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ALS–Resistant Annual Sedge (*Cyperus compressus*) Confirmed in Turfgrass

Patrick E. McCullough, Jialin Yu, J. Scott McElroy, S. Chen, H. Zhang, Timothy L. Grey, and Mark A. Czarnota*

Acetolactate synthase (ALS) inhibitors are widely used for POST control of sedges in turfgrass. A suspected resistant (R) biotype of annual sedge was collected from a bermudagrass turf in Georgia with a history of exclusive use of halosulfuron. Research was conducted to evaluate the resistance level of this biotype to halosulfuron, efficacy of ALS-inhibiting herbicides and other mechanisms of action for control, and the molecular and physiological basis for resistance. In greenhouse experiments, the halosulfuron rate required to reduce shoot biomass 50% in comparison with the nontreated at 8 wk after treatment (WAT) were 8 and $>$ 1,120 g ai ha^{-1} for the S (susceptible) and R biotypes, respectively. Imazapic, sulfosulfuron, and trifloxysulfuron reduced biomass of the S biotype greater than 60% at 8 WAT, but biomass was reduced less than 20% for the R biotype. Glufosinate, glyphosate, MSMA, and sulfentrazone reduced shoot biomass of the R biotype by 93, 86, 97, and 45%, respectively. In laboratory experiments, the halosulfuron concentration required to inhibit ALS activity by 50% in excised leaf tissues was 5.8 and $>$ 1,000 μM for the S and R biotypes, respectively. Gene sequencing of the R biotype revealed a Pro-197-Ser substitution that confers resistance to ALS inhibitors. This is the first report of ALS-inhibitor resistance in annual sedge and herbicide resistance in a sedge species from a turfgrass system.
Nomenclature: Glufosinate; glyphosate; halosulfuron; imazapic; MSMA; sulfentrazone; sulfosulfuron; trifloxysulfuron; annual sedge, *Cyperus compressus* L.; bermudagrass, *Cynodon dactylon* L. (Pers.) × *Cynodon transvaalensis* Burtt-Davy.
Key words: Efficacy, mutation, sedge, sod, turf, sulfonylurea.

ALS inhibitors are the most widely used herbicides for POST control of sedges (*Cyperus* spp.) in turfgrass. These herbicides have low use rates, broad-spectrum weed control, and limited injury potential to tolerant turfgrasses (Blum et al. 2000; Maloy and Christians 1986). The ALS inhibitors flazasulfuron, imazapic, sulfosulfuron, and trifloxysulfuron control sedges in warm-season turfgrasses. These herbicides are highly efficacious for sedge control but are injurious to cool-season grasses (Derr 2012). Halosulfuron is the only ALS inhibitor labeled for sedge control in all major warm and cool-season turfgrasses (Blum et al. 2000; Derr 2012; Fry et al. 1995; Teuton et al. 2008). It controls multiple sedge species (Lowe et al. 2000; Vencill et al. 1995) and can reduce total biomass and reproductive structures (McElroy et al. 2004; Webster and Grey 2014). Practitioners often use halosulfuron for sedge control when multiple turfgrass species are managed in lawns, golf, or sod production.

Resistance to ALS-inhibiting herbicides has increased exponentially during the last decade in various cropping systems (Heap 2015). ALS inhibitor resistance in weeds is attributed to either target site alteration or enhanced herbicide degradation (Riar et al. 2015; Tranel and Wright 2002). Of these two, target site alteration occurs more frequently and results in greater levels of ALS resistance (Shaner 1999; Tranel and Wright 2002; Whaley et al. 2006; Zheng et al. 2011). There are currently five sedge species with ALS inhibitor resistance reported, including shortleaf spike sedge [*Cyperus brevifolius* (Rottb.) Endl. Ex Hassk], smallflower umbrella sedge (*Cyperus difformis* L.), yellow nutsedge (*Cyperus esculentus* L.), rice flatsedge (*Cyperus iria* L.), and fragrant flatsedge (*Cyperus odoratus* L.) (Busi et al. 2006; Heap 2015; Kuk et al. 2004; Merotto et al. 2010; Ortiz et al. 2015; Riar et al. 2015; Tehranchian et al. 2015a,b). Fragrant flatsedge, rice flatsedge, and smallflower umbrella sedge are annuals that reproduce from seed, while the others are perennials that reproduce from seed and tubers. There is very limited information available about annual sedge seed production (Reddi and Reddi 2009;

