**The Identification of Foramsulfuron and Sulfentrazone Resistant Goosegrass (*Eleusine indica*) Populations**

by

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## Abstract

*Eleusine indica,* commonly known as goosegrass, is a $C_4$ weed species infesting turfgrass, cropping systems, and home lawns. Goosegrass is well known for developing herbicide resistance to different herbicide families and modes of action. Populations collected with suspected resistance were submitted to the Herbicide Resistance Diagnostic Lab at Auburn University to evaluate for resistance to six common herbicides used in turfgrass. Research was conducted in greenhouse conditions to assess susceptible (S) goosegrass responses compared to suspected resistant (R) biotypes. Based on the postemergence screen, 30 of the 50 populations were diagnosed as resistant to one or more herbicides. In contrast, 20 populations were diagnosed as susceptible to all treatments compared to the nontreated. Seventeen populations possessed cross-resistance to two or more herbicides.

Since their discovery in 1995 and 1989, respectively, foramsulfuron and sulfentrazone have been widely utilized herbicides in turfgrass management. Despite their extensive use, limited research has been conducted on goosegrass biotypes resistant to these herbicides. Following initial postemergence screening, confirmed resistant biotypes underwent further analysis. Our research aimed to clarify the resistance level of these goosegrass biotypes by conducting dose-response assays with increasing rates of foramsulfuron (1.81 to 115.56 g ai/ha) and sulfentrazone (26.21 to 1677.78 g ai/ha). Visual injury ratings were recorded at 7, 14, 21, and 28 days after treatment (DAT), with aboveground biomass assessed at 28 DAT. Foramsulfuron-resistant populations (R-R1, R-R2, and R-R3) exhibited calculated $I_{50}$ values of 0.12, 0.12, and 0.09, respectively, while the susceptible biotype displayed an $I_{50}$ of 0.02. In

comparison, sulfentrazone-resistant populations (D-R1 and D-R2) demonstrated $I_{50}$ values of 0.11 and 0.21, respectively, whereas the susceptible biotype had an $I_{50}$ of 0.03. Target-site gene sequencing of acetolactate synthase (ALS) and protoporphyrinogen IX oxidase (PPO1 and PPO2) indicated no mutation in the resistant biotypes compared to the susceptible, suggesting a potential non-target site mechanism.

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## List of Abbreviations

| | |
|---|---|
| ACCase | Acetyl CoA Carboxylase |
| ai | active ingredient |
| AL | Alabama |
| ALS | acetolactate synthase |
| CA | California |
| DAT | days after treatment |
| EPSP | 5-enolpyruvylshikimate-3-phosphate |
| EPSPS | enolpyruvyl shikimate phosphate synthase |
| FL | Florida |
| g | grams |
| ha | hectares |
| HI | Hawaii |
| HPPD | *p*-hydroxyphenyl pyruvate dioxygenase |
| HRDL | Herbicide Resistance Diagnostic Lab |
| IL | Illinois |
| IPM | integrated pest management |
| L | liter |
| MOA | mode of action |
| NCBI | National Center for Biotechnology Information |
| NTSR | non-target site resistant |

| | |
|---|---|
| OH | Ohio |
| PBU | Plant Breeding Unit |
| POST | Post-emergence |
| PPIX | protoporphyrin IX |
| PPO | protoporphyrinogen IX oxidase |
| PRE | Pre-emergence |
| PSI | Photosystem I |
| PSII | Photosystem II |
| R | resistant |
| RCBD | Randomized Complete Block Design |
| S | susceptible |
| SC | South Carolina |
| SNP | single nucleotide polymorphism |
| SOA | site of action |
| TN | Tennessee |
| TSR | target site resistant |

# Chapter I

## Literature Review

The term ‘weed’ encompasses any plant that interferes with the growth or value of another plant (Anderson 1996). However, defining weeds remains a subject of debate, as individuals’ circumstances lead to varied perceptions of what constitutes a weed and how they should be defined. One prevalent method of categorizing weeds is based on habitat preferences, distinguishing between agrestals and ruderals. Agrestals typically inhabit tilled land, such as fields cultivated for cereal or root crops, orchards, gardens, and similar cultivated areas. In contrast, ruderals are characterized by their preference for man-made environments, thriving in disturbed areas like construction sites, garbage sites, roadsides, railway lines, and other similar locations where human activity has caused disruption (Hermanutz 1991; Holzer and Numata 2013). In modern, fully mechanized agriculture, agrestals can impede machine harvesting, affecting crop competitiveness and introducing diseases that compromise the quality of the final product. Weed management presents challenges, particularly as weed seedlings often emerge concurrently with crops, complicating control measures without risking harm to desired plants through herbicide applications (Chauhan and Johnson 2008).

The economic ramifications of weed infestations are very significant, with reported annual losses of millions of dollars each year at state and national levels. A prime example of a highly prevalent and economically damaging agronomic weed in the southeastern United States is Palmer amaranth (*Amaranthus palmeri*). Historically, Palmer amaranth has been ranked among the most troublesome weeds in cotton, corn, and soybean production (Webster and

Nichols 2012). In a formative investigation, Ward et al. (2013) demonstrated that the introduction of Palmer amaranth resulted in substantial yield reductions across various crops. Specifically, the presence of Palmer amaranth was associated with estimated yield losses of up to 91% in corn, 65% in cotton, 68% in peanut, and 79% in soybean (Ward et al. 2013).

At times, the influence of weeds extends beyond economic quantification. For instance, weeds in recreational spaces, lawns, athletic fields, and other prominently displayed areas. While they may not directly affect economics via yields, they are often perceived as an undesirable nuisance and can diminish the aesthetic and utility value of landscapes. Turfgrass is a major market that is highly influenced by televised sporting events, making it available to millions worldwide. These perfectly maintained venues with increased popularity have created a demand for more minor local courses to maintain the same conditions. The presence of unwanted weed species greatly diminishes the playability and aesthetics of these courses (McElroy et al. 2013). Visual turf uniformity is often disrupted by many weeds that have a similar clumpy growth habit to dallisgrass *(Paspalum dilatatum*), quackgrass *(Elymus repens*), goosegrass *(Eleusine indica*), or smutgrass *(Sporobolus indicus*). The lighter color and seedheads of annual bluegrass (*Poa annua*) on a golf green can often distract from the aesthetic appeal of the playing surface. Weeds frequently indicate a weakened turf rather than being the root cause of the issue. Growing a vigorous, thick turf is the best way to reduce weed seed germination significantly. Cultural and chemical practices are often used to control weeds in turfgrass. Reducing soil compaction through aerification is an example of cultural practices to prevent unwanted species. The best option is chemical control with the use of synthetic herbicides. Proper weed identification and herbicide selection are crucial components of chemical weed management.

*Eleusine indica,* commonly known as goosegrass, is a grass species with a C4 photosynthetic mechanism that frequently infests agriculture fields and turfgrass. According to Holm et al. (1977), goosegrass has been documented as a significant weed in 46 crops across more than 60 countries. Goosegrass is widely distributed in tropical and subtropical regions in Asia, the Pacific, Africa, and North America (Holm et al. 1977). It is an increasing concern for the orchard production system in California. It competes for water and nutrients, challenging irrigation uniformity and, crucially, harvest operations, particularly during ground-based almond and walnut harvesting (Wolter et al. 2023). In sugarcane, much like other agriculture crops, severe infestations of goosegrass can result in sugarcane yield losses. Additionally, this weed serves as a host for diseases, nematodes, and viruses affecting crops such as maize (*Zea mays* L.), peanuts (*Arachis hypogaea* L.), sugarcane (*Saccharum officinarum* L.), and rice (*Oryza sativa* L.) (Chauhan and Johnson 2008). Goosegrass hosts the *Meloidogyne* species*,* an economically crucial root-knot nematode distributed worldwide (Rich et al. 2009).

Goosegrass is identified by its rosette growth habit and distinct flattened, white/silver base. It is often misidentified as crabgrass (*Digitaria ischaemum*), also a C4 grass species with sprawling stems resembling the legs of a crab (Spencer 2013). Understanding the distinctions between these weeds is crucial for effective management. Crabgrass is notorious for its rapid spread, often dominating extensive areas, while goosegrass develops a deeper fibrous root system. Crabgrass typically features broad leaves, whereas goosegrass exhibits thin, wiry leaves arranged in a rosette shape. Goosegrass stems are flattened and branching with few to no hairs and fleshy at the base. The leaves are normally hairless but may be slightly pubescent at the base. A short jagged membranous ligule is present. The plant can grow up to 3 feet tall and spreads by reseeding itself (Steed et al. 2017). Goosegrass is an aggressively resilient plant due to its

vigorous growth and abundant seed production, often producing up to 140,000 seeds per plant (Holm et al. 1977, Chauhan and Johnson 2008). Additionally, goosegrass populations frequently infest areas characterized by compacted soil, elevated soil moisture, and limited turf competition (McCullough et al. 2013). The characteristics of goosegrass detrimentally affect the growth, quality, and aesthetics of putting greens on golf courses. Goosegrass has the inherent capability to thrive in locations with nutritionally deficient compacted soils, frequent mowing, and drought conditions, thereby diminishing the value of turf.

## Goosegrass Control

Goosegrass is controlled primarily through the use of synthetic herbicides. Both preemergence (PRE) and postemergence (POST) herbicides effectively control goosegrass. PRE herbicides are preferred in turfgrass because they prevent the appearance of the weed from ever emerging. POST herbicides can create voids where the turfgrass must recover after controlling the weed. Across both agriculture and turfgrass, PRE herbicides have been effectively and readily used for goosegrass control. Trifluralin, bensulide, dithiopyr, oxadiazon, pendimethalin, and prodiamine are common PRE herbicides that control goosegrass (Koski 2008; Patton and Elmore 2023). Prodiamine applied at 1.68 kg ai $ha^{-1}$ controlled a known susceptible population greater than 80%, whereas control of the dinitroaniline-resistant biotype was <35% (McCullough et al. 2013). Single and sequential applications of indaziflam at 0.035 kg $ha^{-1}$ have been known to give results of 100% goosegrass control (McCullough et al. 2013). Herbicides containing oxadiazon have demonstrated reliable PRE efficacy against goosegrass (Patton and Elmore 2023;

Johnson and Murphy 1996). In one research study, oxadiazon was applied at 2.2 kg $ha^{-1}$ one week before sprigging a bermudagrass (*Cynodon* dactylon) putting green and resulted in 99 and 100% control without injuring the bermudagrass. In the same study, oxadiazon applications made after sprigging were significantly less effective (Brecke et al. 2010).

Oxadiazon and indaziflam are heavily relied upon for goosegrass control, primarily because the cancellation or severe use restrictions of other herbicides like monosodium methanearsonate (MSMA) have significantly limited the options available for effectively targeting this weed species while maintaining acceptable turfgrass quality. Goosegrass control with dinitroanilines and dithiopyr is generally considered inferior to oxadiazon and indaziflam (McCullough et al. 2013). For instance, Johnson and Murphy (1996) evaluated 37 PRE herbicides for goosegrass control, revealing that only 12 treatments achieved >80% control. Notably, applying prodiamine alone at 0.75 lb/acre and pendimethalin at 3.0 lb/acre resulted in inadequate goosegrass control of 64% and 43%, respectively. Sequential applications of prodiamine and pendimethalin applications were needed to increase the control.

