J. Pestic. Sci., **33**(4), 364–370 (2008)
DOI: 10.1584/jpestics.G08-11

Original Article

Herbicidal activity of a new paddy bleaching herbicide, benzobicyclon

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(Received April 9, 2008; Accepted July 20, 2008)

The herbicidal activity and properties of a novel paddy herbicide, benzobicyclon [3-(2-chloro-4-mesylbenzoyl)-2-phenylthiobicyclo[3.2.1]oct-2-en-4-one], were examined under flooded conditions. Benzobicyclon showed broad-spectrum activity at doses of 200–300 g a.i./ha when applied from pre-emergence to early post-emergence against annual grass, sedge, and broadleaf weeds in paddy. Benzobicyclon has excellent selectivity of transplanted rice. The most significant herbicidal symptom was bleaching. Benzobicyclon had a wide application window and controlled *Scirpus juncoides* up to 5 leaf stages, which is difficult to control throughout the season using other paddy herbicides. Benzobicyclon controlled *S. juncoides* much faster under high temperature (25°C) than under low temperature (15°C); however, the herbicidal efficacy was almost the same, regardless of the temperature, when the leaf stage of the untreated control was the same. The residual activity of benzobicyclon on *S. juncoides* lasted for at least 8 weeks. The herbicidal activity of benzobicyclon at doses of 200–300 g a.i./ha was not affected by the emergence depth (0–3 cm) of *S. juncoides* or soil types (clay loam, loam, light clay, sandy loam, and heavy clay). Moreover, benzobicyclon controlled sulfonylurea herbicide-resistant biotypes as well as wild-type weeds.

Keywords: benzobicyclon, herbicidal activity, *Scirpus juncoides*, bleaching, paddy, sulfonylurea resistant.

Introduction

In the history of paddy herbicide development, new herbicides have been launched in response to growing demands such as a decreased herbicide dose, broad-spectrum activity against various weeds, transition of troublesome weed species, and high weed-crop selectivity. *Scirpus juncoides* is one of the major troublesome weeds in paddy fields in Japan. Recently, the evolution of sulfonylurea herbicide (SU)-resistant weeds, including *S. juncoides*, was reported in various locations, and these weeds have become one of the major problems of weed control in paddy fields in Japan.[1] During our research to develop a herbicide for *Scirpus*, we found a novel bleaching herbicide, benzobicyclon [3-(2-chloro-4-mesylbenzoyl)-2-phenylthiobicyclo[3.2.1]oct-2-en-4-one], which showed potent herbicidal activity against *S. juncoides* (Fig. 1).[2]

Two types of bleaching herbicides that inhibit carotenoid biosynthesis are known: phytoene desaturase (PDS) inhibitors and *p*-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors.[3] PDS inhibitors, such as norflurazon and diflufenican, are commercial herbicides and are mainly used to control grass and broadleaf weeds in cotton and cereals, respectively. However, these herbicides are less selective for transplanted rice. HPPD inhibitors, such as sulcotrione and pyrazolate, are also commercial herbicides and are mainly used in corn and paddy rice, respectively. Unfortunately, sulcotrione is less selective for transplanted rice, and pyrazolate is less effective against sedge weeds such as *S. juncoides*.

In this study, we describe the herbicidal activity and properties of benzobicyclon and its applicability as a paddy herbicide.

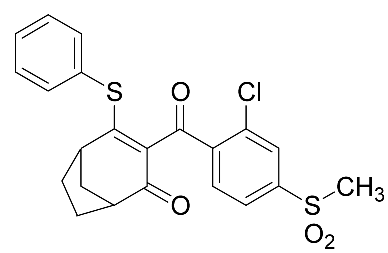

Fig. 1. Chemical structure of benzobicyclon.

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Published online October 10, 2008

Materials and Methods

1. Plant materials

Seeds of *Echinochloa crus-galli* (Ec), *S. juncoides* (Sj), *Monochoria vaginalis* (Mv), and *Lindernia dubia* var. *dubia* (Ld) and tubers of *Cyperus serotinus* (Cs) and *Sagittaria pygmaea* (Sp) were collected from the paddy fields of SDS Biotech, Minori Agricultural Experiment Station, Japan. Seeds of SU-resistant (SU-R) Sj, Mv, and Ld were obtained from the paddy fields of Mie, Akita, and Ibaraki prefectures, respectively. SU-R biotypes of Sj, Mv, and Ld were approximately 1300-, 50-, and 150-fold resistant to bensulfuron-methyl than the susceptible biotypes (wild type), respectively (data not shown). To break dormancy, the seeds were stored at 4°C for 1 month.

