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## Selectivity of Tefuryltrione between Rice and *Eleocharis kuroguwai*

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**ABSTRACT.** Tefuryltrione is a new hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor, which has been recently registered for the use for paddy rice, Korea. Dose-response studies were conducted to compare rice safety and weed control efficacy of tefuryltrione against *Eleocharis kuroguwai*. When rice and *E. kuroguwai* were applied at a range of doses of tefuryltrione, GR<sub>90</sub> values (the dose required to inhibit weed growth by 90%) of *E. kuroguwai* were 82.38-93.39 g a.i. ha<sup>-1</sup> in two independent experiments. The GR<sub>10</sub> values (the dose required to inhibit rice growth by 10%) of tefuryltrione for rice were 297.77-471.54 g a.i. ha<sup>-1</sup>. As a result, the selectivity indices (GR<sub>10</sub> for rice/GR<sub>90</sub> for *E. kuroguwai*) of tefuryltrione were 3.19-5.72. Therefore, these results demonstrate that tefuryltrione has a relatively high selectivity between rice and *E. kuroguwai* with a high herbicidal activity against *E. kuroguwai* and a good rice safety.

**Key words:** *Eleocharis kuroguwai*, Hydroxyphenylpyruvate Dioxygenase, HPPD, Rice, Selectivity, Tefuryltrione

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### Introduction

Tefuryltrione is a new herbicide developed by Bayer CropScience AG and belongs to benzoylcyclohexane-1,3-dione herbicides, which inhibit 4-hydroxyphenylpyruvate dioxygenase (HPPD) (Schultz et al., 1993). This inhibition results in decreased plastoquinone (PQ) in plants and consequential inhibition of carotenoid biosynthesis, leading to plant bleaching and death (Norris et al., 1995). New herbicide products containing tefuryltrione were recently registered for the use in paddy rice, Korea in 2015. Other HPPD inhibitors including benzobicyclon and mesotrione were registered as rice herbicides and have already been used for paddy weed control in Korea (KCPA, 2007).

Since the first acetolactate synthase (ALS) inhibitor resistance was reported in paddy fields in 1999 (Park et al., 1999), many efforts have been made to develop new herbicides with different modes of action. Among many different modes of action, inhibitors targeting HPPD drew many attentions since successful commercialization of mesotrione for maize. These HPPD inhibitor herbicides have been known to control many ALS inhibitor resistant

weeds such as *Schoenoplectus juncooides* (synonym-*Scirpus juncooides*) and *Monochoria vaginalis* (Park et al., 2012; Won et al., 2013). Unlike other HPPD inhibitors, tefuryltrione is known to show good herbicidal efficacy against *Eleocharis kuroguwai* (Personal communication with Bayer CropScience).

*Eleocharis kuroguwai* has been considered as one of most problematic perennial sedge weeds and has been reported as a dominant weed species in Korean paddy fields since 1971. The infestation of *E. kuroguwai* is a serious threat to transplanted rice cultivation due to its high competitiveness, resulting in 62% of rice yield loss when not controlled during the whole rice season (Moon et al., 2014). The economic threshold level of *E. kuroguwai* is estimated to be up to 0.8 plants m<sup>-2</sup> under transplanted rice cultivation (Moon et al., 2010), indicating its significant threat and importance in rice. The critical competition period for *E. kuroguwai* is known to be up to 40 days after rice transplanting based on a 5% of acceptable yield loss (Ku et al., 2003), suggesting that *E. kuroguwai* should be controlled up to 40 days after rice transplanting. Therefore, many studies have been made to find effective control measures for *E. kuroguwai*, particularly using sulfonylurea-based mixture herbicides and sequential herbicide application (Im et al., 2003; Moon et al., 2009; Park et al., 2002). However,

as the continuous use of sulfonylurea-based mixture herbicides has caused the development of herbicide resistance and results in increased herbicide costs, these existing measures are no longer viable and effective for *E. kuroguwai* control. Therefore, we should find alternative herbicides with different modes of action for effective *E. kuroguwai* control, otherwise it will still remain troublesome considering its eco-physiological nature (Kim, 2004; Lee et al., 2005).

Therefore, this study was conducted to evaluate the herbicidal activity of tefuryltrione in controlling *E. kuroguwai* and its safety to rice, and consequently to determine the selectivity of tefuryltrione between rice and *E. kuroguwai*.

## Materials and Methods

Pot experiments were conducted in the glasshouse at the experimental farm station of Seoul National University, Suwon, Korea in 2009 and 2010 to evaluate the effects of tefuryltrione on rice and *E. kuroguwai* by soil application.

