A Bioassay to Determine *Poa annua* Responses to Indaziflam

A Thesis Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

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May 2022

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ACKNOWLEDGEMENTS

I never imagined I would be on the trajectory I am on for attaining graduate-level degrees three years ago. I have my dear friend Devon Carroll and advisor Dr. Jim Brosnan to thank for pushing me to be the best version of myself and continue my education. My passion for the turfgrass industry grows day by day and I would never have found this passion if it weren't for the mentorship of the two mentioned above. I would also like to Greg Breeden and Javier Vargas for their mentorship and assistance from my days as an undergrad research assistant to the present.

Dr. Brosnan, I would like to thank you for the continued opportunities you provide for me as well as the faith you put in me to represent your lab and the cutting-edge research you facilitate in your program. You are one of the reasons that make me proud to be a Tennessee Volunteer. I look forward to continuing to build off this in my Ph.D. and continuing to make you proud as my mentor and boss.

Devon, I will never be able to thank you enough for the friendship, guidance, assistance, and just overall amazing person you have been to me over the past 3 years I have known you. You will always be one of my best friends and I look forward to seeing all the amazing things you will continue to accomplish in your personal and professional life. It has also been an honor to watch all your accomplishments to date in your time at the University of Tennessee. You are going to continue to do amazing things, but you will be missed here at the University of Tennessee. Good thing you'll only be a call away! Finally, Devon "I love having you as a friend."

To my family, thank you all for continuing to support me in my educational journey to achieve my aspirations and goals. I couldn't do it without you all.

Finally, my little buddy Lyla Mae. You're a good dog and I'm glad I always knew I could count on you to interrupt my zoom meetings and bring me smiles and joy throughout every day!

ABSTRACT

Herbicide resistance within *Poa annua* is widespread in managed turfgrass systems. In 2020, a *P. annua* collection from a golf course in the southeastern United States was reported to be resistant to indaziflam as well as six other mode-of-action groups. Considering *P. annua* is the most troublesome weed in turfgrass, a bioassay to screen other collections with putative indaziflam resistance is needed. A dose-response experiment was conducted with ten concentrations of indaziflam (0, 250, 500, 750, 1000, 1250, 1500, 2000, 4500, and 9000 pM) in Gelrite® culture during 2021 and 2022. An herbicide-susceptible (S1) collection of *P. annua*, a resistant standard (Site 3A), and a collection with putative-resistance to indaziflam (Site 18) were included in this experiment. Petri dishes were filled with 80 mL of Gelrite® (3.75 g/L) containing technical grade (≥ 98%) indaziflam (Sigma-Aldrich, St. Louis, MO) and rifampicin (1000 µg mL^{-1}). Each plate was sealed with parafilm after placing 15 seeds of a single collection on the Gelrite® surface. During the experiment, all plates were placed at a 75-degree angle to facilitate gravitropic root growth and stored in a growth chamber set to a constant air temperature of 16°C. At 14 days after seeding (DAS), the length of the radicle (mm) protruding from each seed was recorded with digital calipers. Root length data from each *P. annua* collection were expressed as a percentage of the non-treated and subjected to non-linear regression analysis to calculate indaziflam concentrations required to reduce root growth by 70% (EC_{70}). Statistically significant differences were detected among *P. annua* collections with the EC_{70} for the herbicide-susceptible collection measuring 742 pM [95% confidence interval (CI) = 686 to 803 pM] compared to 2226 pM (CI = 1851 to 2759 pM) for Site 3A and 4263 pM (CI = 3471 to 5382) for Site 18. Overall, these findings indicate that a discriminatory dose of 750 pM can be used to differentiate among susceptible and resistant individuals when screening additional *P. annua* collections from field sites where poor control is observed following broadcast applications of indaziflam.

TABLE OF CONTENTS

CHAPTER ONE INTRODUCTION 1
Poa Annua Biology 1
Poa Annua Resistance 2
Indaziflam as a Cellulose Biosynthesis Inhibiting Herbicide 5
Poa Annua Surviving Treatment with Indaziflam 7
Bioassay Screening for Resistance 8
CHAPTER TWO MATERIALS AND METHODS 11
Method Development 11
Screening Additional Collections of Poa Annua 13
CHAPTER THREE RESULTS AND DISCUSSION 15
Method Development 15
Screening Additional Collections of Poa Annua 20
CHAPTER FOUR CONCLUSIONS 24
LIST OF REFERENCES 25
APPENDIX 36
MICHIGAN STATE GENE EXPRESSION WORK 37
Introduction 37
Materials and Methods 37
Results and Discussion 39
List of References 43
IMPLEMENTING BIOASSAY IN WEED DIAGNOSTICS CENTER 44
VITA 46

LIST OF TABLES

Table 3.1. ANOVA of root length data collected in repeated laboratory experiments at the University of Tennessee evaluating responses of various *Poa annua* collections to increasing concentrations of indaziflam in Gelrite® culture for 14 days during 2021 and 2022. 17

Table 3.2. *Poa annua* collections subjected to increasing doses of indaziflam (0, 250, 500, 750, 1000, 1250, 1500, 2000, 4500, 9000 pM) in Gelrite® culture for 14 days. Root length (mm) measurements were expressed as a percentage of the non-treated (0 pM) for each collection. The concentration of indaziflam to reduce root length 70% (EC_{70}) was used to calculate a resistance index. 19

Table 3.3. Mean root length (mm) data for indaziflam-resistant (Site 3A and Site 18) and -susceptible (S1) collections of *Poa annua* in laboratory assays evaluating root growth in in Gelrite® culture for 14 days. 21

Table A.1. Technical indaziflam dilution math for the 750 pM discriminatory dose as well as the non-treated concentration of 5 pM. All dilutions were completed using dimethylsulfoxide (DMSO), Millipore deionized (DI) water, sterilized glass stirrers, and sterilized glass beakers. These concentrations will be used for future testing to detect indaziflam resistance in *Poa annua*..... 45

LIST OF FIGURES

Figure 3.1. Root length of *Poa annua* collections subjected to increasing concentrations of indaziflam (0, 250, 500, 750, 1000, 1250, 1500, 2000, 4500, 9000 pM) in Gelrite® culture for 14 days. Root length (mm) measurements were expressed as a percentage of the non-treated (0 pM) for each collection. Error bars presented illustrate the standard error of the mean for each collection (N = 150). 18

Figure 3.2. Root length (mm) of *Poa annua* seed 14 days after being plated in Gelrite® culture containing a 750 pM dose of indaziflam known to reduce root length of herbicide-susceptible *Poa annua* by 70%. A total of 105 seedlings per *Poa annua* collection were challenged with the discriminatory dose of indaziflam. Root length of non-treated control plants (0 pM) ranged from 1.9 to 12.7 mm. 23

Figure A.1. *Poa annua* collections subjected to quantitative polymerase chain reaction analysis at Michigan State University (East Lansing, MI) in July 2021. Whole plants were organized into categories [short (S; root length < 6 mm), medium (M; root length 9 to 14 mm), or long (L, root length > 16 mm)] based on the root length (mm) following exposure to 750 pM indaziflam in a gel bioassay. Expression of a cytochrome P450 gene (CYP1174) associated with non-target site resistance in each collection and root category with error bars representing ranges of expression within samples. 41

CHAPTER ONE

INTRODUCTION

Poa Annua Biology

Poa annua is cool-season (C_3) grass species within the Poaceae family (Huff et al. 2003). *Poa annua* is an allotetraploid species that resulted from a hybridization event between the diploid species *Poa supina* (male parent) and *Poa infirma* (female parent) (Mao and Huff 2016). *Poa annua* can be found on all seven continents globally (Chwedorzewska 2008) and is referred to by various common names including: annual meadowgrass, annual bluegrass, wintergrass, junegrass, suffolkgrass, speargrass, and walkgrass (Gibeault 1971). *Poa supina* and *Poa infirma* have been characterized as prostrate perennial and erect annual species, respectively (Darmency, Berti, Gasquez, & Matjicek, 1992). It has been suggested that polyploidy in *Poa annua* has increased its genetic diversity and therefore increased its ability to colonize a wide geography and exhibit variable morphology (Hovin 1957; McElroy, Walker, Wehtje, & van Santen 2004). Morphological variability within *Poa annua* was thoroughly reviewed by Carroll et al. (2021) who questioned why *Poa annua* is often referred to as an annual species given abundant reports of it surviving perennially and that the species does not exhibit monocarpic senescence.

