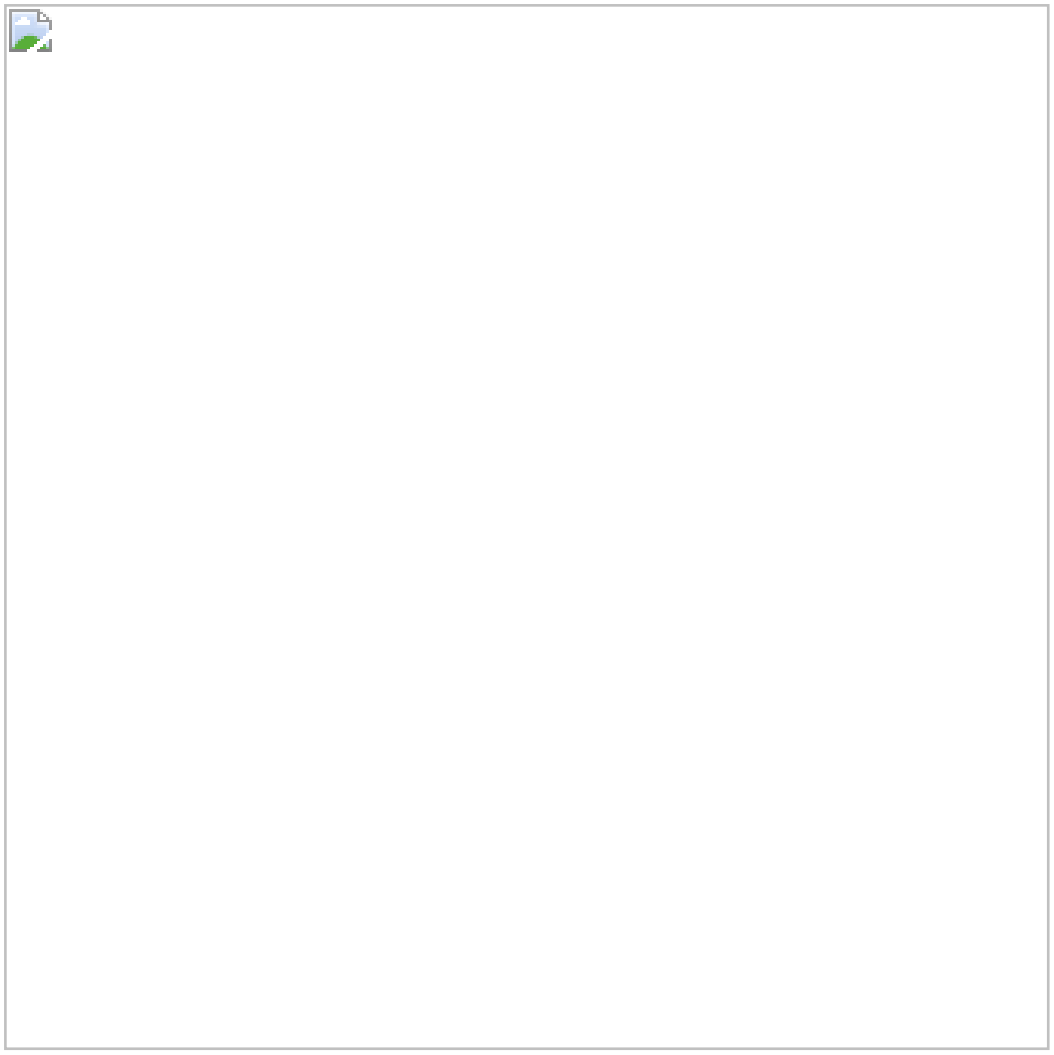

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Target site resistance to acetolactate synthase inhibitors in a fall panicum (*Panicum dichotomiflorum* Michx.) accession from Wisconsin and its response to alternative herbicides

Jose J. Nunes[1], Damilola A. Raiyemo[2], Nicholas J. Arneson[3], Alexandre T. Rosa[4], Patrick J. Tranel[5] and Rodrigo Werle[6]

[1]Graduate Student, Department of Agronomy, University of Wisconsin–Madison, Madison, WI, USA; [2]Graduate Student, Department of Crop Sciences, University of Illinois, Urbana, IL, USA; [3]Outreach Specialist, Department of Agronomy, University of Wisconsin–Madison, Madison, WI, USA; [4]Former Postdoctoral Research Associate, Department of Agronomy, University of Wisconsin–Madison, Madison, WI, USA; [5]Professor, Department of Crop Sciences, University of Illinois, Urbana, IL, USA and [6]Assistant Professor, Department of Agronomy, University of Wisconsin–Madison, Madison, WI, USA

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clethodim; glufosinate; glyphosate; isoxaflutole; mesotrione; nicosulfuron; tembotrione; tolpyralate; quizalofop-p-ethyl; fall panicum; *Panicum dichotomiflorum* Michx.; sweet corn; *Zea mays* L. var. saccharate

Keywords:
ALS-inhibiting herbicide resistance; grass control; molecular biology; weed resistance

Author for correspondence:
Rodrigo Werle, University of Wisconsin, 1575 Linden Drive, Madison, WI 53706. Email: rwerle@wisc.edu

