Weed Science 2013 61:21–25

A Trp_{574} to Leu Amino Acid Substitution in the ALS Gene of Annual Bluegrass *(Poa annua)* Is Associated with Resistance to ALS-Inhibiting Herbicides

J. Scott McElroy, Michael L. Flessner, Zhuoyu Wang, Fenny Dane, Robert H. Walker, and Glenn R. Wehtje*

Annual bluegrass is commonly controlled by acetolactate synthase (ALS)-inhibiting herbicides in managed turfgrass. An annual bluegrass population with suspected resistance to ALS-inhibiting herbicides was collected from Grand National Golf Course in Opelika, AL (GN population). Subsequent testing confirmed resistance of the GN population to foramsulfuron, trifloxysulfuron, bispyribac-sodium (bispyribac), and imazaquin when compared to a susceptible population collected locally at Auburn University (AU population). Sequencing of the ALS gene revealed a point mutation resulting in an amino acid substitution at Trp_{574}. Cloning of the ALS gene surrounding the Trp_{574} region yielded two distinct ALS gene sequences: one producing Trp_{574} and one producing Leu_{574}. Trp_{574} to Leu has been previously correlated with resistance to ALS-inhibiting herbicides. Both AU and GN gene sequences contained other similar silent and missense mutations. This research confirms resistance of annual bluegrass to ALS-inhibiting herbicides with Trp_{574} to Leu amino acid substitution being the most likely mode of resistance based on past literature.

Nomenclature: Bispyribac; foramsulfuron; imazaquin; trifloxysulfuron; annual bluegrass, *Poa annua* L.

Key words: Acetolactate synthase-inhibiting herbicides, herbicide resistance, imidazolinone, pyrimidinyl benzoates, sulfonylurea, target-site mutations, turfgrass management.

ALS-inhibiting herbicides within the sulfonylurea, imidazolinone, and pyrimidinyl benzoate herbicide families are used to control annual bluegrass in managed turfgrass. Sulfonylurea herbicides such as foramsulfuron, trifloxysulfuron, rimsulfuron, and others are commonly used to control annual bluegrass and other weeds in warm-season turfgrasses such as bermudagrass (*Cynodon* spp.), zoysiagrass (*Zoysia* spp.), and centipedegrass [*Eremochloa ophiuroides* (Munro) Hack] (Toler et al. 2007). The pyrimidinyl benzoate herbicide bispyribac-sodium (bispyribac), and the imidazolinone herbicide, imazaquin, can be used to control annual bluegrass; however, multiple applications are needed for acceptable control (Lycan and Hart 2006; McElroy et al. 2011; Rodriguez et al. 2001). ALS-inhibiting herbicides are highly valuable to the turfgrass industry because of their low mammalian toxicity (LD_{50} most $> 5{,}000$ mg kg^{-1}), low use rates (usually < 100 g ai ha^{-1}), and low potential of ground water contamination (Battaglin et al. 2000; Senseman 2007).

ALS-inhibiting herbicides act by specifically inhibiting ALS (E.C. 4.1.3.18) enzyme activity, which is a key enzymatic step in the formation of branched-chain amino acids valine, leucine, and isoleucine. ALS is a nuclear-encoded gene with biosynthetic activity occurring in plant chloroplasts (Chipman et al. 1998; Mazur et al. 1987). The ALS gene contains a chloroplast transit peptide sequence; however, sequencing of this region has revealed a lack of homology between related transit peptide sequences of nuclear-encoded/chloroplast active proteins (Mazur et al. 1987). Many higher plants contain multiple copies of the ALS gene (Chipman et al. 1998). Multiple ALS alleles are potentially due to allopolyploidy from different ancestor species (Chipman et al. 1998). The allotetraploid rapeseed (*Brassica napus* L.) expresses five separate ALS genes, some of which can be correlated to its diploid progenitor species *B. rapa* L. (syn. *B. campestris* L.) or *B. oleracea* L. (Ouellet et al. 1992).

DOI: 10.1614/WS-D-12-00068.1

* First, second, fifth, and sixth authors: Associate Professor, Research Associate III, Professor, and Professor, respectively, Department of Agronomy and Soils, 201 Funchess Hall, Auburn University, Auburn, AL 36849; third and fourth authors: Graduate Research Assistant and Professor, respectively, Department of Horticulture, 101 Funchess Hall, Auburn University, Auburn, AL 36849. Corresponding author's Email: jsm0010@auburn.edu

