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Effective Herbicides for Control of Sulfonylurea-Resistant *Monochoria vaginalis* in Paddy Field

Yong In Kuk*† and Oh Do Kwon**

**Biotechnology Research Institute, Chonnam National University, Gwangju 500-757, Korea*
***Jeonnam Agricultural Research and Extension Service, Naju 520-811, Korea*

ABSTRACT: ***Monochoria vaginalis* is one of the most troublesome resistant weeds in Korean rice culture. Thus, the objectives of this study were to evaluate the response of *M. vaginalis* resistant to sulfonylurea (SU) herbicides and to determine alternative herbicides for the control of resistant *M. vaginalis* in direct seeded and transplanted rice culture in Korea. In greenhouse studies, the resistant biotype was 31-, 38-, 3172-, and 7-fold more resistant to bensulfuron-methyl, cyclosulfamuron, imazosulfuron, and pyrazosulfuron-ethyl, respectively, than the susceptible biotype, indicating cross-resistance to the SU herbicides used in this study. Non-SU herbicides, butachlor, carfentrazone-ethyl, mefenacet, pretilachlor, pyrazolate, and thiobencarb, several SU herbicide-based mixtures, ethoxysulfuron plus fentrazamide, pyrazosulfuron-ethyl plus pyrazolate plus simetryn, and non-SU herbicide-based mixtures, pyrazolate plus butachlor, pyrazolate plus pretilachlor, simetryn plus molinate, carfentrazone-ethyl plus butachlor, and carfentrazone-ethyl plus thiobencarb can be used to control both the resistant and susceptible biotypes of *M. vaginalis* when applied before the second leaf stage. In the field experiment, the resistant biotype of *M. vaginalis* that survived from the paddy fields treated with a SU herbicide-based mixture could effectively be controlled by using mixtures of bentazone plus MCPA, bentazone plus mecoprop-P, and bentazone plus 2, 4-D when applied at 2 or 4 main leaves. Our results suggest that the SU-resistant *M. vaginalis* had not developed multiple resistances to herbicides with different modes of action. In particular, bentazone plus MCPA and bentazone plus mecoprop-P were effective control measures after failure to control resistant *M. vaginalis* in Korean rice culture.**

***Keywords*:** *Monochoria vaginalis* (MOOVA), acetolactate synthase (ALS), herbicide resistance, sulfonylurea herbicide, rice, weed control

Sulfonylurea (SU) herbicides were commercialized in the early 1980s and inhibit acetolactate synthase (ALS), the first enzyme that catalyzes the biosynthesis of branched-chain amino acids, valine, leucine, and isoleucine (Brown, 1990; Ray, 1984). These herbicides are widely used because they control many troublesome weeds at low use rates, provide excellent crop safety, and have low mammalian toxicities (Smith, 1991; Saari *et al.*, 1994). Some annual weeds are tolerant to SU herbicides thus SU herbicide-based mixtures have been widely used in the paddy fields of Korea and other countries to control or suppress a broad spectrum of broadleaf and grass weeds. Repeated use of the same SU herbicide-based mixtures resulted in the development of resistance in several rice weed species.

The first case of resistance to a SU herbicide in Korea was reported *Monochoria korsakowii* in 1999 in monoculture rice fields treated with the same herbicides for eight years (Park *et al.*, 1999). Since then, resistance to SU has been reported in *Monochoria vaginalis* (Kwon *et al.*, 2000), *Rotala indica* (Kuk *et al.*, 2002b), *Lindernia dubia* (Park *et al.*, 2000), *Cyperus difformis* (Kuk *et al.*, 2003), and *Scirpus juncoides* (Kuk *et al.*, 2002a).

The resistance of *M. vaginalis* to SU herbicides can grow as vigorously as the susceptible ones. The occurrence of resistant biotype of *M. vaginalis* in direct-seeded and transplanted rice culture in southeastern Korea caused 70 and 44% rice yield loss, respectively (Kwon *et al.*, 2002). Furthermore, infections of resistant biotypes in rice fields increase weed control cost for the sequential application of foliar herbicides. The objectives of this study were to evaluate the response of *M. vaginalis* resistant to SU herbicides and to determine alternative herbicides for the control of resistant *M. vaginalis* in direct seeded and transplanted rice culture in Korea.