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* First, second, and sixth authors: Associate Professor, Postdoctoral Researcher, and Associate Professor, respectively, Crop and Soil Sciences Department, University of Georgia, Griffin, GA 30223; third, fourth, and fifth authors: Professor, Graduate Research Assistant, and Graduate Research Assistant, respectively, Department of Crop, Soil, and Environmental Sciences, Auburn University, Auburn, AL 36849; seventh author, Associate Professor, Horticulture Department, University of Georgia, Griffin, GA 30223. Corresponding author's E-mail: pmccull@uga.edu

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Sharma and Shiam 1981). Seed production in this outcrossing species would facilitate pollen-mediated gene flow of nuclear ALS-resistant alleles from resistant individuals to previously susceptible populations. This has been noted in multiple species including Palmer amaranth (*Amaranthus palmeri* S. Wats.), wild radish (*Raphanus raphanistrum* L.), ryegrass (*Lolium rigidium* Gaudin), and giant foxtail (*Setaria faberi* Herrm.) (Wise et al. 2009; Volenberg and Stoltenberg 2002; Yu et al. 2003, 2008).

An annual sedge biotype with suspected resistance to ALS inhibitors was identified in a bermudagrass turf in Georgia. The manager noted reductions in annual sedge control in 2014 after using halosulfuron exclusively for over a decade. There have been no reports of ALS-inhibitor–resistant sedges identified from turfgrass systems. The confirmation of an ALS-inhibitor–resistant sedge species could have serious implications for future resistance issues, herbicide selection, and turf management programs. The objectives of this research were to evaluate (1) the level of resistance of this biotype to halosulfuron, (2) efficacy of various herbicides for control, and (3) molecular and physiological mechanisms associated with resistance.

Materials and Methods

Plant Material. Annual sedge plants were collected from a hybrid bermudagrass field in Cook County, GA, on August 12, 2014. The location coordinates will not be disclosed to protect the privacy of the landowner. These plants were uninjured from halosulfuron at a standard use rate, 70 g ai ha^{-1}, that was applied approximately 3 wk prior to collection. Annual sedge was also collected in Griffin, GA, from a susceptible population. The two biotypes were then transplanted to separate plastic pots with 79-cm^2 surface areas and 10-cm depths filled with sand : peat moss (85 : 15 v/v). Pots were placed in a greenhouse set for 32/25 C day/night at the University of Georgia Griffin campus. Approximately 18 plants of each biotype were irrigated to prevent moisture deficiencies and allowed to produce seed heads. Upon maturity, seeds were collected by hand and scattered over pots with 3.8-cm diam and 20-cm depths with the aforementioned soil. Pots were fertigated (MacroN 28–7–14 Sprayable Fertilizer, LESCO Inc., Cleveland, OH) biweekly and allowed to reach a height of ~10 cm before treatments.

Herbicide Response Experiments. The response of the two annual sedges was evaluated from a rate titration of halosulfuron. Treatments were applied in a spray chamber calibrated to deliver 187 L ha^{-1} with a flat-fan nozzle (8002E, TeeJet Spraying Systems Co., Roswell, GA 30075). Halosulfuron-methyl (Sandea 75WG, Gowan Co., Yuma, AZ 85364) was applied at 4.4, 8.8, 17.5, 35, 70, 140, 280, 560, or 1,120 g ai ha^{-1}. Nontreated checks of the two biotypes were included. A nonionic surfactant (Chem Nut 80-20, mixture of alkyl and arkylaryl polyoxyethylene glycol, 80%, Chem Nut Inc., P.O. Box 3706, Albany, GA 31706) was added to the spray solution at 0.25% v/v. Plants were returned to the greenhouse at ~1 h after treatment (HAT) and did not receive irrigation until 24 HAT. Shoot biomass was harvested at 8 WAT, oven-dried for 72 h at 60 C, and then weighed.

In a separate experiment, annual sedges were treated with glufosinate, glyphosate, halosulfuron, imazapic, mesotrione, MSMA, sulfentrazone, sulfosulfuron, and trifloxysulfuron. A nontreated check was included. Application rates and product information are presented in Table 1. All herbicides were applied with the aforementioned surfactant except glyphosate. Shoot biomass was harvested at 8 WAT, oven-dried, and weighed.