Abstract

Owing to the lack of effective POST herbicide options, producers typically rely on nicosulfuron as the main POST grass herbicide in sweet corn production systems. In 2019, a Wisconsin sweet corn producer reported fall panicum control escapes after spraying nicosulfuron. Seeds from mature plants were collected to (1) measure fall panicum response to acetolactate synthase (ALS)-inhibiting herbicides, (2) elucidate the resistance mechanism, and (3) evaluate its response to alternative POST herbicides. Greenhouse and laboratory investigations were conducted to assess fall panicum response to ALS-inhibiting herbicides and elucidate the resistance mechanism. Dose–response results showed that fall panicum was highly resistant to nicosulfuron with a resistance ratio of >12.9-fold (survived rates >254 g ai ha^{-1}, or 8× the field label rate). Molecular and genetic studies indicated that there are multiple ALS gene copies in fall panicum and that resistance was due to a mutation in one copy, resulting in an Asp-376-Glu amino acid substitution. Additional greenhouse experiments indicate that clethodim (105 g ai ha^{-1}), quizalofop-p-ethyl (70 g ae ha^{-1}), glyphosate (864 g ae ha^{-1}), and glufosinate (650 g ai ha^{-1}) are effective POST options to manage the ALS-resistant fall panicum (>90.0% control and 96.8% biomass reduction) in rotational years. The 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides isoxaflutole (105 g ai ha^{-1}), mesotrione (105 g ai ha^{-1}), tembotrione (92 g ai ha^{-1}), and tolpyralate (39 g ai ha^{-1}) did not provide effective POST fall panicum control. Because these herbicides are commonly used for POST weed control in sweet corn, more investigations are required to evaluate combinations of HPPD-inhibiting herbicides with herbicides from other sites of action for POST fall panicum control. Herein we confirm the first case of herbicide resistance in fall panicum in the United States.

Introduction

Sweet corn is one of the most popular vegetable crops in North America, cultivated for both processing and fresh market uses (NASS 2020; Williams et al. 2008). In the United States, Washington, Minnesota, and Wisconsin are the three largest sweet corn–producing states, responsible for 57% of the 158,110 ha of sweet corn harvested in the country in 2019. Weed management represents one of the biggest challenges sweet corn producers face (Williams and Masiunas 2006; Williams et al. 2008). One of the reasons for difficulty in weed control is that sweet corn producers establish their crops at different times within a growing season to extend harvest for market availability, thereby creating a scenario where weed management becomes even more complex and dynamic, given that planting date can affect the competitive advantage of the crop over weeds (Williams 2009; Williams and Lindquist 2007).

Among the several weed species that can impact sweet corn production, fall panicum is one of the most frequently found weeds in sweet corn fields in the U.S. Midwest (Williams et al. 2008). Native to Louisiana, fall panicum is a small-seeded annual grass (Poaceae family) found throughout the United States, infesting agronomic and horticultural crops, turfgrass, nurseries, landscapes, and noncrop areas (Odero et al. 2011; Natural Resources Conservation Service 2006). Fall panicum grows vigorously and reaches an average of 45 to 120 cm in height (Odero et al. 2011) and prolific seed production, with up to 100,000 seeds per plant (Govinthasamy and Cavers 1995).

Germination is favored by warm, alternating temperatures (20/30 C) (Fausey and Renner 1997; Taylorson 1980). Under Wisconsin conditions, most fall panicum emergence is estimated to occur late in the spring (late May and June) (Werle et al. 2014). Besides being a challenge to sweet corn producers in the Midwest, fall panicum is also one of the most troublesome weed species in Florida sugarcane (Odero et al. 2011; Rott et al. 2018) and Louisiana rice production systems (Teló et al. 2018; Webster 2014).

Herbicides are the primary tool adopted for weed management in conventional sweet corn production systems. Though more preemergence and postemergence herbicide options are available today than in the past, atrazine is still the most widely adopted herbicide for weed control in sweet corn (Williams et al. 2010). However, because of groundwater and drinking water contamination concerns and lack of effective control of certain weed species, including fall panicum and other grasses (Brecke and Duke 1980; DATCP 2021; Parochetti 1974; Williams et al. 2008), producers also rely on other chemistries for postemergence weed control in sweet corn, especially of grass weeds (Arslan et al. 2016; Williams and Harvey 2000), and nicosulfuron is one of the few with postemergence grass effectiveness (Choe and Williams 2020; Williams and Harvey 2000). Nicosulfuron is an acetolactate synthase (ALS)-inhibiting herbicide from the sulfonylurea chemical family that kills susceptible plants by inhibiting the ALS (also known as acetohydroxyacid synthase [AHAS]) enzyme and, therefore, blocking the biosynthesis of the branched-chain amino acids leucine, isoleucine, and valine, leading to the depletion of downstream products of this pathway and plant death (Brown and Cotterman 1994; Schloss 1994; Yu and Powles 2014). The selectivity of nicosulfuron to corn is due to the metabolization of the herbicide in the plant by P450 enzymes (Choe and Williams 2020), minimizing detrimental impacts in corn compared to sensitive species (Brown and Cotterman 1994).

Several factors, including low field rates, broad-spectrum weed control, soil residual activity, crop safety, and low mammalian toxicities, contribute to the wide adoption of ALS-inhibiting herbicides in a broad range of cropping systems (Tranel and Wright 2002; Yu and Powles 2014). However, the widespread use of ALS-inhibiting herbicides coupled with numerous mutations conferring ALS resistance have favored the selection and evolution of many resistant biotypes, making ALS-inhibiting herbicides the number one group on the list of weed resistance cases worldwide (Heap 2021; Tranel and Wright 2002; Yu and Powles 2014). The amino acid substitution at the position Asp-376-Glu has been reported to confer resistance to sulfonylureas and imidazolinones herbicides (Heap 2021). To this date, no fall panicum resistance case has been reported to ALS-inhibiting herbicides worldwide (Heap 2021). We received a report from a regional agronomist in 2019 of a population of fall panicum that was not controlled by a postemergence application of nicosulfuron, and we subsequently received seed collected from the surviving plants to fulfill the objectives of this study: to (1) measure fall panicum response to ALS-inhibiting herbicides, (2) elucidate the resistance mechanism, and (3) evaluate its response to alternative POST corn and soybean herbicides.

Materials and Methods

Greenhouse Studies

Plant Material

Fall panicum seeds, putative ALS-resistant (43.4436°N, −88.4145°W, designated here as R) and ALS-susceptible (43.5140°N, −88.3722°W, designated here as SW), were collected from 20 random mature plants in two sweet corn fields located in Fond du Lac County, Wisconsin. The field where the putative ALS-susceptible accession was collected had a history of glyphosate-resistant corn and alfalfa as the main crops cultivated; seeds were collected from plants located in the field margin. As for the ALS-resistant accession, it was collected from a field with a history of nicosulfuron use in sweet corn, and fall panicum plants were observed to survive postemergence application of nicosulfuron during the 2019 growing season; seeds were collected from plants within the field. Collected seeds were packed in paper bags, air-dried at room temperature, manually cleaned, and stored at 4 C until the beginning of the experiments.

