Weed Science 2016 64:231–239

A Target-Site Point Mutation in Henbit (*Lamium amplexicaule*) Confers High-Level Resistance to ALS-Inhibitors

Vijay K. Varanasi, Amar S. Godar, Dallas E. Peterson, Doug Shoup, and Mithila Jugulam*

Henbit is a facultative broadleaf winter annual in the Lamiaceae family. Acetolactate synthase (ALS) inhibitors are primarily used to control a broad spectrum of weeds, including henbit. During 2012 to 2013, field applications of ALS-inhibiting herbicides were ineffective in controlling a henbit population from Marion County, KS (MCK). To confirm field-evolved resistance to ALS inhibitors, response of MCK henbit and a known susceptible henbit population from Kansas (DPS) to varying doses of three different ALS inhibitors were examined: chlorsulfuron, imazamox, and propoxycarbazone. Results of the dose–response experiments suggest that the MCK population is highly resistant to chlorsulfuron (resistance index [R/S] > 1,000) and propoxycarbazone (R/S = 331) but is susceptible to imazamox. A full-length *ALS* gene sequence obtained using the 5′- and 3′- rapid amplification of complementary DNA ends approach revealed a Pro_{197} to Arg point mutation (a common mutation that confers resistance to sulfonylurea herbicides, e.g., chlorsulfuron) in the MCK henbit. No other known resistance-conferring mutations were found in the study. Evolved resistance to major classes of ALS inhibitors in the MCK henbit will reduce herbicide options for its control. To our knowledge, this is the first case of evolution of herbicide resistance in henbit.
Nomenclature: Chlorsulfuron; imazamox; propoxycarbazone; henbit, *Lamium amplexicaule* L.
Key words: 5′- and 3′- RACE, ALS inhibitors, herbicide resistance, winter annual weed.

Increased adoption of no-till production systems (or conservation tillage) in recent years has resulted in a corresponding increase in the prevalence of winter annual weeds, such as common chickweed [*Stellaria media* (L.) Vill.], shepherd's-purse [*Capsella bursa-pastoris* (L.) Medik.], field pennycress (*Thlaspi arvense* L.), purple deadnettle (*Lamium purpureum* L.), and henbit. Henbit is a facultative, winter-annual broadleaf weed that belongs to the Lamiaceae family. It is a major weed in the midwestern and central United States, most notably causing yield losses (13 to 38%) at high densities (82 to 155 plants m^{-2}) in winter wheat (*Triticum aestivum* L.) (Conley and Bradley 2005) and acting as an alternate host of pests, such as soybean cyst nematode in soybeans [*Glycine max* (L.) Merr.] (Nelson et al. 2006; Venkatesh et al. 2000).

ALS or acetohydroxyacid synthase (AHAS) inhibitors are one of the most extensively used classes of herbicides in agronomic crops (Lamego et al. 2009). These herbicides inhibit the ALS enzyme, which catalyzes the first step in the synthesis of branched-chain amino acids Leu, Val, and Ile (Devine and Eberlein 1997). Both target (mutations in the target gene)- and nontarget (reduced absorption or enhanced metabolism)-based mechanisms of resistance to ALS inhibitors have been reported in weeds (Powles and Yu 2010; White et al. 2002). The target-site resistance mechanism for ALS inhibitors involves a single amino acid substitution in the ALS enzyme (Tranel and Wright 2002). Acetolactate synthase is encoded by a nuclear gene and has five highly conserved domains (Saari et al. 1994). Acetolactate synthase is the common target site for all five herbicide families in Group 2 (WSSA classification): sulfonylurea (SU), imidazolinone (IMI), triazolopyrimidine, pyrimidinyl-thiobenzoates, and sulfonylaminocarbonyltriazolinone (SCT).

Acetolactate synthase-inhibiting SU herbicides, such as chlorsulfuron (Glean, E.I. du Pont de Nemours, Wilmington, DE), have been used for controlling both grass and broadleaf weeds, including henbit in winter wheat. Acetolactate synthase inhibitors are popular herbicide options because of their low cost and use rates, high efficacy, environmental safety, minimal mammalian toxicity, and wide crop selectivity (Sari et al. 1994; Yu et al. 2003). Extensive use of ALS-inhibiting herbicides has resulted in evolution of resistance to these herbicides in many weed species, including kochia [*Kochia scoparia* (L.) Schrad.] (Beckie et al. 2011; Varanasi et al. 2015), Palmer amaranth (*Amaranthus palmeri* S. Wats.) (Gaeddert et al. 1997), and common

DOI: 10.1614/WS-D-15-00152.1

* Postdoctoral Research Associate, Postdoctoral Research Associate, Professor, Associate Professor, and Assistant Professor, Department of Agronomy, Kansas State University, Manhattan, KS 66506. Corresponding author's E-mail: mithila@ksu.edu

Table 1. Primers used for amplifying the coding region of *ALS* gene sequence from henbit. The GSPs were used for the 5′- and 3′-RACE PCR reactions, whereas sequencing primers were used to obtain the full-length coding sequence of *ALS* gene from susceptible DPS and resistant MCK henbit.[a] Location of GSP primers is shown in Figure 4.

Primer	Sequence (5′– 3′)	T_m
		C
5′ RACE GSP	CTTGGTAATGGATCGAGTTACCT	55.0
3′ RACE GSP1	AGGTAACTCGATCCATTACCAAG	53.0
3′ RACE GSP2	CTGGGAATGCACGGAACCGTGTA	58.0
Full-length sequence primer (forward)	ACAAGGATGAGACGTTCGCTT	54.0
Full-length sequence primer (reverse)	TCTCCCATCTCCTTCTGTGATC	54.0

[a] Abbreviations: GSP, gene-specific primers; RACE-PCR, rapid amplification of complementary DNA ends-polymerase chain reaction; *ALS*, acetolactate synthase gene; DPS, Dallas Peterson susceptible; MCK, Marion County, KS.

chickweed (Lingenfelter and Curran 2014). Weed resistance to ALS-inhibiting herbicides evolved rapidly compared with other groups of herbicides (Tranel and Wright 2002), following their introduction in the early 1980s. Initial cases of ALS-inhibitor resistance were reported in 1987 in prickly lettuce (*Lactuca serriola* L.) (Mallory-Smith et al. 1990), kochia (Primiani et al. 1990), Russian-thistle (*Salsola tragus* L.) (Stallings et al. 1994) in the United States, and in common chickweed in Canada (Devine et al. 1991). By 2015, resistance to ALS inhibitors was reported in 154 weed species around the world (Heap 2015).

