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Response of a Sulfonylurea (SU)-Resistant Biotype of *Limnophila sessiliflora* to Selected SU and Alternative Herbicides

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Sulfonylurea (SU) herbicides, known for their high herbicidal activity and low mammalian toxicity, were used since 1988 to control *Limnophila sessiliflora* and other broadleaf weeds on rice fields at Sennan Village, Akita Prefecture, Japan. Since 1996, control of *L. sessiliflora* with the SU herbicides was no longer satisfactory. Two greenhouse studies at Tohoku National Agricultural Experiment Station and one experiment in the rice fields at Sennan Village were conducted in 1997 to confirm *L. sessiliflora* resistance to SU herbicides and to compare herbicide treatments for control of SU-resistant *L. sessiliflora*. Greenhouse studies showed that the *L. sessiliflora* biotype from Sennan Village was cross-resistant to four SU herbicides, including bensulfuron-methyl (BSM), pyrazosulfuron-ethyl (PSE), imazosulfuron (IMS), and ethoxysulfuron (ETS). The resistant biotype was 300–900 times more resistant to SU herbicides than the susceptible biotype from Omagari, Akita based on GR_{50} (50% growth reduction) values. Standard treatments of pretilachlor and pentoxazone or mixtures of simetryn + MCPA-thioethyl, ETS + pyrazolate + pretilachlor, BSM + cafenstrole + daimuron, and BSM + daimuron + cafenstrole + azimusulfuron applied postemergence controlled SU-resistant *L. sessiliflora*. In the field experiment, mixtures of IMS + cafenstrole + daimuron and BSM + mefenacet failed to control *L. sessiliflora,* but herbicide treatments controlling SU-resistant *L. sessiliflora* included postemergence applications of mixtures of esprocarb + dimethametryn + PSE + pretilachlor and BSM + thiobencarb + mefenacet and sequential applications of pretilachlor/thiobencarb + simetryn + MCPB, pretilachlor/bifenox + SAP, pretilachlor/MCPA-thioethyl + simetryn, and pretilachlor/BSM + thiobencarb + mefenacet. Our results suggest that the SU-resistant *L. sessiliflora* had not developed multiple resistances to herbicides with different modes of action. In particular, amide or phenoxy herbicides were effective control measures.

INTRODUCTION

Limnophila sessiliflora Blume is a rooted, amphibious aquatic angiosperm, having both submersed and emersed plant parts. In terms of worldwide distribution, it appears to be largely endemic to Asia, particularly Indochina and Malaysia (1–9), and is documented as a major weed problem in paddy rice fields of India, China, Japan, and the Philippines (10–13). With regard to control, very few chemicals registered for use in aquatic systems have been used with success (11, 14), but high levels of 2,4-dichlorophenoxyacetic acid and daily spraying for 8 days with 1000 ppm paraquat (1,1-dimethyl-4,4′ bipyridinum dichloride) gave excellent control of *L. sessiliflora* (11, 14).

Bensulfuron-methyl[2] (BSM) [methyl *a*-[[3-(4,6-dimethoxypyrimidin-2-yl)ureido] sulfony]-*o*-toluate], the first sulfonylurea (SU) used in Japan, is known for its very high herbicidal activity and low mammalian toxicity. The mode of action of the herbicide is through the inhibition of acetolactate synthase (ALS; EC 4.1.3.18)

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[2] Abbreviations used: ALS, acetolactate synthase; BSM, bensulfuron-methyl; CDS-941, BSM + cafenstrole + daimuron; CG-113, pretilachlor; CH-908, BSM + daimuron + cafenstrole + azimusulfuron; ETS, ethoxysulfuron; GR_{50}, 50% growth reduction; HJ-941, ETS + pyrazolate + pretilachlor; Hok-7505, simetryn + MCPA-thioethyl; IMS, imazosulfuron; KPP-314, pentoxazone; PSE, pyrazosulfuron-ethyl; SU, sulfonylurea.

