Society Awards 2019
(on high prospectiveness)

Development of a rice herbicide, fenoxasulfone

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(Accepted June 10, 2019)

Fenoxasulfone is a novel rice herbicide that was discovered and developed by Kumiai Chemical Industry Co., Ltd. It displays excellent herbicidal activity against Echinochloa spp. and other annual weeds at 150–200 g a.i./ha with long residual activity and has a favorable toxicological, ecotoxicological, and environmental profile. Fenoxasulfone’s mode of action was investigated, and it has been shown to inhibit the biosynthesis of very-long-chain fatty acids in plants. Fenoxasulfone was registered in Japan in 2014, and various products containing fenoxasulfone have been launched. With its high efficacy and long residual activity, we believe that fenoxasulfone will contribute to efficient food production in the future.

Keywords: fenoxasulfone, 3-sulfonylisoxazoline, 2-isoxazoline, paddy rice, herbicide, very-long-chain fatty acid elongase.

Introduction

Rice is a staple food in Japan. For the stable production of rice, weed control in paddy fields is essential. Of annual paddy weeds, Echinochloa spp. has been the most troublesome in Japan for a long time. Chemical tools for the control of Echinochloa spp. have been developed by many companies. However, sometimes such herbicides do not display enough activity in a type of soil or water condition. Also, in recent years, weeds that have developed sulfonyleurea (SU) resistance have become apparent, causing much trouble. Under these circumstances, a herbicide that is not affected by soil types or water conditions and is active against SU-resistant weeds is required. Our purpose was, thus, to discover a new stable rice herbicide that would be active under various environmental conditions against not only Echinochloa spp. but also annual weeds and some SU-resistant weeds.

Before we started exploring such rice herbicides, pyroxasulfone (2: Fig. 1) was being developed as a pre-emergence herbicide for corn, soybeans, cotton and wheat.1–3) Pyroxasulfone was discovered by modifying the skeletal structure of novel 3-sulfonylisoxazoline derivatives (1: Fig. 1), of which R1–R4 are the substituents, and Ar is the aromatic ring including heterocyclic groups.

Through this study, we found that compounds with a benzene ring had unique physicochemical properties such as low solubility in water and strong adsorption by soil. We considered that these properties would provide stable efficacy under flooded rice culture systems and prevent the risk of runoff from paddy rice fields. Therefore, we focused on substituents on the benzene ring and evaluated the herbicidal performance of the resulting compounds. Finally, we discovered fenoxasulfone (Fig. 2) with excellent herbicidal activities and long residual activities.4) In this paper, we describe the discovery, physicochemical properties, biological activity, and mode of action of fenoxasulfone.

1. Discovery of Fenoxasulfone

At first, the optimization of the isoxazoline moiety was carried out, and we found that 5,5-dimethyl-4,5-dihydroisoxazole was the best moiety.1,2) Various benzyl halides were prepared to optimize the benzene ring moiety. The synthetic route of these compounds is shown in Fig. 3. We reconsidered the structure–activity relationship of the paddy field herbicide focusing on its physicochemical properties, especially soil adsorption.

1.1. Effects of substituents on the benzene ring—mono-substituted ring

Initially, substituent positions on the benzene ring were replaced with a methyl group and other substituents (Fig.4). As a result, the ortho-substituent derivative expressed stronger herbicidal activity in comparison with those of other substituents (Table 1). Further, substituent groups other than methyl were examined, and the results showed the same tendency. The herbicidal activ-
ity of the 2-substituted compound indicated that the 2-substitution might be involved in a specific molecular recognition. These findings indicated that the introduction of a functional group at the ortho position was necessary to express stronger herbicidal activity. The effect of the substituent group at the ortho position was then examined. The size, electrical properties, and stability were evaluated, and the results are shown in Table 2.

An ethoxy group or a chlorine atom showed better efficacy against weeds and some safety toward rice. However, these mono-substituted derivatives did not exhibit sufficient efficacy and safety for use as herbicides in paddy field rice cultivation. Under the supposition that an ethoxy group and a chlorine atom were effective substituents for this structure, we further investigated the effect of substituents on the benzene ring by introducing a second substituent.

1.2. Effects of substituents on the benzene ring—di-substituted ring

An ethoxy group and a chlorine atom seemed to be favorable substituents of this structure for use in rice cultivation. Therefore, di-substituted benzene rings (a combination of an ethoxy group and a chlorine atom) were investigated. The results are shown in Table 3. The combination of an ethoxy group and a chlorine atom provided higher herbicidal activity compared with mono-substituted analogs. On the basis of these results, 5-chloro-2-ethoxy compound (Fig. 5) was selected as the lead compound.

