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Acetyl-CoA Carboxylase Herbicide Tolerance in Bermudagrass

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ABSTRACT

Contamination of newly planted bermudagrass (*Cynodon* spp.) varieties by undesirable off-type bermudagrass genotypes is an ever increasing concern for turf managers because selective control options are limited. In 2009, a sethoxydim {2-[1-(ethoxyimino) butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one} tolerant bermudagrass genotype (93-175) was identified during herbicide screening at the University of Georgia in Tifton. The objective of this research was to assess the tolerance of 93-175 to three Acetyl-CoA carboxylase (ACCase) herbicides in comparison to the susceptible genotypes Tifway and common bermudagrass. Greenhouse and field trials were performed between August 2011 and April 2013. Factors in the field experiment included ACCase herbicides, application rates, bermudagrass genotypes, and locations. Turfgrass injury ratings taken 42 days after treatment (DAT) and during greenup the following spring supported initial preliminary findings. At the 1x rate of sethoxydim (280 g a.i. ha^{-1}), 93-175 displayed 50 to 87% less injury in comparison to the susceptible genotypes. In the spring of 2013, 93-175 plots treated with a 1x rate of sethoxydim reached 100% recovery during the same time period as non-treated controls, while common and Tifway had only recovered to 48 and 60%, respectively. The tolerance mechanism of 93-175 to sethoxydim did not confer an appreciable reduction of clethodim {(*E,E*)-(6)-2-[1-[[(3-chloro-2-propenyl)oxy]imino]propyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one} or fluazifop {(6)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid} herbicide treatment effects. 93-175 will continue to be studied to determine transferability of herbicide tolerance to progeny and the mechanism of the observed tolerance.

Bermudagrass is a warm-season perennial grass that is used widely in the southern United States, India, Australia, Africa, and South America. It is found in more than 100 countries within tropical and subtropical regions throughout the world. Bermudagrass is generally regarded as having exceptional tolerance to heat and drought, but poor tolerance of shade and freezing temperatures. Improved bermudagrass cultivars provide durable turf on golf courses, sports fields, recreational areas, forages, and home lawns (Beard, 1973). Common bermudagrass [*C. dactylon* (L.) Pers.] is the most prevalent species around the globe and is mostly regarded as an invasive weed in many crops, including improved varieties of bermudagrass. Non-cultivated common bermudagrasses typically are tetraploid ($2n = 4x = 36$), although hexaploid ($2n = 6x = 54$), pentaploid ($2n = 5x = 45$), and triploid ($2n = 3x = 27$) accessions have been collected (Harlan et al., 1970b; Wu et al., 2006). The genetic variability found within this genus of Poaceae has allowed for their widespread adaptation and utilization (Harlan et al., 1970a). Tetraploid genotypes tend to be more fertile than the diploid ($2n = 2x = 18$) species such as African Bermudagrass (*C. transvaalensis* Burtt Davy), which generally do not produce many viable seeds (Taliaferro, 2003).

In most intensely managed turf areas, improved varieties of bermudagrass such as the sterile hybrids made from crosses between *C. dactylon* and *C. transvaalensis* are preferred because of their superior aesthetics and performance throughout many years and in different situations (Bunnell et al., 2005; Webster et al., 2003). These sterile hybrids are clonally propagated and are transplanted as sod or sprigs when established in new turf areas. Currently there are no selective herbicides to control common bermudagrass that has contaminated a hybrid cultivar due to a lack of differential tolerance. Due to the persistence of common bermudagrass, competiveness and difficulty of eradication by fumigation, unwanted infestation is a normal occurrence and can become a large issue if left untreated. The only methods currently available to renovate infested areas are to physically remove all rhizome and sod materials, or by multiple glyphosate [*N*-(phosphonomethyl)glycine] applications followed by replanting of new sod (Bigelow, 2008; Lowe and Foy, 2012).

