First report of Ser653 Asn mutation endowing high-level resistance to imazamox in downy brome (Bromus tectorum L.)

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Abstract

BACKGROUND: *Bromus tectorum* L. is one of the most troublesome grass weed species in cropland and non-cropland areas of the northwestern USA. In summer 2016, a *B. tectroum* accession (R) that survived imazamox at the field-use rate (44 g ha^{-1}) in an imidazolinone-tolerant (IMI-tolerant or Clearfield™) winter wheat field was collected from a wheat field in Carter County, MT, USA. The aim of this study was to determine the resistance profile of the *B. tectroum* R accession to imazamox and other ALS inhibitors, and investigate the mechanism of resistance to imazamox.

RESULTS: The R *B. tectorum* accession had a high-level resistance (110.1-fold) to imazamox (IMI) and low to moderate-levels cross-resistance to pyroxsulam (TP) (4.6-fold) and propoxycarbazone (SCT) (13.9-fold). The R accession was susceptible to sulfosulfuron (SU) and quizalofop and clethodim (ACCase inhibitors), paraquat (PS I inhibitor), glyphosate (EPSPS inhibitor) and glufosinate (GS inhibitor). Sequence analysis of the *ALS* gene revealed a single, target-site Ser653Asn mutation in R plants. Pretreatment of malathion followed by imazamox at 44 or 88 g ha^{-1} did not reverse the resistance phenotype.

CONCLUSION: This is the first report of evolution of cross-resistance to ALS-inhibiting herbicides in *B. tectorum*. A single-point mutation, Ser653Asn, was identified, conferring the high-level resistance to imazamox.

1 INTRODUCTION

Bromus tectorum L. (downy brome) is a self-pollinated, winter annual grass that is widely spread in crop, range, pasture and conservation reserve program (CRP) lands in the Great Plains and Mountain West regions of the USA.[1,2] *Bromus tectorum* has been found to infest almost 23 million hectares in 17 states in this region, including Montana.[3] In recent years, *B. tectorum* has increasingly become a problem weed in the dryland, no-till wheat production systems of this region. *Bromus tectorum* produces copious amount of seed (> 70 million seeds per acre) indicating its potential for invasion and colonizing new areas.[4] Seeds of *B. tectorum* are short lived in the soil, and seedlings mostly emerge during late-summer to early fall and overwinter in a semi-dormant state.[5] A season-long infestation of *B. tectorum* at densities ranging from 54 to 538 plants m^{-2} reduced winter wheat yields by 28–92%.[6,7]

Growers rely heavily on selective herbicides, primarily acetolactate synthase (ALS) inhibitors, for *B. tectorum* control in wheat. Currently, several chemistries from five structurally different ALS-inhibiting herbicide families, including sulfonylureas (SUs), imidazolinones (IMIs), triazolopyrimidines (TPs), pyrimidinylthiobenzoates (PTBs) and sulfonylamino-carbonyl-triazolinones (SCTs) are used for *B. tectorum* control in wheat. The development of IMI-tolerant (Clearfield™) wheat cultivars with the use of imazamox has simplified the *B. tectorum* control since commercialization of this technology in 2001.[8]

Intensive and frequent use of ALS-inhibiting herbicides has resulted in the widespread evolution of ALS-resistant weed biotypes in several different crops globally (reviewed in Tranel and Wright[9]; Powles and Yu[10]). The first report on resistance to ALS inhibitors was confirmed in *Lactuca serriola* L. in 1987.[11] To date, > 250 weed species (147 dicots and 105 monocots) with resistance to ALS inhibitors have been reported worldwide.[12] *Bromus tectorum* accessions with cross-resistance to ALS inhibitors (primisulfuron, sulfosulfuron, imazamox and propoxycarbazone-sodium) have previously been confirmed in Oregon, USA in Kentucky bluegrass (*Poa pratensis* L.) fields;[13,14] however, there is no previous report on occurrence of ALS-resistant *B. tectorum* in wheat.

