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Target-site mutation and enhanced metabolism confer resistance to thifensulfuron-methyl in a multiple-resistant redroot pigweed (*Amaranthus retroflexus*) population

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Research Article

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Abstract

Redroot pigweed (*Amaranthus retroflexus* L.) is a troublesome dicot weed species widely distributed across China. A population of *A. retroflexus* that survived the recommended label rate of thifensulfuron-methyl was collected from the main soybean [*Glycine max* (L.) Merr.] production area in China. Whole-plant dose–response assays indicated that the resistant (R) population was highly resistant (61.80-fold) to thifensulfuron-methyl compared with the susceptible (S1 and S2) populations. In vitro acetolactate synthase (ALS) activity experiments showed that the thifensulfuron-methyl I_{50} value for the R population was 40.17 times higher than that for the S1 population. A preliminary malathion treatment study indicated that the R population might have cytochrome P450–mediated metabolic resistance. The R population exhibited a high level of cross-resistance to representative ALS herbicides (imazethapyr, flumetsulam, and bispyribac-sodium) and multiple resistance to the commonly used protoporphyrinogen oxidase (PPO)-inhibiting herbicides lactofen and fomesafen. Two common mutations, Trp-574-Leu in *ALS* and Arg-128-Gly in *PPO2*, were identified within the R population. This study identified possible enhanced metabolism of thifensulfuron-methyl coexisting with target-site mutations in both *ALS* and *PPO2* in a multiple-resistant *A. retroflexus* population.

Introduction

Acetolactate synthase (ALS; EC 2.2.1.6) is a critical enzyme found in plants, fungi, and bacteria (Duggleby and Pang 2000). It is a plastidic enzyme involved in the biosynthetic pathway of the branched-chain amino acids valine, leucine, and isoleucine (Durner et al. 1990; Umbarger 1978). ALS is also the most common target enzyme for five different classes of herbicides: triazolopyrimidines, sulfonylureas (SUs), pyrimidinylthio-benzoates, imidazolinones (IMIs) and sulfonylamino-carbonyltriazolinones. ALS-inhibiting herbicides have been used on various crops and as highly successful commercial herbicides for more than 30 yr. However, repeated use of these herbicides has resulted in selection of resistant weeds. To date, 165 weed species have evolved resistance to one or multiple ALS-inhibiting herbicide classes globally (Heap 2020).

Target-site mutation has been the predominant mechanism providing resistance to ALS-inhibiting herbicides. It is mostly caused by one of eight mutations in the *ALS* gene that result in amino acid changes (Ala-122, Pro-197, Ala-205, Asp-376, Arg-377, Trp-574, Ser-653, and Gly-654). Enhanced metabolism is another critical mechanism that confers ALS resistance (Devine and Shukla 2000; Yu and Powles 2014). This mechanism has been well documented in different weeds, including blackgrass (*Alopecurus myosuroides* Huds.) (De Prado and Franco 2004), rice barnyardgrass [*Echinochloa phyllopogon* (Stapf) Kosso-Pol.] (Yasuor et al. 2009), wild mustard (*Sinapis arvensis* L.) (Veldhuis et al. 2000), and flixweed [*Descurainia sophia* (L.) Webb ex Prantl] (Yang et al. 2016). Cytochrome P450 monooxygenases (CYP450s) have been reported to be critical in herbicide metabolism (Ma et al. 2013; Powles and Yu 2010).

Redroot pigweed (*Amaranthus retroflexus* L.) is an annual broadleaf weed and is widespread worldwide. It is a highly competitive C_4 species with high seed production (Francischini et al. 2014). It can produce a million seeds that have long seed viability and prolonged germination periods (Karimmojeni et al. 2014). *Amaranthus retroflexus* is widely distributed in crop fields in China, substantially affecting crop yields (Li et al. 2004). Growers rely heavily on herbicides to control this troublesome weed. Because of the intensive application of herbicides, *A. retroflexus* has evolved resistance to three mechanisms of action of herbicides, including photosystem II

Table 1. Information on herbicides used in this study.