Target-site nucleotide mutations in the ALS gene inducing missense mutations in the ALS amino acid protein sequence are the most common mechanism of resistance reported in plant species (Tranel and Wright 2002). There are six-target site amino acids that can be changed to numerous other amino acids most commonly correlated with resistance [numbered according to corresponding sequence of *Arabidopsis thaliana* (L.) Heynh]: Ala_{122}, Pro_{197}, Ala_{205}, Asp_{376}, Trp_{574}, and Ser_{653} (Corbett and Tardiff 2006; Tranel and Wright 2002; Whaley et al. 2007). Sulfonylurea and imidazolinone herbicides have been studied the greatest with respect to influence of target-site mutations and varied herbicide response. Substitutions at Ala_{122}, Ala_{205}, and Ser_{653} confer resistance to imidazolinones, but a low level of resistance to sulfonylureas (Powles and Yu 2010) except for substitution of Ala_{122} to Tyr, which confers a high level of resistance to both families (Han et al. 2012). Substitutions at Pro_{197} confer resistance to sulfonylureas but a low level of resistance to imidazolinones (Park and Mallory-Smith 2004; Powles and Yu 2010; Whaley et al. 2007). Substitutions at Asp_{376} and Trp_{574} confer a high level of resistance to both groups (Powles and Yu 2010). Other potential amino acid locations have been reported at which substitutions can occur and resistance is conferred, e.g., Gly_{654} and Arg_{377} (Massa et al. 2011; Powles and Yu 2010). However, the above six have historical precedent in several species as potential locations of amino acid substitution sites conferring ALS-inhibiting herbicide resistance.

Both homozygous and heterozygous mutations have been identified that confer resistance to ALS-inhibiting herbicides. Patzoldt and Tranel (2002) identified both homozygous and heterozygous Trp_{574} to Leu allele in giant ragweed (*Ambrosia trifida* L.) within a single interbreeding population; however, zygosity did not influence response to sulfonylurea or imidazilinone herbicides as both incurred a similar level of resistance. Different amino acid substitutions can also occur to the same target amino acid site. For instance, amino acid substitutions of Pro_{197} to His, Thr, or Ser in corn poppy (*Papaver rhoeas* L.) confer the same reported level of resistance (Scarabel et al. 2004). Ala_{122} to Tyr confers a higher level of broad-spectrum resistance to ALS-inhibiting herbicides,

whereas Ala_{122} to Thr confers resistance to imidazolinone only (Han et al. 2012).

Annual bluegrass is often cited as one of the most troublesome weeds in turfgrass (Beard et al. 1978, Christians 2006; Webster 2004). Annual bluegrass is an allotetraploid (2n = 4× = 28) with presumed progenitor species of *Poa supina* Schrad. (2n = 2× = 14) and *P. infirma* Kunth. (2n = 2× = 14) (La Mantia and Huff 2011; Tutin 1957). Annual bluegrass is known to have worldwide distribution, with extensive ecotypic variation (McElroy et al. 2002; Tutin 1957). The adaptability of annual bluegrass is most easily illustrated by the fact that it is one of the few species to colonize Antarctica and is also a major weed species in the southeastern United States (Greene and Walton 1975; Olech 1996; Walton and Smith 1973; Webster 2004).

Herbicide-resistant annual bluegrass populations are currently known to exist with the following mechanism of action: photosystem II inhibitors (e.g., atrazine, simazine, diuron), photosystem I inhibitors (e.g., paraquat), inhibitors of very long chain fatty acids (e.g., ethofumesate), mitotic-inhibiting herbicides (e.g., prodiamine, pendimethalin), and 5-enolpyruvate shikimate-3-phosphate synthase inhibitor (e.g., glyphosate) (Brosnan et al. 2012; Heap 2012; Kelly et al. 1999). Because of resistance development to these modes of action, ALS-inhibiting herbicides have become a popular choice for annual bluegrass weed control in turfgrass.

To date, no ALS-resistant annual bluegrass has been reported in the world (Heap 2012). A population of annual bluegrass was reported to not be controlled by foramsulfuron at Grand National Golf Course in Opelika, AL. The objective of this research was to evaluate annual bluegrass from Grand National Golf Course for response to ALS-inhibiting herbicides and determine the mechanism of resistance.

Materials and Methods

Annual bluegrass plants were collected in February 2011 at Grand National Golf Course in Opelika, AL. The turf was treated with foramsulfuron 4 wk prior to collecting to ensure only suspected resistant plants were collected. Collected plants were placed in an isolated greenhouse to prevent cross-pollination with other annual bluegrass plants. Dehiscent panicles were collected, and seed were cleaned, dried, and stored at 2 C for future testing. An annual bluegrass population with no known history of exposure to any ALS-inhibiting herbicides was collected from the Auburn University campus in Auburn, AL, and propagated in a similar fashion. All herbicide bioassays were conducted in a greenhouse at Auburn University Plant Sciences Greenhouses in Auburn, AL (32.35°N, 85.29°W). The greenhouse was maintained at 22/18 C day/night (+2 C) with no additional supplemental lighting. All greenhouse experiments were conducted from September 2011 to February 2012. Populations will henceforth be referred to as GN (Grand National population, suspected resistant) or AU (Auburn University population, suspected susceptible).