An altered target site because of amino acid substitutions is the most common mechanism conferring resistance to ALS inhibitors in weed biotypes.[9,15] It has been found that single amino acid substitution in one of the five highly conserved domains of the matured ALS protein (domain A–E) is sufficient to confer resistance to ALS inhibitors.[13] There are eight confirmed sites in the ALS enzyme, and multiple amino acid substitutions at each site

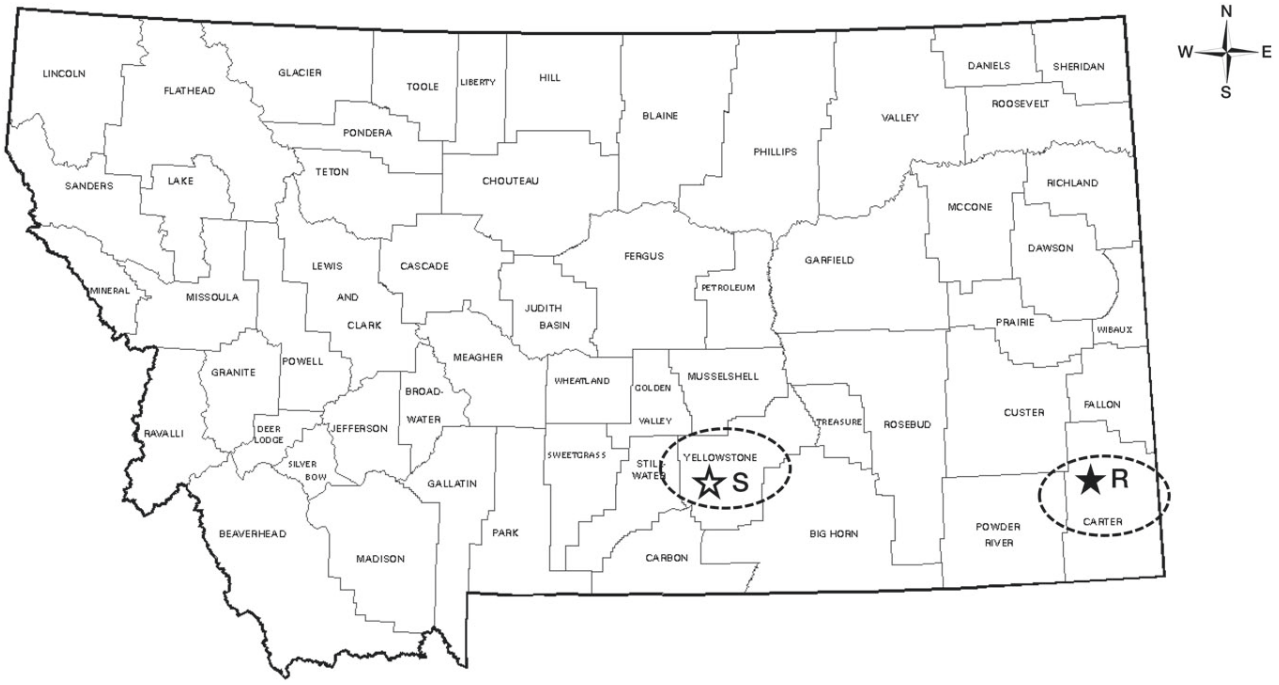

Figure 1. Map of Montana showing locations of Carter and Yellowstone counties, from where R and S *B. tectorum* accessions were collected in summer 2016, respectively.

can confer resistance to the ALS inhibitors: Ala122, Pro197, Ala205, Asp376, Arg377, Trp574, Ser653 and Gly654.[16] In addition to target site mutations, non-target site-based resistance mechanisms, primarily the enhanced rate of metabolism mediated by cytochrome P450 monooxygenases, has also been reported in several ALS-resistant weed biotypes.[10] Furthermore, it is not uncommon to acquire both target and non-target site resistance mechanisms to ALS inhibitors in a single population or in a same plant, as previously observed in ALS-resistant common waterhemp (*Amaranthus tuberculatus*) and Palmer amaranth (*Amaranthus palmeri*).[17,18]

In summer 2016, a control failure of a *B. tectorum* accession from imazamox with MSO adjuvant used at the field-recommended rates was observed in an IMI-tolerant wheat field from Carter County, MT. The objectives of this research were to: (1) investigate the level of resistance of the *B. tectorum* accession to imazamox and cross-resistance to other ALS-inhibiting herbicides (SU, TP, and SCT families) used in wheat; (2) determine the response to other sites-of-action herbicides; and (3) determine the underlying mechanism of resistance to imazamox in the field-collected *B. tectorum* accession.