2. Evaluation of herbicidal activity

A plastic pot (200 cm^2) was filled with paddy soil (clay loam, pH 5.9; organic matter content, 1.55%), and the soil was flooded after the addition of fertilizer. Seeds of Ec, Sj, Mv, and Ld were sown in the pot, and tubers of Cs and Sp were planted on the soil surface and at 3 cm depth from the soil surface, respectively. After the transplantation of rice seedlings (*Oryza sativa* cv. Koshihikari) at the two-leaf stage, the pots were placed in a greenhouse (day/night temperature was 30°C/18°C) and maintained under flooded conditions (4 cm of water). Benzobicyclon was formulated in a flowable (6% w/v), which is registered in Japan (registration No. 20625). The flowable was diluted with water and applied to the flooded surface at the pre-emergence stage, one-leaf stage (L), or 2 L of Ec, and the irrigation water was then leached (2 cm/day) for 3 consecutive days from the day after each application. Forty days after the application, weed control and crop injury were evaluated using a visual rating scale of 0% (no weed control or crop injury) to 100% (complete inhibition).

3. Herbicidal activity against S. juncoides at various leaf stages

Seeds of Sj were sown in a plastic pot (100 cm^2) filled with paddy soil (clay loam), and the soil was flooded. When Sj grew to 1, 2, 2.5, 4, or 5 L, benzobicyclon flowable was applied as described in method 2. Approximately 3, 5, 7, and 9 weeks after application, weed control was evaluated visually (0–100%).

4 Herbicidal activity against S. juncoides at various emergence depths

Seeds of Sj were sown at 0, 1, 2, or 3 cm depth from the soil surface in a plastic pot (200 cm^2) filled with paddy soil (clay loam), and the soil was flooded. Benzobicyclon flowable was applied to the flooded surface at the pre-emergence stage, 1 L or 2 L, and leaching was carried out as described in method 2. Forty days after application, weed control was evaluated visually (0–100%).

5. Herbicidal activity under various soil textures

A plastic pot (200 cm^2) was filled with 5 types of paddy soils (clay loam, loam, light clay, sandy loam, and heavy clay), and each soil was flooded after the addition of fertilizer. The characteristics of the tested soils are shown in Table 1. Seeds of Sj were sown at a depth of 0.5 cm from the soil surface, and benzobicyclon flowable was applied to the flooded surface at the pre-emergence stage or 1 L. Leaching was carried out as described in method 2. Four weeks after application, weed control was evaluated visually (0–100%).

6. Herbicidal activity under various temperatures

A plastic pot (100 cm^2) was filled with paddy soil (light clay), and the soil was flooded. Seeds of Sj were sown at a depth of 0.5 cm from the soil surface, and benzobicyclon flowable was applied to the flooded surface at the 1 L. The pots were placed in a growth chamber (EYELA FLI-1001; TOKYO RIKAKIKAI, Japan) at 25°C, 20°C, or 15°C (light/dark cycle, 12 h/12 h; light intensity, 270 $\mu mol/m^2/sec$) and maintained under flooded conditions. Weed control was evaluated visually (0–100%), and the evaluation was continued until the efficacy value reached 95%.

Table 1. Characteristics of the tested soils[a)]

Soil texture	Coase sand	Fine sand	Silt	Clay	Organic matter (%)	pH (H_2O)
Sandy loam	12.7	53.6	19.1	14.6	0.89	5.7
Loam	13.5	42.6	40.1	3.8	4.60	5.8
Clay loam	4.1	39.1	33.9	22.9	1.55	5.9
Light clay	5.0	20.2	37.0	37.8	2.69	5.2
Heavy clay	1.8	9.7	35.8	52.7	3.84	5.3

[a)] The physical characteristics of the tested soils were analyzed by PALYNO SURVEY Co. Ltd., Japan.

