Point mutation in acetolactate synthase confers sulfonylurea and imidazolinone herbicide resistance in spiny annual sow-thistle [*Sonchus asper* (L.) Hill]

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Park, K. W., Kolkman, J. M. and Mallory-Smith, C. A. 2012. **Point mutation in acetolactate synthase confers sulfonylurea and imidazolinone herbicide resistance in spiny annual sow-thistle [*Sonchus asper* (L.) Hill]**. Can. J. Plant Sci. **92**: 303–309. Suspected thifensulfuron resistant spiny annual sow-thistle was identified near Colfax, Washington, in two fields with a winter wheat and lentil rotation. Therefore, studies were conducted to examine resistance of spiny annual sow-thistle to thifensulfuron and cross-resistance to other acetolactate synthase inhibitors and to determine the physiological and molecular basis for herbicide resistance. Whole-plant bioassay confirmed that the biotype was highly resistant to the sulfonylurea (SU) herbicides, thifensulfuron, metsulfuron, and prosulfuron. The resistant (R) biotype was also highly resistant to the imidazolinone (IMI) herbicides, imazamox and imazethapyr. An in vivo acetolactate synthase (ALS) assay indicated that the concentrations of SU and IMI herbicides required for 50% inhibition (I_{50}) were more than 10 times greater for R biotype compared with susceptible (S) biotype. Analysis of the nucleotide and predicted amino acid sequences for ALS genes demonstrated a single-point mutation from C to T at the *als1* gene, conferring the substitution of the amino acid leucine for proline in the R biotype at position197. The results of this research indicate that the resistance of spiny annual sow-thistle to SU and IMI herbicides is due to on altered target site and caused by a point mutation in the *als1* gene.

Key words: Spiny annual sow-thistle, acetolactate synthase, herbicide resistance, cross-resistance, *als* gene, point mutation

Park, K. W., Kolkman, J. M. et Mallory-Smith, C. A. 2012. **Une mutation ponctuelle de l'acétolactate synthase confère au laiteron rude [*Sonchus asper* (L.) Hill] une résistance aux herbicides à la sulfonylurée et à l'imidazolinone**. Can. J. Plant Sci. **92**: 303–309. Des plants de laiteron rude qu'on soupçonnait résister au thifensulfuron ont été identifiés dans deux champs de blé d'hiver et de lentille cultivés en assolement, près de Colfax, dans l'État de Washington. On a tenté de déterminer l'origine de cette résistance et de voir s'il y avait résistance croisée à d'autres inhibiteurs de l'acétolactate synthase, ainsi que de préciser l'origine physiologique et moléculaire de la résistance aux herbicides. L'analyse biologique de la plante a confirmé la très grande résistance du biotype aux herbicides à la sulfonylurée (SU), tels le thifensulfuron, le metsulfuron et le prosulfuron. Ce biotype résistait aussi considérablement aux herbicides à l'imidazolinone (IMI), comme l'imazamox et l'imazethapyr. Un dosage in vivo de l'acétolactate synthase (ALS) révèle que la concentration d'herbicide SU et IMI requise pour inhiber 50% de la croissance (I_{50}) du biotype résistant dépasse de plus du dix fois celle nécessaire pour obtenir les mêmes résultats avec le biotype sensible. L'analyse des nucléotides et des séquences d'acides aminés prévues pour les gènes codant l'ALS indique une mutation ponctuelle de C à T sur le gène *als1*, qui a entraîné la substitution de la leucine par de la proline à la position 197, dans le génotype résistant. Les résultats de ces travaux indiquent que la résistance du laiteron rude aux herbicides SU et IMI émane d'une modification de la cible, engendrée par la mutation ponctuelle sur le gène *als1*.

Mots clés: Laiteron rude, ALS, résistance aux herbicides, résistance croisée, gène *als*, mutation ponctuelle

Acetolactate synthase (ALS; EC 4.1.3.18) is one of the most important herbicide sites of action and ALS inhibitors are used in many cropping systems because of a broad spectrum of weed control, relatively low use rates, residual activity, and wide crop selectivity (Mazur and Falco 1989; Saari et al. 1994). ALS catalyzes the first common step in the biosynthesis of the branched chain amino acids, leucine, isoleucine, and valine (DeFelice et al. 1974; Ray 1984) and is the primary target enzyme for five structurally distinct chemical classes, sulfonylurea (SU), imidazolinone (IMI), triazolopyrimidine (TP), pyrimidinylthiobenzoate (PTB), and sulfonylaminocarbonyltriazolinone (SCT) herbicides (Tranel et al. 2011).

