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Full Paper

Acetolactate Synthase Activity and Growth of Rice (*Oryza sativa* L.) and Weed Species Treated with the Herbicide Propyrisulfuron

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Propyrisulfuron, a recently developed sulfonylurea herbicide, has shown high activity against broadleaf weeds, sedges, and grasses, unlike older sulfonylureas which are active only against broadleaf weeds and sedges. Greenhouse and laboratory studies were conducted to determine if the differential response of three rice cultivars (Azucena, N22 and IR64) and five weed species (*Ludwigia hyssopifolia*, *Cyperus iria*, *Echinochloa colona*, *Echinochloa crus-galli*, and *Leptochloa chinensis*) to propyrisulfuron is a function of its acetolactate synthase (ALS) activity. Growth and ALS activity were measured from test plants grown in sterilized soil under greenhouse conditions. Propyrisulfuron inhibited *in vivo* ALS activity of the five weed species resulting in reduced shoot fresh weight, growth inhibition and eventual death. *Ludwigia hyssopifolia* and *Cyperus iria* were most susceptible, followed by *Echinochloa colona*, *Echinochloa crus-galli*, and *Leptochloa chinensis*. In weeds, inhibition of ALS activity increased progressively with time, with lowest *in vivo* ALS activity and greatest injury at 6 days after herbicide spraying. In contrast, minimal inhibition of *in vivo* ALS activity was observed in the three rice cultivars, where Azucena (*japonica*) showed lesser tolerance than N22 (*aus*) and IR64 (*indica*). Greater seedling injury and *in vivo* ALS inhibition occurred in plants treated at the two-leaf stage than in plants treated at the five-leaf stage. The I_{50} values of *in vitro* ALS activity were similar in rice and weeds *indica*ting that response to propyrisulfuron cannot be attributed to differences in ALS sensitivity at the target site. The study showed that propyrisulfuron is active not only against *L. hyssopifolia* and *C. iria* but also against *E. colona*, *E. crus-galli* and *L. chinensis,* with high selectivity to rice. The ability of propyrisulfuron to control grasses is a characteristic not found in older sulfonylureas. Hence, propyrisulfuron can be used as an alternative herbicide that can control grasses, broadleaf weeds and sedges in rice.

Keywords: acetolactate synthase, *Cyperus iria*, *Echinochloa colona*, *Echinochloa crus-galli, Leptochloa chinensis*, *Ludwigia hyssopifolia,* propyrisulfuron, rice

INTRODUCTION

Rice is the predominant staple food in 17 countries in Asia and the Pacific, 9 countries in North and South America and 8 countries in Africa. The worldwide area cultivated for rice is about 154 M ha, 88% of which is found in the Asian region (FAOSTAT 2012). In order to maximize rice yield, studies about the sources of yield growth in rice have been done, including the changing pattern in farmers' crop management practices (Mataia et al. 2011).

Weeds commonly reduce rice yields by about 10% in transplanted rice to between 20-30% in direct-seeded rice (Rao et al. 2007). A large share of about USD28 B in global herbicide sales that constitutes 47% of the worldwide total sale of agrochemicals demonstrates the significant economic impact of weeds on agriculture (Singh 2006). Among more than 100 weed species associated with rice, the most troublesome species include the grasses such as *Echinochloa colona, Echinochloa crus-galli* and *Leptochloa chinensis*, the broadleaf weed *Ludwigia hyssopifolia* and the sedge *Cyperus iria* (Rao et al. 2007).

Sulfonylurea herbicides such as pyrazosulfuron, imazosulfuron, azimsulfuron, ethoxysulfuron, cyclosulfamuron and halosulfuron are among the most widely used herbicides for the control of broadleaf weeds and sedges in rice (Takeda et al. 1986). Sulfonylureas inhibit acetolactate synthase (ALS), also referred to as acetohydroxyacid synthase (AHAS), the key enzyme involved in catalyzing the first common step in the biosynthesis of valine, leucine and isoleucine in bacteria, yeast and higher plants (Poston et al. 2002; Mc Court et al. 2006). ALS-inhibiting herbicides block the normal function of the acetolactate synthase thereby preventing plant metabolism and cell division or mitosis that leads to plant death (Yoon et al. 2003).

Propyrisulfuron is a new pyrimidinylsulfonylurea developed in 2008 for weed control in rice (Ikeda et al. 2011a; Ort 2012). Propyrisulfuron and other recently developed sulfonylurea compounds possess a fused heterocyclic moiety bonded to a sulfonyl group (Tanaka et al. 2006; Ikeda et al. 2011a). Unlike the older sulfonylureas, propyrisulfuron has shown high activity not only against broadleaf weeds and sedges but also against grasses (Ikeda et al. 2011a). Other newer sulfonylureas with high activity against grasses and high selectivity to rice are azimsulfuron and flucetosulfuron (Pesticide Manual 2000). In addition to high activity against grasses, broadleaf weeds and sedges, propyrisulfuron also showed high activity against sulfonylurea-resistant weeds which have evolved in California, Australia, Japan, Korea and other rice-growing countries, after continuous use of bensulfuron and older sulfonylureas in the past 10-15 years (Ikeda et al. 2011a).

Data obtained from our study will help elucidate the efficacy and selectivity of propyrisulfuron for weed control in rice. Because it also controls grasses as well as broadleaf weeds and sedges, the use of propyrisulfuron will broaden the weed control spectrum. Additionally, propyrisulfuron can be used to control weeds which have developed resistance to older sulfonylureas.