ALS Herbicide Response Evaluation. Response of AU and GN to ALS-inhibiting herbicides was conducted to assess potential herbicide resistance. Plants were grown in a native Wickham sandy loam (pH 6.3, 2.1% organic matter) in 700 cm^3 pots. Each pot contained five plants at the three-tiller stage when treated. Treatments included foramsulfuron (Revolver, Bayer CropScience, Research Triangle Park, NC) at 25 and 50 g ai ha^{-1}, trifloxysulfuron (Monument 75WG, Syngenta Crop Protection, Greensboro, NC) at 16 and 32 g ai ha^{-1}, bispyribac (Velocity SG, Valent U.S.A. Corporation, Walnut Creek, CA) at 150 and 300 g ai ha^{-1}, and imazaquin (Image 70 DG, BASF Corporation, Research Triangle Park, NC) at 490 and 980 g ai ha^{-1}. These herbicides were selected to encompass three ALS-inhibiting herbicide families, sulfonylurea, pyrimidinyl benzoates, and imidazolinone, and because they are registered for annual bluegrass control in turfgrass at 1X and 2X labeled rates. Herbicide applications were made in an enclosed spray cabinet using a single TeeJet TP8002EVS nozzle (Spraying Systems Co., Wheaton, IL) calibrated to deliver at 280 L ha^{-1}. All treatments included a nonionic surfactant (Induce, Helena Chemical Company, Collierville, TN) at 0.25% v v^{-1}. Nontreated plants were included as control treatments. Each experiment was arranged as a randomized complete block design with three replications and was repeated once. Visible control data were collected 14 and 28 d after treatment (DAT) on a 0 to 100% scale where 0 corresponded to no control, and 100 corresponded to complete plant death. Total above ground biomass (g) to the soil surface was also collected 28 DAT and immediately weighed as fresh weight. These data were transformed to a percent reduction relative to the nontreated for analysis and presentation. Data analyses were performed using SAS (SAS version 9.1, SAS Institute Inc., Cary, NC). Since ANOVA indicated no differences between experimental repetitions, data were pooled across experimental repetitions for subsequent analysis. Contrast statements were used to compare populations for response to each herbicide treatment.

ALS Gene Amplification and Sequencing. Research was conducted to determine the molecular basis of herbicide resistance of GN to ALS herbicides. AU and GN cDNA was isolated via RNA extraction of leaf material (approximately 0.1 g) and reverse transcriptase conversion. RNA was extracted using standard guanidinium thiocynanate-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).

An approximately 1.5 kilobase (kb) section of the ALS gene was amplified using PCR and sequenced to evaluate potential single nucleotide polymorphisms known to confer herbicide resistance utilizing a method previously reported by Beam (2004) with slight modifications. PCR was conducted using plant cDNA amplification in a 25-µl volume using high fidelity Taq polymerase (Phusion High-Fidelity DNA Polymerase, New England Biolabs, Inc., Ipswich, MA). The mixture contained 1X High Fidelity buffer (New England Biolabs), 200 µM dNTPs, 0.5 µM forward and reverse primer, 250 ng of cDNA, and 1 U Taq polymerase. Thermocycle program was one activation step of 98 C for 30 s followed by 35 cycles of 98 C for 20 s, 50 C for 40 s, and 72 C for 1 min, and a final extension step 72 for 5 min. Forward and reverse primers utilized were ALSF (5′-GTCATCACCAACCACCTCTTC-3′) and ALSR (5′-AAAATCTGGATATATCTCACTCTCA-3′) (Beam 2004). PCR product was extracted from a 1% agarose gel using Qiagen QIAquick Gel Extraction Kit (Qiagen N.V., Venlo, Netherlands) and

https://doi.org/10.1614/WS-D-12-00068.1 Published online by Cambridge University Press

Table 1. Comparison AU (suspected susceptible) and GN (suspected resistant) annual bluegrass population response to imazaquin, trifloxysulfuron, foramsulfuron, and bispyribac.[a]

Population	Herbicide[b]	Visible injury		Above ground biomass
		14 DAT[a]	28 DAT	28 DAT
		%		% change[c]
AU[a]	Imazaquin	43.3 ± 3.9	95.9 ± 2.2	−93.8 ± 1.1
	Trifloxysulfuron	52.5 ± 4.1	96.7 ± 1.8	−91.9 ± 1.6
	Foramsulfuron	58.3 ± 4.6	99.6 ± 0.4	−92.8 ± 1.4
	Bispyribac	35.5 ± 2.4	46.8 ± 6.7	−57.8 ± 5.7
GN[a]	Imazaquin	0.0 ± 0.0	0.0 ± 0.0	−11.0 ± 9.4
	Trifloxysulfuron	1.7 ± 1.7	0.0 ± 0.0	0.4 ± 13.3
	Foramsulfuron	0.8 ± 0.8	0.0 ± 0.0	−14.7 ± 12.0
	Bispyribac	0.0 ± 0.0	0.0 ± 0.0	−22.2 ± 8.1
			P value	
Contrasts: AU vs GN	Imazaquin	< 0.001	< 0.001	< 0.001
	Trifloxysulfuron	< 0.001	< 0.001	< 0.001
	Foramsulfuron	< 0.001	< 0.001	< 0.001
	Bispyribac	< 0.001	< 0.001	< 0.001

[a] Abbreviations: AU, Auburn University population; DAT, days after treatment; GN, Grand National population.
[b] Herbicide rate not significant in ANOVA; therefore, data were pooled across rate within herbicide. Herbicide rates were: foramsulfuron at 25 and 50 g ai ha^{-1}, trifloxysulfuron at 16 and 32 g ai ha^{-1}, imazaquin at 490 and 980 g ai ha^{-1}, and bispyribac at 0.15 and 0.30 g ai ha^{-1}.
[c] Negative values indicate a decrease relative to nontreated plants of the individual populations.