Abbreviations: **ALS**, acetolactate synthase; **CPCA**, 1,1-cyclopropanedicarboxylic acid; $\mathbf{GR_{50}}$, herbicide rate required for 50% growth reduction; $\mathbf{I_{50}}$, inhibitor concentration required for 50% inhibition of enzyme activity; **IMI**, imidazolinone; **PCR**, polymerase chain reaction; **PTB**, pyrimidinylthiobenzoate; **R**, resistant; **S**, susceptible; **SCT**, sulfonylaminocarbonyltriazolinone; **SU**, sulfonylurea; **TP**, triazolopyrimidine

Resistance to an ALS inhibitor was first reported in prickly lettuce (*Lactuca serriola* L.) in 1987, only 5 years after introduction of the SU herbicides (Mallory-Smith et al. 1990), and since then, 107 weed species have been documented to be resistant to ALS inhibitors (Heap 2011). A modified ALS with reduced herbicide binding properties is the most common resistance mechanism reported in many ALS inhibitor resistant weed biotypes (Tranel and Wright, 2002). ALS is encoded by a nuclear gene and has five highly conserved domains (Saari et al. 1994). Several different point mutations in the conserved domains have been indentified to be responsible for target-site resistance to ALS inhibitors. These mutations include amino acid substitutions at Ala_{122}, Pro_{197}, Val_{205}, Asp_{376}, Arg_{377}, Trp_{574}, Ser_{653}, and Gly_{654}. Amino acid substitutions in the different domains and different amino acid substitutions at the same mutation site confer different patterns of cross-resistance (Tranel and Wright 2002; Tranel et al. 2011). Eight different amino acid substitutions in Pro_{197}, all possible amino acid substitutions for Pro, have been identified in weed species (Tranel et al. 2011). In most cases, Pro_{197} mutations confer a high level of resistance to SU herbicides. However, the level of resistance to IMI, TP, PTB, and SCT herbicides depends on which amino acids are substituted for Pro_{197}.

Spiny annual sow-thistle [*Sonchus asper* (L.) Hill] is an annual to occasional biennial species belonging to the Asteraceae family (Halvorson 2003). This species is considered to be native to Africa, Asia, and Europe, and is a common weed in North America. Spiny annual sow-thistle occurs in cultivated fields and is also found in roadsides, wastelands, gardens, and pastures. This species is a troublesome weed because it is competitive and produces large numbers of seeds that are easily dispersed by wind (Hutchinson et al. 1984).

SU and IMI herbicides were used to control spiny annual sow-thistle in fields near Colfax, Washington, USA, rotated with winter wheat and lentil crops. In 2000, however, suspected SU-resistant spiny annual sow-thistle biotypes were found in two fields. Triazine and SU herbicide resistant spiny annual sow-thistle were found in France and Canada in 1980 and 1996, respectively (Rashid et al. 2003; Heap 2011). Common sowthistle (*Sonchus oleraceus*) resistant to a SU herbicide, chlorsulfuron, evolved in large areas of wheat fields in Australia (Adkins et al. 1997).

The objectives of this research were to characterize the resistance determining the level of cross resistance to different classes of ALS inhibitors (SU and IMI) in the spiny annual sow-thistle biotypes and to the physiological and molecular basis for resistance to the ALS inhibitors.

MATERIALS AND METHODS

Plant Materials

Seeds (R1 and R2) of suspected thifensulfuron resistant (R) spiny annual sow-thistle biotypes were collected near Colfax, Washington, USA, in 2002 from two fields that had been in winter wheat and lentil rotations since at least 1988. Beginning in 1989, imazethapyr was applied to all lentil crops and thifensulfuron was applied to about one-half of the wheat crops. Seeds of the susceptible (S) biotype were collected near Corvallis, Oregon, USA, from fields in which the spiny annual sow-thistle was known to be controlled with ALS-inhibiting herbicides.

