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Exploring Putative Herbicide Resistance in Two Summer Annual Grassy Weeds

A Thesis

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Requirement

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Abstract

Goosegrass (*Eleusine indica* L. Gaertn) is a summer annual weed of warm-season turfgrass. While goosegrass biotypes have evolved resistance to several herbicidal modes of action, there are few reports of goosegrass resistance to acetolactate synthase (ALS) inhibitors. Plants surviving treatment with foramsulfuron (44 g ha^{-1}) were collected from a bermudagrass (*Cynodon* spp.) bowling green in Ala Moana Beach Park in Honolulu, HI and cultured for seed collection. Foramsulfuron dose-response experiments were conducted at the University of Tennessee (Knoxville, TN) during summer 2019 comparing putative-resistant (PR) goosegrass to a known susceptible (S) biotype. Experiments were repeated in both time and space during 2019. After establishment, pots were thinned to contain three plants and treated with foramsulfuron at 0, 5.5, 11, 22, 44, 88, 176, 352 g ha^{-1} using an enclosed spray chamber calibrated to deliver 215 L ha^{-1}.

Smooth crabgrass (*Digitaria ischaemum*) is a summer annual weed of warm-season turfgrass. Smooth crabgrass plants were collected from a bermudagrass (*Cynodon* spp.) golf course fairway after surviving treatment with oxadiazon (3360 g ha^{-1}). Collected plants were brought to the University of Tennessee (Knoxville, TN) and cultured for seed production. In summer 2019, experiments were conducted at the University of Tennessee comparing the putative resistant (PR) smooth crabgrass to a known susceptible (S) smooth crabgrass. Experiments were repeated in both time and space during 2019. Pots were seeded with both biotypes, then immediately sprayed with oxadiazon at 0, 423, 850, 1,700, 3,400, 6,800, 13,500, 27,000 g ha^{-1} using an enclosed spray chamber calibrated to deliver 215 L ha^{-1}.

Goosegrass and smooth crabgrass control were visually assessed using a 0 (i.e., no control) to 100% (i.e., plant death) scale. Aboveground biomass was harvested at the end of all

studies, dried in a forced-air oven at 55°C, and weighed. In goosegrass experiments, data were subjected to log-logistic regression analysis to determine the relationship between PR and S biotypes to increasing doses of foramsulfuron. Few differences in goosegrass control and aboveground biomass were detected between biotypes and those present were inconsistent across experimental runs. Diversity across experimental runs in smooth crabgrass control and aboveground biomass provided difficult and inconsistent data. We cannot conclude that PR goosegrass is resistant to foramsulfuron based on the data collected in these experiments, nor can we conclude that the smooth crabgrass biotype collected is resistant to the protoporphyrinogen oxidase inhibiting herbicide oxadiazon**.**

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Protoporphyrinogen Oxidase (PPO) and Acetolactate Synthase (ALS) Inhibiting Herbicides

Herbicide resistance is an increasingly problematic phenomena concerning weed management. It is across all forms of agronomic applications. Herbicide resistance is the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type (Vencill et al. 2012). Turfgrass is just one of the many agronomic systems affected by evolved resistance of weeds. Golf course turf encompasses almost all reported cases of resistance in turfgrass (Brosnan and Breeden 2013). Among grassy weeds worldwide, acetolactate synthase (ALS) inhibitors have the most resistance cases per any group of herbicide (Sato et al. 2009). This is in part due to the simplicity of the single or double point mutation needed for resistance to ALS inhibitors (Sato et al. 2009). There are 165 reported weed species resistant to ALS inhibitors alone (Heap 2020). Protoporphyrinogen oxidase (PPO) inhibitors have been used for around 50 years (Matsunaka 1976). With recent developments of glyphosate resistance, PPO inhibitors have become more popular (Legleiter et al., 2009; Salas et al., 2016) in agronomic cropping systems. There are currently 13 weed species resistant to PPO inhibiting herbicides, despite their long existence and increased use (Heap 2020).