sequenced by Auburn Genomics and Sequencing Lab with the ABI3100 sequencer (Applied Biosystems Inc. Foster City, CA). Sequences were aligned using MacVector (version 12.0.3; MacVector, Inc., Cary, NC). Nucleotide sequences were subjected to nucleotide (blastn) and amino acid (blastx) searches using the National Center for Biotechnology Information Basic Local Alignment Search Tool to align to nucleotide and transcribed amino acid sequences. As a means of replication, RNA was extracted, converted to cDNA, and utilized for sequencing from five separate GN plants surviving foramsulfuron treatment from the herbicide screening and five nontreated AU plants. Sequences were identical within each population.

Cloning of Trp_{574} ALS Gene Region. Following the identification of a specific mutation around the Trp_{574} codon, PCR was conducted to isolate the region around the mutation. PCR methods utilized were previously reported by Yu et al. (2008) with slight modifications. Forward and reverse primers utilized were ALSF574 (5′-TGGGCGGCTCAG-TATTACAC-3′) and ALSR574 (5′-ATAGGCAGCACATG-CTCCTG-3′) that amplified a 479-bp region of the ALS gene. cDNA amplification was conducted in a 21-µl volume containing 1X PCR buffer (New England Biolabs), 200 µM dNTPs, 0.2 µM of each primer, 200 ng template cDNA, and 2 U of Taq Polymerase (Taq DNA Polymerase with Standard Taq Buffer, New England Biolabs). Thermocycle program was one activation step of 94 C for 4 min followed by 37 cycles of 94 C for 1 min, 55 C for 1 min, and 72 C for 2 min. A final extension step of 72 C for 5 min was used. PCR product was visualized on 1% agarose gel and excised, extracted, sequenced, and analyzed as previously indicated.

The 479 bp amplicon of the GN population ALS gene containing the Trp_{574} location was cloned using Promega pGEM -T Easy Vector (Promega Corp., Fitchburg, WI) and JM109 competent cells (Promega Corp.). Plaques were propagated on media of LB broth, X-gal, IPTG, and ampicillin following protocol guidelines. Fifteen transgenic white plaques were selected. ALS transgenic amplicons were then sequenced and analyzed as previously described. The 479 bp amplicon of AU population was sequenced after PCR without cloning and used as a comparison.

Results and Discussion

ALS Herbicide Response Evaluation. Within each individual herbicide treatment, rate (high or low) was not significant ($P > 0.05$) for visible control and above ground biomass data (data not shown); therefore, data were pooled across rate within each herbicide treatments (Table 1). Contrast analysis comparing AU (suspected susceptible) and GN (suspected ALS resistant) at both visible control-rating dates and above ground biomass data showed highly significant ($P < 0.001$) values for all herbicides. No herbicide injured GN > 2% at either 14 or 28 DAT. Further, all herbicides, except bispyribac, reduced GN above ground biomass < 15% at 28 DAT. All herbicides injured AU 35 to 58% at 14 DAT. However, all herbicides except bispyribac injured and reduced biomass of AU > 90% at 28 DAT relative to nontreated AU. Bispyribac was the outlier among the herbicides in that it only injured AU 47% and reduced AU above ground biomass 58% relative to nontreated AU. Further, while bispyribac did not injure GN visibly, it did reduce above ground biomass by 22%. Bispyribac is normally applied multiple times in turfgrass since single applications are only marginally effective (Lycan and Hart 2006; McElroy et al. 2011).

These data indicate that GN is less susceptible to the ALS-inhibiting herbicides, trifloxysulfuron, foramsulfuron, bispyribac, and imazaquin compared to the susceptible AU (Table 1). These results confirmed field observations of suspected ALS-inhibiting herbicide resistant annual bluegrass and served as the basis for further investigation.

ALS Gene Sequencing. Our initial sequencing resulted in a 1.5-kb amplicon of the ALS gene coding from amino acid 150 to 650 (complete sequence not shown). Five amplicons of AU and GN resulted in similar sequences within each population, which met our criteria for adequate replication. The amplicon contained highly cited mutations sites of concern except nucleotides coding for Ala_{122} and Ser_{653}. Substitutions at Ala_{122} and Ser_{653} are known to cause a high level of resistance to imidazolinone but little resistance to sulfonylureas (Powles and Yu 2010), except for Ala_{122} to Tyr, which confers resistance to imidazolinone and sulfonylurea (Han et al. 2012). From this sequence, we observed a double peak at the

https://doi.org/10.1614/WS-D-12-00068.1 Published online by Cambridge University Press