ALS Enzyme Inhibition. Experiments were conducted to evaluate halosulfuron inhibition of ALS enzymes with modified methods described by Cross et al. (2013). This evaluation involved converting acetolactate to acetoin after exposing plant tissues to halosulfuron. Acetoin reductions indicate that acetolactate production is inhibited due to less ALS enzyme activity during branched-chain amino acid synthesis. Plants were established in the greenhouse as previously mentioned. Green leaf tissue was harvested from both biotypes and incubated in a 10-ml capped polystyrene culture tube. The incubation mixture consisted of 100 mg of plant tissue in 3 ml of 25% (w/v) Murashige and Skoog salt media (Sigma-Aldrich Corp., basal salt mixtures M5524, St. Louis, MO 63103) containing 500 μM 1,1-cyclopropanedicarboxylic acid and 0.25% (v/v) Triton X-100 (Sigma-Aldrich Corp., basal salt mixtures M5524, St. Louis, MO 63103). Tubes contained technical grade halosulfuron-methyl (99% chemical purity, Chem Service, Inc. West Chester, PA 19381) at 0, 0.01, 0.1, 1, 10, 100, or 1,000 μM concentrations. Incubations were conducted at 25 C for 12 h under 350 μmol m^{-2} s^{-1}) constant light in a growth chamber (Percival, 505 Research Drive

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Table 1. Shoot biomass of two annual sedge biotypes at 8 wk after treatment from nine herbicides used for POST weed control in turfgrass in two greenhouse experiments, Griffin, GA. Results were pooled over experimental runs.

WSSA group[b]	Herbicide	Trade name (manufacturer)	Rate	Shoot biomass reduction[a]	
				Resistant	Susceptible
			g ai ha^{-1}	% from nontreated	
2	Halosulfuron-methyl	Sandea 75WG (Gowan Co., Yuma, AZ; http://www.gowanco.com)	70	−18	62
	Imazapic	Plateau 2 L (BASF Corp., Research Triangle Park, NC; http://www.basf.com)	105	20	63
	Sulfosulfuron	Certainty 74WDG (Monsanto Co., St. Louis, MO; http://www.monsanto.com)	66	−6	80
	Trifloxysulfuron-sodium	Monument (Syngenta Corp., Greensboro, NC; http://syngeta-us.com)	29	18	69
9	Glyphosate	Roundup Pro 3 lb ae gal^{-1} (Monsanto Co., St. Louis, MO; http://www.monsanto.com)	420[c]	86	77
10	Glufosinate	Finale 1L (Bayer Environmental Science, Research Triangle Park, NC; http://www.bayercropscience.us)	1,120	93	86
14	Sulfentrazone	Dismiss 4SC (FMC Corp., Philadelphia, PA; http://www.fmc.com)	420	45	54
17	MSMA	Target 6L (Luxembourg-Pamol Inc., Memphis, TX; http://www.luxpam-usa.com)	2,240	97	95
27	Mesotrione	Tenacity 4SC (Syngenta Corp., Greensboro, NC; http://syngeta-us.com)	280	17	17
			$LSD_{0.05}$	21	

[a] Shoot biomass of the nontreated plants averaged 0.67 (± 0.04) and 0.81 (± 0.09) g $plant^{-1}$ for the resistant and susceptible biotypes, respectively. Negative numbers represent increased biomass in comparison with the nontreated.

[b] Weed Science Society of America group numbers listed represent the following: 2, ALS inhibitors; 9, 5-enolpyruvoylshkimate-3-phosphate synthase inhibitor; 10, glutamine synthetase inhibitor; 14, protoporphyrinogen oxidase inhibitor; 17, cell-division inhibitor; and 27, 4-hydroxyphenyl pyruvate dioxygenase inhibitor.

[c] Glyphosate rate is listed as g ae ha^{-1}.

Perry, IA 50220 USA). After removal from the growth chamber, incubation tubes were placed in a freezer at −80 C until analyzed.

Acetolactate was then converted to acetoin for measurements with procedures developed by Westerfeld (1945) as follows. The filtered samples were acidified using H_2SO_4 (Sigma Aldrich, St. Louis, MO 63103) to a final concentration of 0.5% (v/v). The tubes were then heated in a water bath (Branson Ultrasonic Model 5510, Danbury, CT 06813) for 30 min at 60 C to achieve the decarboxylation of acetolactate to acetoin. All tubes then received a mixture of 1-naphthol and creatine monohydrate solution (Sigma Aldrich) to achieve the final concentration of 20 mg ml^{-1} and 2 mg ml^{-1}, respectively. The solutions were then heated at 37 C for 30 min for color development. For the determination of acetoin, the tubes were centrifuged for 10 min at 9,900 × *g* (Centrifuge 5417 C, Eppendorf AG, Hamburg 22331, Germany) and the absorbance was measured at 530 nm with a spectrophotometer (UV-Vis Spectrophotometer, UV-1700 Series, Nishinokyo Kuwabara-cho, Nakagyo-ku, Kyoto 604-8511, Japan). Acetoin concentrations were then used to determine the levels of acetolactate produced in the presence of halosulfuron.

