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Abstract

During the 2006/07 and 2008/09 growing seasons in Southern Brazil, we evaluated crop selectivity and weed control efficacy of clomazone on rice when applied alone or in a mixture with other pre- and post- emergence herbicides (BRS Querência variety). All herbicide treatments caused some degree of rice injury during both years; however, in no case was the injury still visible 21 days after application. Rice injuries were observed when application rates exceeded the recommended dose, and particularly when the herbicide was applied pre-emergence. Density reduction and panicle sterility served as measures of rice injury. Echinochloa crus-galli was the primary weed; it reduced rice yields by approximately 50% compared treated plots. In both growing seasons, clomazone herbicide (400 g ai ha 1) controlled the weed 87.0%e99.6%, and it provided 8.06 t ha 1 to 9.44 t ha 1 of rice yield.

Introduction

The main rice cultivation areas of Brazil are in Rio Grande do Sul state (RS), where rice is sown over an estimated 1.17 million ha that yielded 7.66 t ha 1 during the 2010/11 cropping season. Intensification of rice cropping systems in this area has favored weed in- festations and led growers to depend increasingly on herbicides for effective weed control in approximately 95% of RS state paddy (Andres and Machado, 2004).

The most troublesome weeds in the rice crop ecosystems of Southern Brazil are weedy rice (Oryza sativa L.) and species from the Echinochloa complex (including Echinochloa crus-galli (L.) Beauv. and Echinochloa colona (L.) Link). Most recently, Aeschyno- mene denticulata Rudd has also been reported as an important weed in several rice areas, particularly in the "Clearfield!" system, which is more than 50% of the area. These weeds are strong competitors of rice (Chauhan and Johnson, 2009; Gibson et al., 1999), resulting grain yield reduction depends on several factors: infestation, rice variety, control method efficacy, and rice man- agement (Agostinetto et al., 2010; Galon and Agostinetto, 2009; Galon et al., 2007; Gibson et al., 2002; Ottis and Talbert, 2007; Richard and Street, 1984).

In the south of Brazil, rice is mostly dry-seeded (90%) and flooding begins after the crop is well established (3e4 leaf stage). Under these conditions, the initial crop environment is potentially favorable for flood-adapted weeds to emerge (Cassol et al., 2008; Chauhan and Johnson, 2009). The critical period of weed compe- tition is early in rice emergence; specifically, weed control applied two weeks post-emergence (POST) produced higher yields than when applied later in the season (Zhang et al., 2003). Perera et al. (1992) found that E. crus-galli competition was higher when the weed/crop relationship was established in the early stages. Galon et al. (2007) pointed out that competitive potential can vary to the same weed among different rice varieties. Gibson et al. (2002) suggested that spacing crop rows closely and controlling weeds early might be key components of a successful integrated rice management strategy. The same authors also put forth that delayed weed emergence might lead to reduced Echinochloa species growth and increased rice grain yields as opposed to conditions in which weeds and rice emerged concurrently. On the other hand, Webster et al. (1999) postulated that long-lasting E. crus-galli interference could reduce rice yields on average 30%.

Clomazone is an active ingredient registered in Brazil for rice weed control since 1991. It is now one of the most used herbicides in Southern Brazil with application on more than 400,000 ha annually. It can be applied pre- and post-emergence to dry-seeded and water-seeded rice to provide effective pre-emergence (PRE) control of E. crus-galli, E. colona, Digitaria horizontalis (Willd., D. bicornis (Lam.) Roem. & Schult.), Eleusine indica (L.) Gaertn., and Brachiaria plantaginea (Link) Hitchc. (Sosbai, 2012). When applied pre-emergence, clomazone can be used alone or in combination with glyphosate; in post-emergence application, it is mixed with cyhalofop, fenoxaprop, bispyribac-sodium, penoxsulam, profox- ydim, propanil, or quinclorac (Andres and Machado, 2004). Taken up from the soil through the roots, clomazone is transported to the leaves via the xylem (Ferhatoglu and Barrett, 2006). In susceptible species, clomazone affects the biosynthesis of chlorophyll and carotenoid pigments and results in white or yellow (bleached) leaves and plants (Duke et al., 1991; Ferhatoglu and Barrett, 2006).