Whole-plant Bioassay

Greenhouse studies were conducted to confirm the resistance of spiny annual sow-thistle to thifensulfuron and investigate the cross-resistance to other ALS inhibitors. Seeds of R1, R2, and S biotypes were planted in 98 cell tray (26 mL per cell) containing a commercial potting mix (Sunshine Mix #1, Sun Gro Horticulture Inc., Bellevue, WA). Plants were grown in a greenhouse with 16-h supplemental lighting and 25/20°C day/night temperature. When plants were 2 wk old, individual plants were transplanted into 267-mL plastic pots (6 cm × 6 cm) containing commercial potting mix. Herbicide treatments were applied when the plants were at the six- to seven-leaf stage with an 8002 even flat fan nozzle and an overhead compressed air sprayer calibrated to deliver 187 L ha^{-1}. SU herbicides (thifensulfuron, metsulfuron, and prosulfuron) and IMI herbicides (imazamox and imazethapyr) were applied at various ranges of rates with a 0.25% (vol/vol) nonionic surfactant (Table 1). Above-ground biomass was harvested 21 d after treatment, dried at 60°C for 72 h, and weighed. Biomass data were reported as the percent of the untreated control within each replication.

The response to thifensulfuron and imazamox was similar between R1 and R2 spiny annual sow-thistle biotypes collected from the two different fields, so seeds of R1 and R2 spiny annual sow-thistle biotypes were combined and used for metsulfuron, prosulfuron, and imazethapyr dose-response studies, the ALS assay and DNA sequence analysis.

In Vivo ALS Assay

ALS enzyme activities were determined from the R and S biotypes using in vivo assay procedure (Hanson et al. 2004). Seedlings of the R and S biotypes were grown in the greenhouse as previously described. When the plants were in the five- to six-leaf stage, leaf discs were removed from the youngest leaf using a 5-mm cork bore. Several pairs of leaf disks were removed from either side of the mid-vein. One disk from each pair was placed into a well (350 μL) in a 96-well microtiter plate as a untreated control which had 120 μL incubation solution

Table 1. Herbicides and rates used in dose-response studies with resistant (R) and susceptible (S) spiny annual sow-thistle *Sonchus asper* biotypes

Chemical class	Common name	Biotype	Herbicide rates
			----------(g ha^{-1})----------
Sulfonylurea	Thifensulfuron-methyl	S	0, 0.1, 0.3, 1, 3, 10, 30, 100
		R	0, 1, 10, 30, 100, 300, 1000, 3000, 10000
	Metsulfuron-methyl	S	0, 0.0001, 0.001, 0.01, 0.1, 1
		R	0, 0.0001, 0.001, 0.01, 0.1, 1, 10
	Prosulfuron	S	0, 0.000001, 0.00001, 0.0001, 0.001, 0.01, 0.1, 1
		R	0, 0.001, 0.01, 0.1, 1, 10, 100, 1000
Imidazolinone	Imazamox	S	0, 0.1, 0.3, 1, 3, 10, 30, 100
		R	0, 1, 3, 10, 30, 100, 300, 1000, 3000, 10000
	Imaazethapyr	S	0, 0.01, 0.1, 1, 10, 100, 1000
		R	0, 1, 10, 100, 1000, 3000, 10000

containing 25% (wt/vol) M&S salt media, 500 μM 1,1-cyclopropanedicarboxylic acid (CPCA) and 0.5% (wt/vol) L-alanine. The other disk from the pair was placed into a well containing 120 μL incubation solution with different concentrations of herbicides. Herbicides were applied at range of 10^{-3} to 10^{3} nM for SU herbicides and 10^{-2} to 3×10^{4} nM for IMI herbicides. Leaf discs in the microtiter plates were incubated at 25°C for 14 h under continuous light. The microtiter plates were placed in a –20°C freezer for 1 h and thawed in a 60°C incubator for 30 min. Samples were acidified by adding 30 μL 2 N sulfuric acid and incubated at 60°C for 30 min. In this step, the product of ALS enzyme, acetolactate, was decarboxylated to form acetoin. Pink color (which is an indicator for acetoin concentration) was developed by adding 150 μL 2.5% (wt/vol) α-naphthol and 0.25% (wt/vol) creatine in 2 N sodium hydroxide into each well and incubating at 60°C for 15 min (Westerfield 1945). After transferring 200 μL of the sample solution to a new microtiter plate, acetoin concentrations were determined by measuring the absorption of the solution at 540 nm with a microtiter plate reader. The absorbance values were corrected by subtracting the average value of the control (no disc) from the values of each well containing a leaf disc. Percent of ALS inhibition was calculated from the ratio of the adjusted absorbance value from each treated leaf disk to the value from each untreated paired leaf disk.