### Tefuryltrione dose-response study

Fifteen-day-old seedlings of rice (*Oryza sativa* L. cv. Chucheong) and sprouted tubers of *E. kuroguwai* were transplanted in a plastic pot (200 cm<sup>2</sup>) containing paddy soil supplemented with a fertilizer (N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O=55-45-45 kg ha<sup>-1</sup>). Rice and *E. kuroguwai* were grown in a submerged paddy condition maintained at 4±1 cm water depth in the glasshouse. At 12 days after rice transplanting, tefuryltrione (0.7% GR, Bayer CropScience AG, Korea) was applied onto the flooded soil in the pot at 0, 26.25, 52.50, 105, 210, 420, 840 and 1680 g a.i. ha<sup>-1</sup>. All the pots were then arranged in a completely randomized block design with three replicates.

Weed control and phytotoxicity were assessed 40 days after application (DAA) using a visual rating scale from 0% (no weed control or rice damage) to 100% (completely killed) (Sekino et al., 2008). At 40 DAA, two rice plants and six *E. kuroguwai* plants were harvested for measuring the aboveground fresh weight.

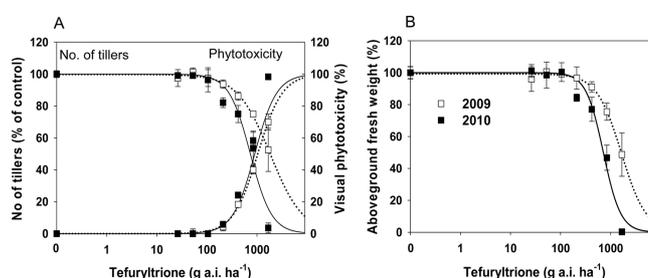
### Statistical analysis

All measurements were initially subjected to analysis of variance (ANOVA). Non-linear regression analysis was conducted by fitting the log-logistic model (Streibig, 1980) to the observed data to estimate the GR<sub>50</sub> value (the dose required for 50% growth reduction) of rice and *E. kuroguwai* and the parameter B (the slope of the dose-response curve). Based on the GR<sub>50</sub> value and the parameter B, the GR<sub>10</sub> (the dose required for 10% growth reduction) value for rice and GR<sub>90</sub> (the dose required for 90% growth reduction) value of *E. kuroguwai* were then estimated. All statistical analyses were conducted using Genstat (Genstat Committee, 2002).

## Results and Discussion

### Rice safety of tefuryltrione

Tefuryltrione showed no or little effect on rice growth at less than the recommended dose of 210 g a.i. ha<sup>-1</sup> until 40 DAA (Fig. 1). When 210 g a.i. ha<sup>-1</sup> of tefuryltrione was applied, rice was visually damaged, resulting in about 4% and 6% visual phytotoxicities at 40 DAA compared with untreated control in 2009 and 2010, respectively. The number of tillers and the fresh weight of rice were also reduced by less than 5% although the bleaching symptom appeared on the leaf and leaf sheath of rice. The bleaching symptom of rice remarkably



**Fig. 1.** The number of tillers and visual phytotoxicity (A) and aboveground fresh weight (B) of rice at 40 days after herbicide application at a range of doses from 0 to 1680 g tefuryltrione a.i. ha<sup>-1</sup> in 2009 (□) and 2010 (■).

**Table 1.** Parameter estimates of the logistic model for the number of tillers, visual phytotoxicity, and aboveground fresh weight of rice in 2009 and 2010. The numbers in parentheses are the standard errors.

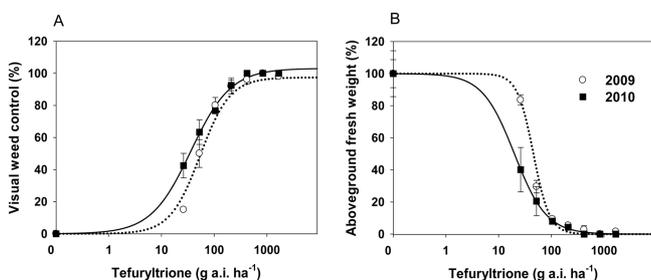
Rice response	Parameter estimates <sup>z</sup>				R <sup>2</sup>	
	GR <sub>50</sub>		B		2009	2010
	2009	2010	2009	2010		
Number of tillers	1839.0 (373.13)	738.0 (74.61)	1.321 (0.476)	2.033 (0.394)	0.74	0.88
Visual phytotoxicity	1031.7 (42.01)	690.4 (0.039)	-1.736 (0.131)	-2.658 (0.241)	0.98	0.98
Aboveground fresh weight	1640.9 (248.11)	714.6 (60.40)	1.762 (0.633)	2.510 (0.491)	0.72	0.91

<sup>z</sup>GR<sub>50</sub>, the dose required 50% growth reduction of rice; B, the slope of the log-logistic model.