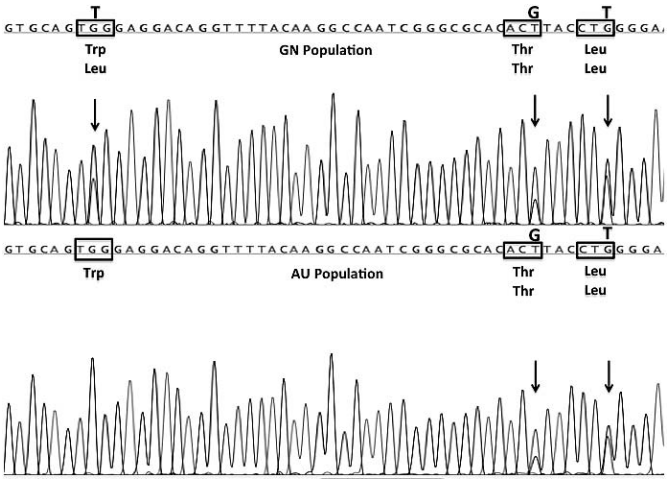

Figure 1. Chromatogram of nucleotide sequence surrounding TGG codon encoding Trp_{574}. The presence of a double peak at this loci possibly indicates a heterozygous amino acid substitution of TTG coding for Leu. Other potential points of heterozygosity are indicated with arrows.

Trp_{574} codon with the middle nucleotide predicted as T or G (Figure 1; complete sequence not shown). Trp_{574} is transcribed from TGG and is a non-ALS resistant amino acid. Leu_{574} is transcribed from TTG and is a known ALS resistant amino acid substitution. Patzoldt and Tranel (2002) observed an identical double-peak chromatogram, which was confirmed to be a heterozygous nucleotide substitution of G to T resulting in a Trp_{574} to Leu amino acid substitution that conferred ALS-inhibiting herbicide resistance in giant ragweed. Additional potentially double peaks surrounding the Trp_{574} loci were observed in the chromatogram that warranted further investigation.

Additional double peaks were observed for both AU and GN in the 1.5-kb amplicon (Figure 1; full sequence not shown). Inspection of these double peaks revealed only one difference : AU did not contain a double peak at Trp_{574}. GN contained a double peak at Trp_{574} suggesting two forms of the ALS gene. Thus, primers were utilized to amplify the Trp_{574} gene region to further investigate potential variants.