This study was conducted to determine if propyrisulfuron has excellent activity against grasses in addition to broadleaf weeds and sedges and selectivity to rice. The general objective was to determine if the differential response to propyrisulfuron in three rice cultivars, three grasses, one broadleaf weed, and one sedge is a function of the activity of the target enzyme, acetolactate synthase (ALS). Specifically, this study determined: i) the effect of different concentrations and times of application of propyrisulfuron on early seedling growth of rice and weeds; ii) the effect of propyrisulfuron on *in vitro* and *in vivo* ALS activities of rice and weeds; and iii) the effect of propyrisulfuron on ALS activity over time.

MATERIALS AND METHODS

Materials

Seeds of rice cultivars IR64 (*indica*), Azucena (*japonica*) and N22 (*aus*) were obtained from International Rice Research Institute (IRRI). Seeds of *Echinochloa colona* (L.) Link, *Echinochloa crus-galli* (L.) P. Beauv, *Leptochloa chinensis* (L.) Nees., *Ludwigia hyssopifolia* (G. Don) Exell. and *Cyperus iria* (L.) were obtained from the weed seed collection of IRRI.

Analytical grade laboratory reagents were from Belman Laboratories (Manila, Philippines), and the commercial formulation of propyrisulfuron 10 SC used was from Sumitomo Chemicals, Inc., Tokyo, Japan.

Time and place of study

All experiments were conducted at the greenhouse and laboratory facilities of the International Rice Research Institute (IRRI) in Los Baños, Laguna, Philippines (latitude 15^{o} 43' N, longitude 121^{o} 13' E) from December 2011 to December 2012.

Effect of propyrisulfuron on seedling growth

Whole plant dose response assay. Seeds of the three rice cultivars and the five weed species were incubated at 45^{0}C for 90 min. The seeds were sown in sterilized soil at 15 mm depth using plastic containers (100 mm x 100 mm x 70 mm), then were kept in the greenhouse under natural light, temperature (27-32 ^{o}C), and watered daily. Six days after emergence, the seedlings were thinned to one plant per container. All test plants were sprayed at the two-leaf stage with propyrisulfuron (10 SC) at the rates of 0, 12.5, 25, 50, 100 and 200 g a.i. ha^{-1} using a research track sprayer (De Vries Manufacturing, Hollandale, MN) with 214 L ha^{-1} spray volume delivery and spray pressure at 140 kPa, fitted with flat fan nozzles (Teejet 80015E, Spraying Systems Co., North Ave., Schmale Rd., Wheaton, IL 60189). The recommended rate for propyrisulfuron (10 SC) is 50 g a.i. ha^{-1}. At 2, 4 and 6 d after herbicide spraying (DAHS), plant height was measured and phytotoxicity symptoms (chlorosis) were assessed using a visual rating scale (Rahman et al. 2011): 1=highly resistant (green shoot and leaves); 2=resistant (green shoot and light leaves); 3=partly resistant (green shoot and pale yellow leaves); 4=susceptible (partial control/ almost dead); 5=highly susceptible (complete control/dead).

At 6 DAHS, seedlings were harvested; the roots were washed free of soil and separated from the shoots. Fresh weights of shoots and roots were recorded using a Mettler Toledo AG204 analytical balance (New Boston St., Woborn, MA). The dose of propyrisulfuron that causes 50% reduction in plant height and shoot fresh weight (GR_{50}) was determined from the dose response curves using a non-linear computer analysis based on the log-logistic model (Seefeldt et al. 1995).

Single dose herbicidal assay. To determine the effect of propyrisulfuron on rice and weeds beyond 6 DAHS, the monitoring of herbicidal activity of 50 g a.i. ha^{-1} of propyrisulfuron was extended until 14 DAHS. Shoot height and visual phytotoxicity rating were monitored every 2 d.

Effect of propyrisulfuron on *in vitro* ALS activity

Seeds of the three rice cultivars and five weed species were grown in the greenhouse as described above. Test plants at the two-leaf stage were harvested and the shoots were separated from the roots. Leaves were cut into small pieces and stored at -20^{o}C prior to ALS extraction and *in vitro* assay of ALS activity.

Acetolactate synthase extraction in test plants. The process of ALS extraction was conducted at 0-4^{o}C. (Forlani et al. 1991; Yoon et al. 2003). Leaves were thoroughly washed with tap water, cut into small pieces and suspended in 5 mL g^{-1} of ice-cold standard buffer (20 mM potassium phosphate (pH 7.5) containing 200 g L^{-1} of glycerol, 1 mM magnesium chloride, 0.25 mM dithiothreitol, 0.1 mM thiamine pyrophosphate (TPP) and 0.01 mM flavin adenine dinucleotide (FAD). The mixture was homogenized using a Teflon-in-glass potter homogenizer and 10 mg mL^{-1} polyvinyl polypyrrolidone was added. The mixture was centrifuged at 20,000 *g* for 15 min and the supernatant containing ALS was collected.