MATERIALS AND METHODS

Plant materials

Seeds of SU-resistant *M. vaginalis* biotypes were collected from a field of the Jeonnam Agricultural Research and Extension Service in Korea. The fields had been treated with a SU-herbicide-based mixture, mainly imazosulfuron (0.25%) plus molinate (5%) and pyrazosulfuron-ethyl (0.07%) plus molinate (5%) for eight consecutive years. The

†Corresponding author: (Phone) +82-062-530-2052 (E-mail) yikuk@chonnam.ac.kr <Received March 3, 2003>

susceptible biotype of *M. vaginalis* seeds was collected from a field where SU herbicides had never been used for the previous three years. The seeds of both biotypes were stored at 4°C for one month to break dormancy.

Response of *M. vaginalis* to SU herbicides

Seeds were sown in plastic pots (280 cm^2 surface area) filled with paddy soil (clay loam), placed in a greenhouse maintained at 30/25°C and a 14/10 h day:night period. When the seedling had reached the two-leaf stage, growing in 3-cm deep simulated flood, the pots were treated with formulation of four SU herbicides, bensulfuron-methyl (0.4, 0.8, 1.6, 12.8, 25.5, 51, 102, 204, 408, and 816 g a.i. ha^{-1}), cyclosulfamuron (0.5, 0.9, 1.9, 3.8, 7.5, 15, 30, 60, 120, 240, 480, and 960 a.i. ha^{-1}), imazosulfuron (0.15, 0.3, 0.6, 1.2, 2.3, 4.7, 9.4, 18.8, 37.5, 75, 150, 300, 600, 1200, and 2400 g a.i. ha^{-1}), and pyrazosulfuron-ethyl (0.15, 0.3, 0.7, 1.3, 2.6, 5.3, 10.5, 21, 42, 84, 168, and 336 g a.i. ha^{-1}). The recommended rates of bensulfuron-methyl, cyclosulfamuron, imazosulfuron, and pyrazosulfuron-ethyl were 51, 60, 75, and 21 g a.i. ha^{-1}, respectively. The responses of *M. vaginalis* were evaluated at 20 days after treatment (DAT) by measuring shoot dry weight. Experiments were repeated two or three times. Data were expressed as a percentage of untreated control to standardize comparisons between resistant and susceptible biotypes. Sigmoid regression (equation 1) or logistic (equation 2) equations

Equation 1: $Y = \dfrac{a}{1 + e - (X - X_{0/b})}$

Equation 2: $Y = \dfrac{a}{1 + (X/X_0)^b}$

were used to describe the response of biotypes to SU herbicides using Statistical Analysis Systems (SAS) software (2000), where Y is the shoot dry weight expressed as a percentage of the untreated control, X is the herbicide rate, and X_0 is the rate at the point of inflection midway between the upper and lower asymptotes. The amount of herbicide that would reduce shoot dry weight by 50% (GR_{50}) was calculated from the regression equations.

Response to non-SU herbicides

Six herbicides, butachlor, carfentrazone-ethyl, mefenacet, pretilachlor, pyrazolate, and thiobencarb with different modes of action (Table 2) were tested for their efficacy on SU-resistant *M. vaginalis*. These herbicides were applied at 1, 2, and 4~5-leaf stages of *M. vaginalis*. These herbicides were applied into the flooded pots in granular form. The shoot dry weight was recorded 30 DAT. Other procedures were the same as those described in the section response of *M. vaginalis* to SU herbicides.

Response to SU herbicide-based mixtures

Six SU herbicides and six non-SU-based mixtures (Table 3) were tested for efficacy on SU-resistant *M. vaginalis*. These herbicides were applied at 2 and 4~5-leaf stages of *M. vaginalis*. Other procedures were the same as those described in the section response of *M. vaginalis* to SU herbicides.

Control of SU-resistant *M. vaginalis* in paddy fields

Field experiments were conducted in paddy fields infested with herbicide-resistant *M. vaginalis* at Chonnam, Chungnam, and Kyounggi, Korea in 2002. The field had been treated with SU herbicide-based mixtures for over five consecutive years.

In water-seeded rice at Chonnam, pyrazosulfuron-ethyl plus molinate at 21 plus 1500 g a.i. ha^{-1} was applied at 10 days after seeding but failed to control *M. vaginalis*. Pregerminated Ilmibyeo seeds (1 to 2 mm radicle) were planted by hand at 50 kg ha^{-1}. Cultural management practices the rice cultivation method of the Rural Development Administration of Korea (1998). Plot size was 3×5 m with random block design with 3 replicates. The herbicides used, application timing, and rates are listed in Table 4. Visual ratings for herbicidal efficacy were recorded at 10, 20, and 30 days after application using an 0-9 scale, were 0 represents no control and 9 being equivalent to complete control. Visual evaluation on crop injury was also recorded during the same periods.