During 2012 to 2013, field applications of chlorsulfuron and propoxycarbazone were ineffective in controlling a henbit population in Marion County, KS (MCK), indicating possible evolution of ALS-inhibitor resistance. The objectives of this study were, therefore, to conduct greenhouse and laboratory studies to confirm ALS-inhibitor resistance and to determine the underlying mechanism in the MCK henbit population.

Materials and Methods

Seed Collection and Plant Material Preparation. The MCK henbit plants were collected from a field that was exposed to frequent (every other year) ALS-inhibitors applications for >10 yr during wheat, soybean, corn (*Zea mays* L.), and sorghum [*Sorghum bicolor* (L.) Moench ssp. *bicolor*] rotation. The field was sprayed with Finesse (E.I. du Pont de Nemours) in the early fall, with a follow-up application of Affinity (E.I. du Pont de Nemours) in December 2012. In May 2013, seeds from 15 to 20 plants were sampled from an area (60.9 m^2) of this field, where there was a lack of henbit control. This sample was used in the greenhouse experiments.

MCK and DPS (known susceptible) henbit plants were grown in Miracle-Gro potting mix (Scotts Miracle-Gro Company, Marysville, OH) in 10- by 10- by 10-cm plastic pots and watered from the top in a greenhouse (25/20 C day/night temperature; 15/9 h day/night light, supplemented with 120 mmol m^{-2} s^{-1} illumination from sodium vapor lamps). Five plants (5 to 6 cm tall) of each MCK and DPS henbit were treated separately with field-use rates and twice the field-use rate of chlorsulfuron (18 and 36 g ai ha^{-1} Glean XP), imazamox (35 and 70 ai ha^{-1} Beyond, BASF Corporation, Research Triangle Park, NC), and propoxycarbazone (44 and 88 g ai ha^{-1} Olympus, Bayer CropScience, Kansas City, MO). All herbicide treatments included 0.25% v/v nonionic surfactant (NIS). Only POST activity of the herbicides was evaluated on henbit. Herbicide treatments were applied with a moving, single-nozzle, bench-type sprayer (Research Track Sprayer, De Vries Manufacturing, Hollandale, MN) equipped with a flat-fan nozzle tip (80015LP TeeJet tip, Spraying Systems Co., Wheaton, IL) delivering 168 L ha^{-1} at 222 kPa in a single pass at 3.2 km h^{-1}.

MCK plants that survived the treatment with 36 g ai ha^{-1} of chlorsulfuron were transferred to individual pots approximately 3 wk after treatment (WAT) and were allowed to self-pollinate. Upon maturity, seeds were collected from the self-pollinated plants and used in dose–response and *ALS* gene-sequencing experiments.

Whole Plant Herbicide Dose–Response Experiments. Progeny seeds from four MCK plants and DPS were germinated in two separate trays in the greenhouse. Individual seedlings were transplanted to 6.5- by 6.5- by 6.5-cm pots in the greenhouse (similar environment as previously described) for whole-plant herbicide dose–response experiments. Six plants of each DPS and MCK henbit (5 cm tall) were treated separately with 0, 0.031, 0.062, 0.125, 0.25, 0.5, 1, and 2 and 0, 0.5, 1, 2, 4, 8, 16, and 32 times the recommended field rate, respectively, of chlorsulfuron and propoxycarbazone.

Additionally, six more plants of each DPS and MCK henbit were also treated with 0, 0.5, and 1 and 0, 1, and 4 times the recommended field use rate of imazamox, respectively. All herbicide treatments included 0.25% v/v NIS and were applied as described previously. Aboveground dry biomass and plant health (composite visual estimation of growth reduction, chlorosis, and other injuries on a scale of 0 [dead] to 100% [not affected]) were determined 2 WAT.

Statistical Analysis. Aboveground dry biomass and plant health (expressed as a percentage of the untreated control) data were analyzed using the *drc* package in R 3.1.2 (R Development Core Team 2014; Ritz and Streibig 2014; Seefeldt et al. 1995). The three-parameter log-logistic model (Equation 1) showed good fit (lack of fit test; $P > 0.81$ for all analyses); thus, the relationship between herbicide rate and biomass or plant health was described as follows:

$$Y = d/(1 + \exp\{b[\log(x) - \log(GR_{50})]\}) \qquad [1]$$

where Y is the response (dry biomass or plant health) expressed as a percentage of the untreated control, d is the asymptotic value of Y at upper limit, b is the slope of the curve around GR_{50} (the herbicide rate giving response halfway between d and the lower asymptotic limit, which was set to 0), and x is the herbicide rate. The resistance index (*Resistance/Susceptibility* [R/S]) was calculated as GR_{50} ratio between the MCK and the DPS henbit populations. There were no significant experimental effects ($P > 0.05$); thus, data from repeated experiments were pooled.

Biomass data, expressed as percentage of the untreated control, for imazamox treatment were analyzed using one-way ANOVA in R version 3.1.2, and the means were compared using Tukey's honestly significant difference test. The data from repeated experiments were pooled because the interaction of herbicide treatment and experiment was not significant ($P > 0.05$).