In contrast, bermudagrass control can be achieved in centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.] and zoysiagrass

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Abbreviations: ACCase, Acetyl-CoA carboxylase; DAT days after treatment.

(*Zoysia japonica* Steud.) through the application of ACCase herbicides that have naturally occurring selectivity between the species (Cox et al., 1999; Johnson, 1987, 1992; Lewis et al., 2010). Herbicide resistances have also been developed in many agronomic crops to control weeds through traditional plant breeding approaches and with biotechnological methods (Mazur and Falco, 1989). Thus far, many of these cultivars were developed using transgenic approaches that are deemed impractical when developing turfgrass resistance systems due to the extensive regulations associated with the production and release of a transgenic crop. Costs also could be extensive, limiting the feasibility for public turfgrass breeding programs to use this approach (Bradford et al., 2005).

Preliminary field trials in Tifton, GA, indicated that 93-175 was tolerant to a labeled rate (280 g a.i. ha^{-1}) of sethoxydim (B.M. Schwartz, unpublished data, 2009). Tolerance was confirmed in a greenhouse dose response experiment in conetainers at varying rates of sethoxydim application from 25 to 800 g a.i. ha^{-1} (B.M. Schwartz, unpublished data, 2011). The evaluation identified Tifway (Burton, 1966) as sustaining 96% injury at the 400 g ha^{-1} rate, whereas 93-175 exhibited no more than 16% injury in the 400 to 800 g ha^{-1} treatments 28 d post-application. The discovery or incorporation of naturally occurring herbicide resistance or tolerance in an improved hybrid bermudagrass cultivar would give managers the ability to selectively control warm-season grassy weeds in their turf without extensive damage or large amounts of labor intensive work. Therefore, the objectives of these experiments were to (i) evaluate the bermudagrasses 93-175, Tifway, and common for response to the ACCase inhibiting herbicides sethoxydim, clethodim, and fluazifop in the greenhouse; (ii) further verify these results under field conditions; and (iii) determine whether point mutations in the ACCase gene, known to confer herbicide tolerance in other crops, are present in the evaluated bermudagrass genotypes.

MATERIALS AND METHODS

Greenhouse and field experiments were conducted between August 2011 and April 2013 at the University of Georgia Coastal Plain Experiment Station in Tifton. Tifway, common, and 93-175 bermudagrasses were obtained for these experiments from the University of Georgia germplasm collection in Tifton. Tifway was included in this research because it is a popular cultivar used on home lawns, sports fields, and golf courses. Common bermudagrass was also evaluated because it and Tifway are presently the primary contaminants of new bermudagrass cultivars after planting. 93-175 is an experimental tetraploid ($2n = 4x = 36$) bermudagrass genotype selected from a variety Tiflawn progeny population in 1993. The population was evaluated for turf quality and seed production. 93-175 did not meet requirements for seed production, but was kept for use as a potential parent due to its turf quality. For this study 93-175 was chosen for its potential herbicide tolerance.

Greenhouse Experiment

A greenhouse experiment was performed at the University of Georgia Tifton Campus from December 2011 to September 2012. The experimental design was a randomized complete block with five replications. Factors included ACCase herbicides (sethoxydim 1x = 280 g a.i. ha^{-1}, clethodim 1x = 300 g a.i. ha^{-1}, fluazifop 1x = 350 g a.i. ha^{-1}), herbicide application rates (0.25x, 0.5x, 1x, 2x, 4x, 6x, 8x,and non-treated), bermudagrass genotype (93-175, Tifway, and common bermudagrass), and run (Run 1 initiated on 26 June 2012 and Run 2 initiated on 19 July 2012).

In 2011 December, plant materials were vegetatively established into 7.65-cm round pots containing commercial potting media, and were grown to 90% coverage at a 3.8 cm canopy height before herbicide application. Plants were fertilized at a monthly rate of 2.4 g N m^{-2} with nutrient solution Miracle-Gro 20–20–20 Water Soluble All Purpose Plant Food (Scotts Miracle-Gro, Marysville, OH) and irrigated as needed to maintain quality growth. The greenhouse was maintained at 35±4°C, with natural sunlight as the light source.