Cloning of Trp_{574} Gene Region. In order to investigate different forms of ALS in annual bluegrass, PCR was utilized to produce a 479-bp amplicon surrounding the Trp_{574} codon. As described previously, the amplicon was cloned and 15 separate plaques were sequenced. Alignment and elimination of dubious sequences at fragment ends yielded a final 416-bp product. The cloned sequences were aligned and compared to AU. Cloning of the amplicon resulted in the sequencing of two distinct nucleotide sequences surrounding the Trp_{574} mutation (Figure 2). Nine clones contained codons TGG coding for Trp_{574} amino acid, and six clones contained codon TTG coding for Leu_{574}. Comparing the two cloned sequence types, nine amino acid substitutions were observed (listed as Trp_{574} wild type or Leu_{574} mutant) T to C at 1698, T to G at 1710, G to T at 1760, T to G at 1797, T to G at 1803, C to T at 1854, G to A at 1857, G to A at 1893, and A to C at 1919 (nucleotides and subsequent amino acids numbered according to *Arabidopsis*). Transcription and alignment resulted in the identification of seven silent and two missense amino acid substitutions (Figures 2 and 3). T to C at 1698, T to G at 1710, T to G at 1797, T to G at 1803, G to T at

```
Trp574 Wild Type     ----------GGAGCAATGGGGTTTGGGTTGCCAGCTGCAGCTGGTGCTGCTGTGGCCAAC
Leu574 Mutant        ----------GGAGCAATGGGGTTTGGGTTGCCAGCTGCAGCTGGTGCTGCTGTGGCCAAC
AU Population        GCTGGTCTGGGAGCAATGGGGTTTGGGTTGCCAGCTGCAGCTGGTGCTGCTGTGGCCAAC
Bromus tectorum      GCTGGTTTGGGGGCAATGGGATTTGGCTTGCCAGCTGCAGCTGGTGCTTCTGTGGCCAAC
Arabidopsis     1561 GGAGGCCTTGGAGCTATGGGATTTGGACTTCCTGCTGCGATTGGAGCGTCTGTTGCTAAC 1620

Trp574 Wild Type     CCAGGTGTCACAGTTGTTGACATTGATGGAGATGGTAGCTTCCTCATGAATATTCAGGAG
Leu574 Mutant        CCAGGTGTCACAGTTGTTGACATTGATGGAGATGGTAGCTTCCTCATGAATATTCAGGAG
AU Population        CCAGGTGTCACAGTTGTTGACATTGATGGAGATGGTAGCTTCCTCATGAATATTCAGGAG
Bromus tectorum      CCAGGTGTTACAGTTGTTGACATTGATGGGGATGGTAGCTTCCTCATGAACATTCAGGAG
Arabidopsis     1621 CCTGATGCGATAGTTGTGGATATTGACGGAGATGGAAGCTTTATAATGAATGTGCAAGAG 1680

                                  I           P
Trp574 Wild Type     TTGGCACTGATTCGTATTGAGAACCTCCCTGTTAAGGTGATGATACTGAACAACCAACAT
                                  I           P
Leu574 Mutant        TTGGCACTGATTCGCATCGAGAACCTCCCGGTTAAGGTGATGATACTGAACAACCAACAT
AU Population        TTGGCACTGATTCGCATTGAGAACCTCCCTGTTAAGGTGATGATACTGAACAACCAACAT
Bromus tectorum      TTGGCGTTGATTCGTATTGAGAACCTTCCAGTGAAGGTGATGATATTGAACAACCAACAT
Arabidopsis     1681 CTAGCCACTATTCGTGTAGAGAATCTTCCAGTGAAGGTACTTTTATTAAACAACCAGCAT 1740

                                        W                                   T
Trp574 Wild Type     CTGGGAATGGTGGTGCAGTGGGAGGACAGGTTTTACAAGGCCAATCGGGCGCACACTTAC
                                        L                                   T
Leu574 Mutant        CTGGGAATGGTGGTGCAGTTGGAGGACAGGTTTTACAAGGCCAATCGGGCGCACACGTAC
AU Population        CTGGGAATGGTGGTGCAGTGGGAGGACAGGTTTTACAAGGCCAATCGGGCGCACACTTAC
Bromus tectorum      CTGGGAATGGTGGTGCAATGGGAGGACAGGTTTTACAAGGCCAATCGGGCACACACCTAC
Arabidopsis     1741 CTTGGCATGGTTATGCAATGGGAAGATCGGTTCTACAAAGCTAACCGAGCTCACACATTT 1800

                      L                                                 A  K
Trp574 Wild Type     CTTGGGAACCCAGAAAATGAGAGTGAGATATATCCAGATTTTGTGACGATTGCCAAGGGG
                      L                                                 A  K
Leu574 Mutant        CTGGGGAACCCAGAAAATGAGAGTGAGATATATCCAGATTTTGTGACGATTGCTAAAGGG
AU Population        CTGGGGAACCCAGAAAATGAGAGTGAGATATATCCAGATTTTGTGACGATTGCCAAGGGG
Bromus tectorum      CTTGGCAACCCAGAAAACGAGAGTGAGATATATCCAGATTTTGTGACGATTGCTAAAGGA
Arabidopsis     1801 CTCGGGGATCCGGCTCAGGAGGACGAGATATTCCCGAACATGTTGCTGTTTGCAGCAGCT 1860

                                              K                   K
Trp574 Wild Type     TTCAATGTTCCTGCTGTTCGTGTGACAAAGAAGAGTGAAGTCCGTGCAGCAATCAAGAAG
                                              K                   L
Leu574 Mutant        TTCAATGTTCCTGCAGTTCGTGTGACAAAGAAAAGTGAAGTCCGTGCAGCAATCAAGACG
AU Population        TTCAATGTTCCTGCTGTTCGTGTGACAAAGAAAAGTGAAGTCCGTGCAGCAATCAAGAAG
Bromus tectorum      TTCAACGTTCCCGCAGTTCGTGTGACAAAGAAGAGTGAAGTACGTGCAGCAATCCAGAAG
Arabidopsis     1861 TGCGGGATTCCAGCGGCGAGGGTGACAAAGAAAGCAGATCTCCGAGAAGCTATTCAGACA 1920


Trp574 Wild Type     ATGCTTGAGACTCCAGGGCCATACTTGTTGGATATCATCGTCCCTCACCAGGAGCATGTG
Leu574 Mutant        ATGCTTGAGACTCCAGGGCCATACTTGTTGGATATCATCGTCCCTCACCAGGAGCATGTG
AU Population        ATGCTTGAGACTCCAGGGCCATACTTGTTGGATATCATCGTCCCTCACCAGGAGAGGGCC
Bromus tectorum      ATGCTTGACACCCCAGGGCCGTACTTGCTGGATATCATTGTCCCGCATCAGGAGCACGTA
Arabidopsis     1921 ATGCTGGATACACCAGGACCTTACCTGTTGGATGTGATTTGTCCGCACCAAGAACATGTG 1980

Trp574 Wild Type     CTGCCTATAATCAC
Leu574 Mutant        CTGCCTATAATCAC
AU Population        TTGCCAAT------
Bromus tectorum      CTGCCTATGATCCC
Arabidopsis     1981 TTGCCGATGATCCC
```

Figure 2. Nucleotide sequence of Grand National Golf Course in Opelika, AL (GN population) alleles and Auburn University (AU) population surrounding the Trp_{574} to Leu substitution. Sequences were aligned with *Bromus tectorum* L. (National Center for Biotechnology Information [NCBI] accession number AF488771.1) and *Arabidopsis thaliana* (NCBI accession number NM_114714). Silent and missense codons for GN Trp_{574} wild type and GN Leu_{574} mutant are underlined. Amino acids are presented above GN codons. Heterozygous double peaks in the AU sequence are underlined.