***In vitro* assay of acetolactate synthase activity.** This assay was conducted following the methods of Westerfield (1945), Fischer et al. (2000), and Hwang et al. (2000). All of the procedures were carried out at 0-4°C. Leaf tissues (0.1 g) were cut into small pieces and placed in vials. A 2-mL reaction mixture was added at a temperature condition of 30°C. The reaction mixture was composed of 1 mL crude enzyme extract and 0.9 mL of 50 mM potassium phosphate buffer (pH 7.5) containing 10 mM magnesium chloride, 0.1 mM TPP, 10 µM FAD and 10 mM sodium pyruvate. The resulting mixture was added with 0.1 mL of 5, 10, 15, 20 and 25 nM propyrisulfuron. To terminate the reaction, 0.05 mL of 3 M H_2SO_4 was added. For the decarboxylation of ALS, the reaction mixture was heated at 30°C for 15 min and then 0.5 mL of 5 g L^{-1} creatine and 50 g L^{-1} 1-napthol dissolved in 100 g L^{-1} NaOH were added. The solution was heated at 60°C for an additional 15 min, centrifuged at 3000 *g* for 3 min, and the absorbance of the supernatant was measured at 530 nm using a UV-Vis Shimadzu 1800 spectrophotometer. To quantify the enzyme reaction products, a standard curve using acetoin was constructed. Specific enzyme activity was determined from the standard curve. One unit of enzymatic activity is defined as the amount of acetoin required to form 1 nmol hr^{-1} of acetolactate. Enzyme concentrations were determined with bovine serum albumin (BSA) as standard using the Bradford method (Bradford 1976). Enzyme solution (50 µL) from each plant sample was pipetted into each microfuge tube. Distilled water (850 µL) and Bradford reagent (100 µL) were added. After 5 min, absorbance at 595 nm was measured using a UV-Vis Shimadzu 1800 spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan).

The concentration of propyrisulfuron that causes 50% inhibition of ALS activity (I_{50}) was derived from the dose response curve using the log-logistic model. The dose response curve shows a plot of % ALS activity and propyrisulfuron dose. Samples were analyzed in triplicate for all experiments.

Effect of propyrisulfuron on *in vivo* ALS activity
This assay was conducted following the procedure of Lovell et al. (1996), Volenberg et al. (2002), and Uchino et al. (2007). Seedlings of the three rice cultivars and five weed species were treated with 0, 12.5, 50, 100 and 200 g a.i. ha^{-1} propyrisulfuron at the two-leaf and five-leaf stages, and ALS activity was analyzed 24 h after treatment with propyrisulfuron. Plants were sprayed with 766 g ha^{-1} of 1,1-cyclopropanedicarboxylic acid (CPCA) containing 2.5 g L^{-1} of the non-ionic surfactant Tween 20 (sorbitan monolaureate) using the research track sprayer 21 h after treatment with propyrisulfuron. Then plants were harvested 3 h after treatment with CPCA, leaves cut into small pieces and frozen at -20°C for 24 h. Frozen leaf tissues (0.1 g) were added with 3 mL distilled water and thawed at 25°C for 45 min by shaking the samples at 15-min intervals in a vortex mixer. Leaf tissue residue was discarded and the 3-mL homogenate aliquot was treated with 50 µL 3 M H_2SO_4 and mixed for 20 sec. The mixture was incubated at 60°C for 30 min to allow conversion of acetolactate to acetoin. To stop the reaction, 1-mL aliquot of creatine and α-naphthol solution (0.9 g L^{-1} and 9 g L^{-1}, respectively) in 2 M NaOH was added to the mixture and the resulting solution was mixed in a vortex mixer for 10 sec. The solution was placed in a 60°C water bath for 30 min to allow the color change from white to pink/red, with higher color intensity indicating higher amount of acetoin, hence, higher ALS activity. Samples were cooled to ambient room temperature and centrifuged at 10,000 *g* for 5 min. Absorbance of the supernatant was measured at 530 nm using a UV-Vis Shimadzu 1800 spectrophotometer. Leaf extracts from untreated control plants (without herbicide and CPCA) were used as comparison for spectrophotometric measurements.The absorption values were converted into µg acetoin hr^{-1} g^{-1} foliage (fresh weight) using a standard curve.

***In vivo* assay of ALS activity in two-leaf stage seedlings over a 6-day period**
To determine the changes in ALS activity over time, the *in vivo* ALS activity was assayed at different time intervals. Plants grown in the greenhouse as described earlier were treated with 50 g a.i. ha^{-1} propyrisulfuron at two-leaf stage. At 2, 4 and 6 DAHS (sampling time), plants were harvested and shoots were separated from the roots. Leaf tissues were cut and stored at -20°C prior to ALS extraction and assay. At each sampling time, the *in vivo* ALS activity was assayed.

Statistical design and data analyses
All experiments were arranged in a completely randomized design with three replications; and the experimental unit was one plant. All data were subjected to one-way analysis of variance and treatment means were compared using Tukey's test. Herbicide concentration causing 50% inhibition (I_{50}) of ALS activity and 50% growth reduction (GR_{50}) of plant height and shoot fresh weight were estimated using the log-logistic model with the aid of Sigma Graph 2005-2010 data graphing and analysis software. To obtain the dose response curves, regression analysis using the following model (Poston 2002) was used:

$$y = C + (D-C)/ [1 + \exp (\log ED_{50} - \log X) b]$$

This model estimates the dose of propyrisulfuron that reduced a certain parameter (ALS activity, shoot height, shoot fresh weight) by 50% (ED_{50}) where: y = parameter of interest (ALS activity, plant height, shoot fresh weight); C = the lower limit of the dose response curve at the highest herbicide concentration; D = the upper limit of the dose-response curve at the lowest herbicide concentration; b = the slope of the dose-response curve around the ED_{50}; ED_{50} = the effective dose concentration that causes 50% reduction in the parameter of interest; X = the herbicide concentration. For all statistical tests, significance was set at P ≤ 0.05.