Target[a]	Herbicide formulation[b]	Manufacturer	Dose g ai ha^{-1}	Website
ALS	Imazethapyr (50 g L^{-1}AS)	BASF, Shanghai, China	100	https://www.basf.com/cn/zh.html
ALS	Flumetsulam (80% WDG)	Ruibang Agrochemical, Jiangsu, China	60	http://www.repont.com
ALS	Bispyribac-sodium (100 g L^{-1}SC)	Jilinhengda Chemical, Jilin, China	10	http://www.jilinhengda.com
PPO	Lactofen (240 g L^{-1} EC)	Fengle Agrochemical, Anhui, China	108	http://flnh.com.cn
PPO	Fomesafen (250 g L^{-1} AS)	Qiaochang Agriculture, Binzhou, China	275	http://www.qiaochang.com
PSII	Bentazone (480 g L^{-1}AS)	BASF, Shanghai, China	1,440	https://www.basf.com/cn/zh.html

[a]ALS, acetolactate synthase; PPO, protoporphyrinogen oxidase; PSII, photosystem II.
[b]AS, aqueous solution; SC, suspending agent; WDG, water-dispersible granules.

(PSII), protoporphyrinogen oxidase (PPO), and ALS. Several mutations at Ala-122, Ala-205, Asp-376, Trp-574, and Ser-653 in ALS afford resistance to ALS-inhibiting herbicides (Chen et al. 2015; Huang et al. 2016), and one mutation (Arg-128-Gly) in *PPO2* conferring PPO-inhibitor resistance in *A. retroflexus* in China has been reported (Huang et al. 2020).

The control of *A. retroflexus* in soybean [*Glycine max* (L.) Merr.] fields has mainly relied on the ALS-inhibiting herbicide thifensulfuron-methyl since its introduction in China in 1999. Thifensulfuron-methyl is very popular because of its high efficacy in broadleaf weed control. In recent years, farmers have found that the efficacy of thifensulfuron-methyl on *A. retroflexus* has greatly decreased in northeast China. The general aim of this research was to determine the status of resistance to thifensulfuron-methyl in an *A. retroflexus* population, to examine the cross- and multiple resistance to a range of common herbicides, and to determine resistance mechanisms present in the *A. retroflexus* population.

Materials and Methods

Plant Materials

Seeds of a putative thifensulfuron-methyl-resistant *A. retroflexus* population were collected in 2018 from a soybean field (49.32°N, 125.53°E) receiving regular thifensulfuron-methyl applications for more than 7 yr. This population was treated with thifensulfuron-methyl (30 g ai ha^{-1}), and 20 surviving seedlings were grown to maturity in a greenhouse. Seeds of the subpopulation (R population) were harvested and used in subsequent experiments. Two known herbicide-susceptible populations (S1 and S2 populations) maintained in our laboratory (Huang, et al. 2016) were used as controls.

Twenty seeds of each S1, S2, and R population were planted into 8.5-cm^2 pots containing 1:1 (v/v) peat:sand mixture. At 20 d after seeding, six evenly sized seedlings per pot were kept and grown in a greenhouse at a day/night temperature of 35/21 C and photoperiod 16/8 h, with regular water and fertilization.

Whole-Plant Dose–Response Experiments

Amaranthus retroflexus seedlings were treated with thifensulfuron-methyl at the 3- to 4-leaf stage in the absence and presence of malathion using a cabinet sprayer (3WP-2000, Nanjing Agricultural Mechanization Research Institute, Nanjing, China). Thifensulfuron-methyl was applied at 0, 1.88, 3.75, 7.5, 15, 30, and 60 g ai ha^{-1} (recommended label rate: 30 g ai ha^{-1}) for the S1 and S2 populations and at 0, 15, 30, 60, 120, 240, and 480 g ai ha^{-1} for the R population. Malathion (1,000 g ai ha^{-1}) was applied 1 h before thifensulfuron-methyl treatment (Preston et al. 1996). The seedlings were grown in a greenhouse after herbicide treatments. At 21 d after thifensulfuron-methyl treatments, the aboveground parts of the plants were cut and oven-dried at 60 C in a drying chamber, and the dry weights were recorded. Plants were recorded as dead if they did not produce new healthy leaves. The experiment was conducted twice, with three replications (6 plants per replication) each.

