Confirmation and Characterization of ALS-inhibitor Resistance in Henbit (*Lamium amplexicaule* L.)

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INTRODUCTION

- Henbit (*Lamium amplexicaule* L.) also known as deadnettle is a facultative winter annual broadleaf weed that belongs to the mint family (Lamiaceae).
- Henbit is a serious problem in no-till production systems in Midwestern and central U.S., most notably causing yield losses in winter wheat (*Triticum aestivum*) (Conley & Bradley, 2005) and acting as an alternate host of pests such as soybean cyst nematode (SCN) in soybeans (*Glycine max*) (Venkatesh et al., 2000).
- Acetolactate synthase (ALS)-inhibiting herbicides such as chlorsulfuron (Glean®) have been the primary herbicides for control of broadleaf weeds including henbit in winter wheat.
- During 2013-2014 season, field applications of ALS-inhibiting herbicides were ineffective in controlling a henbit population in Marion County Kansas (MCK).

OBJECTIVES

- Confirm ALS-inhibitor resistance in MCK henbit.
- Determine the mechanism of ALS-inhibitor resistance in MCK henbit.

MATERIALS & METHODS

Herbicide dose-response studies to confirm ALS-inhibitor resistance in MCK population:

- The MCK and a known susceptible (S) henbit populations were grown in the greenhouse and treated (5 cm tall) with three families of ALS inhibitors: sulfonylurea (SU) (chlorsulfuron - Glean®), sulfonlyaminocarbonyl-triazolinone (SCT) (propoxycarbazone - Olympus®), and imidazolinone (IMI) (imazamox - Beyond®) with recommended adjuvants. The following rates of these herbicides were applied:

- Chlorsulfuron (1X = 18 g ha^{-1}) and propoxycarbazone (1X = 44 g ha^{-1})
  - S: 0, 0.03, 0.06, 0.125, 0.25, 0.5, 1, and 2X
  - MCK: 0, 0.5, 1, 2, 4, 8, 16, and 32X
- Imazamox (1X = 35 g ha^{-1})
  - S: 0, 0.5, and 1X
  - MCK: 0, 1, and 4X
- Aboveground dry biomass was determined 3 weeks after treatment.
- Biomass data were analyzed by non-linear log logistic regression using *drc* package in R v3.1.1 (Knezevic et al., 2007).

Determine the target-site (*ALS* gene) mutation in MCK population:

- Conserved gene specific primers (GSP) (Table 1) were designed by alignment of *ALS* sequences from arabidopsis, amaranthus species, *Bromus tectorum*, *Kochia scoparia*, *Alopecurus myosuroides*, and *Papaver rhoeas* obtained from GenBank.
- The *ALS* gene region spanning known mutation(s) conferring resistance to ALS-inhibitors was amplified from two MCK plants (MCKR1 and R2) and one known susceptible plant (S1) using 5' and 3' RACE (Rapid Amplification of cDNA Ends). The nucleotide sequences were aligned using MultAlin software (Corpet, 1988).