***ALS* Gene Sequencing.** Attempts to obtain the full-length henbit *ALS* gene fragment using the direct polymerase chain reaction (PCR) approach were unsuccessful because there is no *ALS* gene sequence available in the database for the Lamiaceae family. Therefore, a more-sophisticated and relatively complicated rapid amplification of complementary DNA (cDNA) ends (RACE) PCR was employed to obtain a full-length coding sequence for the *ALS* gene from henbit.

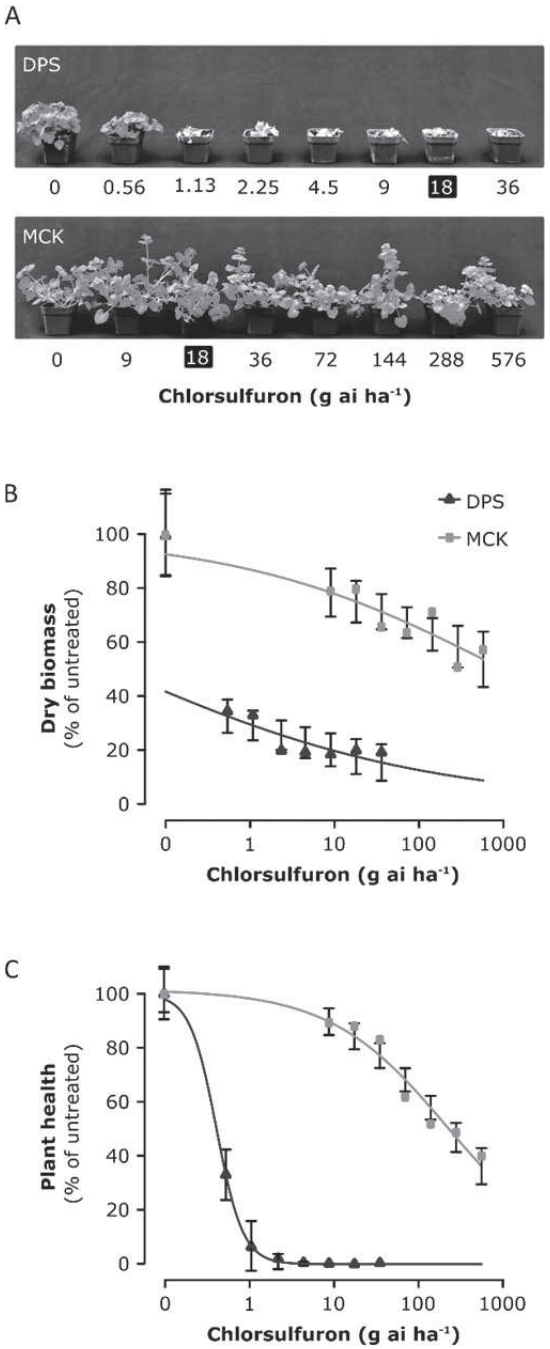

Figure 1. Chlorsulfuron dose–response from a known susceptible henbit population from Kansas (DPS) and from a resistant henbit population from Marion County, KS (MCK) 2 wk after treatment. Photographs of the representative henbit plants for each chlorsulfuron dose (A); highlighted is the recommended rate (18 g ai ha^{-1}). Nonlinear regression analysis is shown of aboveground dry biomass (B) and plant health (C). Symbols in (B) and (C) are an average of eight replicates fitted with a three-parameter log–logistic model (lack of fit test; $P = 0.78$ and 0.86, respectively); model parameters are shown in Table 2. (Color for this figure is available in the online version of this article.)

Total RNA Isolation. Fresh leaf tissue from five MCK plants (MCK 1, 2, 3, 4, and 5) and two DPS plants (DPS 1 and 2) was collected and flash frozen in liquid nitrogen (−196 C). The collected tissue was

Table 2. Summary parameters describing the response of aboveground dry biomass and plant health from DPS (susceptible) and MCK (resistant) henbit to increasing rates of chlorsulfuron treatments 2 WAT. The response was fitted with a three parameter log-logistic model; fitted curves are shown in Figure 1.[a]

Population	Regression parameters[b]		GR_{50} or ED_{50}	R/S
	b	*d*		
			g ai ha^{-1}	
Dry biomass				
DPS	—	100 (8.11)	< 0.56[c]	1
MCK	—	99.9 (7.63)	> 576[c]	$> 1{,}000$
Plant health				
DPS	2.78 (1.11)	100 (4.71)	0.42 (0.06)	1
MCK	0.63 (0.09)	101 (4.27)	224 (48.1)	566 (146)[d]

[a] Abbreviations: DPS, Dallas Peterson susceptible; MCK, Marion County, KS; WAT, weeks after treatment; *b*, relative slope around GR_{50} or ED_{50}; *d*, upper limit of the response; GR_{50}, chlorsulfuron rate causing 50% reduction in aboveground dry biomass; ED_{50}, chlorsulfuron rate causing 50% reduction in plant health; R/S, resistance index (ratio of GR_{50} or ED_{50} to DPS [susceptible] and MCK [resistant] henbit).

[b] Values in parenthesis are $\pm$ 1 standard error.

[c] Estimated value lies beyond range of chlorsulfuron rates (0.56–576 g ai ha^{-1}) tested.

[d] R/S is significantly > 1 at $P < 0.001$.

stored at −80 C for total RNA isolation. The frozen tissue was homogenized in liquid nitrogen using a prechilled mortar and pestle to prevent thawing. The powdered tissue was transferred to a 1.5-ml microcentrifuge tube, and the total RNA was isolated with an RNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA). Ribonucleic acid was treated with DNase 1 enzyme (Thermo Scientific, Waltham, MA) to remove genomic DNA contamination. The isolated RNA was stored at −80 C. The quantity and quality (integrity) of total RNA were determined with a spectrophotometer (NanoDrop 1000, Thermo Scientific) and agarose gel (1%) (Agarose MS) electrophoresis.