2 MATERIALS AND METHODS

2.1 Plant material

Matured seeds of *B. tectorum* plants surviving imazamox at recommended use rate (44 g ha^{-1}) were collected in June 2016 from the IMI-tolerant winter wheat field near in Carter County, MT (Figure 1). The sampled field was under IMI-tolerant wheat production (in rotation with safflower or dry pea) for >4 years, with a history of imazamox use in the spring to control *B. tectorum*. Seeds were collected separately from 50 *B. tectorum* survivors in the field. After cleaning, seeds from individual plants were composited into one sample and stored in a paper bag at 4 °C until used. This putative resistant *B. tectorum* accession was referred as 'R'. By contrast, seeds of a *B. tectorum* accession known to be susceptible to imazamox and other herbicides tested in this study were collected from an organic wheat field near Huntley, MT, and referred as 'S' (Figure 1).

Seeds of both *B. tectorum* accessions were germinated in 53 × 35 × 10 cm plastic trays filled with a commercial potting mixture (VermiSoil™, Vermicrop Organics, Rocklin, CA, USA) during fall 2016 in a greenhouse at the Montana State University-Southern Agricultural Research Center (MSU-SARC) near Huntley, MT. Seedlings from each accession were individually transplanted into 10 cm diameter plastic pots containing the same potting mixture. The greenhouse was maintained at 26/23 ± 3 °C day/night temperatures and 16/8 h day/night photoperiods, and the supplemental photoperiod was provided with metal halide lamps (650 μmol m^{-2} s^{-1}). Seedlings were watered as and when needed to avoid moisture stress and fertilized (Miracle-Gro water-soluble fertilizer (24–8–16), Scotts Miracle-Gro Products, Marysville, OH, USA) regularly to maintain good growth.

2.2 Single dose herbicide resistance testing

The selected *B. tectorum* accessions (R and S) were tested with imazamox and other ALS inhibitors, and with herbicides representing alternative sites of action including inhibitors of acetyl-coenzyme A carboxylase (ACCase), photosystem I (PS I), glutamine synthetase (GS) or 5-enolpyrovylshikimate-3-phosphate synthase (EPSPS) at their recommended field-use rates (Table 1). Seedlings of R and S *B. tectorum* accessions were treated with those herbicides, when seedlings attained the two- to three-leaf stage. All herbicide treatments were applied along with recommended adjuvants using a cabinet spray chamber (Research Track Sprayer, De Vries Manufacturing, Hollandale, MN, USA) equipped with an even flat-fan nozzle tip (TeeJet 8001EXR, Spraying System,

Table 1. Visual injury of R and S *Bromus tectorum* L. accessions with various sites-of-action herbicides at their recommended field-use rates 21 days after treatment (DAT)[a]

					S		R	
Herbicide	Formulation	Manufacturer	Site of action	Rate (g ha^{-1})	% injury			
Quizalofop[b]	EC	Dupont, Wilmington, DE	Inhibition of ACCase	62	99	aA	99	aA
Clethodim[c]	EC	Valent USA, Walnut Creek, CA	Inhibition of ACCase	136	99	aA	98	aA
Mesosulfuron[d]	WDG	Bayer CropScience, Research Triangle Park, NC	Inhibition of ALS	15	54	gA	50	cA
Sulfosulfuron[c]	WDG	Monsanto, St Louis, MO	Inhibition of ALS	35	85	efA	86	bA
Imazapic[c]	SL	BASF, Research Triangle Park, NC	Inhibition of ALS	210	94	bcA	5	eB
Imazamox[d]	L	BASF, Research Triangle Park, NC	Inhibition of ALS	44	98	abA	3	eB
Propoxycarbazone [d]	WDG	Bayer CropScience, Research Triangle Park, NC	Inhibition of ALS	30	89	deA	26	dB
Pyroxsulam[c]	WDG	Dow AgroSciences LLC, Indianapolis, IN	Inhibition of ALS	18	84	fA	31	dB
Glyphosate[e]	L	Monsanto, St Louis, MO	Inhibition of EPSPS	1260	99	aA	99	aA
Paraquat[c]	L	Syngenta Crop Protection, Greensboro, NC	Inhibition of PS I	840	98	abA	98	aA
Glufosinate[e]	SL	Bayer CropScience, Research Triangle Park, NC	Inhibition of GS	593	90	cdA	87	bA

R, *B. tectorum* accession resistant to ALS inhibitors collected from Carter County, MT, USA; S, *B. tectorum* accession susceptible to ALS inhibitors from Huntley, MT, USA. EC, emulsifiable concentrate; WDG, water dispersible granule; SL, soluble liquid; L, liquid.
[a] Treatments were applied at the two- to three-leaf stage of *B. tectorum* plants. Means for a *B. tectorum* accession within a column followed by similar lowercase letters are not significantly different based on Fisher's protected LSD test at $P < 0.05$; means for a herbicide within a row followed by similar uppercase letters are not significantly different based on Fisher's protected LSD test at $P < 0.05$.
[b] Crop oil concentrate (COC) at 0.5% (v/v) was included.
[c] Nonionic surfactant (NIS) at 0.25% (v/v) was included.
[d] Methylated seed oil (MSO) at 1% (v/v) was included.
[e] Ammonium sulfate (AMS) at 2 % (wt./v) was included.