Table 1: Primers used for 5' and 3' RACE PCR

Primer	Sequence	Tm (°C)
5' RACE GSP	5' CTTGGTAATGGATCGAGTTACCT 3'	55
3' RACE GSP	5' AGGTAACTCGATCCATTACCAAG 3'	53

RESULTS & DISCUSSION

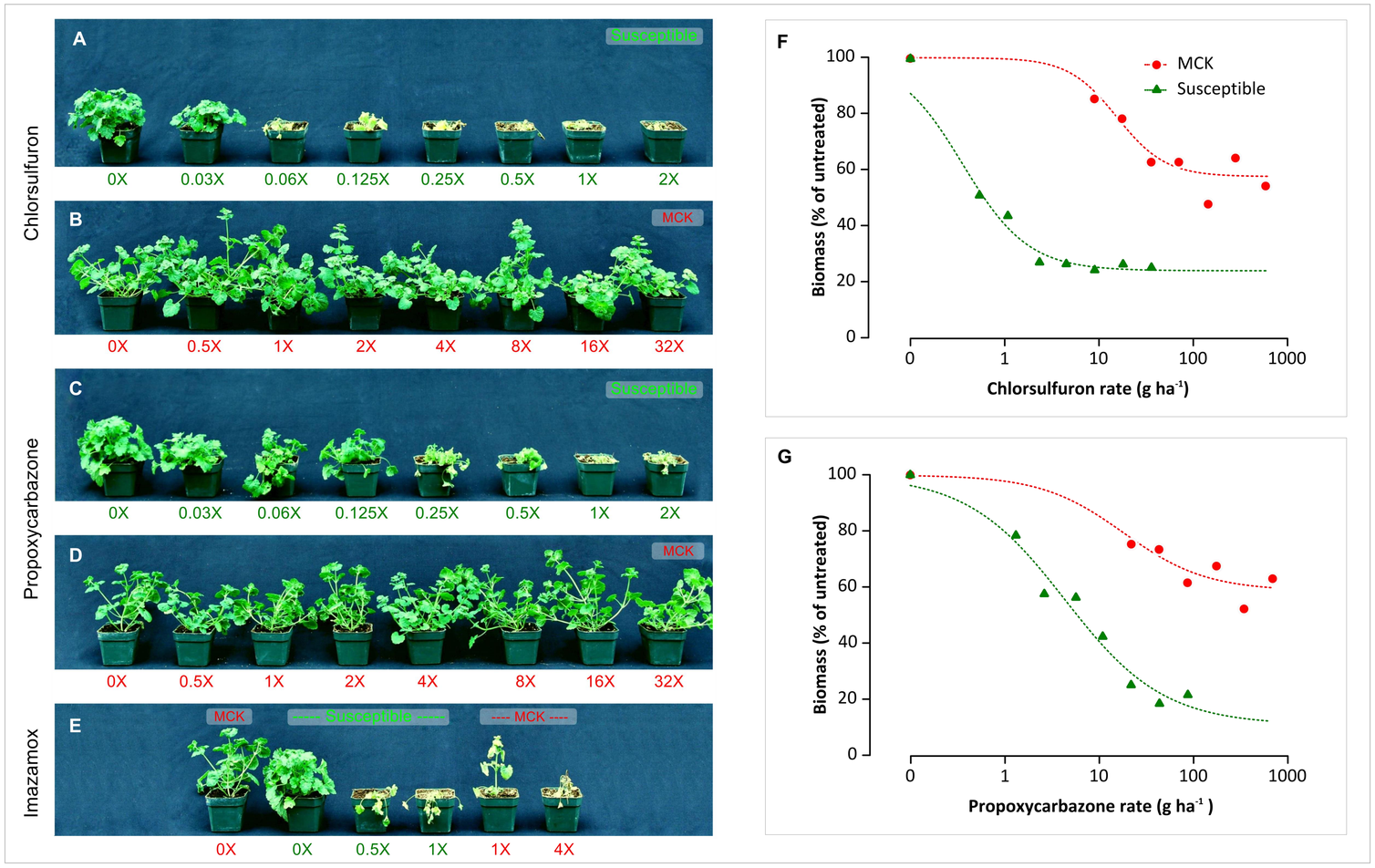

Figure 1 A, B, & F: Dose-response of S and MCK henbit to chlorsulfuron. The MCK henbit survived herbicide rates up to 32X (1X = 18 g ha^{-1}); **C, D, & G:** Dose-response of S and MCK henbit to propoxycarbazone. The MCK henbit survived herbicide rates up to 32X (1X = 44 g ha^{-1}); **E:** Susceptibility of MCK henbit to imazamox.