ALS Gene Sequencing. cDNA was isolated via RNA extraction of leaves (~ 0.1 g) and reverse transcriptase conversion. RNA was extracted via standard guanidinium thiocyanate-phenol-chloroform extraction methodology (TRIzol® LS Reagent, Life Technologies, Carlsbad, CA). RNA was converted to cDNA by reverse transcriptase polymerase chain reaction (PCR) using RETROscript® kit (Life Technologies). Two primer pairs were designed based on the ALS sequence from yellow nutsedge (*Cyperus esculentus*; GenBank accession number:

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Table 2. Primers designed in the study of the ALS gene mutations in annual sedge.

Primer pair	Sequence (5′–3′)	Targeted mutation site
ALSF197	ACGGATGTCTTCGCCTACCC	Pro197
ALSR197	CAGCCAACTGTTGCTGAATGTC	Pro197
ALSF574	AACCGCATGTGTCAATCTGC	Trp574
ALSR574	CTAGTACACAGTCCGGCC	Trp574

KM624613.1) to cover the regions surrounding the Pro_{197} and Trp_{574} codons in S and R biotypes (Table 2). Primers were synthesized by Eurofins (Eurofins MWG Operon LLC, Huntsville, AL 35805). A PCR was conducted using standard protocols in a 25-µl volume that consisted of 250 ng cDNA, 4 µM forward and reverse primer, 0.625 U Taq DNA polymerase, 200 µM dNTP, and 1 × standard Taq reaction buffer. The PCR was run in a Mastercycler (Eppendorf, Hauppauge, NY 11788) with the following profile: 95 C for 1 min; 35 cycles of 95 C (denaturation) for 1 min, 60 C (annealing) for 90 s, and 68 C (extension) for 1 min; followed by a final extension step of 5 min at 68 C. PCR products were electrophoresed on 1.0% agarose gel, stained with ethidium bromide, and visualized under UV light. PCR products were purified using an E.Z.N.A. Gel Extraction Kit (Omega Bio-Tek Inc., Norcross, GA 30071) and then used for Sanger sequencing by the Genomic and Sequencing Laboratory at Auburn University (GSLAU 2015). Sequencing resulted in a 710-bp amplicon surrounding the Trp-574 codon (GenBank accession numbers [KT150718] and [KT150720] for R and S biotypes, respectively) and a 410-bp amplicon surrounding the Pro-197 codon (GenBank accession numbers [KT150717] and [KT150719] for R and S biotypes, respectively).

Experimental Design and Data Analysis. The experimental design in the greenhouse and the laboratory experiment were a randomized complete block with five replications. All experiments were repeated once. A block design was used to reduce the potential variability of greenhouse location on plant responses to herbicides. The block design was chosen for ALS inhibition experiments due to the amount of time required to analyze one replication and to reduce potential variability of laboratory conditions over time.

Data were subjected to analysis of variance with the PROC GLM in SAS (SAS v. 9.3, Cary, NC) to test for treatment by experimental run interaction. For the dose response experiments, data were regressed against the following equation:

$$y = \beta_0^*\{1-[\exp(-\beta_1^* x)]\} \quad [1]$$

where y is shoot biomass, β_0 is the asymptote, β_1 is the slope estimate, and x is halosulfuron rate. For the ALS enzyme analysis, data were regressed against the following equation:

$$y = \beta_0+\beta_1 x \quad [2]$$

where y is ALS activity, β_0 is the intercept, β_1 is the slope estimate, and x is halosulfuron concentration. Models were chosen for regression analysis that characterized the relationship of response curves with herbicide rate after plotting treatment means in figures.

For the evaluation of various herbicides, shoot biomass data were converted to percent reductions in comparison with the nontreated by replication. Means were separated with Fisher's protected LSD test at $\alpha = 0.05$. Treatment-by-experimental run interactions were not detected, and thus, results were combined for presentation.

Results and Discussion

Herbicide Response Experiments. A biotype-by-rate interaction was detected for shoot biomass reductions in comparison with the nontreated, and thus results are presented by biotype. The halosulfuron rate required to reduce biomass 50% measured 8 and $> 1{,}120$ g ai ha^{-1} for the S and R biotypes, respectively (Figures 1 and 2). Biomass of the R biotype was reduced $< 12\%$ from all application rates. Halosulfuron rates $\geqslant 35$ g ai ha^{-1} reduced biomass of the S biotype $> 80\%$. Results support the supposition that the annual sedge biotype was resistant to halosulfuron with an estimated > 140-fold greater level of resistance than the S biotype.