Dose–Response Studies

Dose–response studies were conducted to evaluate the response of both accessions to the ALS-inhibiting herbicides nicosulfuron (sulfonylurea) and imazethapyr (imidazolinone). For nicosulfuron, rates ranging from 0 to 254 g ai ha^{-1} (0, 3.9, 7.9, 15.9, 31.7, 63.5, 127, and 254 g ha^{-1}; Accent® Q, DuPont de Nemours, Wilmington, DE, USA) were evaluated, where 31.7 g ha^{-1} represented the labeled field-use rate for fall panicum control. For imazethapyr, rates ranging from 0 to 560 g ae ha^{-1} (0, 70, 140, 210, 280, 350, 420, 490, and 560 g ha^{-1}; Pursuit®, BASF Corporation, Research Triangle Park, NC, USA) were evaluated, where 70 g ha^{-1} represented the labeled field-use rate of imazethapyr.

POST Herbicide Screen

A herbicide screen was conducted to assess POST herbicide options for fall panicum control. In total, eight herbicides from four different sites of action and different production systems (alfalfa [*Medicago sativa* L.], corn, and soybean [*Glycine max* (L.) Merr.] production) were evaluated at field-use rates (refer to Table 1 for herbicides and rates).

Experimental Design

Studies were conducted in the Walnut Street Greenhouse at the University of Wisconsin in Madison, WI (43.0761°N, −89.4235° W), as a completely randomized design with six replications. Each experimental unit consisted of a plastic Cone-tainer (656 mL Cone-tainer™, Stuewe and Sons, Tangen, OR, USA) filled with potting mix (Pro-Mix® HP Mycorrhizae, Premier Tech Horticulture, Quakertown, PA, USA) containing one fall panicum plant. Seeds of the two accessions were seeded in 20-cm-wide by 30-cm-long by 5-cm-deep aluminum trays with potting mix and transplanted into the Cone-tainers 7 days after sowing, when seedlings were at the one-leaf growth stage. Cone-tainers were stored on benches in the greenhouse and watered daily at the potting mix surface as necessary to maintain adequate growing conditions. Trays with the Cone-tainers were rearranged in the greenhouse every morning to reduce the impact of light variation in the greenhouse. Both studies were conducted twice.

For the nicosulfuron dose–response study and the POST herbicide screen (except for tolpyralate, which was evaluated simultaneously with the imazethapyr dose–response), experimental runs were conducted during August and September 2020. Temperature (average 24.9 C, minimum 21.5 C, maximum 27.7 C) and relative humidity (RH) (average 61.3%, minimum 40.7%, maximum 74.5%) were monitored in the greenhouse with a WatchDog® A150 temperature/RH logger (Spectrum Technologies, Aurora, IL, USA). For the imazethapyr dose–response study and tolpyralate evaluation, experimental runs were carried out during April

Table 1. Herbicides screened for fall panicum POST control, followed by their trade names, manufacturer, address, site of action with the group number in parentheses, and the rate used in the study.[a]

Herbicide[b]	Trade name	Manufacturer	Address	SOA	Rate[c]
Clethodim	Select Max®	Valent	Walnut Creek, CA	ACCase (1)	105
Quizalofop- p-ethyl	Assure® II	DuPont de Nemours	Wilmington, DE	ACCase (1)	70
Glyphosate	Roundup PowerMAX®	Bayer CropScience	St. Louis, MO	EPSPS (9)	864
Glufosinate	Liberty®	BASF Corporation	Research Triangle Park, NC	GS (10)	650
Isoxaflutole	Balance® Flexx	Bayer CropScience	St. Louis, MO	HPPD (27)	105
Mesotrione	Callisto®	Syngenta Crop Protection	Greensboro, NC	HPPD (27)	105
Tembotrione	Laudis®	Bayer CropScience	St. Louis, MO	HPPD (27)	92
Tolpyralate	Shieldex®	SummitAgro	Durham, NC	HPPD (27)	39

[a]Abbreviations: ACCase, acetyl coenzyme A carboxylase; EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase; GS, glutamine synthetase; HPPD, 4-hydroxyphenylpyruvate dioxygenase; SOA, site of action.
[b]Ammonium sulfate (2.2 kg ha^{-1}) and crop oil concentrate (0.5% v/v) were added to the spray solution of all herbicide treatments.
[c]Clethodim, glufosinate, isoxaflutole, mesotrione, tembotrione, and tolpyralate expressed as g ai ha^{-1}; quizalofop and glyphosate expressed as g ae ha^{-1}.

and May 2021. Temperature (average 27.0 C, minimum 18.3 C, maximum 42.2 C) and RH (average 29.8%, minimum 6.5%, maximum 80.3%) were recorded during the two experimental runs in the greenhouse.

Herbicide Applications

In all studies, herbicides were sprayed when fall panicum plants reached the two- to three-leaf stage (<10 cm) using a single-nozzle research track spray chamber (Devries Manufacturing, Hollandale, MN, USA), equipped with an AI9502E flat-fan nozzle (TeeJet® Technologies, Wheaton, IL, USA), calibrated to deliver 140 L ha^{-1} of spray solution at 276 kPa at a speed of 5 km h^{-1}. For all herbicide treatments in all studies, ammonium sulfate at 2.2 kg ha^{-1} and crop oil concentrate (COC) at 0.5% v/v were added to the spray solution. Following herbicide application, plants were moved back to the greenhouse benches and watered daily starting at 24 h after spraying.