In transplanted rice, two trials were conducted in Kyonggi and Chungnam, Korea in 2002. Other procedures were the same as those described in water-seeded rice.

RESULTS AND DISCUSSION

Response of *M. vaginalis* to SU herbicides

M. vaginalis in paddy fields was easily controlled by SU herbicides. However, the efficacy of the herbicides on *M. vaginalis* had declined greatly after application of SU herbicide-based mixtures for 5~8 consecutive years since 1990, and *M. vaginalis* became the dominant weed in paddy fields. It was suspected that the *M. vaginalis* population had developed resistance to SU herbicides.

The susceptible *M. vaginalis* was very sensitive to the herbicides used in this test and was completely controlled at one-fourth of the recommended rates (data not shown). The GR_{50} values of the resistant and susceptible *M. vaginalis* when applied at 2-leaf stages with bensulfuron-methyl, cyclosulfamuron, imazosulfuron and pyrazosulfuron-ethyl are shown in Table 1. The resistant *M. vaginalis* biotype was about 31, 38, 3172 and 7-fold more resistant to bensulfuron-methyl, cyclosulfamuron, imazosulfuron, and pyrazosulfuron-ethyl, respectively than the susceptible biotype. This indicated that resitant *M. vagianlis* biotype showed cross-resistance to the SU herbicides tested, although there was a large difference in the level of resistance among the herbicides tested. Related research has shown that the SU-resistant biotype of *M. vaginalis* was also cross-resistant to other SU herbicides (Hwang *et al.*, 2001). Other SU-resistant weed species have shown different patterns of cross-resistance. For instance, imazosulfuron-resistant *S. juncoides* was cross resistant to three SU herbicides as well as to imidazolinone herbicide, imazapyr, but not to imazaquin (Kuk *et al.*, 2002a). Thus, the occurrence of SU-resistant *M. vaginalis* could truly became a pratical problem in rice fields.

Response to other herbicides with different modes of action

The resistant biotype of *M. vaginalis* did not show multiple resistance to other herbicides with different modes of action (Table 2). Resistnat and susceptible biotypes were controlled completely by recommended rates of butachlor, carfentrazone-ethyl, mefenacet, pretilachlor, pyrazolate, and thiobencarb when applied at 1-leaf stage. Resistant and susceptible biotypes were also controlled completely by carfentrazone-ethyl and mefenacet when applied at 2-leaf stage, but both biotypes were not controlled completely by butachlor, pretilachlor, pyrazolate, and thiobencarb. All of the herbicides tested showed poor control of both resistant and susceptible biotypes when applied at 4~5-leaf stages. The efficiacy of these herbicides was less when applied late (2 and 4~5-leaf stages) than when applied early (1-leaf stage). Related studies showed that SU-resistant *Rotala indica*, *S. juncoides*, *Cyperus difformis*, and *L. dubia* also did not have multiple resistances to other herbicides with different modes of action (Kuk *et al.*, 2002a and b; Kuk *et al.*, 2003). These results show that control of SU-resistant *M. vaginalis* is possible by rotating herbicides with different modes of action when applied early. However, these herbicides could not be applied late to control *M. vaginalis*.

Response to SU herbicide-based mixtures

Although butachlor, carfentrazone-ethyl, mefenacet, pretilachlor, pyrazolate, and thiobencarb can control SU-resistant *M. vaginalis* when applied early, these herbicide do not control perennial weeds. Paddy fields insfected with the resistant biotype of *M. vaginalis* may also be infested with perennial weeds such as *Eleocharis kuroguwai* and *Sagittaria trifolia*. To achieve some control of perennial species in rice paddies, SU herbicides are rountinely used with the herbicides above. Thus, various SU and non-SU herbicide-based mixtures were tested for the control of resistant *M.*

Table 1. Response of *M. vaginalis* resistant (R) and susceptible (S) to sulfonylurea herbicides.

Biotype	GR_{50} [a] (g a.i. ha^{-1})			
	Bensulfuron-methyl	Cyclosulfamuron	Imazosulfuron	Pyrazosulfuron-ethyl
R	50	67	1586	3.67
S	1.83	1.77	0.5	0.49
R/S ratio[b]	31	38	3172	7

[a]GR_{50} values were that the herbicide concentrations reduced shoot dry weight by 50%, calculated from the regression equations.
[b]R/S ratios were calculated relative to the GR_{50} value of the susceptible biotypes of *M. vaginalis*.