Resistance levels to halosulfuron are comparable to previous reports in ALS-inhibitor–resistant sedges. Biotypes of rice flatsedge from Arkansas and Mississippi had > 21-fold levels of resistance relative to an S biotype (Riar et al. 2015). Tehranchian et al. (2015b) reported > 15-fold level of resistance to ALS inhibitors in smallflower umbrella sedge from Arkansas. There have been no reports of ALS-inhibitor–resistant sedges in turfgrass, but researchers have identified similar resistant levels for annual bluegrass (*Poa annua* L.) in turf (Cross et al. 2013; McElroy et al. 2009). Comparable levels of ALS-inhibitor

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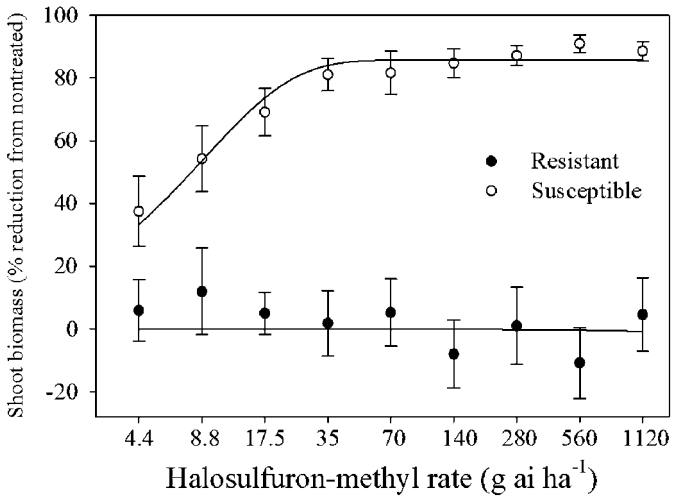


Figure 1. Shoot biomass of acetolactate synthase–inhibitor-resistant and –susceptible annual sedge biotypes at 8 wk after treatment in two greenhouse experiments. Results were pooled over experimental runs. Vertical bars represent standard errors of the mean ($n = 10$). Data were regressed with $y = \beta_0 \times \{1 - [\exp(-\beta_1 \times x)]\}$, where y is shoot biomass reductions, β_0 is the asymptote, β_1 is the slope estimate, and x is halosulfuron-methyl rate. Susceptible regression: $r^2 = 0.42$, $y = 85.72 \times \{1 - [\exp(-0.11 \times x)]\}$. Standard errors for β_0 and β_1 were 2.7 and 0.02, respectively, and 95% confidence limits for β_0 and β_1 were 80 to 91 and 0.08 to 0.15, respectively. Resistant regression: $r^2 = 0.0$, $y = -1.72 \times \{1 - [\exp(-0.01 \times x)]\}$. Standard error for β_0 and β_1 were 10.3 and 0.08, respectively, and 95% confidence limits for β_0 and β_1 were −22.1 to 18.7 and −0.2 to 0.2, respectively.

resistance have been reported in other row-crop weed species including horseweed [*Conyza canadensis* (L.) Cronq.], pigweeds (*Amaranthus* spp.), and rice barnyardgrass [*Echinochloa phyllopogon* (Stapf) Koso-Pol] (Osuna et al. 2002; Whaley et al. 2006; Zheng et al. 2011).

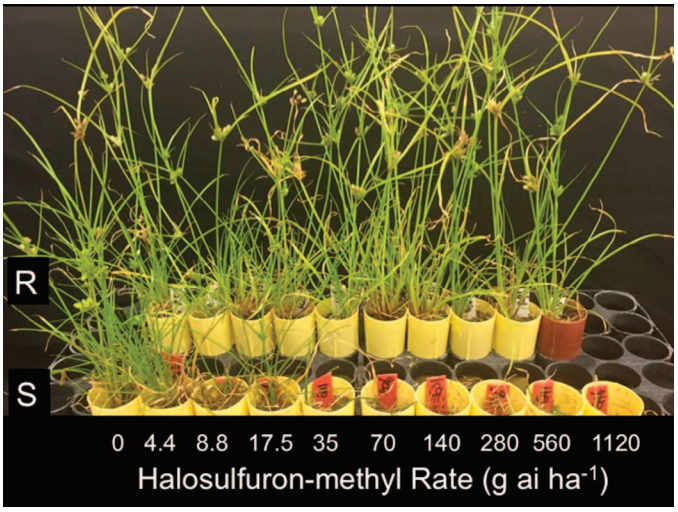


Figure 2. Resistant (R) and susceptible (S) annual sedge biotypes at 8 wk after treatment with halosulfuron-methyl in a greenhouse experiment, Griffin, GA. Photo courtesy of J. Yu.