Goosegrass [*Eleusine indica* (L) Gaertn] and crabgrass (*Digitaria* spp.) are two grassy weeds that often appear in turfgrass systems. Herbicide resistance in goosegrass throughout agronomic systems is well documented in dinitroanilines (Mudge et al. 1984), arloxyphenoxyproprionates and cyclohexanediones (Marshal et al. 1994), glyphosate (Teng and Teo 1999), imidazolinones (Valverde et al. 1993), paraquat (Buker et al. 2002), sethoxydim (Osuna et al. 2012), and fluazifop (Cha et al. 2014). One such case of goosegrass herbicide resistance in turfgrass is metribuzin (Brosnan et al. 2008). However, throughout these cases of resistance, there are no confirmed cases of goosegrass resistance to ALS inhibiting herbicides (Heap 2020). In crabgrass, there has only been one documented case of resistance evolving in managed turfgrass; Derr (2002) confirmed resistance to fenoxaprop in a smooth crabgrass (*Digitaria ischaemum* Schreb) biotype from New Jersey.

Chapter I

Introduction

In turfgrass, goosegrass is a problematic summer annual weed (McCullough et al. 2016). It is seen in warm and cool-season turfgrass established on lawns, golf courses, sports fields, and landscaped areas throughout the United States transition zone southward (McCullough et al. 2012). In warmer temperate regions goosegrass would be considered a summer annual, while in tropical regions it can be perennial (Brosnan et al. 2008).

Within warmer temperate regions preemergence herbicides including prodiamine and oxadiazon are commonly used to control goosegrass (Wiecko 2000). Postemergence herbicides commonly used to control goosegrass include simazine + monosodium methanearsonate (MSMA; Murdoch and Ikeda 1974), diclofop + metribuzin (Nishimoto and Murdoch 1999), foramsulfuron + metribuzin (Busey 2004), foramsulfuron (Nishimoto and Kawate 2003), MSMA + metribuzin (Johnson 1980; McElroy et al. 2007), fluazifop (Johnson 1993, 1994), and fenoxaprop (Dernoeden 1989).

A perennial goosegrass population in Hawaii was suspected to be resistant to foramsulfuron, an ALS inhibiting herbicide. The population was found on a lawn bowling club (Honolulu Lawn Bowls Club, Ala Moana Regional Park, Honolulu, HI), a semi-private lawn bowling field. The site is maintained by a greenskeeper through routine weed management and mowing. Foramsulfuron was used in consecutive seasons at the club for weed management, and they witnessed poor control on goosegrass within that time period. Plants from the site were transplanted to a greenhouse and seed was collected for testing. Pictures below are for reference to the site and surviving goosegrass (Figure 1; Figure 2; Figure 3).

Figure 1. Honolulu Lawn Bowls Club (Honolulu, HI) entrance

Figure 2. Putative resistant goosegrass (Eleusine indica) from Honolulu Lawn Bowls Club (Honolulu, HI)

Figure 3. Playing surface at Honolulu Lawn Bowls Club (Honolulu, HI)

We hypothesized that this goosegrass collection from Hawaii was resistant to foramsulfuron. A series of controlled environment experiments were conducted to evaluate the response of this putative-resistant goosegrass (PR) to increasing doses of foramsulfuron compared to a known susceptible biotype.

Materials and Methods

Goosegrass plants were collected from a bermudagrass (*Cynodon* spp.) bowling (Honolulu Lawn Bowls Club. Honolulu, HI) after surviving multiple applications of foramsulfuron (44 g ha^{-1}). Controlled environment experiments were conducted during 2019 in a glasshouse at the University of Tennessee (Knoxville, TN) and repeated in time and space. In experimental run one, air temperature averaged 25.5 ^{o}C with an average of 375 µmol m^{-2} s^{1} solar radiation, while in experimental run two, air temperature averaged 26.1^{o}C with 301 µmol m^{-2} s^{1} average solar radiation.