Goosegrass has been controlled by several POST herbicide families, including Acetolactate synthase (ALS) inhibitors and acetyl CoA carboxylase (ACCase) inhibitors. POST herbicides should be applied before the plants become well-established or tillering and are still susceptible to herbicides. Even with the application of POST herbicides, goosegrass will persist in producing large numbers of germinations throughout the summer, rendering longevity of control challenging with just a single application. In cool-season turf, fenoxaprop, fluazifop, sulfentrazone, and topramezone are utilized for POST goosegrass control. Sequential applications are often required to manage tillered mature plants effectively. For example, foramsulfuron has excellent bermudagrass safety, but multiple applications are needed for

adequate goosegrass control (Busey 2004). Researchers have documented topramezone resulted in an 83% reduction in goosegrass cover 56 days after treatment (DAT) at 6.14 g $ha^{-1}$ (Cox et al. 2017). Metribuzin has the potential to control goosegrass POST, but it carries the risk of causing unacceptable injury to bermudagrass, and reports of resistance have been documented (Brosnan et al. 2008). In the realm of ornamental plant care, herbicides such as clethodim, fenoxaprop-p-ethyl, fluazifop-p-butyl, and sethoxydim are viable options. These herbicides are designed for application over the top of numerous ornamental plants, effectively targeting grassy weeds such as goosegrass (Steed et al. 2017).

When considering turf management, the selection of herbicides that are both safe and effective is limited. Managing goosegrass in creeping bentgrass (*Agrostis stolonifera* L.), a cool-season turfgrass poses challenges as the most effective herbicides can cause significant injury, particularly in creeping bentgrass tees and fairways. Fenoxaprop is labeled for use in creeping bentgrass, but it should be applied at the lower end of the label rate (0.46 oz / 1000 sq. Ft.; Anonymous 2019). Applying fenoxaprop at a low rate proves effective in controlling small pre-tillered goosegrass plants. Fenoxaprop combined with topramezone applied at a low rate at two-to-three-week intervals, summer goosegrass control can be accomplished as well (Patton and Elmore 2023). In warm-season turfgrasses like bermudagrass and zoysiagrass (*Zoysia spp.*), foramsulfuron, fluazifop, and Tribute Total (a combination of thiencarbazone, foramsulfuron, and halosulfuron) are commonly utilized and frequently combined with triclopyr in tank-mixtures to enhance control (Patton and Elmore 2023).

While herbicides are effective for weed control, herbicide resistance poses a significant problem that can arise when herbicides are the sole means of weed management. Relying solely on herbicides for weed control can lead to the selection of plant species that have developed

mechanisms to withstand herbicide applications, resulting in the development of resistance. Intensive herbicide use has introduced the evolution of herbicide resistance to many modes of action (MOA) in goosegrass populations around the world (Heap 2022). However, there are limited reports documenting resistance to ALS and protoporphyrinogen IX oxidase (PPO) inhibitors from goosegrass biotypes. Among the herbicides examined in this study, foramsulfuron and sulfentrazone fall within this category.

## Foramsulfuron and Sulfentrazone

Foramsulfuron and sulfentrazone are popular herbicides used in turf for POST weed control and have potential for increased use for goosegrass control in turfgrass. Foramsulfuron is a sulfonylurea herbicide that controls a broad range of annual and perennial grasses and some broadleaf weeds (Collins et al. 2001). The chemical formula for foramsulfuron is 2-[(4,6-dimethoxypyrimidin-2-yl)carbamoylsulfamoyl]-4-formamido-N, N-dimethylbenzamide (National Center for Biotechnology Information 2024). It is an ALS-inhibiting herbicide, which inhibits the plants' acetolactate synthase enzyme and has been among the most widely used chemicals since the 1980s (Zhou et al. 2007). The ALS enzyme is the first enzyme to catalyze two reactions in the branched-chain amino acid biosynthesis pathway (Zhou et al. 2007). ALS-inhibiting herbicides are among the largest MOA specifically registered for turfgrass use and have the most evolved resistance (McElroy 2013; Tranel and Wright 2002). These herbicides, like foramsulfuron, are well-liked because they offer broad-spectrum weed control, exhibit soil residual activity, require low application rates, and have low toxicity to mammals (Tranel and

Wright 2002; Saari et al. 2018). Foramsulfuron is safe on bermudagrass and zoysiagrass and can control almost all cool season turfgrasses (McElroy 2013). Because of this, foramsulfuron is often applied to remove overseeded cool-season grasses in the spring (McElroy 2013). Foramsulfuron (Revolver, Bayer Environmental Science, Cary, NC) is labeled for POST goosegrass control in turf used at 0.1 to 0.8 ounces of product per 1000 sq. ft (Anonymous 2015). Sequential applications of foramsulfuron provided <55% goosegrass control and less than 17% seashore paspalum (*Paspalum vaginatum*) phytotoxicity (McCullough 2012). Foramsulfuron has also been reported to control a population of goosegrass resistant to the photosystem II inhibitors metribuzin and simazine, making it a possible option for turf managers to control dinitroaniline-resistant goosegrass POST (Brosnan et al. 2008).

Sulfentrazone is a PPO-inhibitor herbicide from the triazolinone chemical family that is unlike the other herbicides with similar MOAs because it can be applied to the soil PRE in agriculture and POST in turfgrass (McElroy 2013). The chemical formula of sulfentrazone is N-[2,4-dichloro-5-[4-(difluoromethyl)-3-methyl-5-oxo-1,2,4-triazol-1-yl]phenyl]methanesulfonamide (National Center for Biotechnology Information 2024). This herbicide inhibits the PPO enzyme that catalyzes the conversion of protoporphyrinogen IX to protoporphyrin IX (PPIX) (Duke et al. 1991). PPIX formed outside the thylakoids absorbs light and reacts with reactive oxygen species, degrading the cellular structures and eventually killing the plant (Duke et al. 1991; Reddy and Locke 1998). PPO has two isoforms: chloroplast-targeted (PPO1) and mitochondrial-target (PPO2).

Sulfentrazone (Dismiss, FMC Corporation, Philadelphia, PA) is labeled to be applied at 4 – 12 fl oz/acre for control of annual grasses (Anonymous 2017). Sulfentrazone is labeled to control sedges, broadleaf weeds, and goosegrass in turfgrass. A single application of

sulfentrazone alone controlled goosegrass ≤ 35%, but sequential application improved control to ≤ 65% (McCullough 2012). Sulfentrazone provides residual control of both grasses and broadleaf weeds such as Amaranthus species, common lambsquarter *(Chenopodium album* L.*),* green foxtail *(Setaria viridis* L.*),* and velvetleaf *(Abutilon theophrasti* L.*)* (Belfry et al. 2015). In soybeans, tank mixing pyroxasulfone and sulfentrazone improved (> 80%) control of weed species like common lambsquarter, velvetleaf, and green foxtail (Belfry et al. 2015). Currently, there have been no reported cases of resistance to sulfentrazone (Heap 2024).

It has been demonstrated that tank mixtures of herbicides with different MOA have a better potential for controlling goosegrass in turf. Sulfentrazone effectively controls weed biotypes that have developed resistance to herbicides of other MOA. For example, Busey (2004) observed ineffective goosegrass control by foramsulfuron applied at 0.044 kg/ha alone, but tank mixtures with metribuzin applied at 0.0.105 kg/ha increased control to > 85%.

## Introduced Resistance

Herbicide resistance is an example of evolution, as it is developed from an increased selection pressure from exposure to herbicides with the same MOA (Christoffers 1999). This evolution is influenced by many factors, such as the herbicide's structure, the specific weed's genetics, and the selection pressure due to management practices (Christoffers 1999). The first case of herbicide resistance reported in North America was *Daucus carota* to the auxin site of action (SOA) in 1957 (Heap 2022). Today, there are more than 500 unique cases of herbicide resistant weeds globally, with 21 of the 31 known SOA having evolved resistance (Heap 2022).

Much of the southeastern United States is infested with *Palmer amaranth*, which is resistant to many different MOAs, including ALS-inhibiting herbicides, dinitroanilines, triazines, EPSP (5-enolpyruvylshikimate-3-phosphate) inhibitors, and HPPD (4-hydroxyphenylpyruvate dioxygenase) inhibitors (Ward et al. 2013). Sosnoskie and others (2011) described a population of *Palmer Amaranth* from Georgia that has developed multiple resistance to glyphosate and pyrithiobac. Goosegrass herbicide resistance has been reported in 12 countries, with various sites of action exhibiting resistance (Heap 2022). Recently, in Brazil, a goosegrass population was found resistant to EPSPs (glyphosate) and ACCase (haloxyfop-methyl and fenoxaprop-ethyl) inhibiting herbicides, this being the first of its kind (Correia et al. 2022).

Lee and Ngim (2000) confirmed the presence of a first-of-its-kind glyphosate-resistant goosegrass population in Malaysia. To evaluate resistance, 720, 1440, 2880, and 5760 g $ha^{-1}$ glyphosate-isopropylamine salt was applied to plots at the farmer's guava farm in Teluk Intan, as well as another trail where glyphosate-trimesium and glyphosate-isopropylamine salt were used at the same rates to putative resistance biotypes and a known susceptible biotype. At the grower's field, the recommended label rate gave zero control, while the highest rate gave only 25% control, confirming resistance. The susceptible biotypes were controlled. However, the resistant biotypes were not controlled.

Buker et al. (2002) evaluated goosegrass ecotypes, showing a lack of residual control by paraquat in tomato (*Solanum lycopersicum* L.) fields in Manatee County, FL. Two biotypes were utilized in the study; 'Manatee' was collected from tomato fields located in Manatee County, FL, and 'Alachua' was collected in Alachua County, FL, from previous generations that showed no tolerance to paraquat. The experiment was conducted in greenhouses, and paraquat was applied at 0, 0.3, 0.6, 1.1, 2.2, and 4.5 kg/ha to determine the resistance level. To investigate the potential

occurrence of cross-resistance, sethoxydim, clethodim, and metribuzin were applied at the recommended label rate for vegetable production, with or without paraquat. The paraquat rate needed to achieve the $GR_{50}$ (concentration of herbicide that will result in 50% inhibition) for the Manatee biotype was approximately 30 times higher than the $GR_{50}$ rate for the Alachua biotype, leading the investigators to conclude resistance to paraquat. No cross-resistance issues were identified, as both ecotypes were susceptible to sethoxydim, clethodim, and metribuzin (Buker et al. 2002).

Askew (2013) documented the first case of an annual grass exhibiting resistance to a PPO inhibitor. This resistant population was discovered on a golf course in Richmond, Virginia, where oxadiazon had been used for PRE goosegrass management since the early 1990s. Observations showed inadequate goosegrass control even with oxadiazon application rates ranging from 3.4 to 4.5 kg ai/ha.

Understanding the type of resistance is crucial to designing effective weed management systems. The two mechanisms weeds use to survive herbicide applications are split into two categories: target-site (TSR) and non-target-site (NTSR) resistance. TSR includes mechanisms that structurally change the herbicide binding site or increase the expression of the target protein (Délye 2013). NTSR is much broader and less understood, including any other mechanism that can confer resistance. These mechanisms include enhanced metabolism, increased herbicide translocation, and increased herbicide absorption and uptake. NTSR, simply put, is the improved ability of the weed to break down the herbicide. NTSR is considered the most predominant type of resistance occurring worldwide and is most likely underestimated. The primary target resistance mechanisms observed in weeds are typically associated with major classes of

herbicides, including ALS inhibitors, triazine herbicides, ACCase inhibitors, dinitroaniline herbicides, organophosphorus herbicides, and PPO inhibitors.

Specifically, ALS-inhibiting herbicides are notorious for being plagued by the development of herbicide-resistant weeds (Tranel and Wright 2002; Saari et al. 2018). At least 17 different amino acid substitutions in ALS have been identified for imparting resistance to ALS-inhibiting herbicides in an organism, and at least five have been identified in resistant weed populations (Tranel and Wright 2002). Most target-site mutations conferring resistance to PPO inhibitors have been primarily observed in the PPO2 enzyme. However, a recent discovery revealed a novel A212T mutation in the PPO1 enzyme, which conferred resistance to oxadiazon (Bi, 2021).