Broadcast applications of the ACCase inhibiting herbicides were made at the rates previously described using pressurized CO_2 and a mechanical spray chamber calibrated to deliver a volume of 210 L ha^{-1}. At 21, 28, and 60 DAT turf quality and growth were evaluated using visual damage ratings, percent green cover, and clipping weight. Visual herbicide injury ratings were scored using a 1 to 9 scale with 1 = 99% injury and 9 = 0% injury. The green cover of each experimental unit was also assessed using digital photographs that were analyzed with SigmaScan (Systat Software, San Jose, CA) as described by Richardson et al. (2001). Percent injury was then determined by dividing the green cover of the treated pot by the non-treated control within its replication. Clippings above the 3.8 cm canopy height were trimmed and collected from all plants and fresh weights were measured immediately after visual and digital image ratings were completed. The dry weight of each clipping sample was recorded after storage at 60°C for 2 wk. Results were used to identify the appropriate rates of herbicide to be used in subsequent field trials.

Field Experiment

The field experiment was performed at the University of Georgia Tifton Campus from August 2011 to April 2013. The experimental design was a strip-plot with four replications. Factors included bermudagrass genotype (93-175, Tifway, and common bermudagrass organized as whole plots), ACCase herbicide application rate (sethoxydim 1x = 280 g a.i. ha^{-1}, clethodim 1x = 300 g a.i. ha^{-1}, fluazifop 1x = 350 g a.i. ha^{-1} arranged as subplots), and location (Location 1 planted in August 2011 and Location 2 planted in May 2012).

Soil at both locations was Tifton loamy sand (fine-loamy, kaolinitic, thermic Plinthic Kandiudults). Tifway, common, and 93-175 bermudagrasses were established with vegetative plant material at both testing locations. The trials were planted so that a maximum of 15 herbicide/rate subplots could be evaluated per genotype (whole-plot) in each replication along with a control. Each genotype strip was 14.6 by 1.8 m allowing for subplots of 0.9 by 1.8 m. Turf was established to 95% coverage at each location before herbicide treatments began, and was managed with sprinkler irrigation and fertilized monthly at 4.9 g N m^{-2} (Rainbow 16–4–8 Plant Food, Agrium US Inc., Denver, CO) to maintain plant health. The experiments were mown weekly at 3.8 cm with a rotary mower.

Herbicide treatments consisted of sethoxydim (1x, 4x, 6x, 8x, and 10x), clethodim (1x, 2x, 4x, 6x, and 8x), fluazifop (1x, 2x, 4x, 6x, and 8x), and a non-treated control. Herbicides were applied at both locations with a CO_2–pressurized backpack sprayer equipped with flat-fan nozzles delivering 140 L ha^{-1} at 165 kPa and 4.8 km h^{-1} on 11 Sept. 2012. Bermudagrass plots at Location 1 were 407 d old on the day of herbicide application, while those at Location 2 were only 133 d old.

Turf quality and percent injury was evaluated at 14, 21, 28, 35, and 42 DAT. Turf quality was assessed using a 1 to 9 visual scale with 1 = dead, 5 or greater = acceptable, and 9 = excellent. Herbicide injury was rated visually using a 0 to 99% scale. For the digital image analysis (Richardson et al., 2001), percent injury was also determined by dividing percent green cover of the treatment plot by the non-treated control within its replication.

A second application of all herbicide treatments was applied on 8 Nov. 2012 to both trials in the same manner as described above. Turf recovery during spring greenup was measured visually and with digital image analysis during April 2013.