Poa annua is the most troublesome weed of turfgrass (Van Wychen 2020), commonly infesting golf courses, sports fields, and lawns. A survey conducted by the Golf Course Superintendents Association of America reported that *Poa annua* was the third most abundant species found on United States golf courses in 2015, behind *Cynodon* spp. and *Poa pratensis* L. (Gelertner et al. 2017). In Tennessee, new *Poa annua* seedlings emerge in bermudagrass (*Cynodon* spp.) fairways when soil temperatures cool to $\leq 18.3^{\circ}C$ immediately following a period of precipitation (Taylor et al. 2021). This pattern makes *Poa annua* a problematic weed of warm-season turfgrass during winter and early spring that often requires eradication via herbicide treatment (Toler et al. 2007) to minimize reductions in turfgrass aesthetic and functional quality (Beard et al. 1978; Hall and Carey 1992).

Poa Annua Resistance

Sole reliance on herbicides to control *Poa annua* infestations in turfgrass has resulted in extensive selection for herbicide-resistant biotypes, similar to what has been observed in other cropping systems (Tranel and Wright 2002; Yu et al. 2010). Across all cropping systems, *Poa annua* has evolved resistance to ten herbicide mode-of-action groups, including numerous instances multiple resistance (Heap, 2022). The first case of *Poa annua* herbicide resistance in managed turfgrass was reported in Japan in 1983 with simazine, a photosystem-II inhibiting (PS-II) herbicide (Heap, 2022). In the United States, *Poa annua* in an Oregon grass seed production system evolved resistance to ethofumesate, a very long-chain fatty acid synthesis (VLFCA) inhibitor, in 1997, whereas the first report of resistance to a PS-II inhibitor in managed turfgrass was documented on a Mississippi golf course in 1999 (Heap 2022; Kelley et al. 1999). Overall, in managed turfgrass, *Poa annua* had evolved resistance to six mode of action groups by 2020 (Brosnan et al. 2020a).

Acetolactate synthase (ALS) inhibiting herbicide resistance cases are well documented within *Poa annua* infestations of managed turfgrass (Cross et al. 2013; McElroy et al. 2013; Brosnan et al. 2015; Brosnan et al. 2016; Singh et al. 2021). Across these resistance cases a diversity of herbicide families targeting the ALS enzyme have been affected. For example, Cross et al. (2013) described two collections from South Carolina and Georgia golf courses that exhibited resistance to sulfonylurea (foramsulfuron, trifloxysulfuron) and pyrimidinyl benzoate (bispyribac-sodium) herbicides. McElroy et al. (2013) identified a resistant collection from Alabama that survived treatment with sulfonylurea and pyrimidinyl benzoate herbicides, as well as the imidazolinone herbicide imazaquin, via a target site mutation (Trp_{574} – Leu). Brosnan et al. (2016) reported broad spectrum ALS-inhibitor resistance in *Poa annua* via a different target site mutation (Ala_{205} – Phe). Extensive resistance to ALS inhibiting herbicides in turfgrass is not surprising given that the likelihood of resistance evolution, particularly to sulfonylurea herbicides, in cropping systems is high (Tranel and Wright 2002).

Poa annua resistance to mitotic inhibiting herbicide resistance in managed turfgrass was first reported in the United States in 2001 (Lowe et al. 2001). Mitotic-

inhibiting herbicides are frequently used by turfgrass managers for preemergence control of *Poa annua* in warm-season turfgrass, leading to numerous reports of resistance throughout the transition zone and southern United States (Isgrigg et al. 2002; Cutulle et al. 2009; Brosnan et al. 2014; McCullough et al. 2017; Brosnan et al. 2017a; Singh et al. 2020). Despite these reports, information regarding target site mutations conferring resistance to mitotic inhibiting herbicides in *Poa annua* is limited. Rutland et al. (2021) reported a Thr_{239}-Ile substitution on alpha tubulin conferred resistance to prodiamine and dithiopyr; however, mutations were located on different sub-genomes in allotetraploid *P. annua*. This mutation has been shown to confer resistance to mitotic inhibiting herbicides in other weed species including *Eleusine indica*, *Setaria viridis*, and *Lolium rigidum* (Anthony et al. 1999; Délye et al. 2004; Fleet et al. 2018). Resistance to pronamide, a benzamide inhibitor of mitosis, was first reported by McCullough et al. (2017). The researchers suggested that non-target site mechanisms may confer resistance to pronamide in their *Poa annua* collections after documenting reduced absorption and translocation following postemergence applications of C^{14}-pronamide. Singh et al. (2021) documented *Poa annua* resistance to pronamide in Texas and suggested that non-target site mechanisms may be to blame given that no known target site mutations conferring resistance to mitotic inhibiting herbicide were identified in their plants. Considering that select families of cytochrome P450 monooxygenases, particularly Cyp706, in *Arabidopsis* can metabolize dinitroaniline herbicides (Abdollahi et al. 2021), these may also affect a benzamide inhibitor of mitosis such as pronamide. However, exploring non-target site mechanisms of resistance to mitotic inhibiting herbicides can be challenging (Chen et al. 2021). Additionally, it should also be noted that since *Poa annua* is a polyploid and many herbicides target multi-gene families, studies that sequence a limited number of genes may miss mutations that confer resistance.

Resistance to PS-II inhibiting herbicides within *Poa annua* was first identified in the United States in Mississippi (Kelly et al. 1999). This population had a target site mutation in the *psbA* gene that was maternally inherited. Mengistu et al. (2000) reported a different *psbA* mutation that conferred resistance to diuron and metribuzin. In total, there are six mutations linked to PS-II resistance with Ser_{264}-Gly and Val_{219}-Ile being most

common (Tian and Darmency 2006; Beckie and Tardif 2012). PS-II inhibiting herbicides are used to control *Poa annua* in autumn when warm season turfgrasses are transitioning into dormancy (Hutto et al. 2004). Surveys of golf courses in Mississippi and Tennessee reported widespread resistance to simazine, a triazine herbicide that inhibits PS-II (Hutto et al. 2004; Brosnan et al. 2020b). *Poa annua* resistance to the triazolinone herbicide amicarbazone has also been reported (Perry et al. 2012). In this study, amicarbazone effectively controlled herbicide-susceptible *Poa annua* but was ineffective on plants with a mutation conferring target site resistance to the triazine herbicide simazine.

Non-selective herbicides can be used to control *Poa annua* in the United States transition zone where warm-season turfgrasses such as bermudagrass and zoysiagrass (*Zoysia* spp.) are dormant in winter and therefore tolerant of treatment. *Poa annua* resistance to glyphosate, an herbicidal inhibitor of 5-enolpyruvate-shikimate-3-phosphate-synthase (EPSPS), has been documented in managed turfgrass (Binkholder et al. 2011; Brosnan et al. 2012; Cross et al. 2015; Breeden et al. 2017). Binkholder et al. (2011) identified a resistant population from a zoysiagrass fairway on a Missouri golf course that had been treated with glyphosate for 10 years; resulting in a resistance factor (RF) of 5.2. Brosnan et al. (2012) reported glyphosate resistance in *Poa annua* from a bermudagrass golf course fairway in Tennessee with an RF of 12 that exhibited altered shikimate accumulation after treatment. In both cases, RF values were similar to those reported for several other grassy weeds evolving glyphosate resistance (Baerson et al. 2002; Powles et al. 1998; Pratley et al. 1999; Vila-Aiub et al. 2007).

There is only one case of protoporphyrinogen oxidase (PPO) inhibitor resistance evolution in *Poa annua*. Yu et al. (2018) reported that a *Poa annua* biotype from Georgia survived treatment with flumioxazin and sulfentrazone postemergence, a response associated with reduced electrolyte leakage and lipid peroxidation compared to a susceptible control. Interestingly, *Poa annua* plants surviving postemergence applications of flumioxazin and sulfentrazone were effectively controlled with the same active ingredients applied preemergence indicating developmentally timed resistance and hints towards non-target site mechanisms being activated later in *Poa annua* development (Yu et al. 2018).