Then, to improve crop safety, the effect of functional groups other than an ethoxy group and a chlorine atom at the 2- and 5-positions was investigated (Tables 4 and 5). As shown in Tables 4 and 5, the introduction of other functional groups only reduced herbicidal activity, and an improvement in selectivity was not observed. In parallel with greenhouse trials, the assessment of physicochemical properties (log P, soil adsorption) was performed (Fig. 6).
The log \( P \) value of 5-chloro-2-ethoxy compound was lower, and the soil adsorption was weaker than that of commercial products such as fentrazamide and cafenstrole. It was assumed that weaker soil adsorption would lead to less residual activity. It was clear that an improvement in physicochemical properties was necessary for further development as a rice herbicide. To improve crop safety and physicochemical properties, we therefore aimed to introduce further functional groups onto the benzene ring.

1.3. Effects of substituents on the benzene ring—multi-substituted ring

On the basis of the results obtained so far, multi-substituted compounds with an ethoxy group and two chlorine atoms were synthesized and examined. The results are shown in Table 6. The compound with the 2,5-dichloro-4-ethoxy benzene ring (Fig. 2), termed fenoxasulfone, exhibited excellent herbicidal activity and was found to be very safe. This result demonstrated the necessity of combining all substituents on the benzene ring to achieve a high level of herbicidal activity and excellent crop safety. The performance of multi-substituted derivatives with other functional groups was not sufficient (data not shown). The physicochemical properties of fenoxasulfone were also assessed and compared with those of commercial products (Fig. 7). The physicochemical properties of fenoxasulfone were improved as expected with appropriate values for use in paddy field rice cultivation.

Fenoxasulfone exhibited excellent herbicidal activity and sufficient selectivity. In addition, fenoxasulfone had appropriate physicochemical properties for use in paddy fields. Therefore, fenoxasulfone was selected as an agrochemical candidate, which was found to have excellent efficacy and was highly safe.

2. Physicochemical Properties

Common name (ISO name): Fenoxasulfone
Development code: KIH-1419, KUH-071
Chemical name (IUPAC): 2,5-Dichloro-4-ethoxybenzyl 4,5-dihydro-5,5-dimethylisoxazol-3-yl sulfone
CAS registry number: 639826-16-7
Molecular formula: \( \text{C}_{14}\text{H}_{17}\text{Cl}_{2}\text{NO}_{4}\text{S} \)
Molecular weight: 366.26
Appearance (physical state, form, and color): White crystals, odorless
Melting Point: 157.6°C
Solubility in water: 0.17 mg/L (20°C)
\( \log P_{ow} \): 3.30 (25°C)

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| Table 1. Effect of substituent position (g a.i./ha) |
|-----------------|-------------------|-----------------|-----------------|-----------------|
| \( R \) | \( \text{ED}_{20} \) | \( \text{ED}_{90} \) | \( \text{ED}_{20} \) | \( \text{ED}_{90} \) |
| ORYSA | ECHOR | MOOVA | SCPJO |
| 2-Me | 63 | 32 | 250 | 250 |
| 3-Me | 250 | 63 | 1,000 | 250 |
| 4-Me | 250 | 63 | 1,000 | 250 |

Abbreviations: ORYSA, Oryza sativa (Transplanted rice; cv. Kinmaze, 2.0-leaf stage); ECHOR, Echinochloa oryzicola; MOOVA, Monochoria vaginalis; SCPJO, Schoenoplectus juncoides. Treatment: A drop of diluted solution was applied directly into paddy water. Evaluation: 30 days after application (herbicidal activity and crop injury were visually evaluated on the basis of percentage of the growth relative to that of untreated control), \( \text{ED}_{20} \): The dosage of 20% crop inhibition by visual assessment. \( \text{ED}_{90} \): The dosage of 90% weed control by visual assessment.

The log \( P \) value of 5-chloro-2-ethoxy compound was lower, and the soil adsorption was weaker than that of commercial products such as fentrazamide and cafenstrole. It was assumed that weaker soil adsorption would lead to less residual activity. It was clear that an improvement in physicochemical properties was necessary for further development as a rice herbicide. To improve crop safety and physicochemical properties, we therefore aimed to introduce further functional groups onto the benzene ring.