Statistical Analysis

Greenhouse data were subjected to a mixed model ANOVA, with trials and replications as random factors, while bermudagrass genotypes and herbicide rates were fixed factors. The relationship between amount of green cover and herbicide rates fit to a log-logistic regression model,

$$y = c + \left[\frac{d - c}{1 + \left(\frac{x}{I_{50}} \right)^{b}} \right]$$

where y is the amount of green cover, d is the upper limit of the regression, c is the lower limit of the regression, x is the herbicide rate, I_{50} is the herbicide rate that provides median weed control, and b is the slope of the regression at I_{50} (Ritz et al., 2013; Seefeldt et al., 1995). An approximate $R^2_{\text{nonlinear}}$ (Askew and Wilcut, 2001; Jasieniuk et al., 1995) was determined using:

$$R^2_{\text{nonlinear}} = 1 - \left(\frac{\text{Residual sum of squares}}{\text{Corrected total sum of squares}} \right)$$

An analysis of variance was performed on each of the measured variables from the field trials with PROC GLM (SAS Institute Inc., Cary, NC) using a mixed model where trials and replications were considered random effects and genotype, herbicide treatment, and rating dates were fixed effects (Table 1). Data from field experiments were assessed with a histogram and normal probability plot. Pearson correlation coefficients were computed using PROC CORR in SAS (SAS Institute Inc., Cary, NC) to test whether any of the traits were predictors of other characteristics. Genotypic and herbicide treatment means were separated using a Fisher's least significant difference at the 0.05 level of probability where appropriate.

DNA Extraction and Sequencing

Vegetative samples of approximately 0.5 g total of fresh leaf tissue were taken from 10 to 15 leaves of each accession. Fresh leaf tissue was placed in a screw cap microcentrifuge tube with three ceramic grinding beads and 700 µL CTAB buffer and pulverized using a Retsch MM300 mixer mill (RETSCH, Haan, Germany). DNA was extracted according to the CTAB method (Reichardt and Rogers, 1997). DNA quality was verified by running an aliquot of each sample in a 1% agarose gel (DNA grade agarose [FischerBiotech]).

Two gene regions were amplified. The first region contained the 1781 Ile to Leu mutation site and the second contained the 1999 Trp to Cys through 2096 Gly to Ala mutation sites. The region containing the 1781 Ile to Leu mutation was amplified using the sv384f (CGGGGTTTCAGTACATTTAT) forward primer and the sv384r (GATCTTAGGACCACCCAACTG) reverse primer (Heckart et al., 2010). The region containing the 1999 Trp to Cys through 2096 Gly to Ala mutation sites was amplified using the POA3F (CATGTGATCCTCGTGCAG) forward primer and the WS1R (AGAACCTCCAGGAGACTAG) reverse primer (Tate, 2012). All primers were obtained from Integrated DNA Technologies (San Diego, CA, www.idtdna.com). The polymerase chain reaction (PCR) was performed using 20 ng of genomic DNA in a 20 µL total volume with a final concentration of 1X PCR buffer, 0.8 pmol μL^{-1} each primer, 0.2 mM dNTPs, 1.5 mM MgCl2 and 0.05 U μL^{-1} *Taq* DNA polymerase (Promega). The PCR conditions for the sv384f/sv384r primer pair were 4' at 95°C, 35x(30" at 95°C, 30" at 52.7°C, 30" at 72°C), 7' at 72°C (Heckart et al., 2010). The conditions for the POA3F/WS1R primer pair were 5' at 95°C, 35x(30" at 95°C, 30" at 54°C, 30" at 72°C), 7' at 72°C (Tate, 2012).

An aliquot of each PCR product was run in a 1% agarose gel (DNA grade agarose [FischerBiotech]) to verify a single product of the expected size for the primer pair used. The remaining PCR product was then purified using the DNA Clean and Concentrator 5 kit (Zymo Research Inc., Orange, CA). Each purified DNA sample was sequenced both forward and reverse. Briefly, a 15 µL volume containing 10 ng of purified DNA mixed with 25 pmol of corresponding primer were loaded in plates and shipped per instruction of GENEWIZ Inc. (South Plainfield, NJ). Sequences were analyzed using Geneious v5.4 software (Drummond et al., 2011). Sequences were aligned and compared to *Alopecurus myosuroides* Huds. nucleotide sequence AJ310767 as a reference with all known mutation sites annotated. Sequence data from all accessions were then translated and compared with the translation of *Alopecurus myosuroides* AJ310767 as a reference.