Cloning and Sequencing of *als* Genes

Genomic DNA was extracted from the leaf tissue of four-leaf stage R and S biotypes using a DNA isolation kit (DNeasy® Plant Mini Kit, QIAGEN Inc., Valencia, CA). Forward and reverse primers were designed based on homologous regions of *als* gene sequences between two other Asteraceae species, *Xanthium strumarium* L. and *L. serriola* L. Two of these primer sets were successfully used previously in amplifying *als* genes in sunflower (*Helianthus annuus* L.; Kolkman et al. 2004). Polymerase chain reaction (PCR) was conducted to amplify the *als* gene using the previously described primer sets 1. p-AHAS6 (5′-CTGGTCTTCCCGGCG-TMTGT-3′; forward) and p-′AHAS8 (5′-TGAG-CAGCCCACATCTGATG-3 ×; reverse), 2. p-AHAS7 (5′-GGRACNGTTTATGCGAATTATGC′3′; forward) and p-AHAS9 (5′-AATATTTMATTCTGCCRTCGC C-3′; reverse), 3. p-AHAS7 (forward) and p-AHAS8 (reverse), as well as 4. p-AHAS20 (5′-CTSGTCGAAG-CYCTNGARCG-3′; forward) and p-AHAS8 (reverse). For amplification of the *als* gene(s), PCR reaction conditions were the following: 1 × buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 mM each primer, 1.25 U Taq, and 2 ng genomic sowthistle DNA in a total volume of 25 mL. After an initial denaturation step at 94°C for 1 min, a program of 40 cycles was used, consisting of 10 cycles of touch-down PCR (94°C for 30 s, 67°C to 58°C for 30 s, 72°C for 30 s) followed by 30 cycles of the same cycling regime, but with a fixed annealing temperature of 58°C, and a final elongation step of 72°C for 10 min. PCR products were purified using the Concert™ Rapid PCR Purification System (Invitrogen Life Technologies, Carlsbad, CA) or the QiaQuick PCR Purification System (Qiagen Inc., Valencia, CA), and cloned prior to sequencing using the TOPO TA Cloning Kit (Invitrogen Life Technologies, Carlsbad, CA). Colony PCR products were produced using a proofreading polymerase (Platinum Taq DNA Polymerase, High Fidelity, Invitrogen Life Technologies, Carlsbad, CA) and the same PCR protocol as mentioned above. The amplified DNA fragment was sequenced using an automatic DNA sequencer (ABI PRISM® 377, Perkin-Elmer Applied Biosystems, Foster City, CA) with fluorescence dye-labeled dideoxynucleotides.

The 5′- and 3′-RACE (rapid amplification of cDNA ends) PCRs were performed to get the full length sequence of *als* gene using a RACE PCR kit (First-Choice™ RLM-RACE Kit, Ambion, Inc., Austin, TX). Total RNA was isolated from the leaf tissue of each biotype using a RNA isolation kit (RNeasy® Plant Mini Kit, QIAGEN Inc., Valencia, CA). Poly A^+ RNA was selected from the total RNA using poly A^+ RNA purification kit (Oligotex®, QIAGEN Inc., Valencia, CA). Complementary DNA (cDNA) was synthesized

from poly A^+ RNA using M-MLV reverse transcriptase with 5′- and 3′-RACE adapter. Two nested sense and antisense primers specific to 930-bp fragment of *als* gene were designed to amplify the full length of *als* gene with 5′- and 3′-adaptor primers. About 1.3 kb and 0.5 kb fragments were amplified from 5′- and 3′- RACE PCR, respectively. The amplified DNA fragments were sequenced and aligned. Forward and reverse primers were designed at the end of 5′ and 3′ regions of *als* gene. About 2.1 kb fragment was amplified using DNA polymerase (Platinum® Taq DNA Polymerase High Fidelity, Invitrogen, Carlsbad, CA). The amplified DNA fragment was cloned using the TOPO TA cloning kit and sequenced.