Multiple resistance in *Poa annua* is an emerging concern to turfgrass managers (Brosnan et al. 2020a). Reports of *Poa annua* with resistance to ALS and PS-II inhibitors as well as pre- and postemergence (prodiamine and glyphosate, respectively) herbicides have been documented on golf courses in the United States transition zone (Brosnan et al. 2016; Breeden et al. 2017). Multiple resistance to five modes of action was identified in *Poa annua* from an Australian golf course (Barua et al. 2020). In Texas, Singh et al. (2021) identified a population of *Poa annua* resistant to simazine, amicarbazone, trifloxysulfuron, foramsulfuron, as well as preemergence applications of pronamide. Plants contained known target site mutations conferring resistance to PS-II and ALS inhibiting herbicides, whereas the mechanism of resistance to pronamide was suspected to be non-target site based.

Laforest et al. (2021) explored the possibility of simultaneous co-evolution of non-target and target-site resistance within the self-pollinating species *Poa annua*. In working with a *Poa annua* collection that contained target site mutations conferring resistance to both PS-II and ALS inhibiting herbicides, Laforest et al. (2021) confirmed that treatment with an ALS inhibiting herbicide (trifloxysulfuron) resulted in differential expression of genes associated with non-target site resistance, particularly those associated with oxidative stress. Interestingly, resistant plants also exhibited constitutive expression of genes regulating transmembrane transport prior to treatment. The researchers suggested that continued use of an ALS-inhibitor after evolution of target site resistance may lead to an accumulation of resistance mechanisms affecting other modes of action. For example, *Poa annua* plants characterized by Laforest et al. (2021) were less affected by applications of flumioxazin, indaziflam, and pronamide than herbicide-susceptible controls.

Indaziflam as a Cellulose Biosynthesis Inhibiting Herbicide

Cell walls provide structure and shape to plant cells, aid in defending against pathogens, and facilitate communication between the symplast and apoplast (Polko and Kieber 2019). Cell walls are comprised of polysaccharides such as celluloses, hemicelluloses, and pectins, as well as structural proteins and phenolic compounds

(Ivakov and Persson 2012; Lampugnani et al. 2018). Cellulose is a simple polysaccharide molecule with a complex synthesis system (Brabham et al. 2014). Cellulose is comprised of chains of rigid microfibrils that provide up to 70% of the structural strength in a primary cell wall (Delmer and Amor 1995; Shedletzky et al. 1992). Additionally, cell wall dry weight estimates illustrate that approximately 40% of the mass is in the form of cellulose (Delmer and Amor 1995), with deposition important to plant development (Green 1991).

Cellulose biosynthesis inhibiting herbicides (HRAC Group 29) target the cellulose synthase complex (CSC) where cellulose polymers are synthesized and then released to the cell wall (Bashline et al. 2015; Mutwil et al. 2008). Within the CSC, there are 18 to 24 catalytic cellulose synthase A (CESA) proteins that could be targets of cellulose biosynthesis inhibiting herbicides (Brabham et al. 2014; Jarvis 2013; Tateno et al. 2016). Inhibiting the function of any of these proteins leads to radial root swelling caused by the loss of anisotropic growth in cells undergoing expansion (Brabham et al. 2014).

Indaziflam is an alkylazine inhibitor of cellulose biosynthesis with an unknown site-of-action. Brabham et al. (2014) reported that indaziflam increased CESA particle density in the plasma membrane and inhibited polymerization by reducing CESA particle velocity by 65% (Brabham et al. 2014). Indaziflam is also a unique herbicide as it is efficacious on both monocots and dicots, whereas other Group 29 herbicides (e.g., isoxaben) only exhibit dicot activity (Sabba et al. 1999). Furthermore, plants with target-site resistance to isoxaben are not cross resistant to indaziflam, indicating a unique site of action (Brabham et al. 2014). When indaziflam is applied to a susceptible plant, radial root swelling and ectopic lignification will occur leading to chlorosis and eventual mortality (Brabham et al. 2014). In *Arabidopsis*, indaziflam inhibited cellulose production within one hour of treatment in a dose dependent manner (Brabham et al. 2014).

Commercialized in 2010, indaziflam is labeled for preemergence control of weeds in rangeland and pasture systems, managed turfgrass (i.e., golf courses, lawns, sports fields), nursery and landscape production systems, field grown ornamentals, fruit and nut tree production, as well as along roadsides and within forested sites (González-Delgado et al.

2015; Jhala et al. 2012; Sebastian et al. 2017; Tompkins 2010). Indaziflam is a lipophilic herbicide with low water solubility leading to long residual soil activity (Sebastian et al. 2017; Tompkins 2010). Indaziflam has a dissociation constant (pK_a) of 3.5 and an octanol/water partition coefficient of 2.8 at 7 pH (Tompkins 2010). In managed turfgrass, indaziflam (Specticle FLO. Bayer Environmental Sciences, St. Louis, MO) is used to control problematic grassy weeds such as *Poa annua*, *Eleusine indica*, and *Digitaria* spp., as well annual sedges and broadleaf weeds. Considering that few Group 29 herbicides are used for weed control in turfgrass, indaziflam offered turfgrass managers a new mode of action for managing *Poa annua* populations that have evolved resistance to other modes of action (Brosnan et al. 2014, 2015, 2017b). For example, a *Poa annua* population with target site resistance to PS-II inhibiting herbicides was controlled ≥ 98% control with indaziflam (Brosnan et al. 2017b). Similarly, indaziflam effectively controlled *Poa annua* with resistance to mitotic and ALS inhibiting herbicides (Brosnan et al. 2014, 2015).

Poa Annua Surviving Treatment with Indaziflam

The first case of any plant evolving resistance to indaziflam was identified in *Poa annua* collected from a golf course and confirmed via greenhouse and laboratory bioassays (Brosnan et al. 2020c). Plants were identified and collected after escaping preemergence applications of indaziflam (Specticle Flo. Bayer Environmental Sciences. St. Louis, MO) at label rate (48.7 g ha^{-1}) in the field. *Poa annua* surviving indaziflam treatment (applied early-postemergence to non-tillered plants < 2.5 cm height) in greenhouse and laboratory bioassays also exhibited extensive multiple resistance to several other herbicide mode-of-action groups including #2 (foramsulfuron), #3 (pronamide), #5 (metribuzin and simazine), #9 (glyphosate), #10 (glufosinate), and #14 (flumioxazin). Inhibitors of cytochrome P450 monooxygenase including 1-aminobenzotriazole (10 mg L^{-1}), tebuconazole (1510 g ha^{-1}), or malathion (400 g ha^{-1}) could not restore indaziflam susceptibility in these *Poa annua* collections suggesting that metabolism may not be the sole mechanism of resistance in these plants. Interestingly, Brosnan et al. (2020c) demonstrated that concentrations of indaziflam and 1-fluoroethyl triazinediamine (the primary metabolite of indaziflam) were lower in resistant plants

compared to susceptible controls and suggested that altered herbicide absorption may be a mechanism of resistance to be further explored in these collections.

Since confirming indaziflam resistance in *Poa annua*, reports of indaziflam failure in the field are increasing (B. Spesard, personal communication). Given that the target site of indaziflam in weeds is currently unknown (Brabham et al. 2014), mechanisms of indaziflam resistance have not been elucidated. Evolution of indaziflam resistance within *Poa annua* is concerning considering that the herbicide helped turfgrass managers control infestations with resistance to other mode of actions groups (Brosnan et al. 2014, 2015, 2017c). There is a need to develop a bioassay to rapidly screen many collections of *Poa annua* for resistance to indaziflam. A gel-based, *in vitro*, bioassay could help turfgrass managers understand how plants will respond to indaziflam treatment in as few as 14-days compared to the 42 days required for whole-plant testing in a soil medium (Brosnan et al. 2020c). Moreover, a gel bioassay could identify indaziflam resistance regardless of mechanism (Kaundun et al. 2021).

Bioassay Screening for Resistance

Diagnostic assays are a rapid, accurate, and economical way to diagnose resistant weeds from the field (Moss 1995; Beckie et al 2000). Classical dose response experiments are time consuming, require greenhouse space, and large-scale testing is limited (Burgos et al. 2013). Cutulle et al. (2009) found that a plant tissue culture assay simplified measurement of roots and shoots compared to testing putative-resistant weeds via soil-, filter-, or hydroponics-based techniques. Tissue culture assays can detect herbicide resistance whether the mechanism is a function of target site mutation, metabolism, sequestration, or altered biokinetics (Gaines et al. 2020; Kaundun et al. 2021). Furthermore, bioavailability in soil-based assays can vary due to soil properties such as pH, organic matter, and clay content whereas tissue culture assays allow for higher bioavailability when testing (Beckie et al. 2000). This means soil-based and tissue culture assays are not entirely translatable without rigorous testing and consistent control germplasm.