Table 1. Mean squares for herbicide injury measured by digital image analysis of three bermudagrass genotypes measured in two field trials 42 d after herbicide treatment at the University of Georgia Coastal Plain Agricultural Experiment Station in Tifton.

Source	df	Mean squares
Trial (T)	1	10,150**
Error A, Rep(T)	6	631
Genotype (G)	2	118,861*
G × T	2	1,971
Error B, G × Rep(T)	12	424
Herbicide Treatment (H)	15	62,260***
H × T	15	732***
H × G	30	4,297***
H × T × G	30	381*
Error C, H × Rep(T × G)	270	217
Date (D)	4	6,108
D × T	4	1,532***
D × G	8	1,420**
D × H	60	698***
D × T ×G	8	205***
D × T × H	60	86***
D × G × H	120	348***
D × T × G × H	120	58***
Error D, MSE	1,152	10,150
% CV		8.1

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

RESULTS AND DISCUSSION

Results from the greenhouse experiments (data not shown) were used to make the decision to only test herbicide rate treatments of 1x rates and higher for confirmation in larger plots under field conditions similar to those that turfgrass managers could encounter. In the field experiments, a high correlation (0.97, $P < 0.0001$) between visual herbicide injury ratings and digitally analyzed herbicide injury measurements was observed. Therefore, only the results measured using digital image analysis are reported. The overall ANOVA for herbicide injury measured by digital image analysis found the main effects "genotype" and "herbicide treatment" to have considerably larger mean squares than the other sources of variation (Table 1). As may be anticipated for an experiment conducted to test the response of three unique bermudagrass genotypes to three different herbicides applied at different rates over time, all herbicide treatment and "date" interaction terms were also significant. However, the "genotype × trial" interaction was not significant. Therefore, the analyses presented are of means pooled over trials, but separated by each rating date and herbicide treatment. The trends in many of these separate analyses were very similar, therefore four specific analyses that demonstrate the most important interactions are presented.

For a herbicide-tolerant turfgrass cultivar to have practical utility, injury levels and length of recovery must be acceptable for end users (Radko, 1957). In these field experiments, 93-175 had significantly ($P < 0.05$) less injury than both Tifway and common bermudagrass at the 1x field rate (280 g a.i. ha^{-1}) of sethoxydim on all evaluation days (Fig. 1A). At 14 DAT, 93-175 was injured 27.5% compared to the non-treated control, where Tifway (60.5% injury) and common (67.2% injury) exhibited more than twice the level of herbicide damage. At 21, 28, 35, and 42 DAT 93-175 exhibited only 8.7, 1.7, 8.5, and 1.2% injury, respectively. The 3 wk period that 93-175 required to recover to pre-sethoxydim application turf cover is likely an acceptable timeframe for most turf managers who face problematic off-type bermudagrass contamination. At these same rating dates, Tifway (66.4, 76.4, 63, and 47.4%) and common (68.9, 68.2, 58.2, and 50.9%) were more significantly injured and only began to slowly recover at 35 and 42 DAT. Johnson (1987) observed very similar results for Tifway (34 and 52% injury at 220 and 340 g a.i. ha^{-1} sethoxydim, respectively) in a similar timeframe to our 42 DAT rating. Corresponding findings of 46% injury at 300 g a.i. ha^{-1} sethoxydim for common bermudagrass has also been documented (Johnson, 1992). The genotype 93-175 exhibited the ability to recover faster and be actively growing well before the other two genotypes. These responses indicate that turfgrass managers could be able to treat a stand of 93-175 with sethoxydim, reducing the ability of contaminating bermudagrasses to spread and thrive.