7. Herbicidal activity against SU-resistant weeds

Seeds of SU-R Sj, Mv, and Ld were sown in a plastic pot (200 cm^2) filled with paddy soil (clay loam), and the soil was flooded. Benzobicyclon flowable was applied to the flooded surface at the pre-emergence stage or 1 L for each weed. Four weeks after application, weed control was evaluated visually (0–100%).

8. Evaluation of residual activity

A plastic pot (880 cm^2) was filled with paddy soil (clay loam or light clay), and each soil was flooded after the addition of fertilizer. The pots were placed in an outdoor environment, and the examination was conducted by synchronizing with the cultivation time in the Kanto region of Japan. Benzobicyclon flowable was applied to the flooded surface, and irrigation water was leached as described in method 2. The surface of the soil was divided into 12 compartments, and seeds of Sj were sown in the assigned compartment weekly. Four weeks after the completion of seeding in each compartment, weed control was evaluated visually (0–100%).

9. Crop injury in transplanted rice

Rice seedlings (*O. sativa* cv. Koshihikari) at 2 L were transplanted in a plastic pot (200 cm^2) filled with paddy soil (sandy loam) at a planting depth of 3 cm or 1 cm, and the pot was maintained under flooded conditions (4 cm of water). Benzobicyclon flowable was applied on the day of transplantation (+0) or 3 days after transplantation (+3), and the irrigation water was then leached as described in method 2. Four weeks after application, the plants were cut at the soil surface to determine the aboveground fresh weight.

10. Evaluation of soil mobility

A column (8 cm in height) composed of 8 pieces of a vinyl chloride ring (11.3 cm in diameter and 1 cm in height) was buried in a plastic pot (200 cm^2) filled with flooded paddy soil (clay loam, loam, light clay, sandy loam, and heavy clay). Benzobicyclon flowable was applied at a dose of 600 g a.i./ha to the flooded surface. The next day, the irrigated water was then drained (2 cm/day) for 3 consecutive days. After complete drainage, the plastic pot was inverted, the buried column was removed, and the packed soil was then separated into individual layers of 1 cm (100 ml soil). Each soil layer was decanted into a plastic pot (30 cm^2), and the soil was flooded. Seeds of Ec and Sj were sown in the pot, and the pot was maintained under flooded conditions (3 cm of water). Four weeks after seeding, weed control was evaluated visually (0–100%).

Results and Discussion

1. Application timing and herbicidal spectrum

In this study, the herbicidal activity of benzobicyclon against gramineous, sedge, and broadleaf weeds, troublesome weeds in paddy fields, and phytotoxicity to transplanted rice were examined. Benzobicyclon exhibited excellent herbicidal activity against all weeds tested, except Sp, in pre-emergence and 1 L applications at doses of 200–300 g a.i./ha without causing phytotoxic injury to rice. Even in 2 L application, benzobicyclon was active against Sj and Mv. Sp required 600 g a.i./ha in the pre-emergence application for adequate control; therefore, among the weeds tested, Sp was less susceptible to benzobicyclon than the other weed species (Table 2).

Bleaching was the major initial symptom in each weed species treated with benzobicyclon. The weeds emerged with bleached leaves or cotyledons after pre-emergence benzobicy-

Table 2. Herbicidal spectrum and rice crop injury of benzobicyclon

Application timing[a]	Dose (g a.i./ha)	Herbicidal activity[b]						Injury[b]
		Ec[c]	Sj[d]	Mv[e]	Ld[f]	Cs[g]	Sp[h]	Os[i]
Pre-emergence	200	97	99	99	98	90	70	0
(+0)	300	99	100	100	100	95	80	0
	600	100	100	100	100	99	90	5
1 L	200	90	99	95	90	85	55	0
(+7)	300	95	99	97	93	93	70	0
	600	100	100	100	97	97	80	0
2 L	200	30	95	90	75	70	20	0
(+10)	300	60	97	93	80	75	45	0
	600	85	99	95	90	85	65	0

[a] Parentheses show the days after transplantation of rice. [b] Forty days after application. Rating scale, 0% (no weed control or crop injury)−100% (complete inhibition). [c] Ec, *Echinochloa crus-galli*; [d] Sj, *Scirpus juncoides*; [e] Mv, *Monochoria vaginalis*; [f] Ld, *Lindernia dubia* var. *dubia*; [g] Cs, *Cyperus serotinus*; [h] Sp, *Sagittaria pygmaea*; [i] Os, *Oryza sativa* cv. Koshihikari.

