ABSTRACT

ROGERS, RONALD ROBERTSON. Characterization of Herbicide Resistance and Herbicide Efficacy in Turfgrass Systems. (Under the direction of Dr. Travis W. Gannon).

The limited development of novel herbicides and herbicides with new mechanisms of action (MOAs) has intensified the overreliance on existing herbicide options, contributing to the rise of herbicide-resistant weeds, such as annual bluegrass (*Poa annua* L.). This resistance makes effective weed management increasingly difficult. Turfgrass managers are turning to strategies like herbicide safeners, which can protect sensitive turfgrass species from herbicide damage. However, these approaches highlight the need for innovative solutions to effectively manage resistant weeds like annual bluegrass.

The first study evaluated the effect of the herbicide safener metcamifen on the absorption, translocation, and metabolism of ^{14}C-trifloxysulfuron in yellow nutsedge and St. Augustinegrass. Trifloxysulfuron is a post-emergent herbicide used to control weeds in turfgrass systems, and while many warm-season turfgrass species are tolerant, St. Augustinegrass is sensitive. Results showed no effect of metcamifen on ^{14}C-trifloxysulfuron absorption in either plant species. In yellow nutsedge, translocation studies revealed more ^{14}C-trifloxysulfuron remained in the treated leaf (70.9%) when metcamifen was applied, compared to 56.9% when trifloxysulfuron was applied alone. Translocation to other shoots was also reduced in the trifloxysuluforn plus metcamifen treatment (26.6%) compared to 38.9% with trifloxysulfuron alone. Similarly, St. Augustinegrass showed greater ^{14}C-trifloxysulfuron in the treated leaf (98.5%) and less translocation to other shoots (0.8%) when treated with trifloxysulfuron plus metcamifen compared to trifloxysulfuron alone. Metabolism studies revealed St. Augustinegrass metabolized greater ^{14}C-trifloxysulfuron 48, 96, and 192 hours after treatment when treated with

trifloxysulfuron plus metcamifen. However, metcamifen did not affect yellow nutsedge metabolism.

The second study focused on identifying non-target site resistance (NTSR) mechanisms in six annual bluegrass populations resistant to the ALS-inhibiting herbicides. Annual bluegrass is a problematic weed in turfgrass systems. Populations from the Southeastern United States underwent resistance confirmation and absorption and translocation experiments using ^{14}C-trifloxysulfuron. Results showed that the Virginia population absorbed less ^{14}C-trifloxysulfuron (25.7%) compared to the susceptible population (38.3%) 24 hours after treatment (HAT). Trends continued at 96 and 192 HAT. Translocation studies revealed reduced translocation of ^{14}C-trifloxysulfuron from the treated leaf to other shoots in the Florida, Georgia, and South Carolina populations compared to the susceptible population.

The third study evaluated NTSR mechanisms in eight annual bluegrass populations resistant to either photosystem-II (PS-II) or 5-enolpyruvylshikimate-3-phosphatesynthase (EPSPS) herbicides. The PS-II NTSR screening revealed that four populations had reduced ^{14}C-simazine absorption compared to a known susceptible population, with differences observed as early as 12 HAT. Two of these populations showed reduced absorption without the Ser_{264} to Gly mutation associated with PS-II resistance. In contrast, no NTSR mechanisms were found in EPSP-resistant populations, as no reduced absorption or translocation was observed.

The fourth study investigated the influence of trinexapac-ethyl (TE), a plant growth regulator (PGR), on the absorption and translocation of foramsulfuron in annual bluegrass. Foramsulfuron is a postemergence herbicide absorbed primarily through plant foliage. The study found no significant difference in total foramsulfuron absorption between treatments. However, rapid absorption was observed, with 39.5% absorbed 4 HAT and maximum absorption (83.6%)

at 96 hours. Translocation studies showed that tank-mixed foramsulfuron plus TE reduced translocation to other shoots, with 7.5% translocated, compared to 11.3% with foramsulfuron applied alone.

The fifth study investigated the resistance levels of eleven annual bluegrass populations that survived field applications of PS-II, ALS, or EPSPS-inhibiting herbicides. The study found that three populations exhibited a 6 to 15-fold resistance ratio to simazine, four populations had resistance ratios ranging from 3 to 110 for trifloxysulfuron, and three populations showed resistance ratios between 1.9 and 181.4 for glyphosate. These populations were collected from golf courses, athletic fields, and home lawns, reflecting the widespread issue of herbicide resistance in annual bluegrass.

Characterization of Herbicide Resistance and Herbicide Efficacy in Turfgrass Systems

by
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DEDICATION

This dissertation is dedicated to my daughter Penelope Rogers, wife Cassie Rogers, and my parents Ron and Linda Rogers.

BIOGRAPHY

Ronald Robertson Rogers (Tripp) was born in Raleigh, North Carolina. He completed his bachelor's degree in Agricultural Science at North Carolina State University. During his undergraduate studies, he interned under Dr. Charlie Cahoon, who introduced him to the weed science discipline. After graduation, Tripp briefly worked as a foreman with a residential and commercial landscaping firm. He later took a research assistant position under Dr. Travis Gannon, where his work focused on various aspects of pesticide environmental fate and the characterization of herbicide resistance in turfgrass systems. In addition to his research, Tripp actively participated in numerous scientific conferences and extension presentations.

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CHAPTER 1: Effect of Metcamifen on Trifloxysulfuron Absorption, Translocation and Metabolism in St. Augustinegrass (*Stenotaphrum secundatum*) and Yellow Nutsedge (*Cyperus esculentus*)

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Abstract

Trifloxysulfuron is a postemergence herbicide designed to control broadleaf weeds, both annual and perennial grasses, as well as annual and perennial sedges in specific turfgrass systems. Several warm-season turfgrass species are tolerant to trifloxysulfuron; however, St. Augustinegrass is sensitive. The safener metcamifen is known to safen clodinafop-propargyl in rice and maize. Studies were conducted to evaluate the effect of metcamifen on the absorption, translocation, and metabolism of ^{14}C-trifloxysulfuron in yellow nutsedge and St. Augustinegrass. Trifloxysulfuron plus metcamifen did not affect ^{14}C-trifloxysulfuron absorption in yellow nutsedge and St. Augustinegrass compared to trifloxysulfuron applied alone. Yellow nutsedge translocation studies showed more remaining in the treated leaf, with 70.9% of the absorbed ^{14}C-trifloxysulfuron remaining when trifloxysulfuron plus metcamifen was applied, compared to 56.9% when trifloxysulfuron was applied alone. Additionally, reduced translocation to other shoots was observed (26.6%) when treated with trifloxysulfuron plus metcamifen compared to trifloxysulfuron applied alone (38.9%). Similarly, St. Augustinegrass translocation presented greater ^{14}C-trifloxysulfuron in the treated leaf (98.5%) and less ^{14}C-trifloxysulfuron translocation to the other shoots (0.8%) when treated with trifloxysulfuron plus metcamifen compared to trifloxysulfuron alone. Metabolism studies showed a reduction in ^{14}C-trifloxysulfuron recovery when treated with trifloxysulfuron plus metcamifen, with less recovery at 48 hours after treatment (HAT) (76.8%), 96 HAT (67.2%) and 192 HAT (54.8% < 66.8%) compared to trifloxysulfuron applied alone in St. Augustinegrass. The trifloxysulfuron plus metcamifen treatment did not influence ^{14}C-trifloxysulfuron metabolism in yellow nutsedge.

Nomenclature: Trifloxysulfuron, N-[(4,6-dimethoxy-2-pyrimidinyl)carbamoyl]-3- (2,2,2-trifluoroethoxy)-pyridin-2-sulfonamide sodium salt; yellow nutsedge, *Cyperus esculentus* L.; St.

Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze, metcamifen, herbicide, safener; ALS-inhibitor herbicides

Key Words: acetolactate synthase, herbicide, safener, sulfonylurea

Introduction

As the development of new herbicides or herbicides with unique mechanisms of action declines, chemical technologies that can alter the selectivity of existing herbicides have become increasingly important (Casida 2017; Giannakopoulos et al. 2020). Herbicide safeners are a diverse group of chemicals that can prevent herbicidal injury to plants by enhancing a plant's metabolism (Giannakopoulos et al. 2020; Kraehmer 2014). Previous research on cereal crops has shown that increased metabolism is induced by the expression of genes encoding enzymes such as cytochrome P450s (CYPs) and specific glutathione transferases (GSTs), which play a role in detoxifying xenobiotics (Davies & Casely, 1999; Giannakopoulos et al. 2020; Riechers et al. 2010; Rosinger et al. 2019; Ye et al. 2019). Safeners are typically paired for use in specific crops and combined with postemergence herbicides through co-application (Davies and Casely 1999; Giannakopoulos et al. 2020; Kraehmer et al. 2014). Metcamifen is a sulphonamide safener shown to safen clodinafop-propargyl applications in rice (*Oryza sativa* L.) and maize (*Zea mays* L.) (Brazier-Hicks et al. 2019). Additional studies utilizing a metcamifen plus *S*-metolachlor tank-mix did not protect various wheat (*Triticum aestivum* L.) cultivars (Schaeffer et al. 2024).

St. Augustinegrass is a warm-season turfgrass found in tropical, subtropical, and warm humid regions (McCullough et al. 2016). Growth characteristics include a wide leaf blade and an open canopy compared to other turfgrass species (Trenholm et al. 2011). This species heat and drought tolerance make it a popular grass for lawns, parks, and commercial turfgrass areas in the southern United States (McCullough et al. 2016). Despite this species popularity, herbicides are limited for controlling weeds in St. Augustinegrass systems (Wilber et al. 2023a). While some ALS-inhibiting sulfonylurea herbicides like sulfosulfuron may be tolerated by St.

Augustinegrass, application may result in temporary chlorosis or delayed green-up (Anonymous 2016a).

Trifloxysulfuron is a sulfonylurea postemergence herbicide that is efficacious in controlling annual and perennial grasses, broadleaf weeds, and annual and perennial sedge species in select turfgrass systems (Ferrell et al. 2004). Trifloxysulfuron acts by inhibiting the acetolactate synthase (ALS) enzyme, which is a key enzyme in the biosynthesis of branched-chain amino acids (Shaner 2014). Trifloxysulfuron is absorbed by plant shoots and roots, and symptomology includes cessation of growth, chlorosis, and death of apical meristems (Shaner 2014). Whole plant death is observed 1 to 3 wk after initial application (Shaner 2014). Bermudagrass (*Cynodon dactylon* L.) and zoysiagrass (*Zoysia japonica* L.), (*Zoysia matrella* L.), (*Zoysia tenuifolia* L.) are tolerant to trifloxysulfuron; however, St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze) is not tolerant (Ferrell et al. 2004).

Recognition©, a herbicide formulated with trifloxysulfuron plus metcamifen, is labeled for use in St. Augustinegrass systems (Anonymous 2024). Preliminary field studies have demonstrated this formulation is safe for use in St. Augustinegrass and, when combined with other postemergence herbicides, effectively controls troublesome weeds such as dollarweed(*Hydrocotyle verticillate* L.) and dallisgrass (*Paspalum dilatatum* L.) (Wilber et al. 2023b). Additionally, field studies have demonstrated that tank mixes of Recognition© and other herbicides such as fluazifop improved the tolerance of St. Augustinegrass (Wilber et al. 2023b).

Yellow nutsedge (*Cyperus esculentus* L.) is among the world's worst pests (Stoller and Sweet 1987). It is an herbaceous perennial weed that is particularly problematic in various turfgrass and agronomic systems due to its tuber production as well as the longevity of the tubers (Bariuan et al. 1999). In turfgrass systems, yellow nutsedge impacts turfgrass uniformity and

aesthetics and can affect playability on golf courses and athletic fields (Lugi et al. 2021). Chemical control, utilizing ALS inhibiting herbicides has been the most effective means for managing this weed species in tolerant turf species (Techranchian et al. 2015).

While previous studies have documented absorption, translocation, and metabolism of trifloxysulfuron in yellow nutsedge, there is limited information regarding its effects on St. Augustinegrass. Additionally, literature lacks information regarding the impact of safeners, such as metcamifen on herbicide efficacy in turfgrass systems. Therefore, the objectives of this research were to evaluate the effect of metcamifen on the absorption, translocation, and metabolism of ^{14}C-trifloxysulfuron in yellow nutsedge and St. Augustinegrass.

Materials and Methods

Plant Material

Yellow nutsedge tubers and St. Augustinegrass stolons from previously collected field populations were propagated in the North Carolina State University Method Road greenhouses, located at GPS coordinates 35.787ºN, -78.694ºW, to evaluate the effect of metcamifen on trifloxysulfuron absorption, translocation, and metabolism. Greenhouse conditions were maintained at 29/21°C day/night temperatures, respectively. Natural lighting was supplemented with high intensity metal halide lamps at 500 µmol m $^{-2}$ s^{-1} to provide a 16 h photoperiod. The soil medium was composed of 5% Sun Gro Propagation Growing Mix (containing 50-65% Canadian sphagnum peat moss, vermiculite, and 0.0001% silicon dioxide from dolomitic lime) and 95% cement sand by weight. Conetainers with a cell diameter of 3.8 cm and depth of 21 cm were filled with the amended soil. Yellow nutsedge tubers were directly planted into pots. A single shoot was dissected from the St. Augustinegrass stolon and placed directly into separate pots. Plants were irrigated twice daily with overhead irrigation. When yellow nutsedge plants

reached the 6-leaf stage, and St. Augstinegrass plants reached the first tiller stage, plants were transferred to a laboratory growth chamber with 24±2° C temperature. Plants were allowed to acclimate for 1 wk before treatment. Herbicide applications were made using a CO_2-pressurized sprayer equipped with an 8002 EVS nozzle (TeeJet Technologies Spraying Systems Co., Glendale Heights, IL, USA) calibrated to deliver 187 L ha^{-1}. Plants were treated with either trifloxysulfuron (27.75 g ai ha^{-1}) alone or trifloxysulfuron (27.75 g ai ha^{-1}) tank mixed with metcamifen (74.8 g ai ha^{-1}). A non-ionic surfactant (NIS; Induce®, Helena Chemical Co., Memphis, TN) at 0.25% v v^{-1} was added to all treatment solutions. Before treatment, the third youngest fully expanded leaf of yellow nutsedge and St. Augustinegrass plants were marked and covered as described by Troxler et al. (2003).

Absorption, translocation, and metabolism studies were conducted in a completely randomized design with a factorial treatment arrangement with five replicates and two experimental runs. Factorial levels included treatment effects (trifloxysulfuron alone or trifloxysulfuron plus metcamifen), six harvest timings (4, 12, 24, 48, 96, 192 HAT), and four plant parts (treated leaf, other shoots, crown, and roots). Harvest timings, plant parts, and runs were considered fixed effects, while replications were analyzed as random effects as described by Besancon et al. (2017). The 0 h harvest interval was not included in analysis as it ensured the efficacy of the treated leaf wash.

Absorption and Translocation

This study utilized two radioactive treatment solutions, one formulated with trifloxysulfuron alone and the other with non-radiolabeled metcamifen. Technical grade [Pyridinyl-2-^{14}C]-trifloxysulfuron with a specific activity of 2197.8 kBq mg^{-1} and 95.1% radioactive purity was used. The radioactive solutions for each treatment consisted of a 1:1 v v^{-1}

mixture of high-performance liquid chromatography (HPLC)-grade water and methanol plus non-ionic surfactant (0.25% v v^{-1}). A 5µL of radioactive herbicide solution was placed on the adaxial surface of the previously covered third fully expanded leaf for both species delivering 6.59 kBq^{-1} of ^{14}C-trifloxysulfuron .

Plants were harvested 4, 12, 24, 48, 96, and 192 hours after treatment (HAT) and dissected into treated leaf, other leaves (shoots), crown, and roots. In addition, 5 replicates of each species were spotted with ^{14}C-trifloxysulfuron and harvested immediately to evaluate the efficiency of the leaf-wash technique where 100% was recovered. Treated leaves were rinsed slowly in a 20 mL 1:1 mixture of methanol-deionized water and 0.25% v v^{-1} nonionic surfactant to remove any nonabsorbed herbicide (Nandula & Vencill, 2015). A 1-mL aliquot of the leaf-wash solution was added to 20 mL of scintillation fluid (Cabron-14 Cocktail UN2924; Z Scientific, LLC., New City, NY) and radioactivity was quantified by liquid scintillation spectrometry (LSS) using a PerkinElmer Tri-Carb 2800TR Liquid Scintillation Analyzer (PerkinElmer Life and Analytical Sciences, Inc., Waltham, MA). All plant parts were dried for 48 h at 60° C, weighed, and combusted with a biological oxidizer (OX-500 Biological Material Oxidizer, R.J. Harvey Instrument Co., Tappan, NY).

Metabolism

Plants were grown, maintained, and treated as previously described for the absorption and translocation experiment. Plants were harvested 12, 24, 48, 96, and 192 HAT. Only the treated leaf was used in the analysis of parent material for plants harvested 12 and 24 HAT due to the lack of radioactivity detected in the other plant parts. Data obtained from the absorption and translocation experiment demonstrated movement of ^{14}C-trifloxysulfuron out of the treated leaves at 48 HAT. Therefore, to ensure extraction of the applied ^{14}C-trifloxysulfuron, treated

leaves and shoots were analyzed for plants harvested at 48, 96, and 192 HAT. At harvest, plant parts were immediately stored at -20ºC until analysis.

Analyzed plant parts were homogenized by hand utilizing a razor blade. Once homogenized, the matrix was rinsed and vortexed with 5 mL of methanol in a multi-tube vortexer (MultiTube Vortexer 120, Fisher Scientific., Waltham, MA) for 15 min to extract the analyte (Gilevska et al. 2022). This step was repeated twice to ensure any remaining analyte was extracted. The filtrate was combined and evaporated to near dryness using a 12-position analytical nitrogen evaporator (Model 111 Organomation N-EVAP; Berlin, MA). Samples were reconstituted in acetonitrile and placed in an ultrasonic cleaner (Branson 2510 Ultrasonic Cleaner; Branson., Brookfield, Connecticut) for 10 min to ensure all extracted analyte was in solution. The reconstituted samples were then filtered with a polytetrafluoroethylene syringe filter (Whatman® 6874-1304 GD/X 13 mm syringe filter, PTFE filtration medium 0.45µm; Whatman Inc., Clifton, NJ) and vialed for injection and analysis via HPLC-DAD-Beta-RAM using Laura software by LabLogic. The marc from the initial methanol extraction was air dried, combusted and analyzed in LSS to quantify non-extractable radioactivity. Data are presented as the percent of parent herbicide recovered as percentage of total herbicide amount applied.

Statistical Analysis

Statistical analyses were conducted by subjecting data to ANOVA ($\alpha \leq 0.05$) using PROC GLIMMIX in SAS (Version 9.4, SAS Institute, Inc. Cary, NC) and means were separated using Tukey's honestly significant difference test HSD ($\alpha = 0.05$). Absorption and metabolism data followed a two-parameter logarithmic model and a two-parameter exponential decay model, respectively. Absorption and metabolism curves were fitted in SigmaPlot (Systat Software Inc.,

San Jose, CA 95110) to illustrate the effect of harvest period on ^{14}C-trifloxysulfuron absorbed and recovered, respectively.