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MCKR1  ACCGACGTCTTCGCCTACCCCGGCGGCGCGTCGATGGAAATCCACCAGGCACTCACGCGCTCCACCACCATCCGCAACGTCCTCCCTCGCCACGAGCAGGGCGGAGTTTTCGCCGCCGAGGGCTACGCA
MCKR2  ACCGACGTCTTCGCCTACCCCGGCGGCGCGTCGATGGAAATCCACCAGGCACTCACGCGCTCCACCACCATCCGCAACGTCCTCCCTCGCCACGAGCAGGGCGGAGTTTTCGCCGCCGAGGGCTACGCA
S1     ACCGACGTCTTCGCCTACCCCGGCGGCGCGTCGATGGAAATCCACCAGGCACTCACGCGCTCCACCACCATCCGCAACGTCCTCCCTCGCCACGAGCAGGGCGGAGTTTTCGCCGCCGAGGGCTACGCA
                                Alanine122
MCKR1  GCGCCTCCGGCCTACCCGGCGTCTGCATTGCCACCTCTGGCCCCGGCGCCACCAATTTGGTCTCCGGCCTCGCTGACGCTCTCCTCGACAGTGTCCCGATAGTGGCGATTACCGGCCAAGTCCGTCGCC
MCKR2  GCGCCTCCGGCCTACCCGGCGTCTGCATTGCCACCTCTGGCCCCGGCGCCACCAATTTGGTCTCCGGCCTCGCTGACGCTCTCCTCGACAGTGTCCCGATAGTGGCGATTACCGGCCAAGTCCGTCGCC
S1     GTGCCTCCGGCCTACCCGGCGTCTGCATTGCCACCTCTGGCCCCGGCGCCACCAATTTGGTCTCCGGCCTCGCTGACGCTCTCCTCGACAGTGTCCCGATAGTGGCGATTACCGGCCAAGTCCCTCGCC
                                                                                                                                Pro197Arg
MCKR1  GATGATTGGAACCGATGCCGC----------------------------------------------------------------------------------------------------------
MCKR2  GATGATTGGAACCGATGCTGA----------------------GTGATTTGTTGCTCGCATTTGGGGTTAGGTTTGATGATCGCGTAATTGGTAAGTTAGAGGCCGTTTACAAGTAGG
S1     GATGATTGGAACCGATGCCAG----------------------GTGATTTGTTGCTTGCCTTTGGGGTTAGGTTTGATGATCGGGTAACTGGTAAATCGGACACCGTTTGCTACTACG
                  Alanine205                                                    Asp376  Arg377
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Figure 2: Sequencing of *ALS* gene revealed a single mutation involving substitution of proline with arginine at position 197 (yellow box) in henbit MCKR1 and R2 indicating target-site-based resistance to chlorsulfuron. Commonly known *ALS* gene mutation sites are highlighted in blue. MCKR1 and MCKR2 are the two individual plants of MCK henbit, whereas S1 indicates a susceptible henbit plant.

- The MCK population of henbit is resistant to chlorsulfuron and propoxycarbazone (Figure 1 A, B, C & D) and the levels of resistance (GR_{50} ratio of R/S) were 43.0 and 26.90, respectively (Figure 1 F & G); but susceptible to imazamox (Figure 1E).
- A proline to arginine point mutation at position 197, known to confer resistance to SU herbicides, was found in the MCK population (Figure 2).
- Efforts are underway to cover the full length coding sequence of the *ALS* gene to determine if a separate mutation for propoxycarbazone resistance is present in the MCK population.

CONCLUSION & IMPLICATIONS

- The MCK population of henbit is resistant to chlorsulfuron and propoxycarbazone.
- A target-site mutation confers resistance to SU herbicides in the MCK population.
- This is the first report of evolved herbicide resistance in henbit (Heap, 2014).
- The MCK population of henbit can be controlled using IMIs.
- Resistance to other ALS-inhibitors in MCK henbit will reduce control options during the crop growing season.

REFERENCES

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Corpet F (1988) Nucl. Acids Res. 16: 10881-10890.

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