Wheaton, IL, USA) calibrated to deliver 94 L ha^{-1} of spray solution at 276 kPa. The percentage injury of treated plants was visually assessed on a scale of 0 (no injury) to 100 (complete plant death) at 21 days after treatment (DAT). A plant was considered 'dead' if the leaf tissue was necrotic and easily fragmented and there was no new tiller formation. For each herbicide treatment, 60 replicated pots (one plant per pot) from each R and S accession were used. Experiments were set up in a randomized complete block design (blocked by accession) and performed twice.

2.3 Dose response to ALS-inhibiting herbicides

Based on the results from the single-dose herbicide screening, seedlings from putative resistant (R) and susceptible (S) *B. tectorum* accessions were grown in the greenhouse under identical conditions (previously described) to characterize the level of resistance to imazamox and other ALS-inhibiting herbicides, viz., propoxycarbazone sodium and pyroxsulam. Separate dose–response experiments were conducted for each of these three ALS-inhibitor herbicides. Treatments were applied to plants from each accession at the two- to three-leaf stage. Doses for the ammonium salt of imazamox (Beyond® herbicide, BASF, Research Triangle Park, NC, USA) along with 1% (v/v) methylated seed oil (MSO, Southern Agricultural Insecticides, Hendersonville NC, USA) included 0, 11, 22, 44, 88, 176, 264, 352, 440, 528, 880, 1320, 1760 and 2200 g ha^{-1}. Doses for propoxycarbazone sodium (Olympus® herbicide, Bayer CropScience, Research Triangle Park, NC, USA) along with 0.25% (v/v) nonionic surfactant (Southern Agricultural Insecticides) included: 0, 3.75, 7.5, 15, 30, 60, 120, 240 and 480 g ha^{-1}. Doses for pyroxsulam (Powerflex® HL, Dow AgroSciences, Indianapolis, IN, USA) along with R-11®-Spreader Activator Nonionic Surfactant (Wilbur-Ellis, Fresno, CA, USA) included 0, 2.25, 4.5, 9, 18, 36, 72, 144 and 288 g ha^{-1}. All treatments were applied as previously described. At 21 DAT, the percent injury of a treated plant was visually assessed, and the aboveground tissue was clipped and placed in a paper bag, dried at 65 °C for 72 h and weighed to obtain the shoot dry weight. Each dose–response experiment was set up in a randomized complete block (blocked by accession) design, with 10 replicates (pots) per treatment, and repeated over time.

2.4 Effect of malathion on resistance to imazamox

Malathion, an organophosphate insecticide, inhibits the activity of cytochrome P450 monooxygenases involved in detoxification of herbicide molecules, as reviewed in Powles and Yu.[10] The influence of malathion on the resistance status of R *B. tectorum* accession to imazamox was investigated. Plants from R and S *B. tectorum* accessions were grown as previously described, and treated with malathion at 1000 g ha^{-1} at the two- to three-leaf stage. Approximately 30 min after the malathion treatment, plants were sprayed with imazamox at 44 or 88 g ha^{-1} along with MSO at 1% v/v. Malathion and imazamox treatments were applied to the plants individually by using cabinet spray chamber as described previously. A soil drench treatment of 5 mM malathion solution (~ 30 ml per pot) was also applied at 2 days after herbicide treatment. Visual assessment of percent injury was made and reversal (if any) of the resistance phenotype was monitored at 21 DAT. Experiments were conducted in a completely randomized design with five replicates per treatment, and repeated over time.