Results and Discussion

Absorption

In the yellow nutsedge experiments, neither the main effect of treatment nor the main effect of the experimental run had a significant impact on ^{14}C-trifloxysulfuron absorption. Therefore, data from treatments and experimental runs were combined and presented based on the main effect of hours after treatment. Absorption was calculated as described by Troxler et al. (2003), where leaf wash recovery was subtracted from the total ^{14}C recovered from all plant parts. Absorption data followed logarithmic trends (Figure 1.1). Rapid absorption of ^{14}C-trifloxysulfuron was observed by 4 HAT where 36.8% of applied was observed. Total ^{14}C-trifloxysulfuron absorption increased to 58.1% of applied by 12 HAT. Max ^{14}C-trifloxysulfuron absorption occurred at 192 HAT where 60.9% was observed. The trifloxysulfuron absorption pattern was similar to previous reports, such as Troxler et al. (2003), who found that yellow nutsedge rapidly absorbed ^{14}C-trifloxysulfuron by 4 HAT, reaching 63% absorption at 96 HAT. McElroy et al. (2004) reported that green kyllinga (*Kyllinga brevifolia* Rottb.) and false-green kyllinga (*Kyllinga gracillima* L.) readily absorbed ^{14}C-trifloxysulfuron by 4 HAT. Askew and Wilcut (2002) reported that jimsonweed (*Datura stramonium* L.), cotton (*Gossypium hirsutism* L.), peanut (*Arachis hypogaea* L.) and sicklepod (*Senna obtusifolia* L.) showed rapid absorption of ^{14}C-trifloxysulfuron by 4 HAT; however, total absorption was species-specific after 72 HAT with ^{14}C-trifloxysulfuron absorption varying from 30 to 70%.

Similar to the yellow nutsedge, the main effect of treatment and experimental runs were not significant for ^{14}C-trifloxysulfuron absorption in St. Augustinegrass. Thus, treatments were

pooled and presented as the main effect of hours after treatment (Figure 1.2). At 4 HAT, 11.1% of applied ^{14}C-trifloxysulfuron was absorbed. Absorption increased to 28.4% at 12 HAT. Similar observations reported by Gallaher et al. (1999) show that rapid absorption of two sulfonylurea herbicides, primisulfuron and nicosulfuron, occurred within 24 h after application to corn (*Zea mays* L.) and broadleaf signalgrass (*Brachiaria platyphylla).* No other differences in absorption were observed until 192 HAT, at which point the maximum foliar absorption reached 44.2% of ^{14}C-trifloxysulfuron. These data suggest metcamifen does not influence the absorption of ^{14}C-trifloxysulfuron in both St. Augustinegrass and yellow nutsedge.

Translocation

In yellow nutsedge translocation experiments, ANOVA identified a significant two-way interaction between treatment and plant part for translocation of ^{14}C-trifloxysulfuron. Therefore, data are presented as percentage of ^{14}C-trifloxysulfuron absorbed by the plant, pooled over harvest intervals and runs (Table 1.1). Greater ^{14}C-trifloxysulfuron was recovered in the treated leaf for trifloxysulfuron plus metcamifen treatment (70.9%) compared to trifloxysulfuron applied alone (56.9%). Less ^{14}C-trifloxysulfuron was observed in the other shoots for trifloxysulfuron plus metcamifen treatment (26.6%) when compared to trifloxysulfuron applied alone (38.9%).

Significant differences were observed within treatments amongst the partitioned plant parts. When trifloxysulfuron was applied alone, 56.9% ^{14}C-trifloxysulfuron was observed in the treated leaf compared to 38.9% in the other shoots, 2.9% in the crown, and 1.2% in the roots. In the trifloxysulfuron plus metcamfien treatment, 70.9% ^{14}C-trifloxysulfuron was observed in the treated leaf compared to 26.6% in the other shoots,1.7% in the crown, and 0.8% in the roots. Our findings are similar to Troxler et al. (2003) which reported 30% ^{14}C-trifloxysulfuron moving out of the treated leaf area in yellow and purple nutsedge (*Cyperus rotundus* L.). Similarly, McElroy

et al. (2004) reported 22.1% of ^{14}C-trifloxysulfuron recovered in the shoots of green kyllinga (*Kyllinga brevifolia* Rottb.) and false-green kyllinga (*Kyllinga gracillima* L.). Other studies involving additional ALS inhibiting herbicides report greater than 70% of absorbed ^{14}C-chlorimuron and ^{14}C-imazaquin remained in the treated leaf of purple and yellow nutsedge (Nandihalli and Bendixen 1988; Reddy and Bendixen 1988). Besançon et al. (2017) reported more than 76 % ^{14}C-halosulfuron was absorbed in the treated leaf in cucumber (*Cucumis sativus* L.), summer squash (*Cucurbita pepo* L.), pitted morninglory (*Ipomoea lacunosa* L.) and velvetleaf (*Abutilon theophrasti* Medik.). Translocation data indicate that metcamifen may influence ^{14}C-trifloxysulfuron translocation in yellow nutsedge, specifically from the treated leaf to other shoots.

Similar to the yellow nutsedge experiment, the two-way interaction of treatment and partitioned plant parts was significant for ^{14}C-trifloxysulfuron translocation in St. Augustinegrass. Therefore, data are presented as percentage of ^{14}C-trifloxysulfuron absorbed pooled over harvest intervals and runs (Table 1.2). Greater ^{14}C-trifloxysulfuron was recovered in the treated leaf (98.5%) in the trifloxysulfuron plus metcamifen treatment compared to trifloxysulfuron applied alone (90.3%). Less ^{14}C-trifloxysulfuron was observed in the other shoots (0.8%) in the trifloxysulfuron plus metcamifen treatment compared to trifloxysulfuron applied alone (7.5%). No differences were observed in the crown and roots when comparing trifloxysulfuron plus metcamifen to trifloxysulfuron alone.

Significant differences among the partitioned plant parts within treatments were observed. When trifloxysulfuron was applied alone, greater ^{14}C-trifloxysulfuron was observed in the treated leaf (90.3%) compared to 7.5% in the other shoots, 0.9% in the crown and, 1.1% in the root. The trifloxysuflron plus metcamfien treatment showed, 98.5% ^{14}C-trifloxysulfuron in

the treated leaf compared to 0.8% in the other shoots, 0.2% in the crown and, 0.5% in the root. These data indicate that metcamifen may influence ^{14}C-trifloxysulfuron translocation in St. Augustinegrass, specifically out of the treated leaf and into other shoots. Similarly, the trifloxysulfuron plus metcamifen treatment may influence movement of ^{14}C-trifloxysulfuron out of the treated leaf for St. Augustinegrass (Table 1.2).

Metabolism

In yellow nutsedge metabolism experiments, the main effect of treatment was not significant for the amount of ^{14}C-trifloxysulfuron remaining. Therefore, data are pooled over treatments and presented as the main effect of HAT (Figure 1.3). The amount of parent ^{14}C-trifloxysulfuron observed at 12 HAT was 77.8%. By 96 HAT the amount of parent ^{14}C-trifloxysulfuron decreased to 66.3%. By 192 HAT only 53.8% of parent ^{14}C-trifloxysulfuron remained. These data suggest that metcamifen may not accelerate trifloxysulfuron metabolism in yellow nutsedge. Our results are similar to McElroy et al. (2004) where 61% of parent trifloxysulfuron was observed in two kyllinga species 4 through 96 HAT. Additional studies report 60% to 70% of parent trifloxysulfuron was not metabolized in the sensitive species peanut (A*rachis hypogaea*) and sicklepod (*Senna obtusifolia*) (Askew and Wilcut 2002).

In the St. Augustinegrass metabolism experiments, ANOVA identified a significant two-way interaction of treatment and harvest time. The amount of parent ^{14}C-trifloxysulfuron was greater in the trifloxysulfuron treatment when compared to trifloxysulfuron plus metcamifen 48, 96 and 192 HAT (Figure 1.4). At 48 HAT 84.4% parent ^{14}C-trifloxysulfuron was observed in the trifloxysulfuron treatment compared to 79.8% when metcamifen was included with trifloxysulfron. This trend continues to 96 HAT where 74.5 % parent ^{14}C-trifloxysulfuron was observed in the trifloxysulfuron treatment compared to 67.2% for trifloxysulfuron plus

metcamifen. At 192 HAT 66.8% of applied ^{14}C-trifloxysulfuron was observed for the trifloxysulfuron treatment compared to 54.8% for the trifloxysulfuron plus metcamifen.

Previous studies suggest differential metabolism of trifloxysulfuron. Observations reported by Gallaher et al. (1999) depict variable metabolism amongst sulfonylurea herbicides, where less than 10% of nicosulfuron was metabolized compared to greater than 80% metabolized when primisulfuron was applied 72 HAT in broadleaf signalgrass. Askew and Wilcut (2002) reported 31% parent trifloxysulfron remaining in cotton (*Gossypium hirsutum* L,) (a tolerant species) 4 to 72 HAT. Troxler et al. (2007) reported rapid metabolism of trifloxysulfuron, where 12.1% was observed remaining in tobacco at 72 HAT.

Additional studies report metcamifen safening at earlier harvest intervals than observed in St. Augustinegrass. Brazier-Hicks et al. (2019) reported metcamifen safening clodinafop-propargyl applications in two and three stage leaf stage rice (*Oryza sativa* L.) rapidly after application. A 21% decline of clodinafop was observed 5 HAT when metcamifen was applied to two leaf stage rice (Brazier-Hicks et al. 2019). This trend continues with a 72% decline of clodinafop 48 HAT in two-leaf stage rice (Brazier-Hicks et al. 2019). In three-leaf stage rice a 9% loss of clodinafop was observed 5 HAT which continued to a 73% loss 48 HAT (Brazier-Hicks et al. 2019).

Syngenta released the trifloxysulfuron plus metcamifen formulation Recognition© in 2023. The herbicide label along with reports in trade publications cite effective control as well as effective safening in St. Augustinegrass (Anonymous 2024). Preliminary field studies have demonstrated Recognition© tank mixed with other herbicides like fluazifop, improved the tolerance of St. Augustinegrass while not affecting weed control efficacy (Wilber et al. 2023b).

In our study, it is unclear why the addition metcamfien did not show more rapid safening of trifloxysulfron in St. Augustinegrass.

Non-significance between trifloxysulfuron and trifloxysulfuron plus metacmifen treatments in yellow nutsedge suggests that the addition of metcamifen may not accelerate metabolism in this species. Differences observed in trifloxysulfuron plus metcamifen treatment suggest the addition of this safener may accelerate the trifloxysulfuron metabolism in St. Augustinegrass but not in yellow nutsedge. Future research should investigate if metcamifen can safen additional susceptible turfgrass species to trifloxysulfuron.

Acknowledgments

Appreciation is extended to Dr. Patrick Maxwell, Mathieu LeCompte, and Daniel Freund.

Conflicts of Interest

No conflicts of interest have been declared.

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Tables

Table 1.1 Effect of metcamifen on ^{14}C-trifloxysulfuron translocation in yellow nutsedge (*Cyperus esculentus* L.) [a].

Plant Part	Trifloxysulfuron	Trifloxysulfuron + metcamifen
	% of absorbed [b]	
Treated leaf	56.9	70.9
Other shoots	38.9	26.6
Crown	2.9	1.7
Root	1.2	0.8
HSD $_{(0.05)}$ [c]	6.8	

[a] Data pooled over two experimental runs, six harvest timings.

[b] % of absorbed = $\frac{(total\ radioactivity\ in\ plant\ part)}{(total\ radioactivity\ absorbed)} \times 100$

[c] Tukey's honestly significant difference procedure conducted at α= 0.05

Table 1.2 Effect of metcamifen on ^{14}C-trifloxysulfuron translocation in St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze) [a].

Plant Part	Trifloxysulfuron	Trifloxysulfuron + metcamifen
	% of absorbed [b]	
Treated leaf	90.3	98.5
Other shoots	7.5	0.8
Crown	0.9	0.2
Root	1.1	0.5
HSD $_{(0.05)}$ [c]	3.8	

[a] Data pooled over two experimental runs, and six harvest timings.

[b] % of absorbed = $\frac{(total\ radioactivity\ in\ plant\ part)}{(total\ radioactivity\ absorbed)} \times 100$

[c] Tukey's honestly significant difference procedure conducted at α= 0.05

Figures

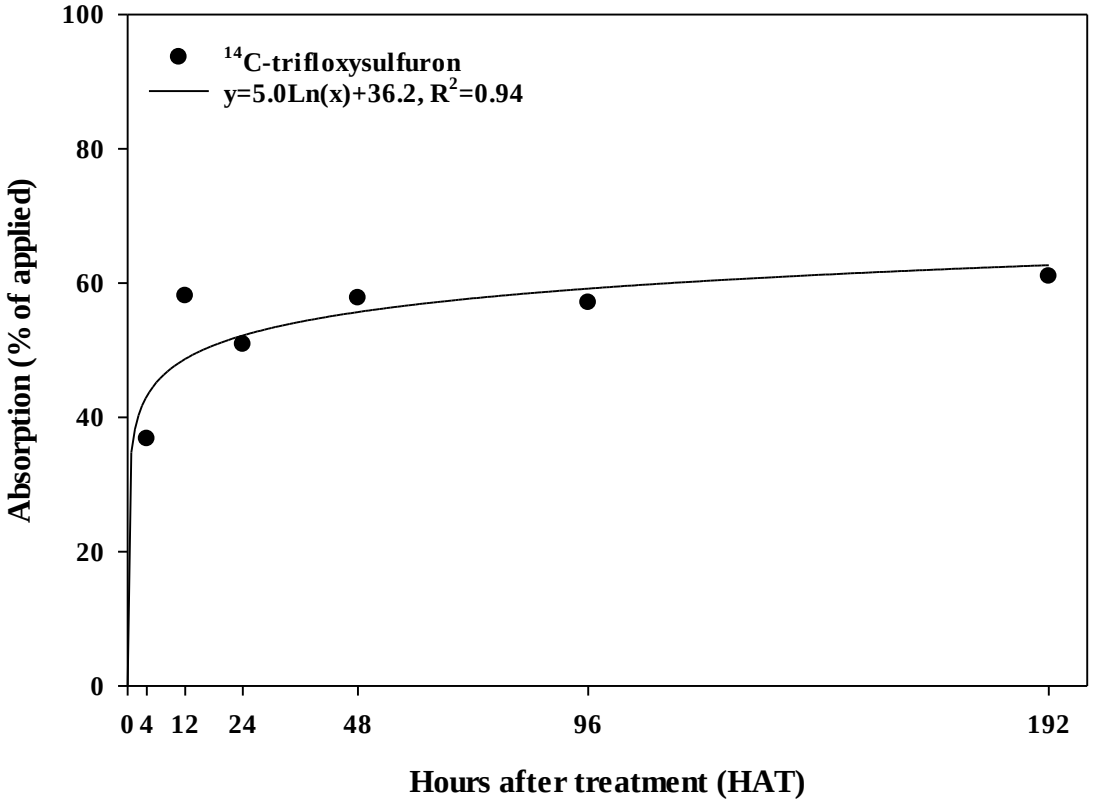

Figure 1.1 Percent absorbed ^{14}C-trifloxysulfuron in yellow nutsedge (*Cyperus esculentus* L.) pooled over treatments and experimental runs. Tukey's honestly significant difference procedure conducted at $\alpha = 0.05$. HSD= 8.3.

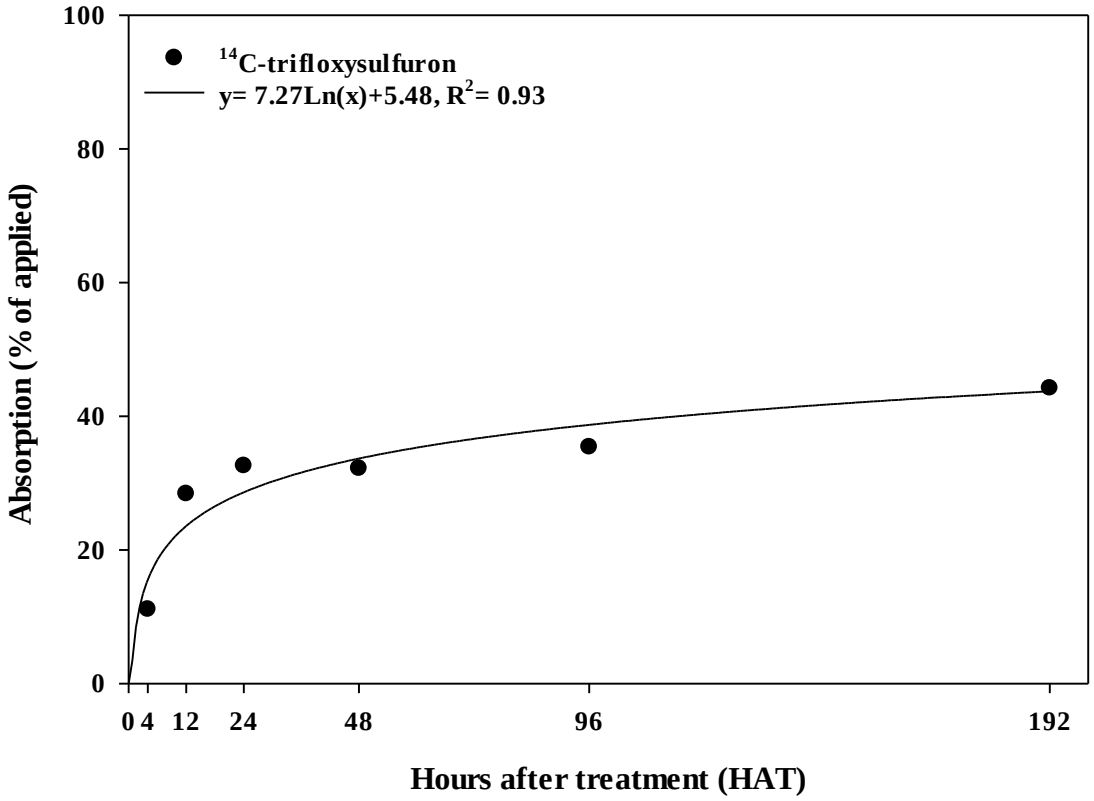

Figure 1.2 Percent absorbed ^{14}C-trifloxysulfuron in St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze) pooled over treatments and experimental runs. Tukey's honestly significant difference procedure conducted at $\alpha = 0.05$. HSD=10.3.

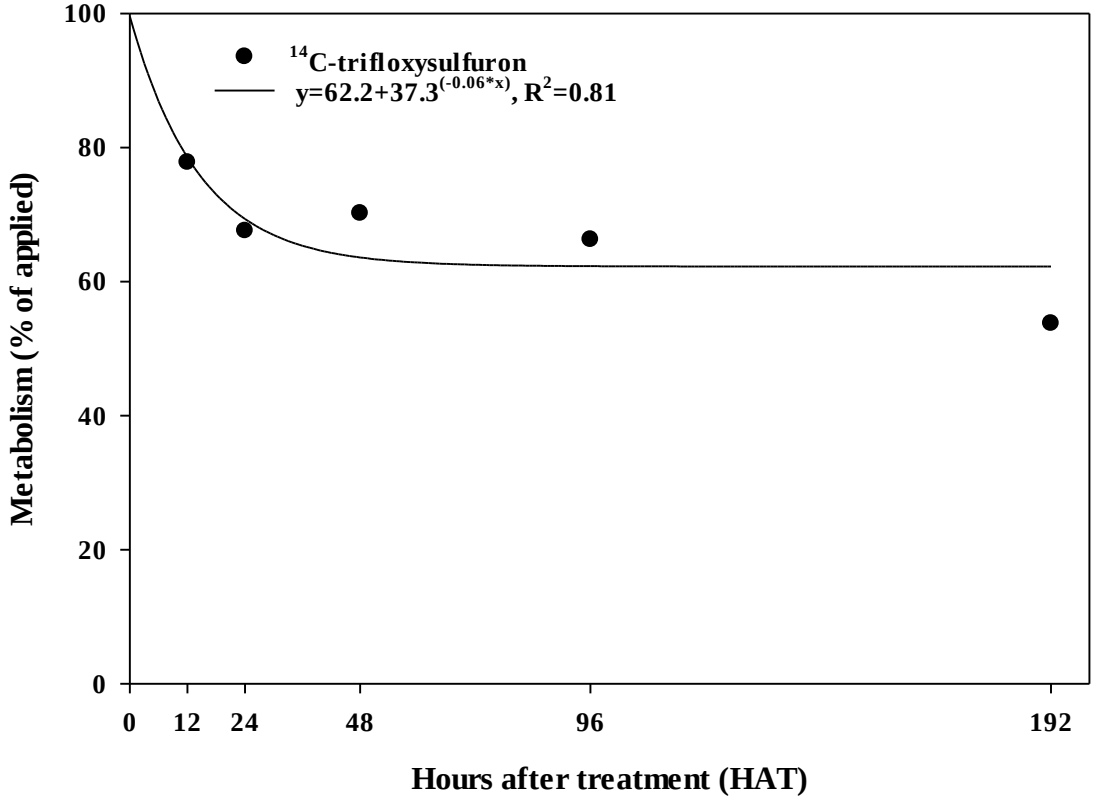

Figure 1.3 Metabolism of ^{14}C-trifloxysulfuron in yellow nutsedge (*Cyperus esculentus* L.) pooled over treatments and experimental runs. Tukey's honestly significant difference conducted at $\alpha = 0.05$. HSD= 8.1.