Table 2. Effects of herbicides with modes of action on the susceptible (S) and resistant (R) biotypes of *M. vaginalis* treated at 1, 2, and 4~5-leaf stages. Shoot dry weight was recorded 30 days after treatment.

Herbcide	Rate (g a.i ha^{-1})	1 Leaf		2 Leaf		4~5 Leaf	
		S	R	S	R	S	R
		% Reduction (based on untreated control)					
Butachlor	1500	100	100	59	50	0	0
Carfentrazone-ethyl	75	100	100	100	100	67	73
Mefenacet	1050	100	100	100	100	0	0
Pretilachlor	600	100	100	70	63	0	0
Pyrazolate	1800	100	100	100	100	33	29
Thiobencarb	2100	100	100	94	87	0	0

Table 3. Effects of sulfonylurea herbicide-based mixtures on the susceptible (S) and resistant (R) biotypes of *M. vaginalis* treated at 2 and 4~5-leaf stage. Shoot dry weight was recorded 30 days after treatment.

Herbicide	Rate (g a.i. ha^{-1})	2 leaf stage		4~5 leaf	
		S	R	S	R
		% Reduction (based on untreated control)			
Bensulfuron-methyl+butachlor	51+750	100	56	–	–
Cyclosulfamuron+molinate	60+2100	100	49	–	–
Ethoxysulfuron+fentrazamide	30+300	100	92	–	–
Imazosulfuron+pretilachlor	75+300	100	52	–	–
Pyrazosulfuron-ethyl+molinate	21+1500	100	22	–	–
Pyrazosulfuron-ethyl+pyrazolate+simetryn	15+900+210	100	100	80	63
Carfentrazone-ethyl+butachlor	75+750	100	100	85	80
Carfentrazone-ethyl+thiobencarb	75+2100	100	100	80	85
Pyrazolate+butachlor	1800+1050	100	100	68	60
Pyrazolate+pretilachlor	1800+450	100	100	60	55
Simetryn+molinate	360+1500	100	100	80	70
Simetryn+thiobencarb	300+2100	100	100	90	85

Table 4. Effects of foliar-application herbicides on the surviving *M. vaginalis* treated at 2 or 4-main leaf stage. The survival individuals of *M. vaginalis* were from the paddy fields treated with the mixture of pyrazosulfuron-ethyl plus molinate at 10 days after seeding or transplanting.

Experimental site	Cropping pattern	Herbicide	Rate (g a.i ha^{-1})	Application timing	Herbicidal efficacy[a]			Rice injury[b]		
					10	20	30 DAA	10	20	30 DAA
Kyonggi	Transplanting	Bentazone	2,400	2-main leaf, 15cm high	4	7	8	0	0	0
		Bentazone+Cyhalofop-butyl	1,650+300		3	7	8	1	1	0
		Bentazone+MCPA	800+120		6	8	8	0	0	0
		Bentazone+MCPA	1,200+180		7	9	9	1	1	0
		Bentazone+MCPA	2,400+360		8	9	9	2	1	1
Chungnam	Transplanting	Bentazone	2,400	4-main leaf, 20cm high	4	8	9	0	0	0
		Bentazone+Cyhalofop-butyl	1,650+300		3	3	6	0	0	0
		Bentazone+MCPA	1,200+180		3	8	9	1	1	1
Chonnam	Wet-seeding	2, 4-D	280	2-main leaf, 15cm high	9	9	9	2	3	3
		Bentazone	1,600		8	7	6	0	0	0
		Bentazone+2, 4-D	1,200+140		9	9	9	1	1	1
		Bentazone+MCPA	930+138		9	9	9	2	2	2
		Bentazone+mecoprop-P	1,000+150		9	9	9	2	2	2
		Bentazone+Cyhalofop-butyl	1,650+300		6	8	8	0	0	0
		Carfentrazon-ethy+mecorop	15+150		8	6	6	2	2	2

[a]A rating of 0 represents no weed control and 9 indicates complete control.
[b]A rating of 0 represents no rice injury and 9 indicates complete killed.
[c]DAA, days after application.

vaginalis (Table 3). Most SU herbicides-based mixtures did not control the resistant biotype when applied at 2-leaf stages. However, mixtures of the SU herbicides, ethoxysulfuron plus fentrazamide and pyrazosulfuron-ethyl plus pyrazolate plus simetryn when applied at 2-leaf stages gave acceptible control (over 95%) of the resistant biotype. Although the mixtures of SU herbicides could be control the resistant biotype, use of these mixtures should be restricted to a special need basis. Herbicides with different modes of action, pyrazolate plus butachlor, pyrazolate plus pretilachlor, carfentrazone-ethyl plus butachlor, carfentrazone-ethyl plus thiobencarb, simetryn plus molinate or simetryn plus thiobencarb, controlled the resistant biotype 100% when applied at 2 leaf stage. However, all herbicides used in this study showed poor control of the resistant biotype when applied at 4~5 leaf stages. Therefore, use of herbicides with different modes of action and application timings are necessary to manage SU-resistant *M. vaginalis.*