ALS Activity Assays

The three *A. retroflexus* populations (S1, S2, and R) were grown as described earlier. Fresh plant tissue was harvested for the ALS activity assays. The procedures for the ALS activity assays were carried out according to the methods described by Yu et al. (2004). Briefly, the frozen shoot tissue was homogenized with a mortar and pestle in 2 volumes of grinding buffer. The homogenate was filtered through a layer of Miracloth (Hebeiwanlian, Hengshui, China) and centrifuged at 27,000 × *g* for 15 min. The supernatant was brought to 50% saturation with $(NH4)_2SO4$. The solution was centrifuged at 27,000 × *g* for 30 min. The pellet was redissolved in 2.5 ml resuspension buffer and desalted on a column. The desalted enzyme extract was immediately used in the assay. This enzyme extract and thifensulfuron-methyl solution (0, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, 1, and 10 μM for the S1 and S2 populations and 0, 10^{-3}, 10^{-2}, 10^{-1}, 1, 10, 100, and 1,000 μM for the R population) were incubated at 37 C for 60 min. The reaction was stopped by adding 40 μl of 6 N H_2SO4 and incubated at 60 C for 15 min. Then, 190 μl of 0.55% creatine solution and 190 μl of α-naphthol solution were added, and the mixture was incubated at 60 C for 15 min. The experiment was conducted twice, with three replications each time.

Susceptibility to Other Herbicides

To study resistance status of the R population, seedlings (3- to 4-leaf stage) of the R, S1, and S2 populations were sprayed with herbicides at the recommended label dose (Table 1). Herbicide treatment methods were the same as those described earlier. Plants producing new leaves were recorded as alive at 21 d after herbicide treatment. The experiment was conducted twice, with four replicates (6 plants per replicate) per treatment. Plant survival rates of 80% to 100%, 20% to 80%, or 0% to 20% were considered highly resistant, poorly resistant, or sensitive, respectively.

ALS and PPO2 Gene Sequencing

Fresh leaves (100 mg) of 20 surviving plants after thifensulfuron-methyl treatments and 3 plants of each untreated S1 and S2 population were sampled individually. Total genomic DNA was extracted from plant samples with a DNA extraction kit (Bioteke Corporation, Beijing, China). The *ALS* gene that covers eight known resistance-endowing mutations was amplified using primers whose sequences have been published (Huang et al.

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2016). PCR was performed as follows: 5 min incubation at 94 C; 30 cycles of 30 s at 94 C, 30 s at 60 C, and 2.5 min (~2.6 kb) at 72 C; and then 5 min at 72 C.

Leaf tissue of R plants surviving the recommended label dose of lactofen and untreated S1 and S2 plants were harvested for RNA isolation using an RNA extraction kit (Bioteke Corporation). After treatment with RNase-free DNase, the RNA was reverse-transcribed to cDNA using PrimeScript™ 1st Strand cDNA Synthesis Kit (Takara, Dalian, China). The *PPO2* gene from 20 plants of the R population and 3 plants each from the S1 and S2 populations was amplified, sequenced, and compared according to the methods of Huang et al. (2020).

The PCR products from the *ALS* gene and *PPO2* gene were sequenced by Biomed (Beijing, China) after gel purification and compared using Vector NTI 12.5 (SigmaPlot Software Inc., Chicago, IL).

Statistical Analysis

Dose–response curves were obtained with a nonlinear log-logistic regression model using SigmaPlot software (v. 12.0, Systat Software, San Jose, CA, USA). The means were compared using Tukey's HSD test at P = 0.05. The data (expressed as a percentage of control) that were not significantly different were pooled and fit to the following equation (Seefeldt et al. 1995):

$$y = C + \left[\frac{D - C}{1 + (x/I_{50})^b}\right] \quad [1]$$

where C and D are the lower and upper limits, respectively, b is the slope of the curve, and I_{50} is the thifensulfuron-methyl dose required to reduce the plant's growth by 50% (GR_{50}) or to reduce ALS activity by 50% (I_{50}). The resistance index (RI) was calculated to quantify the level of resistance by dividing the GR_{50} or I_{50} value of the R population compared with the S1 or S2 population.