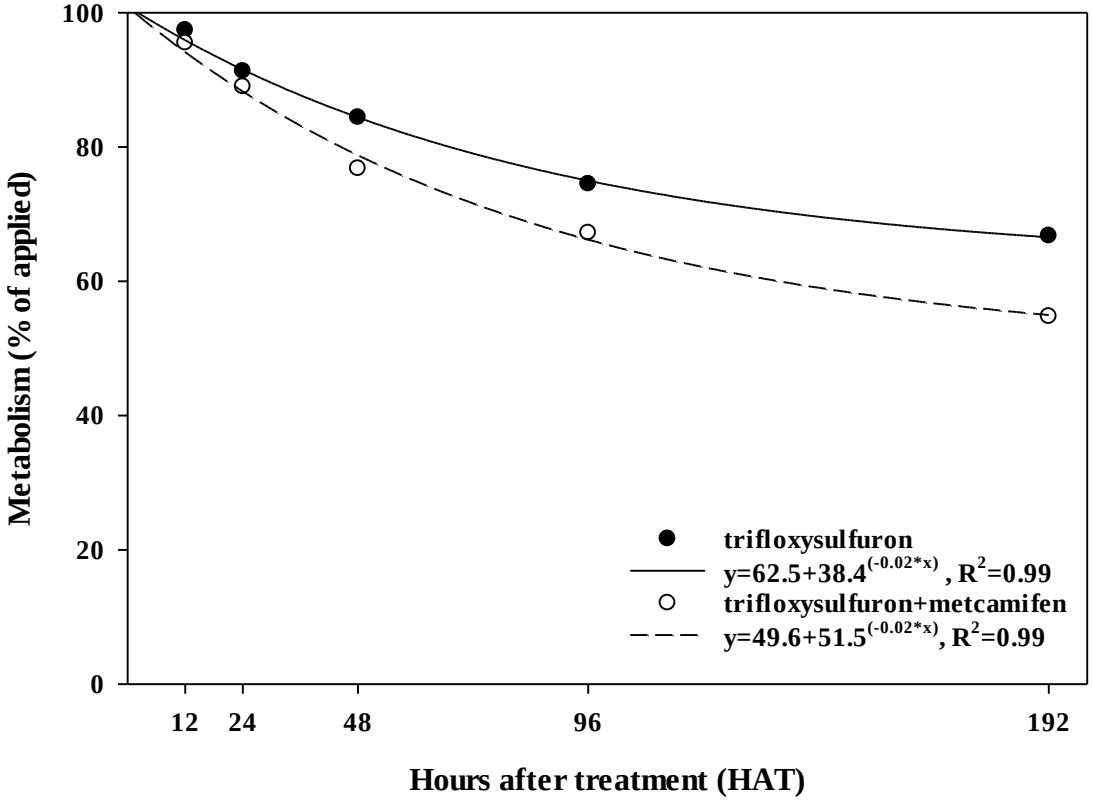

Figure 1.4 Effect of metcamifen on ^{14}C-trifloxysulfuron metabolism in St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze). Tukey's honestly significant difference procedure conducted at $\alpha = 0.05$. HSD= 2.8.

CHAPTER 2: Influence of Reduced Absorption and Translocation of Trifloxysulfuron in ALS Resistant Annual Bluegrass (*Poa annua* L.)

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Abstract

Annual bluegrass is a problematic weed in turfgrass systems. Herbicide resistance in this species to multiple modes of action (MOA's) increases agronomic, economic, and social pressure on turfgrass managers. Herbicide resistant weeds with non-target site resistance mechanisms (NTSR) are a growing threat, as cross-resistance and multiple resistance to herbicides with differing MOA's become more prevalent. The majority of the NTSR and target site resistance (TSR) adaptations have been identified over the past few decades as a response to selection pressures through herbicide use. Trifloxysulfuron is a postemergence ALS-inhibiting herbicide used to control sedges, broadleaf, and grassy weeds in select turfgrass systems. Studies were conducted to identify if NTSR mechanisms were present in six annual bluegrass populations resistant to the ALS-inhibiting herbicide trifloxysulfuron. Populations were collected from the Southeastern United States and underwent a preliminary and full dose rate titration to confirm resistance. Once resistance was confirmed, absorption and translocation experiments utilizing ^{14}C-trifloxysulfuron were conducted to evaluate if NTSR mechanisms were present. Absorption studies identified a population from Virginia that absorbed less ^{14}C-trifloxysulfuron when compared to the susceptible population beginning at 24 HAT (25.7%< 38.3%). This trend continued in the Virginia population at 96 HAT where 35.0% of ^{14}C-trifloxysulfuron was absorbed compared to 50.5% in the susceptible population. At 192 HAT less ^{14}C-trifloxysulfuron absorption was observed in the Virginia population when compared to the susceptible population (33.7% < 47.3%). Translocation studies revealed reduced^{14}C-trifloxysulfuron movement out of the treated leaf and into to the other shoots in the Florida, Georgia, and South Carolina populations when compared to the susceptible population.

Nomenclature: Trifloxysulfuron, N-[(4,6-dimethoxy-2-pyrimidinyl)carbamoyl]-3- (2,2,2-trifluoroethoxy)-pyridin-2-sulfonamide sodium salt; annual bluegrass, *Poa annua* L.; ALS-inhibiting herbicides

Key Words: Annual bluegrass, absorption, translocation, acetolactate synthase, sulfonylurea

Introduction

Annual bluegrass (*Poa annua* L.) is a common cool-season weed that poses challenges in warm and cool-season turf (Mao & Huff 2012; Brosnan et al. 2020b). Native to Europe, annual bluegrass populations can be found worldwide, with great affinity to temperate and sub-tropical areas (Holm 1997). Herbicide resistance is prevalent in this species, making control a challenging task (Svyantek et al. 2016).

In managed turfgrass systems, acetolactate synthase inhibiting herbicides (ALS) are often used to control annual bluegrass (Cross et al. 2015). These herbicides inhibit the branched-chain amino acid production by inhibiting the ALS enzyme (Shaner 2014). Trifloxysulfuron, a postemergence ALS-inhibiting herbicide used to control broadleaf weeds, perennial and annual grasses, and perennial and annual sedge species in select turfgrass systems, is effective in controlling annual bluegrass (Ferrell et al. 2004). Trifloxysulfuron is absorbed by plant shoots and roots, causing symptoms such as cessation of growth, chlorosis, and death of apical meristems, with whole plant death occurring 1 to 3 wks after application (Shaner 2014).

Herbicide resistant annual bluegrass populations have been identified for nine sites of action (SOA) (Heap 2024). The most common global instances of herbicide resistance in this species are related to photosystem II inhibitors (PS-II; e.g., atrazine, simazine), ALS inhibitors (e.g., trifloxysulfuron, foramsulfuron), microtubule inhibitors (MTI; e.g., prodiamine) as well enolpyruvylshikimate-3-phosphate synthase herbicides (EPSP; e.g., glyphosate) (Baura et al. 2020; Binkholder et al. 2011; McElroy et al. 2013).

Globally, reports of ALS-resistant annual bluegrass populations have been documented in the United States and Australia (Heap 2024). The southern and transitional zones of the United States are among the most affected by ALS resistant annual bluegrass (Brosnan et al. 2020), with

cases of ALS resistant populations documented in Alabama, Tennessee, and Mississippi (Heap 2024).

Herbicide resistance of a weed can be divided into two groups, target site resistance (TSR) and non-target site resistance (NTSR) (Gaines et al. 2020). TSR involves mutations in the herbicide's binding site, reducing the herbicide's efficacy (Rigon et al. 2020). In contrast, NTSR weed populations are characterized by reduced absorption and/or translocation, sequestration, or increased metabolism of a herbicide (Rigon et al. 2020). Continued use of a herbicide on a population drives the selection of TSR and NTSR mechanisms, potentially leading to cross-resistance and multiple resistance to other herbicides with different modes of action (Laforest et al. 2021). The majority of NTSR and TSR adaptations have evolved over a few decades as a response to selection pressures through chemical controls (Gaines et al. 2020). Until recently, efforts to characterize ALS resistance have focused on TSR identification (Jugulam & Shyam 2019); however, TSR and NTSR mechanisms have been identified in select populations complicating the management of resistant populations (Gaines et al. 2020; Bai et al. 2019; Chen et al. 2019).

Non-target-site resistance (NTSR) mechanisms have been documented in turfgrass systems. NTSR mechanisms related to reduced translocation have been identified in glyphosate-resistant weeds as well as other herbicides (Gaines et al. 2019). A biotype of *Lolium rigidum* displayed both TSR and enhanced metabolism when treated with acetyl coA-carboxylase inhibiting herbicides (Han et al. 2016). Studies involving glyphosate-resistant annual ryegrass (*Lolium rigidum*) demonstrated 42% of the applied herbicide remained in the tip of the treated leaf of the resistant population compared to 7-12% in the susceptible population (Wakelin et al. 2004). A population of annual bluegrass in Alabama, with no known target site mutation,

exhibited reduced absorption and translocation, and increased metabolism of the PS-II inhibiting herbicide atrazine (Syvantek et al. 2016). These studies outline the complex nature of resistance mechanisms and the importance of gaining a deeper understanding of both TSR and NTSR to develop more effective integrated pest management strategies.

To investigate potential NTSR mechanisms to ALS inhibiting herbicides, six resistant annual bluegrass populations collected from Florida (FL), Georgia (GA), North Carolina (NC), South Carolina (SC), Tennessee (TN), and Virginia (VA) underwent screening to determine if reduced absorption and/or translocation of trifloxysulfuron occurred.

Materials and Methods

Population Selection

Susceptibility and resistance evaluations were conducted with annual bluegrass populations collected from six different states (FL, GA, TN, VA, NC, and SC). A preliminary dose-response assay was completed under greenhouse conditions in which seedlings were sprayed with both a 1x rate (27.75 g ai ha^{-1}) and a 2x rate (55.5 g ai ha^{-1}) of trifloxysulfuron. Non-ionic surfactant (NIS; Induce®, Helena Chemical Co., Memphis, TN) at 0.25% v v^{-1} was added to the treatment solutions. Resistance evaluations were conducted per Burgos (2015) where seedling survival and percent control compared to the non-treated check were evaluated. Populations characterized as resistant (0 to 49% injury) were advanced to a full dose rate titration in which resistant populations were treated with trifloxysulfuron rates ranging from 0.125 x to 8x (3.46, 6.75, 27.75, 55.5 111, and 222 g ai ha^{-1}). The measured response data of annual bluegrass visual injury data were subjected to analysis of variance using PROC GLIMMIX in SAS (SAS/STAT 9.4, SAS Institute, Cary, NC). To test for the significance of the fixed effect of

herbicide rate, Tukey's honestly significant procedure was used to separate means at α=0.05. A four-parameter response curve was used to describe the relationship between plant response (visual estimation of injury) and the herbicide rate (x):

$$Y = Bottom + \frac{(Top - Bottom) * X^{Hillslope}}{EC_{50}^{Hillslope} + X^{Hillslope}} \quad [1]$$

where y is the visual estimation of injury (%), x is the herbicide rate, and the top and bottom are plateaus. The EC_{50} denotes the dose required to produce a 50% response between top and bottom limits. The bottom of the curve is constrained to 0 and the top of the curve was constrained to 100, since the mean maximum response cannot exceed 100. Any dose response level can be expressed by the function EC_{50}:

$$EC_{50} = \frac{EC_x}{[\frac{x}{100-x}]^{\frac{1}{B}}} \quad [2]$$

where EC_x represents the effective dose that elicits x% response between the upper and lower limits of the curve. This equation was used to calculate the effective herbicide rate that produced 90% visual injury (EC_{90}). Curve fitting was performed via nonlinear regression methods with GraphPad Prism (version 10.0.0 for Windows, GraphPad Software, San Diego, CA).

Confirmed resistant populations underwent genotyping experiments at the Herbicide Resistance Diagnostic lab located at Auburn University. TSR mutations consistent with resistance to ALS-inhibiting herbicides, particularly the Trp_{574}-Leu mutation were identified in select populations. This mutation has previously been shown to confer resistance to ALS herbicides such as sulfometuron and imazapyr in other species (Yu et al. 2008). Genotyping experiments identified a Trp_{574} to Leu mutation for the FL, TN, and NC populations while the mutation was not identified in the GA, SC, and VA populations (Table 2.1).

Plant Material

Seeds from the previously confirmed resistant populations of annual bluegrass were planted to evaluate NTSR mechanisms. In addition to the resistant populations selected, a known ALS susceptible population, collected from Mississippi was included for comparison (Table 1). Plants were grown in the North Carolina State University Phytotron chambers located at GPS coordinates 35.787ºN, -78.672ºW. A constant day/night temperature was set at 18.4°C. The lighting source consisted of 18 T5 54W fluorescent lamps averaging 450 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 12h photoperiod. The soil medium consisted of 50% Sun Gro Propagation Growing Mix (Canadian sphagnum peat moss 50-65%, vermiculite, dolomitic lime 0.0001% silicon dioxide) and 50% cement sand by weight. Conetainers with a cell diameter of 3.8 cm and depth of 21 cm were filled with amended soil. Three seeds were planted directly into the pots and later thinned to 1 plant per pot. Plants were irrigated twice daily with overhead irrigation. When plants reached the three-leaf stage, plants were transferred to a laboratory growth chamber with a 20±2°C temperature. Plants were allowed to acclimate for 1 wk before treatment. Herbicide applications were made using a CO_2-pressurized sprayer equipped with an 8002 EVS nozzle (TeeJet Technologies Spraying Systems Co., Glendale Heights, IL, USA) calibrated to deliver 187 L ha^{-1}. Plants were treated with trifloxysulfuron at 27.75 g ai ha^{-1}. A non-ionic surfactant (NIS; Induce®, Helena Chemical Co., Memphis, TN) at 0.25% v v^{-1} was added to treatment solutions. Before treatment, the second youngest fully expanded leaf was marked and covered with aluminum foil as described by Troxler et al. (2003).

Absorption and Translocation

This study utilized technical grade [Pyridinyl-2-^{14}C]-trifloxysulfuron with a specific activity of 2197.8 kBq mg^{-1} and 95.1% radioactive purity. Radioactive solutions for each

treatment consisted of a 1:1 v v^{-1} mixture of high-performance liquid chromatography (HPLC)-grade water and methanol plus non-ionic surfactant (0.25% v v^{-1}) and 7.96 kBq radioactivity of ^{14}C-trifloxysulfuron (Nandula & Vencill, 2015). Once formulated, aluminum foil was removed and a 1µL droplet of the radioactive herbicide solution was placed on the adaxial surface of the previously covered second fully expanded leaf.

Plants were harvested 4, 12, 24, 48, 96, and 192 h after treatment (HAT) and dissected into four distinct parts: treated leaf, other leaves (other shoots), crown, and roots. In addition, 4 replicates of each population were spotted with ^{14}C-trifloxysulfuron and harvested immediately (0 HAT) to evaluate the efficiency of the leaf-wash technique. Treated leaves were rinsed slowly in a 20 mL 1:1 mixture of methanol-deionized water and 0.25% v v^{-1} nonionic surfactant to remove any nonabsorbed herbicide. A 1-mL aliquot of the leaf-wash solution was added to 20 mL of scintillation fluid (Cabron-14 Cocktail UN2924; Z Scientific, LLC., New City, NY) and radioactivity was quantified by liquid scintillation spectrometry (LSS) using a PerkinElmer Tri-Carb 2800TR Liquid Scintillation Analyzer (PerkinElmer Life and Analytical Sciences, Inc., Waltham, MA). After harvest, all plant parts were dried for 48 h at 60° C and combusted with a biological oxidizer (OX-500 Biological Material Oxidizer, R.J. Harvey Instrument Co., Tappan, NY) (Nandula & Vencill 2015). Radioactivity of the oxidized samples were quantified by LSS. Absorption and translocation studies were conducted in a completely randomized design with a factorial treatment arrangement including four replicates and two runs. Factorial levels included seven plant populations, six harvest timings (4, 12, 24, 48, 96, and 192 HAT), and four plant parts (treated leaf, other shoots, crown, and roots).

Statistical Analyses

Harvest timings and plant parts were considered fixed effects, while replications were analyzed as random effects as described by Besancon et al. (2017). The 0 h harvest interval was not included in the analysis as it ensured the efficiency of the treated leaf wash where 100.3% of applied ^{14}C-trifloxysulfuron was recovered. Statistical analyses were conducted by subjecting data to ANOVA ($\alpha = 0.05$) using PROC GLIMMIX in SAS (Version 9.4, SAS Institute, Inc. Cary, NC) and means were separated using Tukey's honestly significant procedure HSD ($\alpha = 0.05$).

Results and Discussion

Absorption

Absorption data were calculated as described by Troxler et al. (2003), where leaf wash recovery was subtracted from the total ^{14}C recovered from all plant parts. Experimental runs were not significant for ^{14}C-trifloxysulfuron absorption thus runs were pooled. ANOVA identified a significant two-way interaction of population by HAT (Table 2.2). For simplification, only the differences observed between resistant populations and the susceptible population will be discussed.

Differences were observed at 4 HAT, where the TN population absorbed 26.2% of ^{14}C-trifloxysulfuron compared to 13.9% in the susceptible population, indicating rapid absorption rates. This pattern aligns with previous studies by McElroy et al. (2004) and Troxler et al. (2003), which reported rapid trifloxysulfuron absorption in green kyllinga (*Kyllinga brevifolia*), false-green kyllinga (*Kyllinga gracillima*), and yellow nutsedge (*Cyperus esculentus* L.). By 12 HAT, the TN population continued this trend, absorbing 36.7% ^{14}C-trifloxysulfuron compared to 24.1% in the susceptible species. No other differences were observed at 4 and 12 HAT.

At 24 HAT, the FL population exhibited higher ^{14}C-trifloxysulfuron absorption (58.5%) compared to the susceptible species (38.3%), while the TN population showed a similar trend with 57.3% ^{14}C-trifloxysulfuron absorbed. In contrast, the VA population absorbed less ^{14}C-trifloxysulfuron (25.7%) than the susceptible species (38.3%), suggesting reduced absorption may contribute to the resistance of this population. Previous research has shown that resistant annual bluegrass populations have exhibited lower absorption rates of ALS herbicides. Specifically, Cross et al. (2013) reported reduced absorbance of trifloxysulfuron, foramsulfuron, and bispyribac-sodium in resistant annual bluegrass populations by 50% when compared to the non-treated control. However, many studies report target-site mutations as the main mechanism conferring resistance to ALS herbicides (Tranel & Wright, 2002). The mutation found in the ALS gene of the FL, TN, and NC populations resulting in a Trp574-Leu substitution, has been widely documented to confer resistance to ALS-inhibiting herbicides in annual bluegrass and other weed species (Singh et al., 2020; McElroy et al., 2013; Yu et al., 2014).

