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Detecting Annual Bluegrass (*Poa annua*) Resistance to ALS-Inhibiting Herbicides Using a Rapid Diagnostic Assay

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Annual bluegrass is the most problematic winter annual weed in managed turfgrass. Acetolactate synthase (ALS)-inhibiting herbicides are effective for annual bluegrass control, but reliance on this mode of action can select for herbicide-resistant biotypes. Two annual bluegrass biotypes not controlled with ALS-inhibiting herbicides were reported at golf courses in South Carolina and Georgia. Research was initiated at Clemson University to verify the level of resistance of these biotypes to ALS inhibitors. Two ALS-susceptible (S) and suspected resistant (SCr, GAr) annual bluegrass biotypes were established in a greenhouse. Dose-response experiments were conducted on mature annual bluegrass plants using trifloxysulfuron, foramsulfuron, and bispyribac-sodium, all ALS-inhibiting herbicides. Additionally, a rapid diagnostic ALS activity assay was optimized and conducted using the same herbicides. For dose-response experiments, the rate of herbicide that reduced shoot biomass 50% (I_{50}) values for the S biotypes were 13.6 g ai ha^{-1} for trifloxysulfuron, 7.0 g ai ha^{-1} for foramsulfuron, and 38.3 g ai ha^{-1} for bispyribac-sodium. Fifty percent shoot biomass reduction was not observed in either the SCr or GAr biotypes at eight times the labeled field rate of all ALS-inhibiting herbicides tested. For in vivo tests of ALS activity, the SCr biotype yielded I_{50} (concentration of herbicide that reduced ALS activity 50%) values 3,650, 3,290, and 13 times the S biotypes following treatment with trifloxysulfuron, foramsulfuron, and bispyribac-sodium, respectively. Similarly, I_{50} values for the GAr biotype were 316, 140, and 64 times greater than the S biotypes following the same herbicide treatments. This research indicates high levels of annual bluegrass resistance to multiple ALS-inhibiting herbicides in South Carolina and Georgia. Future research should focus on the mechanisms of ALS resistance in these annual bluegrass biotypes as well as alternative options for control not targeting the ALS enzyme.

Nomenclature: Bispyribac-sodium; foramsulfuron; trifloxysulfuron; annual bluegrass, *Poa annua* L.
Key words: Bermudagrass, *Cynodon dactylon* (L.) Pers., golf course, herbicide resistance, turfgrass.

Annual bluegrass is the most problematic winter annual weed in managed turfgrass (Beard et al. 1978; McCarty 2011). Its prolific seedhead production regardless of mowing height, clumping growth habit, and lack of tolerance to stress reduces turfgrass aesthetic quality and playability on golf courses (Beard et al. 1978; McCarty 2011). In attempts for annual bluegrass control, herbicides, plant growth regulators, and cultural practices have all been used. However, annual bluegrass control has been inconsistent, likely due to the vast genetic diversity present in the annual bluegrass species (Beard 1970; Lush 1989; Mao and Huff 2012).

Proper herbicide selection and use is an integral component of control programs not only for annual bluegrass, but for all weeds that invade a desirable turfgrass stand (Tranel and Wright 2002). However, continuous use of the same mode of herbicidal action can select for herbicide-resistant weed biotypes (Yu et al. 2010). Cases of evolved resistance to triazines, dinitroanilines, glyphosate, and most recently, acetolactate synthase (ALS) inhibitors, have all been documented in annual bluegrass (Brosnan et al. 2012; Isgrigg et al. 2002; Kelly et al. 1999; McElroy et al. 2013).