Results and Discussion

Thifensulfuron-Methyl Dose–Response Assays

Dose–response assays confirmed that, compared with the S1 and S2 populations, the R *A. retroflexus* population was highly resistant to thifensulfuron-methyl. The S1 and S2 plants were not able to survive in response to 15 g ai ha^{-1}, which was less than half the label rate. However, the R plants were not seriously injured and grew like the untreated plants. According to the GR_{50}, the dry weight in the S1 and S2 populations decreased by 50% in response to thifensulfuron-methyl rates of 3.12 and 4.41 g ai ha^{-1}, respectively, while the GR_{50} of the R population occurred in response to 192.83 g ai ha^{-1}. Therefore, the R population was 61.80-fold more resistant to thifensulfuron-methyl than the S1 population (Figure 1; Table 2).

In this study, we confirm that an *A. retroflexus* population has evolved resistance to thifensulfuron-methyl and other ALS-inhibiting herbicides, including imazethapyr, flumetsulam, and bispyribac-sodium. The same phenomenon was also observed in another *A. retroflexus* population with 126-fold increased resistance to thifensulfuron-methyl in Israel (Sibony et al. 2010). This high thifensulfuron-methyl resistance in the R population indicated that the resistance was likely because of a target-site mutation.

Table 2. Response to thifensulfuron-methyl by different *Amaranthus retroflexus* populations (S1, S2 and R populations) with and without malathion.

Treatments	GR_{50} [a] (SE)			RI[b]
	R	S1	S2	
	g ai ha^{-1}			
Thifensulfuron-methyl	192.83 (22.93)	3.12 (0.65)	4.41 (1.72)	61.80
Thifensulfuron-methyl + malathion	107.35 (11.04)	2.93 (1.21)	3.07 (0.43)	36.64

[a]GR_{50}, herbicide dose causing a 50% growth reduction.
[b]RI, resistance index: RI = GR_{50}(R)/GR_{50}(S1).

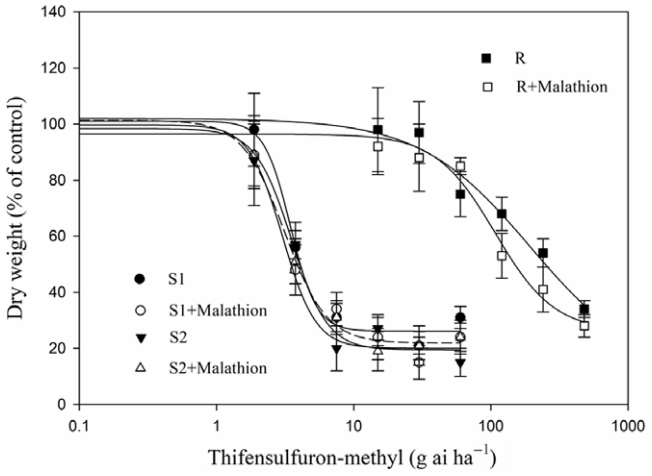

Figure 1. Dose–response curve for plant growth of the S1, S2, and R *Amaranthus retroflexus* populations. Vertical bars represent the SEs (P = 0.05).

Effects of Malathion Pretreatment on Thifensulfuron-Methyl Resistance

Malathion inhibits CYP450 and is usually considered an indicator of metabolic resistance (Nakka et al. 2017). In this study, there were no significant effects on plant growth in the three tested *A. retroflexus* populations when just malathion was applied. However, malathion pretreatment with thifensulfuron-methyl resulted in greater injury to R plants than was observed with herbicide alone. The dry weight of the R plants decreased significantly following malathion plus thifensulfuron-methyl application, and the GR_{50} decreased by 44% (Table 2; Figure 1). In contrast, no significant difference was found in the GR_{50} of the S1 and S2 populations to thifensulfuron-methyl plus malathion pretreatment. These results indicate that enhanced metabolism could be contributing to resistance to thifensulfuron-methyl in the R population.

The members of the family of CYP450 enzymes play a critical role in herbicide metabolism (Duhoux et al. 2017; Yu and Powles 2014). The thifensulfuron-methyl resistance in the R population could be reversed by the CYP450 inhibitor malathion in this study. This observation indicated that CYP450-mediated enhanced metabolism likely occurred in the R population. However, further research is needed to demonstrate this hypothesis. Similar results have also been found in other weed species, such as rigid ryegrass (*Lolium rigidum* Gaudin) (Christopher et al. 1994), large crabgrass [*Digitaria sanguinalis* (L.) Scop.] (Mei et al. 2017), and Palmer amaranth (*Amaranthus palmeri* S. Watson) (Nakka et al. 2017), for which malathion pretreatment significantly reduced the response of the resistant populations to the herbicide. Enhanced herbicide metabolism may afford unpredictable resistance to