By 48 HAT, the FL population absorbed more ^{14}C-trifloxysulfuron (64.8%) compared to the susceptible species (43.3%), while the TN population showed a similar pattern with 64.4% absorption. At 96 HAT, the FL population absorbed more ^{14}C-trifloxysulfuron (62.1%) compared to the susceptible species (50.5%), while the TN population had the highest ^{14}C-trifloxysulfuron absorption at 68.2%. Again, the VA population continued to show lower ^{14}C-trifloxysulfuron absorption rates (35.0%) compared to the susceptible population (50.5%). These findings reinforce the possibility that reduced absorption contributes to resistance in the VA population.

By 192 HAT, the absorption rate in the FL and TN populations remained higher than the susceptible population (47.3%), with FL absorbing 63.6% ^{14}C-trifloxysulfuron and TN absorbing 60.4% ^{14}C-trifloxysulfuron. Again, the Virginia population continued to exhibit lower absorption

of ^{14}C-trifloxysulfuron compared to the susceptible population where only 33.7% was observed. These data suggest that reduced herbicide absorption may be contributing to resistance within the VA population. Our findings align with Brosnan et al. (2015), where nicosulfuron-resistant annual bluegrass biotypes exhibited reduced herbicide absorption when compared to the susceptible biotype.

While uncommon, previous studies have reported ALS-resistant annual bluegrass where a target site mutation is not present, suggesting the involvement of NTSR mechanisms (Singh et al. 2020). Overall, these findings indicate that reduced absorption is not a resistance mechanism in the FL and TN populations, which exhibited an ALS target-site mutation conferring resistance. However, the VA population, lacking the ALS mutation, showed significantly lower absorption rates at 24, 96, and 192 HAT. This absence of a target site mutation, along with reduced ^{14}C-trifloxysulfuron absorption, suggests the possibility of NTSR mechanisms in this particular annual bluegrass population. Unlike target-site mutations, which have been extensively studied, reduced absorption is not a common NTSR mechanism (Gaines et al. 2020). Additionally, the absence of statistical differences in absorption between the GA, NC, and SC populations and the susceptible population suggests that reduced absorption does not contribute to resistance in those populations. Resistance for the NC population is likely due to the presence of the Trp_{574}-Leu mutation. While no mutations were identified for the GA and SC populations, resistance may be caused by the NTSR mechanism of increased metabolism, which was not evaluated in this study.

Translocation

Interaction of plant part by HAT

Experimental runs were not significant for ^{14}C-trifloxysulfuron translocation thus runs were pooled. ANOVA identified a significant two-way interaction of plant part by HAT (Table 2. 3). The data are expressed as the percentage of ^{14}C-trifloxysulfuron absorbed by specific plant parts of all populations. The results indicate notable differences in ^{14}C-trifloxysulfuron distribution among the plant parts and across time points.

A greater amount of ^{14}C-trifloxysulfuron was observed in the treated leaf at 4 and 12 HAT (100%) compared to 24 HAT (90.3%). Similarly, greater ^{14}C-trifloxysulfuron was observed at the 24-hour harvest interval (90.3%) compared to 48 HAT (83.1%). This trend continued, where greater ^{14}C-trifloxysulfuron was observed in the treated leaf at 96 HAT (83.2%) compared to 192 HAT (80.3%).

In the shoots, the amount of ^{14}C-trifloxysulfuron increased numerically over time. At 4 and 12 HAT, 0% ^{14}C-trifloxysulfuron was found in the shoots compared to 24 HAT (9.7%). The highest amount of ^{14}C-trifloxysulfuron in the shoots was observed at 192 HAT (14.7%), surpassing all other time points (0, 12, 24, 48, and 96 HAT).

Detection of ^{14}C-trifloxysulfuron in the crown did not occur until 48 HAT, where 4.5% was observed. This finding differed from the 4, 12, and 24 HAT results. No other significant differences were observed up to 192 HAT. Similarly, the detection of ^{14}C-trifloxysulfuron in the roots was not observed until 48 HAT, where 1.7% was found. No other significant differences were observed between 48 and 192 HAT.

Reduced translocation has not been widely documented as a key resistance mechanism in ALS-resistant weed species with various studies suggesting that translocation of ALS inhibitors,

such as imazethapyr, does not significantly contribute to resistance in select weed species (Saari et al. 1994; Al-khatib et al. 1998). However, findings from this study observed reduced translocation to the other shoots when compared to the susceptible population in select resistant populations.

Interaction of plant part by population

ANOVA identified a significant two-way interaction of population by plant part (Table 2. 4). Data are presented as the percentage of ^{14}C-trifloxysulfuron absorbed into the plant, pooled over harvest intervals. A greater amount of ^{14}C-trifloxysulfuron was observed in the treated leaf of the FL population (92.7%) compared to the susceptible population (89.7%). Additionally, less ^{14}C-trifloxysulfuron was detected in the other shoots of the FL population (5.4%) compared to the susceptible population (7.8%). In contrast to these findings, Rey-Caballero et al. (2017) observed increased translocation of ^{14}C-tribenuron-methyl to other shoots in *Papaver rhoeas* resistant populations, with translocation ranging from 46.8% to 68.9% compared to 25.6% in susceptible populations.

Similarly, the GA population showed greater ^{14}C-trifloxysulfuron in the treated leaf (94.6%) compared to the susceptible population (88.2%), while a lower percentage of ^{14}C-trifloxysulfuron was translocated to the other shoots of the susceptible population (4.2%). Additionally, the SC population also showed greater amounts of ^{14}C-trifloxysulfuron in the treated leaf (96.0%) compared to the susceptible population (88.2%). Similarly, less ^{14}C-trifloxysulfuron was found in the other shoots (2.8%). These results suggest that reduced translocation may be a resistance mechanism in the FL, GA, and SC populations.

The VA population, exhibited a different pattern, with less ^{14}C-trifloxysulfuron retained in the treated leaf (76.8%) compared to the susceptible population (88.2%). Additionally, a

greater amount of ^{14}C-trifloxysulfuron was translocated to the other shoots of the VA population (18.3%). Since the VA population exhibited greater ^{14}C-trifloxysulfuron translocation, the higher accumulation in the other shoots suggests possible sequestration within specific plant tissues. Therefore, the role of reduced translocation related NTSR mechanisms in this population's resistance remains unclear.

In contrast, no translocation differences were observed in the TN and NC populations, suggesting that reduced translocation does not contribute to ALS resistance in these populations. Nonetheless, the findings of this study highlight the potential significance of reduced herbicide translocation in select annual bluegrass populations examined. Specifically, the FL, GA, and SC populations, which retained higher amounts of ^{14}C-trifloxysulfuron in treated leaves, translocating only 4 to 7.3% of the herbicide to other plant parts when compared to a susceptible population (11.8%). This pattern of reduced translocation aligns with previous studies involving glyphosate, across various species, including *Lolium rigidum*, *Conyza canadensis*, *Amaranthus palmeri*, *Amaranthus tuberculatus*, and *Sorghum halepense* (Lorraine-Colwill et al. 2002; Feng et al. 2004; Sammons & Gaines 2014).

Additionally, the GA and SC populations lack target-site mutations generally associated with ALS resistance (Yu et al. 2008). This absence of known target-site resistance mutations suggests that resistance is likely due to an NTSR mechanism such as reduced translocation. However, without definitive evidence separating the effects of herbicide mobility and metabolism from physiological adaptations in the resistant populations, the role of reduced translocation as a resistance mechanism remains unclear. Future research should investigate the effects of enhanced metabolism of ALS inhibiting herbicides in all evaluated populations.

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Tables

Table 2.1 Annual bluegrass (*Poa annua* L.) populations selected for ALS NTSR screening.[a,]

Population	Mutation [b]	EC_{50} (g ai ha^{-1}) [c]	EC_{90} (g ai ha^{-1}) [c]	R^2	Hillslope	R/S Ratio [d]
Florida	Trp_{574}-Leu	142.2	6,714.4	0.91	0.57	36.5
Georgia	None	23.1	1,459.0	0.96	0.53	5.9
North Carolina	Trp_{574}-Leu	54.4	4,406.4	0.98	0.50	13.9
South Carolina	None	4.9	1,190.7	0.98	0.41	1.3
Tennessee	Trp_{574}-Leu	57.8	12,284.2	0.90	0.41	14.8
Virginia	None	15.2	82.4	0.99	1.3	3.9
Susceptible		3.9	60.7	0.96	0.8	N/A

[a] Abbreviations: ALS, acetolactate synthase; NTSR, non-target site resistance.

[b] Genotyping experiments, conducted by the Herbicide Resistance Diagnostic lab at Auburn University

[c] $EC_{50} = \frac{EC_x}{[\frac{x}{100-x}]^{\frac{1}{B}}}$ where EC50 is effective concentration for 50% visual injury (%) and EC_x is effective concentration for x% (90%) Visual injury (%) and *B* is Hill slope

[d] R/S ratio individually compares the EC_{50} values for each resistant population with the susceptible population with a 1x rate of trifloxysulfuron equaling 27.75 g ai ha^{-1}.

Table 2.2 Two-way interaction of population by HAT on ^{14}C-trifloxysulfuron absorption in annual bluegrass (*Poa annua* L.) [a,b,c].

HAT	Florida	Georgia	North Carolina	South Carolina	Tennessee	Virginia	Susceptible
	% absorbed [c]						
4	14.8	15.3	10.5	11.1	26.2	20.7	13.9
12	27.7	23.3	21.1	28.0	36.7	30.9	24.1
24	58.5	40.7	38.3	34.3	57.3	25.7	38.3
48	64.8	52.1	35.0	44.6	64.4	31.9	43.3
96	62.1	56.6	48.4	51.5	68.2	35.0	50.5
192	63.6	52.3	48.1	53.9	60.4	33.7	47.3
$HSD_{(0.05)}$ [e]	11.6						

[a] Abbreviations: HAT, hours after treatment;

[b] The second fully expanded leaf of each population were treated with a 1µL droplet of ^{14}C-trifloxysulfuron dissolved in water, methanol and 0.25% (v/v) nonionic surfactant containing 7.96 kBq radioactivity.

[c] Data pooled over two experimental runs.

[d] % absorbed = $\frac{(total\ radioactivity\ in\ wash) - (\ total\ radioactivity\ in\ all\ plant\ parts)}{(total\ radioactivity\ applied)} \times 100$

[e] Tukey's test conducted at α= 0.05

Table 2.3 Two-way interaction of plant part by HAT on ^{14}C-trifloxysulfuron translocation in annual bluegrass (*Poa annua* L.) [a,b,c].

	HAT					
	4	12	24	48	96	192
Plant Part	% of absorbed [d]					
Treated leaf	100	100	90.3	83.1	83.2	80.3
Other shoots	0	0	9.7	10.7	12.0	14.7
Crown	0	0	0	4.5	3.4	3.7
Root	0	0	0	1.7	1.5	1.3
HSD $_{(0.05)}$ [e]	1.7					

[a] Abbreviations: HAT, hours after treatment;

[b] The second fully expanded leaf of each population were treated with a 1µL droplet of ^{14}C-trifloxysulfuron dissolved in water, methanol and 0.25% (v/v) nonionic surfactant containing 7.96 kBq radioactivity.

[c] Data pooled over two experimental runs, and seven populations.

[d] % of absorbed = $\frac{(total\ radioactivity\ in\ plant\ part)}{(total\ radioactivity\ absorbed)} \times 100$

[e] Tukey's test conducted at α= 0.05

Table 2.4 Two-way interaction of plant part by population on ^{14}C-trifloxysulfuron translocation in annual bluegrass (*Poa annua* L.) [a,b].

Plant Part	Florida	Georgia	North Carolina	South Carolina	Tennessee	Virginia	Susceptible
	% of absorbed [c]						
Treated leaf	92.7	94.6	89.7	96.0	88.4	76.8	88.2
Other shoots	5.4	4.2	6.8	2.8	9.7	18.3	7.8
Crown	1.5	0.9	2.5	0.7	1.3	3.9	2.7
Root	0.4	0.4	1.1	0.5	0.6	1.0	1.3
HSD $_{(0.05)}$[d]	2.2						

[a] The second fully expanded leaf of each population were treated with a 1µL droplet of ^{14}C-trifloxysulfuron dissolved in water, methanol and 0.25% (v/v) nonionic surfactant containing 7.96 kBq radioactivity.

[b] Data pooled over two experimental runs, and six harvest timings.

[c] % of absorbed = $\frac{(total\ radioactivity\ in\ plant\ part)}{(total\ radioactivity\ absorbed)} \times 100$

[d] Tukey's test conducted at α= 0.05

Figures

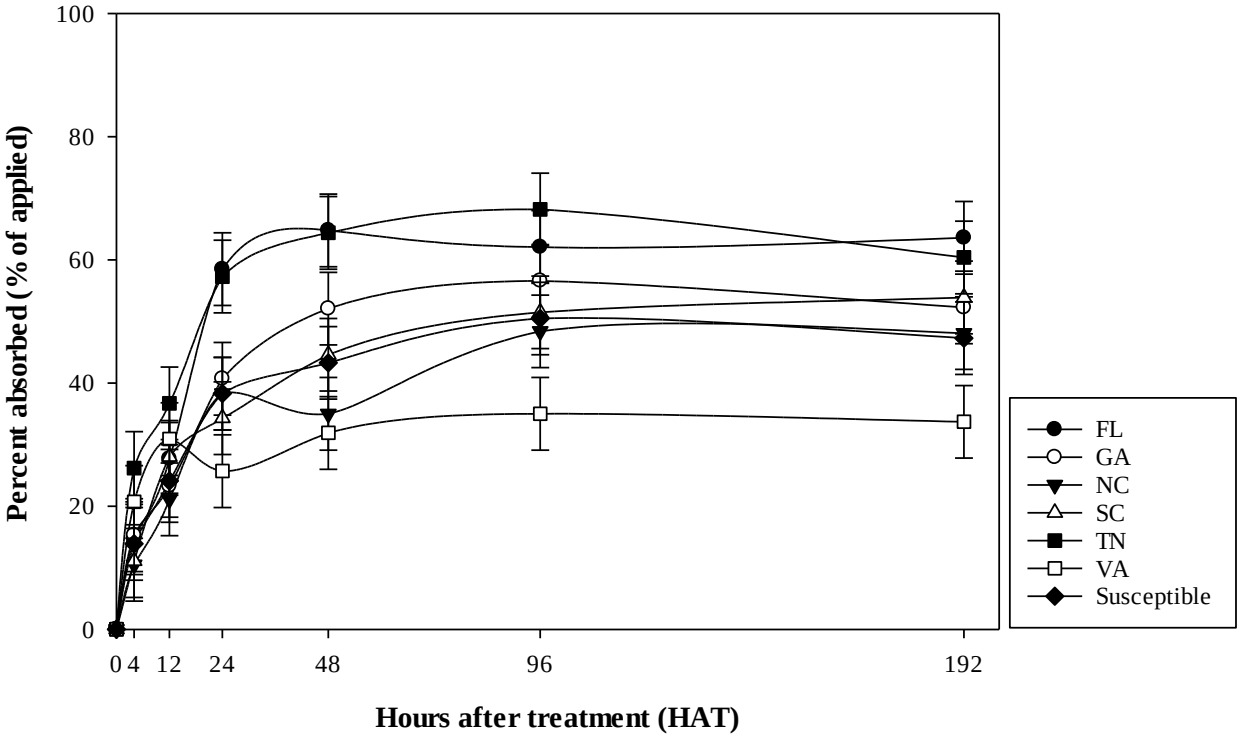

Figure 2.1 Percent absorbed ^{14}C-trifloxysulfuron in annual bluegrass (*Poa annua* L.) pooled experimental runs. Tukey's honestly significant difference procedure conducted at $\alpha = 0.05$. HSD= 11.6.

CHAPTER 3: Reduced Absorption and Translocation of Simazine and Glyphosate in Resistant Annual Bluegrass (*Poa annua* L.) Populations

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Abstract

Annual bluegrass is considered the most troublesome weed in turfgrass systems. Reports of both target site and non-target site herbicide resistance have been identified in this species. Despite its significance, limited reports of non-target site resistance (NTSR) mechanisms have been documented in turfgrass systems; therefore, this study aimed to determine if select annual bluegrass populations exhibited nontarget site resistance NTSR characteristics. Eight unique resistant annual bluegrass populations were identified for NTSR screening to either photosystem-II (PS-II) or 5-enolpyruvylshikimate-3-phosphate synthase (EPSP) herbicides. To evaluate NTSR mechanisms absorption and translocation experiments utilizing ^{14}C-herbicide were conducted. The PS-II NTSR screening identified four populations that exhibited reduced ^{14}C-simazine absorption compared to the susceptible population with differences observed as early as 12 hours after treatment (HAT). Notably, two of these populations showed reduced absorption without exhibiting the Ser_{264} to Gly mutation, which confers known resistance to PS-II inhibitors. In contrast, EPSP NTSR screening did not identify any populations with reduced absorption or translocation relative to the susceptible population. These results suggest that reduced absorption may contribute to resistance in four PS-II resistant populations, while no NTSR mechanisms were observed in the EPSP-resistant populations

Nomenclature: annual bluegrass, *Poa annua* L.; PS-II inhibiting herbicides; EPSP inhibiting herbicides

Key Words: Annual bluegrass, absorption, translocation, photosystem II, 5-enolpyruvylshikimate-3-phosphatesynthase

Introduction

Annual bluegrass (*Poa annua* L.) is a cool season annual grass that is considered the most troublesome weed in turfgrass systems (Van Wychen 2021). Annual bluegrass is typically managed using a combination of pre-emergence and postemergence herbicides (Ignes et al. 2023). However, herbicide-resistant populations of this species have been documented, with reports of resistance to nine different sites of action (SOA) (Heap 2024). Herbicide resistance in this species is frequently associated with photosystem II inhibitors (PS-II), such as atrazine and simazine, as well as enolpyruvylshikimate-3-phosphate synthase (EPSP) herbicides, like glyphosate (Baura et al. 2020; Binkholder et al. 2011; McElroy et al. 2013).

Photosystem II inhibitors (PS-II) are often used to control annual bluegrass in many warm season turfgrass systems (Vargas &Turgeon, 2004). PS-II inhibitors act through the binding of the plastoquinone site on the D1 protein in the PS-II complex of the chloroplast (Gronwald 1994). Blocking in this manner disrupts photosynthesis as the plastoquinone is required for electron transfer from PS-II to PS-I. Thus, this process impedes the generation of essential molecules, such as nicotinamide adenine dinucleotide phosphate (NADPH) and ATP essential for the plant to synthesize sugars and other molecules needed for growth (Jugulam & Shyam, 2019). Without NADPH and ATP, the plant cannot produce energy to survive, which ultimately leads to plant death Simazine (6-chloro-N,N′-diethyl-1,3,5-triazine-2,4-diamine) is a pre and early postemergence PS-II inhibiting triazine herbicide commonly used in turfgrass systems. Simazine is readily absorbed through roots and translocated to shoots via the xylem (Shaner 2014). While simazine is labeled for post-application control of annual bluegrass it is poorly absorbed by leaves and shows little basipetal translocation from the leaves (Shaner 2014).

Glyphosate (N-phosphonomethyl glycine), a non-selective, broad-spectrum herbicide, inhibits EPSP in the shikimate pathway. By substituting for phosphoenolpyruvate, glyphosate blocks the production of aromatic amino acids, such as tryptophan, tyrosine, and phenylalanine, which are essential for plant growth (Duke & Powles, 2008; Jugulam & Shyam, 2019). Typical glyphosate broadcast applications, in maintained turfgrass systems, occur in geographies that allow warm season grasses such as bermudagrass (*Cynodon* spp.) to enter winter dormancy (McCarty & Miller 2002). Additionally, glyphosate can be used in spot applications.