ALS is the first enzyme common to the branched-chain amino acid biosynthetic pathway where leucine, isoleucine, and valine are produced (Umbarger 1978). ALS-inhibiting herbicide applications result in plant starvation of these essential amino acids, the primary cause for plant death (Tranel and Wright 2002). However, 2-ketobutyrate accumulation, as well as protein synthesis and photosynthate transport disruption, may contribute to toxicity of ALS inhibition (Shaner 1991; Tranel and Wright 2002). Among many other uses in turfgrass, ALS-inhibiting herbicides, especially the sulfonylureas, are very effective for control of annual bluegrass in bermudagrass [*Cynodon dactylon* (L.) Pers.]. Toler et al. (2007) reported greater than 95% annual bluegrass control with four sulfonylurea herbicides applied in February. However, sulfonylurea herbicides are highly susceptible to the development of herbicide-resistant weed species (Tranel and Wright 2002). The first case of resistance to ALS-inhibiting herbicides developed following chlorsulfuron applications for control of prickly lettuce (*Lactuca serriola* L.) and kochia (*Kochia scoparia* [L.] Schrad.) only 5 yr after its commercial introduction (Mallory-Smith et al. 1990; Primiani et al. 1990). Currently, there are 129 weed species resistant to ALS inhibitors worldwide, more than to any other herbicidal mode of action (Heap 2013).

Rapid development of resistance to ALS-inhibiting herbicides can be attributed not only to continuous herbicide use without alternating mode of action, but also to characteristics of ALS and ALS genes themselves. The gene encoding ALS is nuclear, thus, resistance can be transmitted through pollen or seed (Tranel and Wright 2002). In addition, the resistant ALS allele is dominant over the susceptible ALS allele, conferring resistance in the heterozygous condition (Tranel and Wright 2002). Resistance to ALS inhibitors in plants is most commonly the result of target-site nucleotide mutations in the ALS gene that lead to changes in the protein amino acid sequence. Substitutions at six positions on the ALS enzyme are frequently associated with varying degrees of resistance to ALS-inhibiting herbicides in numerous plant species (McElroy et al. 2013; Tranel and Wright 2002). However, many other mutations in ALS genes do not adversely affect activity of the protein (Tranel and Wright 2002).

In areas where warm-season grasses are not winter-dormant, use of more economical nonselective herbicides for annual bluegrass control is ruled out, and ALS-inhibiting herbicides are effective alternatives (McElroy et al. 2011; Toler et al.

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2007). However, there are reports of poor control of annual bluegrass with ALS-inhibiting herbicides after repetitive usage on golf courses in South Carolina and Georgia. Few herbicides would be able to selectively control annual bluegrass in bermudagrass should ALS-inhibiting herbicides not be effective due to resistance development. Therefore, the objective of this research was to verify and quantify the level of resistance to ALS-inhibiting herbicides in two biotypes of annual bluegrass using both whole-plant dose-response experiments and a rapid in vivo diagnostic ALS activity assay.

Materials and Methods

Plants of suspected resistant annual bluegrass biotypes were collected from bermudagrass golf course fairways at Calawassie Island Golf Club in Okatie, SC (SCr), and The Ford Plantation in Richmond Hill, GA (GAr), in 2010 using a golf course cup cutter. These areas had been treated with ALS-inhibiting herbicides for at least eight consecutive years (B. Bagwell, N. Caron, personal communication). Additional annual bluegrass plants were obtained from a site where no known previous herbicide applications had been made (Clemson, SC [Scu]) and annual bluegrass seeds were obtained from a commercial source (Valley Seed Service, Fresno, CA [Svs]). These biotypes were susceptible to ALS-inhibiting herbicides and included for comparison. Plants were potted in a commercial potting mix (Fafard 3B Mix, Conrad Fafard Inc., Agawam, MA) and grown to seed in an isolated greenhouse to prevent cross-pollination. Mature seed was harvested from all populations and stored in a −20 C freezer until further tests were conducted.