## Prevention of Resistance and Mitigation Practices

Extensive research has been conducted on the development of herbicide resistance, and there seem to be two common explanations for its evolution. Some believe using high rates of pesticides kills off susceptible biotypes and selects for an herbicide-resistant weed population. Others believe that using low rates will make the population immune to the herbicide and create resistance. It is unclear if either is completely accurate.

On a global basis, herbicide resistant weeds continue to grow and threaten the purity of agriculture and turfgrass, and the overall choices of herbicides are limited. At the most basic level, herbicide resistance is an evolutionary process driven by the intensive use of single MOAs as the primary form of weed control practices. Improved knowledge of weed biology can further

develop weed management technologies to control weeds like goosegrass and combat resistance. Any weed control practices that maintain the susceptible biotypes will be selecting for resistance. Managers need to rotate through different herbicide MOA from year to year to avoid the development of herbicide resistant weed species. Additionally, farmers and superintendents should spend more time understanding the weed species in their fields to match the correct herbicide.

## Research Objectives

The main objective of the thesis is to assess the occurrence and severity of goosegrass herbicide resistance in the United States. This research was primarily initiated due to the concern of oxadiazon resistance in turfgrass. Reports to the Herbicide Resistance Diagnostics Lab were increasing beginning in 2015 and continue to increase. This thesis is split into two parts. The first study focuses on the survey and discovery of goosegrass herbicide resistance in the United States. The second objective was to take the biotypes found resistant to foramsulfuron and sulfentrazone and better understand the resistance level using dose-response assays. By evaluating these objectives, it is hoped a greater understanding of goosegrass herbicide resistance occurrence will be achieved. Weed science researchers and turfgrass managers will use the research results to improve weed management strategies.

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## Chapter II.

## Survey of Goosegrass (*Eleusine indica*) Herbicide Resistance


### Abstract

Reports of herbicide-resistant goosegrass have intensified in recent years. Successful herbicide resistance management programs require early detection of resistance and deployment of alternative control strategies to mitigate the spread of resistance. The Herbicide Resistance Diagnostic Lab (HRDL) at Auburn University developed a protocol using a single herbicide rate for screening suspected herbicide resistant goosegrass populations collected from managed turfgrass. Six different herbicides were utilized that covered different modes of action, including sulfentrazone (PPO-inhibitor), oxadiazon (PPO-inhibitor), foramsulfuron (ALS-inhibitor), sethoxydim (ACCase-inhibitor), metribuzin (PSII-inhibitor), and prodiamine (microtubule assembly inhibitor) in one micromolar solution of hydroponics. As of January 2023, the HRDL has screened 50 populations. Of these, eight were resistant to sulfentrazone, 14 to oxadiazon, 23 to foramsulfuron, six to sethoxydim, and zero to metribuzin. Only 40 populations were screened for prodiamine resistance, and five were found resistant. Thirty populations were diagnosed as resistant to one or more herbicides, and 20 as susceptible to all treatments. Cross-resistance (resistant to two or more herbicides) was identified in 17 populations.



**Additional index words:** goosegrass, turfgrass, herbicide resistance

**Nomenclature:** *Eleusine indica*

## Introduction

Globally, some of the most widespread and economically significant herbicide-resistant weeds include *Amaranthus* spp. (redroot pigweed, A. *retroflexus* L.; Palmer amaranth, A. *palmeri;* and common waterhemp, A. *rudis*), Annual ryegrass (*Lolium rigidum*), goosegrass (*Eleusine indica*), and horseweed (*Conyza canadensis*) (Beckie 2006). Annual weeds like goosegrass and Palmer amaranth are prolific seed producers, genetically diverse, and widely distributed, making them perfect candidates to develop resistance (Chauhan and Johnson 2008). There have been 37 different cases of goosegrass populations exhibiting evolved resistance (Heap 2022). Resistance to 5-enolpyruvylshikimate-3 phosphate synthase (EPSP) and photosystem I (PSI) electron diverters is the most common.

In the family *Poaceae*, goosegrass is a summer annual weed characterized as a prolific seed producer, often capable of producing ≤140,000 seeds per plant, and a nuisance in agricultural fields and turfgrass (Chauhan and Johnson 2008; Jalaludin et al. 2010). It is predominantly present in Africa, America, and intertropical regions of Asia (Takano et al. 2016). As an annual and rhizomatous weed, goosegrass can rapidly propagate in high-traffic areas with compact soils and high soil moisture but is best known for infesting golf courses and athletic fields (McCullough et al. 2013).

Herbicide resistance is a result of the evolutionary adaptation of weeds to herbicide selection and can be categorized as either target-site resistance (TSR) or non-target-site resistance (NTSR). TSR is endowed by gene mutations in target enzymes, such as acetolactate synthase (ALS), acetyl-CoA carboxylase (ACCase), and protoporphyrinogen IX oxidase (PPO)

(Dayan et al. 2014; Kaundun 2014; Yang et al. 2016). NTSR is achieved by mechanisms reducing herbicide concentration reaching the target site (Yang et al. 2016). Some of these mechanisms include enhanced metabolism and reduced translocation.

Early cases of herbicide resistance were highly herbicide-specific; however, today's cases generally involve resistance to multiple modes of action (MOA), known as multiple resistance. The number of documented cases of herbicide resistance has increased from only a few in the early 1970s to over 500 in 2023 (Heap 2023). Herbicide resistance has been reported to all major known herbicide MOA. It will only increase in the future due to a lack of new MOA and some herbicides being taken off the market by environmental restrictions. Hugh J. Beckie, in a study of herbicide-resistant weeds, states, "The main risk factors for the evolution of herbicide-resistant weeds are: (a) recurrent application of highly efficacious herbicides with the same site of action; (b) annual weed species that occur at high population densities, are widely distributed, genetically variable, prolific seed producers, and have efficient gene dissemination; and (c) simple cropping systems that favor a few dominant weed species" (Beckie 2006).

Acknowledging the global issue of goosegrass and the escalating challenge in its control, particularly attributed to herbicide resistance, this study's objectives were to assess the distribution of goosegrass resistance in the United States and analyze the patterns and levels of resistance to different herbicides used in turfgrass management practices. Understanding the distribution and extent of herbicide resistance in goosegrass populations across the United States is crucial for developing effective management strategies. The study aims to contribute to the broader understanding of herbicide resistance in weed species and support the development of sustainable weed management practices in turfgrass.

## Materials and Methods

*Eleusine indica* populations were collected across the United States from golf courses, athletic fields, and city recreational parks. Populations were predominately chosen based on herbicide use history and reports from turfgrass managers indicating a lack of control with a standard herbicide application, raising suspicions of resistance. A total of 50 goosegrass samples submitted by golf course managers were screened for herbicide resistance from 2020 to 2023. Latitude and longitude coordinates were recorded for each sampling location to visualize spatial trends in suspected resistance (Figure 1). Greenhouse experiments were conducted at the Auburn University Herbicide Resistance Diagnostics Lab greenhouse in Auburn, AL. The goosegrass biotypes tested for resistance are listed in Table 2. Weed samples were screened for resistance to various herbicides commercially available to golf course managers (Table 1): sulfentrazone (Dismiss, FMC Corporation, Philadelphia, PA) applied at 0.28 kg/ha, oxadiazon (Ronstar 2G; Bayer Environmental Science, Cary, NC) applied at 1.12 kg/ha, foramsulfuron (Revolver, Bayer Environmental Science, Cary, NC) applied at 0.03 kg/ha, sethoxydim (Segment, BASF Corp., Research Triangle Park, NC) applied at 0.21 kg/ha, metribuzin (Sencor, Bayer Environmental Science, Cary, NC) applied at 0.56 kg/ha, and prodiamine (Barricade, Syngenta Crop Protection, Greensboro, NC) in one micromolar solution of hydroponics. Only the first 40 populations were screened for prodiamine resistance. A nontreated treatment was included in soil and hydroponic system for comparison.

**Postemergence Screening.** Persons submitted 15 to 20 tillering plants for screening. Plants were potted into distinct flats filled with potting media (Miracle-Gro Moisture Control Potting Mix, Scotts Miracle-Gro Company, Marysville, OH) to allow for acclimation to the greenhouse environment. Postemergence testing was then conducted by transplanting ten single tillers into 4-inch by 4-inch pots filled with herbicide-free Marvyn sandy loam soil. The greenhouse consistently maintained an air temperature of 30 ± 2°C throughout the investigation, with additional supplemental light provided. In order to encourage robust growth, the plants were fertilized weekly with approximately 0.25 lb N per 1000 sq feet using a standard fertilizer (28-6-16 Miracle-Gro Water-soluble All-Purpose Plant Food from Scotts Miracle-Gro Products Inc, Marysville, OH). Additionally, they were irrigated three times a day using an overhead misting system. Additional plants were transplanted into distinct flats to generate seeds for storage and progression to subsequent studies as needed. Weed species were sprayed at early growth stages (around two to four leaves) to optimize herbicide efficacy. Treatments were applied using a $CO_2$ pressurized backpack sprayer, calibrated at 280 L $ha^{-1}$ with a handheld four-nozzle boom (TeeJet TP8002 flat fan nozzles with 25 cm spacing; Spraying Systems Company, Wheaton, IL). A nonionic surfactant (Induce, Helena, Chemical Company, Collierville, TN) was included in all the treatments at 0.25 % V/V. Visible ratings of injury on a percent scale, 0% meaning no harm was observed, and 100% representing the plant died from herbicide damage, were taken 14 and 28 days after treatment (DAT).

**Preemergence Screening.** Hydroponics was conducted in 10-liter bins using aquarium pumps and air stones for aeration. Lids to the containers had holes drilled into them so the goosegrass roots could be fully submerged in the water. The treatments included a one-micromolar

prodiamine solution and another non-treated one. Each container included 3.5 grams of hydroponics fertilizer (10-5-14 MaxiGro Water-soluble Plant Food, General Hydroponics, Santa Rosa, CA) to ensure plants had enough available nutrients. Submitted plants were divided into ten individual plants; each bin comprised five plants. The goosegrass roots were washed to remove any soil debris before being put into the hydroponic system. Visual root clubbing associated with mitotic inhibition was taken 14 DAT to determine resistance compared to the non-treated control.

**Data Analysis.** All analyses were performed using R Statistical Software (v4.2.0; R Core Team 2024). R packages that were used included: ggplot, sf, rnaturalearth, rnaturalearthdata, ggspatial, and cowplot (Wickham 2016; Pebesma 2018; Massicotte and South 2023; South et al. 2024; Dunnington 2023; Wilke 2024). All sites were displayed on a map of the United States for a visual representation of goosegrass herbicide resistance spread (Figure 1). Additionally, each herbicide treatment had a map created to display each population resistant to that specific herbicide (Figures 2, 3, 4, 5, and 6). The treatment metribuzin does not have a map because no population resulted in resistance to it.

## Results and Discussion

Results confirmed that goosegrass herbicide resistance is occurring, and many golf course superintendents are seeking help to combat the spread of resistance. A total of 50 populations were received and screened for resistance. Among these, 30 biotypes demonstrated a resistance

mechanism. A particularly problematic aspect of this survey was the extent of multiple resistance observed. Seventeen biotypes had resistance to multiple herbicides and modes of action. Of the total population's collected, 34% presented resistance to two or more herbicide-active ingredients. For example, the population from Wild Dunes Resort, Isle of Palm, SC, possessed cross-resistance to three different herbicides: oxadiazon, foramsulfuron, and sethoxydim. A biotype from Sarasota National Golf Club, Venice, FL, was resistant to sulfentrazone, oxadiazon, foramsulfuron, and sethoxydim.

This information motivates managers to modify their integrated pest management (IPM) programs by incorporating diverse herbicides with various MOA. Effectively controlling turfgrass weeds necessitates employing a variety of MOA to address the diversity of species that may be present. However, resistance evolution will probably not be prevented by annual rotations of weed control methods, including the application of herbicides with different MOA. Nevertheless, even if a manager were to rotate weed control methods successfully, effectively managing resistant weeds, it would not reverse the evolution of resistance. It merely interrupts the selection process, maintaining the existing resistance level rather than allowing it to escalate. This emphasizes the importance of designing management practices based on evidence demonstrating their effectiveness in specific resistance scenarios.