Gene-Specific Primer Designing. Gene-specific primers (GSPs) were designed using OligoAnalyzer 3.1 (IDT SciTools, 2014; Integrated DNA Technologies, Inc., Coralville, IA). Both 5′ and 3′ gene-specific primers (5′ RACE GSP, 3′ RACE GSP1 and GSP2) (Table 1) of the *ALS* gene were designed by alignment of the nucleotide sequences from species representing several plant families available in GenBank (National Center for Biotechnology Information, Bethesda, MD; http://www.ncbi.nlm.nih.gov, accessed December 19, 2014): mouse-ear cress [*Arabidopsis thaliana* (L.) Heynh.] (GenBank NM_114714.2), pigweed (*Amaranthus* sp.) (GenBank U55852.1), downy brome (*Bromus tectorum* L.) (GenBank AF487459.1), kochia (GenBank EU517499.1), blackgrass (*Alopecurus myosuroides* Huds.) (GenBank AJ437300.2), and corn poppy (*Papaver rhoeas* L.) (GenBank AJ577316.1). The nucleotide sequences were aligned using MultAlin (Corpet 1988). Finally, the entire coding sequence for the *ALS* genes from MCK and DPS henbit were amplified from cDNA using full-length sequencing primers F and R (Table 1), designed based on the sequences obtained from 5′ and 3′ RACE. The full-length coding (cDNA) sequences of henbit from MCK and DPS were deposited and are accessible in the GenBank (KR816155 and KR816156, respectively).

RACE-Ready cDNA Synthesis. The 5′- and 3′-RACE-ready cDNA was synthesized from 1 μg of total RNA with the SMARTer RACE 5′/3′ kit (Clonetech Laboratories, Inc., Mountain View, CA) and following manufacturer's instructions. The 5′- and 3′-RACE-ready cDNA samples were stored at −20 C until RACE-PCR.

RACE-PCR. The PCR was performed in a T100 thermal cycler (BioRad Inc., Hercules, CA) using PCR master mix (Promega, Madison, WI). The 50-μl reaction volume consisted of 25 μl of PCR master mix (2 ×), 5 μl of GSP (10 μM), 5 μl of 10 × universal primer mix, 3 μl of cDNA template (5′-RACE-ready cDNA for 5′ RACE-PCR; and 3′-RACE-ready cDNA for 3′ RACE PCR, synthesized above), and 12 μl of nuclease-free water. The 5′ RACE-PCR conditions were 94.0 C for 3 min, 35 cycles of 94.0 C for 30 s, 54.0 C for 45 s, and 72.0 C for 1 min, followed by 72.0 C for 7 min. The 3′ RACE-PCR conditions using GSP1 primer were similar, except for an annealing temperature of 53.0 C. The 3′ RACE-PCR conditions for GSP2 primer were 94.0 C for 3 min, 35

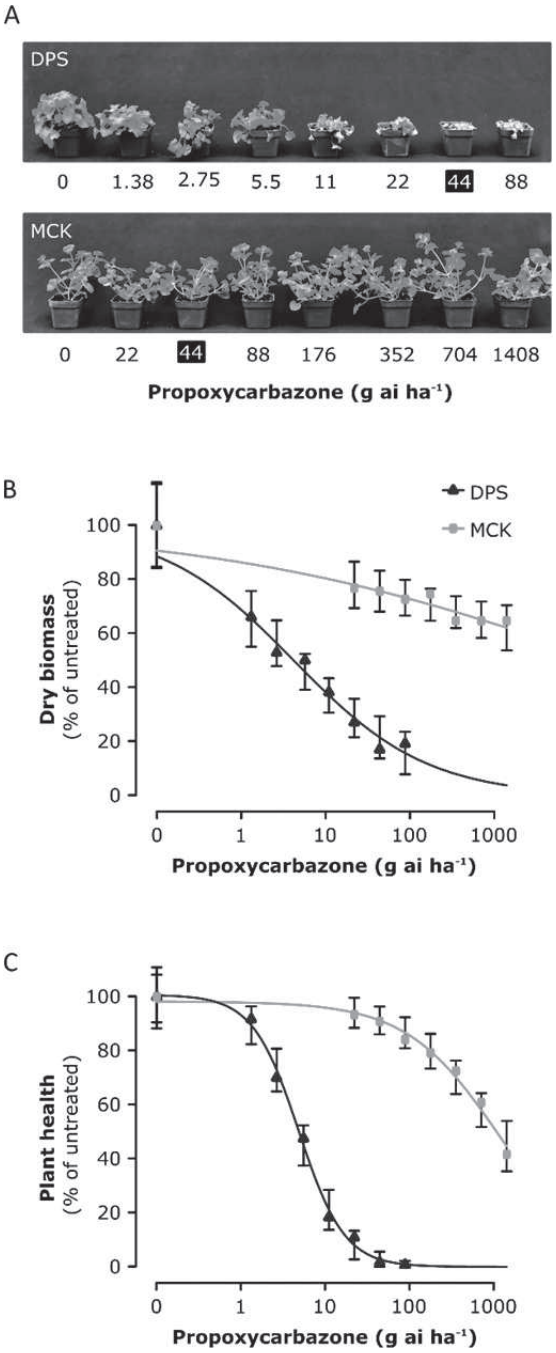

Figure 2. Propoxycarbazone dose–response from a known susceptible henbit population from Kansas (DPS) and from a resistant henbit population from Marion County, KS (MCK) 2 wk after treatment. Photographs are of the representative henbit plants for each propoxycarbazone dose (A); highlighted is the recommended rate (44 g ai ha^{-1}). Nonlinear regression analysis of aboveground dry biomass (B) and plant health (C) is shown. Symbols in (B) and (C) are an average of six replicates fitted with a three-parameter log–logistic model (lack of fit test; P = 0.997 and 0.995, respectively); model parameters are shown in Table 3. (Color for this figure is available in the online version of this article.)

cycles of 94.0 C for 30 s, 58.0 C for 30 s, and 72.0 C for 3 min, followed by 72 C for 7 min. The PCR products were run on 0.8% agarose gel with 100-bp and 500-bp markers to confirm amplicon size.

cDNA Synthesis. cDNA for full-length *ALS* gene sequencing was synthesized from 1 μg of the total RNA using RevertAid First Strand cDNA synthesis kit (Thermo Scientific). The cDNA was then diluted with molecular-grade water in a 1 : 5 ratio and used in a PCR reaction to amplify the coding region of the *ALS* gene from MCK and DPS henbit populations.