Whole-Plant Dose-Response. Annual bluegrass seeds were sown in 3.8 × 21–cm polypropylene containers filled with a mixture of sand and peat (85 : 15 by volume). Plants were grown under greenhouse conditions to tillering stage (> 3 tillers $plant^{-1}$) and treated with three ALS-inhibiting herbicides: trifloxysulfuron (Syngenta Crop Protection Inc., Greensboro, NC) or foramsulfuron (Bayer CropScience, Research Triangle Park, NC) at 0, 3.5, 7, 14, 28, 56, 112 and 224 g ai ha^{-1} or bispyribac-sodium (Valent U.S.A. Corporation, Walnut Creek, CA) at 0, 9.3, 18.5, 37, 74, 148, 296, and 592 g ai ha^{-1}. Labeled rates for trifloxysulfuron and foramsulfuron are 28 g ai ha^{-1} compared to 74 g ai ha^{-1} for bispyribac-sodium (Anonymous 2007, 2009, 2010). Each treatment included a nonionic surfactant (Induce, Helena Chemical Company, Collierville, TN) at 0.25% v/v. Each container consisted of a single annual bluegrass plant. Herbicides were applied using an enclosed spray chamber (DeVries Manufacturing, Hollandale, MN) calibrated to deliver 374 L ha^{-1} through an 8001E flat fan nozzle (Tee Jet Spraying Systems Co., Wheaton, IL).

After herbicide application, plants were maintained in a greenhouse under an approximately 14-h photoperiod of natural light (average intensity 700 μmol m^{-2} s^{-1}) with day/night temperatures maintained near 29/18 C. Twenty-one days after treatment, surviving plants were removed from containers and washed free of soil. Above- and belowground biomass was separated, dried at 80 C for 72 h, and weighed.

In vivo ALS Activity Assay. Annual bluegrass seeds were sown in a commercial potting mix and grown to maturity (> 10 tillers) in a greenhouse growth room with a 12-h photoperiod under 400 μmol m^{-2} s^{-1} and day/night temperatures of 24/15 C. Plants were watered every 48 to 72 h to prevent soil desiccation and fertilized monthly with 4.9 g N m^{-2}.

Determination of ALS activity was conducted according to procedures of Gerwick et al. (1993) with some modifications. Tissue from the apical portion of annual bluegrass leaves (300 mg) was removed from a plant, weighed, and transferred to a 100-ml petri plate for incubation (Gerwick et al. 1993). The incubation solution consisted of 5 ml of 25% Murashige and Skoog salt media (Sigma Aldrich Co., St. Louis, MO), 500 μM 1,1-cyclopropanedicarboxylic acid (CPCA, Sigma Aldrich), 50 mM L-alanine (Sigma Aldrich), and 0.1% v/v nonionic surfactant (Triton X-100, Sigma Aldrich). Trifloxysulfuron, foramsulfuron, or bispyribac-sodium (commercial formulations) were added to the incubation solution at 0, 0.001, 0.01, 0.1, 1, 10, 100, and 1,000 μM concentrations. An incubation solution containing neither CPCA nor ALS-inhibiting herbicides was used as the reference absorbance. To quantify ALS activity in relation to herbicides, the untreated control was a solution which contained CPCA but no ALS-inhibiting herbicide. Incubations were conducted in a growth chamber for 16 to 20 h under 350 μmol m^{-2} s^{-1} of constant light and ambient atmospheric conditions (Uchino et al. 1999). After removal from the growth chamber, plates were wrapped with Parafilm (Bemis Company, Inc., Neenah, WI) and stored in a freezer at −80 C until analyzed.

For analysis, contents of plates were transferred to 15-ml culture tubes and acidified using H_2SO_4 (Sigma Aldrich) to a final concentration of 0.5% (25 μl). Tubes were placed in a water bath and heated to 60 C for 30 min. Acetoin was quantified according to the procedure described by Westerfeld (1945) with modifications (Gerwick et al. 1993; Kuk et al. 2003). A 2-ml aliquot was taken from each sample and transferred into a clean culture tube. A reagent consisting of 1-naphthol (Sigma Aldrich) and creatine monohydrate (Sigma Aldrich) dissolved in 2.5 N NaOH (Sigma Aldrich) was added to each sample for a final concentration of 20 mg ml^{-1} 1-naphthol and 2 mg ml^{-1} creatine monohydrate. Tubes were heated to 37 C in a water bath for 30 min for color development. Samples were measured in a spectrophotometer at 530 nm, and a standard curve was produced to quantify acetoin. A high concentration of acetoin results in a red/pink color upon addition of the reagent to the solution. Thus, solutions with a high absorbance (red/pink color) in the presence of ALS-inhibiting herbicides indicate a resistant annual bluegrass biotype.