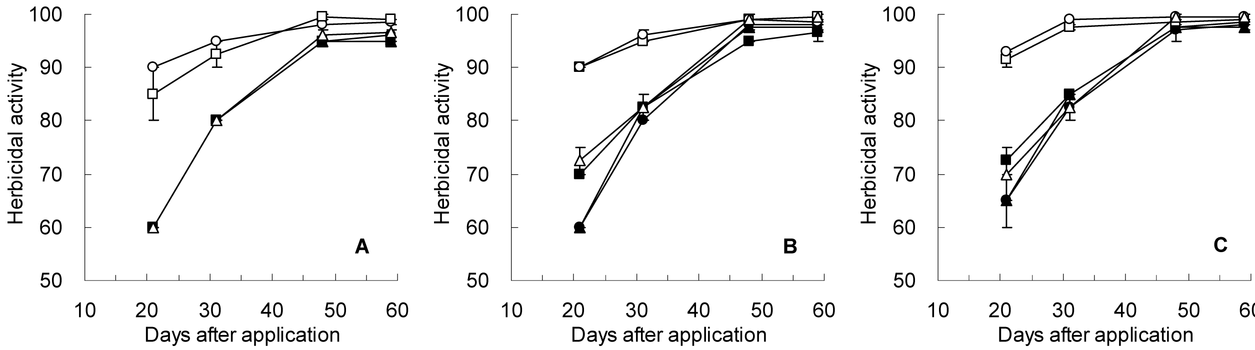

Fig. 2. Herbicidal activity of benzobicyclon against *Scirpus juncoides* in pre-emergence (○), 1-leaf (□), 2-leaf (△), 2.5-leaf (●), 4-leaf (■), and 5-leaf (▲) stage applications at doses of 100 g a.i./ha (A), 200 g a.i./ha (B), and 300 g a.i./ha (C). Rating scale, see Table 2. Data are the mean value of two experiments with two replications. Vertical bars represent ±S.E. Y-axis (Herbicidal activity) covers the range of 50–100%.

clon treatment, and bleaching was observed on newly developed leaves when benzobicyclon was applied post-emergence stage. Bleaching symptoms appeared within 1 week after application, followed by necrosis, leading to weed death. The damage observed in benzobicyclon-treated plants was similar to that caused by pyrazole-type bleaching herbicides such as pyrazolate, pyrazoxyfen, and benzofenap. The herbicidal spectrum of benzobicyclon, however, was quite different from those of pyrazole-type herbicides and benzobicyclon was effective against sedge weeds, especially Sj.[4–6)]

The application window of benzobicyclon against Sj was examined. Benzobicyclon exhibited a wide application window and controlled Sj from pre-emergence up to 5 L at doses of 100–300 g a.i./ha, although the herbicidal rate decreased in higher leaf stages (Fig. 2). Other commercial *Scirpus* herbicides, such as bromobutide and clomeprop, were active when applied from pre-emergence to 2.5 L,[7,8)] but their herbicidal activity decreased in applications over 4 L (data not shown).

2. *Effect of emergence depth of Scirpus juncoides*

Benzobicyclon exhibited excellent herbicidal activity against Sj at doses of 100–300 g a.i./ha, regardless of the emergence depth between 0 and 3 cm. In contrast, the herbicidal activity of dymron depended on the emergence depth, and its herbicidal activity tended to decrease with an increase in the emergence depth of Sj (Table 3). Dymron is mainly absorbed through the root.[9)] The decreased activity of dymron with the increase in the emergence depth may be attributed to the position of the root in the soil. On the other hand, in our preliminary tests, benzobicyclon was absorbed from both shoot and root (data not shown); therefore, the difference in herbicidal activity against deep-emergence Sj between benzobicyclon

Table 3. Effect of emergence depth of *Scirpus juncoides* on herbicidal activity of benzobicyclon

Application timing	Test herbicides	Dose (g a.i./ha)	Herbicidal activity[a)]/Emergence depth			
			0 cm	1 cm	2 cm	3 cm
Pre-emergence	Benzobicyclon	100	99	95	100	95
		200	99	98	99	98
		300	100	100	99	99
	Dymron[b)]	2100	100	98	85	65
1 L	Benzobicyclon	100	99	97	99	96
		200	99	100	100	99
		300	100	100	100	100
	Dymron	2100	95	93	80	65
2 L	Benzobicyclon	100	98	94	93	95
		200	100	97	96	95
		300	99	99	96	99
	Dymron	2100	90	87	70	50

[a)] Rating scale, see Table 2. [b)] Dymron, 1-(1-methyl-1-phenylethyl)-3-*p*-tolylurea applied as flowable (9%, w/v).