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Table 3. I_{50} values of S1, S2, and R *Amaranthus retroflexus* populations treated with thifensulfuron-methyl.[a]

Population	Total ALS activity (SE)	R/S[b]	I_{50}	RI[c]
	—nmol acetoin mg^{-1}protein min^{-1}—		— g ai L^{-1}—	
S1	10.06 (1.24)	–	0.06	–
S2	13.10 (1.87)	1.30	0.10	1.67
R	37.41 (4.71)	3.72	2.41	40.17

[a]I_{50}, herbicide rate that inhibited acetolactate synthase (ALS) activity by 50%.
[b]R, resistant; S, susceptible.
[c]RI, resistance index.

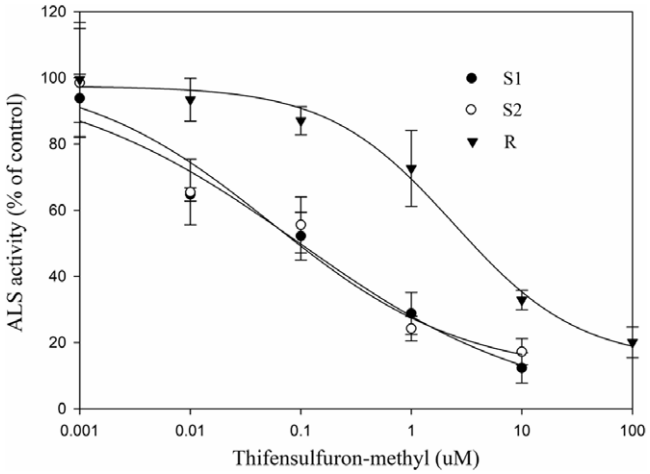

Figure 2. Dose–response curve of acetolactate synthase (ALS) activity in S1, S2, and R populations of *Amaranthus retroflexus*. ALS activity is expressed as a percentage of activity, based on the untreated controls. Data were fit using a four-parameter to the equation: $Y = C + [(D - C)/[1 + (x/I_{50})^b]$. Vertical bars represent the SEs (P = 0.05).

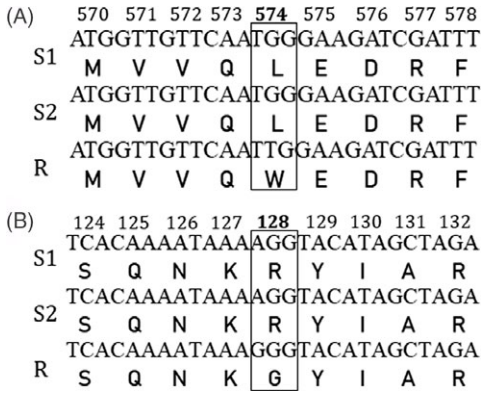

Figure 3. Partial DNA and derived amino acid sequences of *ALS* and *PPO2* from S1, S2, and R *Amaranthus retroflexus* populations. (A) Trp-574-Leu mutation in *ALS* gene in the R population; (B) Arg-128-Gly mutation in *PPO2* gene in the R population.

herbicides with different modes of action (Délye 2013; Yu and Powles 2014). Although malathion pretreatment with PPO-inhibiting herbicides was not performed in this research, the possibility of enhanced herbicide metabolism affording resistance to PPO herbicides cannot be excluded. In a future study, whether enhanced herbicide metabolism plays a role in PPO herbicide resistance and which candidate CYP450 genes are involved in herbicide resistance in the R population will be investigated and identified, respectively.

ALS Activity Assay

In the absence of thifensulfuron-methyl, the activity of the crude ALS enzyme was 10.06, 13.10, and 37.41 nmol acetoin mg^{-1} protein min^{-1} in S1, S2, and R populations, respectively. However, ALS within the R population was significantly resistant to thifensulfuron-methyl in the presence of thifensulfuron-methyl. As shown in Figure 2, the I_{50} values of S1, S2, and R populations were 0.06, 0.10, and 2.41, respectively. These results indicate that the thifensulfuron-methyl I_{50} value for the R population was 40.17 times greater than that of the S1 population (Table 3). These results strongly indicate a target site–based resistance mechanism within the R population. Furthermore, the resistance index of whole-plant dose responses (61.80-fold) was much higher than that of the ALS activity assays (40.17-fold), indicating that other herbicide-resistance mechanisms, such as enhanced herbicide metabolism, are at play in the R population. Enhanced herbicide metabolism to ALS-inhibiting herbicides has rarely been documented in eudicot weeds. The possible reason for this may be that it often affords low-level resistance, and compared with target-site mutation, enhanced metabolism is usually underestimated (Ahmad-Hamdani et al. 2012; Nakka et al. 2017).