Statistical Design and Analysis. The experimental design for both whole-plant and in vivo studies was a completely randomized design with three replications. Treatments consisted of the factorial combination of three ALS-inhibiting herbicides (trifloxysulfuron, foramsulfuron, and bispyribac-sodium), four annual bluegrass biotypes (SCr, GAr, Scu, Svs), and eight herbicide rates or concentrations. Two replications of the whole-plant dose-response and ALS activity assay were conducted with data from each subjected to ANOVA. No treatment by experimental-run interactions were detected for either study; therefore, data from both experimental runs were combined.

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Table 1. I_{50}[a] values and resistance factors for whole-plant dose-response and in vivo ALS enzyme activity assays as affected by annual bluegrass biotype and ALS-inhibiting herbicide.

		Whole-plant dose-response		In vivo ALS activity	
Biotype[b]	Herbicide	I_{50}	RF[c]	I_{50}	RF
		g ai ha^{-1}		μM	
S	Trifloxysulfuron	13.57	—	0.003	—
	Foramsulfuron	7.04	—	0.004	—
	Bispyribac-sodium	38.29	—	0.009	—
SCr	Trifloxysulfuron	> 224*	> 17	10.95*	3,650
	Foramsulfuron	> 224*	> 32	13.16*	3,290
	Bispyribac-sodium	> 592*	> 15	0.122*	13
GAr	Trifloxysulfuron	> 224*	> 17	0.948*	316
	Foramsulfuron	> 224*	> 32	0.559*	140
	Bispyribac-sodium	> 592*	> 15	0.574*	64

[a] Abbreviations: I_{50}, the rate of herbicide that reduced shoot biomass 50% for whole-plant experiments or the concentration of herbicide that reduced ALS activity 50% for in vivo experiments; ALS, acetolactate synthase; S, susceptible biotypes; SCr, resistant biotypes from South Carolina; GAr, resistant biotypes from Georgia; RF, resistance factors.

[b] S biotypes were obtained from a commercial source (Fresno, CA) and a wild-type population where no previous herbicide applications had been made (Clemson, SC) and pooled for comparison to resistant biotypes from South Carolina (SCr) and Georgia (GAr).

[c] RF is a ratio comparing I_{50} values of resistant to susceptible annual bluegrass biotypes quantifying the level of resistance to an individual ALS-inhibiting herbicide.

* Indicates significantly different than the S biotypes at $P < 0.05$ according to Student's *t* test.

Nonlinear regression analysis was used in the dose-response component of each study to estimate the rate of herbicide which reduced shoot biomass 50% or concentration of herbicide which reduced ALS activity 50% (I_{50}, as a percentage of untreated control). The formula used was

$$y = C + (D - C) / \left(1 + 10^{x\text{-}\log I_{50}}\right) \quad [1]$$

proposed by Seefeldt et al. (1995) where, for the dose-response study, y was shoot biomass, x was herbicide rate (g ai ha^{-1}), D was the upper bound of y, and C was the lower bound of y. For the in vivo ALS activity assay, y was ALS activity, x was the herbicide concentration (μM), D was the upper bound of y, and C was the lower bound of y. When ANOVA suggested a significant effect of biotype, I_{50} comparisons between susceptible and resistant annual bluegrass biotypes were conducted using Student's t test at $P < 0.05$. All ANOVA and t test calculations used JMP version 9.0 (SAS Institute Inc., Cary, NC) and nonlinear response modeling used Prism 5.0 (GraphPad Software, Inc., La Jolla, CA).

Results and Discussion

In both experiments, responses of each susceptible (Scu, Svs) annual bluegrass biotype to increasing doses of trifloxysulfuron, foramsulfuron, and bispyribac-sodium were not significantly different (data not shown). Thus, these biotypes were pooled and used for comparison to SCr and GAr plants and are further referred to as "S" biotypes.