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(also called acetohydroxy acid synthase), which catalyzes the first common step in the biosynthesis of the branched-chain amino acids leucine, isoleucine, and valine. In Japan, BSM and other SU herbicides, such as pyrazosulfuron-ethyl (PSE) [ethyl 5-[[3-(4,6-dimethoxypyrimidin-2-yl)ureido]sulfonyl]-1*H*-1-methylpyrazole-4-carboxylate], imazosulfuron (IMS)[1-(2-chloroimidazo[1,2-a]pyridin-3-ylsulfonyl)-3-(4,6-dimethoxypyrimidin-2-yl)urea], and ethoxysulfuron (ETS) [3-(4,6-dimethoxypyrimidin-2-yl)-1-(2-ethoxyphenoxysulfonyl)urea] have been widely used for early postemergence control of broadleaf and some sedge weeds (including *L. sessiliflora*) in cultivated rice (*Oryza sativa* L.) since 1988. In 1996, rice producers in Sennan Village, Akita Prefecture began to observe that *L. sessiliflora* survived SU herbicide application in their fields. Preliminary greenhouse studies in early spring of 1997 showed that *L. sessiliflora* collections from two rice fields in Sennan Village were not controlled by BSM at two times the recommended rate. The objectives of this research were to (1) investigate the whole-plant response of the suspected BSM-resistant biotype of *L. sessiliflora* to BSM, PSE, IMS, and ETS; (2) evaluate the whole-plant response of resistant *L. sessiliflora* to SU and alternative herbicides that do not inhibit ALS activity; and (3) determine the percentage of the grower's farm that was infested and explain the factors that influenced the spread of resistant *L. sessiliflora* to nonoriginal fields.

MATERIALS AND METHODS

Plant Material

Seeds of suspected SU-resistant *L. sessiliflora* were collected in October, 1996 from plants that had survived SU herbicide treatment in a rice field of Sennan Village, Akita Prefecture. To break dormancy, the harvested seeds were stored in water in a cool room at about 4°C; water was changed twice monthly to remove any germination inhibitors. As a control, seeds of SU-susceptible *L. sessiliflora* were collected in Omagari City, Akita Prefecture and stored in the same manner. Both populations were confirmed to be *L. sessiliflora* by Dr. T. Yamazaki, a plant taxonomist from Tokyo University.

Greenhouse Studies

Response to SU herbicide applications. This experiment was conducted in 1997 in 15.8-cm diameter pots filled with gray lowland soil (28.8% sand, 44.3% silt, and 26.9% clay). When the pots were prepared, 2 g of commercial compound fertilizer, Hommuran No. 888, containing 8:8:8% of N:P:K (produced by Mizuhoyokki Co. Ltd., Kobe, Japan), was given to each pot, which is equivalent to the recommended application dose. During the whole experimental period, no further fertilizer was applied. Seeds were sowed on June 1, 1997 in the greenhouse. Seedlings at the one-leaf stage were treated on June 16 with BSM, IMS, PSE, and ETS under submerged conditions with 2 to 4 cm water depth. Herbicide doses used on three replicate pots of each biotype are shown in Table 1. All herbicides were applied as granules. The number of surviving plants was recorded, three plants of each pot were harvested, and fresh weight was measured 2 months after treatment. Regression analysis (15) was used to compute resistance ratios (R/S), $GR_{50\ resistant}/GR_{50\ susceptible}$, for each herbicide.

Response to other herbicides. Seven herbicides having modes of action different from those of the SU herbicides were also tested for their control of the SU-resistant *L. sessiliflora*. The herbicides tested are shown in Table 2. Pretilachlor [2-chloro-2′,6′-diethyl-*N*-(2-propoxyethyl) acetanilide] (CG-113) granule, pentoxazone [3-(4-chloro-5-cyclopentyloxy-2-fluorophenyl)-5-isopropylidene-1,3-oxazolidine-2,4-dione] (KPP-314) granule, ETS + pyrazolate [4-(2,4-dichlorobenzoyl)-1*H*-1,3-dimethyl-5-pyrazolyl-*p*-toluenesulfonate] + pretilachlor (HJ-941) granule, BSM + cafenstrole [1-(diethylcarbamoyl)-3-(2, 4, 6-trimethylphenylsulfonyl)-1,2,4-triazole] + daimuron [1-(*a*,*a*-dimethylbenzyl)-3-(*p*-tolyl)urea] (CDS-941) flowable, and BSM + daimuron + cafenstrole + azimusulfuron [1-(4,6-dimethoxypyrimidin-2-yl)-3-[4-(2-methyl-2*H*-tetrazol-5-yl)-1*H*-pyrazol-5-yl]sulfonylurea] (CH-908) granule were applied