Herbicide resistance is categorized into two primary types: target-site resistance (TSR) and non-target site resistance (NTSR) (Gaines et al. 2020). TSR is commonly reported in resistant weed species (Yang et al. 2016). This manner of resistance is largely caused by gene mutations in target enzymes that affect a herbicide's ability to bind, thus reducing herbicide efficacy (Yang et al. 2016; Rigon et al. 2020). The less common NTSR imparts resistance through mechanisms such as increased herbicide metabolism or reduced absorption and/or translocation (Rigon et al. 2020).

Annual bluegrass has been reported to exhibit resistance to numerous herbicides, including PS-II inhibitors like simazine and non-selective herbicides including glyphosate (Brosnan et al. 2019). Reports of simazine resistance in annual bluegrass found that 43% of golf courses in Mississippi were affected by simazine-resistant populations (Hutto et al. 2004). This discovery led to further investigations, including a subsequent case of simazine resistance documented on a sod farm in Tennessee (Brosnan et al. 2017). Moreover, TSR studies have observed annual bluegrass with an altered *psbA* gene. This occurs when there is a change in the amino acid at position 264, where Serine (Ser) is replaced by glycine (Gly) (Ser_{264} to Gly). Annual bluegrass with this Ser_{264} to Gly mutation have exhibited resistance to the PS-II

inhibitors such as atrazine and amicarbazone (Svyantek et al. 2015). While limited, potential PS-II NTSR mechanisms have been documented in a population of annual bluegrass with the absence of the altered psbA gene (Svyantek et al. 2015).

Reports of glyphosate-resistant annual bluegrass populations have been documented on golf courses and other managed turfgrass systems across the US transition zone (Binkholder et al. 2011; Breeden et al. 2017; Brosnan et al. 2012; Cross et al. 2015). Glyphosate resistance in annual bluegrass is largely associated with TSR where an Ala substitution occurs at the Pro_{106} gene (Perez-Jones et al. 2007; Cross et al. 2015). In extreme instances, both TSR and NTSR mechanisms have been identified in select species making these resistant biotypes more difficult to control (Gains et al. 2020; Bai et al. 2019; Chen et al. 2019).

Despite growing recognition of NTSR mechanisms in herbicide-resistant weed populations, our understanding of these mechanisms in annual bluegrass remains limited. Therefore, to elucidate the prevalence of NTSR mechanisms in annual bluegrass biotypes, six PS-II herbicide resistant populations and two glyphosate resistant populations underwent absorption and translocation experiments utilizing ^{14}C-simazine and ^{14}C-glyphosate.

Materials and Methods

Population Selection

As part of a multi-state survey of golf courses, athletic fields, and sod farms, annual bluegrass populations that survived a field application of either a PS-II inhibiting herbicide or glyphosate were collected. Once collected, a preliminary dose-response evaluation was conducted. Under greenhouse conditions, plants were treated with either a 2.24 kg ai ha^{-1} of simazine or 840 g ae ha^{-1} of glyphosate. Resistance evaluations reflected the methodology of

Burgos (2015) where percent control is compared to the nontreated check. Populations that exhibited resistance (0 to 49% injury) underwent a comprehensive dose response experiment where resistant populations were treated with herbicide rates ranging from 0.125x to 8x rate of simazine or glyphosate.

The visual injury response data for annual bluegrass were analyzed using analysis of variance (ANOVA) with PROC GLIMMIX in SAS (SAS/STAT 9.4, SAS Institute, Cary, NC). Tukey's Honestly Significant Difference (HSD) test was applied to compare means and determine the significance of the fixed effect of herbicide rate at $\alpha = 0.05$. Since experimental runs were not significant, data were pooled. A four-parameter response curve was used to model the relationship between plant response (y) (visual injury estimation) and the herbicide rate (x):

$$Y = Bottom + \frac{(Top - Bottom) * X^{Hillslope}}{EC_{50}^{Hillslope} + X^{Hillslope}} \quad [1]$$

This model describes the relationship between visual injury (%) (y) and herbicide rate (x). It uses a dose-response curve, bounded by a lower plateau (bottom) and an upper plateau (top). The ED_{50} represents the herbicide rate needed to achieve a 50% injury between these plateaus. The model can be used to determine the herbicide rate needed for any desired level of injury, represented by this EC_{50} function:

$$EC_{50} = \frac{EC_x}{[\frac{x}{100-x}]^{\frac{1}{B}}} \quad [2]$$

The EC_x value represents the herbicide dose needed to achieve a specific percentage (x) of the maximum possible effect, considering the minimum and maximum response levels. In this study, nonlinear regression using GraphPad Prism software (version 10.0.0 for Windows, GraphPad Software, San Diego, CA) was used to fit the dose-response curve and calculate the herbicide rate that resulted in 90% visual injury (EC_{90}). Following resistance screening, resistant

populations underwent genotyping experiments, conducted by the Herbicide Resistance Diagnostic lab at Auburn University, where known target site mutations that confer resistance to PS-II and EPSPS-inhibiting herbicides were identified. Several populations in the NTSR screening exhibited the Ser264 to Gly mutation during PS-II screening (Table 3.1) and the Pro106 to Ala substitution for EPSPS screening (Table 3.2), both of which have been observed in PS-II and glyphosate-resistant populations (Brunharo et al. 2019; Singh et al. 2021).

Plant Material

To investigate NTSR mechanisms, annual bluegrass plants from previously identified resistant populations were established in controlled environments. Plants were initially grown in the North Carolina State University Phytotron located at GPS coordinates 35.787ºN, -78.672ºW. A constant 18.4°C (day/night) temperature was set, under a 12-hr photoperiod with light provided by 18 T5 54W fluorescent lamps averaging 450 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The growth medium consisted of a 50/50 mix of Sun Gro Propagation Growing Mix (Canadian sphagnum peat moss, vermiculite, dolomitic lime, and silicon dioxide) and cement sand by weight, filled into conetainers (3.8 cm diameter, 21 cm depth). Three seeds were sown per container and thinned to one plant after germination (Nandula &Vencill 2015). Plants were irrigated twice daily. At the 3-leaf stage, plants were moved to a laboratory growth chamber (20±2°C) for a 1wk acclimation period before herbicide treatment.

Absorption and Translocation

Prior to treatment, the second youngest fully expanded leaf of each plant was covered with aluminum foil, following the method described by Troxler et al. 2003. Herbicide applications were made using a CO_2-pressurized sprayer with an 8002 EVS nozzle, calibrated to deliver 187 L ha. Simazine (2.24 kg ai ha^{-1}) was applied to populations selected for PS-II NTSR

screening, and glyphosate (840 g ae ha^{-1}) was applied to those selected for EPSPS NTSR screening.

For PS-II studies, technical grade [simazine ring-$^{14}C(U)$]-simazine with 592,000 kBq mg^{-1} specific activity, 99% radioactive purity was used. The EPSPS studies used technical grade [glycine-2-^{14}C]-glyphosate with 1,850,000 kBq mg^{-1} specific activity, 99% radioactive purity. Radioactive treatment solutions were prepared as a 1:1 mixture of HPLC-grade water and methanol, plus 0.25% v v^{-1} non-ionic surfactant. The solution contained either 6.26 or 4.84 kBq of ^{14}C-simazine or ^{14}C-glyphosate, respectively. A 1µL droplet of the radioactive solution was applied to the adaxial surface of the previously covered second fully expanded leaf.

Plants were harvested at 4, 12, 24, 48, 96, and 192 hours after treatment (HAT) and divided into treated leaf, other leaves (shoots), crown, and roots. Four replicates of each population, treated with either ^{14}C-simazine or ^{14}C-glyphosate, were also harvested immediately after application (0 HAT) to assess the leaf-wash efficiency. Treated leaves were rinsed with 20 mL of a 1:1 methanol-deionized water solution containing 0.25% v v^{-1} nonionic surfactant. A 1-mL aliquot of the rinse solution was then mixed with 20 mL of scintillation fluid and analyzed by liquid scintillation spectrometry (LSS) using a PerkinElmer Tri-Carb 2800TR liquid scintillation analyzer. All harvested plant parts were dried at 60°C for 48 hours and then combusted using a biological oxidizer (OX-500 Biological Material Oxidizer, R.J. Harvey Instrument Co., Tappan, NY) (Nandula &Vencill 2015). The radioactivity of the combusted samples was also measured by LSS. Absorption and translocation experiments were conducted using a completely randomized design with a factorial arrangement of treatments. Experiments included four replicates and were conducted with two runs. The factors considered were plant populations, six harvest times (4, 12, 24, 48, 96, and HAT), and four plant parts (treated leaf,

other shoots, crown, and roots). Harvest time and plant part were treated as fixed effects, while replicates were considered random effects. Statistical analyses were conducted by subjecting data to ANOVA (α=0.05) using PROC GLIMMIX in SAS (Version 9.4, SAS Institute, Inc. Cary NC) and means were separated using Tukey's honestly significant procedure HSD (α =0.05)

Results and Discussion

^{14}C-simazine Absorption and Translocation

Absorption studies revealed the Alabama, Mississippi, Georgia-49, and Virginia populations absorbed less ^{14}C-simazine than the susceptible populations at several time points (Table 3.3) Beginning at 4 HAT the Mississippi population absorbed only 16.2% ^{14}C-simazine highlighting differences compared to the susceptible population (28.9%). Similarly, the Alabama population absorbed less ^{14}C-simazine (15.6%) compared to the susceptible population. This trend continues, where the Georgia-49 population absorbed less herbicide (12.6%) in comparison to the susceptible population.

Observations at 12 HAT revealed that the Virginia (25.0%), Mississippi (21.3%), Alabama (24.4%), and Georgia-49 (27.4%) populations absorbed less ^{14}C-simazine compared to the susceptible population (40.4%), while Georgia-10 absorbed more ^{14}C-simazine (59.3%) than the susceptible population. No other differences were observed between the susceptible and the Tennessee population at this time point. At 24 HAT the susceptible population absorbed 60.9% ^{14}C-simazine, greater than the Virginia (32.8%), Mississippi (29.5%), Alabama (31.9%), and Georgia- 49 (36.8%) populations. Similarly, at 48 HAT the susceptible population absorbed 60.9% ^{14}C-simazine greater than the Virginia (40.3%), Mississippi (39.1%), Alabama (40.2%), and Georgia- 49 (41.1%) populations.

At 96 HAT, the susceptible population absorbed greater ^{14}C-simazine (69.7%) in comparison to Virginia (28.8 %), Mississippi (34.5%), Alabama (34.4%), and Georgia- 49 (42.2%) populations. Results are consistent with Sing et al. (2015) who reported 33% ^{14}C-atrazine absorbed in seashore paspalum (*Paspalum vaginatum* Sw.) 3 days after application. The decrease in absorption in the Virginia populations from 48 HAT (32.8%) to 96 HAT (28.8%) is attributed to withholding irrigation after ^{14}C-simazine application. Trends continue 192 HAT where less ^{14}C-simazine absorbed in the Virginia (40.2%), Mississippi (37.0%), Alabama (40.1%), and Georgia- 49 (49.3%) populations when compared to the susceptible (75.6%) population. Our findings align with Svyantek et al. (2016) who reported two annual bluegrass biotypes with a Ser_{264} to Gly mutation absorbed on average 43% and 48% ^{14}C-atrazine over 168 hours.

The consistent decreased absorption of ^{14}C-simazine in the Virginia, Mississippi, Alabama, and Georgia-49 populations, when compared to the susceptible population strongly suggest that reduced absorption may contribute to their resistance. Furthermore, the Mississippi, Alabama, and Tennessee populations possess the Ser_{264} to Gly substitution known to confer resistance to PS-II inhibiting herbicides (Dayan et al. 2015; Hutto et al. 2004; Perry et al. 2012; Yu et al. 2013). By contrast, the Virginia and Georgia-49 populations do not exhibit the Ser_{264} to Gly mutation while consistently demonstrating reduced absorption of ^{14}C-simazine compared to the susceptible population. Reduced absorption of ^{14}C-atrazine for a population without a Ser_{264} to Gly substitution was reported by Svyantek et al. (2016) where only 21% absorption was observed.

Translocation data are presented as depicted by Ou et al. (2016) where the total ^{14}C-simazine recovered in individual plant parts is divided by the total radioactivity recovered in the plant. Experimental runs were not significant for ^{14}C-simazine translocation thus runs were

pooled. ANOVA identified a significant two-way interaction of plant part by HAT (Table 3.4) where the percentage of ^{14}C-simazine recovered in specific plant parts is pooled over all populations. Additionally, ANOVA identified a significant two-way interaction of population by plant part (Table 3.5).

Table 3.4 shows the percentage of ^{14}C-simazine recovered by specific plant parts of all populations. These results indicate notable differences in ^{14}C-simazine distribution among the treated leaf and non-absorbed ^{14}C-simazine recovered in the wash. Focusing specifically on radioactivity recovered in the leaf wash, ^{14}C-simazine decreased over time. At 4 HAT , 75.7% ^{14}C-simazine was recovered, which decreased to 62.5% at 12 HAT. Trends continue where 48.3% ^{14}C-simazine was found in the leaf wash 24 HAT. By 48 HAT, 44.4 % radioactivity was recovered which decreased to 37.7% 96 HAT. The lowest recovery was observed at 192 HAT where 31.9% of the ^{14}C-simazine remained non-absorbed. By contrast, the amount of radioactivity found in the treated leaf increased at each sampling time. Initially at 4 HAT, 24.3 % ^{14}C-simazine was recovered, which increased to 37.2% at 12 HAT. Trends continued through the duration of the experiment where 50.9% was observed 24 HAT, 54.6% at 48 HAT, and 59.1% at 96 HAT. The greatest ^{14}C-simazine was observed at 192 HAT where 62.0% was recovered. Translocation to other shoots was observed 96 and 192 HAT where 2.6% and 5.1% were recovered, respectively. These data align with Wilson et al. (2009), which reported 69% of applied ^{14}C-simazine was detected in the whole plant of *Canna hybrida* 7 days after exposure. No other differences were observed amongst the other plant parts (other shoots, crown, and roots).

Differences in ^{14}C-simazine recovered were only significant for the treated leaf wash (non-absorbed) and the treated leaf (Table 3.5). As seen in the absorption data, only 38.3%

simazine was recovered in the treated leaf wash of the susceptible population, less than the percentage recovered in the Virginia (58.2%), Mississippi (34.1%), Alabama (60.7%), the Georgia-49 (56.5%) populations. Less ^{14}C-simazine was observed in the Georgia-10 (33.6%) population when compared to the susceptible population. Additionally, greater simazine was recovered in the treated leaf of the susceptible population (60.7%) when compared to the Virginia (58.2%), Mississippi (34.1%), Alabama (37.0%), Georgia-49 (41.5%) populations. Interestingly greater simazine recovery was observed in the treated leaf of the Georgia-10 where 64.2% was recovered compared to 60.7% in the susceptible population. No other differences were observed amongst the populations in the remaining plant parts (other shoots, crown, and roots). Previous studies report less translocation to the shoots of a PS-II resistant population of annual bluegrass. Svyantek et al. 2016 reported a resistant annual bluegrass translocated 12% less ^{14}C- atrazine to other shoots when compared to a susceptible population. Low translocation out of the treated leaf, as seen in this study, may be attributed to simazine having little to no basipetal translocation from the leaves of plants (Shaner 2014). Since the only observable differences in translocation occurred between the leaf wash and radioactivity in the treated leaf, these data suggest that reduced translocation is not contributing to resistance in these populations.

^{14}C-glyphosate absorption and translocation

The ^{14}C-glyphosate absorption data were calculated in the same manner as previously described, where leaf wash recovery was subtracted from the total ^{14}C recovered from all plant parts. Experimental runs and the main effect of population were not significant for ^{14}C-glyphosate absorption; thus, data were pooled over runs and population. ANOVA identified a significant difference for the main effect of HAT (Table 3.6).

Differences were observed at 4 HAT, where 11.3% ^{14}C-glyphosate was absorbed which increased to 26.4% by 12 HAT. Absorption rates continue to increase over time where 49.3% ^{14}C-glyphosate was absorbed by 24 HAT. Absorption was similar at 48 HAT where 58.8% ^{14}C-glyphosate was absorbed. Absorption rates continued to increase slightly through the duration of the experiment where maximum absorption occurred at 192 HAT where 63.8 % ^{14}C-glyphosate was observed. These data are consistent with Brunharo et al. (2019) where 69% and 67% ^{14}C-glyphosate was absorbed in a susceptible and resistant biotype of annual bluegrass 193 HAT, respectively.

Unlike PS-II, there were no apparent differences in absorption rates in comparing resistant and susceptible populations. Similar to our findings, Baura et al. (2021) reported slight variations in absorption amongst select glyphosate-resistant annual bluegrass biotypes; however, differences were not observed when comparing resistant and susceptible biotypes.While numerical differences were observed, absorption rates were not different between resistant and susceptible populations (Brunharo et al. 2019). With no clear differences between resistant and susceptible annual bluegrass biotypes, these data suggest that reduced absorption is not contributing to glyphosate resistance in the screened populations.

Translocation data were analyzed in the same manner as the ^{14}C-simazine translocation experiments. Experimental runs were not significant for ^{14}C-glyphosate translocation so runs were pooled. ANOVA identified a significant two-way interaction of plant part by HAT (Table 3.7) where the percentage of ^{14}C-glyphosate recovered in specific plant parts is pooled over all populations.

These results indicate notable differences in ^{14}C-glyphosate distribution among the treated leaf and the non-absorbed ^{14}C recovered in the wash. Focusing specifically on radioactivity recovered in the leaf wash, ^{14}C-glyphosate decreased over time, although

differences were not observed after 48 HAT. At 4 HAT 88.6% of the ^{14}C-glyphosate remained unabsorbed. This decreased to 70.4% by 12 HAT. By 24 HAT, 47.6 % ^{14}C-glyphosate was recovered, which decreased to 28.6% by 48 HAT. By 192 HAT, only 23.3% ^{14}C was found unabsorbed in the treated leaf wash.

By contrast, the amount of ^{14}C-glyphosate recovered from the treated leaf increased over time. Initially, 7.5% ^{14}C-glyphosate was recovered by 4 HAT which rapidly increased to 24.5% by 12 HAT. Radioactivity recovery doubled by 24 HAT where 50.8% was found in the treated leaf. At 48 HAT 64.7% ^{14}C-glyphosate was recovered. By 192 HAT, 72.1% of the recovered ^{14}C-glyphosate was found in the treated leaf. These data agree with previous reports where 75 and 68% of recovered ^{14}C-glyphosate was observed in sicklepod (*Senna obtusifolia*) 48 and 96 HAT respectively (Walker & Oliver 2008). Additionally, previous studies show glyphosate tends to accumulate in high amounts in the treated leaf of the plant (Adu-Yeboah et al. 2020; Baruara et al. 2022; Zelaya et al. 2004). No other differences were observed amongst the other plant parts (other shoots, crown, and roots).

While reduced absorption and translocation play a role in glyphosate resistance, reduced translocation is thought to be the primary NTSR mechanism for glyphosate resistance (Baerson et al. 2002). Reduced translocation was not observed in this study; however, previous reports have observed no difference in glyphosate absorption among 11 biotypes of susceptible and resistant *C. canadensis* (Feng et al. 2004). Interestingly, the South Carolina population exhibited resistance but did not contain the Pro_{106} to Ala target site mutation. While it remains unclear why this population is exhibiting glyphosate resistance, the possibility of increased metabolism of glyphosate should be evaluated. The lack of significant differences in translocation between the

resistant and susceptible populations, suggests reduced absorption or translocation does not contribute to glyphosate resistance in these evaluated populations.