**Research Implications.** This study found that goosegrass biotypes responded differently to the selected herbicides. Through the screening process, it was observed that goosegrass exhibited resistance against sulfentrazone, oxadiazon, foramsulfuron, sethoxydim, and prodiamine, while metribuzin did not show evidence of evolved resistance. The biotypes tested were predominantly sourced from golf courses, where bermudagrass dominates and is managed at diverse heights to

obtain putting greens, roughs, and fairways. This issue is predominantly observed in the southern United States, particularly throughout the Southeast, where the range of goosegrass is extensive. Consequently, the scope of this investigation does not encompass the entirety of goosegrass resistance dynamics, notably excluding cropping systems, forages, and home lawns. Herbicide usage diverges significantly between golf courses and other agriculture fields. While some herbicides may share common active ingredients, the application rates, particularly in turfgrass settings, are typically lower to mitigate phytotoxicity rates. PRE herbicides available in turfgrass to control goosegrass include prodiamine, pendimethalin, dithiopyr, oxadiazon, and indaziflam, whereas a broader array of POST herbicides are available, often categorized by their MOA. These herbicides are ACCase inhibitors and can be utilized for PRE and POST goosegrass control. Several POST commercial products like Tribute Total (a combination of thiencarbazone, foramsulfuron, and halosulfuron), Celsius (including thiencarbazone, iodosulfuron, and dicamba), and Blindside (containing sulfentrazone and metsulfuron) serve as effective combinations for weed control. It is important to note that this study only investigated a limited subset of herbicides available on the market for goosegrass management in turfgrass, thus potentially overlooking other resistance mechanisms.

## Products Used

Anonymous (2012) Dismiss® CA, FMC Corporation, Philadelphia, PA.

Anonymous (2007) Ronstar Flo®, Bayer Environmental Science, Cary, NC.

Anonymous (2015) Revolver®, Bayer Environmental Science, Cary, NC.

Anonymous (2010) Segment®, BASF Corporation, Research Triangle Park, NC.

Anonymous (2004) Sencor DF®, Bayer Environmental Science, Cary, NC.

Anonymous (2011) Barricade 4FL®, Syngenta Crop Protection, LLC Greensboro, NC.

Anonymous (2019) Pendimethalin®, BASF Corporation, Research Triangle Park, NC.

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## Tables and Figures

**Table 1.** Herbicide treatments used for the resistance screening process. All rates are standard.

| HRAC | Application Timing | Active ingredient | Active ingredient rate kg ai $ha^{-1}$ | Trade name | Manufacturer | Formulation | Product rate $acre^{-1}$ | NIS (% V/V) |
|---|---|---|---|---|---|---|---|---|
| 14 | POST | sulfentrazone | 0.28 | Dismiss | Bayer Crop Science | 4.0 lb gal-1 | 8 fl oz | 0.25 |
| 14 | POST | oxadiazon | 1.12 | Ronstar Flo | Bayer Crop Science | 3.17 lb gal-1 | 1 lb ai | 0.25 |
| 2 | POST | foramsulfuron | 0.03 | Revolver | Bayer Crop Science | 0.19 lb gal-1 | 17.4 fl oz | 0.25 |
| 1 | POST | sethoxydim | 0.21 | Segment | BASF Corporation | 1.0 gal-1 | 1.5 pt | 0.25 |
| 5 | POST | metribuzin | 0.5 | Sencor 75 DF | Bayer Crop Science | 75% | 0.67 lb | 0.25 |
| 3 | PRE | prodiamine | 1.12 | Barricade 4FL | Syngenta crop protection | 4.0 lb gal-1 | 1 lb ai | 0.25 |

**Table 2.** List of goosegrass *(Eleusine indica)* populations screened for herbicide resistance, and the *s*creening results for each biotype. Red boxes indicate resistance, yellow indicates an intermediate resistance, and green indicates the biotype was found to be susceptible to that herbicide. White boxes mean the population was not screened for resistance against that herbicide.

| Number | Location | sulfentrazone | oxadiazon | foramsulfuron | sethoxydim | metribuzin | prodiamine |
|---|---|---|---|---|---|---|---|
| 1 | Grand National putting green | R | S | R | S | S | S |
| 2 | Sandestin putting green | S | S | R | S | S | S |
| 3 | Mid-Pines #6 | S | S | R | S | S | S |
| 4 | Currituck | S | S | S | S | S | S |
| 5 | Clanton | S | S | S | S | S | S |
| 6 | PBU | S | S | S | S | S | S |
| 7 | NON DR | S | S | S | S | S | S |
| 8 | Naples NON #17 | S | S | S | S | S | S |
| 9 | RB | S | S | R | S | S | S |
| 10 | Innsbrook | S | S | S | S | S | S |
| 11 | Vanderbilt S6 | S | S | S | S | S | S |
| 12 | Vanderbilt N7 | S | S | S | S | S | S |
| 13 | 2 Surf | S | S | S | S | S | S |
| 14 | TCC 1 | S | S | S | S | S | S |
| 15 | TCC 2 | S | S | S | S | S | S |
| 16 | Magnolia GC Houston, Texas | S | S | S | S | S | S |
| 17 | Ballen Isles | S | S | I | S | S | S |
| 18 | Monarch | S | S | S | S | S | S |
| 19 | Ridges | S | R | S | S | S | S |
| 20 | Lebanon | S | R | R | S | S | S |
| 21 | Waiehu Golfcourse dwarf | S | S | R | S | S | S |
| 22 | Troy Country Club | S | S | S | S | S | S |
| 23 | Pine Lakes | S | R | R | S | S | S |
| 24 | GNRTJ Collar | S | R | R | S | S | S |
| 25 | Wild Dunes Clay Breazeale | S | R | R | R | S | S |
| 26 | Surf 2 Clay Breazeale Bayer | S | S | S | R | S | S |
| 27 | Verdae Green Greenville, SC | S | R | S | R | S | |
| 28 | Paris Mt. 18 | S | S | I | S | S | I |
| 29 | Furman University 2 | S | S | S | S | S | R |
| 30 | Furman University 13 | S | S | I | S | S | I |
| 31 | Paris Mt. 12 | R | R | R | S | S | I |
| 32 | CCV | S | R | R | S | S | R |
| 33 | USF | I | S | I | S | S | S |
| 34 | Highland Oaks | I | S | R | S | S | S |
| 35 | Willow Lake CC Turfcat | R | R | R | S | S | S |
| 36 | Willow Lake CC Turfcat 5 | S | S | R | S | S | S |
| 37 | Willow Lake CC Turfcat 9 | S | R | S | S | S | S |
| 38 | Pelican Sound Golf and River | S | S | R | S | S | S |
| 39 | City of Apopka atheletic fields | S | S | S | S | S | S |
| 40 | Florida Rec Davies FL | S | S | R | S | S | S |
| 41 | West Bay Golf Club | S | S | R | S | S | S |
| 42 | Sarasota | R | R | R | I | S | |
| 43 | La Gorce | S | I | R | I | S | |
| 44 | Marion 1 | R | R | S | S | S | |
| 45 | Marion 2 | R | R | S | R | S | |
| 46 | Marion 3 | S | S | S | S | S | |
| 47 | Tallahassee 1 | S | S | S | S | S | |
| 48 | Tallahassee 2 | S | S | S | S | S | |
| 49 | Tallahassee 3 | S | S | S | S | S | |
| 50 | Tallahassee 4 | S | S | S | S | S | |

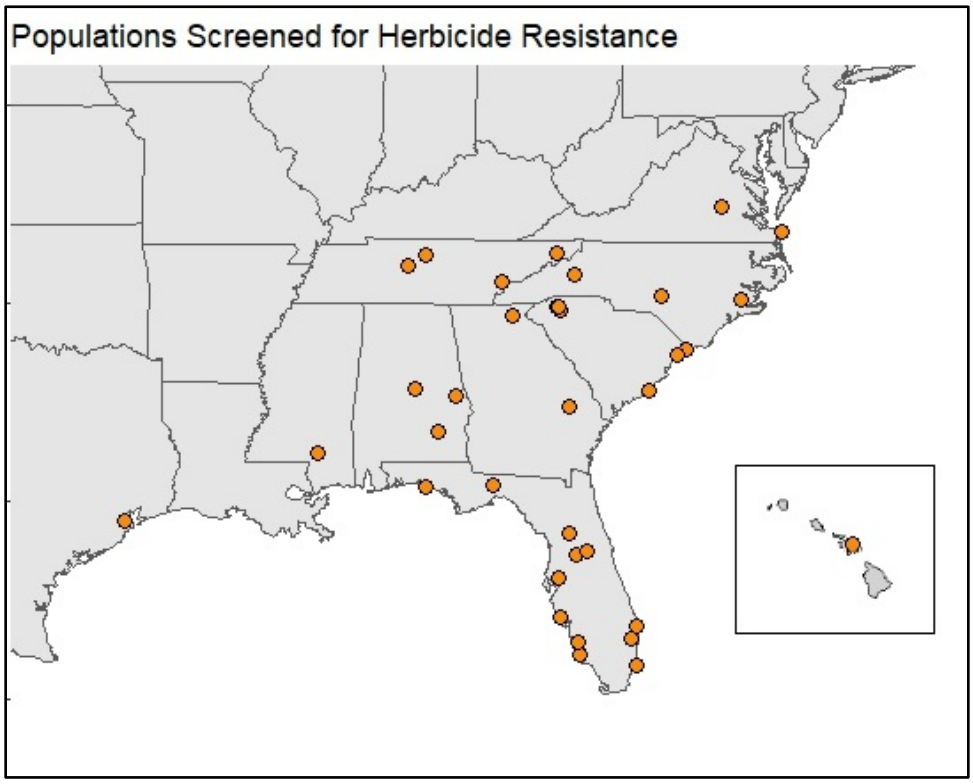


**Figure 1.** The map displays collection sites for all populations of goosegrass (*Eleusine indica*) across the United States. Orange points mark individual populations. However, due to the proximity of collection sites and the presence of multiple biotypes at some locations, not all 50 populations are individually represented on the map.

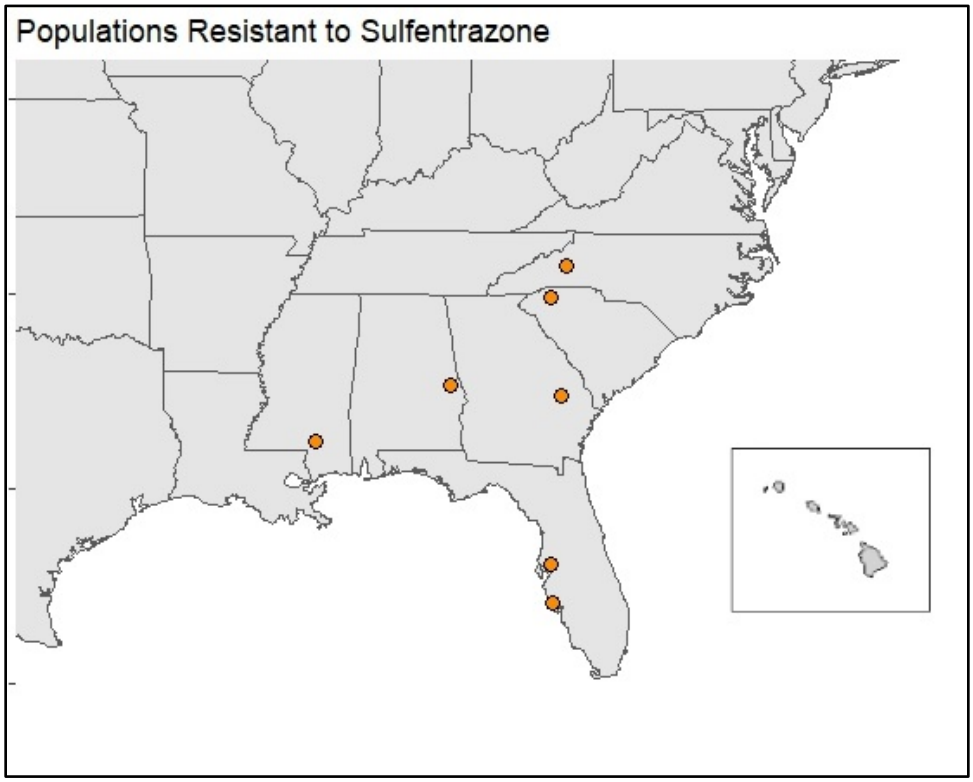


**Figure 2.** Map illustrating suspected resistant goosegrass (*Eleusine indica*) populations to sulfentrazone at a rate of 0.28kg/ha.