Table 4. Effect of soil type on herbicidal activity of benzobicyclon

Application timing	Dose (g a.i./ha)	Herbicidal activity[a]/Soil type				
		Clay loam	Loam	Light clay	Sandy loam	Heavy clay
Pre-emergence	200	98	98	98	95	99
	300	100	98	98	97	99
1 L	200	98	99	98	92	91
	300	100	100	98	95	95

[a] Rating scale, see Table 2.

and dymron is due to the different herbicide-absorption site.

The emergence depth of Sj in paddy fields is mostly within 2 cm but Sj could emerge from the depth of 3–5 cm.[10] Since benzobicyclon could effectively control Sj emerging from the depth of 0–3 cm, it would be effective against most Sj under paddy field conditions.

3. *Effect of soil type*

In general, herbicidal efficacy is affected by various soil factors such as soil texture, organic matter content, clay content, pH, moisture, cation exchange capacity, and phosphate absorption coefficient. These factors influence the adsorption of herbicides by soil particles.[11] Sj was completely controlled by benzobicyclon applied at pre-emergence and 1 L stages at doses of 200 and 300 g a.i./ha in 5 types of paddy soils, namely, clay loam, loam, light clay, sandy loam, and heavy clay (Table 4). Our study results indicated that the herbicidal activity of benzobicyclon against Sj was hardly affected by the soil types.

4. *Effect of temperature*

Sj was completely controlled at all temperatures tested; however, it took longer to reach 90% control at low temperatures. Ninety percent control at 25°C, 20°C, and 15°C was achieved at 14, 22, and 32 days after application, respectively (Fig. 3A). On the other hand, the leaf stage of the untreated control when benzobicyclon showed 90% control was almost the same (5.1 to 5.3 L), irrespective of the temperature (Fig. 3B).

Since official field trials for the registration of benzobicyclon flowable in Japan started in 1994, the JAPR (The Japan Association for Advancement of Phyto-Regulators) has conducted numerous evaluations on the practical application of this herbicide. The variation in the efficacy of this herbicide increased with a decrease in temperature (data not shown). The reason for this variation would be the increased influence of other environmental factors such as water leakage that affects herbicidal activity, resulting in slower herbicidal action at low temperatures.

5. *Herbicidal activity against SU-resistant weeds*

Recently, SU-R weeds have become one of the major problems in paddy fields in Japan.[1] Some herbicides with a different mode of action are available to control SU-R weeds; however, complete control is difficult due to narrow herbicidal

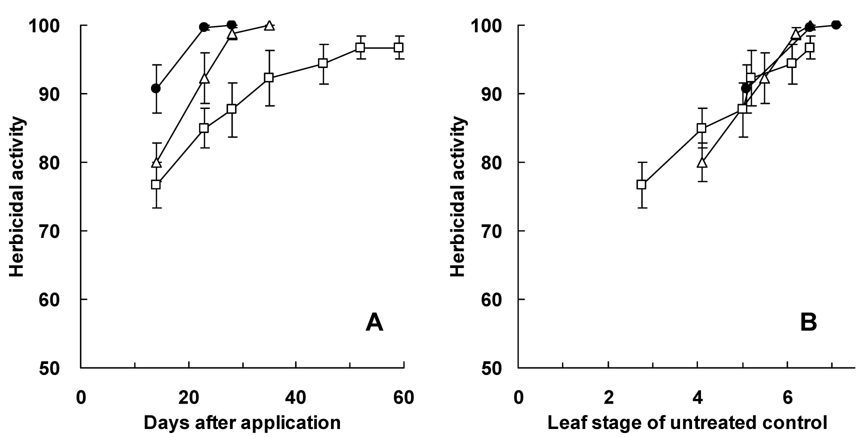

Fig. 3. Herbicidal activity of benzobicyclon against *Scirpus juncoides* in 1-leaf stage application at a dose of 300 g a.i./ha at 25°C (●), 20°C (△), and 15°C (□). (A), Herbicidal activity vs days after application; (B), Herbicidal activity vs leaf stage of untreated control (each temperature) at evaluation timing. Rating scale, see Table 2. Data are the mean value of three replications. Vertical bars represent ±S.E. Y-axis (Herbicidal activity) covers the range of 50–100%.