In conclusion, this study suggests that the Georgia-49, Alabama, Georgia-10, and Mississippi populations exhibited reduced absorption of ^{14}C-simazine compared to the susceptible population, indicating the presence of NTSR mechanisms. The ^{14}C-glyphosate data, however, did not suggest the presence of NTSR mechanisms in the screened populations. Additionally, the findings indicate that the NTSR mechanism of reduced absorption is present in simazine resistant annual bluegrass populations. Furthermore, these data suggest that NTSR and TSR mechanisms may not operate independently in certain populations screened with ^{14}C-simazine, highlighting the complex nature of resistance mechanisms in select populations.

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Conflicts of Interest

No conflicts of interest have been declared.

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Tables

Table 3.1 Annual bluegrass (*Poa annua* L.) populations selected for PS-II NTSR screening [a].

Population	Mutation [b]	EC_{50} (kg ai ha^{-1}) [c]	EC_{90} (kg ai ha^{-1}) [c]	R^2	Hillslope	R/S Ratio [d]
Alabama	Ser_{264} to Gly	2.0	6.9	0.99	1.78	6.1
Georgia-10	None	4.9	21.4	0.98	1.49	16.3
Georgia-49	None	2.6	8.8	0.99	1.80	8.7
Mississippi	Ser_{264} to Gly	1.5	3.7	0.99	2.41	5.0
Tennessee	Ser_{264} to Gly	1.5	4.4	0.99	2.04	5.0
Virginia	None	5.6	11.2	0.99	3.18	16.9
Susceptible	N/A	0.3	0.33	0.89	26.4	N/A

[a] Abbreviations: PS-II, photosystem II; NTSR, non-target site.

[b] Genotyping experiments, conducted by the Herbicide Resistance Diagnostic lab at Auburn University

[c] $EC_{50} = \frac{EC_x}{[\frac{x}{100-x}]^{\frac{1}{B}}}$ where EC_{50} is effective concentration for 50% visual injury (%) and EC_x is effective concentration for x% (90%) Visual injury (%) and *B* is Hill slope

[d] R/S ratio individually compares the EC_{50} values for each resistant population with the susceptible population with a 1x rate of simazine equaling 2.24 kg ai/ha.

Table 3.2 Annual bluegrass (*Poa annua* L.) populations selected for EPSPS NTSR screening [a].

Population	Mutation [b]	EC_{50} (g ae ha^{-1}) [c]	EC_{90} (g ae ha^{-1}) [c]	R^2	Hillslope	R/S Ratio [d]
North Carolina	Pro_{106} to Ala	357.9	2303.7	0.99	1.18	2.5
South Carolina	None	509.3	32,168.5	0.61	0.53	3.5
Susceptible	N/A	141.5	409.01	0.99	2.07	N/A

[a] Abbreviations: EPSPS, enolpyruvylshikimate-3-phosphate synthase; NTSR, non-target site resistance.

[b] Genotyping experiments, conducted by the Herbicide Resistance Diagnostic lab at Auburn University.

[c] $EC_{50} = \frac{EC_x}{[\frac{x}{100-x}]^{\frac{1}{B}}}$ where EC_{50} is effective concentration for 50% visual injury (%) and EC_x is effective concentration for x% (90%) Visual injury (%) and *B* is Hill slope.

[d] R/S ratio individually compares the EC_{50} values for each resistant population with the susceptible population with a 1x rate of glyphosate at 840 g ae ha^{-1}.

Table 3.3 Two-way interaction of population by hours after treatment on ^{14}C-simazine absorption in annual bluegrass (*Poa annua* L.) [a].

HAT	Alabama	Georgia-10	Georgia-49	Mississippi	Tennessee	Virginia	Susceptible
	% absorbed [b]						
4	15.6	34.4	12.6	16.2	38.6	18.3	28.9
12	24.4	59.3	27.4	21.3	45.3	25.0	40.4
24	31.9	66.9	36.8	29.5	66.5	32.8	60.9
48	40.2	67.1	41.1	39.1	58.1	40.3	69.2
96	34.4	65.5	42.2	34.5	70.7	28.8	69.7
192	40.1	70.7	49.3	37.0	68.9	40.2	75.6
$HSD_{(0.05)}$ [c]	10.9						

[a] Data pooled over two experimental runs.

[b] % absorbed = absorbed = $\frac{(total\ radioactivity\ in\ wash) - (\ total\ radioactivity\ in\ all\ plant\ parts)}{(total\ radioactivity\ applied)} \times 100$

[c] Tukey's honestly significant difference test conducted at $\alpha = 0.05$

Table 3.4 Two-way interaction of plant part by hours after treatment on ^{14}C-simazine translocation in annual bluegrass (*Poa annua* L.) [a,b].

	HAT					
	4	12	24	48	96	192
Plant Part	% of recovered [c]					
Leaf wash	75.7	62.5	48.3	44.4	37.6	31.9
Treated leaf	24.3	37.2	50.9	54.6	59.1	62.0
Other shoots	0	0.3	0.8	0.7	2.6	5.1
Crown	0	0	0	0.2	0.4	0.5
Root	0	0	0	0.1	0.2	0.4
HSD $_{(0.05)}$ [d]	2.9					

[a] The second fully expanded leaf of each population were treated with a 1µL droplet of ^{14}C-simazine dissolved in water, methanol and 0.25% (v/v) nonionic surfactant containing 6.26 kBq radioactivity.

[b] Data pooled over two experimental runs and seven populations.

[c] % of recovered = $\frac{(total\ radioactivity\ in\ plant\ part\ or\ leaf\ wash)}{(total\ radioactivity\ recovered\ in\ plant\ and\ leaf\ wash)} \times 100$

[d] Tukey's honestly significant difference test at α= 0.05

Table 3.5 Two-way interaction of plant part by population on ^{14}C-simazine translocation in annual bluegrass (*Poa annua* L.) [a,b].

Plant Part	Alabama	Georgia-10	Georgia-49	Mississippi	Tennessee	Virginia	Susceptible
	% of recovered [c]						
Leaf wash	60.7	33.6	56.5	65.1	38.0	58.2	38.3
Treated leaf	37.0	64.2	41.5	34.1	59.7	38.9	60.7
Other shoots	2.0	1.3	1.8	0.7	1.7	2.8	0.7
Crown	0.2	0.4	0.0	0.1	0.4	0.1	0.1
Root	0.1	0.3	0.0	0.0	0.2	0.0	0.2
HSD $_{(0.05)}$[d]	3.2						

[a] The second fully expanded leaf of each population were treated with a 1µL droplet of ^{14}C-simazine dissolved in water methanol and 0.25% v v^{-1} nonionic surfactant containing 6.26 kBq radioactivity.

[b] Data pooled over two experimental runs, and six harvest timings.

[c] % of recovered = $\frac{(total\ radioactivity\ in\ plant\ part\ or\ leaf\ wash)}{(total\ radioactivity\ recovered\ in\ plant\ and\ leaf\ wash)} \times 100$

[d] Tukey's honestly significant difference test conducted at α= 0.05

Table 3.6 Main Effect of hours after treatment on ^{14}C-glyphosate absorption in annual bluegrass (*Poa annua* L.) [a,b].

HAT	% of absorbed [c]
4	11.3
12	26.4
24	49.3
48	58.8
96	60.6
192	63.8
$HSD_{0.05)}$[d]	10.5

[a] The second fully expanded leaf of each population were treated with a 1µL droplet of ^{14}C-glyphosate dissolved in water, methanol and 0.25% (v/v) nonionic surfactant containing 4.84 kBq radioactivity.

[b] Data pooled over two experimental runs, and three populations.

[c] % of absorbed = absorbed = $\frac{(total\ radioactivity\ in\ wash) - (\ total\ radioactivity\ in\ all\ plant\ parts)}{(total\ radioactivity\ applied)} \times 100$

[d] Tukey's honestly significant difference test conducted at $\alpha = 0.05$

Table 3.7 Two-way interaction of plant part by hours after treatment on ^{14}C-glyphosate translocation in annual bluegrass (*Poa annua* L.) [a,b].

	HAT					
	4	12	24	48	96	192
Plant Part	% of recovered [c]					
Leaf wash	88.6	70.4	47.6	28.6	28.5	23.3
Treated leaf	7.5	24.5	50.8	64.7	67.1	72.1
Other shoots	1.9	2.8	0.5	5.3	1.4	1.6
Crown	0.7	0.9	0.4	0.5	1.3	1.4
Root	0.9	1.4	0.7	0.9	1.6	1.6
HSD $_{(0.05)}$ [d]	6.3					

[a] The second fully expanded leaf of each population were treated with a 1µL droplet of ^{14}C-glyphosate dissolved in water methanol and 0.25% v v $^{-1}$ nonionic surfactant containing 4.84 kBq radioactivity.

[b] Data pooled over two experimental runs and three populations.

[c] % of recovered = $\frac{(total\ radioactivity\ in\ plant\ part\ or\ leaf\ wash)}{(total\ radioactivity\ recovered\ in\ plant\ and\ leaf\ wash)} \times 100$

[d] Tukey's honestly significant difference test conducted at α= 0.05

Figures

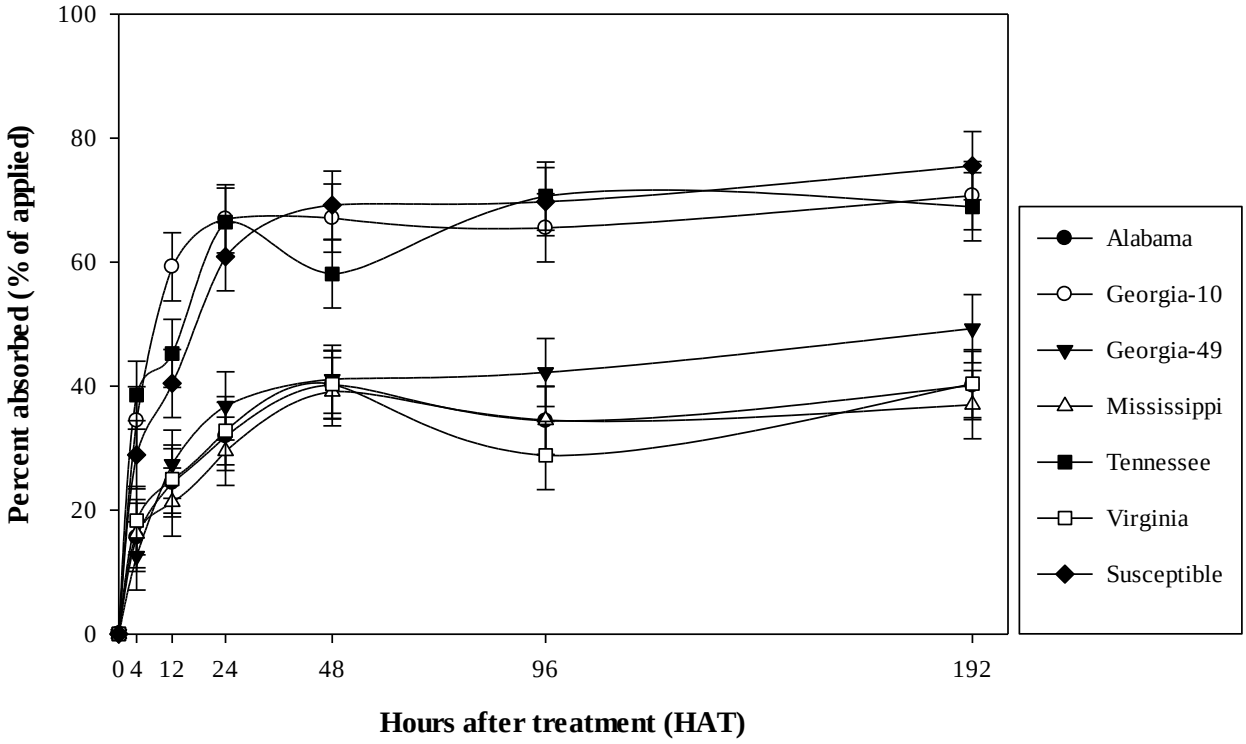

Figure 3.1 Percent ^{14}C-simazine absorbed in annual bluegrass (*Poa annua* L.) pooled over two experimental runs. Tukey's honestly significant difference procedure conducted at $\alpha = 0.05$. HSD= 10.9.

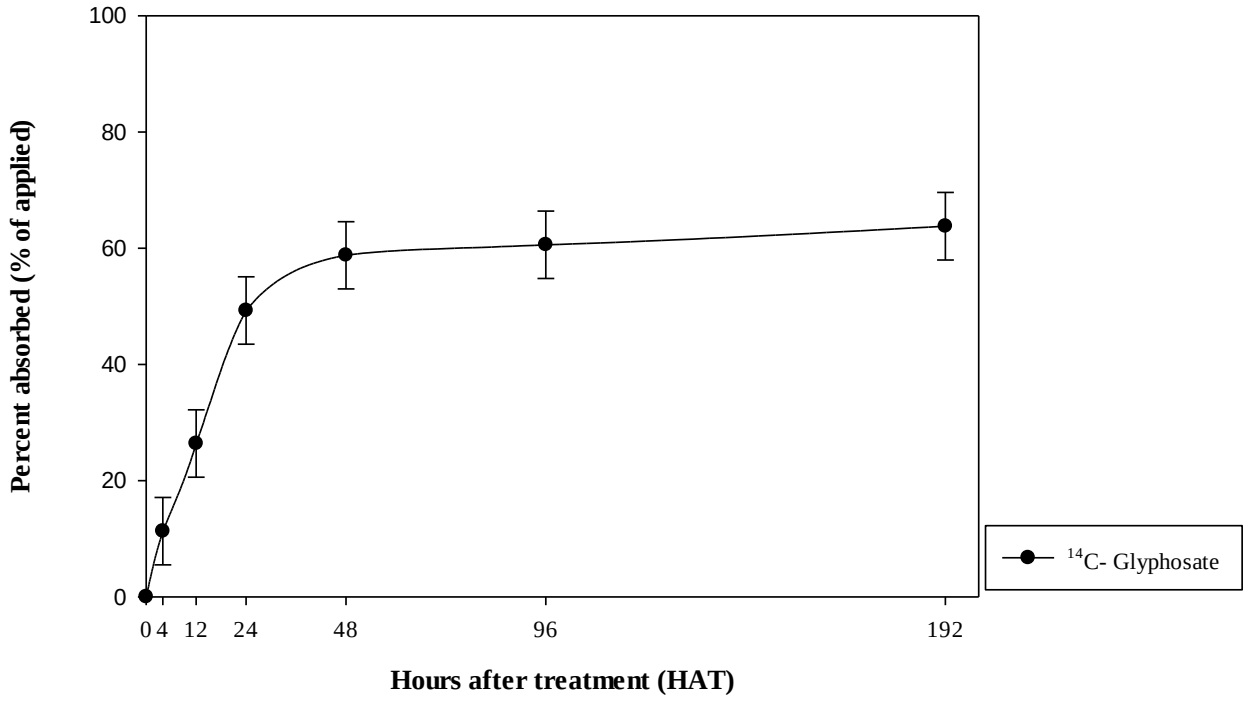

Figure 3.2 Percent ^{14}C-glyphosate absorbed in annual bluegrass (*Poa annua* L.) pooled over two experimental runs and three populations. Tukey's honestly significant difference procedure conducted at $\alpha = 0.05$. HSD= 10.8.

Chapter 4: Effect of Trinexapac-ethyl on Foramsulfuron Absorption and Translocation in Annual Bluegrass (*Poa annua* L.)

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Abstract

Foramsulfuron is a postemergence herbicide used to control broadleaf and grassy weeds in select turfgrass systems. Many warm-season turfgrass species exhibit tolerance to foramsulfuron; however, unlike many other sulfonylurea herbicides, foramsulfuron is absorbed primarily through plant foliage. Annual bluegrass (*Poa annua* L.) is a cool season grass that is problematic in turfgrass systems. Plant growth regulators (PGRs) are commonly used in turfgrass management to reduce mowing frequency and suppress seedhead production in turfgrasses and annual weeds such as annual bluegrass. Trinexapac-ethyl (TE), a commonly used PGR, inhibits shoot growth and suppresses seedhead production. The TE label suggests its use for seedhead suppression or renovation of areas infested with annual bluegrass. Previous studies have reported conflicting effects of tank-mixing PGRs with herbicides, ranging from synergistic to antagonistic or no effect. This study evaluated the influence of TE on the absorption and translocation of foramsulfuron in annual bluegrass. Results indicated no significant difference in total foramsulfuron absorbed between treatments; however, rapid foliar absorption was observed, with 39.5% absorbed by 4 hours after treatment (HAT) and maximum absorption (83.6%) achieved at 96 HAT. Translocation studies revealed a significant treatment effect. Foramsulfuron combined with TE showed greater retention in the treated leaf (90.3%) compared to foramsulfuron applied alone (85.5%). Additionally, TE reduced translocation to other shoots, with 7.5% translocated when tank mixed with foramsulfuron compared to 11.3% in the foramsulfuron alone treatment. These findings suggest TE influences foramsulfuron translocation in annual bluegrass, potentially altering its efficacy.

Nomenclature: Foramsulfuron, Trinexapac-ethyl, annual bluegrass, *Poa annua* L., herbicide, plant growth regulators; ALS-inhibitor herbicides

Key Words: acetolactate synthase, herbicide, plant growth regulators, sulfonylurea

Introduction

Annual bluegrass (*Poa anna* L.) is a problematic weed in turfgrass systems (Tang et al. 2020). Prolific seedhead production from annual bluegrass is known for reducing turfgrass aesthetics, functionality, and overall quality (McCullough & Hart 2010; Carroll et al. 2021). Single plants have been reported to produce up to 2200 seeds per season (McCarty 1999). The high fecundity of annual bluegrass leads to significant seed accumulation in the soil seedbank, further complicating management efforts (Busey 2003; Shem-tov & Fennimore 2003).

Plant growth regulators (PGRs) are chemicals frequently employed by professional turfgrass managers to suppress plant growth or seedhead production (Fagerness & Penner 1998). Trinexapac- ethyl (TE) [ethyl 4-cyclopropyl [hydroxy]methylidene)-3,5-dioxocyclohexane-1-carboxylate] is a common PGR that inhibits gibberellic acid biosynthesis as well as ethylene formation, which reduces cell elongation and ultimately shoot growth (Bearss et al. 2020). Additionally, TE is labeled for conversion, renovation, or seedhead suppression of annual bluegrass (Anonymous 2020).

The acetolactate synthase inhibiting (ALS) sulfonylurea herbicide, foramsulfuron, is labeled for POST control of annual bluegrass (Anonymous 2024). Foramsulfuron can be safely applied in select warm-season turf, with previous reports observing no injury in bermudagrass cover (McElroy et al. 2005). Previous foramsulfuron studies have shown rapid absorption ranging between 55% and 70% into the treated leaf within 48 hours in various weed species (Henry et al. 2008; Bunting et al. 2004). Foramsulfuron is mobile in the xylem and phloem; however, translocation has been reported to be minimal with less than 10% moving from the treated leaf to other plant parts (Henry et al. 2008; Shanner 2014).

Current management strategies in turfgrass systems recommend the rotation of herbicides with differing modes of action (MOAs), as well as utilizing herbicides with multiple MOAs within each growing season (Beckie & Reboud, 2009). Due to the limited MOAs available in turfgrass systems, practitioners must adopt a multifaceted approach to weed management strategies, rather than relying solely on herbicides (Allen et al. 2022).