Data Collection

At 28 days after treatment (DAT), visual control was assessed (0%, no control to 100%, complete plant death) and aboveground biomass was harvested (clipped at the potting mix level) and dried to constant weight in a forced-air oven at 60 C. For dose–response, the raw biomass weight (grams per Cone-tainer) was subjected to statistical analysis, as suggested by Keshtkar et al. (2021). As for the herbicide screen, dry biomass weight was converted to the percentage of biomass reduction (BR) compared to the non-treated check of each accession using the equation:

$$\text{BR} = 1 - \frac{\text{Dry biomass of treated plants (g per pot)}}{\text{Dry biomass of untreated check (g per pot)}} \times 100 \quad [1]$$

Molecular Investigations

DNA Extraction and ALS Gene Sequencing

Molecular investigations were conducted through greenhouse and lab experiments at the Plant Care Facility and Edward R. Madigan Laboratory, located at the University of Illinois, Urbana, to elucidate the resistance mechanism. Besides the two fall panicum accessions from Wisconsin described earlier (R and SW), another susceptible accession from Illinois was utilized only for the molecular studies (designated here as SI). The three accessions were used to compare sequences of the *ALS* gene. To confirm resistance, seeds from each accession were grown in the greenhouse in Cone-tainers filled with a growing medium that included Sunshine® LC1 (Sun Gro® Horticulture, Agawam, MA, USA) growing mix, soil, peat, and torpedo sand (3:1:1:1 by wt). Plants were sprayed at the two- to three-leaf stage (<10 cm) with 2 and 4 times the nicosulfuron field-use rate (63 g ha^{-1}; Accent Q). All herbicide applications included 1% (v/v) COC and 28% urea ammonium nitrate. Herbicide treatments were applied using a spray chamber equipped with an 80015 even flat-fan nozzle calibrated to deliver 187 L ha^{-1}. Following visual assessment 3 wk after application, individuals surviving herbicide treatments were phenotyped resistant, and those seriously damaged (dead) were phenotyped sensitive.

Young leaf tissues from individuals of each accession collected prior to the resistance confirmation were then subjected to genomic DNA isolation following a standard cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1990). DNA integrity was determined using a spectrophotometer (NanoDrop™ 1000 spectrophotometer, Thermo Fisher Scientific, Waltham, MA, USA) and then diluted to 1 to 100 ng µl^{-1} for downstream molecular investigations. Owing to the unavailability of the *ALS* sequence for fall panicum, nucleotide sequences of other grass species—*Panicum hallii* Vasey (NC_038042.1, NC_038049.1, NC_038042.1), *Avena fatua* L. (JN175309.1), *Lolium rigidum* Gaudin (EF411170.1), *Lolium multiflorum* Lam. (AF310684.2), *Hordeum vulgare* L. (AF059600.1), *Triticum aestivum* L. (AY210406.1), *Alopecurus myosuroides* Huds. (AJ437300.2), *Zea mays* L. (X63554.1), and *Echinochloa crus-galli* (L.) P. Beauv. (LC006063.1)—available in the GenBank database of the National Center for Biotechnology Information were obtained and aligned using a CLC sequence viewer 8.0. Regions of *ALS* showing high conservation were employed in primer design using the IDT OligoAnalyzer™ tool (https://www.idtdna.com/pages/tools/oligoanalyzer).

Preliminary experiments were carried out to determine optimal polymerase chain reaction (PCR) conditions for different primer sets. Finally, the primer sets ALS122–377A_FOR and ALS1340bp_REV, capturing domains with five known mutations, and the primer sets ALS890bp_FOR and ALS574_654B_REV, capturing domains with three known mutations, were used in optimized PCR reactions (Table 2; Figure 1). The PCR reaction mixture consisted of 12.3 µl of biology-grade water, 5 µl of 5X Green GoTaq™ Flexi Buffer (Promega, Madison, WI, USA), 2 µl of 10 mM dNTP mix (New England Biolabs, Ipswich, MA, USA), 2.5 µl of 25 mM $MgCl_2$ (Promega), 1 µl each of forward and reverse primer diluted to a concentration of 10 µM (Integrated DNA Technologies, Coralville, IA, USA), 1 µl DNA, and 0.2 µl Taq polymerase (Promega). The thermocycler settings for PCR amplification included the following steps: initial

Table 2. List of primers used to amplify the *ALS* gene of fall panicum.[a]

Primer name	Primer sequence (5′–3′)	Expected amplicon size	Included mutation sites
		bp	
ALS122_377A_FOR	AAGGGCGCCGACATCCTCGTC	1,340	Ala122 Pro197 Ala205 Asp376 Arg377
ALS_1340bp_REV	CATGAGGAAGCTGCCATCCCATC		
ALS890bp_FOR	CGTGTGACAGGGAAAATTGAGGC	890	Trp574 Ser653 Gly654
ALS574_654B_REV	TACACGGTCCTGCCATCACCATCC		

[a]Abbreviation: ALS, acetolactate synthase.

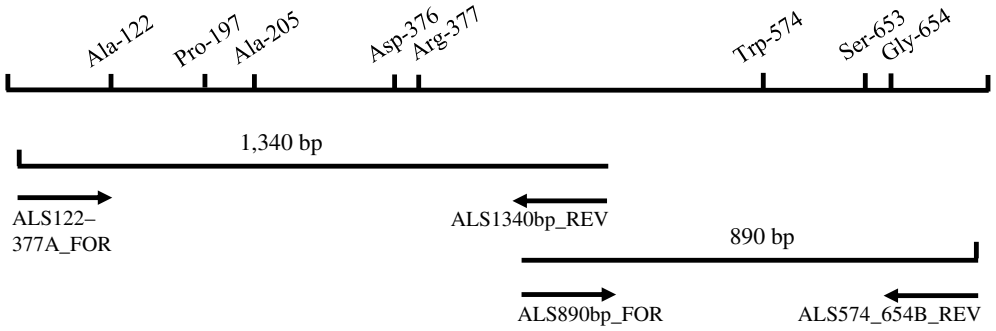

Figure 1. Schematic of Sanger sequencing approach for ALS gene in fall panicum.