The genotype 93-175 was also less injured than the other two bermudagrass genotypes at the 4x rate sethoxydim treatment (1120 g a.i. ha^{-1}) on all evaluation dates, exhibiting the ability to recover to a 9.7% injury level at 42 DAT with a peak injury level at 14 DAT of 59.7% (Fig. 1B). At 14 DAT, Tifway and common bermudagrass were 89.9 and 81.8% injured, respectively, with peak injuries not observed until 21 DAT. When treated with 680 g a.i. ha^{-1} sethoxydim, Johnson (1987) found Tifway to be 78% damaged during the same interval after treatment. 93-175 appears tolerant to greater than field labeled sethoxydim rates, which would allow turf managers greater application flexibility if sprayer overlap or miscalibration occur. Even though 93-175 did not show a practical level of tolerance at 1x applications to either fluazifop (Fig. 1C) or clethodim (Fig. 1D), it was significantly ($P < 0.05$) less injured than either common bermudagrass or Tifway on all evaluation dates except at 35 DAT in the clethodim herbicide treatment.

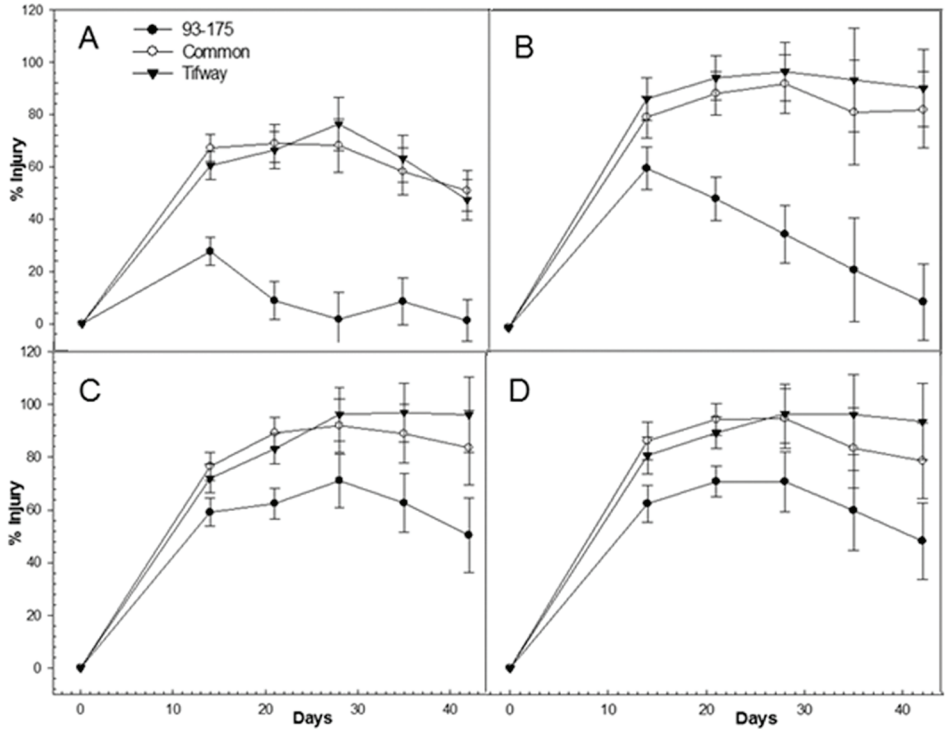

Fig. 1. Percent herbicide injury (compared to the non-treated control) of three bermudagrass genotypes over 42 d after herbicide treatments with (A) sethoxydim at 280 g a.i. ha^{-1}, (B) sethoxydim at 1120 g a.i. ha^{-1}, (C) fluazifop at 350 g a.i. ha^{-1}, and (D) clethodim at 300 g a.i. ha^{-1} in two field trials conducted in Tifton, GA. Vertical bars represent Fisher's LSDs ($P < 0.05$) on each respective rating date.