Specific tank mixes have been reported to improve herbicide efficacy (Dodds et al. 2007). Previous studies have investigated the effect of tank mixing herbicides with TE. McCullough and Hart (2010) reported increased uptake of the ALS-inhibiting herbicide bispyribac-sodium when tank mixed with TE, thus providing a synergistic effect. Conversely, Bearss et al. (2020) reported TE mixed with the POST herbicides fenoxaprop, quinclorac, or mesotrione did not affect smooth crabgrass (*Digitaria ischaemum*) control. Additionally, a study involving PGR's tank mixed with glyphosate and metsulfuron reported antagonistic effects in alligatorweed (*Alternanthera philoxeroides*) control (Clements et al. 2012). These conflicting results raise questions as to whether TE would provide a synergistic or antagonistic effect on foramsulfuron and other herbicides commonly used in grass systems. To further explore, a study was conducted to evaluate the impact of TE on foramsulfuron absorption and translocation in annual bluegrass.

Materials and Methods

Plant Material

Annual bluegrass seeds, susceptible to ALS- inhibiting herbicides, Plants were grown in the North Carolina State University Phytotron C-chambers located at GPS coordinates 35.787ºN, -78.672ºW. A constant day/night temperature of 18.4°C was set. The lighting source consisted of 18 T5 54W fluorescent lamps averaging 450 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 12h photoperiod. The soil

medium consisted of 50% Sun Gro Propagation Growing Mix (Canadian sphagnum peat moss 50-65%, vermiculite, dolomitic lime 0.0001% silicon dioxide) and 50% cement sand by weight. Conetainers, with a cell diameter of 3.8 cm and depth of 21 cm were filled with the amended soil. Three seeds were planted directly into the pots and later thinned to 1 plant per conetainer. When plants reached the two-leaf stage, plants were transferred to a laboratory growth chamber with a 20±2°C temperature. Plants were allowed to acclimate for 1 week before treatment. Before treatment, the second youngest leaf was marked and covered with aluminum foil as described by Nandula & Vencill (2015). TE and herbicide applications were made using a CO_2-pressurized sprayer equipped with an 8002 EVS nozzle (TeeJet Technologies Spraying Systems Co., Glendale Heights, IL, USA) calibrated to deliver 187 L ha. Half of the plants were treated with TE at 94.6 g ai ha^{-1} tank mixed with foramsulfuron at 28.9 g ai ha^{-1}. The remaining plants were treated with foramsulfuron alone at 28.9 g ai ha^{-1}.

Absorption and Translocation

Two solutions were utilized in this study, one formulated with foramsulfuron alone and another formulated with a mix of foramsulfuron and TE. For both solutions, technical grade [phenyl-UL-^{14}C]-foramsulfuron with a specific activity of 2,008,730 kBq mg^{-1} and 99.0% radioactive purity was used. Radioactive solutions for each treatment consisted of a 1:1 v v^{-1} mixture of high-performance liquid chromatography (HPLC)-grade water and methanol plus non-ionic surfactant (0.25% v v^{-1}) and 3.10 kBq radioactivity of ^{14}C-foramsulfuron. A 1µL droplet of radioactive herbicide solution was placed on the adaxial surface of the previously covered second fully expanded leaf.

Absorption and translocation studies were conducted in a completely randomized design with a treatment arrangement including four replicates and two runs. Factorial levels included

two populations, six harvest timings (4, 12, 24, 48, 96, and 192 HAT), and four plant parts (treated leaf, other shoots, crown, and roots). Four replicates of each treatment were treated with ^{14}C-foramsulfuron and immediately harvested (0 HAT) to assess the efficiency of the leaf-wash technique. Treated leaves were rinsed with 20 mL of a 1:1 methanol-deionized water mixture containing 0.25% v v^{-1} nonionic surfactant to remove any non-absorbed herbicide. A 1-mL aliquot of the leaf-wash solution was pipetted into 20 mL of scintillation fluid (Cabron-14 Cocktail UN2924; Z Scientific, LLC., New City, NY), and radioactivity was measured using liquid scintillation spectrometry (LSS) with a PerkinElmer Tri-Carb 2800TR Liquid Scintillation Analyzer (PerkinElmer Life and Analytical Sciences, Inc., Waltham, MA).

Following harvest, all plant parts were dried at 60°C for 48 hours and combusted using a biological oxidizer (OX-500 Biological Material Oxidizer, R.J. Harvey Instrument Co., Tappan, NY). Radioactivity in the oxidized samples was quantified by LSS.

Statistical Analyses

Harvest timings and plant parts were treated as fixed effects, while replications were analyzed as random effects, as described by Besancon et al. (2017). The 0-hour harvest interval was excluded from the analysis, as it validated the effectiveness of the treated leaf wash, recovering 100% of the applied ^{14}C-foramsulfuron. Statistical analyses were performed using ANOVA ($\alpha = 0.05$) in PROC GLIMMIX (SAS Version 9.4, SAS Institute, Inc., Cary, NC), and mean comparisons were conducted using Tukey's Honestly Significant Difference (HSD) test ($\alpha = 0.05$).

Results and Discussion

Absorption

Absorption data were calculated as described by Troxler et al. (2003), where leaf wash recovery was subtracted from the total ^{14}C recovered from all plant parts. Experimental runs and treatment effects were not significant for ^{14}C-foramsulfuron absorption, thus runs and treatments were pooled. ANOVA identified the main effect of HAT on ^{14}C-foramsulfuron absorption (Figure 4.1).

Rapid absorption of ^{14}C-foramsulfuron was observed by 4 HAT (35.9%). By 12 HAT, the total ^{14}C-foramsulfuron absorption increased to 54.1%. By 24 HAT, ^{14}C-foramsulfuron absorption increased to 67.5%. Absorption trends continue to increase where at 48 HAT 77.3% ^{14}C-foramsulfuron was observed. Maximum foliar absorption was observed at 96 HAT where 83.6% ^{14}C-foramsulfuron was observed. While absorption rates varied slightly between 48 and 192 HAT, no other statistical differences were observed through these harvest intervals.

These data are similar to Henry et al. (2008) where an accumulation of foramsulfuron was found to be 69% at 48 HAT in dallisgrass (*Paspalum dilatatum* L.) pretreated with foramsulfuron. Moreover, Bunting et al. (2004) documented 65 to 70% absorption of ^{14}C-foramsulfuron after 24h in two hybrid corn varieties. Additionally, absorption trends are similar to reports of other sulfonylurea herbicides, where McElroy et al. (2004) documented that green kyllinga (*Kyllinga brevifolia* Rottb.) and false-green kyllinga (*Kyllinga gracillima* L.) readily absorbed ^{14}C-trifloxysulfuron during the initial 4-hour period following application. Absorption rates were similar to the results described by McCullough & Hart (2010), which reported that 30% ^{14}C-bispyribac-sodium absorption in annual bluegrass was observed 8 HAT. Reports of rimsulfuron absorption in black nightshade (*Solanum nigrum* L.), eastern black nightshade

(*Solanum ptycanthum* L.), and hairy nightshade (*Solanum physalifolium* L.) ranged between 54 and 71% ^{14}C 48 HAT (Ackley et al. 1999). Additional reports show high absorption of nicosulfuron and primosulfuron in broadleaf signalgrass (*Brachiaria platyphylla* L.) where 61 and 79% was observed 72 HAT, respectively (Gallaher et al. 1999).

While these data show similar absorption rates to previous studies using ^{14}C-foramsulfuron, the similarities between the TE plus foramsulfuron treatment and foramsulfuron applied alone suggest that TE does not influence foramsulfuron absorption in annual bluegrass. Findings are further supported by Bearss et al. (2020), who reported that tank mixes of TE and fenoxaprop, quinclorac, and mesotrione did not affect smooth crabgrass control.

Translocation

Experimental runs were not significant for ^{14}C-foramsulfuron translocation, thus runs were pooled. ANOVA identified a significant two-way interaction of plant part by HAT (Table 4. 1). Data are presented as the percent of ^{14}C-foramsulfuron observed in specific plant parts, pooled across both treatments (Table 4.1). Additionally, ANOVA identified a significant two-way interaction of treatment by plant part (Table 4.2). All translocation data are presented as percentage of ^{14}C-foramsulfuron absorbed into the plant as described by Besancon et al. (2017)

The significant two-way interaction of plant part by HAT indicate notable differences in ^{14}C-foramsulfuron translocation across different time points (Table 1). Differences were observed starting at 12 HAT where the percentage of ^{14}C-foramsulfuron in the treated leaf decreased from 100% to 90.2% at 24 HAT. This trend continued, with ^{14}C-foramsulfuron in the treated leaf declining to 78.0% at 48 HAT. However, by 96 HAT the amount of ^{14}C-foramsulfuron in the treated leaf increased to 82.8% before decreasing again to 76.6% at 192 HAT.

Translocation of ^{14}C-foramsulfuron to the other shoots was detected 24 HAT, where 9.7% ^{14}C-foramsulfuron was observed. This trend continues where the translocation of ^{14}C-foramsulfuron to the other shoots increased to 15.6% 48 HAT. The amount of ^{14}C-foramsulfuron translocated to the crown increased from 0% at 24 HAT to 4.5% 48 HAT. No other differences were observed in the other shoots, crown or roots at any subsequent time point.

The two-way interaction of treatment by plant part (Table 2), showed differences in the translocation between TE plus foramsulfuron and foramsulfuron applied alone. The TE plus foramsulfuron treatment showed greater ^{14}C-foramsulfuron in the treated leaf (90.3%) compared to foramsulfuron applied alone (85.5%). Conversely, greater radioactivity was observed in the other shoots of the foramsulfuron alone treatment (11.3%) compared to the TE plus foramsulfuron (7.5%). These data suggest the addition of TE may impact the amount of foramsulfuron translocated out of the treated leaf.

While translocation of foramsulfuron to the other shoots was slightly lower than the tank mix of TE plus foramsulfuron, the total amount of foramsulfuron translocation was still higher than what has been reported in previous studies. Henry et al. (2008) reported only 0.62% foramsulfuron translocating in other shoots below the treated leaf in dallisgrass. Additionally, only 0.7% to 1.5% foramsulfuron was translocated out of the treated leaf of two hybrid corn varieties (Bunting et al. 2004). In comparison, translocation findings are more consistent with other sulfonylurea herbicides such as metsulfuron where translocation in barley (*Hordeum vulgare* L.) and wheat was observed to be 15 and 10%, respectively, 6 hours after treatment (King et al. 2003). Similarly, McElroy et al. (2004) reported 36.3% of halosulfuron and 22.1% of trifloxysulfuron translocation to the other shoots of green kyllinga and false green kyllinga, respectively.

Findings of this study suggest that the addition of TE to a foramsulfuron application does not affect foramsulfuron absorption by annual bluegrass. However, these data suggest that translocation out of the treated leaf may be decreased if TE is included with foramsulfuron application. It remains unclear if decreased translocation of foramsulfuron will influence herbicide efficacy. Further research is needed to evaluate the impacts of TE on foramsufluron metabolism in annual bluegrass. Additionally, field studies are needed to assess whether the inclusion of TE influences foramsulfuron efficacy in controlling annual bluegrass and other weeds.

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Conflicts of Interest

No conflicts of interest have been declared.

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Tables

Table 4.1 Two-way interaction of plant part by HAT on ^{14}C-foramsulfuron translocation in annual bluegrass (*Poa annua* L.) [a,b,c].

	HAT					
	4	12	24	48	96	192
Plant Part	% of absorbed [d]					
Treated leaf	100	100	90.2	78.0	82.8	76.6
Other shoots	0	0	9.7	15.6	13.6	17.3
Crown	0	0	0	4.5	2.2	3.8
Root	0	0	0	2.1	1.3	2.4
HSD $_{(0.05)}$ [e]	4.3					

[a] Abbreviations: HAT, hours after treatment.

[b] The second fully expanded leaf of each population were treated with a 1µL droplet of ^{14}C-foramsulfuron dissolved in water-methanol and 0.25% v v $^{-1}$ nonionic surfactant containing 3.1 kBq radioactivity.

[c] Data pooled over two experimental runs, and two treatments.

[d] % of absorbed = $\frac{(total\ radioactivity\ in\ plant\ part)}{(total\ radioactivity\ absorbed)} \times 100$

[e] Tukey's honestly significant difference procedure conducted at $\alpha = 0.05$

Table 4.2 Effect of trinexapac-ethyl on ^{14}C-foramsulfuron translocation in annual bluegrass (*Poa annua* L.) [a,b].

Plant Part	Foramsulfuron	Trinexapac-ethyl + foramsulfuron
	% of absorbed [c]	
Treated leaf	85.5	90.3
Other shoots	11.3	7.5
Crown	1.9	1.5
Root	1.3	0.7
LSD $_{(0.05)}$[d]	2.4	

[a] Data pooled over two experimental runs, and six harvest timings.

[b] The second fully expanded leaf of each population were treated with a 1µL droplet of ^{14}C-foramsulfuron dissolved in water-methanol and 0.25% v v $^{-1}$ nonionic surfactant containing 3.1 kBq radioactivity.

[c] % of absorbed = $\frac{(total\ radioactivity\ in\ plant\ part)}{(total\ radioactivity\ absorbed)} \times 100$

[d] Tukey's honestly significant difference procedure conducted at $\alpha = 0.05$

Figures

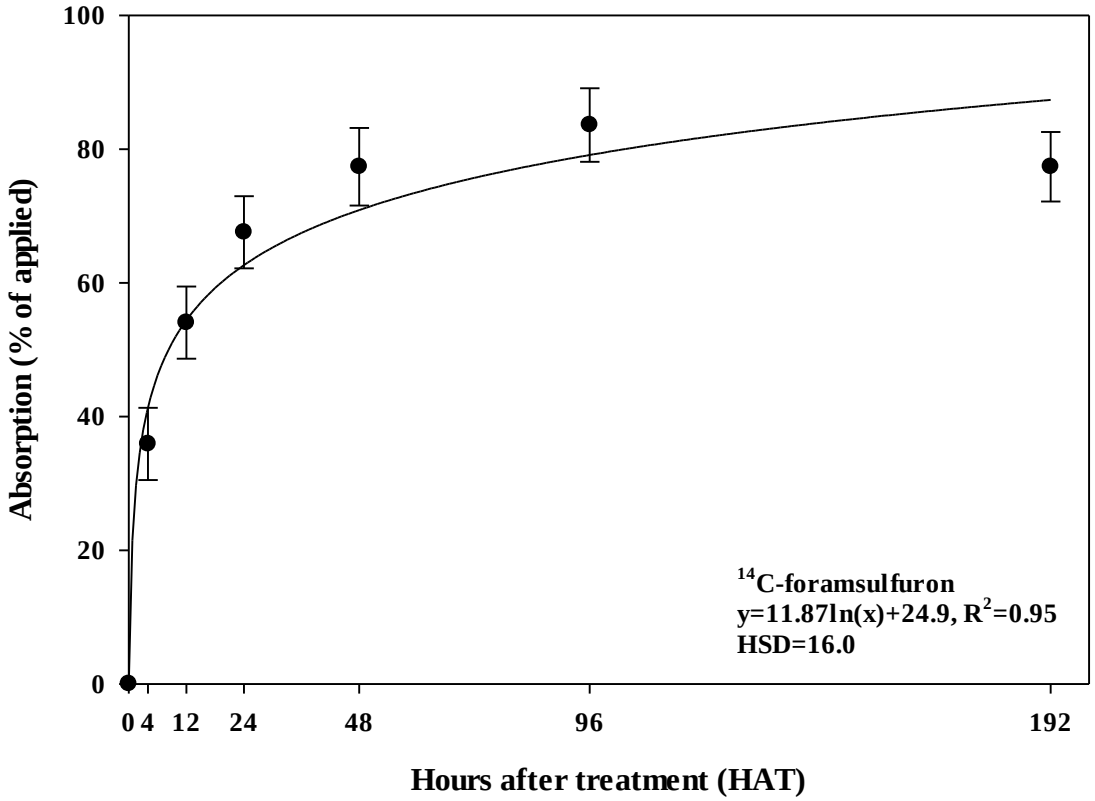

Figure 4.1 Percent absorbed ^{14}C-foramsulfuron in annual bluegrass (*Poa annua* L.) pooled over treatments and experimental runs. Tukey's honestly significant difference procedure conducted at $\alpha = 0.05$. HSD=16.0.

Chapter V: Characterization of Herbicide Resistant Annual Bluegrass Populations in North Carolina (*Poa annua* L.)

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Abstract

Annual bluegrass is a problematic weed in managed turfgrass systems. Photosystem II (PS-II), Acetolactate synthase (ALS), and enolpyruvylshikimate-3-phosphate synthase (EPSPS) inhibiting herbicides are commonly used to control annual bluegrass in turfgrass systems. Reports of ALS-resistant annual bluegrass have been documented at golf courses in South Carolina, Georgia, and Tennessee. Furthermore, reports of PS-II and glyphosate resistant populations have been documented in much of the southeastern United States. Following a statewide survey, research was initiated at North Carolina State University to determine resistance levels of eleven unique annual bluegrass populations that survived a field application of either a PS-II, ALS, or EPSPS-inhibiting herbicide. Biotypes were established in a greenhouse and subsequently treated in dose-response experiments using either simazine, trifloxysulfuron, or glyphosate. Simazine dose response screenings revealed three populations that exhibited between a 6 and 15-fold resistance ratio when compared to the susceptible population. Trifloxysulfuron visual injury data revealed four populations with resistance ratios ranging between 3 and 110. Glyphosate visual injury data revealed three populations exhibiting resistance ratios between 1.9 and 181.4. The populations screened represented not only golf courses but also athletic fields and home lawns. The documented resistance levels across diverse turf environments further emphasize the significance of this issue and the potential challenges for effective weed control in the future. Additionally, these findings highlight the widespread issue of herbicide resistance in annual bluegrass populations in North Carolina.

Nomenclature: annual bluegrass, *Poa annua* L.; ALS-inhibiting herbicides, PS-II inhibiting herbicides; EPSPS inhibiting herbicides

Key Words: Annual bluegrass, acetolactate synthase, sulfonylurea, glyphosate, photosystem II, herbicide resistance

Introduction

Annual bluegrass (*Poa annua* L.) is a pervasive and problematic weed in warm-season and cool-season turfgrass systems (Brosnan et al. 2015). Annual bluegrass is considered the most problematic herbicide-resistant weed in turfgrass systems, with resistance reported to nine unique modes of action (MOAs) (Heap 2024). Herbicide-resistant biotypes of annual bluegrass ultimately compromise turfgrass systems, where they reduce turfgrass aesthetic, quality, and playability (Beard et al. 1978; McCarty 2011; Brosnan et al. 2020).

Herbicide resistance in annual bluegrass has been observed for many herbicide modes of action (MOAs), including photosystem II inhibitors (e.g., atrazine, simazine), acetolactate synthase (ALS) inhibitors (e.g., trifloxysulfuron, foramsulfuron), microtubule inhibitors (e.g., prodiamine), and enolpyruvylshikimate-3-phosphate synthase (EPSPS) inhibitors (e.g., glyphosate) (Baura et al. 2020; Binkholder et al. 2011; McElroy et al. 2013). Among these, ALS inhibitors are commonly used in managed turfgrass systems, particularly in the southern and transitional regions of the United States, where resistance is widespread (Brosnan et al. 2015; Brosnan et al. 2020; Cross et al. 2015). Cases of ALS-resistant populations have been documented in Alabama, Tennessee, and Mississippi (Heap 2024), with resistance often associated with target-site mutations on the ALS gene (McElroy et al. 2013). ALS-inhibiting herbicides such as trifloxysufluron are effective in controlling annual bluegrass, as well as sedge, broadleaf, and grassy weeds (Ferrell et al. 2004). However, continuous use of the same herbicidal mode of action without rotation has led to the selection of resistant biotypes (Tranel and Wright 2002; Yu et al. 2010). The development of resistance to ALS-inhibiting herbicides is further influenced by the inherent characteristics of ALS enzymes and their corresponding genes (Vijayarajan et al. 2023).