Data Analyses

Whole-plant bioassay and in vivo ALS assay experiments were conducted in a completely randomized design with three replications and were repeated. Data from both experiments had similar magnitudes and constant variances in the whole-plant bioassay and in vivo ALS assay; therefore, the data were combined and analyzed. Dose response curves for the whole-plant bioassay were obtained by a nonlinear regression using log-logistic equations (Streibig 1988; Seefeldt et al. 1995):

$$y = C + \frac{D - C}{1 + \exp[b(\log(x) - \log(GR_{50}))]}$$

where y represents aboveground biomass (percentage of control) at herbicide rate x and C, D, b, and GR_{50} are empirically derived constants. C is the lower limit, D is the upper limit, b is the slope at the GR_{50}, and GR_{50} is herbicide rate required for 50% growth reduction. In vivo ALS assay data also were analyzed using the log-logistic equations. Herbicide rates required for 50% ALS enzyme activity inhibition (I_{50}) were calculated using the equations. The regression parameters from the R and S biotypes were compared to test significant difference using a sum of square reduction test. The level of resistance from the whole-plant bioassay or in vivo ALS assay was determined by calculating the ratio of GR_{50} or I_{50} of the resistant biotypes to GR_{50} or I_{50} of the susceptible biotypes, respectively. Statistical computations were carried out using SAS/STAT NLIN (SAS Institute, Inc. 1987).

RESULTS AND DISCUSSION

Whole Plant Bioassay

Resistance in the R biotype was confirmed from the whole-plant bioassay. Above-ground biomass of the S biotype was reduced by 80% by thifensulfuron at 30 g a.i. ha^{-1} (Fig. 1). However, the R biotype was not affected at this rate. The rate of thifensulfuron required for 50% growth reduction (GR_{50}) was 1090 g a.i. ha^{-1} for the R biotype but only 2.3 g a.i. ha^{-1} for the S biotype (Table 2). The R biotype also was highly resistant to metsulfuron and prosulfuron (Fig. 1 and Table 2). The level of resistance calculated by the estimated GR_{50} values showed that the R biotype was 479-, 99-, and 127-fold more resistant than the S biotype to the thifensulfuron, metsulfuron, and prosulfuron, respectively (Table 2). The R biotype also displayed high level resistance to IMI herbicides, imazamox and imazethapyr (Fig. 1). Based on the GR_{50} values for the R and S biotypes, the R biotype was 14- and 83-fold more resistant than the S biotype to imazamox and imazethapyr, respectively (Table 2). The dose response revealed that the R biotype was highly resistant to both SU and IMI herbicides tested in this study.

In Vivo ALS Assay

The herbicide concentration by biotype interaction was highly significant, indicating different response of biotypes to herbicide concentrations (Fig. 2). The rate of thifensulfuron required for 50% ALS activity inhibition (I_{50}) was 74 nM for the R biotype but only 0.12 nM for the S biotype (Table 2). The level of resistance calculated by the estimated I_{50} values showed that the R biotype was 624-, 41-, and 13-fold more resistant than the S biotype to the thifensulfuron, metsulfuron, and prosulfuron, respectively (Table 2). The R biotype also was 17- and 108-fold more resistant than the S biotype to imazamox and imazethapyr, respectively (Table 2). With the exception of metsulfuron and prosulfuron, the ALS enzyme assays for the R biotype was consistent with the results of whole plant dose-response experiments (Table 2). The level of resistance calculated from the whole plant dose-response experiments was 2.4 and 9.7 times higher than that from the ALS enzyme assays for metsulfuron and prosulfuron, respectively. Although we did not investigate the difference in the resistance level calculated from the whole plant dose-response study and ALS enzyme assay, these results suggest that altered target enzyme was a main resistance mechanism in the R biotype.

Cloning and Sequencing of *als* Genes

Three partial *als* genes (*als1*, *als2*, *and als3*) were amplified, cloned and sequenced from the R and S biotypes using four primer pair combinations, three of which were previously used in *als* amplification (Kolkman et al. 2004). The *als* gene family in the related sunflower was also composed of three *als* genes located in different regions of the genome. In sunflower, there is strong homology between all three genes, making amplification of single genes difficult, yet distinguishable by the presence of a 9 bp and 3 bp indel between the *als1 gene* and the *als2* and *als3 genes*, respectively (Kolkman et al. 2004). The *als1* sequence contained the resistance polymorphism, and is thereby considered the target gene for resistance mechanisms. The role of *als2* and *als3* remains unclear, and was therefore not considered further for sequence analysis in this study. We did not pursue amplification of full length *als2* and