TABLE 1
Doses of SU Herbicides Used in Greenhouse Study[a]

Herbicide	R-type (g a.i./ha)	S-type (g a.i./ha)
BSM	0, 7.5, 18.8, **75**, 150, 300, 600, 1200, 2400, 4800, 9600	0, 0.375, 0.75, 2.5, 7.5, 18.8, **75**
PSE	0, 2.1, 5.25, **21**, 42, 84, 168, 336, 672, 1344, 2688	0, 0.105, 0.21, 0.7, 2.1, 5.25, **21**
IMS	0, 9, 22.5, **90**, 180, 360, 720, 1440, 2880, 5760, 11520	0, 0.45, 0.9, 3, 9, 22.5, **90**
ETS	0, 2.1, 5.25, **21**, 42, 84, 168, 336, 672, 1344, 2688	0, 0.105, 0.21, 0.7, 2.1, 5.25, **21**

[a] Recommended use rates are shown in boldface.

5 days after sowing, and simetryn [2,4-bis(ethylamino)-6-methylthio-1,3,5-triazine] + MCPA-thioethyl [*S*-ethyl (4-chloro-*o*-tolyoxy) thioacetate] (Hok-7505) granule and BSM + mefenacet [2-(benzothiazol-2-yloxy)-*N*-methylacetanilide] (DPX-84T) granule were applied 20 and 10 days after sowing, respectively, at their recommended rates. This experiment was conducted in the summer of 1997 with pot preparation and materials as described above. The number of plants surviving each herbicide treatment was recorded 2 months after sowing.

Field Experiment

A field experiment was conducted during the rice season of 1997 at Sennan Village, Akita Prefecture. Rice fields with uniform natural infestations of *L. sessiliflora* that survived sulfonylurea treatments in the previous year were selected. The experiment consisted of nine plots in which plots 1 to 4 were used for single one-time treatments, plots 5 to 8 were used for sequential treatments, and plot 9 was a control. Herbicides and application timings used in this experiment are shown in Table 3 and the recommended rates were used. The number of plants surviving each herbicide treatment was recorded 40 days after rice planting.

Field Survey

A field survey was conducted in early July in 1997 on the 489 fields of Sennan Village to determine the proportion of the resistant biotype infestation in the area where the rice fields were treated with SU-based herbicides. The density of *L. sessiliflora* was divided into four classes: high (100 to 200 individuals m^{-2}), middle (10 to 100 m^{-2}), low (1 to 10 m^{-2}), and absent.

RESULTS

Greenhouse Studies

Response to SU herbicide applications. L. sessiliflora from a Sennan Village rice field that had been successively treated with SU-based herbicides for 7 to 8 years exhibited a high level of resistance to BSM, IMS, PSE, and ETS. Even

TABLE 2
Herbicides with Modes of Action Different from Those of the Sulfonylureas Used in Greenhouse Study

Herbicide	Formulation
CG-113	Pretilachlor (4.0%)
KPP-314	Pentoxazone (1.5%)
Hok-7505	Simetryn (4.5%) + MCPA-thioethyl (2.1%)
HJ-941	ETS (0.21%) + pyrazolate (12%) + pretilachlor (4.5%)
CDS-941	BSM (1.4%) + cafenstrole (5.5%) + daimuron (10%)
CH-908	BSM (0.3%) + daimuron (6%) + cafenstrole (3%) + azimusulfuron (0.06%)
DPX-84T	BSM (0.75%) + mefenacet (10%)
Control	—

TABLE 3
Herbicides and Application Timings Used in Field Experiment and the Effect on the Survival of an SU-Resistant *Limnophila sessiliflora* Population