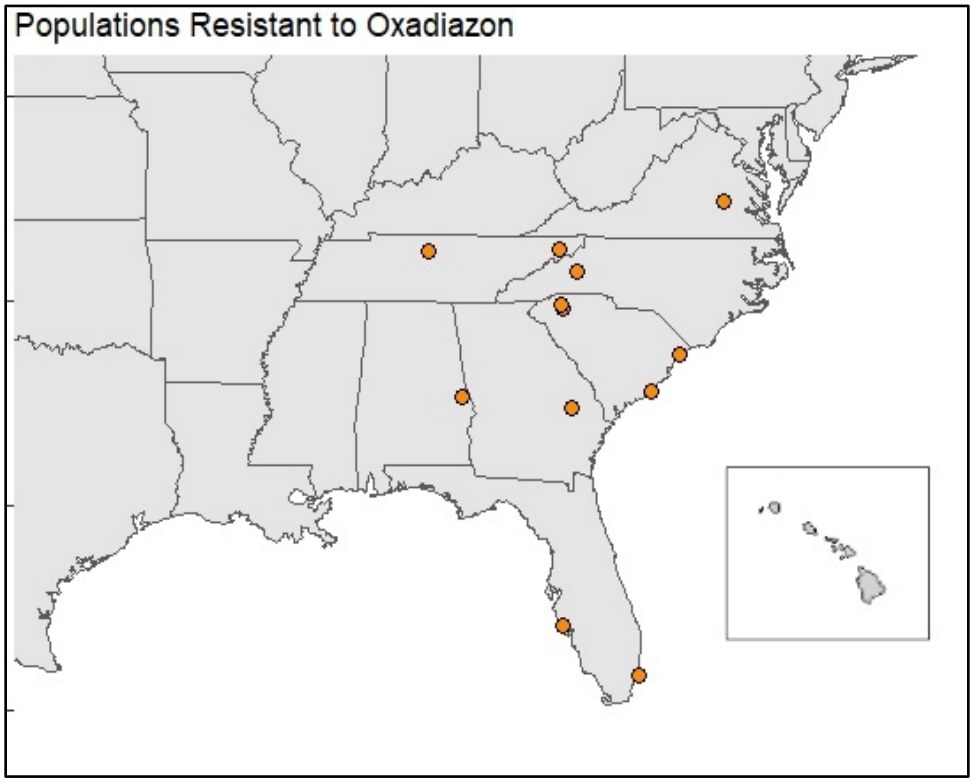


**Figure 3.** Map illustrating suspected resistant goosegrass (*Eleusine indica)* populations to oxadiazon at a rate of 1.12 kg/ha.

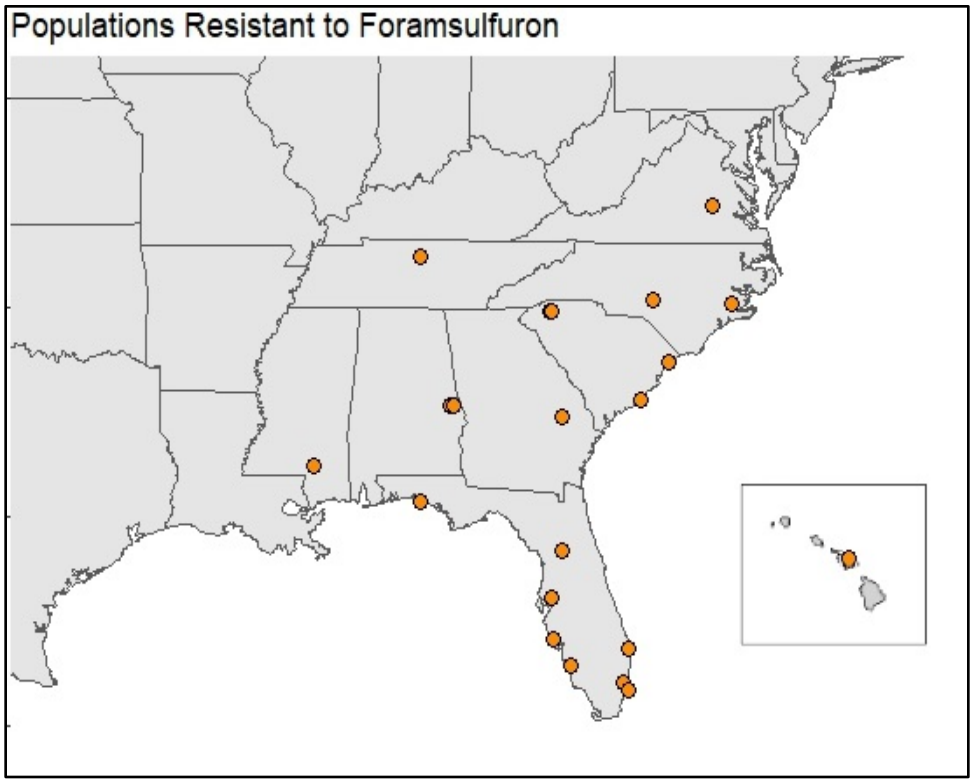


**Figure 4.** Map illustrating suspected resistant goosegrass (*Eleusine* indica) populations to foramsulfuron at a rate of 0.03 kg/ha.

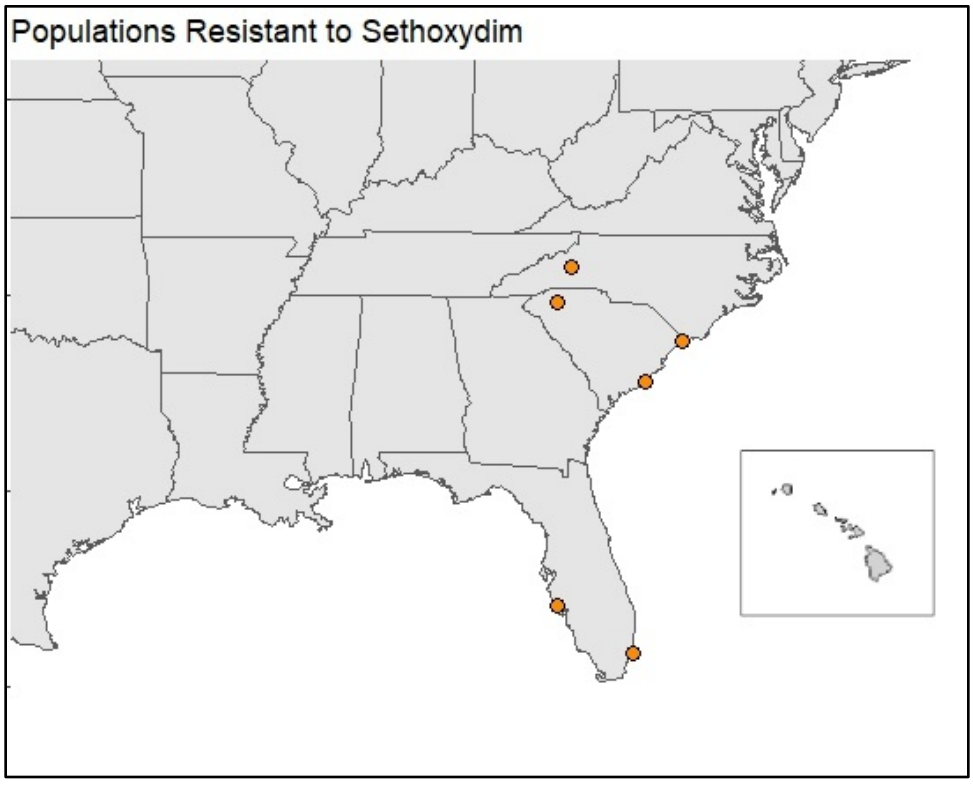


**Figure 5.** Map illustrating suspected resistant goosegrass (*Eleusine* indica) populations to sethoxydim at a rate of 0.21 kg/ha.

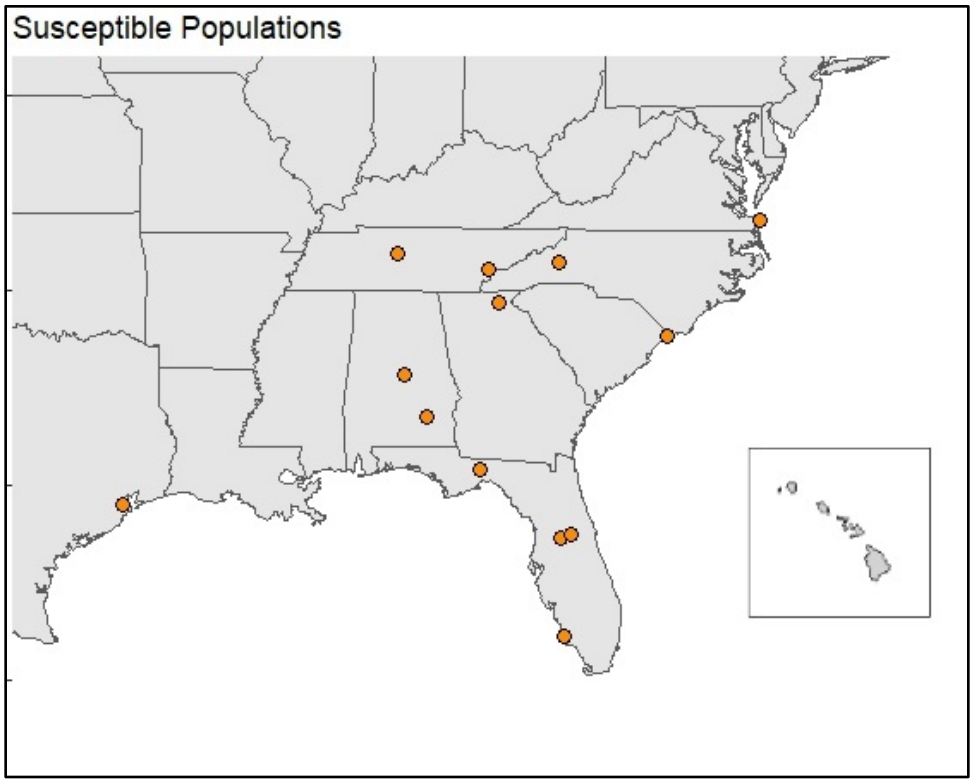


**Figure 6.** Map depicting all the goosegrass (*Eleusine* indica) biotypes that were found to be susceptible to all herbicides evaluated (sulfentrazone, oxadiazon, foramsulfuron, sethoxydim, metribuzin, and prodiamine).