An agar-based assay was developed to detect resistance to dinitroaniline herbicides in *Lolium rigidum* (McCalister et al. 1995). Since then, in-season agar assays have been developed to screen weeds for resistance to EPSP synthase, VLCFA-, acetyl co-A carboxylase (ACCase-), ALS-, mitotic-, and PS-II inhibiting herbicides (Bourgeois et al. 1997; Claude et al. 2004; Cutulle et al. 2009; Kaundun et al. 2011; Kaundun et al. 2014; Messelhasuer et al. 2021). The first agar assay developed to screen turfgrass weeds was used to discern PS-II inhibitor herbicide resistance in *Poa annua*; 0.05 millimolar simazine reduced fresh weight of a susceptible population 50% whereas 63 millimolar was needed to elicit the same response in resistant biotypes (Kelly et al. 1999). Recently, an on-farm assay to detect ACCase resistance in *Lolium multiflorum* failed due to seed dormancy and herbicide degradation (Perez et al. 2021).

In turfgrass, herbicide resistance assays have been developed for four mode-of-action groups. Cutulle et al. (2009) developed an agar assay to screen *Poa annua* for resistance to mitotic inhibiting herbicides such as prodiamine, dithiopyr, and pendimethalin; however, a hydroponics technique was preferred because it returned results within 10 days of harvesting mature plants whereas the agar assay required seed. A rapid assay was developed to detect *Poa annua* resistance to ALS inhibitors by measuring acetoin concentrations in resistant and susceptible biotypes with a spectrophotometer (Cross et al. 2013). Resistant plants accumulated higher concentrations of acetoin in the presence of an ALS inhibiting herbicide yielding a distinct visible color change. Brosnan et al. (2017c) developed an agar assay to detect *Poa annua* resistance to EPSPS- and ALS-inhibitors. Polycarbonate plant culture boxes were filled with agar containing discriminatory doses (µM) of trifloxysulfuron or glyphosate for 10 days. The researchers determined that 12.5 µM and 100 µM could be used as discriminatory doses for trifloxysulfuron and glyphosate, respectively.

Widespread herbicide resistance within *Poa annua* warrants the development of a bioassay to screen collections for resistance to herbicides, particularly those for which target sites (and subsequent mutations conferring resistance) have not been elucidated. Indaziflam is an ideal candidate for such an assay given that the target site for this herbicide has not been elucidated and the one documented case of resistance to date is

speculated to be the result of non-target site mechanisms. The objective of this research is to develop a bioassay to determine *Poa annua* responses to indaziflam that can be used to discern amongst resistant and susceptible accessions.

CHAPTER TWO

MATERIALS AND METHODS

Method Development

Laboratory research was conducted at the University of Tennessee in 2021 and 2022 to develop a bioassay to screen *Poa annua* germplasm response to indaziflam. Herbicide susceptible (S1) and one resistant biotype (Site 3A) used in this experiment were the same as those described by Brosnan et al. (2020c). Collections S1 and Site 3A are from University Park, PA and a golf course fairway in Cummings, GA respectively. Additionally, a collection of *Poa annua* with putative resistance to indaziflam (Site 18) was also included. This collection was submitted to the University of Tennessee Weed Diagnostics Center after escaping treatment with preemergence applications of indaziflam (Specticle Flo; Bayer Environmental Sciences, St. Louis, MO) on a golf course fairway in Thomasville, GA. Plants were cultured in a glasshouse to produce seed required for research. Plants were placed in greenhouse pots filled with a peat moss growing media (PRO MIX BX General Purpose; Premier Tech Horticulture, Quakertown, PA, USA) and treated with complete fertilizer (20 N–20 P_2O_5-20 K_2O. Peter's 20–20–20. JR PETERS, Incorporated. Allentown, PA, USA) at 49.5 kg N ha^{-1} wk^{-1}. Mature seed from each collection were harvested with scissors, dried in a forced-air oven (Laboratory Oven; The Grieve Corporation, Round Lake, IL), and sieved (Standard Test Sieve; Fisher Scientific Company LLC, Hampton, NH) to remove chaff. After processing, seed were stored in glass vials (40 mL Scintillation Vial; Thermo-Fisher Scientific, St. Louis, MO, USA) at -20˚C for a minimum of four weeks.

A natural gelling agent (Gelrite®; Duchefa Biochemie, Haarlem, The Netherlands) was used to culture plants in this research. This agent was prepared by adding 7.5 g Gelrite® to 8.9 g MS Salts & Vitamins (Murashige & Skoog M519 Basal Medium with Vitamins; Phyto Tech Labs, Lenexa, KS) in 1966 mL of distilled H_2O in sterilized Erlenmeyer flasks; MS Salts & Vitamins was added to distilled H_2O prior to the Gelrite®. The pH of this solution was measured with a pH conductivity meter (Multimeter Model 250; Denver Instrument, Bohemia, NY) and adjusted to 6.5 by adding either hydrochloric

acid (HCl) or sodium hydroxide (NaOH) to lower or raise the pH, respectfully. The resultant solution went through a liquid 20 cycle that raised temperature to 121˚C in an autoclave (Amsco Century V120; Steris, Mentor, OH) for 20 minutes. After the autoclave cycle was finished, sealed flasks were placed on a hot plate (FisherBrand Stirring Hot Plate; Thermo Orion Incorporated, Chelmsford, MA) and stirred until cooling to 80˚C. Rifampicin (1000 µg mL^{-1}; Rifamipicin Molecular Biology Reagent; Alfa Aesar, Ward Hill, MA) and technical indaziflam (≥ 98%; Sigma-Aldrich, St. Louis, MO) were added at this temperature to create ten indaziflam concentrations for evaluation: 0, 250, 500, 750, 1000, 1250, 1500, 2000, 4500, 9000 picomolar (pM). These concentrations were selected based results of Brosnan et al. (2020c) who reported that early-postemergence exposure to 633 pM indaziflam in agar culture could differentiate between indaziflam resistant and susceptible collections of *Poa annua*. Technical indaziflam (150 mg) was diluted in 99.5 mL dimethyl sulfoxide with a concentration of 5000 µM. This stock solution was frozen to keep concentrations uniform across all trials. On the day of trial initiation, this bottle was allowed to thaw to facilitate adding the appropriate amount of indaziflam to each flask for each concentration. Serial dilutions using deionized H_2O were used to deliver intended pM concentrations of indaziflam to each autoclaved solution.

After adding rifampicin and indaziflam, 80 mL of the resultant solution was added to square petri dishes (120 x 15 mm) within a sterile fume hood (1300 Series A2 Fume Hood; Thermo-Fisher Scientific, St. Louis, MO, USA) using a dispensette (Dispensette S Bottle Top Dispenser; BrandTech Scientific Incorporated, Essex, CT). Plates were left open in the fume hood for 12 min in order to cool before being covered, sealed with parafilm (2 IN All-Purpose Laboratory Film; Amcor, Menasha, WI), and placed in a growth chamber (G1000-Germinator; Conviron, Winnipeg, Manitoba, Canada) set to constant 16˚C temperature and 16 h photoperiod. Plates were unsealed to facilitate adding *Poa annua* seed (15 seed per collection) to the Gelrite® surface using sterilized tweezers; seed were pressed into the Gelrite® surface with sterilized spatulas after plating. Each petri dish was then re-sealed with parafilm after seeding and placed in the aforementioned growth chamber at a constant temperature of 16˚C.