Reverse Transcription PCR. Reverse transcription–polymerase chain reaction (RT-PCR) was performed in a T100 thermal cycler (BioRad Inc.) using PCR master mix. The 25-μl reaction volume consisted of 12.5 μl of PCR master mix (2 ×), 2.5 μl of forward primer (5 μM), 2.5 μl of reverse primer (5 μM), 3 μl of cDNA template, and 4.5 μl of nuclease-free water. PCR conditions were 94 C for 3 min, 35 cycles of 94 C for 30 s, 54 C for 45 s, and 72 C for 2 min, followed by 72 C for 7 min. The PCR products were run on 0.8% agarose gel with 100-bp and 500-bp markers to confirm amplicon size.

PCR Purification and Sequencing. Both RACE-PCR and RT-PCR products were purified using GeneJet PCR purification kit (Thermo Scientific) and were quantified with a Nanodrop spectrophotometer. The purified PCR products (25 to 50 ng μl^{-1}) were sequenced with an ABI 3730 DNA analyzer (Applied Biosystems, Grand Island, NY).

Results and Discussion

The MCK henbit exhibited minimal response to increasing rates (up to 32 times the field use rate, 576 g ai ha^{-1}) of chlorsulfuron at 2 WAT (Figure 1). Representative plants treated with each rate of chlorsulfuron are shown in Figure 1A. The chlorsulfuron rate that caused 50% reduction in the biomass of DPS (GR_{50}) was less than the lowest rate (0.56 g ai ha^{-1}), whereas for MCK, it was greater than the highest rate (576 g ai ha^{-1}) used in this study. Thus, the R/S is estimated to be greater than 1,000 fold (Figure 1B; Table 2). Although the accurate GR_{50} values for chlorsulfuron could not be calculated, these results suggest that the MCK henbit is highly resistant to chlorsulfuron. The biomass data were slightly inflated because all plants accumulated approximately 20% of the final biomass of untreated plants at the time of herbicide treatment. However, the chlorsulfuron rate that caused 50% reduction in plant health (ED_{50}) values lay within the chlorsulfuron rates used (Figure 1C; Table 2). Based on a visual estimation of plant health, the MCK henbit was 566 times more resistant ($P < 0.001$) to

Table 3. Summary parameters describing the response of aboveground dry biomass and plant health in DPS (susceptible) and MCK (resistant) henbit populations to increasing rates of propoxycarbazone treatments 2 WAT.[a] The response was fitted with a three-parameter log–logistic model; fitted curves are shown in Figure 2.

Population	Regression parameters[b]		GR_{50} or ED_{50}	R/S
	b	*d*		
			g ai ha^{-1}	
Dry biomass				
DPS	0.54 (0.11)	99.9 (7.82)	4.25 (1.71)	1
MCK	—	100 (7.86)	$>$ 1,408[c]	$>$ 331
Plant health				
DPS	1.60 (0.24)	101 (5.12)	4.85 (0.61)	1
MCK	0.79 (0.20)	98.2 (5.02)	1069 (235)	220 (55.9)[d]

[a] Abbreviations: DPS, Dallas Peterson susceptible; MCK, Marion County, KS; WAT, wk after treatment; *b*, relative slope around GR_{50} or ED_{50}; *d*, upper limit of the response; GR_{50}, propoxycarbazone rate causing 50% reduction in aboveground dry biomass; ED_{50}, propoxycarbazone rate causing 50% reduction in plant health; RS, resistance index (ratio of GR_{50} or ED_{50} of DPS [susceptible] and MCK [resistant] henbit populations).

[b] Values in parenthesis are ± 1 standard error.

[c] Estimated value lies beyond the maximum rate of propoxycarbazone (1,408 g ai ha^{-1}) tested.

[d] R/S is significantly $>$ 1 at $P < 0.001$.

chlorsulfuron than was the DPS henbit. Similar to chlorsulfuron, the MCK henbit was also not controlled by increasing rates of propoxycarbazone (Figure 2). The GR_{50} for propoxycarbazone were estimated at 4.25 and $>$ 1,408 g ai ha^{-1}, for DPS and MCK henbit, respectively (Table 3). Thus, the MCK henbit was also highly resistant to propoxycarbazone (R/S $>$ 331, based in GR_{50}; R/S = 220, based on ED_{50}).