The R biotype of annual sedge was not controlled by other ALS inhibitors evaluated. Halosulfuron, imazapic, sulfosulfuron, and trifloxysulfuron reduced biomass of the R biotype ≤ 20% in comparison with the nontreated at 8 WAT (Table 1). Conversely, these herbicides reduced biomass for the S biotype by 62 to 80%. Similar cross-resistance to ALS inhibitors was noted in rice flatsedge and smallflower umbrella sedge in rice fields (Merotto et al. 2010; Riar et al. 2015; Tehranchian et al. 2015b).

Glufosinate, glyphosate, and MSMA reduced biomass 77 to 97% for both biotypes, while sulfentrazone reduced biomass of both biotypes by 45 to 54% relative to the nontreated. Mesotrione caused initial visual injury on both biotypes (PE McCullough, unpublished data), but biomass was only reduced 17% at 8 WAT. Rotating herbicides to non–ALS inhibitors effectively controlled R biotypes of rice flatsedge and smallflower umbrella sedge in previous investigations (Riar et al. 2015; Tehranchian et al. 2015).

The R biotype may be selectively controlled in bermudagrass with MSMA or sulfentrazone. These herbicides are important tools for managing ALS resistance but have significant limitations for end-users. In the United States, MSMA has restricted uses in golf course, highway rights-of-way, and sod farms (U.S. EPA 2013). One spot application of MSMA, not to exceed 25% of the total area per year, is allowed at golf courses, whereas two applications are allowed in sod and roadsides. Residential lawns and athletic fields have lost all uses of MSMA. These results emphasize the importance of MSMA use in turfgrass for managing herbicide-resistant weeds. The implications for losing organic arsenical herbicides could limit the mechanisms of action available to turf managers for selective POST weed control, and could further exacerbate the spread of resistant biotypes.

Sulfentrazone is a protoporphyrinogen oxidase inhibitor that may provide PRE and POST control of annual sedge, yellow nutsedge, and *Kyllinga* spp. (Senseman 2007). This herbicide may provide an alternative mechanism of action to ALS inhibitors for use in sequential programs for POST control of annual sedge (Brecke et al. 2005; McElroy et al. 2005; Wehtje et al. 1997). Sulfentrazone was less efficacious for controlling the R and S biotypes than MSMA, and sequential applications may be required for complete control of annual sedge. Moreover, cool-season turfgrasses cannot be treated with greater than 280 g ha^{-1} of sulfentrazone in a single application due to greater injury potential than

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warm-season grasses (Anonymous 2012). This rate restriction may limit efficacy for POST control of sedges and could warrant sequential applications that increase turfgrass injury potential.

Glufosinate and glyphosate use would be limited to spot applications in turfgrass for annual sedge control since these herbicides are nonselective and cause significant injury to actively growing turfgrasses. Nonselective herbicides would be important if practitioners have inconsistent control from other mechanisms of action or have exceeded the annual use rates from other herbicides. The R biotype of annual sedge was cross-resistant to all ALS inhibitors tested. With the exception of mesotrione, all other herbicides tested have potential to control this ALS R biotype.

ALS Enzyme Inhibition. A biotype-by-halosulfuron concentration interaction was detected for ALS enzyme inhibition, and results are presented across all combinations. The halosulfuron concentration required to inhibit isolated ALS enzymes 50% (I_{50}) measured 5.8 and > 1,000 μM for the S and R biotypes of annual sedge, respectively (Figure 3). Differences in target site susceptibility have been reported in sedges with resistance to ALS inhibitors. The I_{50} values for R and S biotypes of rice flatsedge were 2,600 and 200 μM of halosulfuron, respectively (Singh et al. 2015). In yellow nutsedge, Tehranchian et al. (2014) reported the ALS enzyme from an R biotype was 2,540 times less susceptible to inhibition by halosulfuron than the S biotype. Similar differences in target site inhibition were reported in a halosulfuron-resistant biotype of smallflower umbrella sedge (Tehranchian et al. 2015b).