RESULTS AND DISCUSSION

Effect of propyrisulfuron on seedling growth of rice and weeds

Responses of the test plants to propyrisulfuron were assessed using the visual phytotoxicity rating and the effect on plant height and shoot fresh weight. The visual phytotoxicity rating scale developed by Rahman et al. (2011) considers chlorosis in assessing plant symptoms. Using that scale, the three rice cultivars showed a visual phytotoxicity rating of 1 (green shoot and leaves) to propyrisulfuron even at the highest rate (200 g a.i. ha^{-1}) of application (Table 1).

Plant heights of the rice cultivars were reduced by an average of 20% (lowest rate) to 44% (highest rate). Javier et al. (2005) also showed that herbicide treatment can reduce growth in rice cultivars. Among the rice cultivars, N22 was the most tolerant (Table 2; Figure 1) with only 24% height reduction even at the highest rate of propyrisulfuron. Azucena was the least tolerant with greater reduction in height than either IR64 or N22.

Similar to plant height, shoot weight of N22 was the least affected (Table 3; Figure 2), with 5-20% shoot weight reduction at the lower rates and 35% at the highest rate of propyrisulfuron (Table 3; Figure 2). IR64 showed 56% reduction in shoot fresh weight even at the highest rate of propyrisulfuron. Azucena was most affected (Table 3; Figure 2) with 68% shoot fresh weight reduction at 50 g a.i. ha^{-1} and about 72% reduction at the two highest rates. Less injury in *indica* rice than *japonica* rice might be due to faster herbicide metabolism, resulting in greater tolerance of the *indica* varieties (Beyer et al. 1988). These data indicates genetic variation of rice cultivars in response to propyrisulfuron application. Six days after herbicide spraying, rice plants started to recover and by 14 days after herbicide spraying no height differences were apparent in rice (Figure 3).

Based on phytotoxicity ratings of the five weed species, *L. chinensis* showed tolerance above 50 g a.i. ha while others showed moderate to high susceptibility to propyrisulfuron, especially at the three highest rates (Table 1). Whole plant dose response assay showed that as the rate of herbicide increased, reduction in height and shoot biomass of *E. colona, E. crus-galli, L. chinensis, C. iria* and *L. hyssopifolia* also increased (Tables 2 and 3; Figures 1 and 2). High activity against grasses is not observed in previously developed sulfonylureas like chlorsulfuron and bensulfuron (Ray 1984).

Height of *L. hyssopifolia* and *C. iria* were reduced by 78-95 % at the highest rates while those of *E. colona* and *E. crus-galli* were reduced by 55-72% (Table 2; Figure 1). Plant height of *L. chinensis* was reduced by only 20-29%, even at the highest rate of propyrisulfuron (Table 2; Figure 1).

Table 1. Phytotoxicity rating of rice cultivars and weed species at 6 days after herbicide spraying at different rates of propyrisulfuron

Propyrisulfuron (g a.i. ha^{-1})	Rating[a]							
	E. col	*E. crus.*	*L. chin*	*C. iria*	*L. hyssop.*	IR64 (*ind.*)	Azucena (*jap*)	N22 (aus)
12.5	2	2	1	4	4	1	1	1
25	3	3	1	4	4	1	1	1
50	4	4	2	5	5	1	1	1
100	5	5	2	5	5	1	1	1
200	5	5	2	5	5	1	1	1

[a]Rating scale [Rahman et al. 2011]: 1 = highly resistant (green shoot and leaves); 2 = resistant (green shoot and light leaves); 3= partly resistant (green shoot and pale yellow leaves); 4 = susceptible (partial control/ almost dead); 5 = highly susceptible (complete control/dead)

Table 2. Effect of different rates of propyrisulfuron on height reduction of rice cultivars and weeds under greenhouse conditions [#]

Propyrisulfuron (g a.i. ha^{-1})	Reduction in plant height (%)							
	E. col	*E. crus.*	*L. chin*	*C. iria*	*L. hyssop.*	IR64 (*ind.*)	Azucena (*jap*)	N22 (aus)
0	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{abcd}
12.5	43^{b}	34^{b}	0^{a}	80^{bc}	81^{bc}	21^{b}	19^{b}	20^{ab}
25	60^{bc}	54^{c}	27^{ab}	82^{cd}	86^{c}	24^{bc}	24^{bc}	21^{ab}
50	65^{c}	58^{c}	20^{ab}	83^{cd}	90^{de}	28^{bcd}	33^{cd}	22^{ab}
100	67^{c}	55^{c}	26^{ab}	78^{d}	90^{de}	28^{bcd}	34^{cd}	24^{bcd}
200	72^{bc}	65^{c}	29^{bc}	83^{d}	95^{e}	35^{bcd}	44^{cd}	24^{bcd}
P-value	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0004*	<0.0001*	0.0478
SEM	5.25	7.22	18.09	4.1	1.15	14.71	10.31	31.62