2.5 Statistical analyses

All data from single-dose and whole-plant dose–response experiments were subjected to analysis of variance (ANOVA) using PROC MIXED in SAS 9.3 (SAS Institute, Cary, NC, USA) to test the significance of the fixed effects, i.e., accession, treatment (herbicides in single-dose experiments or doses of a herbicide in whole-plant dose–response), and their interactions. Random effects in the model statement included experimental run and replication (nested within experimental runs) (SAS 9.3). The residual analysis was performed using PROC UNIVARIATE, and homogeneity of variance assumption was checked. All data met those

Table 2. *Bromus tectorum* L. visually assessed injury and shoot dry weight response to imazamox 21 DAT using a three-parameter log-logistic model. The tested accessions were ALS inhibitor-resistant (R) and inhibitor-susceptible (S). All treatments included 1% (v/v) methylated seed oil and 10% (v/v) urea ammonium nitrate. Standard error of means are given in parentheses

	Dose–response model parameter estimates				
Accessions	*D* Injury (%) or shoot dry weight plant^{-1} (% of mean nontreated)	*B*	I_{50} or GR_{50} Imazamox (g ha^{-1})	95% CI	RF
Injury (%)					
R	99.9 (± 2.0)	−1.2 (± 0.2)	1059.4 (± 40.9)	763–1355	93.7
S	99.7 (± 1.0)	−2.5 (± 0.4)	11.3 (± 0.5)	6–16	
Shoot dry weight					
R	96.4 (± 2.3)	0.7 (± 0.1)	242.3 (± 24.1)	194–290	110.1
S	100.0 (± 2.5)	0.4 (± 0.1)	2.2 (± 0.7)	0.5–3.5	

D, upper asymptote; *B*, relative slope around I_{50} or GR_{50}; I_{50} or GR_{50}, effective dose of imazamox causing 50% injury and 50% reduction in the shoot dry weight relative to nontreated plants, respectively; RF, resistance factor (I_{50} or GR_{50} of R over S accession).

ANOVA requirements. For dose–response experiments, data on percent injury or shoot dry weight (percentage of nontreated control) for each accession were regressed over herbicide doses using a three-parameter log-logistic model.[19,20]

$$Y = \{D/1 + \exp[B(\log X - \log E]\} \quad (1)$$

Where *Y* is the response variable (percent injury or shoot dry weight as a percentage of nontreated), *D* is the upper limit, *B* is the slope of each curve, *E* is the herbicide dose required to cause 50% response (i.e. 50% injury referred as I_{50} or 50% reduction in shoot dry weight referred as GR_{50}), and *X* is the herbicide dose. Nonlinear regression parameter estimates, standard errors, 95% confidence intervals (CI) for each accession were determined using the *drc* package in *R* software.[21] The resistance factor (RF) (resistant to susceptible ratio) based on I_{50} or GR_{50} values was estimated to determine the level of resistance in the R relative to the S *B. tectorum* accession for each of the three ALS-inhibitor herbicides tested.

2.6 *ALS* gene sequencing

Young green leaf tissues from each plant (10 plants per accession) were collected, flash frozen, and stored at −80 °C for genomic DNA (gDNA) extraction. The gDNA was extracted using DNeasy Plant Mini kit (Qiagen, Valencia, CA, USA) in accordance with the manufacturer's instructions. Each DNA sample was quantified on an Epoch2 microplate spectrophotometer (Biotek, Winooski, VT, USA). The forward and reverse primers were designed from DNA sequences of the *ALS* gene for *B. tectorum* obtained from the GenBank (accession number AF487459.1) using a Primer 3 Plus software. The forward and reverse primer sequences were 5′-GTCGACGTCTTCGCCTACC-3′ and 5′-AATATTCGATCCTGCCATCA-3′, respectively. These primers were used to amplify a 1675-bp fragment of the *ALS* gene in R and S *B. tectorum* plants. In addition, an *ALS* amplicon of <200 bp containing the conserved region of domain E was amplified with another set of primers: 5′-GAAGTACGTGCAGCAATCCA-3′ and 5′-TATTCGATCCTGC CATCACC-3′.