A known susceptible (S) and PR goosegrass biotype were surface seeded into greenhouse pots (8.9 cm^{3}) filled with Sequatchie silt loam (fine-loamy, siliceous, semiactive, thermic, humic, Hapludult). Soil was amended with calcined clay at a 4:1 soil to clay ratio. Pots were overhead irrigated twice daily (06:00 and 13:00). After emergence, plants were supplied nutrients with complete fertilizer (20-20-20 JR Peters Inc., Ellington, PA) at 49 kg N ha^{-1}. Five weeks after seeding, pots were thinned to three plants. At the three-tiller stage, pots were treated with foramsulfuron (Revolver. Bayer Crop Sciences. St. Louis, MO) using an enclosed spray chamber (Generation III Research Sprayer, DeVries Manufacturing, Hollandale, MN) calibrated to deliver 215 L ha^{-1} via a single flat-fan nozzle (8004EVS. TeeJet Spraying Systems, Wheaton, IL). Foramsulfuron rates were at 0, 5.5, 11, 22, 44, 88, 176, 352 g ha^{-1} respectively which represented 0.125 to 8 times the maximum label rate of 44 g ha^{-1}, respectively. Goosegrass control was visually assessed using a 0 (i.e., no control) to 100% (i.e., plant death) scale. Assessments were made at the end of the study: 55 days after treatment (DAT) in the first experimental run and 63 DAT in the second. Following visual rating, aboveground biomass was harvested, dried in a forced air oven (Laboratory Oven Model LR-271C, The Grieve Corporation, Round Lake, IL) for two days at 55^{o} C, and weighed. Aboveground biomass data were expressed as a percentage of non-treated controls in each replication. Pots were arranged in a randomized complete block design with four replications. Data were subjected to non-linear regression analysis in Prism (Version 8.2.1. GraphPad Prism. La Jolla, CA.) using a log-logistic model (Seefeldt et al. 1995). A global sums-of-squares F-test was used to detect differences between goosegrass biotypes at $\alpha = 0.05$.

Results and Discussion

Results for this experiment were varying. The first experimental run reported no significant differences in goosegrass control between the susceptible and putative resistant (PR) biotypes (Figure 4). However, there was a statistically significant difference in biomass reduction among the goosegrass biotypes as foramsulfuron dose increased (Figure 5). Dose titration response visually was captured (Figure 8). Interestingly, the PR collection from Hawaii exhibited a differing growth habit compared to the susceptible collection, this is particularly evident in non-treated controls treated with 0 g ha^{-1} foramsulfuron (Figure 9). This could be from management conditions at the bowling green or from the environmental climate in Hawaii where the plants grown perennially.

In the second experimental run, there was a significance difference in the dose of foramsulfuron required to control S and PR goosegrass 50% (IC50) (Figure 6). IC50 values for S and PR goosegrass were 23 and 60 g ha^{-1}, respectively. Interestingly, the susceptible goosegrass biotype was controlled with foramsulfuron doses > 22 g ha $^{-1}$, whereas the putative resistant biotype survived 176 g ha $^{-1}$ in some cases (Figure 6; Figure 9).This difference among susceptible and PR goosegrass was not evident in biomass data (Figure 7) and may be related to differences in overall growth habit in the absence of herbicide.