Annual bluegrass has been reported to exhibit resistance to PS-II inhibitors like simazine and non-selective herbicides like glyphosate (Brosnan et al. 2020). The first domestic report of herbicide-resistant annual bluegrass on a golf course was documented in Mississippi where the populations survived an application of the PS-II inhibiting herbicide simazine (Kelly et al. 1999). Reports of simazine resistance in annual bluegrass found that 43% of golf courses in Mississippi were affected by simazine-resistant populations (Hutto et al. 2004). This discovery led to further investigations, including a subsequent case of simazine resistance documented on a sod farm in Tennessee (Brosnan et al. 2017). In Texas, an annual bluegrass population exhibited three-way resistance to trifloxysuflruon, simazine, and pronamide (Singh et al. 2017). Similarly, a population collected in Australia exhibited resistance to simazine, rimsulfuron, foramsulfuron, endothall, and pinoxaden herbicides (Barua et al. 2020). In Tennessee, a population collected from a golf course exhibited resistance to prodiamine and glyphosate (Breeden et al. 2017).

In North Carolina, annual bluegrass populations have been reported to exhibit resistance to PS-II inhibiting herbicides and dinitroaniline herbicides (Isgrigg et al. 2002). While ALS, EPSPS-resistant annual bluegrass has been documented in neighboring states, the prevalence and extent of ALS, PS-II and EPSPS-resistant biotypes in North Carolina remains unclear. Therefore, the objective of this research was to quantify the level of annual bluegrass resistance to three herbicides including simazine, trifloxysulfuron, and glyphosate. Suspected resistant biotypes of annual bluegrass were collected from golf courses, athletic fields, and one home lawn following the survival of a field application of ALS-inhibiting, PS-II or EPSPS herbicide. Selected biotypes were then evaluated through a whole-plant dose-response experiment.

Materials and Methods

Population Collection

A statewide survey was conducted to collect potentially resistant biotypes of annual bluegrass. Efforts focused on collecting samples from golf courses, athletic fields, sod farms, and home lawns. The populations collected survived prior field applications of either an ALS-inhibiting herbicide (e.g., foramsulfuron, trifloxysulfuron), PS-II inhibiting herbicide (e.g. simazine, atrazine.), or glyphosate. Two populations selected for PS-II dose-response were collected from unique golf course fairways (Table 5.1). An additional population collected from an athletic field was included in PS-II resistance screenings. Among the biotypes collected for ALS dose-response screening, three populations from warm-season golf course tees exhibited survival after field applications of foramsulfuron (Table 5.2). An additional population was collected from a professionally maintained home lawn after surviving a field application of trifloxysulfuron (Table 5.2). Three of the populations selected for glyphosate resistance screening were collected from athletic fields. An additional population from a golf course fairway was included for resistance screening (Table 5.3).

Dose-Response Study Under Greenhouse Conditions

Greenhouse experiments were conducted at the North Carolina State University Method Road greenhouse facilities located at GPS coordinates 35.787ºN, -78.694ºW. Five seeds from the suspected resistant biotypes were sown into separate 10 by 10 cm pots containing a soil mixture. The soil medium consisted of 5% Sun Gro Propagation Growing Mix (Canadian sphagnum peat moss 50-65%, vermiculite, dolomitic lime 0.0001% silicon dioxide) and 95% cement sand by weight. Plants were irrigated twice daily to maintain optimal growing conditions. A known susceptible annual bluegrass population was included for comparison purposes for all MOAs.

Greenhouse conditions were maintained at 24°C/18°C day/night temperatures with a 12-hour photoperiod, supplemented with artificial lighting. Before herbicide treatment, pots were thinned to one plant per pot. A randomized complete block design was used, with herbicide rates blocked within populations. Each treatment was replicated four times per herbicide rate, and the experiment was repeated twice.

Herbicide Application

Herbicide applications were made using a CO_2-pressurized sprayer equipped with an 8002 EVS nozzle (TeeJet Technologies Spraying Systems Co., Glendale Heights, IL, USA), calibrated to deliver 187 L ha^{-1}. Plants were treated with subsequent rates of herbicide ranging from 0 to 32x the maximum labeled rate of herbicide. Plants screened for PS-II inhibiting herbicides were treated with simazine at 0, 1.2, 2.2, 4.8, 9.6, 19.3, 38.7, 77.44 kg ai ha^{-1}. Populations screened for ALS resistance were treated with trifloxysulfuron applied at 0, 0.013, 0.027, 0.05, 0.11, 0.22, 0.44, 0.88 kg ai ha^{-1}, and a non-ionic surfactant (NIS; Induce®, Helena Chemical Co., Memphis, TN) at 0.25% (v v^{-1}). For EPSPS resistance screening glyphosate was applied at 0, 0.35, 0.7,1.4,2.8,5.6,11.2 and 22.4 kg ai ha^{-1}.

Data Collection and Statistical Analyses

Visual estimates of herbicide injury were recorded at 7, 14, 21, and 28 days after treatment (DAT). Injury was visually estimated on a percent scale from 0% (no injury) to 100% (complete plant death). At 28 DAT, aboveground vegetation was harvested and weighed to obtain fresh biomass data. The fresh biomass was then oven-dried at 60°C for 96 hours to determine dry biomass weights. Dry-weight biomass was converted to percent reduction using the equation:

$$\%reduction = \left[\frac{NT-T}{NT}\right] \text{x } 100 \quad [1]$$

Statistical analyses were performed using ANOVA (α = 0.05) in PROC GLIMMIX in SAS (Version 9.4, SAS Institute, Cary, NC). Means were separated using Tukey's Honestly Significant Difference (HSD) procedure (α = 0.05). Percent dry biomass reduction data identified a significant two-way interaction between population and herbicide rate for PS-II and ALS screenings. Additionally, the ALS and EPSPS dose response screenings identified a significant two-way interaction between herbicide rate and population for percentage visual control 28 days after treatment (DAT). A four-parameter response curve was used to describe the relationship between the herbicide rate (x) and the measured plant response (y).

$$Y = Bottom + \frac{(Top-Bottom)*X^{Hillslope}}{EC_{50}^{Hillslope}+X^{Hillslope}} \quad [2]$$

For PS-II, ALS, and EPSPS dose-response screening, y represents the % biomass reduction, and x represents the herbicide rate. For ALS and EPSPS dose-response screening, the visual estimation of injury (%) is represented by y and the herbicide rate is represented by x. The top and bottom are plateaus. The EC_{50} denotes the dose required to produce a 50% response between top and bottom limits. The dose-response level can be expressed by the function EC_{50}:

$$EC_{50} = \frac{EC_x}{[\frac{x}{100-x}]^{\frac{1}{B}}} \quad [3]$$

Where EC_x represents the effective dose that elicits x% response between the upper and lower limits of the curve. This equation was used to calculate the effective herbicide rate that provided 90% visual injury or 90% dry biomass reduction (EC_{90}). Curve fitting was performed via nonlinear regression methods with GraphPad Prism (version 10.0.0 for Windows, GraphPad Software, San Diego, CA).

Results and Discussion

PS-II Dose Response

Hill slopes, EC_{50} and EC_{90} values are presented for the measured responses by herbicide rate for each population (Tables 4-6). Table 4 presents the PS-II dry biomass reduction data. Annual bluegrass control varied among the susceptible and suspected resistant populations screened for simazine resistance. The EC_{50} values for simazine were 1.8, 3.4, and 4.5 kg ai ha^{-1} for the P-706, P-709, and P-744 biotypes, respectively (Table 5.4). The calculated R/S ratios for the tested biotypes ranged from 6 to 15. In contrast, resistance to PS-II herbicides in the United States has been documented at much higher levels, sometimes exceeding 1,000-fold (Brosnan et al. 2015; Kelly et al. 1999; Syvantek et al. 2016).

The EC_{90} values followed a similar trend among the suspected resistant biotypes, with the P-744 population exhibiting the highest EC_{90} rate at 8.9 kg ai ha^{-1}. Additionally, the EC_{90} values align with Brosnan et al. (2015), who reported that simazine application between 0.14 and 9.0 kg ai ha^{-1} resulted in $\leq$ 20% control of certain annual bluegrass biotypes. Much of the previous research on PS-II resistant annual bluegrass has focused on golf course populations. Specifically, a survey of Mississippi golf courses revealed the presence of simazine resistant annual bluegrass in 18 of 20 courses screened (Hutto et al. 2004). Interestingly, the P-744 population screened in this study was collected from an athletic field, suggesting that resistance to simazine is not limited to golf courses in North Carolina.

ALS Dose Response

Table 5.5 presents the ALS dry biomass reduction data. The EC_{50} values were 0.12, 0.79, 1.06, and 0.07 kg ai ha^{-1} for the A-361, A-413, A-432, and A-473 populations, respectively. The

calculated R/S values ranged from 3.5 to 39.5. EC_{90} values follow similar trends to the EC_{50} values with the suspected resistant populations ranging from 0.13 to 1.75 kg ai ha^{-1}.

ANOVA identified a significant two-way interaction between trifloxysulfuron rate and population for percent visual injury (Table 5.6). The EC_{50} values were 0.19, 2.2, 0.06, and 0.08 kg ai ha^{-1} for the A-361, A-413, A-432, and A-473 populations, respectively (Table 5.5). These values are consistent with previous reports that found trifloxysulfuron applications ranging from 3.5 to 223 g ai ha^{-1} provided only 40% control of resistant annual bluegrass populations (Brosnan et al. 2015). The calculated R/S values ranged from 3 to 110. These values align with previous findings, such as Singh et al. (2020), who reported an 84-fold increase in resistance in an annual bluegrass population collected from a Texas golf course. Similarly, Yu et al. (2010) reported a high resistance factor (>1,333) for ALS-inhibiting herbicides in a rigid ryegrass population.

The EC_{90} values followed trends similar to the EC_{50} values, with suspected resistant populations ranging from 0.2 to 534.6 kg ai ha^{-1}. Similarly, McElroy et al. (2013) reported limited efficacy of four ALS-inhibiting herbicides on an annual bluegrass population from Alabama. Twenty-eight days after treatment with trifloxysulfuron, foramsulfuron, bispyribac-sodium, and imazaquin at rates of 0.032, 0.05, 0.3, and 0.98 kg ai ha^{-1}, respectively, no visible injury was observed (McElroy et al. 2013). Similarly, resistant annual bluegrass populations, with high half maximal inhibitory concentration (IC_{50}) values have been reported exceeding 224 g ai ha^{-1} (Cross et al.2015). Sulfonylurea herbicides are prone to the development of herbicide-resistant weed species (Tranel and Wright, 2002). Notably, the A-473 population was collected from a home lawn, suggesting that ALS resistance is present in turfgrass systems other than golf courses.

EPSPS Dose Response

Dry biomass reduction data, presented in table 5.7, depicts EC_{50} values of 1.2, 12,046, 1.1 and 0.6 kg ai ha^{-1} for the G-703, G-704, G-705, and G-712 populations, respectively. The G-703, G-705, and G-712 populations have lower EC_{50} values than previously reported levels of glyphosate resistance in annual bluegrass. Reports of glyphosate applications at rates of 6.27 kg ha^{-1} resulted in less than a 60% biomass reduction in resistant annual bluegrass populations (Binkholder et al. 2011). While the majority of these populations exhibited low levels of resistance, the G-704 population exhibited very high resistance levels. Additionally, the calculated R/S ratio ranged from 2 to 40,153 with the G-704 population exhibiting the highest resistance ratio.

Similarly, ANOVA identified a significant two-way interaction between herbicide rate and population for the percent visual injury to glyphosate (Table 5.8) The EC_{50} values were 25.4, 0.99, 2.6, and 1.6 kg ai ha^{-1} for the G-703, G-704, G-705, and G-712 populations, respectively. The calculated R/S values for the tested biotypes ranged from 7.1 to 181.4. Previous studies have reported much lower levels of glyphosate-resistant annual bluegrass, with resistance levels ranging from 1.3- to 2.5-fold that of a susceptible population (Barua et al. 2021). The ED_{90} values ranged from 2.5 to 4.33 kg ai ha^{-1}.

The findings of this study document multiple instances of annual bluegrass resistance in different types of use sites. While varying levels of resistance were observed within each MOA, the presence of resistant populations in non-golf course environments, such as athletic fields and home lawns, demonstrates the widespread incidence of herbicide resistance. These findings indicate that commonly used herbicides may no longer be effective in managing annual bluegrass. Additionally, resistance found in different use sites raises concerns about resistance

transfer to unaffected sites through seed dispersal. These findings further emphasize the need for effective resistance management strategies, such as herbicide rotation and incorporation of multiple MOA's within herbicide applications.

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Conflicts of Interest

No conflicts of interest have been declared.

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Tables

Table 5.1 Use site and herbicide history of suspected PS-II resistant annual bluegrass populations (*Poa annua* L.) [a].

Population	Use Site	Herbicide History [b]
P-706	Golf course fairway	Simazine (09/2021), glyphosate (02/2022)
P-709	Golf course Fairway	Simazine (10/2018), simazine (12/2018)
P-744	Athletic field	Simazine (11/2021)

[a] Abbreviations: PS-II, photosystem II.

[b] Herbicide history and application dates retrieved through personal communication with turfgrass manager

Table 5.2 Use site and herbicide history of suspected ALS resistant annual bluegrass (*Poa annua* L.) populations [a].

Population	Use Site	Herbicide History [b]
A-361	Golf course tee	Prodiamine x2 (10/2018 & 12/2018) foramsulfuron (2/2019)
A-413	Golf course tee	Foramsulfuron (2/2019)
A-432	Golf course tee	Foramsulfuron, trifloxysulfuron, MSMA (10/2018)
A-473	Home lawn	Trifloxysulfuron (10/2018)

[a] Abbreviations: ALS, acetolactate synthase.

[b] Herbicide history and application dates retrieved through personal communication with turfgrass manager

Table 5.3 Use site and herbicide history of suspected glyphosate resistant annual bluegrass (*Poa annua* L.) populations.

Population	Use Site	Herbicide History [a]
G-703	Athletic field	Glyphosate+pendimethin (2/22)
G-704	Athletic field	Glyphosate+pendimethin (2/22)
G-705	Athletic field	Glyphosate+pendimethin (2/22)
G-712	Golf course	Prodiamine (10/18), simazine + glyphosate (1/19)

[a] Herbicide history and application dates retrieved through personal communication with turfgrass manager

Table 5.4 Hill slopes and EC_{50} and EC_{90} values of simazine on dry biomass reduction (% of control) in annual bluegrass (*Poa annua* L.).

Population	EC_{50} (kg ai ha^{-1}) [a]	EC_{90} (kg ai ha^{-1}) [a]	R^2	Hillslope	R/S Ratio [b]
P-706	1.8	4.7	0.99	2.3	6
P-709	3.4	5.6	0.97	4.4	11.3
P-744	4.5	8.9	0.90	3.2	15
Susceptible	0.3	0.33	0.89	26.4	N/A

[a] $EC_{50} = \frac{EC_x}{[\frac{x}{100-x}]^{\frac{1}{B}}}$ where EC50 is effective concentration for 50% visual injury (%) and EC_x is effective concentration for x% (90%) Visual injury (%) and *B* is Hill slope

[b] R/S ratio individually compares the EC_{50} values for each resistant population with the susceptible population with a 1x rate of simazine equaling 2.24 kg ai ha^{-1}.

Table 5.5 Hill slopes and EC_{50} and EC_{90} values of trifloxysulfuron on dry biomass reduction (% of control) in annual bluegrass (*Poa annua* L.).

Population	EC_{50} (kg ai ha^{-1}) [a]	EC_{90} (kg ai ha^{-1}) [a]	R^2	Hillslope	R/S Ratio [b]
A-361	0.12	0.13	0.79	17.9	6
A-413	0.79	1.75	0.21	2.75	39.5
A-432	1.06	1.22	0.48	15.2	53
A-473	0.07	N/A [c]	0.96	-19.5	3.5
Susceptible	0.02	N/A [c]	0.96	-14.59	

[a] $EC_{50} = \frac{EC_x}{[\frac{x}{100-x}]^{\frac{1}{B}}}$ where EC50 is effective concentration for 50% visual injury (%) and EC_x is effective concentration for x% (90%) Visual injury (%) and *B* is Hill slope

[b] R/S ratio individually compares the EC_{50} values for each resistant population with the susceptible population with a 1x rate of trifloxysulfuron equaling 0.027 kg ai ha^{-1}

[c] EC_{90} values are not disclosed. Negative hillslope values produce ED_{90} values that are below calculated EC_{50} values.

Table 5.6 Visual estimate of injury of trifloxysulfuron on annual bluegrass (*Poa annua* L.) populations selected for ALS resistance screening.

Population	EC_{50} (kg ai ha^{-1}) [b]	EC_{90} (kg ai/ ha^{-1}) [b]	R^2	Hillslope	R/S Ratio [c]
A-361	0.19	0.7	0.99	1.7	9.5
A-413	2.2	534.6	0.96	0.4	110
A-432	0.06	0.4	0.98	1.23	3
A-473	0.08	0.2	0.98	2.2	4
Susceptible	0.02	0.6	0.96	1.9	

[a] Abbreviations: ALS, acetolactate synthase.

[b] $EC_{50} = \frac{EC_x}{[\frac{x}{100-x}]^{\frac{1}{B}}}$ where EC50 is effective concentration for 50% visual injury (%) and EC_x is effective concentration for x% (90%) Visual injury (%) and *B* is Hill slope

[c] R/S ratio individually compares the EC_{50} values for each resistant population with the susceptible population with a 1x rate of trifloxysulfuron equaling 0.027 kg ai ha^{-1}

Table 5.7 Hill slopes and EC_{50} and EC_{90} values of glyphosate on dry biomass reduction (% of control) in annual bluegrass (*Poa annua* L.).

Population	EC_{50} (kg ai ha^{-1}) [a]	EC_{90} (kg ai/ ha^{-1}) [a]	R^2	Hillslope	R/S Ratio [b]
G-703	1.2	142.4	0.94	0.46	4
G-704	12,046	31,985	0.94	2.25	40,153
G-705	1.1	2.9	0.90	2.25	3.6
G-712	0.6	3.3	0.96	1.28	2
Susceptible	0.3	2.8	0.98	0.98	

[a] $EC_{50} = \frac{EC_x}{[\frac{x}{100-x}]^{\frac{1}{B}}}$ where EC50 is effective concentration for 50% visual injury (%) and EC_x is effective concentration for x% (90%) Visual injury (%) and *B* is Hill slope

[b] R/S ratio individually compares the EC_{50} values for each resistant population with the susceptible population with a 1x rate of glyphosate equaling 0.7 kg ai ha^{-1}

Table 5.8 Visual estimate of injury to glyphosate on annual bluegrass (*Poa annua* L.) populations selected for EPSPS dose response [a].

Population	EC_{50} (kg ai ha^{-1}) [b]	EC_{90} (kg ai ha^{-1}) [b]	R^2	Hillslope	R/S Ratio [c]
G-703	25.4	586.1	0.95	0.7	181.4
G-704	0.99	2.5	0.89	2.4	7.1
G-705	2.6	4.33	0.86	4.3	18.6
G-712	1.6	3.0	0.95	3.5	11.4
Susceptible	0.14	0.44	0.99	2.1	

[a] Abbreviations: EPSPS, enolpyruvylshikimate-3-phosphate synthase.

[b] $EC_{50} = \frac{EC_x}{[\frac{x}{100-x}]^{\frac{1}{B}}}$ where EC50 is effective concentration for 50% visual injury (%) and EC_x is effective concentration for x% (90%) Visual injury (%) and *B* is Hill slope

[c] R/S ratio individually compares the EC_{50} values for each resistant population with the susceptible population with a 1x rate of glyphosate equaling 0.7 kg ai ha^{-1}