Reduced target site susceptibility has been associated with ALS-inhibitor resistance in other weeds. Annual bluegrass resistant to ALS inhibitors required greater than 3,000 times higher concentrations of trifloxysulfuron and foramsulfuron to inhibit ALS by 50% compared to an S biotype (Cross et al. 2013). Reduced levels of ALS inhibition by sulfonylureas have been noted in R biotypes of shattercane [*Sorghum bicolor* (L.) Moench] and eastern black nightshade (*Solanum ptychanthum* Dunal) compared to their respective S biotypes (Anderson et al. 1998; Carey et al. 1997). These differences could further exacerbate the spread and competition of R biotypes in polyculture with S biotypes when ALS-inhibitors are applied.

Comparative Sequence Analysis of the Genes Encoding ALS Enzymes. Pro-197 and Trp-574 are common amino acid substitution sites that yield a high level of resistance to sulfonylurea herbicides and have been identified in the past to confer resistance in other sedge species (Tehranchian et al. 2014, 2015b; Riar et al. 2015), and were evaluated in this work. A mutation (CCC to TCC) was identified resulting in a Pro-197 to Ser amino acid substitution codon in the resistant population (Figure 4). A mutation was not detected in the Trp-574 codon. Previous studies have shown that Pro-197-Ser substitution in ALS confers strong resistance to chlorsulfuron, cloransulam, chlorimuron, imazethapyr, and bispyribac (Guttieri et al. 1995; Tal and Rubin 2004; Uchino and Watanbe 2002). Thus, results confirm that the amino acid substitution of Pro-197 to Ser confers target-site resistance to ALS inhibitors in this R annual sedge biotype.

Amino acid substitutions at Pro-197 for weed species have been well documented in the literature with cases in kochia [*Kochia scoparia* (L.) Schrad.] (Legere et al. 2013), wild radish (Li et al. 2012; Yu et al 2003), shepherd's purse [*Capsella bursa-pastoris* (L.) Medik.] (Jin et al. 2011), and many other species, including smallflower umbrella sedge (Tehranchian 2015b). Cross-resistance to sulfonylureas and imidazolinones was noted when ALS genes from a mutant line of *Arabidopsis thaliana* (L.) were inserted into cultivated tobacco (*Nicotiana tabacum* L.) via genetic

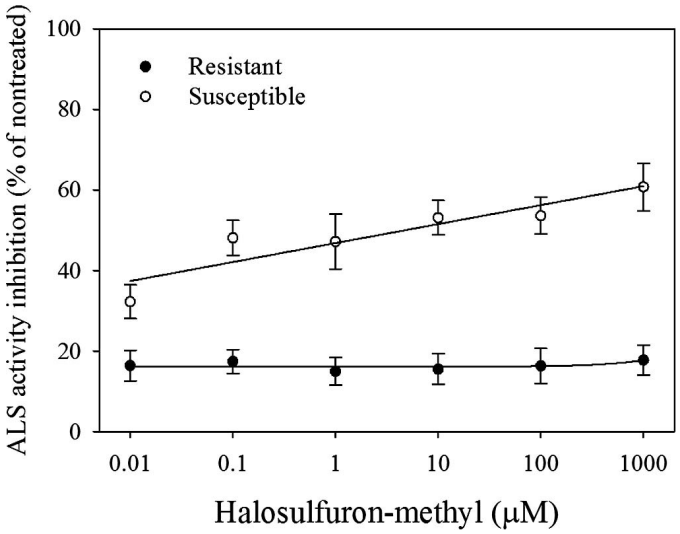


Figure 3. Reductions in acetolactate synthase (ALS) enzyme activity by halosulfuron-methyl for ALS-resistant and -susceptible biotypes of annual sedge in two laboratory experiments. Results were pooled over experimental runs. Vertical bars represent standard errors of the mean (n = 8). Data were regressed with, $y = \beta_0 + \beta_1$, where y is ALS enzyme inhibition, β_0 is the intercept, β_1 is the rate, and x is the common logarithmic concentration of halosulfuron-methyl. Resistant biotype regression: $r^2 = 0$, y = 16.3 + 0.11x. Susceptible biotype regression: $r^2 = 0.21$; y = 46.8 + 4.73x.

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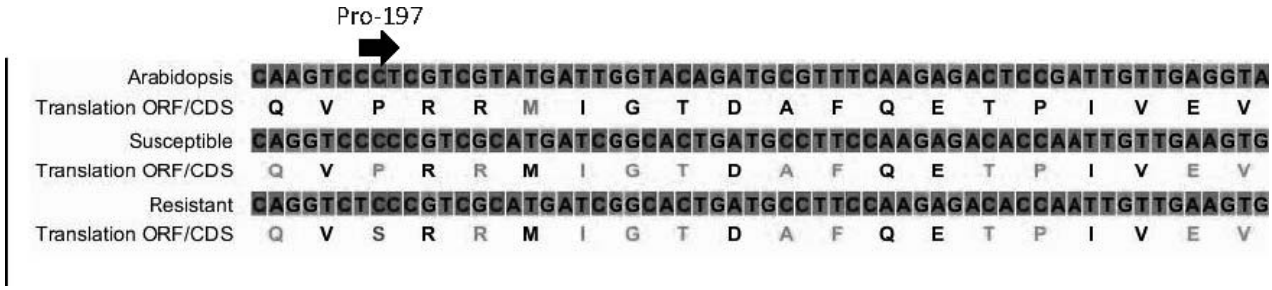