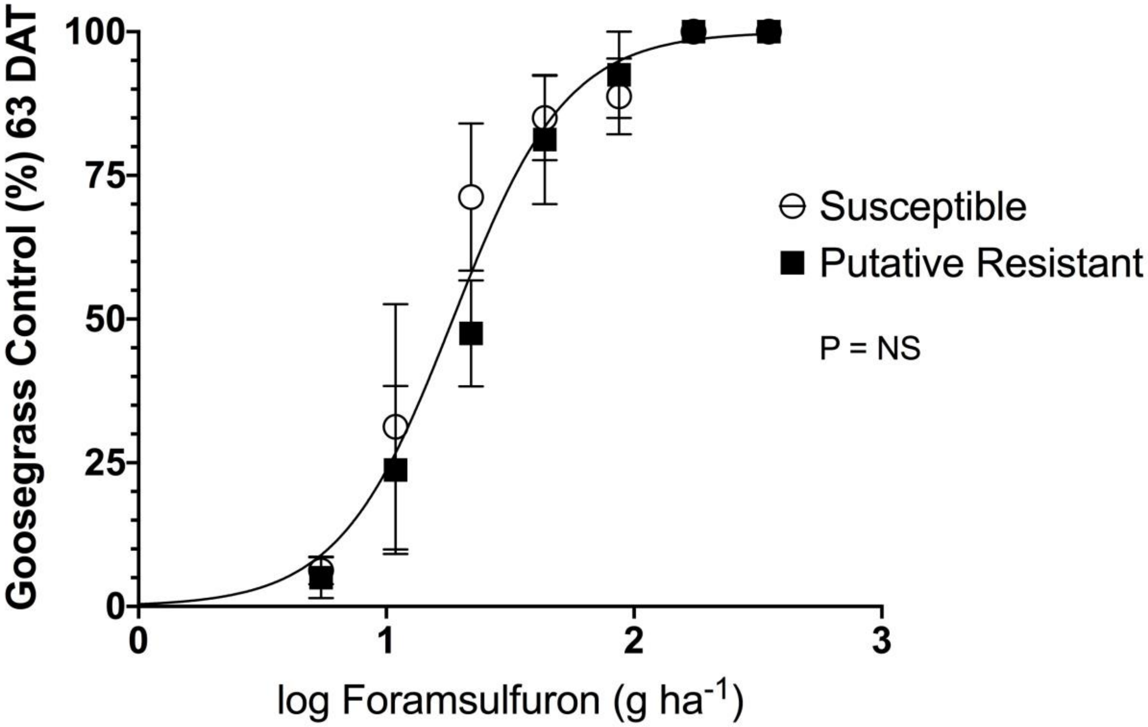

Figure 4. Non-linear regression analysis of goosegrass control data during a glasshouse experiment conducted in Run 1: Knoxville, TN from July 16, 2019 to September 9, 2019. Foramsulfuron rates were log transformed prior to log-logistic regression analysis. Vertical bars represent standard error of the mean.

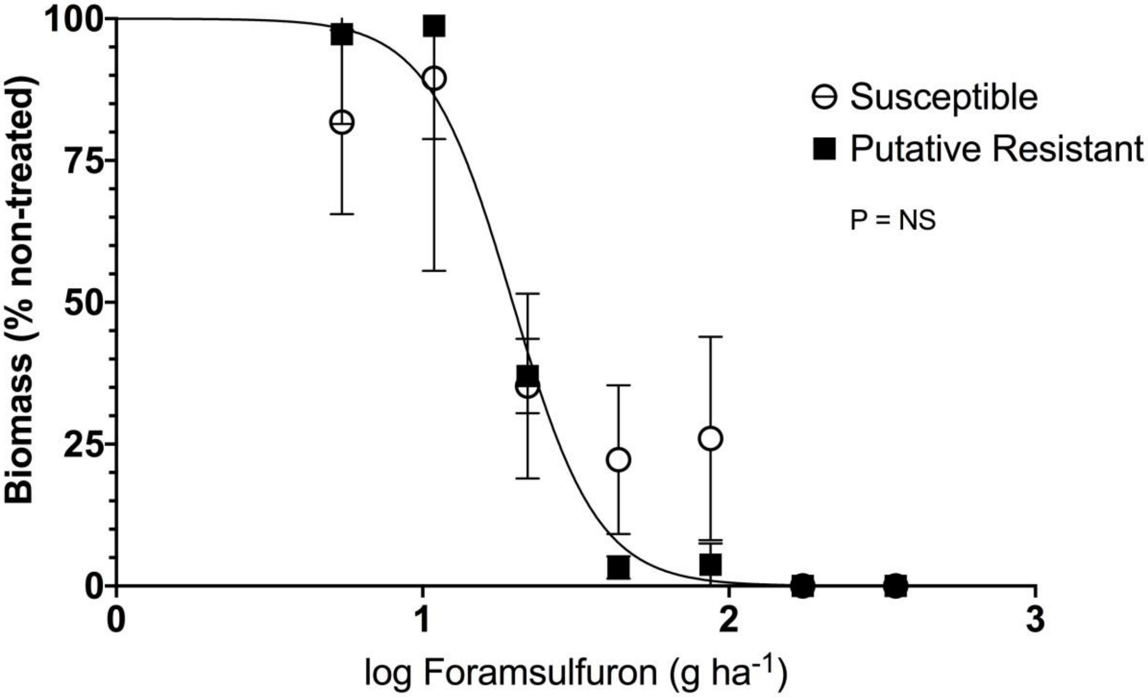

Figure 5. Non-linear regression analysis of goosegrass biomass data during a glasshouse experiment conducted in Run 1: Knoxville, TN from July 16, 2019 to September 9, 2019. Foramsulfuron rates were log transformed prior to log-logistic regression analysis. Vertical bars represent standard error of the mean.