Table 2. Effect of different ortho substituents (g a.i./ha)

| ortho position | \( \text{ED}_{20} \) | \( \text{ED}_{90} \) | \( \text{ED}_{20} \) | \( \text{ED}_{90} \) |
|----------------|-----------------|-----------------|-----------------|-----------------|
| OMe | 250 | 63 | 1,000 | 250 |
| OEt | 250 | 16 | 250 | 63 |
| OCF2H | 32 | 16 | 125 | 32 |
| Cl | 250 | 16 | 500 | 500 |
| CF3 | 63 | 16 | 250 | 125 |
| CN | 63 | 63 | 500 | 125 |
| COOMe | >1,000 | 250 | >1,000 | >1,000 |
| MeSO2 | 250 | 250 | >1,000 | 250 |

See Table 1 for abbreviations and conditions.
3. Biological Properties

3.1. Biological activity

Greenhouse experiments were conducted at the Life Science Research Institute of Kumiai Chemical Industry Co., Ltd. in Shizuoka, Japan, to evaluate the herbicidal efficacy of fenoxasulfone against *Echinochloa oryzicola* and *Echinochloa crus-galli*. Fenoxasulfone exhibited excellent herbicidal activities against *E. oryzicola* and *E. crus-galli*, from pre-emergence to 3.0-leaf stage at doses of 50–200 g a.i./ha (Fig. 8).

After treating *E. oryzicola* with fenoxasulfone, the first observations were shrinkage of new leaves, darkening of the green color of the body, and suppression of growth. It finally led to death in about 2–3 weeks (Fig. 9).

In addition to *Echinochloa* spp., efficacy of fenoxasulfone against major annual broadleaf weeds in Japanese paddy fields was also examined and it showed excellent herbicidal activities against *Monochoria vaginalis*, *Lindernia* spp., *Gratiola japonica*, *Rotala indica*, and *Ludwigia epilobioides*, which sometimes acquire SU resistance and have presented serious problems in recent years (Fig. 10).

3.2. Residual activity of fenoxasulfone

Residual activity is one of the key factors required in herbicides for paddy field rice cultivation. To confirm the residual activ-
ity, a greenhouse experiment was conducted at the Life Science Research Institute of Kumiai Chemical Industry Co., Ltd. in Shizuoka, Japan. Fenoxasulfone applied at 200 g a.i./ha provided excellent control against *E. oryzicola*, *M. vaginalis*, and *Lindernia dubia* about 60–70 days after application (Fig. 11). It is confirmed that fenoxasulfone has long residual activity as a paddy herbicide.

3.3. Stable effect during overflow conditions

During rice cultivation in Japan, heavy rainfall occurs frequently and sometimes causes overflow of paddy water. Therefore, the herbicidal efficacy and residual activity of fenoxasulfone in overflow conditions were examined. In general, overflow reduces efficacy to some extent; however, significant reduction in efficacy of fenoxasulfone against *E. oryzicola* at 3.0-leaf stage and *M. vaginalis* at 1.0-leaf stage was not observed between the overflow and non-overflow conditions (Fig. 12) and its longer residual activity against *E. oryzicola* in both conditions compared to that of commercial standards was confirmed (Figs. 13). This stable performance is enabled probably because fenoxasulfone has physicochemical properties of strong soil adsorption and low water solubility, and, thus, is less susceptible to water movement. This could also be beneficial for the prevention of its runoff from a paddy rice field and could contribute to ecofriendly...
3.4. Crop safety

To examine the phytotoxicity of fenoxasulfone, greenhouse experiments were conducted at the Life Science Research Institute of Kumiai Chemical Industry Co., Ltd. in Shizuoka, Japan. Transplanted rice shows good tolerance to fenoxasulfone when applied 0–10 days after transplanting at a planting depth of 2 cm or more (Fig. 14). Shallow planting depth (less than 2 cm) may cause damage, including a reduction in rice growth.

3.5. Field trials

Field trials of fenoxasulfone have been conducted officially at multiple test sites in the Tohoku area of Japan as KUH-071 2% granule since 2008. KUH-071 showed excellent control of *Echinochloa* spp., *M. vaginalis*, annual broadleaf weeds, annual sedges, and so on, from 3 days after transplantation to 2.5-leaf stage in the field (Fig. 15). Sufficient levels of crop safety were also observed with rice (Fig. 16). Through a large number of field trials, the applicability of fenoxasulfone for use in paddy fields has been proven.

4. Mode of Action

Herbicidal symptoms of fenoxasulfone were similar to those of VLCFAE-inhibiting herbicides containing pyroxasulfone. The action mechanism of fenoxasulfone was studied by examining the inhibitory effects of this herbicide on the biosynthesis of very-long-chain fatty acids (VLCFAs) (Fig. 17).

Fenoxasulfone treatment decreased the content of VLCFAs—such as C20:0, C20:1, C22:0, C24:0, C24:1, and C26:0 fatty acids—in barnyard millet cultured cells and increased that of long-chain fatty acids and medium-chain fatty acids—such as C18:0 and C15:0—which are precursors of VLCFAs (Table 7, Fig. 13.
Fenoxasulfone potently inhibited the activity of VLCFA elongase (VLCFAE) in the microsomal fraction of etiolated barnyard millet seedlings, which catalyzes the elongation steps from C22:0 to C24:0 and C24:0 to C26:0 (Fig. 19). These results strongly suggest that fenoxasulfone is a potent inhibitor of plant VLCFAEs and should be categorized within group K3 of the Herbicide Resistance Action Committee.