incubation at 94 C for 30 s, 35 cycles of denaturation at 94 C for 15 s, annealing at 69 C for 15 s and extension at 72 C for 20 s, and a final extension at 72 C for 1 min. PCR products were analyzed by separation in 1% agarose gel stained with GreenGlo™ Safe DNA Dye (Thomas Scientific, Swedesboro, NJ, USA) and visualized under an ultraviolet light illuminator. The PCR products showing expected band sizes were purified with a GeneJET PCR purification kit (Thermo Fisher Scientific) following the manufacturer's protocol. Purified products were then subjected to Sanger sequencing using a BigDye™ Terminator v3.1 cycle sequencing kit (Thermo Fisher Scientific), with reaction mixture consisting of 1.8 µl of biology-grade water, 1 µl of BigDye mix, 2 µl of 5X sequencing buffer, 5.2 µl of 12.5% glycerol, 2 µl of primer (Figure 1), and 1 µl of template DNA. The PCR products were submitted to the Roy J. Carver Biotechnology Center at the University of Illinois, Urbana-Champaign for sequencing. *ALS* fragments obtained from the sequencing facility were edited and aligned using Sequencher 5.4 to obtain consensus *ALS* sequences.

Molecular Diagnosis of Asp-376-Glu Mutation with Cleaved Amplified Polymorphic Sequence Assay

A cleaved amplified polymorphic sequence (CAPs) assay was developed for an *Mbo*I restriction enzyme polymorphism at the *ALS* 376 codon. A 200-bp fragment of the *ALS* sequence containing codon 376 was amplified with primer sets POS376B_200bp_F (5′-CTT CCC CAG TAC GAC CCG CT-3′) and POS376B_200bp_R (5′-AAT CTC AGC CGG ATC AAT GTC A-3′). Thermocycler settings were identical to those described in the previous section, except the annealing temperature was changed to 61 C. Upon PCR amplification and visualization of expected band sizes with gel electrophoresis, digestion was carried out with the following reaction mixture: 12.7 µl of PCR product, 2 µl 10X CutSmart® buffer (New England Biolabs), and 0.3 µl *Mbo*I restriction enzyme for 1 h at 37 C. Digested products were then separated on 3% MetaPhor® agarose gel (Lonza, Rockland, ME, USA) stained with GreenGlo Safe DNA Dye and visualized under an ultraviolet light illuminator.

To confirm whether double peaks observed in sequence chromatograms were due to heterozygosity of alleles or the presence of multiple copies of *ALS*, progenies from a single, self-fertilized individual were evaluated using the CAPs assay described earlier. DNA was extracted from 20 progenies, and plants were sprayed at <10 cm with 4 times the labeled use rate of nicosulfuron. The extracted DNA were then subjected to molecular screening using the CAPs assay.

Statistical Analyses

For dose–response studies, the three-parameter Weibull-1 nonlinear regression model was fitted to visual control and biomass weight data using the DRC package in R (Ritz et al. 2015). The three-parameter Weibull model is given by the equation:

$$f(x) = 0 + (d - 0)\exp(-\exp[b(\log(x) - \log(e))]) \quad [2]$$

where $f(x)$ is the percentage of control or biomass, b is the relative slope at the inflection point, d is the upper limit or asymptote, and e is the inflection point of the curve. The effective herbicide doses required for 50% (ED_{50}) and 90% (ED_{90}) control/biomass weight reduction were estimated using the *ED()* function, and the resistance ratio (RR) was calculated by dividing the ED_{50} values for control or biomass reduction of the resistant accession by the ED_{50} values of the susceptible accession. Furthermore, the three parameters of the curves (b, d, and e) from the two accessions were compared using the *compParm()* function. All functions are from the DRC package in R software.

For herbicide screen, visual control and percentage of biomass reduction were subjected to analysis of variance using the generalized mixed model *glmmTMB()* from the GLMMTMB package (Brooks et al. 2017), where herbicide treatments and accessions were treated as fixed effects and the experimental run as a random

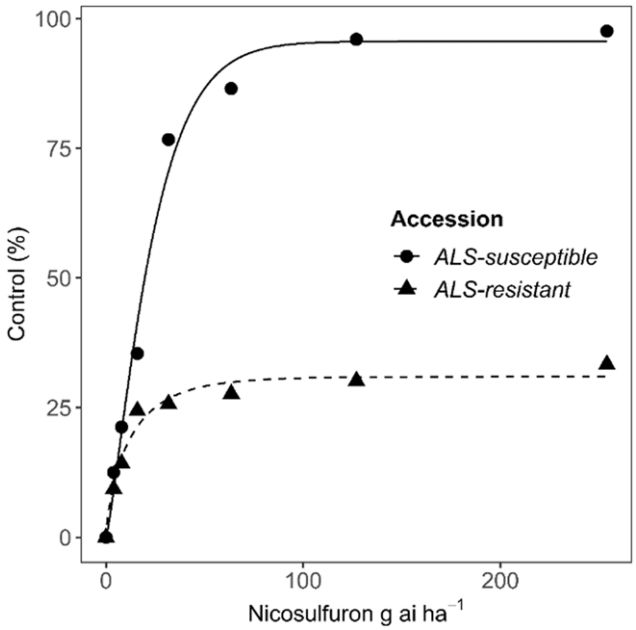

Figure 2. Dose–response curves for fall panicum visual control (%) as a function of nicosulfuron rates. Solid and dashed lines indicate fall panicum acetolactate synthase-susceptible (SW) and resistant accessions, respectively.

