</i> -metolachlor + glyphosate	768 + 1860	EPOST	96	99	97	96
<i>S</i> -metolachlor + glyphosate	1152 + 1860	EPOST	98	100	98	98
<i>S</i> -metolachlor + glyphosate	1680 + 1860	EPOST	98	100	98	98
Untreated check	-	-	N/A	N/A	N/A	N/A
LSD (0.05)	-	-	11	9	16	22

<sup>a</sup>Abbreviation: PRE, preemergence; EPOST, early postemergence. <sup>b</sup>Data were presented as a percent of red rice control compared with the untreated check (no herbicide).

tolachlor provided residual activity in soil preventing red rice emergence throughout the soybean growing season.

## 4. Conclusion

These results indicate that PRE applications of *S*-metolachlor effectively control imidazolinone-resistant red rice. The association of *S*-metolachlor with glyphosate does not improve red rice control in PRE applications. However, associations of *S*-metolachlor with glyphosate significantly improve red rice control in EPOST applications. Soybean injury increases in EPOST applications but is less than 10% at 28 days after herbicide application.

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