[#]Data are means from three replicates; [a-e]Means in columns with the same letters do not differ significantly according to Tukey's test; *Significant at $P \leq 0.05$

Table 3. Effect of different rates of propyrisulfuron on shoot fresh weight reduction of rice cultivars and weed species under greenhouse conditions#

Propyrisulfuron (g a.i. ha^{-1})	Reduction in shoot fresh weight (%)							
	E. col	*E. crus.*	*L. chin*	*C. iria*	*L. hyssop.*	IR64 (*ind.*)	Azucena (*jap*)	N22 (aus)
0	0^a	0^a	0^a	0^a	0^a	0^a	0^a	0^a
12.5	45^b	39^b	27^b	39^b	44^{ab}	6^b	14^b	0^b
25	55^c	54^c	34^c	57^c	62^{ab}	43^c	27^c	5^c
50	98^d	96^d	67^d	96^d	94^d	50^d	68^d	20^d
100	98^d	96^d	80^e	96^e	94^d	56^e	72^e	30^e
200	98^d	96^d	80^e	96^e	94^d	56^e	72^e	35^f
P-value	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
SEM	2.4	0.7	1.67	0.7	1.07	1.72	0.87	2.43

#Data are means from three replicates; a-fMeans in columns with the same letters do not differ significantly according to Tukey's test; *Significant at $P \leq 0.05$

Table 4. GR_{50} values for shoot height and fresh weight of rice and weed species treated with different rates of propyrisulfuron at the two-leaf stage

Plants	GR_{50} value[a]	
	Shoot height (g a.i. ha^{-1})	Shoot weight (g a.i. ha^{-1})
E.colona	8.27 ± 2.10	18.91 ± 0.12
E.crus-galli	10.23 ± 1.43	21.75 ± 0.07
L.chinensis	40.48 ± 3.90	37.83 ± 2.13
C.iria	7.40 ± 0.58	20.80 ± 0.16
L.hyssopifolia	6.96 ± 0.25	18.44 ± 0.43
IR-64	79.82 ± 2.11	64.30 ± 1.28
Azucena	60.04 ± 3.70	40.66 ± 0.76
N22	88.78 ± 4.77	86.42 ± 1.16

[a]GR_{50} value indicates effective herbicide dose that reduced height of shoot by 50% relative to non-treated plants; Data are means from three replicates

Table 5. I_{50} values of *in vitro* and *in vivo* ALS activity of rice cultivars and weed species treated with different rates of propyrisulfuron at the two-leaf and five-leaf stages

Plants	I_{50} [a]		
	In vitro, nM (two-leaf stage)	*In vivo,* g a.i. ha^{-1} (two-leaf stage)	*In vivo,* g a.i. ha^{-1} (five-leaf stage)
E.colona	22.40 ± 0.27	67.93 ± 2.32	75.32 ± 1.83
E.crus-galli	22.22 ± 0.17	66.88 ± 2.53	74.04 ± 0.77
L.chinensis	22.10 ± 0.12	73.01 ± 2.52	76.58 ± 2.67
C.iria	22.16 ± 0.27	42.02 ± 4.42	67.87 ± 4.51
L.hyssopifolia	19.03 ± 0.47	31.68 ± 1.39	65.34 ± 4.57
IR-64 (*indica*)	22.05 ± 0.23	80.21 ± 2.30	86.94 ± 2.32
Azucena (*japonica*)	21.81 ± 0.71	72.74 ± 1.58	81.04 ± 4.84
N22 (*aus*)	21.93 ± 0.68	87.15 ± 3.43	92.63 ± 3.16

[a]I_{50} indicates the effective herbicide dose that inhibited ALS activity by 50% plants; Data are means from three replicates

Shoot fresh weights of all weeds were reduced by more than 90% at 50 g a.i. ha^{-1} propyrisulfuron, except for *L. chinensis*, with showed a reduction of only 67% (Table 3; Figure 2). These data show that *L. chinensis* was less susceptible to propyrisulfuron. Furthermore, it took 100-200 g a.i. ha^{-1} to reduce the shoot fresh weight of *L. chinensis* by 80% (Table 3). GR_{50} values for plant height and shoot fresh weight (Table 4) of all three rice cultivars were greater than all weed species tested. Among the rice cultivars N22 had the greatest GR_{50} value for shoot height (88.78 ± 4.77) while Azucena had the least (60.04 ± 3.70). The results indicate differences in sensitivity to propyrisulfuron among IR-64 (*indica*), Azucena (*japonica*) and N22 (*aus*), and their tolerance to the herbicide compared to the different weed species.

Our results show a similar pattern with those of previous studies involving different weed species that reported a 70-140 g a.i. ha^{-1} propyrisulfuron rate to be effective against *Cyperus serotinus*, *Cyperus difformis*, *Monochoria vaginalis* and *Echinochloa crus-galli* from emergence until the three-leaf stage (Ikeda et al. 2011a; Ikeda et al. 2011b; Ort et al. 2012). These same studies also indicated rice to be tolerant to propyrisulfuron up to 200 g a.i. ha^{-1}, which is similar to our results.