Whole-Plant Dose-Response. I_{50} (mean ± SE) values for S biotypes were 13.57 ± 1.16 g ai ha^{-1} for trifloxysulfuron, 7.04 ± 0.57 g ai ha^{-1} for foramsulfuron, and 38.29 ± 4.85 g ai ha^{-1} for bispyribac-sodium (Table 1; Figure 1). All three herbicides were applied up to eight times labeled field rates, but 50% shoot biomass reductions were not achieved for the SCr and GAr biotypes. Thus, the I_{50} values for these biotypes were concluded to be greater than 224 g ai ha^{-1} for trifloxysulfuron and foramsulfuron and 592 g ai ha^{-1} for bispyribac-sodium, which indicate high levels of resistance.

McElroy et al. (2013) reported minimal activity of four ALS-inhibiting herbicides on an annual bluegrass population from Alabama. No visible injury and minimal (< 23%) decrease in above ground biomass were observed 28 d after treatment with trifloxysulfuron, foramsulfuron, bispyribac-sodium, and imazaquin at 32, 50, 300, and 980 g ai ha^{-1}, respectively. Similarly, sulfometuron-methyl provided little control of a rigid ryegrass (*Lolium rigidum* Gaud.) population at rates as high as 64 g ai ha^{-1} (Christopher et al. 1992). Even higher rates were required to achieve 50% aboveground biomass reduction in an accession of monochoria (*Monochoria vaginalis* [Burm. f.] Kunth), where the I_{50} for imazosulfuron was 1,586 g ai ha^{-1} (Kuk et al. 2003).

In vivo ALS Activity Assay. This in vivo ALS activity assay was previously optimized for resistance diagnosis in numerous weed species (Gerwick et al. 1993; Kuk et al. 2003; Uchino et al. 1999), but never for annual bluegrass. With cases of annual bluegrass resistance to ALS-inhibiting herbicides on the rise, the ability to confirm resistance with multiple methods would be beneficial.

Based on the dose-response analysis from the in vivo ALS activity assay, I_{50} (mean ± SE) values for ALS activity in S biotypes were 0.003 ± 0.0008, 0.004 ± 0.0009, and 0.009 ± 0.003 μM for trifloxysulfuron, foramsulfuron, and bispyribac-sodium, respectively (Table 1; Figure 2). Acetolactate accumulated in the SCr and GAr biotypes regardless of ALS-inhibiting herbicide or concentration. The SCr biotype exhibited I_{50} (mean ± SE) values for ALS activity of 10.95 ± 4.96 μM for trifloxysulfuron, 13.16 ± 6.2 μM for foramsulfuron, and 0.12 ± 0.06 μM for bispyribac-sodium. In comparison, the GAr biotype exhibited I_{50} (mean ± SE) values of 0.95 ± 0.39 μM for trifloxysulfuron, 0.56 ± 0.26 μM for foramsulfuron, and 0.57 ± 0.3 μM for bispyribac-sodium. Thus, resistance factors for the SCr biotype were 3,650 for trifloxysulfuron, 3,290 for foramsulfuron, and 13 for bispyribac-sodium (Table 1). For the GAr biotype, resistance factors were 316, 140, and 64 for trifloxysulfuron, foramsulfuron, and bispyribac-sodium, respectively. These were the herbicide concentrations required to reduce acetolactate accumulation (absorbance), and, in theory, in vivo ALS activity, by 50% of the untreated control. These values cannot be assumed to represent the true herbicide concentrations inhibiting ALS activity in vivo