Control of resistant *M. vaginalis* in field experiments

In this study, soil application of the mixture of pyrazosulfuron-ethyl plus molinate was treated to the fields at 10 days after seeding, but the resistant biotype of *M. vaginalis* was not controlled in the fields. For controlling the survival individual of *M. vaginalis*, the paddy fields were treated with foliar-application herbicides again (Table 4).

Considering the growth stage of the surviving *M. vaginalis*, bentazone, cafentrazone-ethyl, and 2, 4-D were chosen as foliar-application herbicides. Bentazone and 2, 4-D alone at their respective recommended rates (1600 and 280 g a.i ha^{-1}) had 70 and 100% controlling effects on the surviving *M. vaginalis*, respectively at Chonnam experiment field. Bentazone at 2400 g a.i. ha^{-1}, higher than recommended rate gave 100% control in both Chungnam and Kyonggi experiment fields. Although 2, 4-D at 280 g a.i ha^{-1} could be completely control on the surviving *M. vaginalis*, it caused unacceptible phytotoxicity (Table 4) and an increased tillering angle on rice plants (data not shown). Thus, we applied mixtures of bentazone plus MCPA, bentazone plus cyhalofop-butyl, bentazone plus mecoprop-P at various rates, and 2, 4-D at 1/2 of the recommeded rate in order to control the surviving *M. vaginalis*. After failure of *M. vaginalis* control by a SU herbicide-based mixture, the surviving *M. vaginalis* could be controlled by bentazone at 2400 g a.i. ha^{-1}. However, the efficacy on *M. vaginalis* by bentazone application was very low unless paddy fields drained perfectly before the herbicide applied (data not shown) because bentazone is a contact herbicide. In fact, complete drain is very difficulty during rice culture, thus we treated bentazone with MCPA, mecoprop-P, and mecoprop which are hormone-type compounds with systemic action (Herbicide Handbook, 1994) to control *M. vaginalis* at 2 or 4-main leaf. Excellent control of *M. vaginalis* was obtained by the treatment of bentazone plus MCPA and bentazone plus mecoprop-P, regardless of the application rates and experimental sites. There was slight crop injury with the treatment of bentazone plus MCPA observed at 10, 20, and 30 days after application. Bentazone plus cyhalopfop-butyl also gave satisfactory control. The controlling effects on the survived *M. vaginalis* of the mixtures used in this study were all acceptible, regardless of the experimental sites, cropping patterns, rates, and application timing.

We concluded that the *M. vaginalis* accession collected from Jeonnam province in Korea was resistant to imazosulfuron. The resistant biotype was cross-resistant to other SU-herbicides such as bensulfuron-methyl, cyclosulfamuron, and pyrazosulfuron-ethyl. The resistant biotype of *M. vaginalis* does not have multiple resistances. Non-SU herbicides, butachlor, carfentrazone-ethyl, mefenacet, pretilachlor, pyrazolate, thiobencarb, several SU herbicide-based mixtures, ethoxysulfuron plus fentrazamide, pyrazosulfuron-ethyl plus pyrazolate plus simetryn, non-SU herbicide-based mixtures, pyrazolate plus butachlor, pyrazolate plus pretilachlor, simetryn plus molinate, simetryn plus thiobencarb, carfentrazone-ethyl plus butachlor, and carfentrazone-ethyl plus thiobencarb can be used to control both the resistant and susceptible biotypes of *M. vaginalis* when applied before the second leaf stage. Although some mixtures of SU herbicides could be used to control both biotypes, use of these mixtures should be restricted to a special-need basis. However, the resistant biotype of *M. vaginalis* that survived from the paddy fields treated with SU herbicide-based mixtures could effectively be controlled by using mixtures of bentazone plus MCPA, bentazone plus 2,4-D, and bentazone plus mecoprop-P, irrespective of experimental sites, cropping patterns, application timing, and rates. SU resistance can be managed by using an integrated system, which utilizes other herbicides as well as mechanical, cultural, and biological weed control methods. Continuous monitoring of SU-resistant *M. vaginalis*, including its distribution and spread, is imperative for resistance management.

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