Sequencing of the ALS and PPO Gene

To investigate the molecular mechanism for thifensulfuron-methyl resistance, the *ALS* gene covering eight resistance-endowing mutations was amplified from the R, S1, and S2 populations. A comparison of the *ALS* gene showed that all 20 R individuals contained a nucleotide change of TGG to TTG resulting in a Trp to Leu substitution (Trp-574-Leu) at amino acid position 574 (Figure 3). The *ALS* sequence chromatograms exhibited double peaks at position 574 (in 13 out of 20 cases), indicating that 7 mutants were homozygous and 13 mutants were heterozygous (data not shown). The molecular results showed that Trp at position 574 of the *ALS* was replaced with Leu in the R population in this study. This is the same amino acid substitution documented for ALS resistance in previous studies, including waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] (Patzoldt and Tranel 2007), *A. retroflexus* (Chen et al. 2015; McNaughton et al. 2005; Scarabel et al. 2010), and corn gromwell (*Lithospermum arvense* L.) (Wang et al. 2019).

As the R population was multiple resistant to PPO inhibitors fomesafen and lactofen, the herbicide target *PPO2* genes from the R, S1, and S2 populations were amplified. Sequencing analysis showed an Arg-128-Gly substitution in most individuals (14 out of 20) of the R population, and 6 remaining individual plants did not have any reported *PPO2* mutation (Figure 3). It should be noted that 6 plants of the R population did not have the 128 mutation or other reported *PPO2* mutations. These results indicate that target-site mutation may not occur in these plants and that a non–target site resistance mechanism could contribute to resistance to PPO herbicides.

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Table 4. Survival rate of different *Amaranthus retroflexus* populations (S1, S2, and R populations) after herbicide treatments.

Target[a]	Herbicide	Survival rate for S1	Survival rate for S2	Survival rate for R
			%	
ALS	Imazethapyr	0	0	93
ALS	Flumetsulam	0	0	88
ALS	Bispyribac-sodium	0	0	79
PPO	Lactofen	0	0	84
PPO	Fomesafen	0	0	95
PSII	Bentazone	10	7	8

[a]ALS, acetolactate synthase; PPO, protoporphyrinogen oxidase; PSII, photosystem II.

Susceptibility to Other Herbicides

To characterize the resistance status of the R population, the responses of these plants to ALS-, PPO-, and PSII-inhibiting herbicides at the label rate were investigated. The results presented in Table 4 indicated that the R population exhibited a high level of cross-resistance to the ALS-inhibiting herbicides imazethapyr, flumetsulam, and bispyribac-sodium and multiple resistance to the PPO-inhibiting herbicides lactofen and fomesafen. However, the R population was susceptible to the PSII-inhibiting herbicide bentazone. The point mutation resulting in the Trp-574-Leu amino acid change has been found to confer resistance to all five classes of ALS inhibitors (Tranel et al. 2020). Previous research using a whole-plant dose–response assay in another *A. retroflexus* population demonstrated that resistance to imazethapyr was also due to the Trp-574-Leu substitution (Chen et al. 2015). These results were in agreement with those of a study conducted by Ferguson et al. (2001), who reported that the Trp-574-Leu substitution provided cross-resistance to both IMI and SU inhibitors.