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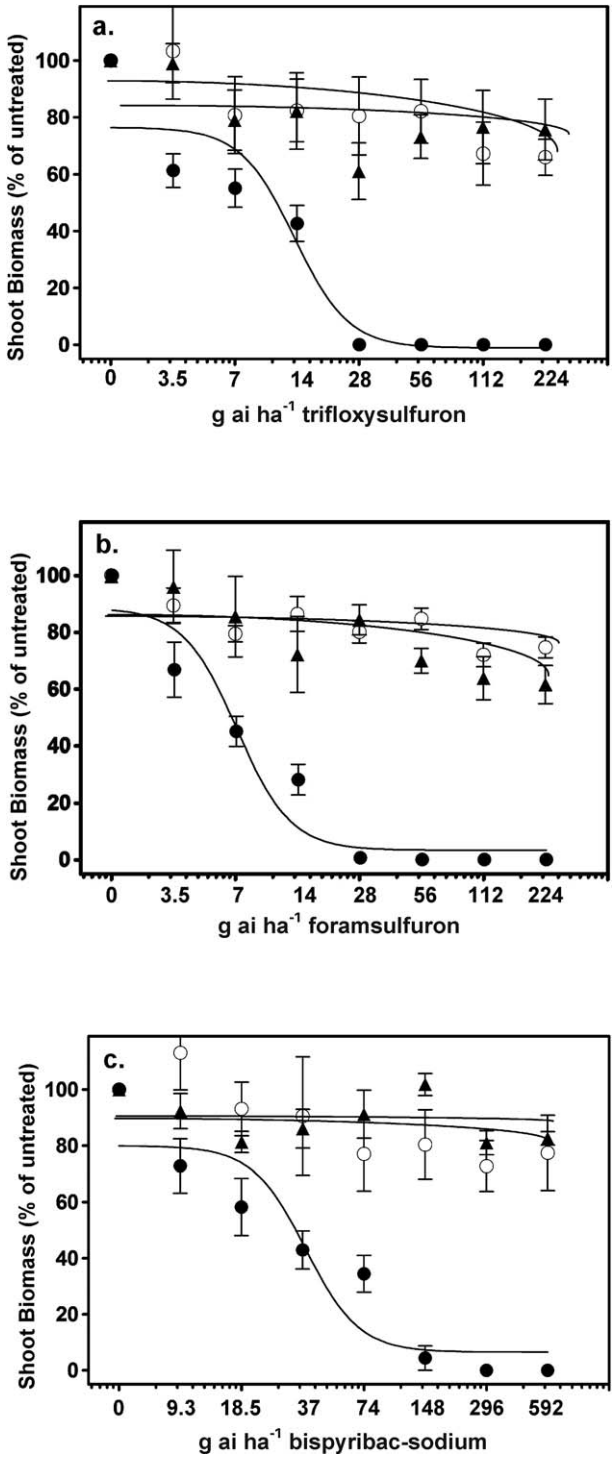

Figure 1. Effect of (a) trifloxysulfuron, (b) foramsulfuron, and (C) bispyribac-sodium on shoot biomass of susceptible (S, ●) and suspected resistant annual bluegrass biotypes from South Carolina (SCr, ○) and Georgia (GAr, ▲). Vertical bars represent standard errors of the mean. Shoot biomass values were calculated as a percentage of the untreated control and analyzed using the nonlinear regression equation $y = C + (D - C)/(1 + 10^{(x\text{-}\log I_{50})})$ where y was shoot biomass, x was herbicide rate (g ai ha^{-1}), D was the upper bound of y, C was the lower bound of y, and I_{50} was the rate of herbicide that reduced shoot biomass 50%. Estimates for equation parameters (C, D, I_{50}) and adjusted R^2 for each herbicide and the S biotype were as follows: trifloxysulfuron (−1.1, 76.5, 13.57, 0.80); foramsulfuron (3.3, 88.6, 7.04, 0.80); bispyribac-sodium (6.5, 80.0, 38.29, 0.65). Fifty percent shoot biomass inhibition was not achieved for the SCr and GAr biotypes, and thus, estimates for all equation parameters could not be obtained. However, I_{50} values for both biotypes are greater than 224 g ai ha^{-1} for trifloxysulfuron and foramsulfuron, and 592 g ai ha^{-1} for bispyribac-sodium.