A polymerase chain reaction (PCR) was performed in a T100 thermal cycler (Bio-Rad, Hercules, CA, USA) using EconoTaq PLUS 2X PCR master mix (Lucigen, Middleton, WI, USA). Each reaction contained 10 μl of Master Mix, 2.5 μl each of the forward and reverse primers (5 μM), 5 μl of gDNA template (10 ng μl^{-1}), and 5 μl of nuclease-free water. For PCR amplification, following thermal conditions were used: 95 °C for 3 min, 30 cycles of 95 °C for 30 s, 58 °C for 30 s and 72 °C for 90 s, followed by 72 °C for 10 min. To confirm the amplicon size, PCR products were examined on a 1.0% agarose gel stained with GelRed™ nucleic acid gel stain (Biotium, Fremont, CA, USA) using 500 and 100 bp markers. Samples were electrophoresed in 1× TAE buffer at 95 V and imaged using Gel Doc EZ Imaging System (Bio-Rad). PCR products were purified using QIAquick PCR Purification Kit (Qiagen) using the manufacturer's protocol, and quantified using an Epoch2 microplate spectrophotometer. The purified PCR products were sequenced using an ABI™ 3130 × L genetic analyzer with same primers used for amplification. By using a MultAlin software, the sequence reads of the *ALS* genes were aligned to a reference *B. tectorum ALS* sequence, to analyze the presence of any known target-site mutation(s) that confer(s) resistance to the ALS inhibitors. The experiment was repeated over time.

3 RESULTS

3.1 Single-dose herbicide resistance testing

Compared with the S accession, control of the R accession with the ALS inhibitors, imazamox, imazapic, pyroxsulam, and propoxycarbazone was 3, 5, 26, and 31% at 21 DAT respectively (Table 1). However, the R accession was susceptible to the SU herbicide, sulfosulfuron (86% control), and to quizalofop and clethodim (ACCase inhibitors), glyphosate (EPSPS inhibitor), paraquat (PS I inhibitor), and glufosinate (GS inhibitor) herbicides, consistent with the S accession. Control of both S and R accessions with mesosulfuron was inadequate (< 60% at 21 DAT).

3.2 Whole-plant dose response to ALS inhibitors

Dose–response studies indicated that the R *B. tectorum* accession was resistant to imazamox, propoxycarbazone, and pyroxsulam (Tables 2–4). Based on the visual assessment of percent injury at 21 DAT, the dose of imazamox causing 50% injury (I_{50} values) of R *B. tectorum* accession was 1059.4 g ha^{-1}, which was greater than the 11.3 g ha^{-1} for the S accession (Table 2). Based on the I_{50} values, the R accession had 93.7-fold resistance to imazamox, compared with the S accession. The lethal dose of imazamox required to reduce 50% shoot dry weight (GR_{50} values) of R and

Table 3. *Bromus tectorum* L. visually assessed injury and shoot dry weight response to propoxycarbazone 21 DAT using a three-parameter log-logistic model. The tested accessions were ALS inhibitor-resistant (R) and inhibitor-susceptible (S). All treatments included 0.25% (v/v) nonionic surfactant. Standard error of means are given in parentheses

	Dose–response model parameter estimates				
Accessions	*D* injury (%) or shoot dry weight plant^{-1} (% of mean nontreated)	*B*	I_{50} or GR_{50} Propoxycarbazone (g ha^{-1})	95% CI	RF
Injury (%)					
R	102.6 (± 3.9)	-0.6 (± 0.1)	93.2 (± 2.9)	72–114	22.1
S	90.4 (± 1.6)	-3.4 (± 0.7)	4.2 (± 0.2)	2–6	
Shoot dry weight					
R	100.2 (± 5.5)	0.4 (± 0.1)	18.1 (± 1.1)	194–290	13.9
S	100.2 (± 5.5)	0.6 (± 0.2)	1.3 (± 0.7)	0.6–3.5	

D, upper asymptote; *B*, relative slope around I_{50}; I_{50} or GR_{50}, effective dose of propoxycarbazone causing 50% injury or 50% reduction in shoot dry weights relative to non-treated individuals, respectively; RF, resistance factor (I_{50} or GR_{50} of R over S accession).

Table 4. *Bromus tectorum* L. visually-assessed injury and shoot dry weight response to pyroxsulam 21 DAT using a three-parameter log-logistic model. The tested accessions were ALS inhibitor-resistant (R) and inhibitor-susceptible (S). All treatments included 0.25% (v/v) nonionic surfactant. Standard error of means are given in parentheses

	Dose–response model parameter estimates				
Accessions	*D* injury (%) or shoot dry weight plant^{-1} (% of mean nontreated)	*B*	I_{50} or GR_{50} pyroxsulam (g ha^{-1})	95% CI	RF
Injury (%)					
R	91.5 (± 2.1)	–1.3 (± 0.1)	16.0 (± 1.1)	13–19	5.1
S	99.0 (± 1.3)	–1.5 (± 0.1)	3.1 (± 0.1)	1–6	
Shoot dry weight					
R	100.9 (± 5.2)	0.4 (± 0.1)	28.6 (± 1.1)	18–39	4.6
S	100.2 (± 5.4)	0.3 (± 0.1)	6.1 (± 0.7)	2–10	

D, upper asymptote; *B*, relative slope around I_{50}; I_{50} or GR_{50}, effective dose of pyroxsulam causing 50% injury or 50% reduction in shoot dry weights relative to non-treated individuals, respectively; RF, resistance factor (I_{50} or GR_{50} of R accession over S accession).