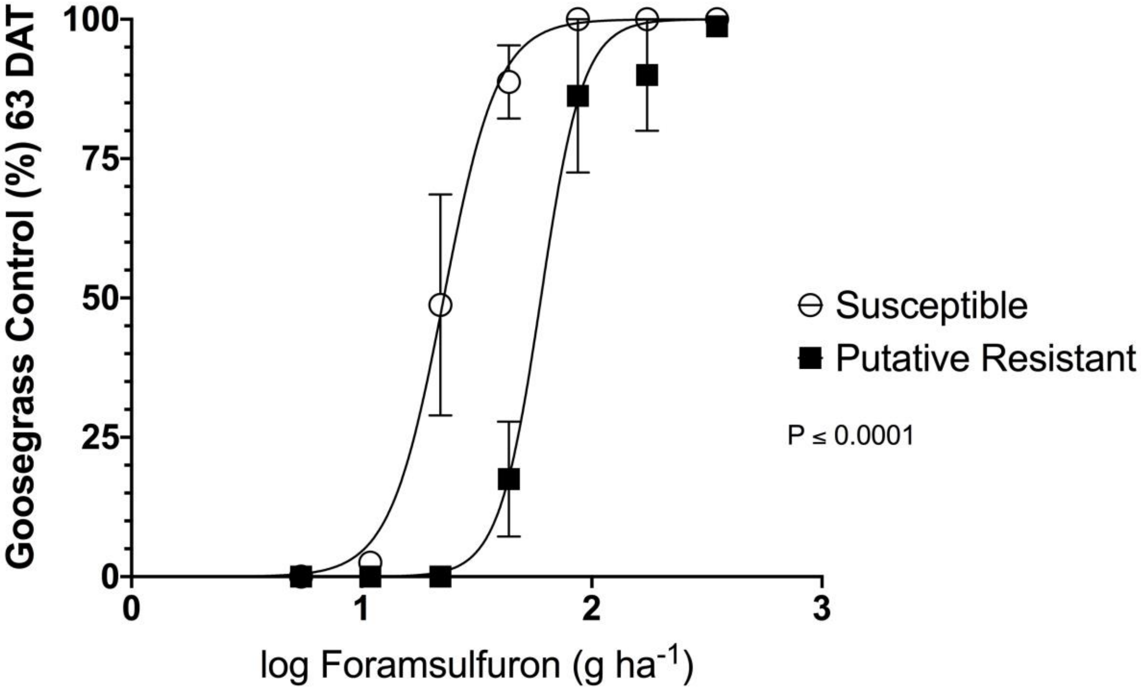

Figure 6. Non-linear regression analysis of goosegrass control data during a glasshouse experiment conducted in Run 2: Knoxville, TN from September 3, 2019 to November 5, 2019. Foramsulfuron rates were log transformed prior to log-logistic regression analysis. Vertical bars represent standard error of the mean.