Effect of propyrisulfuron on *in vitro* and *in vivo* acetolactate synthase activity

In vitro ALS activity. *In vitro* ALS activities of the three rice cultivars and the five weed species were inhibited by propyrisulfuron at all rates, with almost similar I_{50} values, ranging 19-22 nM (Figure 4, Table 5), indicating that differential response is not due to differences in ALS activity at the target site. This agrees with previous reports that ALS enzymes in tolerant and susceptible plant species were equally sensitive to sulfonylureas (Ray 1984). Park et al. (1993) reported similar I_{50} values of *in vitro* ALS activity of two rice cultivars [IR74 (*indica*; more

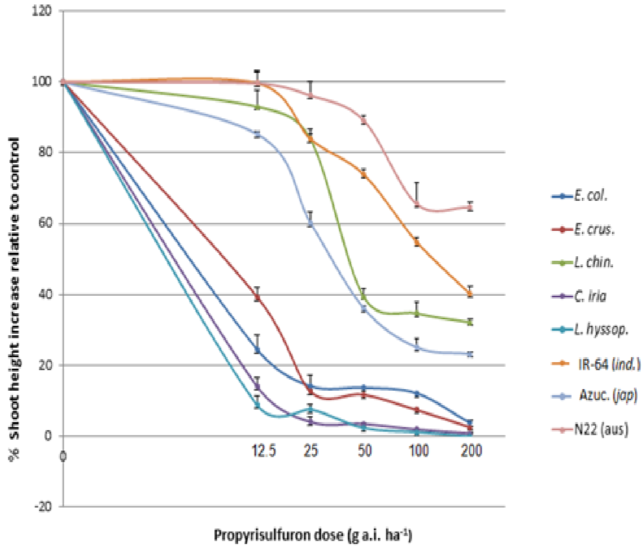

Figure 1. Dose response curves for plant height of three rice cultivars and five weed species treated at the two-leaf stage with different rates of propyrisulfuron

Data are means from three replicates. Vertical bars represent standard error (SE) of the mean.

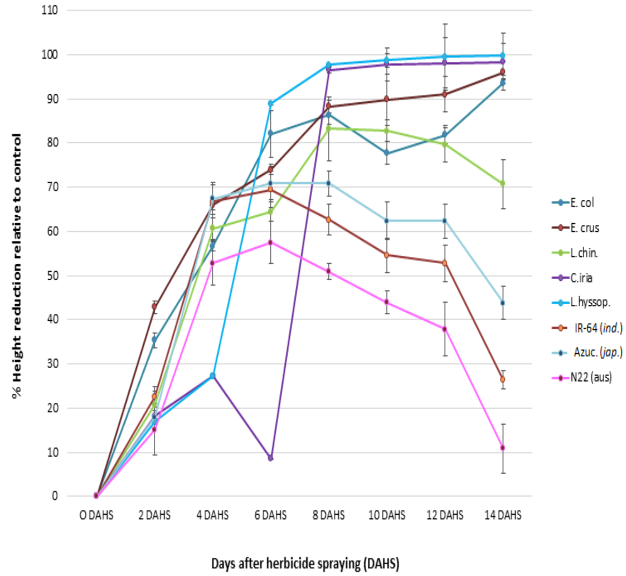

Figure 3. Plant height reduction in three rice cultivars and five weed species from 0 to 14 days after treatment with 50 g a.i. ha^{-1} propyrisulfuron at the two-leaf stage

Data are means from three replicates. Vertical bars represent standard error (SE) of the mean.

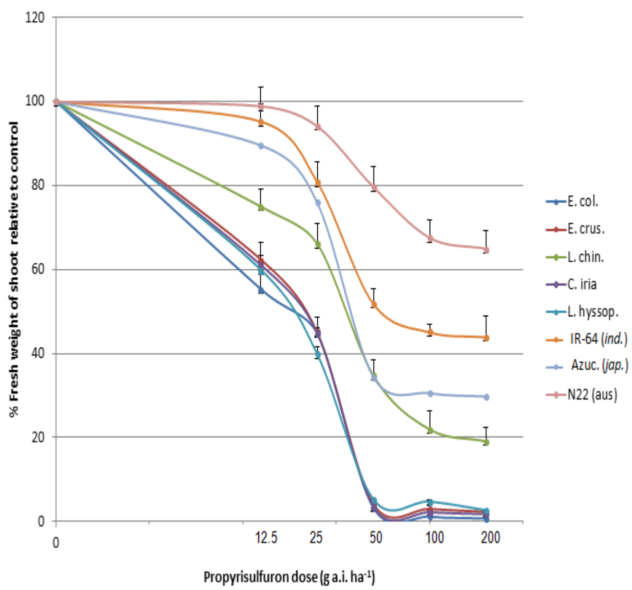

Figure 2. Dose response curves for shoot fresh weight of three rice cultivars and five weed species treated at the two-leaf stage with different rates of propyrisulfuron

Data are means from three replicates. Vertical bars represent standard error (SE) of the mean.