Fourteen days after seeding (DAS) responses to indaziflam were evaluated by assessing the presence (or absence) of root or shoot growth (i.e., 1 = yes, 0 = no). Additionally, the length of the radicle (mm) protruding from each seed was measured via digital calipers (CD-6" AX Digimatic Caliper; Mitutoyo Corporation, Kawasaki, Kanagawa, Japan). The experiment was arranged in a completely randomized design with five replications of each indaziflam concentration. Within a given concentration, the 15 seed per collection were considered sub-samples and used to calculate mean root length values for each *Poa annua* collection that were expressed as a percentage of the non-treated. The experiment was repeated as previously described in January 2022 using five replications of each indaziflam concentration and 15 sub-samples. Data from each experimental run were combined following ANOVA in R (v. 3.6.2) with root length values subjected to non-linear regressions in Prism (Version 9.0.0. GraphPad Prism. La Jolla, CA.) using the 'EC Anything' model described below:

$$Y=Bottom + (Top-Bottom)/(1+(EC_{70}/X)^{HillSlope})$$

In this model, Bottom and Top represent asymptotes that were constrained to 0 and 100 percent, respectively. Y represented root growth as a percentage of the non-treated, and HillSlope represented the steepness of the curve. X represented the indaziflam concentration to reduce root length 70% (EC_{70}). Best-fit parameters for modeled responses were compared using a global sums-of-squares F-test at $\alpha = 0.05$ with 95% confidence intervals used to compared EC_{70} concentrations of indaziflam among resistant and susceptible *Poa annua* collections.

Screening Additional Collections of Poa Annua

The efficacy of this bioassay was evaluated on ten collections of *Poa annua* that escaped treatment with indaziflam on golf courses, sports fields, and lawns during autumn 2019; progeny of these collections survived a challenge of early-postemergence indaziflam treatment (48.7 g ha^{-1}) in a glasshouse at the University of Tennessee (Knoxville, TN) during 2020. Plants surviving treatment were cultured using methods described previously. Seed of each accession was stored at room temperature in glass vials (40 mL Scintillation Vial; Thermo-Fisher Scientific, St. Louis, MO, USA). Vials for

each accession were placed in a -20˚C freezer (Countertop Laboratory Freezer; American Biotech Supply, Alpine, UT) for a minimum of 28 days prior to indaziflam screening in Gelrite® culture.

Plates containing 750 pM indaziflam in Gelrite® culture were prepared as previously described and replicated five times per *Poa annua* collection. Two non-treated control plates (0 pM) were prepared per *Poa annua* collection as well. Plates were seeded as previously described with root length assessed 14 days after seeding as previously described. Root length values were analyzed using a violin plot in Prism to determine the number of *Poa annua* seed within each accession that grew new root tissue following exposure to a discriminatory concentration of indaziflam (750 pM) known to reduce root length of indaziflam-susceptible plants by 70%.

CHAPTER THREE

RESULTS AND DISCUSSION

Method Development

Significant *Poa annua* collection-by-indaziflam concentration interactions were detected in root length data from each experimental run (Table 3.1); therefore, data from both were combined before being subjected to non-linear regression analysis. A non-linear model fit the combined data set with a global sums-of-squares F-test detecting highly significant ($F = 117$, $P < 0.0001$) differences among *Poa annua* collections (Figure 3.1).

While root length reduced for all *Poa annua* collections as indaziflam concentration increased, there were statistically significant differences in the concentration of indaziflam required to reduce root length 70% (EC_{70}) among *Poa annua* collections in this experiment (Table 3.2). EC_{70} values for the herbicide-susceptible collection measured 742 pM [95% confidence interval (CI) = 686 to 803 pM] compared to 2226 pM (CI = 1851 to 2759 pM) for Site 3A and 4263 pM (CI = 3471 to 5382) for Site 18. (Table 3.2). These values can be used to calculate resistance indices (i.e., R/S ratios) of 3.0 and 5.7 for the Site 3A and Site 18 *Poa annua* collections, respectively. Low-level resistance within the *Poa annua* collections evaluated in this study provides further evidence that non-target site mechanisms may be affecting indaziflam efficacy given that non-target site mechanisms are often continuous as opposed to discreet for target-site mutations (Tranel and Wright 2002; Yu and Powles 2014).

Indaziflam resistance indices in the current study align with previous research using Site 3A and S1 *Poa annua* germplasm (Brosnan et al. 2020c); the researchers determined indaziflam concentrations required to reduce root growth of newly emerged *Poa annua* plants 50% (I_{50} values) and calculated a resistance index of 6.8 for Site 3A. The difference in resistance factor for collection Site 3A between experiments can most likely be attributed to differences in plant maturity at the time of indaziflam exposure. Brosnan et al. (2020c) calculated a resistance index following early-postemergence

indaziflam exposure to emerged seedlings, whereas preemergence exposure was evaluated in this research. Additionally, EC_{70} values were used to calculate resistance

Table 3.1. ANOVA of root length data collected in repeated laboratory experiments at the University of Tennessee evaluating responses of various *Poa annua* collections to increasing concentrations of indaziflam in Gelrite® culture for 14 days during 2021 and 2022.

Experimental Run 1				Experimental Run 2			
Source	DF	F	P	Source	DF	F	P
Collection	2	77.8	< 0.0001	Collection	2	66.2	< 0.0001
Concentration	8	18.2	< 0.0001	Concentration	8	7.2	< 0.0001
Collec. x Conc.	16	10.6	< 0.0001	Collec. x Conc.	16	8.7	< 0.0001
Residual	1998			Residual	1998		

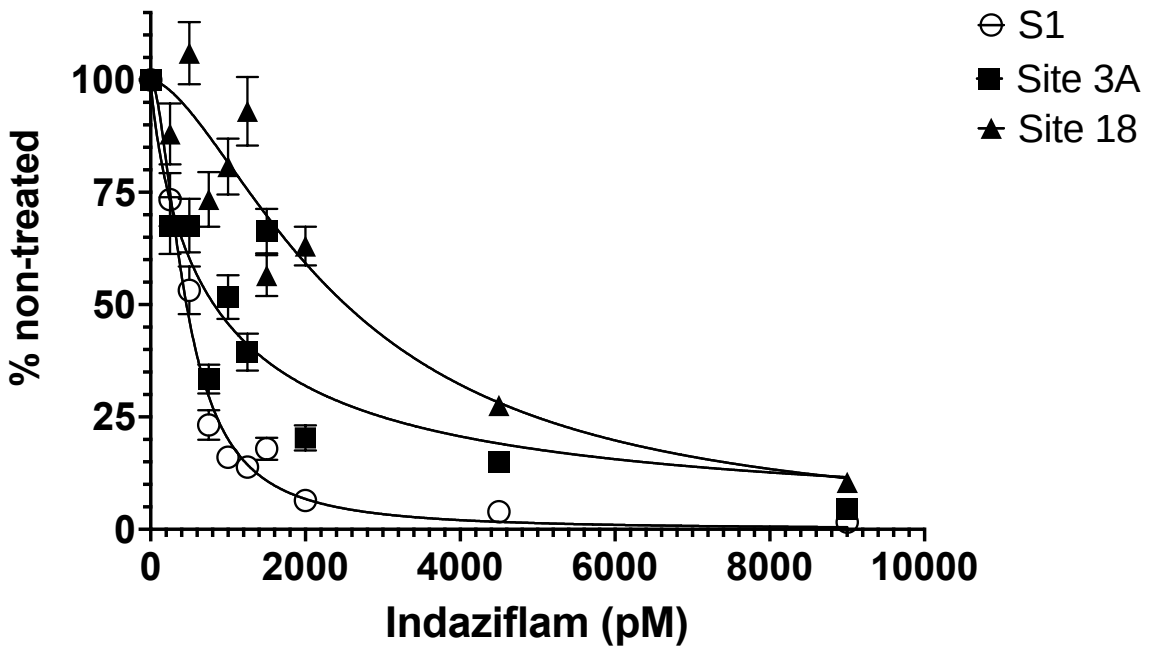


Figure 3.1. Root length of *Poa annua* collections subjected to increasing concentrations of indaziflam (0, 250, 500, 750, 1000, 1250, 1500, 2000, 4500, 9000 pM) in Gelrite® culture for 14 days. Root length (mm) measurements were expressed as a percentage of the non-treated (0 pM) for each collection. Error bars presented illustrate the standard error of the mean for each collection (N = 150).

Table 3.2. *Poa annua* collections subjected to increasing doses of indaziflam (0, 250, 500, 750, 1000, 1250, 1500, 2000, 4500, 9000 pM) in Gelrite® culture for 14 days. Root length (mm) measurements were expressed as a percentage of the non-treated (0 pM) for each collection. The concentration of indaziflam to reduce root length 70% (EC_{70}) was used to calculate a resistance index.