Soil type is the primary determinant of recommended applica- tion rates (O'Barr et al., 2007). This is particularly true in Rio Grande do Sul state where the herbicide is applied mostly during pre-emergence to dry-seeded rice paddies. Soil differences in the area require that rates vary between 300 g ha 1 in sandy soils and 700 g ha 1 in clay soils (Sosbai, 2012). Webster et al. (1999) observed between 8% and 12% rice injury in fine clay soil 7 days after rice emergence when clomazone was applied during pre- emergence at rates of 450, 560, and 670 g ha 1. Zhang et al. (2004) who worked with nine rice varieties in fine silt loam soil type, applied clomazone pre-emergence at a rate of 1120 g ha 1 and reported rice injury in the range of 27%e51% at 14 days after treatment. These symptoms declined 28 days later. In three of the nine varieties, clomazone reduced the rice density while rice yield fell significantly in only one variety. Wu et al. (1998) put forth that this behavior was likely due to the ability of rice to compensate for lower plant density by producing more reproductive tillers per plant. Sanchotene et al. (2010) observed no differences in rice injury between Brazilian varieties, with no differences between rates and soil types. O'Barr et al. (2007) deduced from his work that the application rate is more critical when clomazone is applied in presence of higher sand content in comparison with soils with more clay.

The purpose of this research was to evaluate rice injury and weed control efficacy of clomazone applied alone or mixed with other herbicides in Southern Brazil.

2. Materials and methods
2.1. Study site
Field studies were conducted during the rice-growing seasons of 2006/07 (Experiment 1) and 2008/09 (Experiment 2) at the Embrapa Temperate Agriculture, Terras Baixas Experimental Station, located in Capão do Leão city, RS, Brazil (31 4901100S; 52 2801800W). Table 1 presents the main soil characteristics of the area, which is classified as Haplic Planossoil, Albaqualf (Santos et al., 2006).

2.2. Chemicals
The treatments under consideration differed by rate of cloma- zone (2-(2-chlorobenzyl)-4,4-dimethyl-1,2-oxazolidin-3-one) app- lied alone, pre-emergence or combined either, post-emergence (POST) with propanil (30 ,40 -Dichloropropananilide), or bispyribac- sodium (sodium 2,6-bis(4,6-dimethoxypyrimidin-2-yloxy)benzo- ate), or penoxsulam (3-(2,2-difluoroethoxy)-N-(5,8-dimethoxy 1,2,4]triazolo[1,5-c]pyrimidin-2-yl)-a,a,a-trifluorotoluene-2-sulfo namide) (all commercial market formulations). In Experiment 2 the maximum rate of clomazone was established at 400 g ai ha 1 on the basis of the results obtained in the Experiment 1, which showed high rice injury level at 500 g a ha 1.

2.3. Experimental design and procedures
The experimental design was a randomized complete block with four replicates in both experiments. Plots consisted of nine, 6 m rows. Soil preparation consisted of fall plowing, precision leveling, and field harrowing before sowing. Cultural practices were iden- tical across the two experiments and were

in accordance with official recommendations provided by the "Sociedade Sul-Brasileira de Arroz Irrigado" (Sosbai, 2012). In both experiments the seeded rice variety used was BRS Querência, characterized by a 110-day cycle from emergence to ripening. Rice was drill-seeded in rows spaced every 17.5 cm with an experimental grain seeder set to deliver 500 seeds m 2. Tables 2 and 3 report characteristics of the various treatments. All herbicide treatments were applied with a pressurized CO_2 backpack sprayer calibrated to deliver 125 L ha 1, equipped with four nozzles DG Teejet 110.015 spaced in 0.5 m. Soil fertility was managed each year with a pre-seeding application of 300 kg ha 1 of 5e20e20 (NePeK) followed by a pre-flood appli- cation of urea (50 kg ha 1 N). A second urea application of 50 kg ha 1 N was done at rice panicle differentiation.