In summary, this research illustrates for the first time the presence of both target site–based (Trp-574-Leu mutation in *ALS*) and likely non–target site based (enhanced herbicide metabolism) resistance to thifensulfuron-methyl coexisting in a single multiple-resistant *A. retroflexus* population. Further studies are underway to identify the candidate CYP450 genes that are involved in metabolic herbicide resistance in *A. retroflexus*. The evolution and occurrence of target-site resistance and non–target site resistance in the resistant *A. retroflexus* population poses a serious challenge to weed control. Moreover, multiple resistance to PPO inhibitors afforded by the Arg-128-Gly mutation in *PPO2* was also found in the same *A. retroflexus* population. Therefore, management of this troublesome weed before it becomes a terrible threat in cropping systems is critical. Fortunately, bentazone, a PSII inhibitor, still had a satisfactory control effect on the R population. Previous studies have shown that diversity in weed control may disrupt and delay the development of herbicide resistance (Powles and Gaines 2016). In practice, farmers should integrate various weed management strategies, such as employing herbicides with different sites of action, using different tillage or cultural practices, and rotating crops to control herbicide-resistant weeds.

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References

Ahmad-Hamdani MS, Yu Q, Han H, Cawthray GR, Wang SF, Powles SB (2012) Herbicide resistance endowed by enhanced rates of herbicide metabolism in wild oat (*Avena* spp.) Weed Sci 61:55–62

Chen JY, Huang ZF, Zhang CX, Huang HJ, Wei SH, Chen JC, Wang X (2015) Molecular basis of resistance to imazethapyr in redroot pigweed (*Amaranthus retroflexus* L.) populations from China. Pestic Biochem Physiol 124:43–47

Christopher JT, Preston C, Powles SB (1994) Malathion antagonizes metabolism-based chlorsulfuron resistance in *Lolium rigidum*. Pestic Biochem Physiol 49:172–182

Délye C (2013) Unravelling the genetic bases of non-target-site-based resistance (NTSR) to herbicides: a major challenge for weed science in the forthcoming decade. Pest Manag Sci 69:176–187

De Prado RA, Franco AR (2004) Cross-resistance and herbicide metabolism in grass weeds in Europe: biochemical and physiological aspects. Weed Sci 52:441–447

Devine MD, Shukla A (2000) Altered target sites as a mechanism of herbicide resistance. Crop Prot 19:881–889

Duggleby RG, Pang SS (2000) Acetohydroxyacid synthase. J Biochem Mol Biol 33:1–36

Duhoux A, Carrère S, Duhoux A, Délye C (2017) Transcriptional markers enable identification of rye-grass (*Lolium* sp.) plants with non-target-site-based resistance to herbicides inhibiting acetolactate-synthase. Plant Sci 257:22–36

Durner J, Gailus V, Boger P (1990) New aspects on inhibition of plant acetolactate synthase by chlorsulfuron and imazaquin. Plant Physiol 95: 1144–1149

Ferguson GM, Hamill AS, Tardif FJ (2001) ALS inhibitor resistance in populations of Powell amaranth and redroot pigweed. Weed Sci 49:448–453

Francischini AC, Constantin J, Oliveira RS Jr, Santos G, Franchini LHM, Biffe DF (2014) Resistance of *Amaranthus retroflexus* to acetolactate synthase inhibitor herbicides in Brazil. Planta Daninha 32:437–446

Heap IM (2020) International Herbicide-Resistant Weed Database. http://www.weedscience.com. Accessed: August 23, 2020

Huang ZF, Chen JY, Zhang CX, Huang HJ, Wei SH, Zhou XX, Chen JC, Wang X (2016) Target-site basis for resistance to imazethapyr in redroot amaranth (*Amaranthus retroflexus* L.). Pestic Biochem Physiol 128:10–15

Huang ZF, Cui HL, Wang CY, Wu T, Zhang CX, Huang HJ, Wei SH (2020) Investigation of resistance mechanism to fomesafen in *Amaranthus retroflexus* L. Pestic Biochem Physiol 165:104560

Karimmojeni H, Bazrafshan AH, Majidi MM, Torabian S, Rashidi B (2014) Effect of maternal nitrogen and drought stress on seed dormancy and germinability of *Amaranthus retroflexus*. Plant Spec Biol 29:e1–e8

Li XJ, Zhang HJ, Ni HW (2004) Review on the biological characters and control of redroot pigweed (*Amaranthus retroflexus* L.). Pesticide Sci Adm 25:13–16. Chinese

Ma R, Kaundun SS, Tranel PJ, Riggins CW, McGinness DL, Hager AG, Hawkes T, McIndoe E, Riechers DE (2013) Distinct detoxification mechanisms confer resistance to mesotrione and atrazine in a population of waterhemp. Plant Physiol 163:363–377