Collection[a]	EC_{70} [b]	Resistance Index[c]
S1	742	-
Site 3A	2226	3.0
Site 18	4263	5.7

[a] S1 collection was herbicide-susceptible standard from University Park, PA with no exposure history to indaziflam. Site 3A and Site 18 were collected from hybrid bermudagrass (*Cynodon dactylon* (L.) Pers. x *Cynodon transvaalensis* Burtt Davy, cv. 'Tifway') on golf courses after surviving field applications and challenges with 48.7 g ha^{-1} in the glasshouse

[b] EC_{70} = concentration of indaziflam required to reduce root growth from seed by 70% in Gelrite® culture.

[c] Resistance indices calculated by dividing the EC_{70} value for Site 3A and Site 18 by the EC_{70} value for the susceptible control (S1).

indices in the current study compared to EC_{50} values commonly used by other researchers (Cutulle et al. 2009; Kaundun et al. 2014; Brosnan et al. 2020c). Given that there is only a single report of indaziflam resistance in published literature to date (Brosnan et al. 2020c), EC_{70} values were selected to provide a more stringent filter among putative resistant and susceptible *Poa annua* collections.

Overall, findings of this experiment suggest that 750 pM can be used as a discriminatory concentration of indaziflam to screen other accessions of *Poa annua* for putative resistance to indaziflam in Gelrite® culture. After screening 1,500 plants across 10 concentrations, the 750 pM dose consistently separated resistant collections from a susceptible control. Sebastian et al. (2017) reported that 251 pM indaziflam reduced root growth of feral rye (*Secale cereale* L.) in agar culture by 50%. In the current study, 463 pM of indaziflam was required to yield a similar reduction in root growth of herbicide susceptible *Poa annua* in gel culture. A limitation of this study is that only a single susceptible collection was included in the dose-response experiments. Future work to refine this assay using additional susceptible *Poa annua* collections is warranted.

In addition, it is worth noting that root length values for both indaziflam-resistant collections (Site 3A and Site 18) were greater than the susceptible *Poa annua* collection used in this study (Table 3.3). When placed on non-treated control plates (0 pM), Site 3A and Site 18 yielded root length values of > 13.94-mm roots compared to only 5.97 mm for the susceptible collection (S1). Indaziflam may be selecting for *Poa annua* plants with enhanced root growth vigor from seed. Future research exploring phenotypical traits associated with mechanisms of indaziflam resistance in *Poa annua* is warranted.

Screening Additional Collections of Poa Annua

Nine *Poa annua* collections surviving indaziflam treatment in the field were identified in spring 2020. Progeny of these collections survived a challenge via a labeled early-postemergence application of indaziflam at 48.7 g ha^{-1} in a glasshouse study. The previously described Gelrite® assay was able to discern all of these collections from indaziflam susceptible controls using a discriminatory dose of 750 pM.

Table 3.3. Mean root length (mm) data for indaziflam-resistant (Site 3A and Site 18) and -susceptible (S1) collections of *Poa annua* in laboratory assays evaluating root growth in in Gelrite® culture for 14 days.

	Indaziflam Concentration (pM)									
Collection[a]	0	250	500	750	1000	1250	1500	2000	4500	9000
	mm									
S1	5.97	4.38	3.18	1.39	0.96	0.83	1.07	0.38	0.23	0.10
Site 3A	13.94	9.43	9.42	4.66	7.21	5.50	9.25	2.83	2.11	0.62
Site 18	14.14	12.43	14.97	10.38	11.41	13.16	7.98	8.91	3.90	1.47

[a] S1 collection was herbicide-susceptible standard from University Park, PA with no exposure history to indaziflam. Site 3A and Site 18 were collected from hybrid bermudagrass (*Cynodon dactylon* (L.) Pers. x *Cynodon transvaalensis* Burtt Davy, cv. 'Tifway') on golf courses after surviving field applications and challenges with 48.7 g ha^{-1} in the glasshouse.

Root growth in the presence of a discriminatory dose of indaziflam is presented in Figure 3.2. Across all nine *Poa annua* collections, new root growth in the presence of 750 pM indaziflam was markedly greater than observed on non-treated control plates devoid of indaziflam (0 pM). For example, TN-3-LC-02, TN-3-LC-03, and TN-3-LC-11 seedlings grew new root tissue ranging in length from 1.82 to 51.51 mm, 0.95 to 43.01 mm. and 2.50 to 37.68 mm, respectively. Comparatively, root length for TN-3-LC-02, TN-3-LC-03, and TN-3-LC-11 on non-treated plates (0 pM) ranged from 0.92 to 42.41, 1.19 to 2.99, and 1.31 to 21.04 mm respectively.

The fact that the previously described Gelrite® assay was able to discern *Poa annua* collections surviving labeled applications of indaziflam from an herbicide-susceptible control provides evidence that it could aid turfgrass managers in adjusting their *Poa annua* management plans in-season. This assay could also prove useful considering that coevolution of both target- and non-target site mechanism can evolve in *Poa annua* reducing efficacy of other modes of action (Laforest et al. 2021). The Gelrite® methodology herein would be able to discern amongst indaziflam resistant and susceptible accessions of *Poa annua* regardless of mechanism. As instances of multiple resistance in *Poa annua* increase, future work discerning the response of those plants to indaziflam using the Gelrite® assay documented herein is warranted.

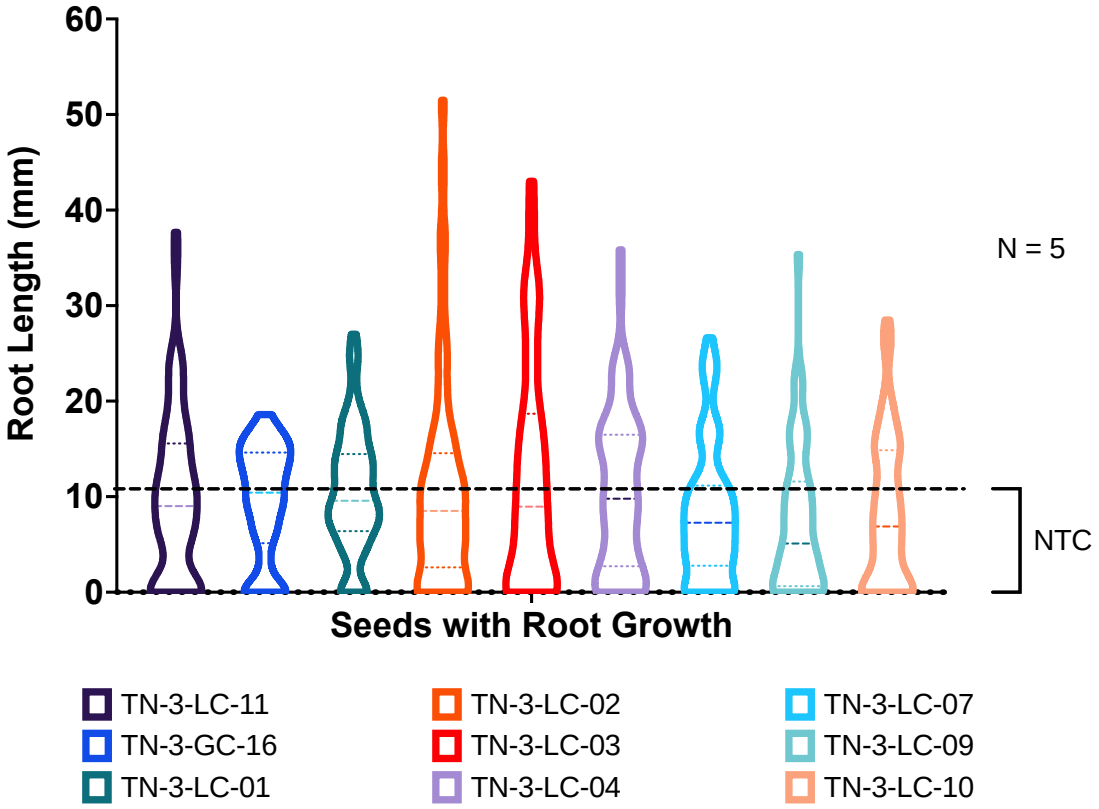


Figure 3.2. Root length (mm) of *Poa annua* seed 14 days after being plated in Gelrite® culture containing a 750 pM dose of indaziflam known to reduce root length of herbicide-susceptible *Poa annua* by 70%. A total of 105 seedlings per *Poa annua* collection were challenged with the discriminatory dose of indaziflam. Root length of non-treated control plants (0 pM) ranged from 1.9 to 12.7 mm.