2.4. Data collection
Weed population was assessed 15 days after planting (DAP) in eight randomly selected quadrats of 625 cm2 (25 cm 25 cm) each. Weed control was evaluated visually at 7, 21, and 40 DAE (days after emergence) PRE and at 7, 21, and 40 DAT in POST according to a scale, in which 0 1⁄4 no weed control and 100 1⁄4 complete weed control.

In both experiments rice injury (bleaching) was determined visually at 7, 21, and 40 days after rice emergence (DAE) PRE and at 7, 21, and 40 days after treatment (DAT) in POST using a scale in which 0 1⁄4 absence of bleached foliage and 100 1⁄4 completely bleached foliage. The number of culms (linear meter), plant height, and % sterile florets were all determined at pre-harvest. Rice height was measured from soil surface to the tip of the extended panicle on ten plants per plot. Rice panicle sterility was recorded by counting the filled and empty grains and expressed as a percent of sterility (empty*100/empty þ filled). The five central rows of each plot were collected when grain moisture was between 18% and 22%. The final grain yield was adjusted to 13% moisture.

2.5. Statistical analysis
Rice yield, weed control, rice injury, crop density, and sterility data were subjected to analysis of variance (ANOVA), and the means were compared using contrast analysis at the $P < 0.05$ level of significance. Data from distinct years were not pool-analyzed. In order to homogenize the variance weed control (%), rice injury (%), and sterility (%) results were previously transformed in arc sin (x). The ANOVA and contrast analysis were performed using statistical software SPSS2 (version 16).

3. Results and discussion
3.1. Rice injury
In each study year, some degree of rice injury was observed in all herbicide treatments. The first assessment of foliar bleaching (at 7 DAT or DAE) averaged 13.3% in Experiment 1 and 10.8% in Experi- ment 2. At the second assessment (21 DAT or DAE), foliar bleaching averaged 6% in the two experiments (Tables 4 and 5).

In Experiment 1, rice injury data at 7 DAE showed that BRS Querência (Table 4) suffered more injury for PRE treatments than in POST treatments. In the same cropping season, as expected, the high clomazone dose used PRE (500 g ai ha 1) caused high injury levels at the initial assessment. Rice injury was also more severe in PRE than in POST treatments (Table 5) in experiment 2 even though the highest rate of clomazone applied PRE was reduced to 400 g ai ha 1. In both experiments, the second assessment of rice injury (21 DAE) by PRE herbicides declined noticeably (Tables 4 and 5) and showed a recovery behavior similar to that described by Zhang et al. (2004, 2005). Morever, rice plants recovered completely from damage by 40 days after treatment (data not shown), as is consistent with work by Jordan et al. (1998) and Scherder et al. (2004).

Experiment 2 experienced weak bleaching when adverse climate conditions delayed its PRE application; consequently, rice plants had attained a more advanced growth stage. In this instance, and as Jordan et al. (1998) had suggested, herbicide was applied after the rice had already germinated for about four days, which likely minimized seed absorption of clomazone.

3.2. Crop density

In Experiment 1, non-treated plots had lower culm density than those with herbicide treatments (Table 4). This finding, consistent with results reported by Gibson et al. (1999), indicated that a portion of the plants died or produced fewer culms due to weed competition. No rice density differences were found between treated and untreated plots (Table 5) in Experiment 2. Differences in culm density of untreated plots between experiments were attributed to differential weed infestation levels.

In the first year of this study, the number of culms counted at rice pre-harvest in the PRE and POST treatments was 85.0 and 78.5 culms per row meter, respectively, which suggested that better weed control conditions permitted the production of more culms; in addition, the higher crop injury caused by PRE application of clomazone could have opened room for a higher tillering by sur- viving plants (Table 4). These results agree with those of Bond et al. (2007) and Mudge et al. (2005) on the effects of clomazone and ALS-inhibiting herbicides in rice, under good weed control conditions.