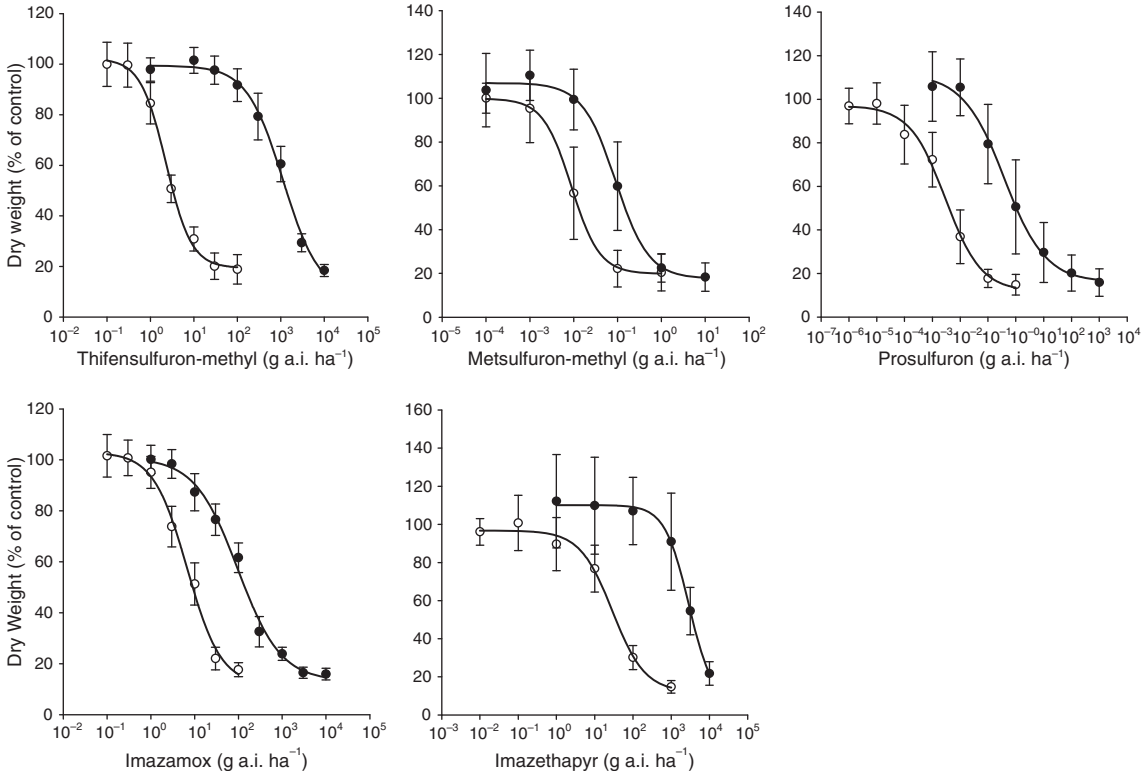

Fig. 1. Effects of ALS inhibitors on the shoot dry weight of the resistant (●) and susceptible (○) spiny annual sow-thistle biotypes. Data were analyzed using the log-logistic equation. Vertical bars represent 95% confidence interval of the mean at each rate.

als3 genes because of the limited relevance of these two genes to resistance (Kolkman et al. 2004). It remains important for future studies, however, that the presence of the three highly similar *als* genes are at a minimum acknowledged in order to avoid false negatives.

As in sunflower, the *als1* gene in spiny annual sow-thistle was distinguished from the *als2* gene by a 9 bp insertion in the reading frame. The a*ls1* gene was distinguished from *als3* in spiny annual sow-thistle by the 3 bp insertion in the same location of the reading frame as the indel between *als1* and *als2*, and is also similar to that found in sunflower.

The full length *als1* gene was cloned and sequenced from the R and S biotypes using 5′- and 3′-RACE PCR and have been deposited into GenBank database under Banklt1104390 and Banklt1104403 respectively. The *als1* sequence that was amplified in spiny annual sow-thistle included 1977-bp coding region, which encoded 658 amino acid residues. The nucleotide sequences of the spiny annual sow-thistle *als1* gene showed over 70%

Table 2. GR^z_{50} and I^y_{50} values of the resistant and susceptible spiny annual sow-thistle *Sonchus asper* biotypes to ALS inhibitors from the whole-plant bioassay and in vivo ALS enzyme assay