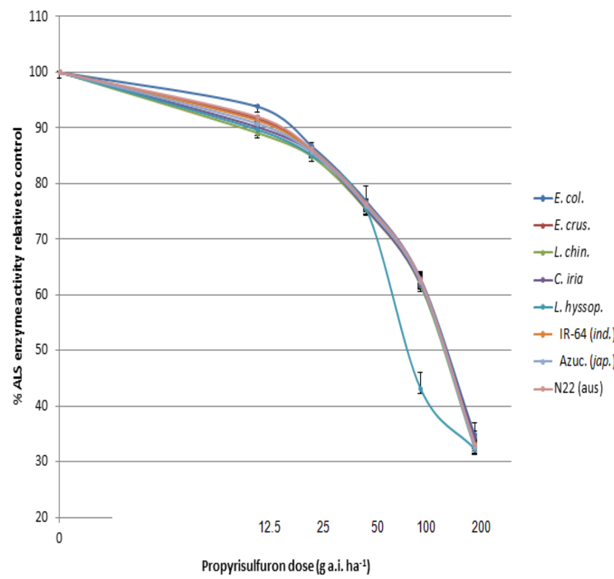

Figure 4. *In vitro* ALS activity in three rice cultivars and five weed species treated at the two-leaf stage with different rates of propyrisulfuron

Data are means from three replicates. Vertical bars represent standard error (SE) of the mean.

tolerant) and Hwajinbyeo (*japonica*)], and by Tanaka and Yoshikawa (1998) in tolerant rice and susceptible *Echinochloa oryzicola* Vasinger and *Cyperus serotinus* Rottb. treated with imazosulfuron. An analog of flucetosulfuron also showed similar I_{50} values for *in vitro* ALS activity in rice (tolerant) and *E. crus-galli* (susceptible) (Hwang et al. 2000). Results from other studies suggested that the selectivity of imazosulfuron was not due to differences in sensitivity of ALS to imazosulfuron but due to the differential metabolism of imazosulfuron by rice and the two weed species.

***In vivo* ALS activity.** Increasing rates of propyrisulfuron resulted in a corresponding decrease in the *in vivo*

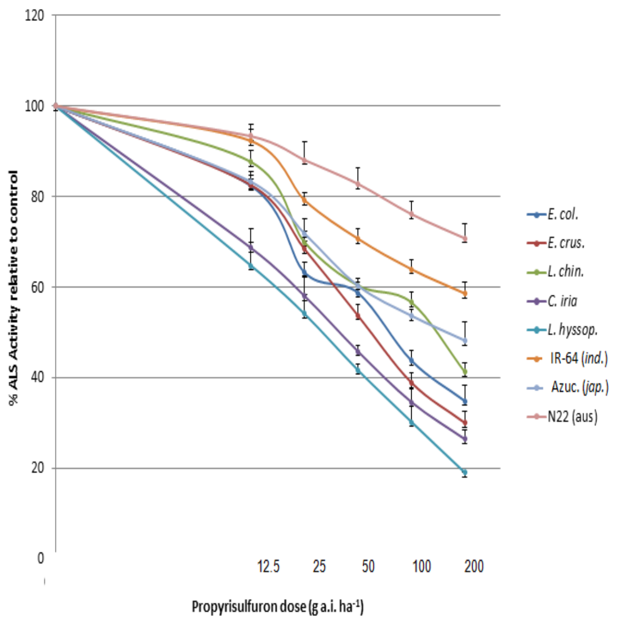

Figure 5. *In vivo* ALS activity in three rice cultivars and five weed species at the two-leaf stage treated with different rates of propyrisulfuron

Data are means from three replicates. Vertical bars represent standard error (SE) of the mean.

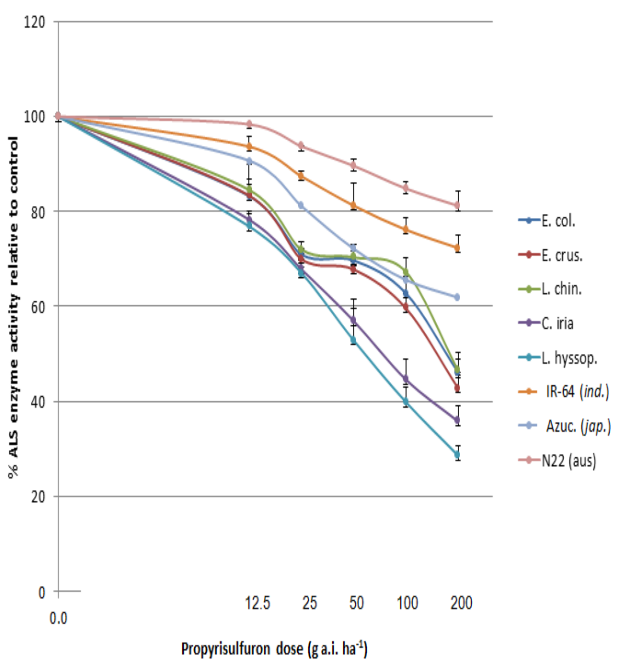

Figure 6. *In vivo* ALS activity in three rice cultivars and five weed species at the five-leaf stage treated with different rates of propyrisulfuron

Data are means from three replicates. Vertical bars represent standard error (SE) of the mean.