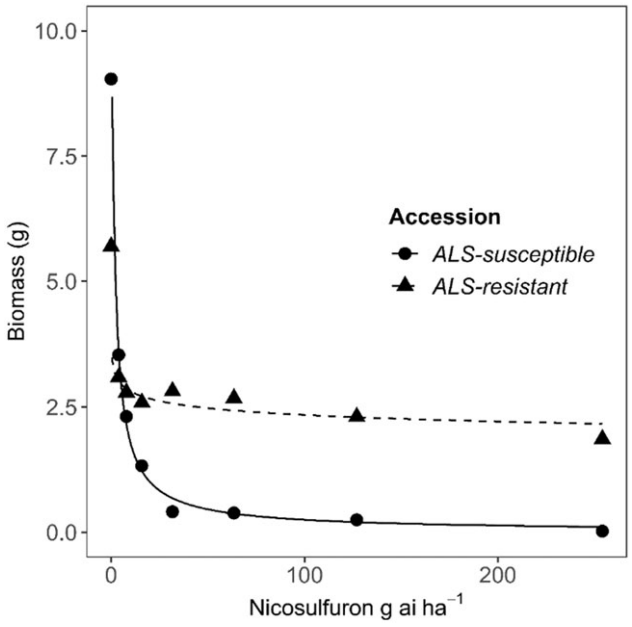

Figure 3. Dose–response curves for fall panicum biomass weight (g) as a function of nicosulfuron rates. Solid and dashed lines indicate fall panicum acetolactate synthase-susceptible (SW) and resistant accessions, respectively.

effect. When significant ($P \leq 0.05$), means were estimated using the *emmeans()* function (EMMEANS package; Length 2021) and separated with Sidak's adjustment at $\alpha = 0.05$. All statistical analyses were performed in R software Version 4.0.2 (R Development Core Team 2021).

Results and Discussion

Greenhouse Study: Nicosulfuron Dose–Response

Nicosulfuron failed to control the resistant fall panicum accession, which displayed resistance ratios of >12.9-fold for control and 2.8-fold for biomass weight reduction compared to the SW susceptible accession. Eight times the label rate (254 g ha^{-1}) of nicosulfuron provided <90% control or biomass weight reduction of the resistant accession. For the susceptible accession, estimated rates of 19.6 and 58.1 g ha^{-1} of nicosulfuron resulted in 50% and 90% control, respectively, whereas 2.6 and 22.6 g ha^{-1} of nicosulfuron provided 50% and 90% biomass weight reduction, respectively (Figures 2 and 3; Table 3).

Owing to the limited response of the resistant accession to nicosulfuron, the nonlinear model failed to estimate ED_{50} and ED_{90} for control and ED_{90} for biomass weight reduction. Therefore the three parameters of the model equation were compared to provide insights regarding the differences between the two accessions. As seen in Table 4, the responses of the two accessions were different ($P < 0.05$) from each other in all three parameters: *b* (relative slope), *d* (upper limit), and *e* (inflection point). Moreover, the upper limit (highest control estimated by the model) of the resistant accession was estimated at 30.9%, about 3 times lower than the susceptible accession treated with nicosulfuron, estimated at 95.6%. Besides the differences between the two accessions in visual control, the resistant accession accumulated lower biomass than the susceptible accession in the absence of the herbicide. The resistant accession had an estimated upper limit average of 5.7 g, whereas the susceptible accession had a 9.0 g estimated upper limit average, a difference of 1.5-fold higher for the susceptible accession (Table 4).

Table 3. Estimated doses of nicosulfuron required for 50% (ED_{50}) and 90% (ED_{90}) control or biomass weight reduction of fall panicum and the ED_{50} resistance ratio at 28 DAT.[a]

	Control (%)			Biomass (g)		
Accession	ED_{50} (±SE)	ED_{90} (±SE)	RR[b] (ED_{50})	ED_{50} (±SE)	ED_{90} (±SE)	RR[b] (ED_{50})
	g ai ha^{-1}			g ai ha^{-1}		
ALS-susceptible (SW)	19.65 (0.61)	58.13 (4.12)	–	2.60 (0.63)	22.60 (6.33)	–
ALS-resistant	>254	>254	>12.9	7.33 (9.24)	>254	2.8

[a]Abbreviations: ALS, acetolactate synthase; ED, estimated dose; RR, resistance ratio.
[b]Resistance ratio when dividing the ED_{50} of the resistance by the ED_{50} of the susceptible accession.

The imazethapyr dose–response was conducted to confirm whether fall panicum also had cross-resistance to ALS-inhibiting imidazolinones; however, given the low efficacy of imazethapyr to control fall panicum, as previously reported in the literature (Curran et al. 1999), poor control (<70.0%) and biomass reduction levels (<55.0%) were observed even with the highest rate tested (560 g ha^{-1}; data not shown) for both the resistant and susceptible accessions. Moreover, despite slight differences in visual control between the two accessions, with a lower response from the resistant accession, it did not translate into significant differences in ED_{50} for control ($P = 0.885$) and biomass reduction ($P = 0.192$) (data not shown).

Out of the current 661 cases of weed resistance to ALS-inhibiting herbicides reported worldwide, 53 cases have been confirmed to cause resistance to nicosulfuron in broadleaf and grass weed species. However, none of the aforementioned cases have been described to affect fall panicum, because the only reported case

Table 4. Nonlinear regression parameters from the fall panicum nicosulfuron dose–response.[a,b]

Accession	Parameter					
	Control			Biomass		
	b (±SE)	*d* (±SE)	*e* (±SE)	*b* (±SE)	*d* (±SE)	*e* (±SE)
	%			g		
ALS-susceptible (SW)	1.23 (0.06)	95.60 (1.10)	25.05 (0.91)	−0.89 (0.17)	9.03 (0.41)	1.85 (0.50)
ALS-resistant	0.76 (0.18)	30.95 (1.41)	12.96 (2.58)	−0.10 (0.05)	5.73 (0.39)	0.27 (0.61)
P-value[c]	0.017	<0.001	<0.001	<0.001	<0.001	0.049

[a]Abbreviations: *b*, relative slope; *d*, upper limit; *e*, inflection point.
[b]Values between parentheses indicate the standard error of each parameter.
[c]Statistics comparison between the regression parameters of each accession. P > 0.05 means a nonsignificant difference between accessions.