Genotypes were evaluated for spring recovery from dormancy on 24 Apr. 2013 following a second application of all herbicide treatments during November 2012. By this date, all non-treated control plots had reached full greenup after the winter. In previous research, Johnson (1987) observed the spring recovery of Tifway plots treated with 220 and 340 g a.i. ha^{-1} sethoxydim to be 76 and 41%, respectively. The following year, spring recovery was 13 and 3%, respectively, on the same plots. Only after two consecutive years of treatment with rates of sethoxydim at 340 g a.i. ha^{-1} and higher was Tifway completely killed. In a comparable study, multiple applications of sethoxydim at 300 g a.i. ha^{-1} over consecutive years never resulted in common bermudagrass eradication (Johnson, 1992). 93-175 displayed more green cover than either Tifway or common bermudagrass at all rates of sethoxydim application (Table 2). Recovery of 93-175 in the 280 g a.i. ha^{-1} treatment was higher than the spring greenup of the non-treated control, indicating that this herbicide treatment actually stimulated growth in this genotype, mimicking the response we observed in the greenhouse evaluation (data not shown). The November 2012 herbicide treatments were applied before the onset of winter dormancy, and the resulting injury was similar to bermudagrass plant response to cooler temperatures, in which the turf turned off-color to a golden brown. This late fall application timing could be a strategy used by turf managers to conceal the injury caused by sethoxydim application from turfgrass users by sending the turf into pseudodormancy before the first frost.

The spring greenup of both common bermudagrass and Tifway on 24 Apr. 2013 was severely inhibited by all fluazifop herbicide treatments, as was the recovery of 93-175 at 4x and higher fluazifop application rates (Table 3). This likely was related to the high levels of injury that were sustained in these treatments during fall fluazifop herbicide applications. Johnson (1987) found that two consecutive years of fluazifop applications at 200 g a.i. ha^{-1} and 280 g a.i. ha^{-1} resulted in complete kill of Tifway bermudagrass, where Johnson (1992) was only able to suppress common bermudagrass to 7% coverage in a mixed stand with variety Emerald zoysiagrass with fluazifop at 300 g a.i. ha^{-1} for two consecutive years. The only marginally acceptable level of spring greenup in the fluazifop treatments was observed at the 1x rate on 93-175 (74.8%), indicating that it may be possible to slowly transition a mixed stand of 93-175 and common or Tifway bermudagrass towards a solid stand of 93-175 over time with consecutive annual 1x fluazifop applications. Greenup in 93-175 was statistically greater than for Tifway at all application rates of clethodim, but never statistically greater than in common bermudagrass or at a level that would allow turfgrass managers to use these treatments successfully. Although the common bermudagrass used in this research may not be genetically identical to common bermudagrasses found elsewhere, its response to the ACCase herbicides studied herein was consistent with prior research (Johnson, 1992) and preliminary screening of more than 800 bermudagrass genotypes during 2009 in Tifton, GA (B.M. Schwartz, unpublished data, 2009).

No mutations known to be associated with ACCase herbicide resistance in other weed and crop species were found when sequencing targeted gene regions of 93-175. Differences were not found in the 1781 Leu to Ile sequences of 93-175 and Tifway or the 1999 Trp to Cys through 2096 Gly to Ala region. In other crops there have been reports of mutations conferring resistance to several herbicides

Table 2. Percent green turf cover during spring greenup on 23 Apr. 2013 when control plots reached 100% recovery of three bermudagrass genotypes treated with sethoxydim in two field trials.

	Application rates of sethoxydim (1x = 280 g a.i. ha^{-1})				
Genotype	1x	4x	6x	8x	10x
	% green turf cover				
93-175	109.4a†‡	86.3a	86.7a	60.5a	81.7a
Common	48.5b	35.0b	22.1b	23.3b	15.9b
Tifway	60.8b	9.5c	7.6b	3.9b	4.1b

† Percent of the non-treated control.

‡ Means followed by the same letter are not significantly different according to Fisher's LSD (P = 0.05).