Mei Y, Si C, Liu MJ, Qiu, LH, Zheng MQ (2017) Investigation of resistance levels and mechanisms to nicosulfuron conferred by non-target-site mechanisms in large crabgrass (*Digitaria sanguinalis* L.) from China. Pestic Biochem Physiol 141:84–89

McNaughton KE, Letarte J, Lee EA, Tardif FJ (2005) Mutations in ALS confer herbicide resistance in redroot pigweed (*Amaranthus retroflexus*) and Powell amaranth (*Amaranthus powellii*). Weed Sci 53:17–22

Nakka S, Thompson, CR, Peterson DE, Jugulam M (2017) Target site-based and non-target site based resistance to ALS inhibitors in palmer amaranth (*Amaranthus palmeri*). Weed Sci 65:681–689

Patzoldt WL, Tranel PJ (2007) Multiple ALS mutations confer herbicide resistance in waterhemp (*Amaranthus tuberculatus*). Weed Sci 55: 421–428

Powles SB, Gaines TA (2016) Exploring the potential for a regulatory change to encourage diversity in herbicide use. Weed Sci 64:649–654

Powles SB, Yu Q (2010) Evolution in action: plants resistant to herbicides. Annu Rev Plant Biol 61:317–347

Preston C, Tardif FJ, Christopher JT (1996) Multiple resistance to dissimilar herbicide chemistries in a biotype of *Lolium rigidum* due to enhanced activity of several herbicide degrading enzymes. Pestic Biochem Physiol 54: 123–134

https://doi.org/10.1017/wsc.2020.93 Published online by Cambridge University Press

Scarabel L, Varotto S, Sattin M (2010) A European biotype of *Amaranthus retroflexus* cross-resistant to ALS inhibitors and response to alternative herbicides. Weed Res 47:527–533

Seefeldt SS, Jensen JE, Fuerst EP (1995) Log-Logistic analysis of herbicide dose-response relationships. Weed Technol 9:218–227

Sibony M, Michel A, Haas HU, Rubin B, Hurle K (2010) Sulfometuron-resistant *Amaranthus retroflexus*: cross-resistance and molecular basis for resistance to acetolactate synthase-inhibiting herbicides. Weed Res 41:509–522

Tranel PJ, Wright TR, Heap IM (2020) Mutations in Herbicide-Resistant Weeds to ALS Inhibitors. http://www.weedscience.com. Accessed: August 23, 2020

Umbarger HE (1978) Amino acid biosynthesis and its regulation. Annu Rev Biochem 47:533–606

Veldhuis LJ, Hall LM, O'Donovan JT, Dyer W, Hall JC (2000) Metabolism-based resistance of a wild mustard (*Sinapis arvensis* L.) biotype to ethametsulfuron-methyl. J Agric Food Chem 48:2986–2990

Wang Q, Ge LA, Zhang LL, You LD, Wang DD, Liu WT, Wang JX (2019) A Trp-574-Leu mutation in the acetolactate synthase (ALS) gene of *Lithospermum arvense* L. confers broad-spectrum resistance to ALS inhibitors. Pestic Biochem Physiol 158:12–17

Yang Q, Deng W, Li X, Yu Q, Bai L, Zheng M (2016) Target-site and non-target-site based resistance to the herbicide tribenuron-methyl in flixweed (*Descurainia sophia* L.). BMC Genomics 17:551

Yasuor H, Osuna MD, Ortiz A, Saldain NE, Eckert JW, Fisher A (2009) Mechanism of resistance to penoxsulam in late watergrass [*Echinochloa phyllopogon* (Stapf) Koss]. J Agric Food Chem 57:3653–3660

Yu Q, Frisesen LSS, Zhang XQ, Powles SB (2004) Tolerance to acetolactate synthase and acetyl-coenzyme A carboxylase inhibiting herbicides in *Vulpia bromoidesis* conferred by two co-existing resistance mechanisms. Pestic Biochem Physiol 78:21–30

Yu Q, Powles S (2014) Metabolism-based herbicide resistance and cross-resistance in crop weeds: a threat to herbicide sustainability and global crop production. Plant Physiol 166:1106–1118

https://doi.org/10.1017/wsc.2020.93 Published online by Cambridge University Press