CHAPTER FOUR
CONCLUSIONS

Evolution of indaziflam resistance in *Poa annua* is concerning given that many turfgrass managers have effectively used this herbicide to control *Poa annua* biotypes that evolved resistance to other modes of action, including those with multiple resistance. A gel bioassay was developed to discern *Poa annua* responses to indaziflam in a 14-day period once seed is available. This bioassay allows turfgrass managers to screen *Poa annua* plants for indaziflam resistance within the same season of management to determine is indaziflam will be an effective component of their *Poa annua* management plans. The gel bioassay described herein could be useful in screening *Poa annua* collections with unknown mechanisms of resistance, particularly those that are not target-site based. Future work should be conducted to determine the mechanism of resistance in the indaziflam-resistant *Poa annua* collections described in this research. Doing so would allow for molecular assays to identify indaziflam resistance to be developed that could return results even more rapidly (Beckie et al. 2000). Use of this assay to determine if indaziflam can be a component of an effective herbicide mixture for *Poa annua* control is also warranted. Hulme (2022) explored multiple resistance across an array of weed species and reported that certain mode of action groups cluster together, with a main cluster including ALS and PS-II inhibitors as well as glyphosate. Given widespread reports of resistance to these herbicides in *Poa annua*, challenging resistant plants with indaziflam using this assay would help determine if the herbicide could be a component of an effective mixture for resistance management or falls within the same multiple resistant cluster.

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APPENDIX

MICHIGAN STATE GENE EXPRESSION WORK

Introduction

Dr. Eric Patterson's Lab at Michigan State University (East Lansing, MI) was conducting research exploring the genetic basis of indaziflam resistance in *Poa annua* during 2021. The gel bioassay described herein was used to identify indaziflam resistant collections of *Poa annua* that were candidates for genetic sequencing. Cryogenically frozen plant material surviving a discriminatory dose of indaziflam (750 pM) was transported to Michigan State University in July 2021 for genetic analysis.

Materials and Methods

Plant material surviving a discriminatory dose of indaziflam (750 pM) was harvested from the Gelrite® surface and placed in whirlpacks (Whirl Pak®; Nasco, Madison, WI). Within each *Poa annua* collection, plants surviving the discriminatory dose were sorted into three groups based on root length: short (S; root length < 6 mm), medium (M; root length 9 to 14 mm), or long (L, root length > 16 mm). *Poa annua* collections producing longer roots in the presence of a discriminatory indaziflam dose were hypothesized to yield higher expression of CYP1174.

Liquid nitrogen was poured in the whirlpacks to flash freeze plant material. Whirlpacks were kept on ice throughout the evaporation process of the liquid nitrogen. Whirlpacks were quickly sealed then placed in a freezer at -20˚C (EV161NZTQ02; Whirlpool, Benton Harbor, MI). Plant material was then transported to Michigan State for analysis.

Whirlpacks were transported to Michigan State in a cooler (Tundra 65; Yeti, Austin, TX) filled with dry ice. Dry ice was added to the cooler the night before transport to ensure the cooler was chilled before adding the samples. Samples were in transit for 10 hours then placed in a -80˚C freezer (TSX Series; Thermo-Fisher Scientific, St. Louis, MO, USA) until RNA extraction. Next, the samples were transferred to 1.5 mL RNase free microcentrifuge tubes using tweezers and spatulas while keeping samples in liquid nitrogen. Tissue was ground using commercial equipment (TissueLyser II, Qiagen, Hilden, Germany) and autoclaved stainless-steel balls.

Once samples were transferred and ground, the RNeasy® Mini Kit by Qiagen (RNeasy® Mini Kit (250); Qiagen, Hilden, Germany) was used to extract RNA for analysis. First, the samples were stabilized by adding a solution of RLT buffer and beta-mercaptoethanol then vortexed vigorously for 1 minute. The lysate would then be transferred to the purple spin column in a 2 mL collection tube and centrifuged (Centrifuge 5425; Eppendorf AG, Hamburg, Germany) for 2 minutes at 15,060 rpm. The supernatant of the flow was then transferred to another 1.5 mL RNase free microcentrifuge tube. The original spin column and collection tube were disposed. Next 220 µL of 100% ethanol was added to the supernatant via pipette. The resultant solution was transferred to a pink spin column in a 2 mL collection tube and centrifuged for 20 seconds at 10,000 rpm. Supernatant was discarded and 350 mL of RWI buffer was added to the center of the spin column and centrifuged at 10,000 rpm for 20 seconds. Supernatant was discarded and 80 µL of a mixture solution (10 µL DNase1 + 70 µL RDD buffer) was added to the center of the spin column then let to sit for 15 minutes at room temperature. Once complete 350 µL RWI buffer was added to the spin column and centrifuged at 10,000 rpm for 20 seconds. Supernatant was discarded and 500 µL RPE buffer was added to the center of the spin column and centrifuged at 10000 rpm for 20 seconds. Supernatant was discarded again and another 500 µL RPE buffer was added to the center of the spin column and centrifuged at 10,000 rpm for 2 minutes. Resultant solution was then placed in a new collection tube and centrifuged for 1 minute at 15,060 rpm. Finally, resultant solution was placed in 1.5 mL RNase free microcentrifuge tube, 50 µL of RNase free water was added and then centrifuged for 1 minute at 10,000 rpm. All these steps were done while keeping samples on ice.

RNA was isolated in 50 µL aliquots from each in 1.5 mL tube. RNA was analyzed using a NanoDrop™ (One/One^{C} Microvolume UV-Vis Spectrophotometer; Thermo-Fisher Scientific, St. Louis, MO, USA) for concentration, while quality was tested with an aliquot of the RNA on denaturing agarose gel stained with ethidium bromide. Once passing concentration and quality benchmarks, RNA samples were diluted to a standard concentration of 15 µg µL^{-1}. This allowed for gene expression in qualitative polymerase chain reaction (qPCR) analysis to be comparable between samples. Primers

were developed for a candidate cytochrome P450 gene associated with non-target site resistance (CYP1174), whereas actin and GADPH were included in qPCR analysis as reference genes. During qPCR, 17 µL of the reductase for each gene was added to the qPCR plate, then 2 µL of each sample would be added to each cell for a total of 19 µL. The kits used to prepare samples for the qPCR were a first strand synthesis system (SuperScript™ III First-Strand Synthesis System; Thermo-Fisher Scientific, St. Louis, MO, USA) with gene specific primers and fluorescent mix (SsoAdvanced Universal SYBR Green Supermix; Bio-Rad Laboratories Inc., Hercules, CA). The cDNA transformation and qPCR analysis were run through a thermal cycler (CFX96 Real-Time PCR Detection System; Bio-Rad Laboratories Inc., Hercules, CA). Forty-four samples were analyzed during July 2021. Analyzed samples of each *Poa annua* collection were comprised of multiple seedlings surviving the discriminatory dose of indaziflam, whereas previous qPCR research with indaziflam only included root tips where indaziflam is known to be absorbed and active (González-Delgado and Shukla 2020).