Mudge et al. (2005) and Zhang et al. (2004) reported that with PRE application, first-to-emerge rice plants generally have a competitive advantage over weeds which emerge later, which was also observed by Gibson et al. (1999) and Perera et al. (1992). Thus, in this trial, surviving plants from PRE applications were benefited to be free from competition with weeds at initial stages of devel- opment, being able to tiller vigorously, which resulted in higher tillering compared to the untreated (weedy) check. de Vida et al. (2006) and Balbinot Jr. et al. (2003) also showed that the ability of rice to shadow soil was the variable most closely related to its competitive potential, and that this characteristic was associated with rice shoot mass 15 days after seeding.

Experiment 2 highlighted other variable relationships also. For example, there were fewer rice plants when clomazone was applied during PRE, as opposed to POST (Table 5). Also, we found that clo- mazone applied at 400 g ai ha 1 reduced the final plant population compared to the typical Southern Brazil rate of 300 g ha 1, which opposed the work of Sanchotene et al. (2010) who found no differ- ences in rice injury among the main Brazilian varieties, but noted instead differences between doses and soil types.

On the whole, our results indicated not only that clomazone may damage rice and reduce crop density, but also that BRS Querência variety is able to recover from the initial injury and maintain its grain yield potentials as shown by other authors (Jordan et al., 1998; Mudge et al., 2005; Zhang et al., 2004). As TenBrook and Tjeerdema (2005) emphasized, further research is needed to describe how rice can recover from clomazone injury and go on to produce normal yields.

3.3. Weed control

In the control plots, E. crus-galli (L.) P. Beauv and A. denticulata Rudd were found at densities of 815 and 7 plants m 2 (Experiment 1) and 1235 and 8 plants m 2 (Experiment 2), respectively. This research showed that rice crop tolerates clomazone well when it is applied at levels that effectively control E. crus-galli. In this work, E. crus-galli was controlled 87.0%e99.6% at pre-harvest (Tables 4 and 5). In previous work with season-long competition between barnyardgrass ranging from zero to 320 plants m 2, competing with the rice variety IRGA 416 at density of 320 plants m 2, we found that each uncontrolled Echinochloa plant per square meter reduced the rice yield by 64 kg ha 1 (Andres and Menezes, 1997). The variety used in this experiment presents similar features to that used in the previous experiment. In the present study there were no differences in percent of control of E. crusgalli among clomazone PRE rates (Tables 4 and 5). Moreover, PRE and POST application comparisons showed no differences in the two experiments.

The level of A. denticulata control differed according to the orthogonal contrasts when POST applications of clomazone þ propanil (w94% and w90% of control) were compared with clomazone þ ALS-inhibiting herbicides (w98% and w99% of con- trol), respectively for experiment 1 (Table 4) and experiment 2 (Table 5). Both growing seasons exhibited higher POST versus PRE application control of A. denticulata. This remarks the existence of a combination effect of clomazone þ ALS-inhibitors

that broadened the weed control spectrum by effectively limiting E. crus-galli and A. denticulata (Tables 4 and 5). When mixed to clomazone, ALS-inhibiting herbicides were more effective than propanil; when the effect of increasing rates of clomazone was considered, there was a proportional rice yield increase despite crop toxicity. As indicated from our POST application results, and described by Richard and Street (1984) and Galon et al. (2007), successful early stage weed control (4-leaf stage) is associated with early water management, which could be useful in weed control aimed at A. denticulata in paddy rice in particular.

3.4. Plant height
In this study, plant height at harvest was similar across treatments. No differences were detected between PRE and POST application (data not shown), which agreed with the observa- tions of Mudge et al. (2005). In contrast, Zhang et al. (2004) re- ported that clomazone applied at higher rates than those used in this study, reduced rice plant height in North American rice varieties.