	GR_{50}			I_{50}		
Herbicide	R	S	Ratio (R/S)[x]	R	S	Ratio (R/S)[x]
	(g a.i. ha^{-1})			(nM)		
Thifensulfuron	1090 (285)[w]	2.3 (0.24)	479	74 (18)	0.12 (0.04)	624
Metsulfuron	0.9 (0.23)	0.01 (0.002)	99	5.0 (0.14)	0.12 (0.05)	41
Prosulfuron	0.4 (0.20)	0.003 (0.001)	127	0.5 (0.14)	0.04 (0.02)	13
Imazamox	97 (13)	6.7 (0.97)	14	1524 (132)	88 (23)	17
Imazethapyr	2410 (611)	29 (8.8)	83	823 (314)	7.6 (2.4)	108

[z]Herbicide dose required for 50% reduction in shoot dry weight.
[y]Herbicide concentration required for 50% ALS enzyme activity inhibition.
[x]The levels of resistance were calculated from the ratio of GR_{50} or I_{50} of the resistant biotype to the GR_{50} or I_{50} of the susceptible biotype.
[w]Asymptotic standard errors for estimated GR_{50} or I_{50} are in parenthesis.

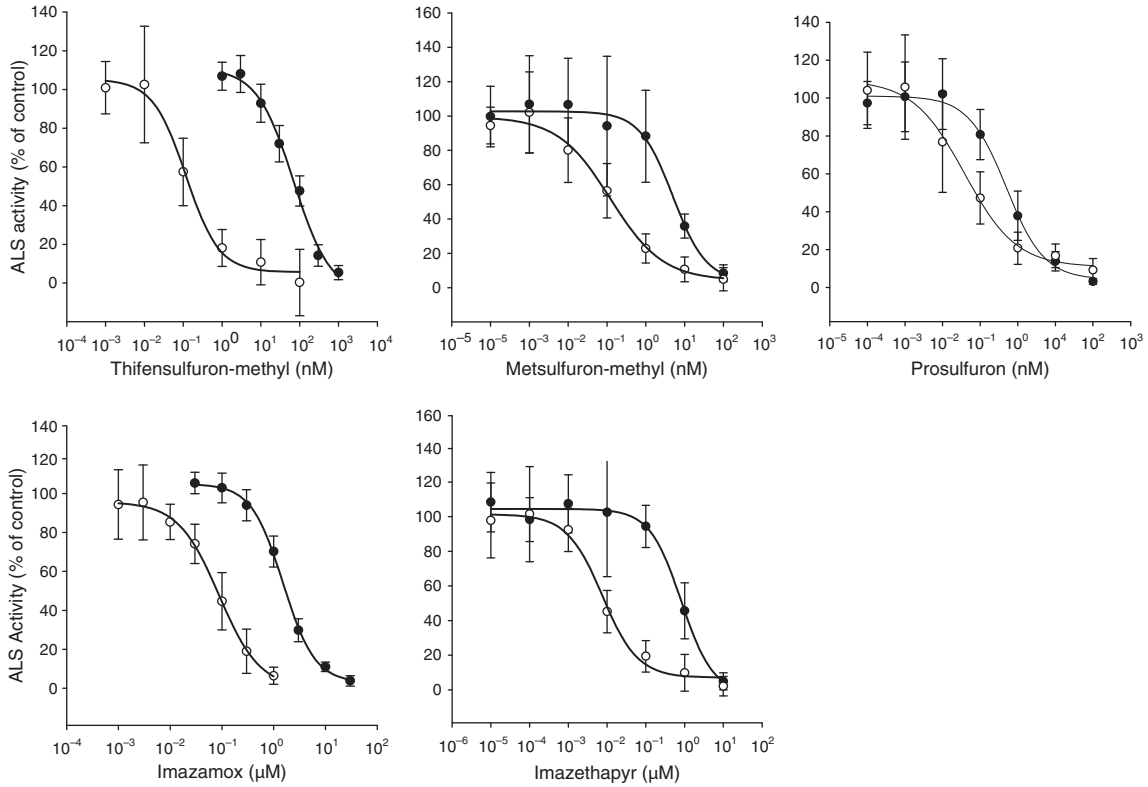

Fig. 2. Effects of ALS inhibitors on the *in vivo* ALS activity in the resistant (●) and susceptible (○) spiny annual sow-thistle biotypes. Data were analyzed using the log-logistic equation. Vertical bars represent 95% confidence interval of the mean at each concentration.

homology with other Asteraceae family *als* nucleotide sequences (Altschul et al. 1997).