Treatment	Application timing (days after rice planting): 4 days	14 days	Survival (plants/m^2)
Single one-time treatment A		Esprocarb (15%) + dimethametryn (0.6%) + PSE (0.3%) + pretilachlor (4.5%)	1
Single one-time treatment B		IMS (0.9%) + cafenstrole (3%) + daimuron (15%)	120
Single one-time treatment C		BSM (0.75%) + mefenacet (10%)	171
Single one-time treatment D		BSM (0.25%) + thiobencarb (15%) + mefenacet (3%)	0
Sequential treatment A	Pretilachlor (4.0%)	BSM (0.25%) + thiobencarb (15%) + mefenacet (3%)	0
		24–25 days	
Sequential treatment B	Pretilachlor (4.0%)	Simetryn (1.5%) + thiobencarb (10%) + MCPB (0.8%)	0
Sequential treatment C	Pretilachlor (4.0%)	Bifenox (6%) + SAP (5%)	0
Sequential treatment D	Pretilachlor (4.0%)	Simetryn (1.5%) + MCPA-thioethyl (0.7%)	0
Control		—	178

at 4 to 8 times the recommended use rate, mortality was identical to plants in the untreated control (Fig. 1). Growth, flowering, and fruiting were also identical. Even at the 32 times the recommended rate, a substantial number of plants survived. In contrast, all the plants from the susceptible population died at the recommended use rates. The GR_{50} values for the resistant biotype treated at the one-leaf stage with BSM, PSE, IMS, and ETS were more than 895, 334, 655, and 737 times the GR_{50} for the susceptible biotype, respectively (Table 4). The resistance of *L. sessiliflora* to four SU herbicides, ranked from highest to lowest levels of resistance, was BSM > ETS > IMS >> PSE.

Response to other herbicides. Plants from the SU-resistant population of *L. sessiliflora* were effectively killed by pretilachlor, pentoxazone, MCPA-thioethyl, pyrazolate, and cafenstrole applied at recommended rates (Table 5). These tests suggest that control of SU-resistant *L. sessiliflora* is possible with herbicides having a different mode of action applied at the early stage or middle stage of growth.

Field Experiment

Results of the 1997 field experiment are shown in Table 3. The SU-resistant *L. sessiliflora* was not controlled with BSM + mefenacet in single one-time treatment C, whereas BSM + mefenacet + thiobencarb [*S*-(4-chlorobenzyl)-diethylthiocarbamate] gave 100% control (single one-time treatment D). This indicates that thiobencarb is effective in controlling the *L. sessiliflora* even if applied 14 days after rice planting. When SU herbicides were mixed with cafenstrole and daimuron applied 14 days after rice planting in single one-time treatment B, only 33% of the *L. sessiliflora* were controlled (Table 3), although 100% of the *L. sessiliflora* were controlled in the greenhouse study (Tables 2 and 5). There are two likely explanations for this result. One is due to the difference in the timing of treatment. Cafenstrole can control the *L. sessiliflora* more effectively applied at the early stage (one-leaf stage) than at the middle stage of growth. The other is due to the difference of conditions between the greenhouse and the field experiments. Since the experimental field in Sennan Village was located in a spring water area, the action of the herbicide may have been influenced by movement of spring water and by rainfall after herbicide application. Herbicidal efficacy is often greater in the greenhouse than in the field. Moreover, judging from the result of this experiment, the effect of thiobencarb in controlling *L. sessiliflora* was much more stable than cafenstrole under the same condition in the field experiment. To control SU-resistant *L. sessiliflora* effectively, it was necessary to apply