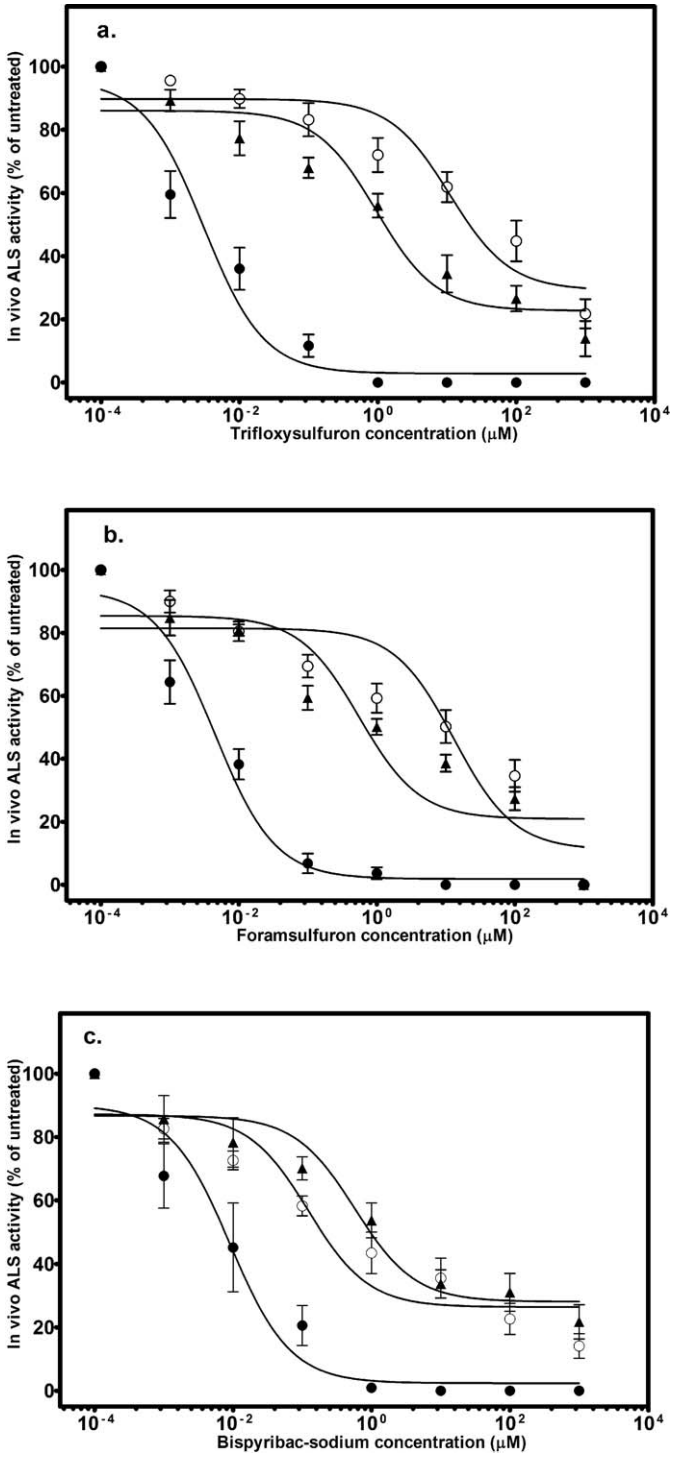

Figure 2. Effect of (a) trifloxysulfuron, (b) foramsulfuron, and (c) bispyribac-sodium concentration on in vivo acetolactate synthase (ALS) activity of susceptible (S, ●) and resistant annual bluegrass biotypes from South Carolina (SCr, ○) and Georgia (GAr, ▲). Vertical bars represent standard errors of the mean. In vivo ALS activity values were calculated as a percentage of the untreated control as determined by spectrophotometric analysis at 530 nm and analyzed using the nonlinear regression equation $y = C + (D - C)/(1 + 10^{(x\text{-}\log I_{50})})$ where y was shoot biomass, x was herbicide rate (g ai ha^{-1}), D was the upper bound of y, C was the lower bound of y, and I_{50} was the concentration of herbicide that reduced ALS activity50%. Estimates for equation parameters (C, D, I_{50}) and adjusted R^2 for each biotype and herbicide were as follows: trifloxysulfuron: S (2.9, 95.7, 0.003, 0.85), SCr (29.2, 89.8, 10.95, 0.79), GAr (22.8, 86.1, 0.95, 0.83); foramsulfuron: S (1.8, 93.9, 0.004, 0.89), SCr (11.1, 81.5, 13.16, 0.78), GAr (20.9, 85.4, 0.559, 0.80); bispyribac-sodium: S (2.4, 90.0, 0.009, 0.70), SCr (26.4, 87.2, 0.122, 0.80), GAr (28.1, 86.7, 0.574, 0.77).