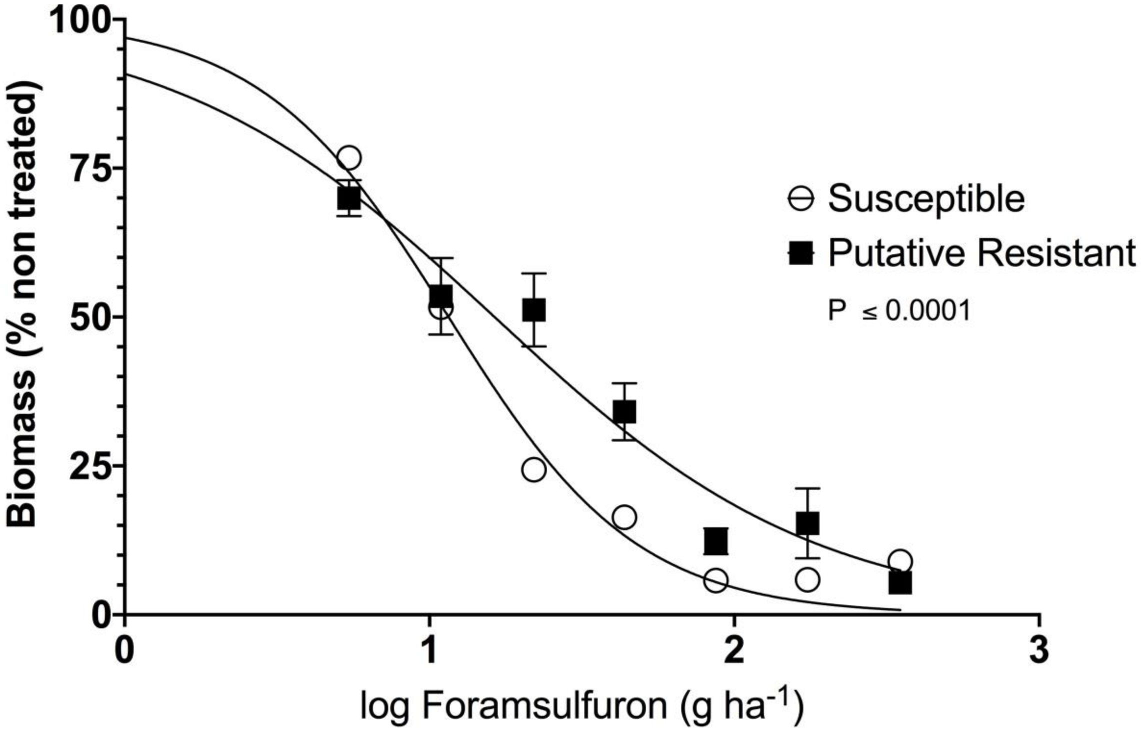

Figure 7. Non-linear regression analysis of goosegrass biomass data during a glasshouse experiment conducted in Run 2: Knoxville, TN from September 3, 2019 to November 5, 2019. Foramsulfuron rates were log transformed prior to log-logistic regression analysis. Vertical bars represent standard error of the mean.

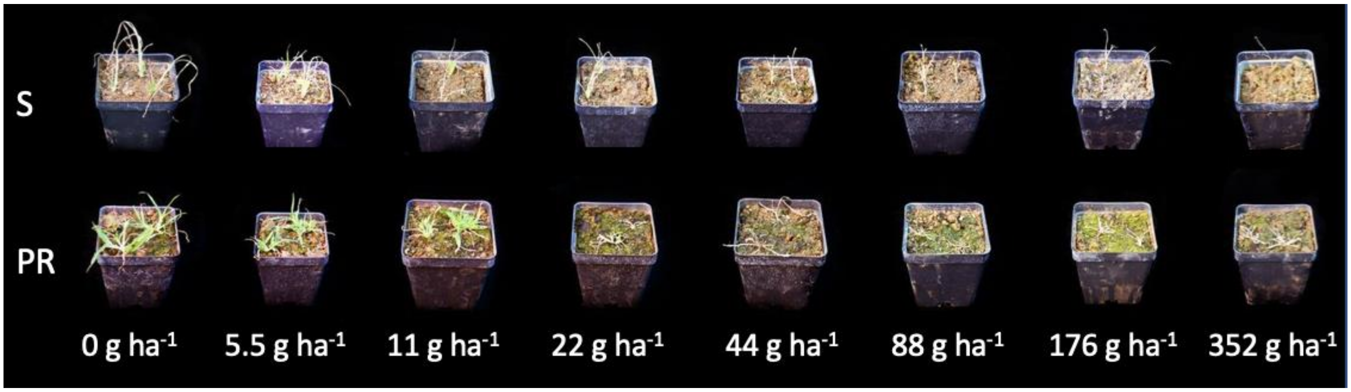

Figure 8. Response of susceptible (S) and putative-resistant (PR) goosegrass to increasing doses of foramsulfuron (g ha-1) during a glasshouse experiment conducted in Run 1: Knoxville, TN from July 16, 2019 to September 9, 2019. Picture was taken 55 days after treatment.