Table 5. Herbicidal activity of benzobicyclon against sulfonylurea herbicide-susceptible (SU-S) and resistant (SU-R) biotypes of *Scirpus juncoides*, *Monochoria vaginalis*, and *Lindernia dubia* var. *dubia*

Herbicides	Application timing	Dose (g a.i./ha)	Herbicidal activity[a)]/Emergence depth							
			S. juncoides				*M. vaginalis*		*L. dubia*	
			SU-S		SU-R		SU-S	SU-R	SU-S	SU-R
			0 cm	3 cm	0 cm	3 cm	0 cm	0 cm	0 cm	0 cm
Benzobicyclon	Pre-emergence	200	99	99	99	99	99	99	98	98
		300	100	100	100	100	100	100	100	100
	1 L	200	99	99	99	99	95	95	90	90
		300	100	100	100	100	97	98	95	95
Bensulfuron-methyl[b)]	Pre-emergence	150	100	99	20	20	99	20	100	10

[a)] Rating scale, see Table 2. [b)] Bensulfuron-methyl, methyl α-(4,6-dimethoxypyrimidine-2-ylcarbamoylsulfamoyl)-*o*-toluate applied as granules (0.25%).

spectra and low residual activities. Benzobicyclon exhibited excellent herbicidal activity against all SU-R biotypes tested as well as SU-susceptible biotypes (Table 5). The wide herbicidal spectrum of benzobicyclon would be an advantageous property.

6. *Residual activity*

In outdoor trials, in which the cultivation time was synchronized with that of the Kanto region in Japan, Sj was controlled for approximately 8 weeks after the application of benzobicyclon at doses of 200 or 300 g a.i./ha in clay loam and light clay soils (Fig. 4). During this period, newly emerged Sj was bleached and eventually died, and then the residual activity gradually decreased irrespective of the herbicide dose and soil type. This result indicated that benzobicyclon has good residual activity and continues to exert its activity over a longer Sj emergence period.

7. *Rice crop safety*

Benzobicyclon exhibited excellent rice crop safety at doses of 200–600 g a.i./ha under usual (3 cm) transplantation at +0 and +3 application. Moreover, there was only a 10–20% loss in fresh weight at 600 g a.i./ha dose under shallow (1 cm) transplantation at +0 application even in sandy loam soil in which herbicides may cause severe crop injury (Fig. 5). Benzobicyclon exhibited excellent herbicidal activity and broad-herbicidal spectrum when applied from +0 (pre-emergence) to 1 L of Ec, without causing injury to transplanted rice.

8. *Soil mobility*

The downward mobility of benzobicyclon in soil columns was within 1 cm, except for in sandy loam soil, although even here the mobility of benzobicyclon was within 1–2 cm (Table 6). With such low mobility in sandy loam soil, benzobicyclon may not cause adverse effects on shallow transplanted rice at