Concluding Remarks

In Japan, the number of agricultural workers is decreasing, and the aggregation of farmland is progressing. In this situation, agricultural workers cannot pay much attention to weed management and a herbicide that exhibits high performance under various conditions is required. Consumers also have increased interest in food safety, and there is high demand for the development of pesticides that are safe not only for people and animals but also for the environment.

This paper has described the discovery and biological aspects of fenoxasulfone. Fenoxasulfone exhibits excellent efficacy against grass weeds and broad-spectrum weed control in paddy field rice cultivation with outstanding residual activity. In addition, its physicochemical properties were strongly suggested to contribute to its stable performance under a variety of environmental conditions and would lead to less environmental impact. Fenoxasulfone has such excellent efficacy and unique characteristics that it is believed to fit the needs of agricultural production today and for the near future. Since 2014, six products have been launched for rice and one product for turf. Also, some products are about to be launched in the market. We expect fenoxasulfone to further contribute to the stable production of food in future.

Acknowledgements

The authors would like to thank everyone involved in the practical evaluation tests and those who offered advice regarding the development of fenoxasulfone from the Japan Association for Advancement of Phyto-regulators and multiple prefectural agricultural experiment stations. The authors also wish to acknowledge National Agriculture and Food Research Organization for the grant to the development of fenoxasulfone. The authors are thankful for the help of everyone in Kumiai Chemical
Fig. 17. Biosynthetic pathway of VLCFAs in plants. VLCFAs catalyze the elongation steps shown by arrows.

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Table 7. Fatty acid contents in barnyard millet cultured cells

| Fatty acid | Content (µg/g Fresh Weight) | Fenoxasulfone |
|-----------|----------------------------|---------------|
|           | 0  | 10⁻⁷ M | 10⁻⁶ M | 10⁻⁵ M |
| C14:0     | 24.6±2.58 | 25.2±6.15 | 23.4±4.28 | 30.3±10.8 |
| C15:0     | 9.35±1.41  | 12.6±2.85  | 32.1±5.99  | 57.6±9.05  |
| C16:0     | 810±84.8  | 765±63.6   | 820±42.2   | 743±98.8   |
| C16:1     | 28.4±4.95 | 33.0±10.3  | 32.1±10.3  | 25.7±9.24  |
| C18:0     | 17.0±1.75  | 15.2±2.49  | 18.5±4.17  | 29.0±5.21  |
| C18:1     | 267±35.0  | 256±29.1   | 300±28.7   | 292±46.0   |
| C18:2     | 1490±138 | 1470±118   | 1570±180   | 1720±157   |
| C18:3     | 104±9.24  | 109±12.3   | 131±12.7   | 132±23.7   |
| C20:0     | 43.3±2.28 | 40.0±3.46  | 35.8±4.72  | 15.2±2.36  |
| C20:1     | 3.75±0.97  | 2.70±0.86  | 1.98±0.30  | 1.58±0.36  |
| C22:0     | 27.1±3.77 | 24.2±3.43  | 20.8±3.19  | 9.14±1.78  |
| C22:1     | n.d.⁶       | n.d.⁶       | n.d.⁶       | n.d.⁶       |
| C24:0     | 75.8±9.31 | 52.5±9.91  | 32.8±5.79  | 18.9±3.06  |
| C24:1     | 14.3±2.79 | 15.6±2.30  | 11.8±3.32  | 9.51±2.62  |
| C26:0     | 22.0±3.52 | 15.4±4.34  | 7.55±1.06  | 7.30±1.03  |

⁶) Data are expressed as the mean±S.D. of 6 independent experiments.

Fig. 18. Inhibitory effects of fenoxasulfone on the biosynthesis of VLCFAs in barnyard millet cultured cells. Relative fatty acid contents in barnyard millet cultured cells treated with 10⁻⁵ M of fenoxasulfone as compared to that in the control without fenoxasulfone are shown. Each data set is expressed as the mean±S.D. of 6 independent experiments.

Fig. 19. Inhibitory effects of fenoxasulfone on VLCFAE activities in etiolated barnyard millet seedlings. Inhibition of VLCFAE in barnyard millet under the condition of 10min pre-incubation of microsomal fractions with fenoxasulfone. VLCFAE activities, which catalyze the elongation steps from C22:0 to C24:0 and C24:0 to C26:0, were 3.0pmol/30min/20µL suspensions.