# Chapter III.

## Identification of Foramsulfuron and Sulfentrazone Resistant Goosegrass (*Eleusine indica)* Populations


## Abstract

As part of a single-rate screening of putative herbicide resistant goosegrass (*Eleusine indica*) populations identified differential responses to foramsulfuron and sulfentrazone. It was determined that further research was needed to quantify the potential resistance level. Foramsulfuron resistant goosegrass populations were from Grand National Golf Course, Opelika, AL (R-R1) (R-R3), and Waiehu Golf Course, Maui County, Wailuku, HI (R-R2). Sulfentrazone-resistant goosegrass populations were from Grand National Golf Course, Opelika, AL(D-R1), Paris Mountain Country Club, Greenville, SC (D-R2). The susceptible population was taken from the Alabama Agricultural Experiment Station, Plant Breeding Unit near Tallassee, AL (S). Resistance was determined by visual injury ratings of plant response to the standard rate of foramsulfuron (28.89 g ai/ha) and sulfentrazone (279.63 g ai/ha) compared to a susceptible standard. Research was then conducted to assess the resistance level of the five suspected resistant populations to increasing rates of foramsulfuron (1.81 to 115.56 g ai/ha) and sulfentrazone (26.21 to 1677.78 g ai/ha). Visual injury ratings were taken at 7, 14, 21, and 28 days after treatment (DAT), and aboveground biomass at 28 DAT. The dose-response evaluation showed that resistant populations needed a much higher herbicide concentration to control it than the susceptible biotypes. Target-site gene sequencing of ALS and PPO1/2 revealed no mutation

in the resistant biotypes compared to the susceptible, indicating a possible non-target site mechanism.

**Additional index words:** goosegrass, dose-response, herbicide resistance, turfgrass

**Nomenclature:** *Eleusine indica*

## Introduction

Goosegrass [*Eleusine indica* (L.) Gaertn.] is a widely distributed summer annual weed whose presence results in complications with turfgrass areas and agricultural landscapes. Several reports of herbicide resistance in goosegrass have occurred worldwide (Russell et al. 2022; Cox 2014). Herbicide resistance is the ability of a plant to survive and reproduce after a standard herbicide application, which is normally lethal to a susceptible plant (Brosnan and Breeden 2013). According to the International Herbicide-Resistant Weed Database, as of June 2022, there are reports of goosegrass biotypes being resistant to eight different groups of herbicides: Photosystem I (PSI) electron diverters (Group 22), inhibition of glutamine synthase (Group 10), microtubule assembly inhibitors (Group 3), acetolactate synthase (ALS) inhibitors (Group 2), acetyl-CoA carboxylase (ACCase) inhibitors (Group 1), protoporphyrinogen oxidase (PPO) inhibitors (Group 14), Photosystem II (PSII) D1 series 264 binders (Group 5), and enolpyruvyl shikimate phosphate synthase (EPSPS) inhibitors (Group 2) (Takano et al. 2016; Heap 2022). Two common herbicides used in turfgrass management practices to control goosegrass are foramsulfuron and sulfentrazone. Foramsulfuron is an ALS inhibitor, and sulfentrazone is a PPO inhibitor. Previous research has revealed no *Eleusine indica* biotypes declared resistant to foramsulfuron or sulfentrazone.

Weed presence in turfgrass decreases its aesthetic quality and usability. In golf courses putting greens specifically, weeds alter the playability of the grass by adding uneven surfaces and altering the natural movement of the ball (McElroy 2013). Control of goosegrass in bermudagrass (*Cynodon* spp.*)* putting greens is complex, and it can be done with pre- and post-

emergent herbicides. Preemergent (PRE) herbicides rely greatly on seed germination, and application timing is critical for a successful herbicide application. Goosegrass seed germination begins mid-spring when soil temperatures rise above 55° - 60°F for 7 to 10 days (Ricigliano 2016). PRE herbicides should be applied two to four weeks before these temperatures are achieved for successful control. Obtaining adequate goosegrass control can be difficult, and a sequential application is often needed to control the later germinating plants fully. Examples of PRE herbicides utilized to control goosegrass include trifluralin, bensulide, dithiopyr, oxadiazon, pendimethalin, and prodiamine (Koski 2008). Postemergence (POST) goosegrass control is much more difficult because they are less reliable, and turfgrass phytotoxicity is an issue on some grasses like creeping bentgrass (*Agrostis stolonifera*) and Kentucky bluegrass (*Poa pratensis*). POST programs for turfgrass are limited to site specifications and decisions regarding chemical control are left at the discretion of superintendents and managers. The most consistent goosegrass control can be achieved with a substantial PRE application, followed by a POST application. Foramsulfuron and sulfentrazone are two POST herbicides commonly used in combination or as a single treatment to control post-emergent goosegrass (Cox 2014).

Foramsulfuron is a POST systemic sulfonylurea herbicide that inhibits the ALS enzyme (Ashton and Monaco 1991). The ALS enzyme is an early enzyme important in the biosynthesis of branched chained amino acids (valine, leucine, and isoleucine) (Zhou et al. 2007). It catalyzes two essential reactions: the condensation of two pyruvate molecules producing $CO_2$ and a-acetolactate and the condensation of pyruvate and a-ketobutyrate forming $CO_2$ and 2-acetohydroxybutyrate (Duke 1990). Since the introduction of ALS-inhibiting herbicides in the 1980s, more weed species have developed resistance to ALS-inhibiting herbicides than any other group (Tranel and Wright 2002; Whitcomb 1999). The rise in resistance can be linked to their

widespread adoption because of their low usage rates, low toxicity to mammals, broad spectrum selectivity, and high efficacy (Saari et al. 2018).

Sulfentrazone is a PPO-inhibiting herbicide in the triazolinone herbicide family. PPO is the last enzyme on the pathway to the biosynthesis of chlorophyll and heme compounds (Matzenbacher et al. 2014). When PPO is inhibited, protoporphyrin IX accumulates and causes light-dependent membrane damage (Li and Nicholl 2005). While developed in the 1960s, resistance to PPO inhibitors started to surface around 2004 (Li and Nicholl 2005). Sulfentrazone is labeled for use in many cold and warm-season turf species (FMC Professional Products 2008a). Uniquely, in turfgrass sulfentrazone can be applied PRE or POST (Brosnan et al. 2010).

Both foramsulfuron and sulfentrazone are selective herbicides that should exert low selection pressure when using the standard rates. High selection pressure is the control of weeds with a single application. Low selection pressure is the need for multiple applications to control a weed and a single application being largely ineffective. These herbicides have shown that multiple applications are often needed to achieve the desired control. According to their labels, it is recommended that both foramsulfuron and sulfentrazone have numerous applications for best results (Anonymous 2012; Anonymous 2015). These herbicides would be characterized as weak selectors because they require multiple applications, whereas a strong selector would only need one application for complete control of the population.

Many resistance-testing protocols have been created for numerous herbicides and weed species (Saari et al. 2018). Herbicide resistance screening is often conducted after complaints by superintendents for herbicide application failures. A lack of control, or resistance, is usually observed after repeated usage of synthetic herbicides with the same mode of action (MOA) without alternative weed control. Annual weeds that are prolific seed producers and are

repeatedly exposed to a single herbicide mode of activity are the most likely to develop resistance (Heap 1994). Seed or plant samples are collected and evaluated using whole plant bioassays when resistance is suspected. Bioassays are a reliable method for identifying new cases of herbicide resistance (Heap 1994). Dose-response assays include applying a range of herbicide rates to a single biotype to observe the sample's reaction to each rate.

Herbicide resistance is often categorized into target (TSR) and non-target site resistance (NTSR). TSR occurs when mechanisms structurally change the herbicide binding site, resulting in decreased sensitivity of the target site to herbicide inhibition (Saari et al. 2018). NTSR can result from many factors limiting herbicide uptake; some common ones are enhanced metabolism, lack of herbicide absorption, and translocation to the site of action (SOA; Saari et al. 2018). The majority of discovered herbicide resistant biotypes are TSR because resistance can be conferred by a single, significant phenotypic effect on a dominant gene. The initial observation of resistance in weeds resulting from treatment with ALS inhibitor herbicides occurred in *Lactuca serriola* L. in 1987 (Mallory-Smith et al. 1990). As of 2024, documented populations of 174 weed species have developed resistance to ALS inhibitors (Heap 2024). There are over 20 amino acid substitutions at eight sites conferring resistance to ALS herbicides. Mutations include Ala122, Pro197, Ala205, Asp376, Arg377, Trp574, Ser653, and Gly654 (Beckie 2012; Powles and Yu 2010). In goosegrass, substitutions of Thr239lle and Met268Thr have been identified as mechanisms of evolved resistance (Beckie 2012; Vaughn et al. 1987). PPO-inhibiting herbicides have a much lower risk for resistance selection, with only 16 species populations exhibiting resistance (Heap 2024). Resistance to PPO-inhibiting herbicides in *Amaranthus tuberculatus* has been linked to a unique mechanism characterized by a three-base pair deletion (Gly210) in the PPX2L gene (Beckie 2012; Patzoldt et al. 2006).

An announcement was distributed to turfgrass managers and pesticide sales personnel throughout the southeast to submit samples to the Herbicide Resistance Diagnostics Lab (HRDL) for screening. Goosegrass biotypes, primarily from golf courses with suspected herbicide resistance, were submitted in 2020-2023. Over 50 goosegrass populations were tested against seven common herbicides used in turfgrass management practices. The herbicides applied were sulfentrazone, oxadiazon, foramsulfuron, sethoxydim, metribuzin, and prodiamine at their standard labeled rates (Table 3). This survey led to the discovery of 30 resistant biotypes. Of these, 23 were resistant to foramsulfuron, and eight were resistant to sulfentrazone. Multiple herbicide resistance was also observed, with one population from a golf course in North Carolina exhibiting resistance to sulfentrazone, oxadiazon, and sethoxydim. Another biotype from a country club in South Carolina had cross-resistance to sulfentrazone, oxadiazon, and foramsulfuron.

Little research has yet to be conducted on resistance goosegrass against foramsulfuron and sulfentrazone. Initial screening research to evaluate suspected resistant populations showed that the standard rates for foramsulfuron (29 g ai/ha) and sulfentrazone (280 g ai/ha) did not control the resistant populations. This research aimed to determine the resistance levels to foramsulfuron and sulfentrazone in the suspected resistant biotypes using dose-response assays.

## Material and Methods

The research was conducted to evaluate three suspected populations resistant to foramsulfuron (Revolver, Bayer Environmental Sciences, Cary, NC) and two to sulfentrazone

(Dismiss, FMC Corporation, Philadelphia, PA). Suspected foramsulfuron resistant populations include Grand National Golf Course, Opelika, AL (R-R1) (R-R3), and Waiehu Golf Course, Maui County, Wailuku, HI (R-R2). Suspected sulfentrazone-resistant goosegrass populations were from Grand National Golf Course, Opelika, AL(D-R1), Paris Mountain Country Club, Greenville, SC (D-R2). The susceptible population (S) previously collected from the Alabama Agricultural Experiment Station, Plant Breeding Unit near Tallassee, Alabama, is referred to as "PBU." The HRDL (http://resistancelab.org) acquired plants due to suspected resistance. Seeds collected were combined, dried at 60 C for 24 h, and stored at 4 C until use.