Figure 4. Alignment of acetolactate synthase (ALS) sequences flanking codon Pro-197 from *Arabidopsis thaliana* and the sedge populations. The black arrow indicates the Pro-197 codon in *A. thaliana.* The resistant populations show a mutation (CCC to TCC) conferring Pro-197-Ser substitution in ALS enzymes.

transformation (Charest et al. 1990). Resistance to ALS inhibitors was also confirmed when mutant ALS genes isolated from *Brassica napus* (L.) were expressed in tobacco transformants (Wiersma et al. 1989).

In another species, rigid ryegrass, multiple substitutions at Pro-197 were identified in ALS-resistant biotypes from Australia (Yu et al. 2008). These mutations conferred resistance to a sulfonylurea, sulfometuron, with low or no resistance to an imidazolinone, imazapyr. Although the Pro-197 to Ser substitution in annual sedge conferred resistance to sulfonylureas and imazapic, the level of resistance to imidazolinones was not quantified. Perhaps, the level of resistance differs from that of sulfonylureas, and warrants further investigation.

Implications from These Findings. The repeated use of halosulfuron or other ALS inhibitors could exacerbate the spread of resistant sedges, particularly species with substantial outcrossed seed production. Annual sedge reproduces via seed in florets (Sharma and Shiam 1981), with each flower having three anthers producing 180 to 320 pollen grains (Reddi and Reddi 2009). Annual sedge seeds have exhibited germination densities of 50 to 200 seedling m^{-2} in field tillage trials (Singh et al. 2015). Turfgrass managers will need to develop integrated weed management programs that prevent the establishment and spread of ALS-resistant biotypes. Hand-weeding may be a practical approach to controlling annual sedge. Turf managers could physically remove or dig plants from the ground after germination in spring to prevent seed development. However, this approach may not be feasible in large areas with dense populations, such as on sod farms. Moreover, the spread of annual sedge over years could contribute to the presence of abundant seed in the soil of infested areas.

Applications of PRE herbicides in turfgrass will be critical for managing ALS-resistant annual sedge, especially for turfgrass species susceptible to injury from other POST herbicides. Dinitroaniline herbicides used for PRE control of annual grassy weeds in turfgrass generally have limited efficacy on sedges. Turf managers may need to incorporate dimethenamid, oxadiazon, sulfentrazone, or *S*-metolachlor in PRE control programs to effectively manage annual sedge populations. These herbicides may have significant limitations for use, such as cost, turfgrass injury, and restrictions on labeled use areas. Selecting the appropriate PRE herbicide may be critical for controlling annual sedge and minimizing the spread of R biotypes.

Sulfentrazone was less efficacious than MSMA, glufosinate, and glyphosate for controlling ALS-resistant annual sedge. Sequential applications for complete POST control of annual sedge could be a limitation to using sulfentrazone in turfgrass. Sulfentrazone has residual activity and may be applied for PRE or POST control of annual sedge. The potential loss of organic arsenical herbicides, including MSMA, could limit the mechanisms of action available to turf managers, and further increase the occurrence of ALS-resistant sedges. Bentazon is a photosystem II inhibitor that offers turf managers an alternative to ALS inhibitors for annual sedge control. Many turfgrass managers have replaced bentazon in spray programs with ALS inhibitors due to improved efficacy, turfgrass safety, and fewer applications required for controlling sedges. However, bentazon should be used alone or in tank-mixtures with ALS inhibitors for managing herbicide resistance in annual sedge populations. These alternative mechanisms of action will be important for controlling ALS-resistant biotypes in turfgrass and other cropping systems.

This is the first report of resistance to ALS inhibitors in annual sedge and the first report of a herbicide-resistant sedge species from a turfgrass system. The repeated use of ALS inhibitors may select for R biotypes of annual sedge in turfgrass, and

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demonstrate the importance of rotating mechanisms of action. The importance of educating practitioners about herbicide rotation should be emphasized for sustainable management as new species are confirmed with resistance in turfgrass.

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