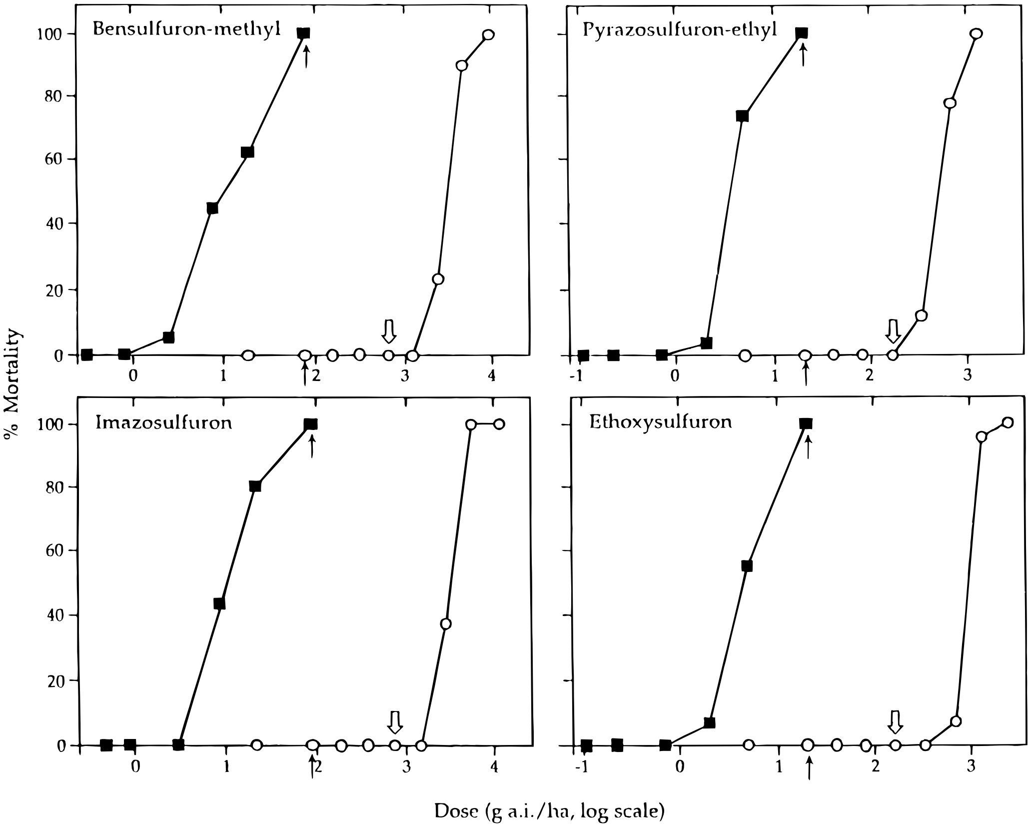

FIG. 1. Effect of four SU herbicides on mortality of *Limnophila sessiliflora* from resistant (—○—) and susceptible (—■—) populations. ↑ shows the recommended rate and ⇩ shows eight times the recommended rate.

pretilachlor sequentially with BSM + thiobencarb + mefenacet 14 days after rice planting or with simetryn + thiobencarb + MCPB [4-(4-chloro-*o*-tolyloxy)butyric acid], bifenox[methyl 5-(2, 4-dichlorophenoxy)-2-nitrobenzoate] + SAP [*S*-2-benzenesulfonamidoethyl *O*,*O*-di-iso-propyl phosphorodi-thioate], or simetryn + MCPA-thioethyl 24–25 days after rice planting (Table 3).

Field Survey

Resistant biotypes were observed at only 10 (2%) of 489 fields surveyed in Sennan Village. Of these, there were 5 fields with over 100 individuals/m^2, 2 fields with 10–100 individuals/m^2, and 4 fields with 1–10 individuals/m^2.

DISCUSSION

The current evidence suggests that the mechanism of SU resistance in rice weeds in Japan is an altered site of action (the ALS enzyme), which is inhibited less in resistant than in susceptible biotypes by SU herbicides (16, 17), although this has not been confirmed for *L. sessiliflora*. Mechanisms of resistance other than changes in target site sensitivity may also result in weeds being resistant to SU herbicides (18). A diclofop-methyl-resistant biotype of annual ryegrass (*Lolium rigidum* Gaud.) is cross-resistant to metsulfuron-methyl and chlorsulfuron (19). However, this biotype has ALS activity with the same sensitivity to ALS-inhibiting herbicides as ALS from the susceptible biotype (20), suggesting a more general resistance mechanism, such as

TABLE 4
Response of *Limnophila sessiliflora* to Four SU Herbicides

Herbicide	GR_{50} R-type (g a.i./ha)	GR_{50} S-type (g a.i./ha)	Resistant ratio
BSM	1316.42	1.47	895.52
PSE	110.32	0.33	334.30
IMS	1244.71	1.90	655.11
ETS	228.70	0.31	737.74

metabolism of the herbicide to nonphytotoxic products. This type of mechanism could explain the observed cross-resistance to herbicides with completely different modes of action (18). The results of the current research showed that the *L. sessiliflora* biotype from a rice field from Sennan Village was resistant to SU herbicides. Moreover, this biotype showed cross-resistance to the four SU herbicides tested but was controlled by herbicides with different modes of action, such as amide or phenoxy herbicides. This suggested that SU resistance in *L. sessiliflora* is not due to differences in herbicide absorption, translocation, or metabolism. A mutation in the ALS genes seems to be implicated. Based on these results, to avoid further increases in the resistant biotype, a number of control measures have already been implemented by local agencies. These include changes in the recommended herbicide use patterns, such as reduction in use rates and number of applications of the same herbicide in a growing season, and elimination of the use of the same herbicide in successive seasons.