**Dose-Response Assay.** Experiments were conducted in a glasshouse from August to September 2021. Greenhouse conditions included air temperature maintained at 30 ± 2 C throughout the investigation, and supplemental light was provided. To establish experimental populations, approximately 100 seeds were sown in plastic greenhouse flats filled with potting media (Miracle-Gro Moisture Control Potting Mix, Scotts Miracle-Gro Company, Marysville, OH). To promote healthy growth, the plants were fertilized (28-6-16 Miracle-Gro Water-soluble All-Purpose Plant Food, Scotts Miracle-Gro Products Inc, Marysville, OH.) weekly and irrigated three times daily by an overhead misting system. Seed germination occurred about 7 to 10 days after sowing. Seedlings developed for another 7 to 10 days and were transplanted into 4-inch pots filled with Marvyn sandy loam soil. Plants then grew for an additional 14 days or until the 3-4 leaf/2 tiller stage was developed. Dose-response experiments were performed on the five resistant populations and one susceptible (S) using foramsulfuron and sulfentrazone. Doses for each herbicide were 0 (non-treated control), 0.125, 0.25, 0.5, 1, 2, 4, and 6 times the standard rate. Foramsulfuron rates were 0.002, 0.004, 0.008, 0.016, 0.029, 0.058, and 0.0116 kg ai/ha. Sulfentrazone rates were 0.026, 0.053, 0.105, 0.21, 0.42, 0.89, and 1.68 kg ai/ha. All herbicide

treatments were applied with a CO2-pressurized backpack sprayer, calibrated to deliver 280 L $ha^{-1}$ with a handheld four-nozzle boom (TeeJet Tp8002 flat fan nozzles with 25 cm spacing; Spraying Systems Company, Wheaton, IL). A nonionic surfactant (Induce, Helena Chemical Company, Collierville, TN) was included in all treatments at 0.25% V/V. The experiment was conducted in a randomized complete block design (RCBD) with four replications, and the study was repeated in time. Data collected included percent injury on a 0% to 100% scale, where 0% is no visual injury, and 100% is total plant death. Percent injury was rated 7, 14, 21, and 28 days after treatment (DAT). Fresh plant aboveground biomass was weighed at 28 DAT by clipping plants at the soil surface and weighing immediately.

**Gene Sequencing**. Transcriptome sequencing was conducted to determine whether any known target-site mutations were present. All sequencing was performed with Novogene (Beijing, China) using an Illumina NovoSeq 6000. Transcriptomes were assembled using Trinity RNA-Seq de novo transcriptome assembly (https://github.com/trinityrnaseq/trinityrnaseq) and annotated using Trinotate (https://github.com/Trinotate/Trinotate). *ALS, PPO1,* and *PPO2* gene sequences were extracted from each transcriptome and visualized for single nucleotide polymorphisms (SNP) using CLC Genomics Workbench 21 (Qiagen, Hilden, Germany). Known *ALS*, *PPO1,* and *PPO2* gene sequences were downloaded from NCBI and were aligned against R-R1, R-R2, R-R3, D-R1, D-R2, and S gene sequences for SNP identification (KU720629, MK040459, and MK573538, respectively).

**Data Analysis**. Herbicide dose-response data were subjected to ANOVA using PROC GLM in SAS v. 9.4 (SAS Institute, Cary, NC). Factors tested were replications, experimental runs, herbicide rate, biotype, and aboveground biomass. Population response to foramsulfuron and

sulfentrazone was graphed using Prism v 9.0.0 (Prism, GraphPad, http://www.graphpad.com). The herbicide rates (including the non-treated) were transformed into a logarithmic scale to ensure equal spacing, with the untreated set to -2.0. The log-transformed rates were -2.0, -1.5, -1.0, -0.5, 0, 0.5, 1.0, 1.5, corresponding to 1.81, 3.61, 7.22, 14.44, 28.89, 57.78, and 115.56 g ai/ha for foramsulfuron and 26.21, 52.43, 104.86, 209.72, 419.44, 838.89, and 1677.78 g ai/ha for sulfentrazone. The sigmoidal dose-response (variable slope) equation used to graph the goosegrass injury is as follows:

$$Y = Bottom + \frac{(Top - Bottom)}{1 + 10^{(LogEC50 - X) \cdot HillSlope}} \quad [1]$$

Where Y is the percentage injury (%), and X is the log rate of the herbicide. The Top and Bottom are plateaued, the LogEC50 is the concentration needed to inhibit 50% of the plant's growth, and the HillSlope is the curve's steepness. The log (inhibitor) vs. normalized response equation used to graph the aboveground biomass of the goosegrass is as follows:

$$Y=100/(1+10^{(X-LogIC50)}) \quad [2]$$

Where Y is the aboveground biomass (g), and the LogIC50 is the concentration needed to inhibit 50% of the plant's growth.

## Results and Discussion

**Dose-Response Assay.** Our initial hypothesis stated that the suspected R populations would resist foramsulfuron and sulfentrazone because the initial screenings failed to control them. Experimental results show that the R populations responded differently than the S populations to both herbicides. The R populations showed higher tolerance than the S population when treated with foramsulfuron and sulfentrazone (Figure 7. and Figure 8.) The R population also amassed more aboveground biomass than the S population when treated with foramsulfuron and sulfentrazone (Figure 9. and Figure 10.). The foramsulfuron R populations, R-R1, R-R2, and R-R3, had a calculated $I_{50}$ of 0.12, 0.12, and 0.09, respectively, whereas the susceptible biotype had a 0.02 $I_{50}$. The sulfentrazone populations D-R1 and D-R2 had a calculated $I_{50}$ of 0.11 and 0.21, respectively, whereas the susceptible biotype had a 0.03 $I_{50}$. Based on the $I_{50}$ values for both injury and biomass, the foramsulfuron R biotypes are highly resistant and require a higher concentration of foramsulfuron to control. However, the resistance level of the sulfentrazone R biotypes is low and requires a lower concentration to control. While this is the first report of foramsulfuron and sulfentrazone resistant goosegrass in peer-reviewed literature, it is not the first instance of goosegrass exhibiting tolerance to these herbicides (Brosnan 2009; Pritchard 2020; Bi 2021).

**Gene Sequencing**. Understanding the type of herbicide resistance, non-target (NTSR), or target site (TSR) resistance mechanism, is crucial for designing effective weed management systems. This study specifically focused on comparing the ALS gene associated with target site resistance to sulfonylureas (foramsulfuron). Additionally, we examined the PPO1 and PPO2 genes related to target site resistance to triazolinones (sulfentrazone). No mutations were discovered on any of the biotypes to confirm target-site resistance (Figure 11 and Figure 12). The resistance observed in these goosegrass biotypes is classified as NTSR.

This research demonstrates two points. First, sulfentrazone and foramsulfuron resistant biotypes exist at the managed turfgrass locations tested. Second, non-target site resistance to sulfentrazone and foramsulfuron exists. Based on our findings, we consider foramsulfuron and sulfentrazone to be marginally effective herbicides for controlling goosegrass. These herbicides require multiple applications to achieve effective control, often at maximum rates. This scenario promotes the evolution of NTSR rather than TSR because the plants tend to survive after one or two applications. Therefore, if complete control is not achieved, there could be a rapid selection of plants that are more susceptible. The population will transition more quickly due to the marginal efficacy of foramsulfuron and sulfentrazone. Further research is needed to determine whether the resistance to foramsulfuron and sulfentrazone in our greenhouse dose-response screens translates to variation in control in a field setting.

**Research Implications.** This research exclusively focused on two herbicides, foramsulfuron and sulfentrazone, and their role in causing resistance in goosegrass. It is important to note that there are numerous herbicides available for goosegrass control. POST herbicide options include topramezone, metribuzin, fluazifop, fenoxaprop, and mesotrione. Additionally, a commercial product known as Speedzone, which contains carfentrazone, 2,4-D, MCPP, and dicamba, is another product available for goosegrass control. While there is a possibility that resistance to these herbicides may be occurring, they were not evaluated in this study. Foramsulfuron and sulfentrazone are two primary herbicides that can be used in bermudagrass and zoysiagrass in golf course turf, sports fields, and commercial lawns. Although there was hope that foramsulfuron could have been developed as a primary herbicide for controlling goosegrass, this

research indicates the presence of inherited herbicide resistance. Effective control of goosegrass biotypes requires sequential applications of sulfentrazone and foramsulfuron. Repeated applications at a lower rate will allow a higher amount of the genetic material to survive into the next generation of weed species, thereby increasing the selection pressure, and accelerating the evolution of resistance. The low selection pressure of foramsulfuron and sulfentrazone poses a significant threat to the rapid evolution of resistance in goosegrass via NTSR mechanisms.

## Products Used

Anonymous (2012) Dismiss ® CA, FMC Corporation, Philadelphia, PA.

Anonymous (2015) Revolver ®, Bayer Environmental Science, Cary, NC.

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## Tables and Figures

**Table 3**. Herbicide treatments used for the resistance screening process. All rates are standard.

| HRAC | Active ingredient | Trade name | Active ingredient rate kg ai $ha^{-1}$ | Formulation | Product rate $acre^{-1}$ | Manufacturer | NIS (% V/V) |
|---|---|---|---|---|---|---|---|
| 14 | sulfentrazone | Dismiss | 0.28 | 4.0 lb gal–1 | 8 fl oz | Bayer Crop Science | 0.25 |
| 14 | oxadiazon | Ronstar Flo | 1.12 | 3.17 lb gal–1 | 1 lb ai | Bayer Crop Science | 0.25 |
| 2 | foramsulfuron | Revolver | 0.03 | 0.19 lb gal–1 | 17.4 fl oz | Bayer Crop Science | 0.25 |
| 1 | sethoxydim | Segment | 0.21 | 1.0 gal–1 | 1.5 pt | BASF Corporation | 0.25 |
| 5 | metribuzin | Sencor 75 DF | 0.5 | 75% | 0.67 lb | Bayer Crop Science | 0.25 |
| 3 | prodiamine | Barricade 4FL | 1.12 | 4.0 lb gal–1 | 1 lb ai | Syngenta crop protection | 0.25 |

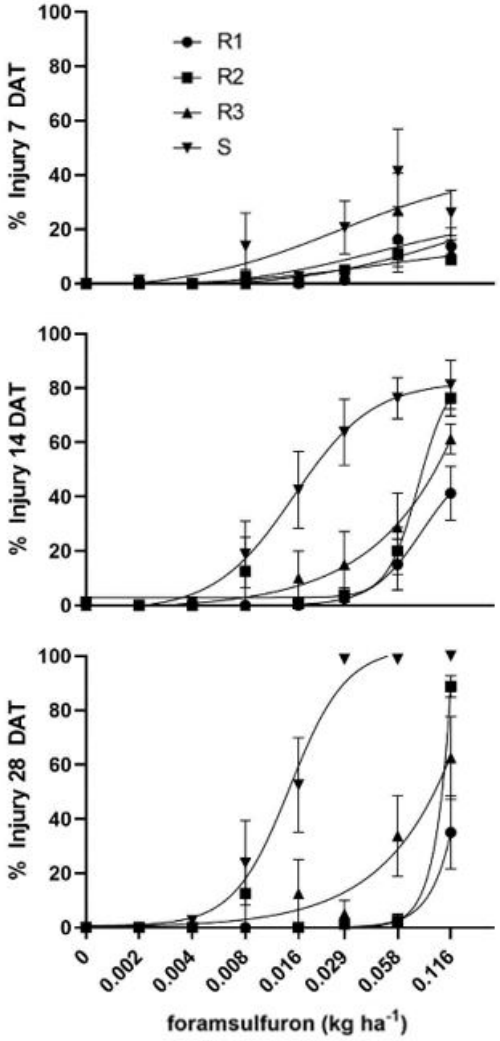


**Figure 7**. Injury response of resistant (R1, R2, and R3) and susceptible (S) biotypes to increasing rates of foramsulfuron at 7, 14, and 28 days after treatment (DAT). Injury was rated on a 0% to 100% scale, where 0% is no injury caused by the herbicide application, while 100% is total plant death.