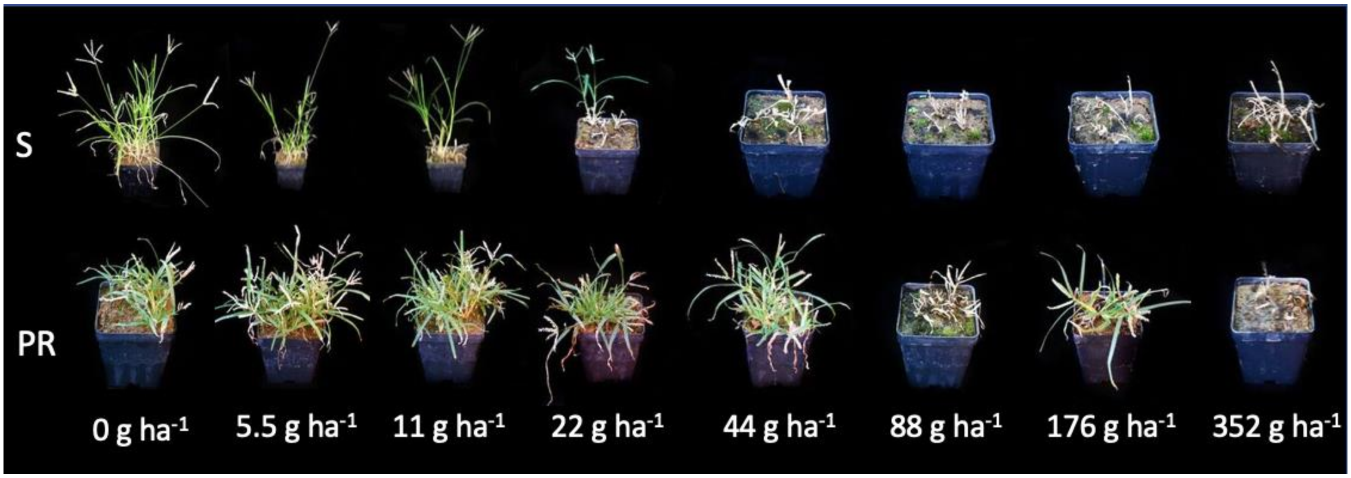

Figure 9. Response of susceptible (S) and putative-resistant (PR) goosegrass to increasing doses of foramsulfuron (g ha-1) during a glasshouse experiment conducted in Run 2: Knoxville, TN from September 3, 2019 to November 5, 2019. Picture was taken 63 days after treatment.

Conclusion

We cannot conclude that PR goosegrass is resistant to foramsulfuron based on the data collected in these experiments. Few differences in control and aboveground biomass were detected and those present were inconsistent across experimental runs. In Hawaii, foramsulfuron tolerance was observed in older plants grown in full tropical sun at sea level. Edaphic factors in the field seem to be a possible reasoning behind the goosegrass surviving herbicide treatment. Foramsulfuron is known to be affected by various edaphic factors such as soil moisture content and air temperature (Wendt et al. 2017). Increasing temperature also increases plant availability of herbicides (Eleftherohorinos et al. 2004); this increase in temperature correlates to higher uptake and stronger metabolism (Kudsk and Kristensen 1992). Relative humidity along with air temperature explained significant differences in foramsulfuron efficacy in previous research with foramsulfuron applications to ryegrass (*Lolium* spp.) stands (Willis 2008). However, foramsulfuron degradation is not impacted by varying soil pH (Szmigielski et al. 2012). The greenskeeper at the Honolulu Lawn Bowls Club was not questioned about added adjuvants or fertilizers upon application, but foramsulfuron performs better when applied with methylated seed oil and nitrogen fertilizer (Bunting et al. 2004). Every one of these environmental or application variables could affect foramsulfuron efficacy at this field site. This information is beneficial for turfgrass managers in tropical climates such as Hawaii showing that lack of efficacy in particular herbicides may be due to reasons other than resistance. This means that they would need to alter their application management to increase efficacy in their herbicides of choice.

Future Research

Additional research exploring these factors on PR and S goosegrass is warranted. Information on some edaphic factors are explored in the conclusions section, however, there were other conditions that are not well documented in literature. Soil texture has been proven to be a determining factor influencing cotton (*Gossypium* spp.) injury from fomesafen (Li 2014), meaning the effects on foramsulfuron should be explored. There has been documentation of inconsistent efficacy of foramsulfuron when targeting goosegrass, and tank mixing foramsulfuron with another herbicide such as metribuzin may help at the Hawaii site (Busey 2004). The presence of organic matter on site could be a possible factor affecting efficacy of foramsulfuron. Herbicide efficacy in general can be affected by soil moisture due to changing absorption, translocation, and metabolism of plants (Reynolds et al. 1988). Finally, considering the climate in Honolulu, HI, excessive solar radiation and the presence of spray water pH might be impactful on the efficacy of the foramsulfuron for goosegrass control.

Appendix

Crabgrass is an annual weed found in golf courses, athletic fields, and landscape turf throughout the United States (McCarty et al. 2005). Soil temperatures of 10.4 to 12.5 C facilitate crabgrass germination, but major emergence flushes typically do not occur in the field until soil temperatures exceed 19.5 C (Fidanza et al. 1996). The researchers used growing degree days (GDD) to predict crabgrass emergence and reported initial germination were between 42 and 78 GDD, while the major germination period occurred between 140 and 230 GDD (Fidanza et al. 1996). In Tennessee, that initial criterion of 42 to 78 GDD was met starting on March 10th when summarizing 10 year historical data (Soil Temperature Maps 2020). There are two species of crabgrass that are found on turfgrass systems: large crabgrass (*Digitaria sanguinalis*) and smooth crabgrass (*Digitaria ischaemum*). Crabgrass species are a problem in bermudagrass (*Cynodon* spp.) as their color, texture, and seedheads reduce turf aesthetic and quality, in addition to competing with the bermudagrass for water and nutrients (Hall et al. 1994).