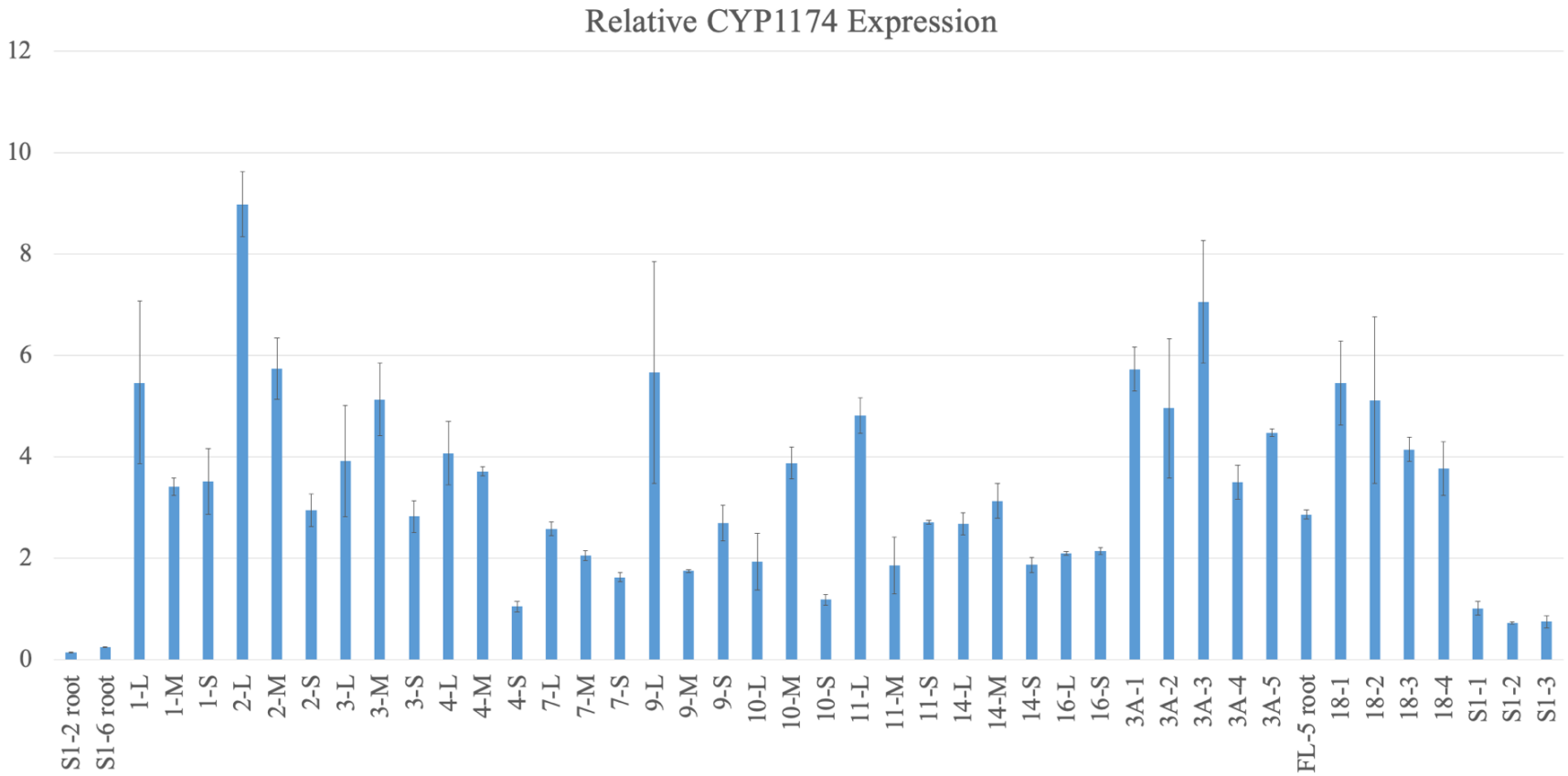
Results and Discussion

Relative expression of the candidate cytochrome P450 gene is presented in Figure A.1. All samples of resistant plant material (i.e., those producing new roots following exposure to 750 pM indaziflam in Gelrite® culture) exhibited higher expression of CYP1174 than herbicide susceptible *Poa annua*. However, there was not a clear relationship among root length categories within each resistant collection with several selections in the medium- and short root length categories yielding similar relative expression values. Interestingly, there was a general trend across resistant collections for plants in the long-root length category to yield relative expression values of CYP1174 that were greater than or equal to those in the medium- or short- root length categories. Plants within resistant Collection 2 that fell within the long- root length category (2-L) exhibited the highest expression of any sample tested (8.99) whereas collection 4-S exhibited the lowest expression of any resistant sample tested (1.05).

Two samples, S1-2 Root and S1-6 Root, were included from previous testing to compare whole plant expression of CYP1174 to that observed only in root tissue.

Expression values for root-only samples were 0.14 and 0.25, respectively. Comparatively, expression of CYP1174 was 3 to 7 times greater using whole plant samples with values for S1-1, S1-2, and S1-3 measuring 1.02, 0.73, and 0.75, respectively.

Figure A.1. *Poa annua* collections subjected to quantitative polymerase chain reaction analysis at Michigan State University (East Lansing, MI) in July 2021. Whole plants were organized into categories [short (S; root length < 6 mm), medium (M; root length 9 to 14 mm), or long (L, root length > 16 mm)] based on the root length (mm) following exposure to 750 pM indaziflam in a gel bioassay. Expression of a cytochrome P450 gene (CYP1174) associated with non-target site resistance in each collection and root category with error bars representing ranges of expression within samples.


Relative CYP1174 Expression
12
10
8
6
4
2
0
S1-2 root
S1-6 root
1-L
1-M
1-S
2-L
2-M
2-S
3-L
3-M
3-S
4-L
4-M
4-S
7-L
7-M
7-S
9-L
9-M
9-S
10-L
10-M
10-S
11-L
11-M
11-S
14-L
14-M
14-S
16-L
16-S
3A-1
3A-2
3A-3
3A-4
3A-5
FL-5 root
18-1
18-2
18-3
18-4
S1-1
S1-2
S1-3

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IMPLEMENTING BIOASSAY IN WEED DIAGNOSTICS CENTER

Putative-resistant *Poa annua* collections submitted to the University of Tennessee Weed Diagnostics Center in the future will be cultured for seed as previously described and screened using the bioassay described herein. The bioassay will deploy three Petri plates containing 750 pM indaziflam in Gelrite® culture, as well as three non-treated control plates (5pM) per *Poa annua* collection. Details for dilution and solution math are outlines in Table A.1. A standard resistant and susceptible collection, Site 18 and S1, respectfully, will be plated along with putative-resistant collections. All collections tested should be placed in cold storage (-20˚C) for four weeks minimum. Plates will be seeded as previously described and stored in a controlled-environment growth chamber for 14 days after seeding. Root length will be assessed as previously described with values for putative resistant collections compared to indaziflam resistant and susceptible standards using a two-Sample t-test in R (v. 3.6.2) at $\alpha = 0.05$.

Table A.1. Technical indaziflam dilution math for the 750 pM discriminatory dose as well as the non-treated concentration of 5 pM. All dilutions were completed using dimethylsulfoxide (DMSO), Millipore deionized (DI) water, sterilized glass stirrers, and sterilized glass beakers. These concentrations will be used for future testing to detect indaziflam resistance in *Poa annua*.

5 pM	**750 pM**
Solution A: 3 mL DMSO + 150 mg technical indaziflam	Solution A: 3 mL DMSO + 150 mg technical indaziflam
Solution B: 3 mL Solution A + 96.54 mL DMSO (5000 μM)	Solution B: 3 mL Solution A + 96.54 mL DMSO (5000 μM)
Solution C: 1 mL Solution B + 999 mL DI water (5000 nM)	Solution C: 1 mL Solution B + 999 mL DI water (5000 nM)
Solution D: 2 mL Solution C + 148 mL DI water (75 nM)	Solution D: 2 mL Solution C + 148 mL DI water (75 nM)
Solution E: 1 mL Solution D + 99 mL DI water (750 pM)	-
5 pM Solution: 13 mL Solution E + 13.65 mL Rifampicin Antibiotic + 1923.45 mL Gelrite® culture	750 pM Solution: 20 mL Solution D + 14 mL Rifampicin Antibiotic + 1966 mL Gelrite® culture

VITA

Benjamin D. Pritchard was born on December 25th, 1998 in Bluff City, TN. He lived there until he moved to Knoxville, TN in Fall 2017 to attend the University of Tennessee. Ben graduated from the University of Tennessee in May 2020 with Bachelor of Science in Plant Sciences. He also graduated with Chancellor's honors as well as two chancellor's citations for extraordinary academic achievement and extraordinary professional promise. Ben also was named the Plant Science department outstanding senior of 2020. In Fall 2020, Ben began his Master's in turfgrass weed science. His project was developing a bioassay to detect indaziflam resistant collections of *Poa annua* to assist turfgrass managers in identifying this problem in a timelier manner. This will provide them with answers as to what they should apply for control of the troublesome weed of turfgrass *Poa annua*. Ben wrote his thesis and defended it on March 29th, 2022. Then graduate on May 20th, 2020. He will pursue a PhD at the university studying turfgrass weed science beginning June 2022.