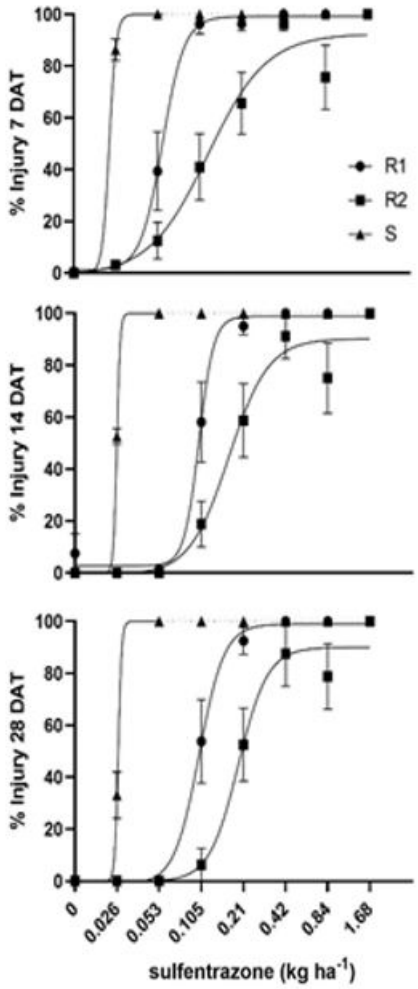


**Figure 8**. Injury response of resistant (R1 and R2) and susceptible (S) biotypes to increasing rates of sulfentrazone at 7, 14, and 28 days after treatment (DAT). Injury was rated on a 0% to 100% scale, where 0% is no injury caused by the herbicide application, while 100% is total plant death.

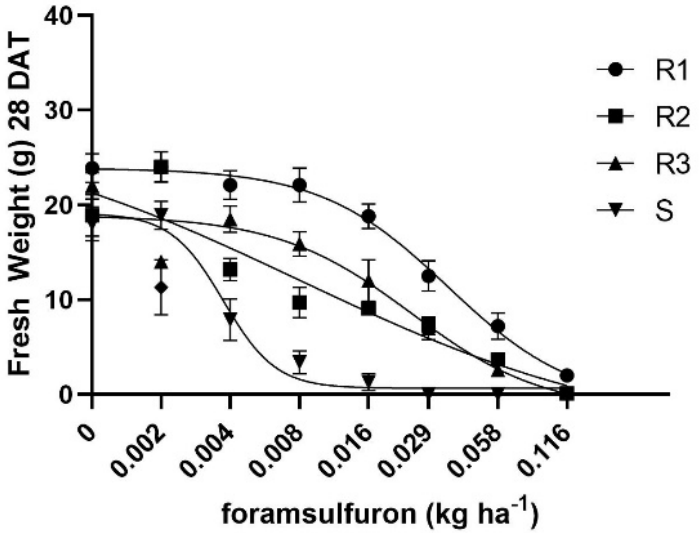


**Figure 9.** Aboveground biomass (g) of resistant (R1, R2, and R3) and susceptible (S) biotypes to increasing rates of foramsulfuron at 28 days after treatment (DAT).

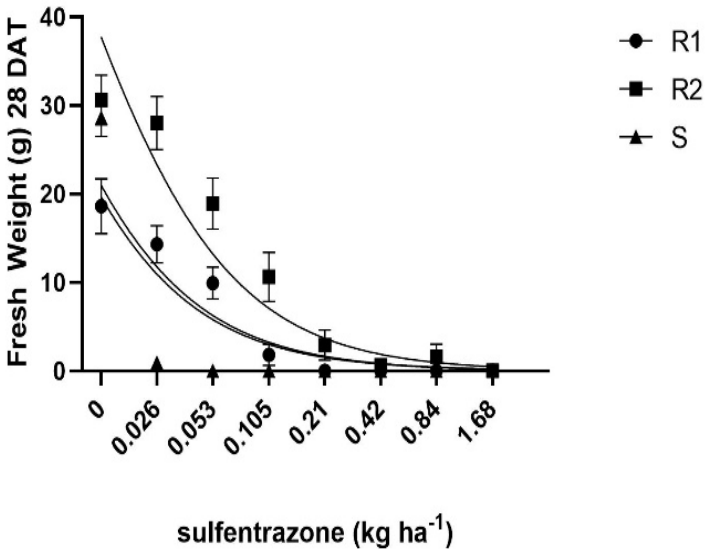


**Figure 10.** Aboveground biomass (g) of resistant (R1 and R2) and susceptible (S) biotypes to increasing rates of sulfentrazone at 28 days after treatment (DAT).

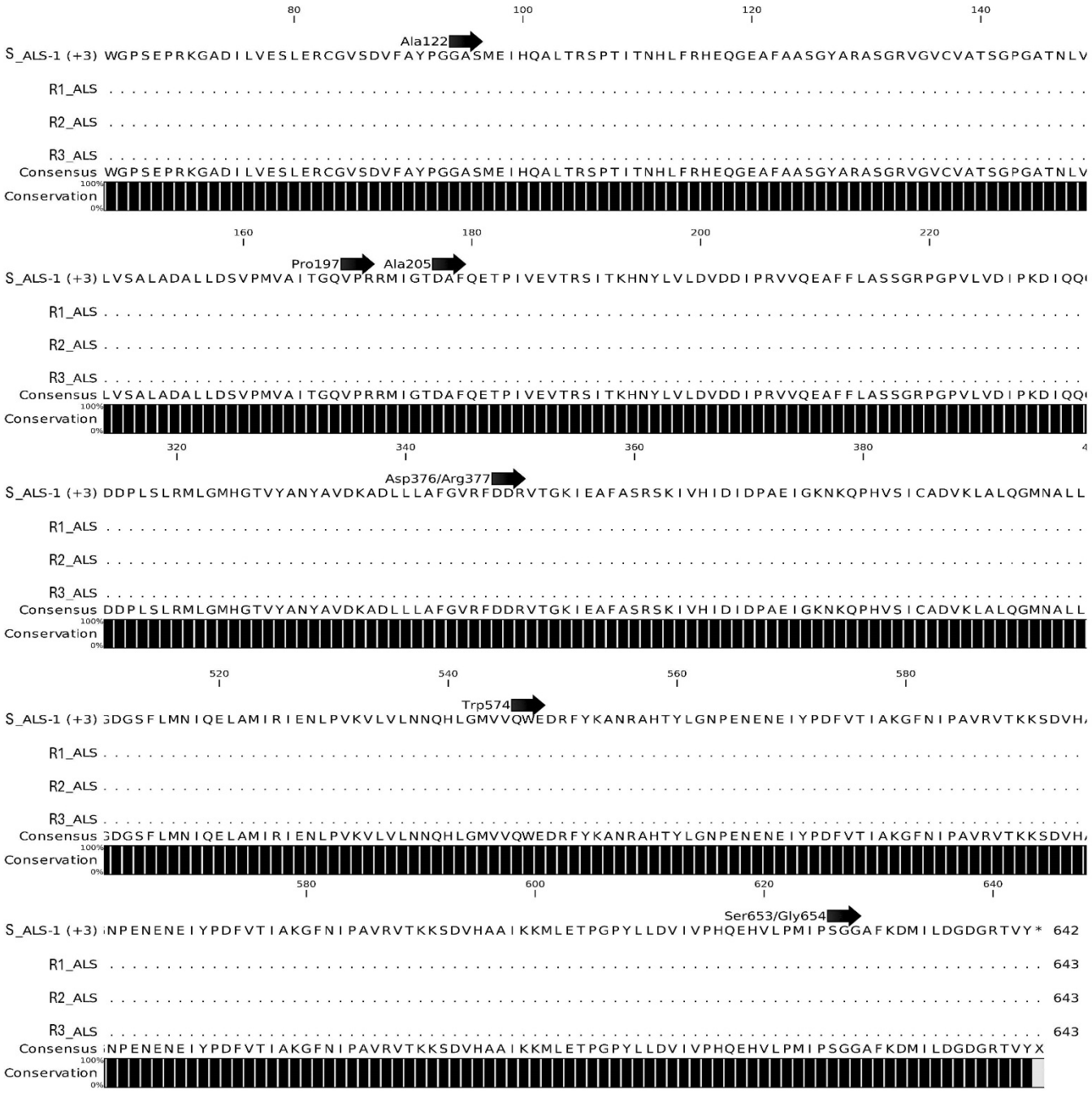


**Figure 11.** Read mapping of transcriptome sequencing data of ALS from foramsulfuron susceptible (S) and resistant populations (R1, R2, and R3) with SNP of known mutation locations at Ala122, Pro197, Ala205, Asp376, Arg377, Trp574, Ser653, and Gly654.

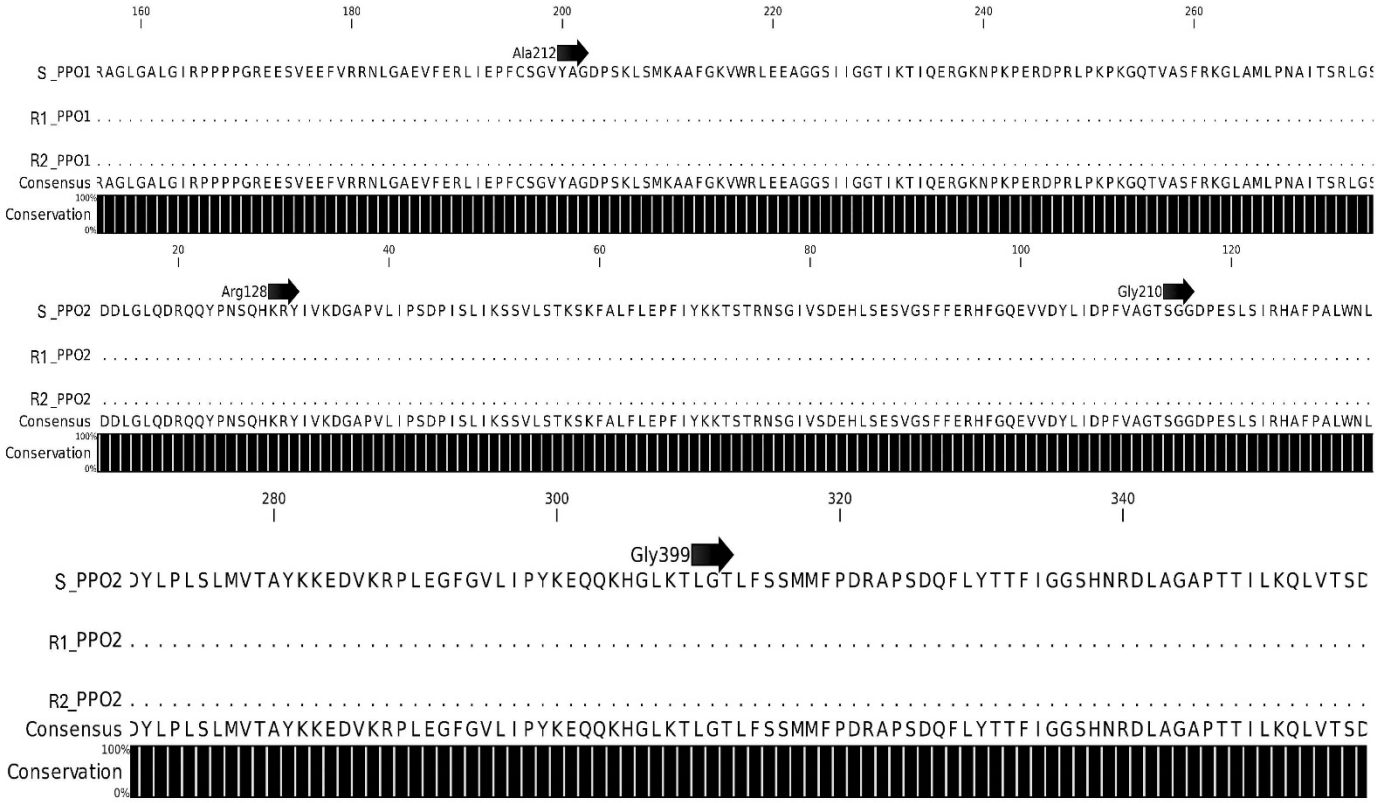


**Figure 12.** Results of read mapping of transcriptome sequencing data of PPO1 and PPO2 from sulfentrazone susceptible (S) and resistant populations (R1 and R2) with SNP of known mutation locations at Ala212, Arg128, Gly210, and Gly399.