DNA sequence analysis of the *als1* gene demonstrated a single-point mutation from C to T at nucleotide 554, conferring the exchange of the amino acid Pro_{197} to Leu in the R biotype (Fig. 3). These data indicate that the proline to leucine change in Domain A of the *als1* gene is responsible for the ALS inhibitor resistance in the R

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S   1 CTCACCCGGTCTAACACCATCCGAAATGTCCTCCCCCGCCATGAACAGGGAGGCGTTTTC
R     ------------------------------------------------------------
S 130 L  T  R  S  N  T  I  R  N  V  L  P  R  H  E  Q  G  G  V  F
R     -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -

S  61 GCCGCCGAAGGCTACGCTCGCGCCTTGGGTAAACCCGGCGTATGCATCGCCACATCCGGC
R     ------------------------------------------------------------
S 150 A  A  E  G  Y  A  R  A  L  G  K  P  G  V  C  I  A  T  S  G
R     -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -

S 121 CCCGGAGCAACCAATCTGGTAAGCGGCCTTGCAGATGCGCTGCTTGACAGTGTCCCAATC
R     ------------------------------------------------------------
S 170 P  G  A  T  N  L  V  S  G  L  A  D  A  L  L  D  S  V  P  I
R     -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -

S 181 GTTGCCATCACCGGCCAAGTCCCCCGGAGAATGATCGGAACTGATGCATTTCAAGAAACC
R     --------------------CTC-------------------------------------
S 190 V  A  I  T  G  Q  V  P  R  R  M  I  G  T  D  A  F  Q  E  T
R     -  -  -  -  -  -  -  L  -  -  -  -  -  -  -  -  -  -  -  -

S 241 CCAATCGTGGAGGTAACGCGTTCCATTACTAAACACAATTACCTTGTTTTGGATGTGGAA
R     ------------------------------------------------------------
S 210 P  I  V  E  V  T  R  S  I  T  K  H  N  Y  L  V  L  D  V  E
R     -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -

S 301 GACATCCCCCGTGTCGTCCATGAAGCTTTCTATCTCGCAACTTCCGGCCGACCTGGTCCT
R     ------------------------------------------------------------
S 230 D  I  P  R  V  V  H  E  A  F  Y  L  A  T  S  G  R  P  G  P
R     -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -
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Fig. 3. Comparison of nucleotide and deduced amino acid sequences of *als1* gene from the resistant (R) and susceptible (S) spiny annual sow-thistle biotypes. The shaded amino acid indicates a Pro to Leu mutation at amino acid 197 in Domain A (box). Numbering of amino acids is based on the precursor ALS from *Arabidopsis thaliana*. The GenBank accession numbers are Banklt1104390 and Banklt1104403 for wild type and mutant *als* genes in spiny annual sow-thistle, respectively.

biotype. This mutation has been identified as the basis for ALS inhibitor resistance in nine other weed species including *Kochia scoparia* (L.) Roth, *Amaranthus retroflexus* L., *H. annuus* L., *Scirpus juncoides* var. ohwianus, and *Thlaspi arvense* L. (Tranel et al. 2011). Resistance found in *A. retroflexus*, which had a Pro_{197} to Leu mutation, exhibited high level of resistance to SU, IMI, PTB, and TP herbicides based on the whole-plant and in vitro ALS assay (Sibony et al. 2001). However, cross resistance patterns and level of resistance in *S. juncoides*, and *T. arvense* were different from those in *A. retroflexus* (Akira et al. 2007; Beckie et al. 2007). According to the results from this study as well as from additional weed species, this point mutation generally confers very high levels of resistance to SU herbicides. However, the level of resistance to the other classes of ALS inhibitors, IMI, PTB, and TP, depends upon the weed species. Resistance to IMI herbicides differed in *A. retroflexus*, *S. juncoides*, and *T. arvense*. *Thlaspi arvense* exhibited a low level of resistance to an IMI herbicide (Beckie et al. 2007), while *S. juncoides* was susceptible to an IMI herbicide (Akira et al. 2007). Although we did not investigate cross resistance to PTB, TP, and SCT herbicides, high levels of resistance to SU and IMI herbicides in this study were consistent with the results from resistance found in *A. retroflexus*.

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