In Japan, since the first confirmed case of SU resistance was reported in *Monochoria korsakowii* Regel et Maack collected from Hokkaido (21) resistance to ALS inhibitors has been reported in seven additional paddy weed species, varieties, or subspecies: *Lindernia micrantha* D. Don (22), *L. pyxidaria* Pennell, *L. dubia* Pennell var. *dubia*, *L. dubia* var. *major* Pennell (23), *Elatine triandra* Schk. (24), *Rotala indica* (Wild.) Koehn (25), and *Schoenoplectus juncoides* (Roxb.) Palla subsp. *juncoides* (26). Of these, *Lindernia* spp. have wide distribution in the surveyed areas (27–31). In Kawanishi Town, Yamagata Prefecture, SU-resistant *L. micrantha* were observed in 76 (11.3%) of 673 fields; moreover, in Yuza Town, the *Lindernia* spp. were observed in as many as 229 of 671 fields surveyed, amounting to 34.1%. In contrast, SU-resistant *L. sessiliflora* was distributed in only 2% of rice fields in Sennan Village. This suggests that SU-resistant *L. sessiliflora* may spread more slowly than resistant *Lindernia* or that the selective pressure on *L. sessiliflora* may be less than that on *Lindernia* under similar herbicide use patterns.

At 8 to 16 times of the recommended rate of SU herbicides, the mortalities of SU-resistant *Lindernia* spp. were at or near 100% (22, 23). However, a substantial number of the *L. sessiliflora* used in this study survived and grew normally even at 32 times the recommended dose (Fig. 1). Therefore, the level of resistance to SU in *L. sessiliflora* was higher than that in *Lindernia* spp., but the percentage distribution was in inverse proportion to the level of resistance.

In some rice fields, seed spread of resistant *Lindernia* spp. might be facilitated by agricultural machinery. In the field, resistant *Lindernia* spp. are patchy in distribution around agricultural machinery's access to the rice field but could be rarely found in the area far away from the access. *Lindernia* spp. produce tiny seeds with diameters of less than 0.5 mm, which makes

TABLE 5
Effect of Several Herbicides on the Survival of *Limnophila sessiliflora* from the SU-Resistant Population

Herbicide	Product rate (per ha)	Plants surviving (per pot)
CG-113	10 kg	0
KPP-314	30 kg	0
Hok-7505	10 kg	0
HJ-941	10 kg	0
CDS-941	10 *L*	0
CH-908	10 kg	0
DPX-84T	10 kg	18
Control	—	20

them easily spread to other fields by the machinery (29–32). Similarly, the seeds of *L. sessiliflora* may also be spread by agricultural machinery, but the establishment of its resistant biotype in nearby fields may be constrained by mid-season drainage that normally occurs in mid July. It is likely that the growth and reproduction of *L. sessiliflora* may more often be limited by water availability during the mid-season drainage than *Lindernia* spp. Our field survey showed that the presence of *L. sessiliflora* was associated with spring water. All 10 rice fields where *L. sessiliflora* is distributed have spring water, whereas no *L. sessiliflora* were found in those with no spring water. This indicates that spring water may be an important ecological factor that is responsible for the existence and evolution of the *L. sessiliflora* resistant biotype. This suggests that the resistant biotype could probably be contained ecologically to some extent by mid-season drainage in fields without spring water.

L. sessiliflora and several other broadleaf weeds that have so far developed resistant biotypes to SU herbicides may not be a very serious problem in Japan, since they may be easily controlled by various herbicides with different modes of action. However, SU-resistant *S. juncoides* (Roxb.) Palla subsp. *juncoides* was found in Hokkaido in 1997 (26). This was a serious weed in the 1970's and 1980's until SU herbicides became widespread in the early 1990's. Continuous monitoring of herbicide-resistant weeds, including their distribution and spread, and studies on ecological fitness of resistant biotypes will be imperative for continued refinement of strategies for their control.

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