ALS activity in both the rice and weed species used in this study (Figure 5). Greater inhibition in *in vivo* ALS activity was observed in plants treated with propyrisulfuron at the two-leaf stage (Figure 5) compared to plants treated at the five-leaf stage (Figure 6). The

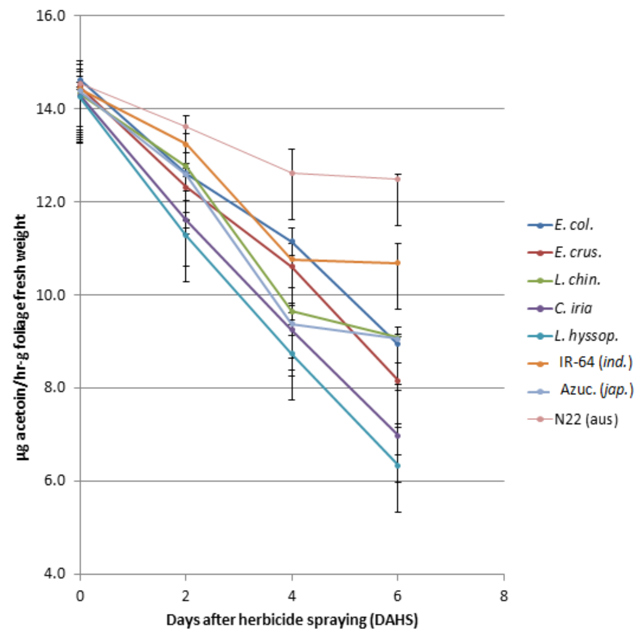

Figure 7. *In vivo* ALS activity in three rice cultivars and five weed species at the two-leaf stage treated with different rates of propyrisulfuron

Data are means from three replicates. Vertical bars represent standard error (SE) of the mean.

greatest inhibition of *in vivo* ALS activity was observed in *L. hyssopifolia* and *C. iria* followed by *E. crus-galli* and *E. colona*. *L. chinensis* showed the least reduction in *in vivo* ALS activity (Figures 5 and 6). Our study showed that the susceptibility of weeds (*E. colona, E. crus-galli, L. chinensis, C. iria* and *L. hyssopifolia*) and the tolerance of rice cultivars (IR-64, Azucena and N22) to propyrisulfuron were due to plant differences in *in vivo* ALS activity (Table 5). Treatment with imazosulfuron has also been reported to result in higher *in vivo* ALS activity in older rice plants than in the younger rice plants (Tanaka and Yoshikawa 1998). The ALS activity of maize (*Zea mays* L.) kernel was also shown to correspondingly increase with age (Muhitch et al. 1987).

Effect of propyrisulfuron on *in vivo* ALS activity over a 6-day period

Inhibition of *in vivo* ALS activity in the three rice cultivars and the five weed species treated with 50 g a.i. ha^{-1} propyrisulfuron at the two-leaf stage increased progressively with time, as reflected in the decreasing amounts of acetoin produced per g fresh weight of leaf tissue from 2-6 d after treatment with propyrisulfuron (Figure 7). In the three rice cultivars, *in vivo* ALS activity also decreased but remained higher than those of the five weed species (Figure 7). Our results suggest tolerance of the three rice *cultivars* and susceptibility of the five weed species to propyrisulfuron. Of the three rice cultivars, N22 (aus) showed the least reduction in *in vivo* ALS activity, followed by *indica* rice (IR64) while *japonica* rice (Azucena) exhibited the greatest reduction in *in vivo* ALS activity.

Reduction in height and fresh weight of the plants could be attributed to the declining ALS activity as a function of herbicide dose. Previous studies showed that ALS inhibitors such as the sulfonylureas decreased ALS activity consequently inhibiting the synthesis of branched chain amino acids, which in turn results in the reduction of plant height and shoot biomass, and in the overall inhibition of growth in susceptible weeds (Tanaka and Yoshikawa 1998; Maja and Branko 2011).

The *indica* cultivar IR64 was more tolerant than the *japonica* cultivar Azucena, but the aus cultivar N22 was more tolerant than either the *indica* or *japonica* cultivars. This genetic variation could potentially be due to differences in translocation, degradation in the roots or metabolic inactivation, which have also been observed in studies using cinosulfuron and bensulfuron (Profelis et al. 1992; Park et al.1993).

Our study shows that propyrisulfuron is active not only against broadleaf weeds such as *L. hyssopifolia* and sedges such as *C. iria* but also against grasses such as *E. colona*, *E. crus-galli* and *L. chinensis,* with high selectivity to different rice cultivars. This is similar to a previous study that reported 90 g a.i. ha^{-1} propyrisulfuron showing high activity against grasses such as *Echinochloa oryzicola* Vasinger and *Echinochloa crus-galli* (L.) P. Beauv*,* sedges such as *Cyperus difformis* L. and broadleaf weeds such as *Monochoria vaginalis* (Burm. f.) C. Presl ex Kunth at the one- to six-leaf stages (Park et al. 1993).

CONCLUSIONS

Propyrisulfuron can control broadleaf weeds, sedges and grasses as indicated by reduced height, shoot biomass and *in vivo* ALS activity. The recommended rate of 50 g a.i. ha^{-1} propyrisulfuron is active not only against *L. hyssopifolia* and *C. iria* but also against *E. colona*, *E. crus-galli,* with high selectivity to rice. However, propyrisulfuron at 100 g a.i. ha^{-1} can be used to control *L. chinensis*. The ability of propyrisulfuron to control grasses is a characteristic not found in older sulfonylureas. This study provided proof that the early effects of propyrisulfuron on rice, though not so severe, could be until 14 DAHS only, indicating that the herbicide has no adverse impacts on consequent growth of rice. Greater seedling injury and *in vivo* ALS inhibition occurred in plants treated at the two-leaf stage than in plants treated at the five-leaf stage. Hence, propyrisulfuron can be used as an alternative herbicide that can control not only of broadleaves and sedges but also of grasses, though further work would be required to determine the species range.

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