3.5. Rice yield and panicle sterility
The high density of weeds, and E. crus-galli in particular, affected rice yields substantially. On average, grain yields recorded in un- treated plots were w51% lower than those obtained in treated plots for both experiments. These losses likely relate, in part, to the higher panicle sterility recorded in untreated plots (Tables 4 and 5).
Herbicide application timing (PRE versus POST) resulted in no yield effects in either experiment (Tables 4 and 5). These results agreed with those reported by Scherder et al. (2004), Zhang et al. (2004) and, Webster et al. (1999).
In Experiment 2, rice yields decreased as the PRE application rate of clomazone rate increased from 300 to 400 g ai ha 1 (Table 5), which is attributable to increased panicle sterility and reduced rice culm density (Table 5). Previous research (Bollich et al., 2000; Jordan et al., 1998), had already linked high rates of cloma- zone (840; 1100; 1700 and 2200 g ai ha 1) to greater rice injury and reduced grain yields. In this study, favorable climate conditions also may have contributed to fast seedling recovery from clomazone injury and therefore, maintaining grain yield potential due to a better plant establishment.
The results of this study showed that clomazone applied alone, pre-emergence at a rate equal to or lower than 400 g ai ha 1 can effectively control E. crus-galli with no lasting injure to the BRS Querência variety. In drill-seeded cultivation, the rice injury due to clomazone application, assessed 7 days after emergence, was no longer visible 28 days after flooding (53 and 44 days after emer- gence, to experiment 1 and 2, respectively). Flooding could be responsible for the reduction of herbicide concentration in the soil, reducing its effect on rice plants. This reduction can be due to simple dilution, herbicide transport (leaching, volatility or runoff), due to higher degradation in anaerobic soil or a combination of all the factors (Martini et al., 2012). According to Tomco et al. (2010), the half-life of clomazone in flooded (anaerobic) soils is 7.9 days while it is 47.3 days in dry (aerobic) soils.
In this study, we found that clomazone rates as high as 400 g ai ha 1 did not cause neither significant bleaching nor affected rice yields; this was attributed to a higher ability of the variety BRS Querência to recover from initial injury and expressing its yield potential.

4. Conclusion
Clomazone caused more injury to rice crop when it was applied during pre- rather than during post-emergence, and injury increased with dose. The highest rice yields were recorded at PRE application trials when rates did not exceed 350 g ai ha 1 due to the ability of the crop to react to clomazone injury by producing more culms. Early E. crus-galli control achieved through pre-emergence application was more important to rice yield than the amount of herbicidal injury at certain levels of herbicide.
In addition, for POST applications, either ALS-inhibiting herbi- cides (bispyribac-sodium and penoxsulam) or propanil may be safely tank-mixed with clomazone, allowing broader weed spec- trum

and increased control of already emerged seedlings of weed species. In Europe, where some E. crus-galli populations demon- strated to be resistant to ALS-inhibitors (Panozzo et al., 2013), clomazone can be a useful tool for managing these populations. Thus, these results might be profitably extended to other rice growing regions of the world where barnyardgrass is among the most important weeds in rice, such as in Asia, European lowlands, and other countries in the Americas.

Acknowledgments

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Table 1. Main physical-chemical features of soil.

Soil characteristic	Experiment 1[a]	Experiment 2[a]
pH_{water} (1:1)	5.3	5.1
Organic matter (g/dm^3)	12	13
Sand (g kg^{-1})	417.7	414.1
Clay (g kg^{-1})	131.7	130.1
Silt (g kg^{-1})	450.7	455.8
P (mg/dm^3)	10.9	11.1
K (mg/dm^3)	38.0	38.0

a: experiment 1=2006/07; experiment 2=2007/08.

Table 2. Main agronomic features.