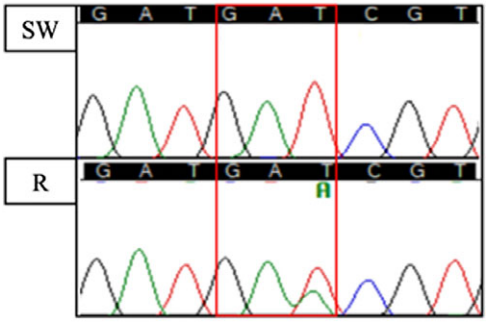

Figure 4. Partial sequence chromatogram of susceptible (SW) and resistant (R) fall panicum accessions. The codon in the red box shows position 376; the change from T to A causes aspartic acid to glutamic acid substitution. The polymorphism shown was the only one observed between the two accessions.

of herbicide resistance for this species was in Spain in a population that was less sensitive to atrazine, but the mechanism of resistance in that population has not been described (Heap 2021). In Florida, three fall panicum populations exhibited reduced sensitivity to asulam, the main POST herbicide for grass control in sugarcane; however, researchers did not describe the lower sensitivity as resistance (Fernandez et al. 2018). Therefore, even though fall panicum can be a troublesome weed species in various cropping systems (Odero et al. 2011; Rott et al. 2018; Teló et al. 2018; Webster 2014; Williams et al. 2008), this confirmation of ALS resistance is novel in agriculture.

Molecular Investigations: DNA Extraction and ALS Gene Sequencing

A target site mutation (Asp-376-Glu) is the most likely explanation of resistance to nicosulfuron in the resistant fall panicum accession. Amplification of the gene sequence yielded a 1,630-bp sequence covering the eight known mutation sites previously reported to confer resistance to ALS inhibitors: Ala122, Pro197, Ala205, Asp376, Arg377, Trp574, Ser653, and Gly654. Following manual inspection of the *ALS* sequence chromatogram, the nucleotide sequence GATC/GAAC and GATC was observed in resistant and sensitive individuals, respectively. The single nucleotide substitution T to A results in a predicted amino acid change from aspartic acid (GAT) to glutamic acid (GAA) (Figure 4; Supplementary Figure S1). This codon change at position 376 [numbering follows the *Arabidopsis thaliana* (L.) Heynh. *ALS* sequence AAK68759.1] was the only change that was consistently different between all R and all S plants analyzed.

CAPs assay revealed the cleavage of the wild-type GATC sequence by *Mbo*I, thus resulting in two bands (120 bp and 80 bp) for sensitive individuals, while the mutant-type GATC/GAAC had an undigested 200-bp band (GAAC) in addition to two bands. Three distinct bands were therefore observed for resistant individuals (Supplementary Figure S3). The presence of double peaks in the *ALS* sequence chromatogram led us to hypothesize that there is either heterozygosity of alleles or multiple copies of *ALS* in fall panicum (Supplementary Figure S2). Fall panicum is a polyploid species with varying reports of chromosome number. Brown (1948) reported a tetraploid chromosome number $2n = 36$, whereas Hamoud et al. (1994) reported a hexaploid chromosome number $2n = 6\times = 54$. Similarly, *Echinochloa crus-galli* (L.) P. Beauv. was reported to have a hexaploid chromosome number $2n = 6\times = 54$ and also multiple copies of *ALS* (Riar et al. 2013). The fact that all resistant fall panicum plants we initially analyzed appeared to have both GAA and GAT 376 codons suggested the presence of at least two different *ALS* genes, rather than heterozygosity at a single locus. If multiple copies of *ALS* exist, perhaps due to polyploidy, all progenies from a self-pollinated individual should carry the mutant-type GATC/GAAC sequence. However, if resistant plants are heterozygous at several loci, including Asp376, progenies should segregate in a Mendelian ratio of 1:1; that is, 50% of the progenies should have the GATC sequence, while the other 50% should have the GAAC sequence. To investigate this further, progenies were obtained from a self-pollinated individual. All progenies were resistant (data not shown), inconsistent with the parent being heterozygous at the resistance locus. Furthermore, a CAPs assay showed that all 20 progenies also carried both GAA and GAT 376 codons (Supplementary Figure S3). We therefore conclude that the primers we used amplified at least two *ALS* copies and that the resistant plants were homozygous for the GAT 376 codon in one of the copies.

Since the earliest characterization of Asp-376-Glu in smooth pigweed (*Amaranthus hybridus* L.) (Whaley et al. 2007), 12 additional weeds have now been reported to be resistant to ALS-inhibiting herbicides as a result of Asp-376-Glu mutation (Tranel et al. 2021). Weeds with the Asp-376-Glu mutation possess varying levels of resistance to classes of herbicides inhibiting ALS: sulfonylurea, imidazolinone, triazolopyrimidine, pyrimidinyl-thiobenzoates, and sulfonyl-aminocarbonyl-triazolinone (Tranel and Wright 2002; Yu and Powles 2014). In *Amaranthus hybridus* L., the mutation confers a high level of resistance to the five classes of ALS-inhibiting herbicides (Whaley et al. 2007), whereas in *Raphanus raphanistrum* L., it confers a high level of resistance to sulfonylurea and triazolopyrimidine herbicides but a moderate level of resistance to imazamox and imazethapyr and susceptibility to imazapyr (Yu et al. 2012). Reports of fitness cost between weed biotypes that are resistant or susceptible to ALS-inhibiting herbicides have been inconclusive (Tranel and Wright 2002). Menegat et al. (2016) reported a significant reduction in root biomass of the *Lolium perenne* L. genotype with Asp-376-Glu mutation relative to a wild type, although no significant impact on shoot biomass was observed. However, in the nicosulfuron dose–response study, resistant plants accumulated less biomass than susceptible plants when no herbicide was applied. In addition, susceptible plants flowered a month earlier than resistant plants under the same growing conditions (data not shown). However, this could be due to other biotypic differences unrelated to the *ALS* mutation.