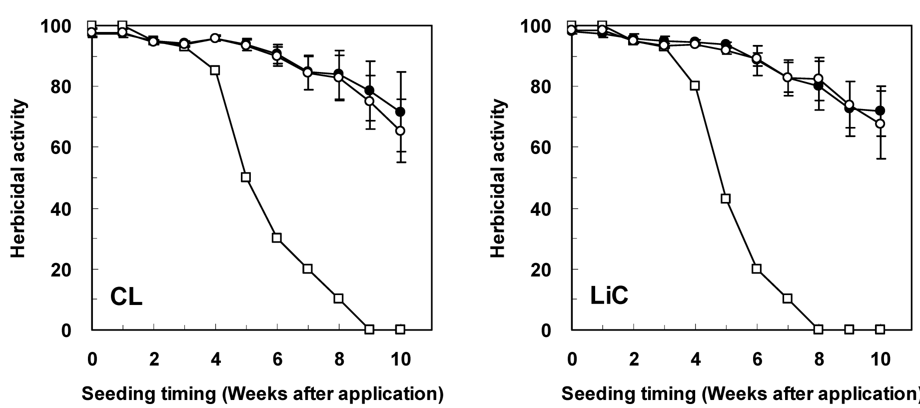

Fig. 4. Residual activity of benzobicyclon against *Scirpus juncoides* in clay loam soil (CL) and light clay soil (LiC) at doses of 200 g a.i./ha (○) and 300 g a.i./ha (●). Rating scale, see Table 2. Data are the mean value of two experiments with two replications. Vertical bars represent ±S.E. Open rectangle (□) represent reference *Scirpus* herbicide, bromobutide; 2-bromo-3,3-dimethyl-*N*-(1-methyl-1-phenylethyl)butyramide (1,800 g a.i./ha) applied as wettable powder (10%).

Table 6. Mobility of benzobicyclon in soil column

Soil layer (cm)	Herbicidal activity[a)]/Soil type									
	E. crus-galli					*S. juncoides*				
	CL[b)]	L[c)]	LiC[d)]	SL[e)]	HC[f)]	CL	L	LiC	SL	HC
0–1	98	93	97	99	97	95	90	95	99	94
1–2	20	0	0	60	0	18	5	40	80	5
2–3	0	0	0	40	0	5	0	20	25	0
3–4	0	0	0	10	0	0	0	0	18	0
4–5	0	0	0	0	0	0	0	0	3	0
5–6	0	0	0	0	0	0	0	0	0	0
6–7	0	0	0	0	0	0	0	0	0	0

[a)] Rating scale, see Table 2. Gray parts represent herbicidal activity over 50%. [b)] CL, clay loam; [c)] L, loam; [d)] LiC, light clay; [e)] SL, sandy loam; [f)] HC, heavy clay.

+0 application (Fig. 5). This result indicated that the soil mobility of benzobicyclon is hardly affected by the soil type and that benzobicyclon is adsorbed tightly by soils; therefore, this property of benzobicyclon would contribute to the stabilization of the herbicide-treated layer in soil, which affects residual activity and prevents groundwater contamination.

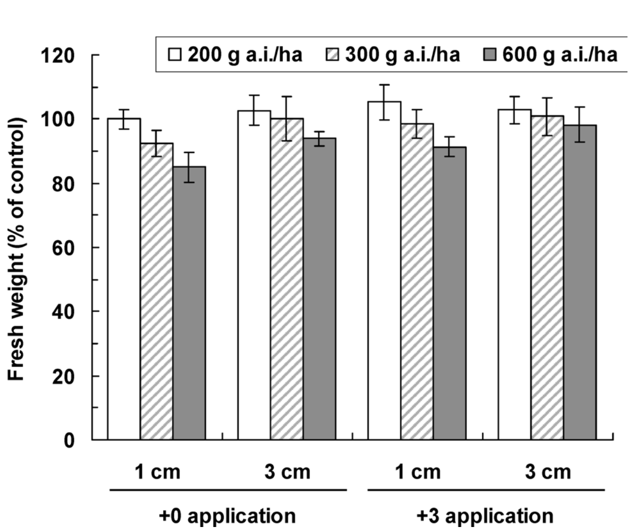

Fig. 5. Crop injury of benzobicyclon in transplanted rice (cv. Koshihikari) under shallow (1 cm) and usual (3 cm) transplantations at +0 and +3 applications. Data are the mean value of two experiments with two replications. Vertical bars represent ±S.E. Fresh weight (g/pot) in untreated control of each experiment: 1 cm-depth transplantation (17.4 and 15.6), 3 cm-depth transplantation (17.0 and 17.9).

Conclusion

Benzobicyclon appears to be an effective herbicide for controlling grass, sedge, and broadleaf weeds when applied from pre-emergence to early post-emergence in paddy fields. Benzobicyclon had a wide application window and good residual activity against Sj. Moreover, benzobicyclon exhibited excellent herbicidal activity against SU-R Sj, Mv, and Ld (Table 5). Accordingly, herbicide mixtures containing benzobicyclon were used widely to control SU-R weeds in 358,500 ha of paddy fields in 2007.

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