Planting information	Experiment 1	Experiment 2
Planting date	Oct. 26, 2006	Nov. 07, 2008
Variety	BRS Querência	BRS Querência
Date of emergence	Nov. 03	Nov. 14
Pre-emergence herbicide application	Oct. 27	Nov. 11
Rain 7 days before application (mm)	4	0
Rain 7 days after application (mm)	8	14.8
Rain from application to flood establishment (mm)	131.4	45.3
Post-emergence herbicide application	Nov. 28	Nov. 26
Days after emergence (DAE)	25	12
Air temperature (°C)	17.8	20
Relative humidity (%)	93.8	92.0
Rain 7 days before application (mm)	0	7.1
Rain 7 days after application (mm)	3.4	21.9
BBCH plant stage at herbicide application (Hess et al., 1997)		
O. sativa	14–15	13–14
E. crus-galli	13–14	12–13
A. denticulata	13	12
Density of weeds (m^{-2})		
Echinochloa crus-galli	815	1235
Aeschynomene denticulata	7	8
Interval between herbicide application (days) and flood establishment		
Date of flooding	Nov. 28	Nov. 30
PRE and irrigation	32	19
POST and irrigation	0 (8 h)	4

5 mm after 1 h application.

Table 3. Treatments, rates and intervention periods.

	Treatment	Rate (g ai ha^{-1})	Intervention period
Experiment 1[a]			
1.	Check	e	e
2.	Clomazone	300	PRE
3.	Clomazone	400	PRE
4.	Clomazone	500	PRE
5.	Clomazone þ Propanil	300 þ 2160	POST
6.	Clomazone þ Propanil	400 þ 2160	POST
7.	Clomazone þ Penoxsulam þ crop oil	300 þ 48 þ 0.5% v/v	POST
8.	Clomazone þ Bispyribac-sodium þ mineral oil	300 þ 32 þ 0.5% v/v	POST
Experiment 2[a]			
1.	Check	e	e
2.	Clomazone	300	PRE
3.	Clomazone	400	PRE
4.	Clomazone þ Propanil	300 þ 2160	POST
5.	Clomazone þ Propanil	400 þ 2160	POST
6.	Clomazone þ Penoxsulam þ crop oil	300 þ 48 þ 0.5% v/v	POST
7.	Clomazone þ Bispyribac-sodium þ mineral oil	300 þ 32 þ 0.5% v/v	POST

[a] Experiment 1 ¼ 2006/07; Experiment 2 ¼ 2007/08.

Table 4. Rice injury, culms, sterility, grain yield, weed control, in cv. BRS Querência. Experiment 1.

Treatments	Rate (g ha^{-1})	Herbicide timing	Injury (%) 7	Injury (%) 21	Culms linear meter	Sterility %	Yield kg ha^{-1}	ECHCG[a] Control (%)	AESDE
Check			0	0	67.0	28.1	4586	0	0
Clomazone	300	PRE	7.3	5.5	79.3	15.7	9764	96.5	60.0
Clomazone	400	PRE	18.0	8.0	88.1	20.0	9439	99.0	61.0
Clomazone	500	PRE	26.0	10.5	87.5	20.5	9190	99.0	70.0
Clomazone + Propanil	300 +2160	POST	8.8	5.0	75.5	20.2	9074	94.7	90.8
Clomazone + Propanil	400 + 2160	POST	11.3	5.5	79.1	21.3	9162	95.0	93.3
Clomazone + Penoxsulam + crop oil	300 + 48 + 0.5% v/v	POST	10.5	3.5	83.8	20.0	9264	98.8	98.8
Clomazone + Bispyribac-sodium + mineral oil	300 + 32 + 0.5% v/v	POST	11.0	3.0	75.5	17.0	8742	98.3	100.0
Orthogonal contrasts									
Untreated vs Treated			−13.1**	−5.8**	13.5**	8.4**	−4647**	−97.3**	−82.0**
PRE vs POST			6.4**	4.2**	6.1**	ns	ns	ns	−32.0**
Clomazone PRE 300 vs 400/500			−14.2**	−3.8**	ns	ns	ns	ns	ns
Clomazone PRE 400 vs 500			8.0**	ns	ns	ns	ns	ns	ns
Clomazone: Propanil vs inhibit. ALS			ns	ns	ns	ns	ns	ns	−7.4**

**Difference between treatments evaluated by orthogonal contrasts ($P < 0.05$).
[a] ECHCG − *E. crus-galli*; AESDE − *A. denticulata*.