appeared at the double rate of the recommended dose at 10 DAA, and diminished within the 30-day period and resulted in 18% and 24% of visual phytotoxicity at 40 DAA in 2009 and 2010, respectively. In addition, the number of rice tillers was reduced by 14% and 25%. The growth of rice sharply decreased with increasing dosage from 840 to 1680 g a.i. ha<sup>-1</sup> of tefuryltrione. As rice was severely affected by bleaching effects of tefuryltrione at 10 DAA, the damage of rice was not recovered even at 40 DAA, resulting in almost 50% phytotoxicity in both years. Our results thus suggest that tefuryltrione can be safely used for rice up to the double rate of its recommended dose although bleaching may occur. By fitting the log-logistic model to the aboveground fresh weight of rice, GR<sub>50</sub> values of rice were estimated to be 1640.90 g a.i. ha<sup>-1</sup> and 714.58 g a.i. ha<sup>-1</sup> in 2009 and 2010, respectively (Table 1). This year variation may be attributed to air temperature difference during the experiment in 2009 and 2010 with average temperature of 25.4°C and 26.8°C, respectively. Higher temperature in 2010 increased susceptibility of rice to tefuryltrione, which is often found in herbicide activity (Hammerton, 1967).

### Herbicidal activity of tefuryltrione against *Eleocharis kuroguwai*

Tefuryltrione showed strong weed control activity against *E. kuroguwai* even at the half of the recommended dose (210 g a.i. ha<sup>-1</sup>) until 40 DAA (Fig. 2). The weed control of *E. kuroguwai* visually remained more than 77% at 40 DAA in both years, while the aboveground fresh weight of *E.*



**Fig. 2.** Visual weed controls (%) (A) and aboveground fresh weights (B) of *Eleocharis kuroguwai* at 40 days after herbicide application at a range of doses from 0 to 1680 g tefuryltrione a.i. ha<sup>-1</sup> in 2009 (□) and 2010 (■)

*kuroguwai* was inhibited to less than 10% of untreated control, resulting in over 90% *E. kuroguwai* control. Even at 52.5 g a.i. ha<sup>-1</sup>, a quarter dose of the recommended dose of tefuryltrione, *E. kuroguwai* was effectively controlled by over 70% of visual weed control at 10 DAA. The growth of *E. kuroguwai*, however, recovered by 30% and 21% of untreated control at 40 DAA in 2009 and 2010, respectively. Our results suggest that tefuryltrione at the half of its recommended dose can effectively control *E. kuroguwai*, but *E. kuroguwai* can regrow and retain its competitiveness at lower doses than the half of the recommended dose. By fitting the log-logistic model to the aboveground fresh weight of *E. kuroguwai*, GR<sub>50</sub> values of *E. kuroguwai* were estimated to be 40.97 g and 19.95 g a.i. ha<sup>-1</sup> in 2009 and 2010, respectively (Table 2). As seen in rice experiment in the above section, tefuryltrione showed greater *E. kuroguwai* control effects in 2010 when air temperature was higher than 2009 during the experiment. Sekino et al. (2008) reported that HPPD inhibitor benzobicyclon effectively controlled *Scirpus juncooides* much faster under high temperature (25°C) than under low temperature (15°C). The higher the air temperature, the greater herbicidal activity of tefuryltrione against *E. kuroguwai* is. However, caution must be given as higher air temperature may decrease rice safety of tefuryltrione as commonly observed in many other herbicides regardless of their modes of action.

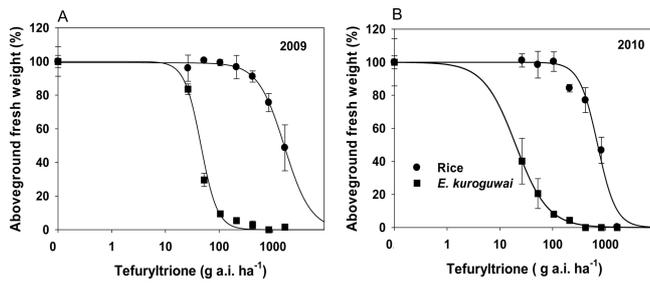
### Selectivity of tefuryltrione between rice and *Eleocharis kuroguwai*

Tefuryltrione showed good efficacy against *E. kuroguwai* even at the half rate of its recommended dose and good safety to rice even at the double rate of its recommended dose. To clarify its selectivity between rice and *E. kuroguwai*, we compared GR<sub>50</sub> values, which were estimated by fitting the log-logistic model to the aboveground fresh weights of rice and *E. kuroguwai* (Fig. 3). The GR<sub>50</sub> values of rice and *E. kuroguwai* were 1640.90 and 40.97 g a.i. ha<sup>-1</sup> in 2009, and 714.58 and 19.95 g a.i. ha<sup>-1</sup> in 2010, respectively (Table 1 and 2). The comparison showed that the GR<sub>50</sub> value of rice was over 40-folds greater than that of *E. kuroguwai* in both years, suggesting that rice is more tolerant to tefuryltrione than *E. kuroguwai*. Although the GR<sub>50</sub> value of rice was much greater

**Table 2.** Parameter estimates of the logistic model for visual weed control and aboveground fresh weight of *Eleocharis kuroguwai* (ELEKU) in 2009 and 2010. The numbers in parentheses are the standard errors.