Investigating target site resistance in polyploids presents additional challenges in that multiple copies of the gene involved may be expressed or differentially expressed, or some may become silenced, or pseudogenes (Yu and Powles 2014). Two imazamox-resistant biotypes of barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] were found to have varying morphological responses to field-use rate of the herbicide, perhaps due to differential expression of various copies of the *ALS* gene (Riar et al. 2013). Further studies will be required to ascertain the number of *ALS* copies in fall panicum. From a management standpoint, evaluating the response or cross-resistance patterns of fall panicum to other ALS-inhibiting herbicides would be required for effective recommendations.

This study reports the first case of Asp-376-Glu amino acid substitution in the *ALS* of fall panicum likely conferring a high level of resistance to a sulfonylurea herbicide. However, in vitro enzyme assays or other experiments are required to functionally validate this conclusion.

Greenhouse Study: POST Herbicide Screen

As for the herbicides evaluated for POST fall panicum control, no interaction between herbicides and accessions was observed. Therefore control and biomass reduction data were pooled across accessions, and the effect of herbicides was analyzed. Out of eight herbicides evaluated, ACCase-inhibiting herbicides clethodim and quizalofop-p-ethyl, along with glyphosate, provided the highest control and biomass reduction of fall panicum, at >97.0% for both response variables (Table 5). Such herbicides could provide excellent control of fall panicum in rotational years, and glyphosate could serve as an option for POST applications on glyphosate-resistant sweet corn hybrids.

Glufosinate provided reasonable POST control (90.7%) and high biomass reduction (96.8%) of fall panicum, similar to the reduction observed by the Group 1 herbicides and glyphosate (Table 5). However, regrowth was observed 28 DAT in some fall panicum plants treated with glufosinate (data not shown). Previous research reported that grasses are more tolerant to glufosinate than broadleaf weed species due to a lower absorption and translocation of glufosinate by grasses (Takano et al. 2019). Therefore glufosinate adoption should strictly follow label recommendations for effective weed control, such as appropriate weed size and application technology to provide adequate foliar coverage, the time of day of spraying, and environmental conditions that favor its uptake and translocation (Culpepper et al. 2000; Takano et al. 2019; Takano and Dayan 2020).

The HPPD-inhibiting herbicides tembotrione, mesotrione, isoxaflutole, and tolpyralate applied POST to fall panicum did not provide effective control, with means ranging from 15.8% to 34.3% and from 9.7% to 61.3% of visual control and biomass reduction, respectively (Table 5). Even though fall panicum plants displayed injury symptoms from the HPPD-inhibiting herbicides (i.e., bleaching of new leaves) and their growth was slightly inhibited, plants kept developing and accumulating biomass and would likely have produced seeds if their life cycle had not been interrupted by the biomass collection. Similar results were reported by Soltani et al. (2012), who observed only suppression of fall panicum 14 d after mesotrione (100 g ai ha^{-1}) and topramezone (12.5 g ai ha^{-1}) application, reaching 70% biomass reduction with no significant difference between the two herbicides.

Besides not being effective for POST fall panicum control when used alone, HPPD-inhibiting herbicides are typically sprayed below recommended label rates in sweet corn due to crop safety concerns and crop rotation restrictions (Williams et al. 2010), likely resulting in lower fall panicum control and imposing additional selection for resistance evolution (Vieira et al. 2020). Nevertheless, research has demonstrated that the application of atrazine with low rates of HPPD-inhibiting herbicides increased weed/grass control and sweet corn yield (Bollman et al. 2008; Williams et al. 2011). Therefore the use of HPPD-inhibiting herbicides with atrazine may be another strategy for managing ALS-resistant fall panicum in sweet corn production systems where atrazine use is allowed.

These results confirm the first case of fall panicum resistance to ALS-inhibiting herbicides in the United States. Molecular studies indicate that resistance is due to the Asp-376-Glu amino acid substitution in the ALS enzyme and confers high levels of resistance to nicosulfuron. ACCase-inhibiting herbicides (clethodim and quizalofop-p-ethyl), glyphosate, and glufosinate were effective POST options for the ALS-resistant fall panicum. Nevertheless, glyphosate is labeled for POST applications only on glyphosate-resistant sweet corn hybrids. Therefore, given that HPPD-inhibiting herbicides are widely used in sweet corn production, evaluating tank mixtures of atrazine + HPPD-inhibiting herbicides may identify additional options to control fall panicum. Owing to the limited POST effective herbicide options on fall panicum control, sweet corn producers are encouraged to adopt PRE herbicides for early-season weed control, rotate herbicides with different sites of action, and alleviate the selection pressure of POST herbicides. Additionally, crop rotation, nonchemical weed control practices like cover crops, favorable planting time, selection of hybrids with higher competitive potential against weeds, and periodic scouting of fields to mitigate and manage herbicide-resistant weeds are fundamental to an integrated weed management program.

Table 5. Fall panicum postemergence control and biomass reduction provided by the different herbicides at 28 DAT.[a],[b]

Herbicide	Rate[c]	Control (±SE)	Biomass reduction (±SE)
		%	
Clethodim	105	97.9 (0.44) a	97.0 (0.62) a
Quizalofop	70	97.9 (0.44) a	97.0 (0.62) a
Glyphosate	864	97.8 (0.45) a	97.1 (0.63) a
Glufosinate	650	90.7 (1.34) b	96.8 (0.65) a
Isoxaflutole	105	34.3 (3.03) c	61.3 (3.08) b
Mesotrione	105	26.3 (2.68) d	59.1 (3.12) b
Tembotrione	92	20.0 (2.28) de	44.4 (3.17) c
Tolpyralate	39	15.8 (1.94) e	9.7 (1.48) d
P-value		<0.001	<0.001

[a]Abbreviation: DAT, days after treatment.
[b]Means followed by the same letter in the columns are not different at the 5% level according to Sidak's adjustment test.
[c]Clethodim, glufosinate, isoxaflutole, mesotrione, tembotrione, and tolpyralate are expressed as g ai ha^{-1}; quizalofop and glyphosate are expressed as g ae ha^{-1}.

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