1854, G to A at 1857, and C to A at 1893 resulted in silent mutations at Ile_{553}, Pro_{557}, Thr_{586}, Leu_{588}, Ala_{605}, Lys_{606}, and Lys_{616}, respectively. G to T at 1760 and A to C at 1919 resulted in missense mutations of Trp_{574} to Leu and Lys_{627} to Thr. All GN silent mutations and the missense Lys_{627} to Thr mutation were present as double peaks in the AU sequence indicating these nucleotide polymorphisms are also present in the susceptible AU population. The only observed difference was a missense mutation resulting in an amino acid substitution at Trp_{574} to Leu in GN and not AU.

Trp_{574} amino acid substitution is the result of a common missense mutation conferring resistance to ALS-inhibiting herbicides (Patzoldt and Tranel 2002; Powles and Yu 2010; Tranel and Wright 2002; Yu et al. 2008). This is the first report of annual bluegrass resistance to ALS-inhibiting herbicides and the first report of the specific mutation conferring resistance. This research presents evidence of two ALS gene sequences that are distinct with respect to missense and silent mutations. Annual bluegrass is an allotetraploid derived from suspected progenitors of *P. supina* and *P. infirma* (Tutin 1957). In our research, we elected to utilize cDNA in order to sequence transcripts responsible for producing the

https://doi.org/10.1614/WS-D-12-00068.1 Published online by Cambridge University Press

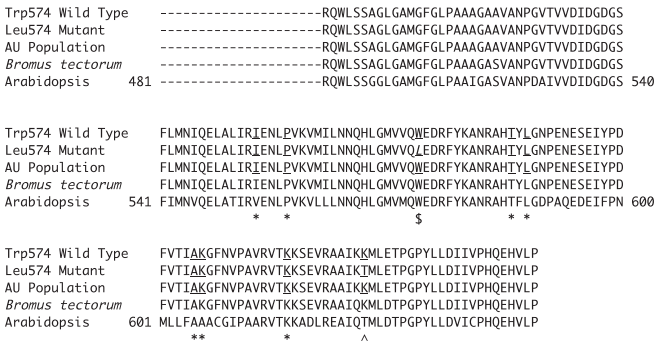

Figure 3. Amino acid sequence of Grand National Golf Course in Opelika, AL (GN population) alleles and Auburn University (AU) population surrounding the Trp_{574} to Leu substitution. Transcribed amino acid sequences were aligned with *Bromus tectorum* (National Center for Biotechnology Information [NCBI] accession number AF488771.1) and *Arabidopsis thaliana* (NCBI accession number AEE78430). "*" denotes silent mutations. "$" denotes the missense Trp_{574} to Leu substitution present only in GN. "^" denotes the missense Lys_{627} to Thr substitution present in both GN and AU.

ALS enzyme. It is plausible that ALS mRNA is derived from the separate progenitor genomes and that other ancestral ALS copies lie dormant within the genome. Such has been demonstrated within the ancestral genome of the allotetraploid *B. napus*, which expresses five ALS genes correlated with its diploid progenitor species *B. campestris* or *B. oleracea* (Ouellet et al. 1992). Regardless of their genetic origin, these distinct ALS isoforms are the most likely explanation for the observed resistance to ALS-inhibiting herbicides.

ALS-inhibiting herbicides can thus be added to the list of modes of action to which annual bluegrass is resistant. Chemical control options to which annual bluegrass is not resistant are limited. Other herbicides and their respective mechanism of action that are registered for use in turfgrass include: indaziflam (cellulose biosynthesis inhibitor), glufosinate (glutamine synthetase inhibitor), paclobutrazol (plant growth regulator inhibiting gibberellin biosynthesis), and oxadiazon (protox inhibitor). Given the propensity of annual bluegrass to adapt to herbicide treatments, it would benefit researchers and turfgrass managers to consider nonchemical annual bluegrass control options. However, little research has been conducted on nonchemical annual bluegrass control and such control practices are virtually nonexistent (Busey 2003).

Acknowledgments

The authors wish to thank Dr. David Weaver, Department of Agronomy and Soils, and Dr. Mark Liles, Department of Biological Sciences, both of Auburn University for their thoughts and ideas on the development of this project.

Literature Cited

Battaglin, W. A., E. T. Furlong, M. R. Burkhardt, and C. J. Peter. 2000. Occurrence of sulfonylurea, sulfonamide, imidazolinone, and other herbicides in rivers, reservoirs and ground water in the Midwestern United States, 1998. Sci. Total Environ. 248:123–133.

Beam, J. B. 2004. Prohexadione calcium for turfgrass management and Poa annua control and molecular assessment of the acetolactate synthase gene in *Poa annua*. Ph.D dissertation. Blacksburg, VA: Virginia Polytechnical Institute and State University. 85 p.

Beard, J. B., P. E. Rieke, A. J. Turgeon, and J. M. Vargas. 1978. Annual bluegrass (*Poa annua* L.) description, adaptation, culture and control. Res. Rep. 352. East Lansing, MI: Michigan State Univ. Agric. Exp. Stat. 32 p.

Brosnan, J. T., G. K. Breeden, and T. C. Mueller. 2012. A glyphosate-resistant biotype of annual bluegrass in Tennessee. Weed Sci. 60:97–100.

Busey, P. 2003. Cultural management of weeds in turfgrass: a review. Crop Sci. 43:1899–1911.