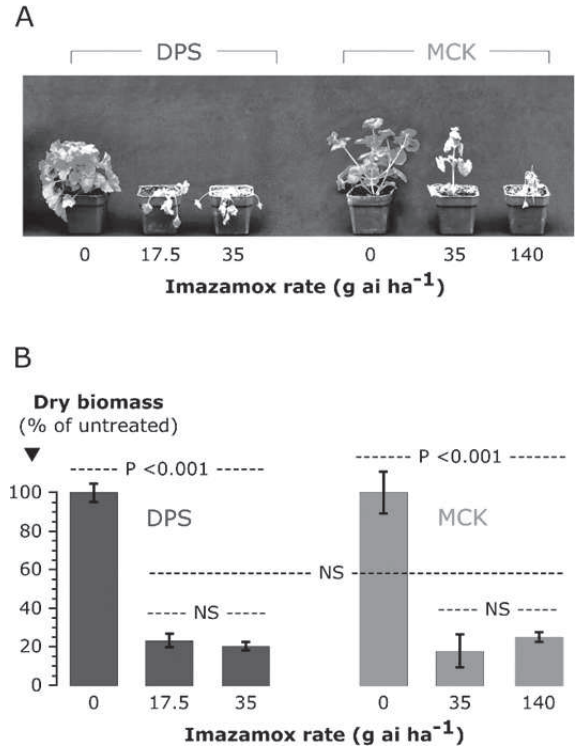

Figure 3. Response from a known susceptible henbit population from Kansas (DPS) and from a resistant henbit population from Marion County, KS (MCK) 2 wk after imazamox treatment. Photographs are of the representative henbit plants for each imazamox rate (A); the field dose is 35 g ai ha^{-1}. A bar graph of aboveground dry biomass is also shown (B). Vertical bars are the averages of six replicates, and error bars are ± 1 standard error of the mean. NS, nonsignificant ($P > 0.05$). (Color for this figure is available in the online version of this article.)

Although resistant to chlorsulfuron and propoxycarbazone, the MCK henbit was susceptible to imazamox (Figure 3). Dry biomass was similar for imazamox-treated plants, regardless of the henbit population or the rate applied ($P > 0.05$). Similar high levels of resistance (R/S = 340 to 400) to SU herbicides sulfosulfuron and sulfometuron and susceptibility to imazamox were detected in hare barley [*Hordeum murinum* L. ssp. *leporinum* (Link) Arcang.] population of Australia (Yu et al. 2007). The high-resistance levels to SU herbicides were attributed to the presence of a Pro to Ser point mutation at position 197 in the *ALS* gene of hare barley. The Pro_{197}–Ser mutation conferring ALS-inhibitor resistance was also reported in kochia (Varanasi et al. 2015; Warwick et al. 2008) and wild mustard (*Sinapis arvensis* L.) (Warwick et al. 2005).

A Pro_{197} to Arg substitution, because of a point mutation in the nucleotide sequence of the *ALS* gene, known to confer resistance to SU herbicides, was found in the MCK population (Figure 4). There were no known IMI-specific mutations (at Ala_{122} and Ala_{205}) found in the MCK population, indicating target-site sensitivity to imazamox, which was confirmed by dose–response studies (Figure 3). In most cases, mutations at Pro_{197} confer resistance to SU but not to IMI herbicides (Yu and Powles 2014). The Pro_{197} position on the *ALS* gene exhibits the highest variability in amino acid substitutions contributing to SU resistance in weeds. So far,