Crabgrass is often controlled using pre or postemergence herbicides including indaziflam (Brosnan et al. 2011), dithiopyr (Johnson 1996), prodiamine (Senseman 2007), pendimethalin (Watschke and Welterlen 1982), and oxadiazon (Johnson 1976). Postemergence herbicides used to control crabgrass control include monosodium methanearsonate (MSMA; Johnson 1996), mesotrione (McCurdy et al. 2008), fenoxaprop (Derr 2002), quinclorac (Dernoeden and Krouse 1990), topramezone (Johnson et al. 2008), and pinoxaden (Henry et al. 2019).

A population of smooth crabgrass was collected from a bermudagrass golf course in Alcoa, TN after surviving preemergence treatment with oxadiazon (3,400 g ha^{-1}) in two consecutive years. This site has an extensive, documented history of herbicide resistance in annual grass weeds, particularly annual bluegrass (*Poa annua* L.) and goosegrass (*Eleusine* *indica* L. Gaertn.) (Breeden et al. 2017a, 2017b). We hypothesized that smooth crabgrass collected from this location had evolved resistance to oxadiazon. A series of controlled environment experiments were conducted to evaluate response of this putative resistant collection to increasing doses of oxadiazon compared to a known susceptible biotype.

Controlled environment experiments were conducted during 2019 in a glasshouse at the University of Tennessee (Knoxville, TN) and repeated in time and space. In experimental run one, air temperature averaged 25.5 ^{o}C with an average of 375 µmol m^{-2} s^{1} solar radiation. In experimental run two, air temperature averaged 26.1^{o}C with 301 µmol m^{-2} s^{1} average solar radiation. Finally in experimental run three, air temperature averaged 72 ^{o}C with 1000 µmol m^{-2} s^{1} average solar radiation. A known susceptible (S) and PR smooth crabgrass biotype were surface seeded into greenhouse pots (8.9 cm^{3}) filled with Sequatchie silt loam (fine-loamy, siliceous, semiactive, thermic, humic, Hapludult). Soil was amended with calcined clay at a 4:1 soil to clay ratio. Pots were overhead irrigated twice daily (06:00 and 13:00). Within twenty-four hours after seeding, pots were treated with oxadiazon (Ronstar Flo. Bayer Crop Sciences) using an enclosed spray chamber (Generation III Research Sprayer, DeVries Manufacturing, Hollandale, MN) calibrated to deliver 215 L ha^{-1} via a single flat-fan nozzle (8004EVS. TeeJet Spraying Systems, Wheaton, IL). Oxadiazon rates were at 0, 423, 850, 1,700, 3,400, 6,800, 13,500, 27,000 g ha^{-1} respectively which represented 0.125 to 8 times the maximum label rate of 3,400 g ha^{-1}.

Smooth crabgrass control was visually assessed using a 0 (i.e., no control) to 100% (i.e., plant death) scale. Assessments were made at the end of the study: 61 days after treatment (DAT) in the first experimental run, 83 DAT in the second, and 56 DAT in the third. Following visual rating, aboveground biomass was harvested, dried in a forced air oven (Laboratory Oven

Model LR-271C, The Grieve Corporation, Round Lake, IL) for two days at 55° C, and weighed. Aboveground biomass data were expressed as a percentage of non-treated controls in each replication. Pots were arranged in a randomized complete block design with four replications in the first two experimental runs, and eight replications in experimental run three. Data were subjected to non-linear regression analysis in Prism (Version 8.2.1. GraphPad Prism. La Jolla, CA.)

Significant differences among experimental runs were detected. In the first experimental run, there was a significant difference in the dose of oxadiazon required to control S and PR crabgrass 50%.. This difference was absent in both the second and third experimental runs. Differences in germination (in the absence of herbicide) were noted across experimental runs. For example, high germination for both biotypes was excellent in the second experimental run whereas germination in the third experimental was nearly 0% for both biotypes. We are unsure of the reasoning for this response but postulate that varying environmental conditions during each run may have influenced results, Overall, we can't conclude that the smooth crabgrass biotype collected in Alcoa, Tennessee is resistant to the PPO inhibiting herbicide oxadiazon. Field tests are going to be conducted at the golf course in spring 2020 to assist the superintendent in determining why oxadiazon is not controlling smooth crabgrass on site.

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