Chipman, D., Z. Barak, and J. V. Schloss. 1998. Biosynthesis of 2-aceto-2-hydroxy acids: acetolactate synthases and acetohydroxyacid synthases. Biochimica et Biophysica Acta 1385:401–419.

Christians, N. 2006. Control options: what's next for *Poa annua* control? Grounds Mainten. 41:28–30.

Corbett, C. L. and F. J. Tardif. 2006. Detection of resistance to acetolactate synthase inhibitors in weeds with emphasis on DNA-based techniques: a review. Pest Manag. Sci. 62:584–597.

Greene, S. W. and D.W.H. Walton. 1975. An annotated check list of the sub-Antarctic and Antartic vascular flora. Polar Rec. 17:473–484.

Han, H., Q. Yu, E. Purba, M. Li, M. Walsh, S. Friesen, and S. B. Powles. 2012. A novel amino acid substitution Ala-122-Tyr in ALS confers high-level and broad resistance across ALS-inhibiting herbicides. Pest Manag. Sci. 68:1164–1170.

Heap, I. 2012. The International Survey of Herbicide Resistant Weeds. http://www.weedscience.com. Accessed: March 20, 2012.

Kelly, S. T., E. Coats, and D. S. Luthe. 1999. Mode of resistance of triazine-resistant annual bluegrass (*Poa annua*). Weed Technol. 13:747–752.

La Mantia, J. M. and D. R. Huff. 2011. Instability of the greens-type phenotype in *Poa annua* L. Crop Sci. 51:1784–1792.

Lycan, D. W. and S. E. Hart. 2006. Seasonal effects on annual bluegrass (*Poa annua*) control in creeping bentgrass with bispyribac-sodium. Weed Technol. 20:722–727.

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

Mazur, B. J., C. F. Chui, and J. K. Smith. 1987. Isolation and characterization of plant genes coding for acetolactate synthase, the target enzyme for two classes of herbicides. Plant Physiol. 85:1110–1117.

McElroy, J. S., G. K. Breeden, and G. Wehtje. 2011. Evaluation of annual bluegrass control programs for bermudagrass turf overseeded with perennial ryegrass. Weed Technol. 25:58–63.

McElroy, J. S., R. H. Walker, and E. van Santen. 2002. Patterns of variation in *Poa annua* populations as revealed by canonical discriminant analysis of life history traits. Crop Sci. 42:513–517.

Olech, M. 1996. Human impact on terrestrial ecosystems in West Antarctica. Proc. NIPR Symp. Polar Biol. 9:299–306.

Ouellet, T., R. G. Rutledge, and B. L. Miki. 1992. Members of the acetohydroxyacid synthase multigene family of *Brassica napus* have divergent patterns of expression. Plant J. 2:321–330.

Park, K. W. and C. A. Mallory-Smith. 2004. Physiological and molecular basis for ALS inhibitor resistance in *Bromus tectorum* biotypes. Weed Res. 44:71–77.

Patzoldt, W. L. and P. J. Tranel. 2002. Molecular analysis of chloransulam resistance in a population of giant ragweed. Weed Sci. 50:299–305.

Powles, S. B. and Q. Yu. 2010. Evolution in action: Plants resistant to herbicides. Annu. Rev. Plant Biol. 61:317–347.

Rodriguez, I. R., L. B. McCarty, and J. K. Higginbottom. 2001. Postemergence annual bluegrass control in dormant common bermudagrass. Intl. Turfgrass Soc. 9:1041–1044.

Scarabel, L., N. Carraro, M. Sattin, and S. Varotto. 2004. Molecular basis and genetic characterisation of evolved resistance to ALS-inhibitors in *Papaver rhoeas*. Plant Sci. 166:703–709.

Senseman, S. A. 2007. Herbicide Handbook, 9th ed. Lawrence, KS: Weed Sci. Soc. Amer. 458 p.

Toler, J. E., T. G. Willis, A. G. Estes, and L. B. McCarty. 2007. Postemergence annual bluegrass control in dormant nonoverseeded bermudagrass turf. HortSci. 42:670–672.

Tranel, P. J. and T. R. Wright. 2002. Resistance of weeds to ALS-inhibiting herbicides: what have we learned? Weed Sci. 50:700–712.

Tutin, T. G. 1957. A contribution to the experimental taxonomy of *Poa annua* L. Watsonia 4:1–10.

Walton, D.W.H. and R.I.L. Smith. 1973. Status of the alien vascular flora of South Georgia., 36:79–97.

Webster, T. M. 2004. Weed survey: southern states. Grass crop subsection. Proc. South. Weed Sci. Soc. 57:404–426.

Whaley, C. M., H. P. Wilson, and J. H. Westwood. 2007. A new mutation in plant ALS confers resistance to five classes of ALS-inhibiting herbicides. Weed Sci. 55:83–90.

Yu, Q., H. Han, and S. B. Powles. 2008. Mutations of the ALS gene endowing resistance to ALS-inhibiting herbicides in *Lolium rigidum* populations. Pest Manag. Sci. 64:1229–1236.

Received May 4, 2012, and approved July 4, 2012.