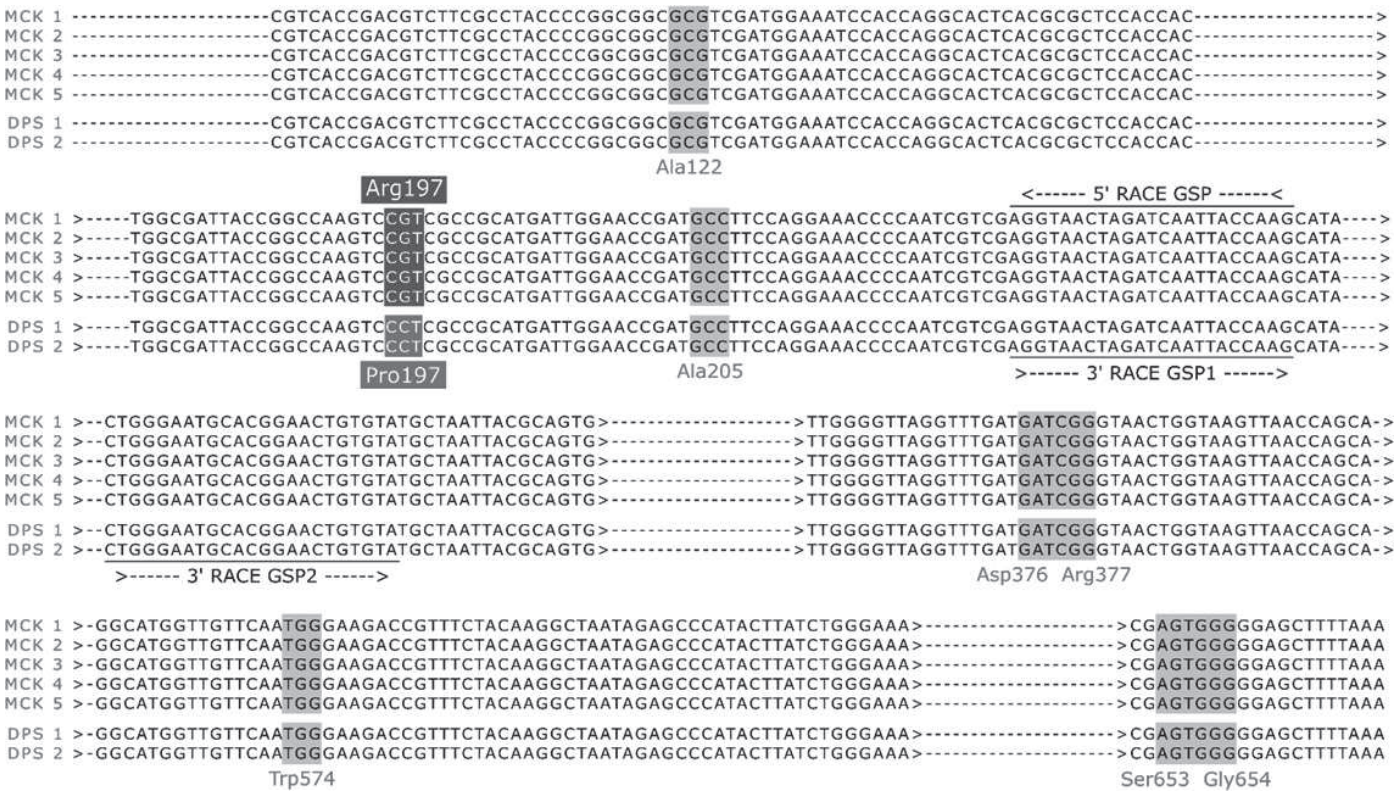

Figure 4. Nucleic acid sequence alignment of *ALS* gene from five Marion County, KS (MCK) (resistant) and two known susceptible (DPS) henbit individual plants. The Pro197 to Arg mutation is highlighted, and the nucleotides are identified in white, whereas other commonly known ALS mutations conferring resistance to *ALS* inhibitors are highlighted in light gray. Also shown are the locations of the primers and the direction of 5′- and 3′-RACE-PCRs. The figure shows only the regions of the *ALS* gene sequence at which point mutations have been reported in other weed species and is not the complete sequence. The dotted lines indicate identical sequence information for all resistant and susceptible lines. For complete coding sequence information, please refer to the GenBank KR816155 and KR816156. (Color for this figure is available in the online version of this article.)

11 substitutions (Thr, Ser, Arg, His, Leu, Gln, Ala, Ile, Asn, Tyr, and Glu) have been reported for the Pro_{197} loci in the *ALS* gene, conferring SU resistance in various weed species (Heap 2015). The Pro_{197} to Arg substitution from the present study has been reported in weed species such as kochia, rigid ryegrass (*Lolium rigidum* Gaudin), and corn poppy but not in any winter annual broadleaf weed species (Heap 2015).

Point mutations in the different conserved domains of the *ALS* gene and substitutions of amino acids at a specific mutation site could confer different cross-resistance patterns (Tranel and Wright 2002; Tranel et al. 2015). Many of the point mutations at Pro_{197} in the ALS enzyme, which confer resistance to chlorsulfuron, can lead to cross-resistance to other ALS inhibitors (Wright and Penner 1998). The level of cross-resistance to ALS family herbicides, such as IMIs, triazolopyrimidines (TPs), pyrimidinylthiobenzoates, and SCTs depends on the specific amino acid that is substituted at the Pro_{197} position in the ALS enzyme (Park et al. 2012).

Park and Mallory-Smith (2004) reported resistance to SUs (primisulfuron and sulfosulfuron) and SCTs (propoxycarbazone-sodium) in downy brome from substitution of amino acid Pro to Ser at position 197 in the *ALS* gene sequence. The Pro_{197} to Ser mutation resulted in cross-resistance to SCTs in downy brome. In another study, the occurrence of resistance in crown daisy (*Chrysanthemum coronarium* L.) to ALS-inhibiting herbicides, such as tribenuron, iodosulfuron, chlorsulfuron, sulfometuron, imazethapyr, flumetsulam, pyrithiobac-sodium, and propoxycarbazone-sodium, was due to the substitution of Pro_{197} with either Ser or Thr (Tal and Rubin 2004). Krysiak et al., in 2011, also reported strong resistance to SUs as well as SCTs in silky windgrass [*Apera spica-venti* (L.) Beauv.], a winter annual, because of substitution from Pro_{197} to either Ser or to Thr. These studies suggest that *ALS* gene mutations resulting in the substitution of Pro_{197} with amino acids Ser or Thr would lead to resistance to both SUs and SCTs in weed species. Massa et al. (2011) found that a Leu mutation at Trp_{574} and a

His mutation at Arg_{377} endow resistance to SUs, SCTs, and TPs in silky windgrass. The MCK henbit didn't have the Pro_{197} to Ser or Thr point mutations nor did it have any known mutations at Trp_{574} and Arg_{377}. Cross-resistance to SCTs from Pro_{197}–Arg mutation has not been studied in other weed species (Heap 2015). The Pro_{197} to Arg mutation in MCK population could possibly endow cross-resistance to SCTs. Cross-resistance patterns depend on various factors, such as the type of mutations, the chemical family of the ALS inhibitors, the specific herbicide within a family, and weed species (Yu and Powles 2014). Further research is needed to determine the cross-resistance patterns to SUs and SCTs from different *ALS* gene mutations (if any) in henbit. Other resistance mechanisms, such as reduced uptake-translocation or increased metabolism of SUs, were not investigated in this study. Hence, the possibility of such nontarget site mechanisms cannot be ruled out in henbit ALS-inhibitor resistance.

In summary, this study reports the first documented case, to our knowledge, of field-evolved resistance in henbit to ALS inhibitors. We have demonstrated that a population from Marion County, KS, is highly resistant to ALS-inhibiting herbicides, such as chlorsulfuron and propoxycarbazone, primarily because of an amino acid substitution from the Pro to the Arg at position 197 in the *ALS* gene sequence. The MCK henbit can be controlled using imazamox and probably with herbicides that have other modes of action recommended for use in winter wheat. Resistance of henbit to ALS inhibitors will reduce weed control options in wheat and no-till crop production.

Acknowledgments

The authors thank Dr. Junjun Ou for his assistance in preparing plant materials used in this study.

Literature Cited

Beckie HJ, Warwick SI, Sauder CA, Lozinski C, Shirriff S (2011) Occurrence and molecular characterization of acetolactate synthase (ALS) inhibitor–resistant kochia (*Kochia scoparia*) in Western Canada. Weed Technol 25:170–175

Conley SP, Bradley KW (2005) Wheat (*Triticum aestivum*) yield response to henbit (*Lamium amplexicaule*) interference and simulated winterkill. Weed Technol 19:902–906

Corpet F (1988) Multiple sequence alignment with hierarchical clustering. Nucleic Acids Res 16:10881–10890

Devine MD, Eberlein CV (1997) Physiological, biochemical and molecular aspects of herbicide resistance based on altered target sites. Pages 159–185 *in* Roe RM, Burton JD, Kuhr RJ, eds. Herbicide Activity: Toxicology, Biochemistry, and Molecular Biology. Amsterdam, Netherlands: IOS