Table 3. Percent green turf cover during spring greenup on 23 Apr. 2013 when control plots reached 100% recovery of three bermudagrass genotypes treated with fluazifop and clethodim in two field trials.

	Application rates of fluazifop (1x = 350 g a.i. ha^{-1})				
Genotype	1x	2x	4x	6x	8x
	% green turf cover				
93-175	74.8a†‡	35.7a	7.9a	1.6a	1.7a
Common	14.6b	1.9b	1.3ab	1.1a	2.4a
Tifway	5.7b	0.5b	0.3b	0.5a	0.2a

	Application rates of clethodim (1x = 300 g a.i. ha^{-1})				
	1x	2x	4x	6x	8x
	% green turf cover				
93-175	45.5a	23.5a	9.9a	21.6a	10.6b
Common	32.9a	25.5a	11.8a	22.6a	18.4a
Tifway	6.5b	4.8b	5.0b	3.0b	1.6c

† Percent of the non-treated control.

‡ Means followed by the same letter are not significantly different according to Fisher's LSD (P = 0.05).

in the ACCase family, an example being blackgrass (*Alopecurus myosuroides* Huds.) where resistance to fluazifop at (Leu-1781-Ile), clethodim at (Asp-2078-Gly), and sethoxydim at (Leu-1781-Ile) has been observed (Powles and Yu, 2010). The genotype 93-175 only had reduced injury to the three different herbicide chemistries and not complete resistance, suggesting a metabolic response or reduction in uptake of ACCase herbicide applications as the mechanism of tolerance rather than a point mutation. Further study of 93-175 regarding the fate of radio-labeled ACCase herbicides after application or the inheritance of herbicide tolerance in offspring may clarify the mechanism of tolerance and provide a better understanding of the value of this genotype to turfgrass managers and the University of Georgia's turfgrass breeding program.

CONCLUSION

Renovating and installing turf can be an expensive and time consuming task. A new variety which has the potential to be managed with ACCase herbicides to limit contamination by grassy weeds would benefit turf users by increasing the potential for successful establishment and extending the timeframe between replacement. Additionally, many golf and sporting facilities demand that the turfgrass playing conditions remain excellent over time (Radko, 1957). Without control, bermudagrass off-types can often reduce the quality, uniformity, and performance of these turf surfaces below acceptable levels. In this study, the spring greenup of 93-175 was only reduced by 25% or less when two fall applications of sethoxydim at the 1x, 4x, and 6x rates were made, and with the 1x fluazifop treatment. The spring injury observed for common bermudagrass and Tifway in the same treatments ranged from 94 to 39%, yet neither of these genotypes were ever completely killed, even by the highest herbicide application rates. Further research of the long-term tolerance, recovery, and competiveness of the three bermudagrasses studied in mixed stands over consecutive years under ACCase herbicide pressure is needed.

This research demonstrated 93-175 has the ability to tolerate herbicide applications that significantly injured non-tolerant genotypes. While 93-175 does have greater turf quality characteristics, that is, density, uniformity, and growth habit, than other tetraploid bermudagrasses, much of its value lies in that it is fertile and may have the ability to transfer ACCase herbicide tolerance to offspring. Currently, 93-175 is being used as a parent in crosses at the University of Georgia and its progeny will be evaluated for inheritance of herbicide tolerance. Incorporation of ACCase herbicide tolerance into sterile triploid bermudagrass hybrids in the future, assuming the trait is transmissible, would be a desired form of release for this technology. Strategies that prevent or reduce the likelihood that ACCase herbicide tolerance is transferred to weedy bermudagrasses should be pursued so that the utility of this trait is not lost for turf managers. Further understanding of whether the mechanism of tolerance is based on mutations that confer a change of the active binding sites of ACCase inhibiting herbicides, or a genetic makeup that allows 93-175 to metabolize ACCase herbicides with less injury than other bermudagrasses is needed. This information would help in transferring tolerance traits to progeny and should be researched in the future.

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