ELEKU response	Parameter <sup>z</sup>				R <sup>2</sup>	
	GR <sub>50</sub>		B		2009	2010
	2009	2010	2009	2010		
Visual weed control	53.68 (4.30)	34.54 (4.20)	-2.080 (0.296)	-1.274 (0.175)	0.97	0.96
Aboveground fresh weight	40.97 (2.45)	19.95 (6.09)	3.149 (0.451)	1.426 (0.541)	0.97	0.87

<sup>z</sup>GR<sub>50</sub>, the dose required for 50% growth reduction of *Eleocharis kuroguwai* by 50%; B, the slope of the log-logistic model.



**Fig. 3.** Aboveground fresh weights of rice (●) and *Eleocharis kuroguwai* (■) at 40 days after herbicide application at a range of doses from 0 to 1680 g tefuryltrione a.i. ha<sup>-1</sup> in 2009 (A) and 2010 (B).

**Table 3.** GR<sub>90</sub> values of tefuryltrione for *Eleocharis kuroguwai* (ELEKU) control and GR<sub>10</sub> value for rice safety and the selectivity indices in 2009 and 2010.

Year	GR <sub>90</sub> for ELEKU (A)	GR <sub>10</sub> for rice (B)	Selectivity index (B/A)
2009	82.38	471.54	5.72
2010	93.39	297.77	3.19

than that of *E. kuroguwai*, it is difficult to say if tefuryltrione can be practically used for *E. kuroguwai* control in rice field as the herbicide dose-response curves are not parallel. The GR<sub>10</sub> value (the dose required for 10% crop injury or crop growth inhibition) of crop and GR<sub>90</sub> value (the dose required for 90% weed control or weed growth inhibition) of weed are practically acceptable indicators in determining herbicide selectivity between crop and weed (Kim et al., 2006). The GR<sub>10</sub> value of rice and the GR<sub>90</sub> value of *E. kuroguwai* were estimated and then compared to estimate the selectivity index, which was calculated by dividing the GR<sub>10</sub> value of rice by the GR<sub>90</sub> value of *E. kuroguwai*. The selectivity index greater than 1 indicates that the tested herbicide can be practically used to manage the weed in the crop cultivation (Kim et al., 2006). In our study, the GR<sub>10</sub> values of rice were 471.54 and 297.77 g a.i. ha<sup>-1</sup> in 2009 and 2010, respectively, while the GR<sub>90</sub> values of *E. kuroguwai* were 82.38 and 93.39 g a.i. ha<sup>-1</sup> (Table 3). The selectivity index (GR<sub>10</sub>/GR<sub>90</sub>) of tefuryltrione was 5.72 and 3.19 for *E. kuroguwai* in 2009 and 2010, respectively. Therefore, our results clearly demonstrate that tefuryltrione has a good safety to rice with an excellent weed control against *E. kuroguwai*.

#### Further studies

Our results suggest that tefuryltrione can be effectively used for the control of *E. kuroguwai* with acceptable rice safety even at the double rate of its recommended dose. Previous studies reported that HPPD inhibitor herbicides such as benzobicyclon and mesotrione caused significant damage to

rice depending on rice cultivars (Kim et al., 2012; Kim et al., 2014; Yang et al., 2014). Most of HPPD inhibitors caused greater damage to tongil (indica × japonica) rice than japonica rice, except for tefuryltrione, which is known to cause less damage than the other HPPD inhibitors regardless of cultivars (Kwon et al., 2012). Although tefuryltrione showed a good safety to rice at the recommended dose in our study, we need to consider rice cultivars and cultural practices. As our study revealed that tefuryltrione becomes more active under high temperature condition, if farmers use a fixed rate of tefuryltrione, the rate may cause unacceptable level of rice damage under unexpectedly high temperature, which we often experience nowadays due to global warming. Further studies should be conducted to evaluate rice safety and weed control of tefuryltrione for other rice cultivars and cultural practices such as water depth and timing of herbicide application under various environmental conditions. Year and regional variations should not be ignored to establish a guideline for nationwide safe use of new herbicides.

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