Devine, MD., Marles MAS, Hall LM. (1991) Inhibition of acetolactate synthase in susceptible and resistant biotypes of *Stellaria media*. Pestic Sci 31:273–280

Gaeddert, JW, Peterson DE, Horak MJ (1997) Control and cross-resistance of an acetolactate synthase inhibitor-resistant Palmer amaranth (*Amaranthus palmeri*) biotype. Weed Technol 11:132–137

Heap I (2015) The International Survey of Herbicide Resistant Weeds. http://www.weedscience.org. Accessed May 13, 2015

IDT SciTools. (2014) A Suite for Analysis and Design of Nucleic Acid Oligomers. https://www.idtdna.com/calc/analyzer. Accessed June 20, 2014

Krysiak M, Gawroński SW, Adamczewski K, Kierzek R (2011) *ALS* gene mutations in *Apera spica-venti* confer broad-range resistance to herbicides. J Plant Prot Res 51:261–267

Lamego FP, Charlson D, Delatorre CA, Burgos NR, Vidal RA (2009) Molecular basis of resistance to ALS-inhibitor herbicides in greater beggarticks. Weed Sci 57:474–481

Lingenfelter DD, Curran W (2014) Another ALS-resistant species: common chickweed and its control. Page 15 *in* Proceedings of the 69th North Central Weed Science Society, Minneapolis, MN: Weed Science Society of America

Mallory-Smith CA, Thill DC, Dial MJ (1990) Identification of herbicide resistant prickly lettuce (*Lactuca serriola*). Weed Technol 4:163–168

Massa D, Krenz B, Gerhards R (2011) Target-site resistance to ALS-inhibiting herbicides in *Apera spica-venti* populations is conferred by documented and previously unknown mutations. Weed Res 51:294–303

Nelson KA, Johnson WG, Wait JD, Smoot RL (2006) Winter-annual weed management in corn (*Zea mays*) and soybean (*Glycine max*) and the impact of soybean cyst nematode (*Heterodera glycines*) egg population densities. Weed Technol 20:965–970

Park KW, Kolkman JM, Mallory-Smith CA (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

Park KW, Mallory-Smith CA (2004) Physiological and molecular basis for ALS inhibitor resistance in *Bromus tectorum* biotypes. Weed Res 44:71–77

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

Primiani MM, Cotterman JC, Saari LL (1990) Resistance of kochia (Kochia scoparia) to sulfonylurea and imidazolinone herbicides. Weed Technol 4:169–172

R Development Core Team. (2014) A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org/. Accessed November 15, 2014

Ritz C, Streibig JC (2014) drc: Analysis of Dose–Response Curve Data. Version 2.3-96. http://cran.r-project.org/web/packages/drc/drc.pdf. Accessed July 12, 2015

Saari, LL, Cotterman JC, Thill DC (1994) Resistance to acetolactate synthase-inhibiting herbicides. Pages 83–139 *in* Powles SB, Holtum JAM, eds. Herbicide Resistance in Plants: Biology and Biochemistry. Boca Raton, FL: Lewis

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

Stallings GP, Thill DC, Mallory-Smith CA (1994) Sulfonylurea-resistant Russian thistle (*Salsola iberica*) survey in Washington State. Weed Technol 8:258–264
Tal A, Rubin B (2004) Occurrence of resistant *Chrysanthemum coronarium* to ALS inhibiting herbicides in Israel. Resist Pest Manag Newsl 13:31–33
Tranel PJ, Wright TR (2002) Resistance of weeds to ALS-inhibiting herbicides: what have we learned? Weed Sci 50:700–712
Tranel PJ, Wright TR, Heap IM (2015) Mutations in Herbicide-Resistant Weeds to ALS Inhibitors. http://weedscience.com. Accessed July 21, 2015
Varanasi VK, Godar AS, Currie RS, Dille JA, Thompson CR, Stahlman PW, Mithila J (2015) Field-evolved resistance to four modes of action of herbicides in a single kochia (*Kochia scoparia* L Schrad.) population. Pest Manag Sci 71:1207–1212
Venkatesh R, Harrison SK, Riedel RM (2000) Weed hosts of soybean cyst nematode (*Heterodera glycines*) in Ohio. Weed Technol 14:156–160
Warwick SI, Sauder C, Beckie HJ (2005) Resistance in Canadian biotypes of wild mustard (*Sinapis arvensis*) to acetolactate synthase inhibiting herbicides. Weed Sci 53:631–639
Warwick SI, Xu R, Sauder C, Beckie HJ (2008) Acetolactate synthase target-site mutations and single nucleotide polymorphism genotyping in ALS-resistant kochia (*Kochia scoparia*). Weed Sci 56:797–806
White AD, Owens MDK, Hartzler RG, Cardina J (2002) Common sunflower resistance to acetolactate synthase-inhibiting herbicides. Weed Sci 50:432–437
Wright TW, Penner D (1998) In vitro and whole-plant magnitude and cross-resistance characterization of two imidazolinone-resistant sugarbeet (*Beta vulgaris*) somatic cell selections. Weed Sci 46:24–29
Yu Q, Nelson JK, Zheng MQ, Jackson M and Powles SB (2007) Molecular characterisation of resistance to ALS-inhibiting herbicides in *Hordeum leporinum* biotypes. Pest Manag Sci 63:918–927
Yu Q, Powles SB (2014) Resistance to AHAS inhibitor herbicides: current understanding. Pest Manag Sci 70:1340–1350
Yu Q, Zhang XQ, Hashem A, Walsh MJ, Powles SB (2003) ALS gene proline (197) mutations confer ALS herbicide resistance in eight separated wild radish (*Raphanus raphanistrum*) populations. Weed Sci 51:831–838

Received September 3, 2015, and approved November 25, 2015.

Associate Editor for this paper: Patrick J. Tranel, University of Illinois.