S accessions was 242.3 and 2.2 g ha^{-1}, respectively, indicating a 110.1-fold resistance of the R relative to the S accession. The lethal dose to cause 50% injury or reduce 50% shoot dry weight (I_{50} or GR_{50} values) of the R *B. tectorum* accession was 13.9–22.1 times greater for propoxycarbazone (Table 3), and 4.6–5.1 times greater for pyroxsulam relative to the S accession (Table 4).

3.3 Effect of malathion on imazamox resistance

The pretreatment of malathion at 1000 g ha^{-1} followed by imazamox applied at either 44 or 88 g ha^{-1} did not reverse the resistance phenotype of the R accession (data not shown), indicating that the enhanced metabolism of imazamox herbicide by cytochrome P450 monooxygenases may not be involved in conferring the high-level resistance to imazamox. The response of R *B. tectorum* accession to propoxycarbazone-sodium and pyroxsulam with a pretreatment of malathion was not assessed in this study; therefore, the role of metabolism-based mechanism causing low- to moderate-level resistance to these herbicides cannot be ruled out.

3.4 *ALS* gene sequencing

The DNA sequence analyses of the *ALS* gene in R *B. tectorum* accession revealed a single-point mutation at Ser653 position (Figure 2). Specifically, a single nucleotide substitution of AGC to AAC at codon 653 was observed in all R relative to S plants. The nucleotide change of G to A at the second base of the codon 653 resulted in an exchange of amino acid from serine to asparagine (Figure 2). Apart from Ser627Asn, Ser653Asn mutation is also known to confer resistance to IMI herbicides in two of three *ALS* genes (imi1 and imi2) in IMI-tolerant wheat.[22] To the best of our knowledge, this research confirms the first report of Ser653Asn mutation conferring high-level resistance to imazamox in *B. tectorum*.

4 DISCUSSION

This research confirms the first case of a *B. tectorum* population with cross-resistance to ALS-inhibiting herbicides evolved from a wheat field. The R accession had a relatively high-level resistance to the IMI herbicide (imazamox), and low- to moderate-levels resistance to SCT (propoxycarbazone-sodium) and TP (pyroxsulam) herbicides. *Bromus tectorum* biotypes with different cross-resistance patterns to ALS-inhibiting herbicides have been previously documented from two different *P. pratensis* fields in Oregon, USA.[13] In that study, one biotype (AR) was highly resistant to primisulfuron (317-fold), sulfosulfuron (263-fold),

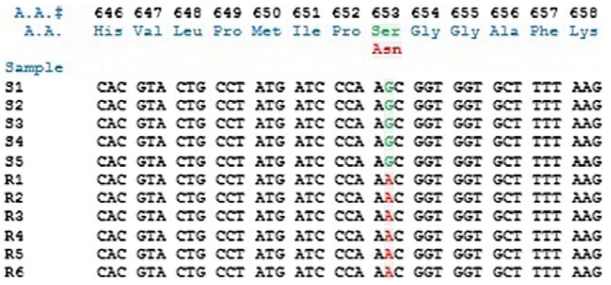

Figure 2. Partial nucleotide and amino acid sequences for *ALS* genes from R and S *B. tectorum* plants. The shaded amino acid (A.A) indicates a Ser to Asn mutation at position 653 in R individuals. The amino acid numbering based on the precursor *ALS* gene from *Arabidopsis thaliana* L.

and propoxycarbazone-sodium (425-fold), but susceptible to imazamox. The other biotype (MR) had low- to moderate-levels resistance to primisulfuron (18-fold), sulfosulfuron (9-fold), propoxycarbazone-sodium (40-fold), and imazamox (14-fold).[13] Resistance to ALS inhibitors in the AR biotype of *B. tectorum* was conferred by a single-point mutation ($Pro_{197}Leu$) in the ALS enzyme; however, the rapid rate of metabolism endowed resistance to the SCT herbicide in the MR biotype.[13,14] In our study, resistance in the R accession collected from a IMI-tolerant wheat field in MT was specific to IMI, SCT, and TP families of ALS inhibitors, and the efficacy of SU herbicides (mesosulfuron and sulfosulfuron) did not differ between R and S accessions.