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because nothing is known about this assay concerning herbicide uptake, translocation, and metabolism. However, the values do provide clear evidence that ALS enzymes for the SCr and GAr are still active in the presence of high concentrations of ALS-inhibiting herbicides.

Most cases of resistance to ALS-inhibiting herbicides in other weed species have been the result of ALS enzyme mutations because of numerous amino acid connections involved in binding ALS-inhibiting herbicides (Duggleby et al., 2008). A second mechanism of resistance to ALS inhibitors is enhanced metabolism and detoxification of the herbicide (Tranel and Wright 2002). This bioassay is an enzyme activity assay, and thus, limitations for determining resistance to ALS-inhibiting herbicides are possible. However, because living annual bluegrass tissue is used to conduct the assay, both target-site and non–target-site resistance could be detected. For example, if an ALS-inhibiting active ingredient did not reach the target site due to metabolism/detoxification, the ALS enzyme would remain active and results would indicate a resistant annual bluegrass plant, assuming the detoxification occurred in the leaf tissue being used for analysis. Further research, including in vitro ALS enzyme assays and ALS gene sequencing, would provide further insight for the detection of resistance using the in vivo assay.

Without further studies, a conclusion cannot be made concerning the mechanisms conferring resistance in the SCr and GAr annual bluegrass biotypes. However, high resistance factors indicate the possibility a nucleotide mutation in the ALS gene has led to an amino acid substitution conferring resistance to multiple ALS-inhibiting herbicides (Table 1). Substitutions at Trp_{574} in plants, the most commonly observed substitution conferring ALS resistance, results in high levels of resistance to multiple chemical classes of ALS inhibitors (Duggleby et al. 2008; Tranel and Wright 2002). In yeast, a substitution at Trp_{586} (equivalent to Trp_{574} residue in plants) results in resistance factors of approximately 10^4 to multiple sulfonylurea herbicides (Duggleby et al. 2003). Similarly, Yu et al. (2010) reported a high resistance factor ($>$ 1,333) for a rigid ryegrass population with a Trp_{574} substitution. Other amino acid substitutions on the ALS enzyme confer varying levels of resistance, and may differ in their resistant characteristics to other ALS-inhibiting chemical classes. For example, four rigid ryegrass populations with different substitutions at Pro_{197} resulted in resistance factors ranging from 95 to 241 for sulfometuron, but these plants were controlled with imidazolinone herbicides (Yu et al. 2010).

A Trp_{574} to Leu substitution was recently confirmed in an annual bluegrass population in Alabama and, like other weed species, appears to be the most likely target site mutation conferring resistance to ALS-inhibiting herbicides in annual bluegrass (McElroy et al. 2013). Within a biotype, variation in resistance factors is likely due to structural differences in the active ingredient molecules. Duggleby et al. (2008) noted significant variation in the potency of ALS inhibition within the sulfonylurea chemical class. The researchers surmised that this variability was from the numerous connections between herbicide molecules and the ALS enzyme as well as general fit of the molecule into the active site.

Results from the ALS enzyme activity assay, which indicated ALS activity in the presence of ALS-inhibiting herbicides in two annual bluegrass biotypes, support evidence from the whole-plant dose-response experiment. Thus, this rapid diagnostic procedure is useful for determining and quantifying annual bluegrass resistance to ALS-inhibiting herbicides. Results can be obtained in as quickly as 72 h, providing an additional method which can be used in confirming annual bluegrass resistance to ALS-inhibiting herbicides.

Herbicides with modes of action other than ALS inhibition would be required at these sites to control annual bluegrass. Future research should focus on determining the mechanisms of resistance in these annual bluegrass biotypes to improve the diagnostic assay as well as explore control options using alternative modes of action.

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