Resistance to ALS inhibitors is generally conferred by single point mutations leading to amino acid substitutions at the herbicide-binding site of the ALS enzyme, causing reduction in the sensitivity to the herbicide.[9,23] In the current study, the Ser653Asn mutation was detected in individuals from R accession and absent in individuals from S accession. Our results agree with the findings of Patzoldt and Tranel,[24] who also reported the Ser653Asn mutation causing high-level resistance to IMI herbicides in a common waterhemp (*Amaranthus tuberculatus* (Moq.) *Sauer*) population from Illinois, USA. However in that study, the Ser653Asn mutation in the *A. tuberculatus* population did not endow any cross-resistance to TP and SU herbicides, while the resistance to SCT herbicides was not detected.[24] There are three possible lines of evidence indicating that resistance to IMI herbicides in the R *B. tectorum* accession in our study is due to a target-site mutation: (1) the relatively high-level of resistance to imazamox, as indicated by slight decline in shoot dry weight of R accession at high rates of imazamox; (2) a consistent observation of single point mutation (> 98% R individuals had Ser653Asn mutation); and (3) non reversal of imazamox resistance phenotype by malathion treatment.[23]

The Ser653Asn mutation causing high levels of resistance to IMI herbicides has also been reported in ALS-resistant biotypes of other weed species including *Avena fatua* L., *Galium spurium*, *Setaria viridis*, *Amaranthus palmeri*, and *Amaranthus hybridus*.[25–29] Furthermore, the Ser653Asn mutation conferring high-level resistance (> 10-fold) to PTB, but low- to moderate- levels resistance (< 10-fold) to SU herbicides in ALS-resistant *A. hybridus* and *S. viridis* biotypes has been reproted.[27–29] In the current study, no other known mutations were detected at Ala122, Pro197, Ala205, Asp376, Arg377, Val560, Trp574, and Gly654 amino acid positions in the R *B. tectorum* accession (data not shown). Although the possibility of non-target site-based mechanisms (reduced herbicide uptake or translocation) conferring resistance to imazamox in the R *B. tectorum* accession cannot be ruled out, the results reported here are consistent with previous studies identifying the target site-based mechanisms of weed resistance to imazamox.[25–29]

The evolution of *B. tectorum* accession with a high-level resistance to imazamox is a recent phenomenon, and poses a serious challenge to cereal producers, and a potential threat to the long-term sustainability of the IMI-tolerant wheat technology. This technology with the use of imazamox has been very effective so far for *B. tectorum* control in wheat. Furthermore, the evidence of cross-resistance to other ALS-inhibiting herbicides, including propoxycarbazone and pyroxsulam, widely used in wheat, would potentially limit herbicide options to control the R *B. tectorum* in wheat production fields. Growers can possibly target the ALS-resistant *B. tectorum* seed bank with alternative, effective sites-of action herbicides (Table 1) in no-till chemical fallow (glyphosate, glufosinate, or paraquat) or in rotational crops (e.g. quizalofop and clethodim use in pulse crops and safflower) in the dryland production systems of this region. All possible efforts should be made to prevent any seed production from the ALS-resistant *B. tectorum* plants. An IWM program, including the use of competitive crops, tillage, crop rotation, or other tools that deplete the soil seed bank of *B. tectorum* might be adopted.[30]

Future research will investigate the possibility of any non-target site-based mechanism (alteration in uptake, translocation, or metabolism) endowing cross-resistance to propoxycarbazone-sodium and pyroxsulam in the R *B. tectorum* accession. Studies to elucidate the inheritance pattern, population genetics, and relative fitness of ALS-resistance alleles in the R vs. S accession will allow us to determine the population dynamics and potential spread of ALS-resistant *B. tectorum* in wheat production fields in this region.

ACKNOWLEDGEMENTS

The authors greatly appreciate Dr Jared Unverzagt (Technical Service Representative at BASF), extension agents, and the grower from Carter County, Montana for their valuable assistance in seed collection. We thank Anjani J., Charlemagne A. Lim and Fanny Liu for their technical assistance in conducting this research. We also thank Montana Wheat and Barley Committee (Grant # 4 W6485) for funding this research.

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